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THESE

Pour obtenir le grade de Docteur en sciences agronomiques

Spécialité : Amélioration des productions végétales

TELLAH Sihem

Etude des mécanismes agrophysiologiques, morphologiques et moléculaires impliqués dans la tolérance au stress hydrique chez quelques populations locales d'arachide (Arachis hypogaea L.)

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Jury

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Avant-propos

Les travaux présentés dans cette thèse ont été réalisés en partie au sein du département d'agriculture (Dipartimento di Agraria), laboratoire de« Chimie Agricole » (Biologia Applicata ai Sistemi Agro Alimentari e Forestali, curriculum: Ecofisiologia delle specie vegetali) à l'Université Méditerranéenne de Reggio de Calabre, Italie et en partie au laboratoire « d'amélioration des légumineuses alimentaires» à l'Ecole Nationale Supérieure Agronomique d'El Harrach, Alger, Algérie. Ce travail a été soutenu par trois projets:

Un projet PNR : sous le thème "AMELIORATION DES LEGUMINEUSES ALIMENTAIRES POUR LA TOLERANCE AU STRESS HYDRIQUE."

Un projet CNEPRU portant sur « L'amélioration de la culture de l'arachide sous contrainte hydrique ».

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L'objectif de ces projets de recherche est l'étude de comportement en milieu contrôlé des associations (plante-rhizobium) vis-à-vis du stress hydrique et cela par une étude morphologique, physiologique, agronomique, moléculaire et technologique des populations d'arachide collectées et leurs rhizobia.

Les perspectives de ce projet visent à définir une stratégie dynamique de conservation et une exploitation raisonnée de la diversité génétique de ces populations collectées à travers le territoire algérien.

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Sihem

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-Laboratoire de ***Chimie Agricole****, Département d'Agriculture, Université méditerranéenne de Reggio de Calabre, Italie.*

-Laboratoire de ***Physiologie Végétale****, Département des sciences biologiques et de l'environnement, Université de Messine, Italie.*

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Sihem

Etude des mécanismes impliqués dans la tolérance au stress hydrique chez quelques populations agrophysiologiques, morphologiques et moléculaires locales d'arachide (Arachis hypogaea L.)

Résumé

Le déficit hydrique affecte considérablement la croissance des plantes : c'est, sans doute (avec la température), l'un des facteurs majeurs qui limite la production végétale en conditions naturelles. En dépit du niveau de résistance assez important développé par l'arachide (*Arachis hypogaea* L.), sa production dans les écosystèmes secs est affectée par des périodes de déficit hydrique survenant au cours du cycle de croissance. L'objectif de cette thése est de mettre en évidence, les réponses au déficit hydrique faible, modéré puis sévère de dix populations d'arachides d'origine locale inoculées par un isolat rhizobien, en vue d'identifier des critères pour la sélection au sein de ces populations locales qui proviennent de différentes conditions pédoclimatiques représentatives du territoire algérien, celles les mieux adaptées à la sécheresse.

Les mécanismes étudiés ont porté principalement sur les aspects agro-physiologiques (hauteur de la tige, surface foliaire, nombre de ramifications ou branches, nombre de feuilles, poids frais et sec des feuilles, teneur totale de la chlorophylle (Chl)), aspects morphologiques et topologiques profonds des racines (la longueur, le diamètre, et la surface des racines), aspects morphologiques et topologiques des nodules (le diamètre, le nombre, la forme, le poids frais et sec des nodules), aspects hydriques de la plante (la teneur relative en eau (TRE), potentiel hydrique foliaire (PHF), résistance stomatique (RES),température du couvert végétal (TCV), la quantité en dioxyde de carbone (CO_2) et la vapeur d'eau des taux de change,la concentration en CO_2 ambiant et la densité des flux photosynthétiques,la conductance foliaire et le potentiel hydrique de la feuille au point de perte de turgescence (Ψtlp) et le potentiel osmotique à la pleine turgescence par des courbes pression-volume, aspects biochimiques (les niveaux et les activités de métabolites antioxydants, y compris l'acide ascorbique, le glutathion réduit (GSH) ainsi que des enzymes antioxydantes, tel que la superoxide dismutase (SOD) ont étaient mesurés, aspects moléculaires (pour l'enzyme antioxydante, à savoir la superoxide dismutase SOD, les effets des traitements sur l'abondance des transcrits d'ARNm ont étaient également évalués, des isolements ont étaient réalisés selon les techniques d'amplification et de séquençage des séquences nucléotidiques spécifiques classiques et cela par des analyse moléculaires et des protocoles standardisés. Les principaux résultats obtenus montrent que les deux populations Berrihane et Tonga, montrent une tolérance au stress hydrique, contrairement aux populations Oum Tboul, Boumalek et El

Frin qui se sont avérées sensibles aux conditions de déficit hydrique. En raison des différentes provenances de ces populations d'arachide étudiéess, il y a une ample variabilité au sein de la collection pour cela elle peut être considérée comme un inventaire utile des stratégies et des mécanismes agro-physiologiques, morphologiques et moléculaires déployés pour la tolérance au stress hydrique. De plus, la variabilité génétique du matériel végétal vis-à-vis du stress hydrique ne semble pas liée à l'origine géographique des populations d'arachides, mais plutôt à leur phénologie et à leur morphologie. Cette étude pourrait servir à la définition de critères pertinents de résistance à la sécheresse utilisables en sélection variétale. Ce travail a permis de constituer la première collection d'arachide (*Arachis hypogaea* L.) dédiée à l'étude de l'adaptation des plantes aux conditions environnementales stressantes.

Mots clés: Arachide, diversité, populations naturelles, stress hydrique, tolérance, sélection

Study of agrophysiological, morphological and molecular mechanisms involved in tolerance to water stress in some local populations of peanut (Arachis hypogaea L.)

Abstract

The water deficit significantly affect plant growth: it is probably (with temperature), one of the major factors limiting crop production under natural conditions. Despite the fairly large resistance level developed by the peanut (*Arachis hypogaea* L.), its production in dry ecosystems is affected by periods of water deficit occurring during the growth cycle. The objective of this thesis is to highlight the responses to the low water deficit, moderate and severe of ten local peanut populations from local origin inoculated by one rhizobial isolate, to identify criteria for selection within of these populations, who come from different soil and climatic conditions representative of the Algerian territory, those best adapted to drought.

The studied mechanisms focused on agro-physiological aspects (stem height, leaf area, number of branches, number of leaves, fresh and dry weight of leaves, total content of chlorophyll (Chl)), deep morphological and topological aspects of the roots (length, diameter, and root surface), morphological and topological aspects of nodules (diameter, number, shape, fresh and dry weight of nodules), water aspects of the plant (the relative water content (RWC), leaf water potential (Ψ_{Lmin}), stomatal resistance (r), leaf canopy temperature, the amount of carbon dioxide (CO_2) and vapor water exchange rate, the ambient CO_2 concentration and density photosynthetic flux, leaf conductance and leaf water potential at turgor loss point (Ψtlp) and osmotic potential at full turgor by curves of pressure- volume, biochemical aspects (the levels and activities of antioxidant metabolites, including ascorbic acid, reduced glutathione (GSH) and antioxidant enzymes, such as superoxide dismutase (SOD) were measured,molecular aspects (for antioxidant enzyme, namely the superoxide dismutase SOD, the effects of treatments on the abundance of mRNA transcripts have also been evaluated, isolations were performed according to amplification techniques and sequencing conventional specific nucleotide sequences by molecular analysis and standardized protocols.

The main results show that both Tonga and Berrihane populations show a tolerance to water stress, unlike populations of Oum Tboul, Boumalek and El Frin which proved sensitive to water deficit conditions. Due to the different origins of these studied peanut populations, there is a wide variability in the collection of peanut, for this reason, it can be considered as a useful inventory of strategies and agro-physiological, morphological and molecular mechanisms deployed for tolerance to water stress. In addition, the genetic variability of plant material

towards water stress does not seem related to the geographical origin of peanut populations, but to their phenology and morphology. This study could be used to define relevant criteria of drought resistance for using in varietal selection. This work allowed to create the first collection of peanut (*Arachis hypogaea* L.) dedicated to the study of plant adaptation to stressful environmental conditions.

Keyswords: Peanut, diversity, natural populations, water stress, tolerance and selection.

دراسة

ملخـــص

دراسة الآليات الزراعية – الفيزيولوجية – المورفولوجية – والجزيئية التي تشارك في مقاومة الإجهاد المائي عند بعض السلالات المحلية للفول السوداني *(Arachis hypogaea L.)* .

نقص الماء يؤثر تأثيرا كبيرا على نمو النباتات وربما هو (مع درجة الحرارة) واحد من العوامل الرئيسية التي تحد إنتاج المحاصيل في ظل الظروف الطبيعية.

وعلى الرغم من مستوى المقاومة إلى حد كبير التي يضعها الفول السوداني *(Arachis hypogaea L.)* يبقى مردوده في النظم الإيكولوجية الجافة متأثرا بفترات الجفاف التي تحدث أثناء دورة النمو.

الهدف من هذا البحث العلمي هو تسليط الضوء على مقاومة الإجهاد المائي المنخفض ، المتوسط والشديد لعشرة سلالات محلية من الفول السوداني ملقحة بسلالة بكتيرية وذلك لتحديد معايير الاختبار داخل هذه السلالات المحلية الآتية من مختلف المناطق الجزائرية ، تلك الأفضل تكيفا مع الجفاف.

الآليات المدروسة تركز على الجوانب الزراعية ، الفيزيولوجية (طول الساق، مساحة الورقة، عدد الفروع، عدد الأوراق، الوزن الرطب والجاف للأوراق والمحتوى الكلي للكلوروفيل) الجانب المورفولوجي والطوبوغرافي العميق للجذور (طول قطر ومساحة الجذور) الجانب المورفولوجي والطوبوغرافي للعقيدات (القطر، العدد، الشكل ،الوزن الرطب والجاف) الجانب المائي للنبتة (المحتوى النسبي للماء , (RES) (TRE),(PHF) ودرجة الحرارة للغطاء النباتي (TCV) كمية ثاني أكسيد الكربون (CO_2) كثافة تدفق التركيب الضوئي و(Ψ_{tlp}) الجانب البيوكيميائي مثل قياس مستويات بعض مضادات الأكسدة كحمض الأسكوربيك والجلوتاثيون (GSH) وبعض الأنزيمات المضادة للأكسدة مثل (SOD) وفي الأخير دراسة الجانب الجزيئي ، مثل قياس وتقييم كمية أنزيم (SOD) عن طريق التحليل البيو جزيئي.

أظهرت النتائج الرئيسية أن كلا من (BER) Berrihane, (AD) (TO)Tonga, (OT) Oum Tboul Adrar, , (BMK) Boumalek أظهروا مقاومة للإجهاد المائي عكس (OS) Oued Souf, (MET)Metlili , Sebseb (SEB) , (FR) El Frin, (TIM) Timimoun الذين أظهروا أنهم حساسون للإجهاد المائي، ونظرا إلى الأصول والأماكن المختلفة لهذه السلالات المدروسة من الفول السوداني فهناك تباينا كبيرا من هذه السلالات لهذا يمكن اعتبارها مثل مخزن نافعا في الاستراتيجيات والآليات الزراعية – الفيزيولوجية – المرفولوجية والجزيئية المخصصة لمقاومة الجفاف، وبالإضافة إلى ذلك فإن التباين والاختلاف الوراثي للنبات بالنسبة لمقاومة الجفاف لا تبدو مرتبطة بالأصل الجغرافي لسلالة الفول السوداني ولكن للمورفولوجية والفيزيولوجية.

يمكن استخدام نتائج هذه الدراسة في تحديد المعايير لمقاومة الجفاف التي يمكن استخدامها في الاختبار النباتي، هذا العمل ساعد على انشاء أول مجموعة من الفول السوداني*(Arachis hypogaea L.)* المخصصة لدراسة تكيف النبات للظروف البيئية الصعبة .

الكلمات المفتاحية : الفول السوداني ، التنوع ، السلالات الطبيعية ، الجفاف ، المقاومة ، الاختبار.

Liste des abbréviatins

AD: Adrar
ADN : Acide désoxyribonucléique
ADNc : ADN complémentaire
APX: Ascorbate Peroxidase
ARN: Acide ribonucléique
ARNm: ARN messager
AsA: Ascorbic Acid
ATP: Adénosine Tri-Phosphate
BER: Berrihane
BLAST: Basic Local Alignment Ssearch Tool
BMK: Boumalek
CAT: Catalase
CTAB: Hexadecyltrimethyl-Ammonium Bromide
DAS: Days After Sowing
DHA: Dehydroascorbate
DHAR: Dehydroascorbate Reductase
dNT: Désoxy Nucléotide Tri Phosphate
DTT : Dithiothreitol
EDTA : Ethylène Diamine Tétra Acétate
EST : Expressed Sequence Tag
FAO : Food and Agriculture Organization
FR: El Frin
FSN : Fixation Symbiotique d'Azote
GR: Glutathione Reductase
GSH: Reduced Glutathione
H_2O_2: Hydrogen Peroxide
kDa: kiloDalton
LEA: Late Embryogenesis Abundant
m/v : Masse/Volume
MDA: Malondialdehyde
MDHAR: Monodehydroascorbate Reductase
MET: Metlili
NBT: Nitro Blue Tetrazolium
NCBI : National Center of Biotechnology Information
$O_2^{\bullet-}$: Superoxide Anion Radical
$^1O_2$: Singlet Oxygen
OS: Oued Souf
OT: Oum Tboul
pb : Paire de Base
PCR : Polymerase Chain Reaction
PVPP: Polyvinylpoly-Pyrrolidone
RACE: Rapid Amplification of cDNA Ends
RNase: Ribonuclease
ROS: Reactive Oxygen Species
SDS: Sodium Dodecyl Sulfate
SEB: Sebseb
SOD: Superoxide Dismutase
SSTE : Sodium Chloride SDS TE
TAE : Tris acide Acétique EDTA

Liste des abbréviatins

TBS : Tris Buffer Saline
TCA: Trichloroacetic Acid
TE: Tris EDTA
TIM: Timimoun
TO: Tonga
UV : Ultra-Violet
v/v : Volume/Volume
X-Gal: 5-Bromo-4-Chloro-3-Indolyl-ß-D-Galactoside

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Introduction générale

Introduction générale

L'eau a un rôle fondamental dans la vie des plantes, dans la mesure où elle conditionne leurs activités physiologiques et métaboliques et sert de de vecteur aux éléments nutritifs de la plante **(Riou, 1993)**. ; elle est, de ce fait, le principal facteur limitant de la production végétale dans les régions arides et semi-arides.

Au cours de leur cycle végétatif, les plantes subissent les conditions du milieu ambiant dans lequel elles vivent. Elles réagissent en s'adaptant à la variation de ces conditions lorsqu'elles deviennent défavorables. La variation des conditions de croissance a pour origine les stress qui sont de nature biotiques ou abiotiques. Dans les milieux arides et semi-arides les stress abiotiques tels que les déficits hydriques imposent des limites au développement de la plante. La résistance à ces stress est dépendante du génotype qui développe des mécanismes morphologiques, physiologiques et/ou biochimiques pour échapper (esquiver), éviter ou tolérer la contrainte **(Levitt, 1982).**

Chez les légumineuses ces contraintes s'appliquent non seulement sur la plante mais aussi sur leurs symbiotes. Les deux partenaires réagissent différemment en réponse au stress hydrique. Les nodosités qui ne possèdent pas de mécanismes efficaces de tolérance sont les premières affectées et souvent leur capacité à fixer l'azote est inhibée de façon irréversible **(Ounane, 2004)**. En effet, la contrainte hydrique et les perturbations osmotiques qui en découlent, peuvent affecter la symbiose rhizobium-légumineuse, en diminuant la croissance et la survie des rhizobiums dans le sol, en réduisant la colonisation de la rhizosphère ou en inhibant totalement ou partiellement le processus d'infection et de développement des nodosités.
L'arachide (*Arachishypogaea*L.) est l'une des plus importantes oléagineuses cultivées dans le monde, notamment dans les régions arides et semi-arides de l'Afrique **(Wani *et al.*, 1995)**.

Plastique et rustique, l'arachide présente l'avantage agronomique d'exiger peu d'intrants, de couvrir très vite et donc de protéger le sol par un épais tapis herbacé (sur une hauteur de 20 à 70 cm).Son succès réside également dans le fait que c'est une oléo-protéagineuse aux nombreuses utilisations alimentaires et fourragères (graine, huile, tourteaux, fanes et coques). Elle peut fournir jusqu'à 30 % de l'azote nécessaire à la plante mais la quantité d'azote fixée est très variable car l'activité symbiotique est influencée par plusieurs facteurs tels que la souche bactérienne, la plante hôte et les conditionsdu milieu **(Wani *et al.*, 1995)**. La recherche de couplesplante/rhizobium tolérants capables de fonctionner dans ces conditions difficiles permet

d'identifier et de comprendre les paramètres et les mécanismes physiologiques liés à ce caractère, et pouvant servir de base à l'établissement de critères de sélection de cette association.

La plupart des travaux effectués sur l'arachide dans le cadre de l'amélioration génétique de la tolérance au stress hydrique, se sont donnés pendant longtemps pour objectif primordial l'augmentation de la productivité, une approche basée sur les performances agronomiques. Actuellement, les programmes d'amélioration de l'arachide s'intéressent de plus à l'amélioration génétique de la tolérance au stress hydrique. Cette amélioration exige d'étudier, d'identifier et de vérifier les caractères phénologiques, morpho physiologiques et biochimiquesliés au rendement en condition de stress hydrique **(Pfeiffer *et al.*, 2000)** . De même, l'étude génétique par la recherche de marqueurs moléculaires du mode de transmission et de l'héritabilité des caractères repérés, comme bons indicateurs de la tolérance au stress hydrique est nécessaire pour faciliter l'utilisation de ces caractères dans les programmes de sélection **(Pfeiffer *et al.*, 2000)**.

L'objectif de ce travail est de caractériser les mécanismes agro morphologiques, physiologiques, biochimiques et moléculaires pouvant être à l'origine d'une tolérance accrue au stress hydrique des plantes par l'évaluation de leur comportement sous cette contrainte à travers des essais en conditions contrôlées tout en essayons de répondre à certaines questions de recherche à savoir :

-Existe-t-il une variabilité de réponses (agro-physiologiques et morphologiques) chez les différentes populations d'arachide étudiées vis-à-vis de la contrainte hydrique?

-La capacité de développement racinaire constitue-t-elle un mécanisme important d'adaptation à la sécheresse ?

-Quels sont les mécanismes antioxydants impliqués dans la tolérance ou la sensibilité vis à vis du stress hydrique?

-Et enfin, quels sont les gènes différentiellement exprimés entre ces populations sensibles voir tolérant lors du stress hydrique?

Ce travail nous permettra donc de rechercher les populations d'arachide les plus performantes, ayant la capacité de tolérer les déficits hydriques qui peuvent se produire à un stade critique de développement de cette espèce. Il porte essentiellement sur :

♣ L'étude de la variabilité de réponses (agro-physiologiques, morphologiques, biochimiques) chez les différentes populations d'arachidevis-à-vis de la contrainte hydrique ;

♣ L'identification des populations d'arachide les plus tolérantes à la sécheresse et l'ètude de leur capacité de développement racinaire qui constitue un mécanisme important d'adaptation à la sécheresse ;

♣ La caractérisation des mécanismes de réponse au déficit hydrique, ayant trait aux systèmes antioxydants,

♣ La détermination des mécanismes antioxydants potentiellement impliqués dans la tolérance ou la sensibilité vis à vis du stress hydrique ;

♣ L'analyse de l'expression des gènes induits par la contrainte hydrique.

Le présent travail comporte cinq chapitres. Le premier chapitre rapporte une synthèse bibliographique sur la problématique du déficit hydrique et seseffets sur la croissance et le développement des plantes en général et de la symbiose arachide-rhizobia en particulier. Le deuxième chapitre traiteles mécanismes agrophysiologiques et morphologiques qui contribuent à la tolérance au stress hydrique chez l'arachide (*Arachishypogaea* L.) Le troisième chapitre porte sur l'implication des mécanismes antioxydants enzymatiques et non enzymatiques dans la tolérance au stress hydrique. Ensuite, le quatrième chapitre est consacréà l'étude de l'expression des gènes impliqués dans la réponse austress hydrique. Enfin, « L'intérêt agricole, socio-économique et cultural des espèces autochtones, en particulier les populations locales de légumineuses alimentaires, en Afrique du Nord » constitue le cinquième chapitre de cette thèse de doctorat.

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Chapitre I

Synthèse bibliographique

Synthèse bibliographique

Dans le contexte de l'adaptation des légumineuses alimentaires au stress abiotique plus particuliérement le stress hydrique, ce travail de synthèse a pour but de résumer les connaissances actuelles concernant l'importance de l'arachide d'une part en tant que légumineuse à graines très consommée dans le monde, mais aussi pour son extéme plasticité et rusticité **(arachisracin.htm)**. L'arachide peut se contenter de sols très légers et même très pauvres. Fixatrice d'azote, l'arachide peut même enrichir le substrat en cet élément souvent déficient dans les sols. La symbiose rhizobium-légumineuses situe souvent l'arachide dans la succession des cultures en tant que culture restauratrice d'une certaine fertilité. On la cultive donc après des plantes plus "épuisantes" **(arachisracin.htm)**. Dans le cadre d'une agriculture durable, le développement de cultivars tolérants et performants est l'une des approches les plus envisageables pour réduire les effets délétères de diverses contraintes biotiques et abiotiques. L'adaptation aux différentes contraintes abiotiques est un phénomène complexe qui intègre aussi bien des modifications agromorphologiques que des ajustements physiologiques biochimiques et moléculaires dont le stress hydrique, par exemple, représente une thématique largement abordée par les équipes d'Ecophysiologie **(Pfeiffer *et al.,* 2000)**.

1. Importance des légumineuses alimentaires

Les légumineuses alimentaires, telle que l'arachide *(Arachis hypogaea* L*)* sont des cultures importantes dans les pays en développement des tropiques, en particulier en Afrique sub-saharienne, en Asie et en Amérique Centrale et du Sud **(Singh *et al.,* 1997)** et dans certaines zones tempérées, y compris la région de la Méditerranée et le sud des États-Unis **(Pasquet, 2000)**.

Les légumineuses alimentaires constituent une composante essentielle pour la nutrition humaine et animale ainsi que pour le maintien de la fertilité des systèmes de production dans les zones arides et semi arides. Elles représentent une famille d'une grande importance économique et occupent le second rang après les céréales comme culture alimentaire dans le monde **(Rochester *et al.,* 2001)**. Leur intérêt réside dans leur richesse en protéines de qualité, et constituent en association avec les céréales, la base de l'alimentation de milliards de personnes et une source importante de fourrage et de produits naturels **(Werner et Newton, 2005)**. Depuis la restructuration du secteur agricole et sa libéralisation en 1987, ces cultures ont connu des perturbations dont les conséquences se traduisent par une réduction de la superficie, une chute

de la productivité et une marginalisation accentuée par le manque de soutien et d'encouragement à ce secteur jusqu'à l'avènement du plan national de développement agricole en 2000.

En parallèle, les importations ne cessent d'augmenter pour satisfaire les besoins de plus en plus croissants de la consommation humaine en accentuent ainsi notre dépendance vis-à-vis de l'extérieur pour notre approvisionnement.

Les légumineuses alimentaires sont cultivées sur l'ensemble des zones agro-écologiques d'Algérie. En effet, de la zone littorale jusqu'aux hauts-plateaux, on peut trouver l'ensemble des espèces telles que la fève et la féverole, le pois chiche, le pois, le haricot, la lentille ainsi que l'arachide.

La production de l'arachide en Algérie n'est pas négligeable mais connaît des fluctuations, un minimum de 25 510 Qx est enregistré en 2013 et un maximum de 46 210 Qx en 2001 avec une moyenne dépassant 34 921 Qx entre 2000 et 2013. Ces fluctuations sont liées essentiellement à des variations des superficies consacrées à cette culture, mais ces dernières n'ont pas beaucoup influè sur l'évolution des rendements durant la même période. Celles-ci tourne autour de 11,54 Qx/Ha **(MADR, 2013)**. Les régions d'El-Tarf, El-Oued, Ghardaïa, Adrar, Bechar et el Bayadh sont les régions productrices d'arachide en Algérie. La mauvaise répartition des précipitations dans l'espace et dans le temps représente le principal facteur limitant la production en culture pluviale. En année sèche, des surfaces considérables sont déclarées non productives, ce qui provoque des fluctuations inter-annuelles des surfaces emblavées. Cette situation rend, dans certains cas, les agriculteurs hostiles à l'introudction des légumineuses alimentaires dans leur assolement. Le tableau I.1. donne l'evolution des superficies, des productions et des rendements des légumineuses alimentaires en Algérie durant la période 2000-2013 d'aprés les statistiques de la **FAOSTAT,2015**.

Tableau I.1. *Evolution des superficies, des productions et des rendements des légumineuses alimentaires en Algérie durant la période 2000-2013* ***(FAOSTAT, 2015).***

Périodes / Espèces	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Superficie (ha)														
Lentille	690	920	920	840	862	1 090	1 218	873	1 369	2 588	4 230	6 900	6 250	5 546
Pois-chiche	19 480	19 290	19 330	22 850	23 079	23 348	21 252	20 681	20 361	22 274	25 520	32 000	30 580	29 321
Fève	34 250	31 450	33 610	34 050	36 777	35 082	33 537	31 284	30 688	32 278	34 210	34 500	36 858	37 676
Pois sec	7 170	6 570	7 070	8 680	9 328	8 299	9 157	9 184	7 556	8 487	8 860	9 200	9 900	11 096
Haricot sec	1 280	1 180	1 190	1 560	1 992	1 206	1 496	1 394	1 040	1 616	1 210	1 200	1 575	1 427
Arachide	4 020	4 250	3 750	3 380	4 080	2 830	2 720	2 680	2 840	2 570	2 520	2 558	2 293	2 249
Production (T)														
Lentille	194	458	435	490	613	423	654	561	1 081	2 693	4 590	7 000	5 738	6 318
Pois-chiche	6 661	12 312	14 971	19 102	16 367	13 727	12 706	14 294	11 211	17 840	23 470	29 000	27 675	34 980
Fève	12 895	21 230	22 933	30 700	32 053	26 886	24 298	27 974	23 521	36 495	36 620	37 000	40 507	42 386
Pois sec	1 593	3 674	4 304	6 342	7 371	5 339	5 381	6 243	3 618	5 969	6 610	7 300	9 178	10 586
Haricot sec	419	734	864	1 096	1 581	666	915	917	544	1 159	840	800	1 024	1 362
Arachide	3 894	4 621	4 616	3 842	4 269	3 304	2 995	3 319	3 222	3 057	3 385	3 037	2 778	2 551
Rendement (qx/ha)														
Lentille	2.18	4.97	4.72	5.83	7.11	3.88	5.36	6.42	7.89	10.40	10.85	10.14	9,18	11.39
Pois-chiche	3.41	6.38	7.74	8.36	7.09	5.87	5.97	6.91	5.50	8.00	9.19	9.09	9.05	11.93
Fève	3.76	6.75	6.82	9.01	8.71	7.66	7.24	8.94	7.66	11.30	10.70	10.72	10.99	11.25
Pois sec	2.22	5.59	6.08	7.30	7.90	6.43	5.87	6.79	4.78	7.03	7.46	7.93	9.27	9.79
Haricot sec	3.27	6.22	7.26	7.02	7.93	5.52	6.11	6.57	5.23	7.17	6.94	6.66	6.50	9.54
Arachide	9.68	10.87	12.30	11.36	10.46	11.64	11.01	12.38	11.34	11.89	13.43	11.90	12.11	11.34

La culture des légumineuses alimentaires en Algérie est soumise à un certain nombre de contraintes tant techniques et économiques qu'organisationnelles qui limitent sa production, son développement et son extension **(INRAA, 2006)**, (Figure I.1.).

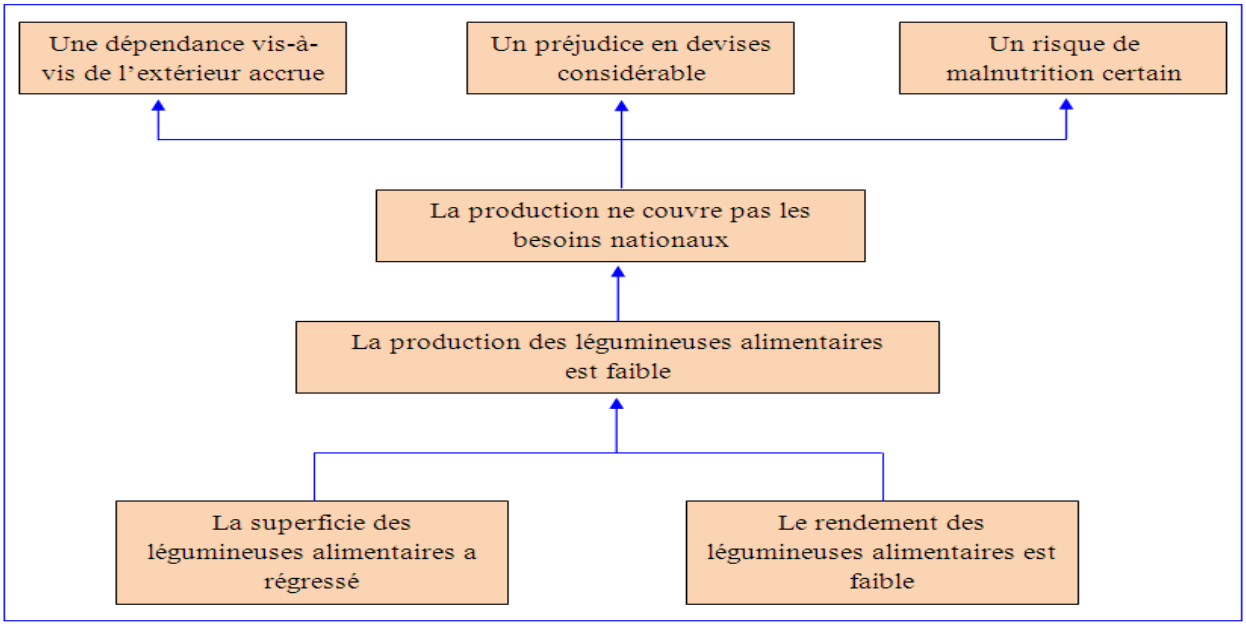


Figure I.1. *Les conséquences de faible rendement des légumineuses alimentaires en Algérie* ***(INRAA, 2006).***

2. L'arachide (*Arachis hypogaea* L.)

Les légumineuses sont des plantes de la famille des *Leguminosae* ou *Fabaceae*, qui ont une distribution mondiale estimée entre 18 000 et 20 000 espèces reparties dans environ 750 genres **(Vincent, 1970)**. Les taxonomistes, sur la base de différences florales, ont divisé cette famille en trois sous familles distinctes, les Caesalpinacées (3%), les Mimosacées (13%) et les Papilionacées (84 %) **(De Faria *et al.*, 1989)**. La famille des Leguminosae renferme de nombreuses espèces comestibles (arachide, fève, pois chiche, pois, soja, haricot), ornementales (genêt, glycine, etc.), fourragères (trèfle, sainfoin, luzerne, gesse). Les légumineuses sont souvent cultivées en alternance avec d'autres espéces, car elles sont capables de fixer l'azote de l'air et de réduire ainsi les apports d'engrais chimiques.

2.1. Origine et diffusion mondiale

L'Arachide est originaire de l'Amérique tropicale. Sa dissémination s'est faite à partir de la côte Péruvienne en direction de l'Extrême – Orient d'une part, et à partir de la côte Brésilienne en direction de l'Afrique de l'Ouest d'autre part. La première introduction en Europe aurait été faite à partir du Brésil **(Clavel et Gautreau, 1997; Schilling *et al.*, 1997)**.

Par la suite, elle a couvert la totalité des zones tropicales à partir de deux centres de diversifications secondaires de l'espèce, l'Afrique de l'Ouest et le Sud-est Asiatique. Actuellement, cette culture déborde très largement son aire d'origine, puisqu'on la retrouve jusqu'aux 40 ème parallèles nord et sud et sur tous les continents lorsque les étés chauds permettent à la plante de boucler son cycle malgré la latitude élevée **(Schilling, 2003)**.

2.2. Caractéristiques génétiques, systématique et types variétaux

L'espèce sauvage *Arachis monticola* tétraploïde, est considérée comme l'ancêtre probable de l'arachide cultivée **(Clavel et Gautreau, 1997)**.

D'après **Krapovickas et Rigoni (1957)** cités par **Clavel et Gautreau (1997) et Schilling *et al.* (1997)**; l'arachide cultivée est un allotétraploïde (2n=4x=40), hybride interspécifique, entre deux parents sauvages diploïdes inconnus, stabilisé par un doublement chromosomique.

Toutes les variétés cultivées appartiennent à l'espèce *Arachis hypogaea*, c'est la seule espèce cultivée du genre *Arachis* et sa variabilité intraspécifique est très grande.

Elle même, divisée en deux sous espèces, *hypogaea hypogaea* correspondant au type Virginia, et *hypogaea fastigiata*; correspondant aux types Valancia et Spanish **(Demol *et al.*, 2002; Schilling, 2003).**

La diversification variétale est considérable, 17 000 variétés maintenues dans la collection

mondiale. L'ensemble des variétés cultivées est généralement classé, en fonction de critères aisés à déterminer, en trois grands types; Virginia, Spanish et Valencia, dont les caractéristiques et la position taxonomique sont données par le tableau I.2.

Le type Virginia diffère sensiblement des types Valencia et Spanish de point de vue disposition des organes reproducteurs. Ceux-ci sont distribués sur les rameaux latéraux de façon alternée chez le type Virginia faisant succéder deux bourgeons végétatifs, deux bourgeons fructifères, alors que chez les types Valencia et Spanish on notera une série de bourgeons fructifères **(Schilling, 2003)**.

Tableau I.2. Classification et principales caractéristiques de l'espèce Arachis hypogaea (Schilling, 2003).

Genre	***Arachis***		
Espèce	***Hypogaea***		
Sous-espèces	***hypogaea***	***Fastigiata***	
Variétés	***Hypogaea***	***Vulgaris***	***Fastigiata***
Types	Virginia	Spanish	Valencia
Port	Érigé/rampant	Érigé	Érigé
Ramification	Alterné	Séquentielle	Séquentielle
Fleurs sur tige principale	Non	Oui	Oui
Couleur feuillage	Vert foncé	Vert clair	Vert clair
Cycle	120-150 J	90 J	90 J
Dormance	Oui	Non	Non
Gousses (cavités)	2 c.	2 c.	3-4 c.

2.3. Morphologie et développement de la plante

L'arachide cultivée est une légumineuse annuelle, herbacée appartenant à la famille des *Fabacées* et la sous-famille des *Papilionacées*.

Une famille qui renferme plus de 17 000 espèces, constituant ainsi le groupe de végétaux supérieurs le plus abondant et le plus diversifié.

La partie aérienne comprend une tige principale toujours érigée et deux ramifications primaires,

érigées ou rampantes (Figure I.2.). Le système racinaire comporte un pivot à chevelu abondant, qui peut s'enfoncer à plus de 1,30 mètre de profondeur et peut porter des nodules sur les racines primaires et secondaires, essentiellement sur les quinze premiers centimètres (Figure I.3.). Leur nombre peut varier entre 800 à 4 000 et leur dimension peut aller de la taille d'une tête d'épingle à environ 4 mm **(Gillier et Sylvestre, 1969)**. Les feuilles sont nombreuses, pennées avec deux paires de folioles elliptiques opposées et subsessiles. Les fleurs jaunes - orangées papilionacées, prennent naissance à l'aisselle des feuilles. La fécondation a lieu avant l'épanouissement des fleurs (Cleistogamie) bien qu'il existe un certain pourcentage d'allogamie de 1 à 4.

Après la fécondation, la base de l'ovaire s'allonge pour donner naissance à un organe appelé gynophore qui porte l'ovaire vers le bas **(Wissuwa *et al.*, 2001)**. La gousse prend une position horizontale entre 2 à 7 cm sous la surface du sol. Elle est composée d'une coque indéhiscente contenant de 1 à 4 graines **(Gillier et Sylvestre, 1969)**.

La graine est dormante dans le groupe Virginia, non dormante chez les Valencia et Spanish. Elle lève au bout de trois à cinq jours. La plante a un développement végétatif limité jusqu'au début de la floraison (25 à 30 jours après le semis). Il s'intensifie ensuite avec émission de fleurs puis formation des gousses. La floraison dans les conditions normales de croissance passe par un maximum entre 40 et 60 jours après semis pour ensuite décroître, sans cesser totalement, jusqu'à la récolte. La plante émet de 400 à 1 000 fleurs dont 10 à 20 % donneront des gousses qui ne parviendront pas toutes à maturité **(Schilling, 2003)**. Seules les premières formées, correspondant à la floraison « Utile ».

Dans les conditions optimales des cultures pluviales, l'arachide achève son cycle en 90 jours pour les variétés hâtives, en 120 jours pour les semi-tardives et en 140 jours pour les tardives **(Schilling *et al.*, 1997)**.

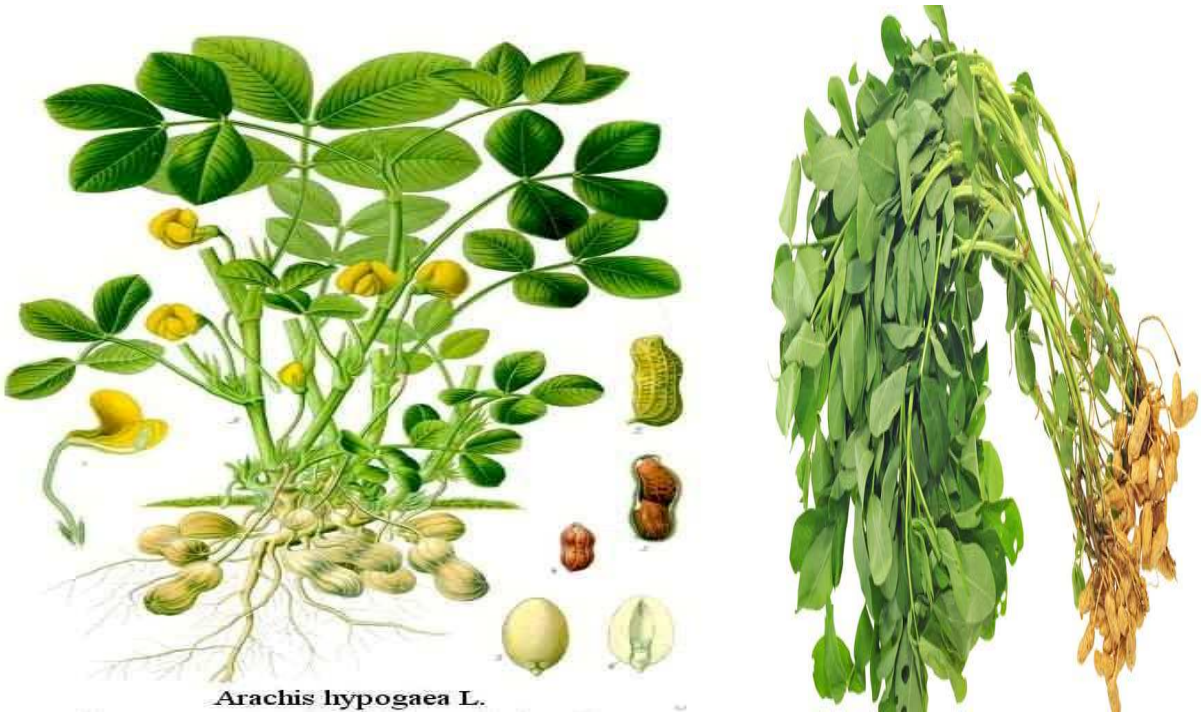


Figure I.2. *Plant d'arachide à ramifications érigées* ***(Encyclopédie, 2015).***

Figure I.3. *Nodulation provoquée par Bradyrhizobium sp. chez l'arachide* ***(Encyclopédie, 2015).***

2.4. Utilisation et importance économique de l'arachide

L'arachide est une oléoprotéagineuse qui se prête à de nombreuses utilisations alimentaires. Sa graine à la fois riche en huile (50%) et en protéine (25%) fait l'objet d'emplois très diversifiés selon les pays producteurs **(Schilling *et al.*, 1997; Clavel et Gautreau, 1997)**. Son fruit connu sous la dénomination de « cacahuète » peut être consommé directement (sans aucune préparation

particulière) ou après une très légère torréfaction. L'huile d'arachide est très appréciée pour la fabrication de certains produits alimentaires en raison de sa très bonne tenue aux hautes températures et sa stabilité **(Schilling *et al.*, 1997)**. Le tourteau, contenant prés de 50% de protéines est un aliment du bétail apprécié des éleveurs et des fabricants d'aliments concentrés **(Clavel et Gautreau, 1997)**. Les coques vides, résultant du traitement des gousses, est un sous produit non négligeable.

En Afrique de l'Ouest, elles fournissent une partie de l'énergie aux huileries locales (1Kg de coque= 1KWh) **(Schilling *et al.*, 1997).**

La valeur fourragère des fanes d'arachide est équivalente à celle de la luzerne **(Schilling *et al.*, 1997)**. Leur valeur marchande peut avoisiner celle de l'arachide en coque à certaines périodes de l'année dans les régions tropicales et subtropicales **(Clavel et Gautreau, 1997)**.

La quasi totalité des transactions internationales en graines ou en coques porte sur l'arachide de bouche, l'arachide d'huile étant le plus souvent triturée sur place dans les pays producteurs **(Schilling *et al.*, 1997)**.

Les superficies occupées par la culture de l'arachide s'étendent sur prés de 25 millions d'hectares dans l'ensemble de la zone tropicale, mais aussi en zone tempérée **(FAOSTAT, 2015)**. Sa remarquable plasticité face aux températures et aux besoins en eau explique l'extension de sa culture dans des zones marginales **(Schilling *et al.*, 1997; Clavel et Gautreau, 1997*).***

La production mondiale d'arachide non décortiquées (en coque) a dépassé 45 millions de tonnes en 2013 avec un rendement de 18 qx/ ha. Elle a connu une progression remarquable ces dernières années. Elle est dominée par cinq pays qui couvrent à eux seuls 61% de la production mondiale. La chine vient en première position et fournit 32% de cette production, elle dédie à cette culture prés de 5 millions d'hectares. L'Inde vient en deuxième position et contribue pour 15%, avec une superficie de plus de 5 millions d'hectares. Le Nigeria, les USA et l'Indonésie viennent ensuite et contribuent respectivement avec 7%, 4% et 3% à la production mondiale.

Pour ce qui est du rendement, la moyenne mondiale pour les cinq dernières années est d'environ 17 qx/ ha.

2.5. Situation de la culture d'arachide en Algérie

La culture d'arachide est très ancienne. En Algérie, elle s'est développée au cours des années 80, sans pratiquement aucun appui de la part de l'Etat. Cependant, il s'agit de culture des variétés d'arachide de bouche dont le débouché naturel n'est pas la transformation par l'industrie. Selon les statistiques du **MADR (2003-2013)** (Tableau I.3.), on constate que la production d'arachide

est en fluctuations continue d'une année à une autre, surtout pour les années 2012 et 2013, où l'on constate une diminution nette de la production, due à un rétrécissement presque de moitié des superficies réservées à cette culture par rapport aux autres années.

La culture de cette espèce est menée durant la période estivale dans toutes les régions d'Algérie. Cependant, les surfaces dédiées à cette culture sont principalement localisées au Nord-Est (El Tarf), au Sud-Est dans les Oasis (Ghardaïa), au Sud-Ouest (El Bayadh) et au Sud (Adrar, Bechar et El-Oued) où la nature des sols et le régime thermique répondent le mieux aux exigences de l'arachide (Tableau I.4. et Figure I.4.).

Tableau I.3. Evolution des superficies, rendements et productions d'arachides en Algérie (MADR, 2003-2013).

Année	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Superficie (ha)	3380	4081	2837	2718	2680	2840	2570	2520	2558	2293	2249
Rdt. (qx/ha)	11,40	10,50	11,60	11,00	12,38	11,34	11,89	13,43	11,90	12,11	11,34
Pro. (qx)	38.420	42.690	33.040	29.950	33.190	32.220	30.570	33.850	30.370	27.780	25.510

Tableau I.4. Evolution des superficies, rendements et productions d'arachides au niveau des principales zones productrices en Algérie (MADR, 2013).

Wilaya	2013		
	Superficie (ha)	Production (qx)	Rendement qx/ha
Adrar	164	2 614	15,90
Bechar	3	50	16,70
El- Bayadh	2	20	10,00
El- Tarf	1 250	11 500	09,20
El-Oued	564	7 340	13,00
Ghardaia	266	3 990	15,00
Total Algérie	**2 249**	**25 514**	**11,30**

3. Généralités sur les rhizobiums

3.1. Définition et intérêt

Rhizobium signifie étymologiquement « ce qui vit dans les racines »; ce sont des bactéries du sol appartenant à la famille des *Rhizobiaceae* (classe des *Proteobacteria*). Elles sont capables de reconnaître, d'infecter et de noduler les racines des légumineuses pour établir une interaction symbiotique dans le but de fixer biologiquement l'azote. L'infection se traduit par la formation d'un organe appelé nodule, dans lequel elles vivent comme endosymbiotes et réduisent l'azote atmosphérique en ammoniac **(Schultze et Kondorosi, 1998 ; Albrecht *et al.*, 1999)**, forme facilement assimilable par les plantes, en échange la plante hôte procure à la bactérie un microhabitat favorable et les substrats carbonés issus de la photosynthèse **(Dommergues *et al.*, 1999)**.

Cette association est largement exploitée dans les pays où les sols sont très pauvres en azote et l'utilisation des engrais azotés chimiques, très coûteuse **(Leena, 2002)**. Dans ce contexte l'association rhizobiums-légumineuses peut remplacer efficacement les engrais azotés chimiques à la fois très onéreux et polluants **(Canadian, 1993)**.

3.2. Principales caractéristiques

Les rhizobiums constituent 0.1 à 8 % de la flore bactérienne totale du sol **(Sadorwsky et Graham, 1998)**, ils se présentent sous forme de coccobacilles ou en bâtonnets réguliers de 0.6 à 0.8 µm de large sur 1 à 4 µm de long **(Dommergues et Mangenot, 1970 ; Vincent, 1974)**, aérobies strictes **(Pelmont, 1993)**, Gram négatives et asporulés **(Jordan, 1984 ; Bekki, 1983)**, généralement très mobiles quand elles sont jeunes grâce à la présence d'un seul flagelle polaire ou 2 à 6 flagelles péritriches **(Bergey's, 1984)**.

Ces bactéries se trouvent soit à l'état libre ou à l'état symbiotique sous forme de bactéroïdes avec une taille dix fois plus grande. Ces derniers ont une forme en X, Y et T **(Dommergues et Mangenot, 1970)**.

Sur milieu YEM gélosé **(Vincent, 1970)**, ces bactéries forment des colonies de 2 à 4 mm de diamètre après 3 à 5 jours d'incubation, de couleur blanchâtres ou beiges, circulaires, convexes, semi translucides ou opaques, élevées et mucilagineuses (Figure I.5.). Les rhizobiums sont des bactéries mésophiles, leur température optimale de croissance se situe entre 25 et 30 °C **(Elkan, 1992)**. Certaines espèces peuvent se développer à des températures allant de 40,5 °C à 42 °C, c'est le cas de *Rhizobium meliloti* qui peut se développer à 42 °C **(Affianha et Alexander, 1992)**.

D'autres souches isolées des légumineuses ligneuses au Kenya, en Inde et au Pakistan peuvent croître à des températures variant entre 44°C et 50°C **(Zahran, 1999)**. Cependant, la température de croissance des rhizobiums varie en fonction de l'espèce et de la région d'isolement **(Cacciari *et al.*, 2003).**

Si la plupart des rhizobiums préfèrent la neutralité **(Jordan, 1984)**, d'autres au contraire tolèrent des pH très bas **(Vincent, 1977)**, c'est le cas de *Bradyrhizobium japonicum* qui supporte des pH de l'ordre de 3.5 à 4 **(Dommergues et Mangenot, 1970)**.

D'autre part il a été montré que des souches de *Rhizobium* peuvent croître à des pH alcalins allant jusqu'à 12 **(Kulkarni *et al.*, 2000)**.

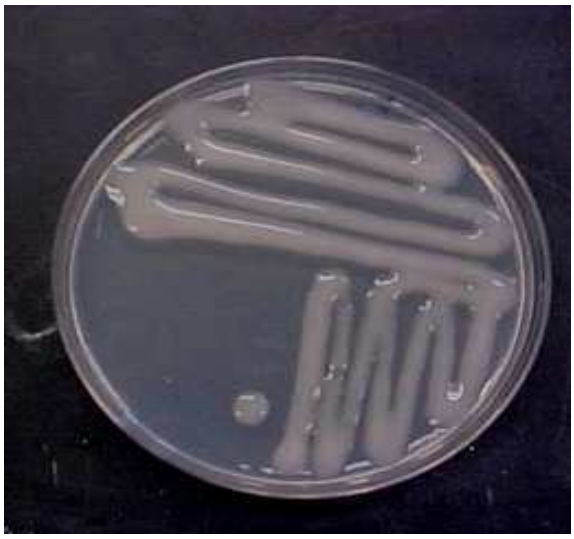


Figure I.4. *Aspect des colonies de Rhizobium sur milieu YEM gélosé.*

On distingue deux groupes de rhizobium sur la base de la vitesse de croissance :

Les rhizobiums à « ***croissance rapide*** », dont les colonies apparaissent trois à cinq jours après l'inoculation sur milieu YEMA. Ce groupe de rhizobium est caractérisé par une forte production de polysaccharides extracellulaires et très souvent, ils acidifient le milieu de culture **(Vincent, 1979)**.

Les rhizobiums à « ***croissance lente*** », dont les colonies apparaissent après sept jours d'incubation sur milieu YEMA. Elles ont une tendance à alcaliniser le milieu de culture et produisent peu de polysaccharides extracellulaires **(Vincent, 1979)**.

4. Généralités sur les légumineuses

Les Légumineuses représentent une superfamille chez les angiospermes, comprenant plus de 750 genres et entre 16000 et 19000 espèces **(Dommergues *et al.*, 1999)**. Elles sont divisées en trois

sous-familles : les Mimosoïdées, les Caesalpinoïdées et les Papilionoïdées **(Udvardi *et al.*, 2005)**. De nombreuses légumineuses constituent une source majeure de protéines, d'huiles végétales et une importante source de nourriture humaine **(Graham et Vance, 2003)**.
C'est la raison pour laquelle, elles sont largement cultivées sur l'ensemble de la planète. On peut citer par exemple : le haricot (*Phaseolus vulgaris*), le soja (*Glycine max*), le pois (*Pisum sativum*), le pois chiche (*Cicer arietinum*), la fève (*Vicia faba*), l'arachide (*Arachis hypogaea* L.)….etc.

Ainsi, les légumineuses couvrent globalement 66% des besoins de subsistance des communautés rurales dans les pays en voie de développement, tout en assurant, un maintien durable de la fertilité des sols, utilisées comme précédent cultural des céréales, et un équilibre des écosystèmes **(Dommergues *et al.*, 2006)**.

La plus grande partie des légumineuses (88% des espèces étudiées) forme des nodules fixateurs d'azote **(Hirsch *et al.*, 2001 ; de Faria *et al.*, 1989)**. De ce fait, les légumineuses sont parmi les plantes les plus étudiées **(Patriarca *et al.*, 2004 ; Gage *et al.*, 2004 ; Stacey *et al.*, 2006)**, notamment, l'émergence de deux plantes modèles : *Lotus japonicus* **(Handberg et Stougaard, 1992 ; Udvardi *et al.*, 2005)** et *Medicago truncatula* **(Barker *et al.*, 1990)** a permis d'accélérer l'étude des mécanismes de mise en place de la symbiose.

5. Aperçu gènèral sur le processus de la symbiose fixatrice d'azote

Le processus d'une symbiose fixatrice d'azote se traduit par la capacité des rhizobiums à induire la formation de nodosités au niveau des racines ou des tiges d'une plante hôte particulière. La présence de nodosités chez les légumineuses était historiquement bien connue, mais leur origine était controversée. **Woronin (1866)** fut le premier à signaler l'observation des micro–organismes ressemblant aux bactéries dans les nodosités de *Lupinus mutabilis*. **Hellriegel et Wilfarth (1988)** ont montré que la formation des nodosités est le résultat d'une infection externe chez les espèces *Lupinus*, *Phaseolus*, *Ornithopus*, *Vicia*, et *Trifolium*. Mais, c'est Beyerinck qui a fourni la première preuve que les bactéries sont à l'origine de la formation des nodosités, en préparant des cultures pures d'organismes provenant des nodosités de *Vicia faba* et en infectant avec ces mêmes cultures des plants de fève cultivés sur un sol stérile **(Beyerinck, 1888, 1890)**.
En fait, la formation de nodosités survient quand les rhizobiums pénètrent leurs hôtes d'une manière strictement coordonnée et contrôlée. Les exigences génétiques de la reconnaissance spécifique sont partagées entre le rhizobium et la plante hôte. Chacun des deux partenaires

possède des gènes qui ne sont exprimés que dans la présence de l'autre **(Djordjevic *et al.*, 1987)**.

5.1. Etablissement de la symbiose fixatrice d'azote

On connait l'importance de l'azote dans la constitution d'un végétal (la chlorophylle, les acides aminés, les alcaloïdes…ect.). On admet que l'approvisionnement en azote assimilable est si fréquemment, un facteur limitant pour la croissance des végétaux, que l'on a souvent et peut être trop couramment même, parlé de ***« faim d'azote »*** pour expliquer les croissances retardées **(Bouillard *et al.*, 1962)**. Cependant, il faut envisager le cas particulier des légumineuses qui, grâce à une symbiose avec des microorganismes fixateurs d‟azote moléculaire, appelés *Rhizobium*, synthétisent directement leurs tissus.

La symbiose se caractérise par la présence d'organes particuliers, situés au niveau des racines, appelés nodosités, au sein desquels la bactérie réduit l'azote atmosphérique en ammoniac, assimilable par la plante. En contrepartie, la plante fournit à son symbiote une niche écologique et les substrats carbonés issus de la photosynthèse, nécessaires à son métabolisme **(Kondorosi et Kondorosi, 2000)**.

L'établissement de cette interaction symbiotique entre bactérie et plante est très spécifique et résulte d'un dialogue moléculaire entre les deux partenaires **(Broughton *et al.*, 2003)**, schématisé dans la Figure I.5. La formation des nodosités suit plusieurs étapes (Figure I.6.):

5.1.1. Préinfection

Dans un premier temps, les Rhizobiums sont attirés vers les poils racinaires par une large gamme de substances, principalement par les Phénylpropanoides exsudés par la racine **(Kape *et al.*, 1991)**. Une production plus importante est remarquée en condition de carence azotée **(Coronado *et al.*, 1995)** .

Les flavonoïdes présents dans les exsudats racinaires induisent l'expression des gènes *Nod* bactériens qui gouvernent la production des facteurs *Nod*, qui sont des lipochitooligosaccharides, dont les formes variables déterminent la spécificité de l'hôte **(Perret *et al.*, 2000)** .

Ces facteurs induisent des changements morphologiques, physiologiques et moléculaires chez la plante hôte **(Wood et Newcomb, 1989).** Les facteurs Nod, isolés de différentes espèces de *Rhizobium*, possèdent tous la même structure de base : ce sont des lipo-chito-oligosaccharides. **(Dénarié *et al.*, 1996)**.

5.1.2. Infection

Au cours de l'infection, la pénétration de la bactérie est facilitée par la courbure du poil racinaire qui crée une zone confinée dans laquelle la bactérie est entourée par la paroi végétale. Un cordon d'infection qui est une structure tubulaire qui croît à l'intérieur de la cellule et dans laquelle la bactérie prolifère est initié à partir de ce point par hydrolyse de la paroi, invagination de la membrane végétale et production de matériel pariétal par la plante **(Gage et Margolin, 2000 ; Mateos *et al.*, 2001 ; Gage, 2004).**

5.1.3. Développement des nodosités

A la faveur d'une lyse localisée de la paroi, les bactéries colonisent le cordon d'infection, qui croit, progresse et se ramifie de cellule en cellule jusque dans le parenchyme cortical de la racine. Les cellules du cortex reprennent leurs divisions et édifient une nodosité **(Foucher et Kondorosi, 2000).**

Au bout du cordon d'infection, les bactéries entrent dans les cellules en s'entourant d'une membrane péribacteroïde, dérivée du plasmalemme, se transforment alors en bactéroïdes (augmentation de leur taille et déformation). Ces derniers, possèdent en effet une enzyme, la nitrogénase qui catalyse la réduction de l'azote atmosphérique en azote ammoniacal utilisable par les cellules végétales **(Duhoux et Nicole, 2004)**. L'azote offert par les bactéries est directement utilisé par la plante, ainsi, il est moins susceptible à la volatilisation, la énitrification et au lessivage **(Garg et Geetanjali, 2007)**.

Les nodosités renferment un pigment rouge très voisin de l'hémoglobine, appelé léghémoglobine. La dégradation de ce dernier correspond à l'arrêt de la fixation de l'azote, et son apparition dans les nodosités coïncide avec le démarrage de la fixation. Cette pigmentation régle la pénétration de l'oxygène qui se localise dans le cortex nodulaire interne et se dirige vers le bactéroïde **(Dommergues et Mangenot, 1970)**. Les nodosités ainsi formés peuvent adopter plusieurs formes (Figure I.7.) :

-***Nodosités à croissance indéterminée*** où l'activité méristématique se maintient (cas du pois et de la luzerne) et de nouvelles cellules apicales sont continuellement infectées pour donner une forme cylindrique à la nodosité.

-***Nodosités à croissance déterminée*** où l'activité méristématique cesse tôt (cas du soja, de l'haricot et de l'arachide). Les cellules infectées engendrent d'autres cellules infectées et la nodosité en grandissant par expansion acquiert une forme sphérique.

-***Un troisième type intermédiaire*** a été identifié chez le genre *Lupinus* et *Sesbania rostrata*. (Les divisions cellulaires se font soit dans le cortex externe soit dans le cortex interne, conduisant à la formation de nodosités soit déterminées soit indéterminées) **(Hirsch, 1992, Hirsch *et al.*, 2001)**.

5.2. Infection chez l'arachide

Le mode d'infection ‘***'Crack entry*** ‘', est un mode d'infection intercellulaire spécifique aux légumineuses tropicales comme *Sesbania rostrata* et les légumineuses appartenant à la tribu des *Aschynomeneae* (Figure I.8.). Le microsymbiote infecte l'hôte par voie fissurale ou par les blessures épidermiques **(Chandler *et al.*, 1982; Uheda *et al.*, 2001)**. Les fissures se situent, généralement au point d'émergence des racines latérales des cellules corticales **(Allen et Allen, 1950 cité par Dommergues et Mangenot, 1970 ; Schilling *et al.*, 1997).** Il n'y a pas de formation du cordon d'infection. Les rhizobiums pénètrent les cellules du cortex externe vraisemblablement par le processus ***'' d'endocytose''***. Ce mécanisme serait vraisemblablement celui de l'arachide **(Uheda *et al.*, 2001)**.

Les nodules sont de forme ovoïde, ou arrondie (type déterminé) (Figure I.9.).

5.3. Rhizobia associés à l'arachide

Les souches de rhizobium associées à l'arachide appartiennent au genre *Bradyrhizobium* dans lequel plusieurs espèces sont actuellement reconnues, *Bradyrhizobium japonicum* **(Jordan, 1982)**, *Bradyrhizobium elkanii* **(Kuykendall *et al.*, 1992)**, *Bradyrhizobium liaoningense* **(Xu *et al.*, 1995)**. Ces trois espèces nodulent le soja.

Une nouvelle espèce *Bradyrhizobium yuanmingene* a été isolée du genre *Lespedeza* **(Yao *et al.*, 2002; Euzéby et Findall, 2004)**. Les autres espèces reconnues de ce groupe sont *Bradyrhizobium betae* **(Rivas *et al.*, 2004)** et *Bradyrhizobium canariense* **(Vinuesa *et al.*, 2005)**. D'autres souches à croissance lente nodulant *Arachis hypogea* **(Urtz et Elkan, 1996)**, *Lupinus* **(Barrera *et al.*, 1997)**, *Astragalus*, *Oxytropis* et *Onobrychis* **(Laguerre *et al.*, 1997)**, *Amphicarpaea* **(Sterner et Parker, 1999)** et autres sont encore non classées. Elles sont désignées aussi par *Bradyrhizobium* sp. suivi du genre de la plante hôte entre parenthèses. Par ailleurs, des travaux ont montré que l'arachide est également nodulée par des souches à croissance rapide **(Taurian *et al.*, 2006)**.

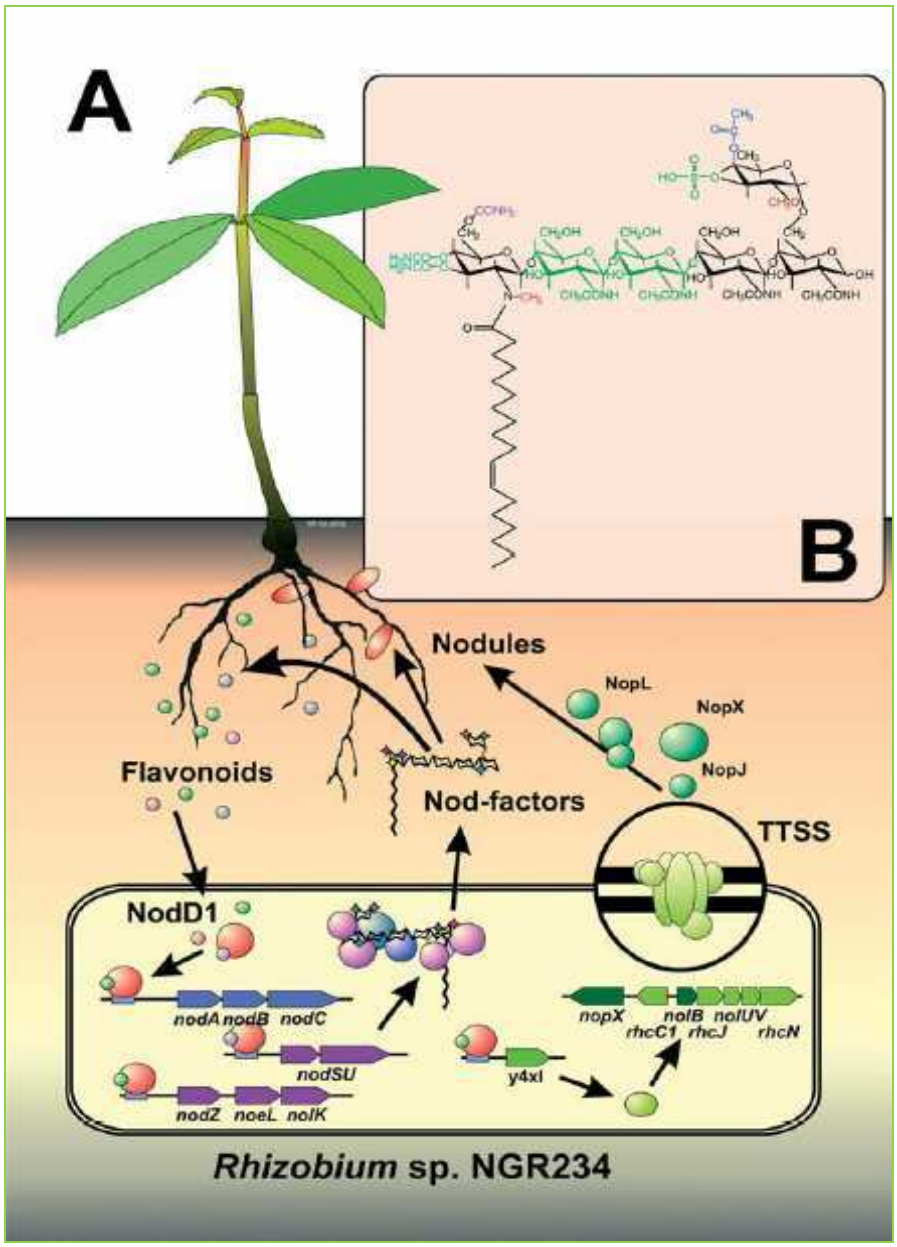


Figure I.5. *Dialogue moléculaire entre la plante et la bactérie lors de la mise en place d'une association symbiotique fixatrice d'azote* ***(Broughton et al., 2003).***

I.5.A*: Echange de signaux entre la bactérie et la plante.*

I.5.B *: Formule chimique des Flavonoïdes.*

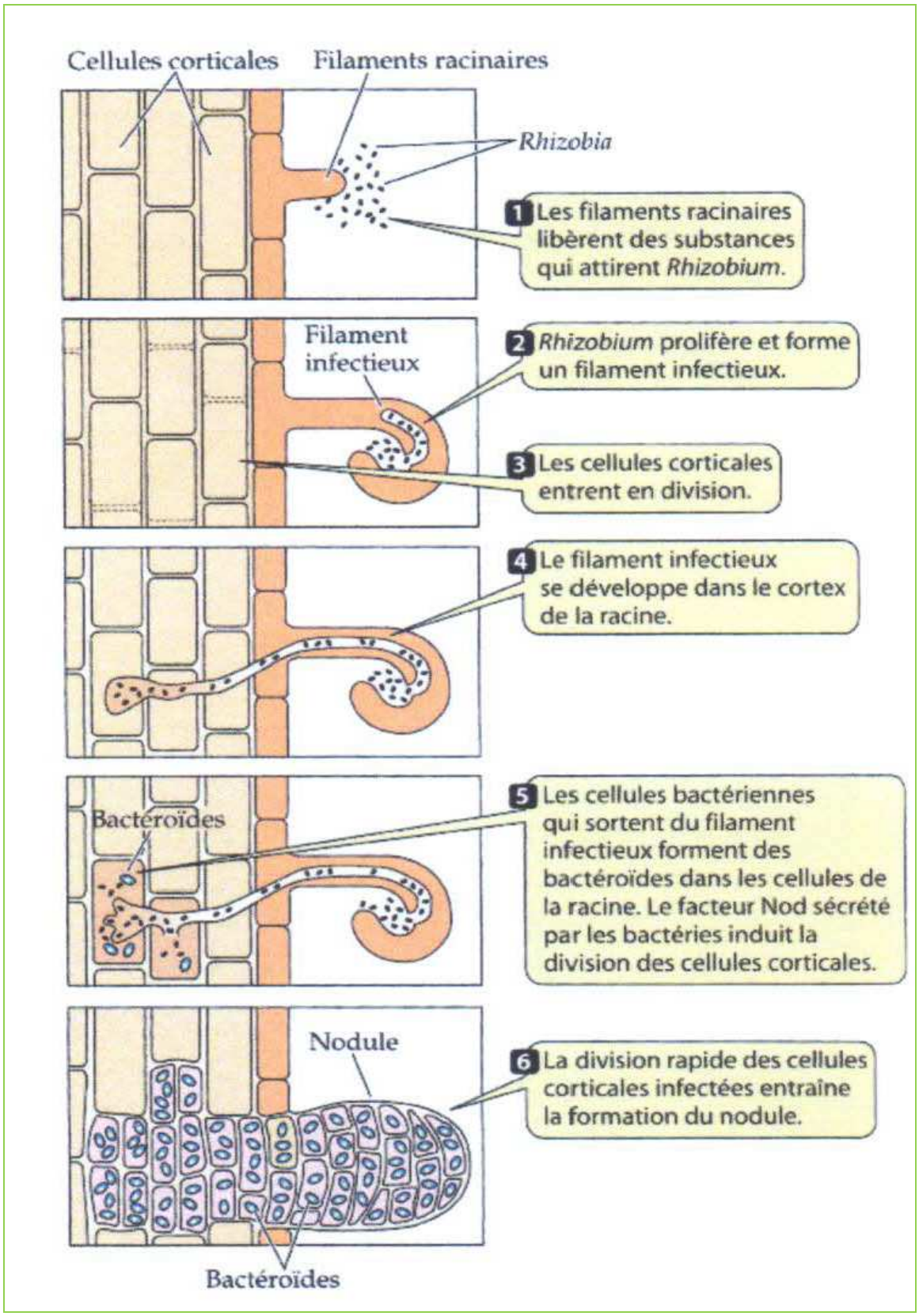


Figure I.6. *Schéma des différentes étapes conduisant à la formation du nodule.*
(Perry et al., 2004).

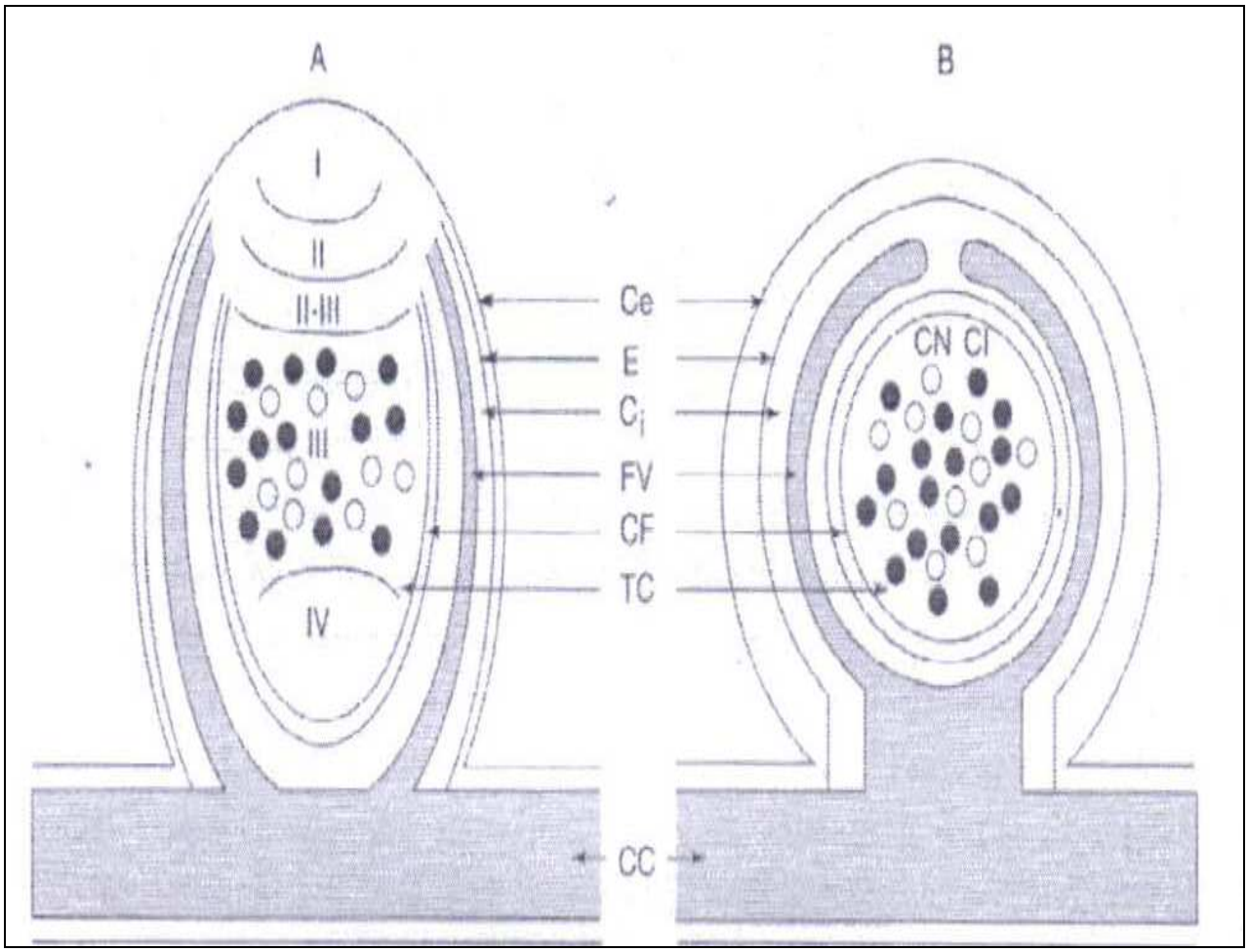
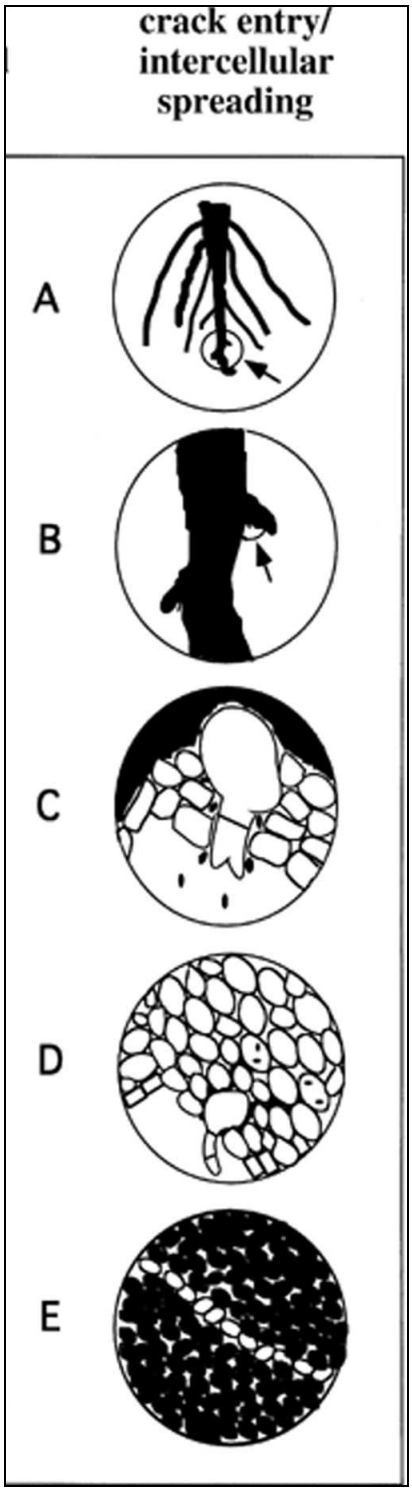


Figure I.7. *Coupe longitudinale schématique d'une nodosité de type indéterminé (A) et de type déterminé (B).*

I= Zone méristématique, II= zone d'infection, II-III= zone fixatrice d'azote, IV= zone de sénescence. Ce= cortex externe, E= endoderme, Ci= cortex interne, FV= faisceau vasculaire, CF= couche frontière, TC= tissu central, CC= cylindre central de la racine, CI= cellule infectée, CN= cellule noninfectée d'après ***Franssen et al., (1992).***



(**A**) Primary and lateral root sytems;

(**B**) Normal root hairs and axillary root hairs;

(**C**) Root hair and crack entry infection;

(**D**) Infection thread spreading and intercellular spreading;

(**E**) Central infected cores with small dispersed uninfected cells and with an array of uninfected cells.

Figure I.8. *Les différentes étapes du mode d'entrée d'infection '* ***« Crack entry »'*** *chez l'arachide d'aprés* ***Fred et al., (1997).***

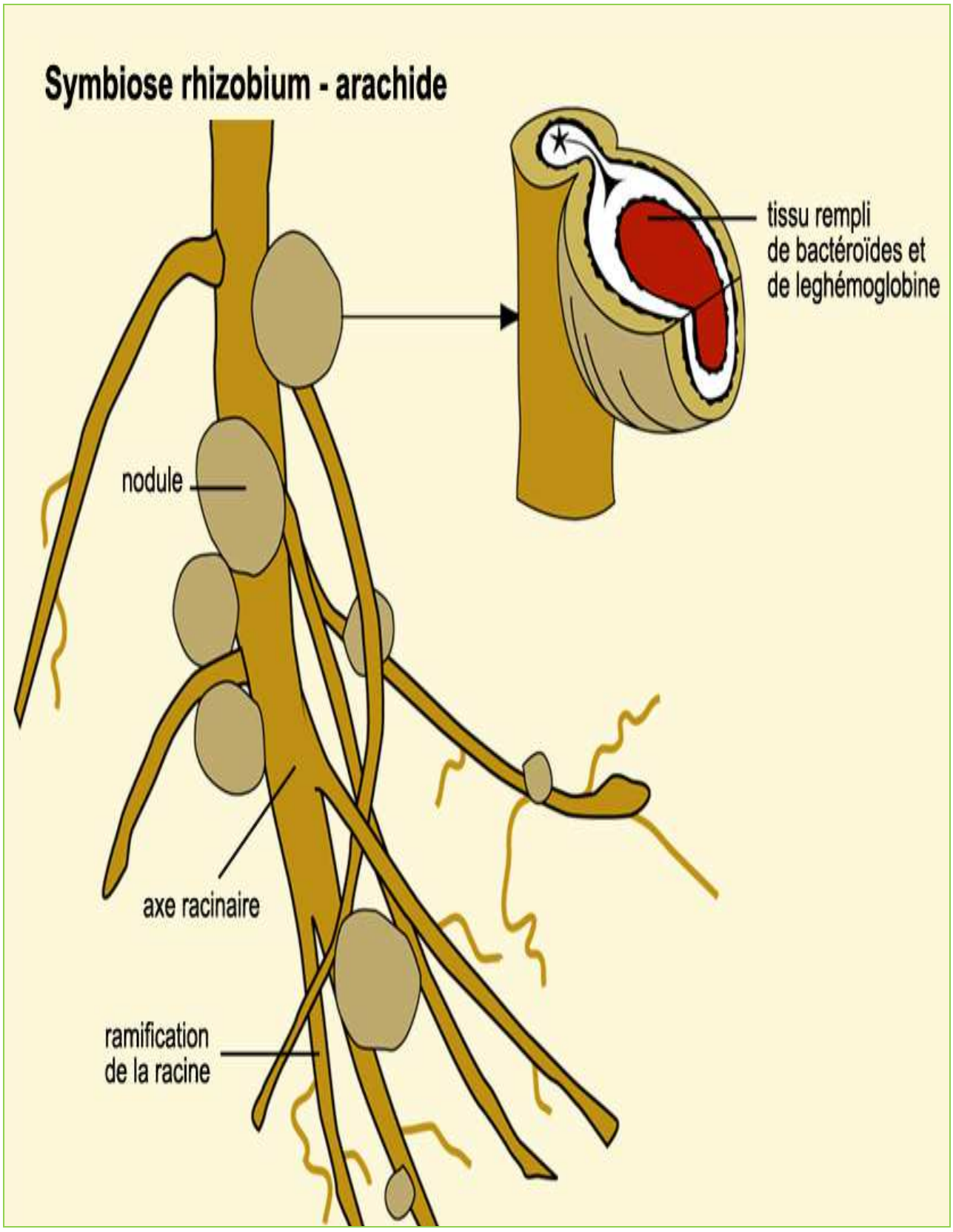


Figure I.9. *La symbiose rhizobium-arachide* (**arachisracin.htm**).

6. L'inoculation pour l'agriculture et l'environnement

Dans les écosystémes semi-arides et arides, la sécheresse et le manque de nutriments notamment l'azote et le phosphore assimilables rendent les sols peu fertiles, ajouté au pobléme de salinité **(Zahran, 1997)**. Ces facteurs rendent la récolte médiocre et détruisent le couvert végétal. Il existe des moyens pour palier à ces contraintes et tenter d'augmenter la fertilité de ces sols pour les rendre plus productifs, tant en respectant la durabilité de ces ecosystémes.

En effet, les légumineuses capables symbiotiquement de fixer l'azote de l'air semblent une voie prometteuse pour s'affranchir de l'untilisation des engrais azotés chimiques. Mais ceci n'est possible que si les légumineuses peuvent s'associer efficacement avec des rhizobia adaptées aux contraintes du milieu et spécifiques à la plante hôte. Souvent, les souches effectives ne préexistent pas toujours dans ces sols. Il convient alors d'inoculer soit le sol, soit les graines qu'on séme.

Obaton et Blachére (1965), estiment que l'inoculation peut etre raisonnablement conseillée lorsque l'on veut établir une luzerniére en sol acide, chaulé depuis peu. La bactérisation des semences à fait de gros progrés dans les pays developpés.

Donc, l'inoculation est une opération de bactérisation des semences ou du sol en vue de l'amélioration de la fixation biologique de l'azote chez une légumineuse à faible activité symbiotique ou nouvellement introduite. Le recours à cette technique est expliqué par l'absence des rhizobia spécifiques à cette légumineuse dans le sol ou la nécessité de substituer les souches indigènes par une nouvelle souche plus efficiente.

D'après **Baraibar (2000)**, l'inoculation est la pratique qui consiste à introduire les souches de *rhizobia* ou *Bradyrhizobia* dans l'écosystème plante-sol ; un inoculum étant une formulation des souches en porteur solide ou liquide.

6.1. Choix des souches de rhizobia pour l'inoculation

Selon **Herridge, (2008)**, la recherche de nouvelles souches de rhizobia pour l'inoculation est continue et conduite dans le but de :

1- fournir les rhizobia aux nouveaux cultivars et espèces de légumineuses ;
2- développer la culture des légumineuses dans des conditions environnementales hostiles ;
3- optimiser la productivité des espèces cultivées.

Brockwell *et al.*, (1995), ont énuméré les caractères suivants comme désirables pour les souches des rhizobia utilisées dans l'inoculation :

1-formation des nodules effectifs capables de fixer l'azote avec la légumineuse inoculée ;

2-concurrencer les rhizobia existants déjà dans le sol pour la formation des nodules ;

3-formation des nodules fixateurs d'azote même en présence de nitrate du sol ;

4-persistance dans le sol, surtout pour les légumineuses pérennes ;

5-colonisation du sol même en absence de la plante hôte ;

6-tolérance aux stress environnementaux ;

6.2. Différentes techniques de l'inoculation

L'inoculation des légumineuses par les rhizobia peut se faire par l'inoculation de la graine ou du sol, bienque la méthode d'application de l'inoculum change selon différentes pratiques et espèces de légumineuses.

6.2.1. La méthode de mélange tourbe-inoculum (Desting)

Avant le semis, la graine est habituellement mélangée avec un substrat à base de tourbe **(Deaker *et al.*, 2004)**, mais cette technique d'inoculation n'est pas recommandée, parce que la majeure partie de l'inoculum ne colle pas aux graines bien que le mélange soit la plupart du temps fait manuellement dans des boites ou dans des bétonnières. L'inoculation d'une grande quantité de semences peut être également automatisée en utilisant des semoirs équipés d'un réservoir d'inoculum, d'une pompe et d'une chambre de mélange semence-inoculum **(Deaker *et al.*, 2004)**. La plupart des semences de légumineuses fourragères sont inoculées à l'aide d'un tambour rotatif, vue la rentabilité et la simplicité de cette technique **(Scott *et al.*, 1997)**.

6.2.2. La méthode de la boue (Slurry)

La technique d'inoculation recommandée pour l'inoculation des graines est la méthode de la boue. D'abord, l'inoculum est mélangé avec une solution contenant l'adhésif, cette boue est mélangée aux graines jusqu'à ce qu'une couverture uniforme soit réalisée. Les graines sont séchées dans des conditions fraiches avant le semis **(Hungria *et al.*, 2005)**. Les adhésifs généralement utilisés incluent le sucrose (10 %), un fongicide, un bactéricide, la gomme arabique (40 %), la cellulose méthyle-éthylique et la cellulose méthyle-hydroxyle-propylique (environ 2 à 4 %) **(Horikawa et Ohtsuka, 1996a)**. Le volume de boue dans le cas du soja ne devrait pas excéder 300 ml par 50 kilogrammes de graines.

6.2.3. Enrobage des graines

L'enrobage des graines est employé quand les conditions de semis sont moins favorables (hautes températures ou pH du sol acide). Dans ce procédé, la graine est inoculée en utilisant un adhésif fort, tel que la gomme arabique 40 %, qui est alors mélangé avec du carbonate de calcium, du phosphate naturel ou de l'argile finement broyé **(Smith, 1992 ; Thompson et Stout, 1992 ; Horikawa et Ohtsuka ; 1996b)**.

En Australie, des micronutriments spécifiques ont été également ajoutés aux graines enrobées dans le cas des sols acides **(Hungria *et al.*, 2005)**.

Les graines inoculées peuvent être stockées aussi longtemps (1-2 ans) avant leur utilisation, mais cette durée de conservation change selon les espèces de légumineuses **(Smith, 1992 ; Gemell *et al.*, 2005)**.

Gemell *et al.*, (2005) recommandent une durée de conservation des graines inoculées de 6 mois pour la luzerne pérenne et les médics, 6 semaines pour le trèfle souterrain et 2 semaines pour le trèfle blanc, rouge et les autres espèces de légumineuses.
Le nombre de rhizobia par graine varie en fonction de la taille de la graine, 10^3 rhizobia par graine pour les légumineuses de petites graines comme le trèfle, les médics, la luzerne pérenne et 10^5 rhizobia /graine pour les légumineuses à grosses graines comme le soja et le haricot **(Smith, 1992 ; Lupwayi *et al.*, 2000)**.

6.2.4. L'inoculation du sol

Pour les légumineuses à grosses graines tels que le soja, le haricot, et en particulier l'arachide, l'inconvénient de l'inoculation de la graine est l'incompatibilité entre les rhizobia contenus dans l'inoculum et les fongicides et insecticides appliqués à la graine. Pour surmonter cette contrainte, l'inoculum peut être appliqué directement dans le sol sous forme de granules, tourbe, ou liquide. Les inoculums ne sont pas mélangés aux engrais, qui peuvent être nuisibles aux rhizobia, mais séparément réunis dans le sol. L'inconvénient majeur de ce procédé est le coût plus élevé parce que la quantité d'inoculum utilisée est plus élevée que celle utilisée pour l'inoculation des graines **(Hungria *et al.*, 2005)**. La profondeur à laquelle l'inoculum est placé est également importante ; la nodulation du soja est supérieure quand l'inoculum est appliqué en sillon à une profondeur de 2,5 cm au-dessous de la graine comparée à une profondeur d'application de 5 cm **(Smith, 1992)**.

L'inoculation directe des graines à la période de semis avec les inoculums liquides ou granulaires évite d'endommager la graine fragile et surmonte l'effet nuisible des pesticides et fongicides appliqués à la graine enrobée par les rhizobia **(Deaker *el al.*, 2004)**.
L'inoculation liquide du soja au moment du semis est également mieux réussie que l'inoculation de la graine avec la boue qui produit une nodulation plus précoce **(Brockwell *et al.*, 1988)**.

6.3. Origine des échecs de l'inoculation

Comme la fixation de l'azote est un processus symbiotique, des facteurs environnementaux affectant la plante hôte ainsi que les *rhizobia* doivent être optimaux pour l'établissement d'une symbiose effective **(Somasegaran et Hoben., 1994)**.
L'inoculation des plantes avec des *Rhizobium* sélectionnés ne donne pas toujours l'effet positif escompté sur les légumineuses fixatrices d'azote sur lesquelles elle a été appliquée **(Brunck *et al.*, 1991)**.
Une mauvaise connaissance du devenir des inoculums au champ est l'une des causes des échecs observés. En effet, la réponse à l'inoculation peut dépendre :

De la qualité de l'inoculum :

- Un inoculum de mauvaise qualité : une densité insuffisante des microorganismes vivants, des souches non compétitives ou bien non infectives ni effectives.
- Contact de l'inoculum avec les engrais ou pesticides toxiques.

Des propriétés du sol :

Milieu-sol défavorable : pH élevé ou faible, humidité insuffisante, température anormale, excès d'azote combinés, carence en certains éléments (P, Ca, Mo, Co, B) mais aussi des propriétés physiques défavorables.
Des caractéristiques symbiotiques de la plante : plante hôte non réceptrice, ou l'échec peut être dû à l'ensemble des composantes du système sol-plante-microorganismes **(Brunck *et al.*, 1991)**.

7. Effet du stress hydrique et les stratégies d'adaptation à la sécheresse

7.1. L'eau dans la plante

Parler de l'eau chez les végétaux est quelque chose de commun. Chacun sait que pour se développer une plante a besoin d'eau et quand celle-ci vient à manquer les conséquences peuvent

être graves **(Bernard, 2006)**. Elle s'y trouve naturellement à l'état liquide, mais aussi sous forme de vapeur d'eau dans les chambres sous stomatiques des feuilles **(Laberche, 2004)**. La richesse en eau des plantes est variable selon les espèces, les organes et les milieux de vie. En effet, une salade peut contenir 90 à 93% d'eau, une feuille est composée souvent de 80 à 90% d'eau et le bois fraîchement coupé peut renfermer 30 à 50 % d'eau **(Leclerc, 1999)**. Il faut 1 500 litres d'eau pour obtenir 1 Kg de blé, 500 litres d'eau pour 1 Kg de maïs et 4 500 litres d'eau pour 1 Kg de riz **(Bernard, 2006)**.

Les rôles multiples assurés par l'eau au sein des plantes en font le premier facteur limitant leur fonctionnement. Parmi ces rôles, on peut citer **(Laberche, 2004)**:

- l'eau contribue au maintien de la structure de la cellule et en particulier de la structure colloïdale du cytoplasme.
- elle est le siège des réactions métaboliques en intervenant dans les réactions métaboliques comme l'hydrolyse ou la photosynthèse.
- elle permet la turgescence des cellules et par là même des tissus et des organes.
- elle véhicule les nutriments minéraux et les produits du métabolisme.
- par son rejet dans l'atmosphère sous forme de vapeur, elle emprunte à la plante sa chaleur latente de vaporisation. Elle permet à celle-ci de supporter les rayonnements solaires et les divers échauffements climatiques.

7.2. Notion de stress

Selon les définitions, le stress chez les plantes apparaît avec des significations différentes en biologie, qui convergent principalement vers n'importe quel facteur environnemental défavorable pour une plante **(Levitt, 1980)**. Selon **Jones *et al.*, (1989)**, un stress désigne à la fois l'action d'un agent agresseur et les réactions qu'il entraîne dans l'organisme agressé, une force qui tend à inhiber les systèmes normaux. D'autre part, les stress environnementaux nés de la fluctuation des facteurs abiotiques (sécheresse, salinité, température) affectent les conditions de croissance, le développement et le rendement des plantes **(Madhava Rao *et al.*, 2006)**.

7.3. Le stress hydrique

Le stress hydrique est l'un des stress environnementaux les plus importants, affectant la productivité agricole **(Boyer, 1982)**. C'est un problème sérieux dans beaucoup d'environnements arides et semi-arides, où les précipitations changent d'année en année et où les plantes sont soumises à des périodes plus ou moins longues de déficit hydrique **(Boyer, 1982)**.

Il y'a autant de définition du stress hydrique qu'il y a d'utilisation de l'eau **(Heathcotes, 1973 cité par Bergaoui et Alouini, 2001)**. En agriculture, il est défini comme un déficit marqué et ce compte tenu des précipitations qui réduisent significativement les productions agricoles par rapport à la normale pour une région de grande étendue **(Mckay, 1985 in Bootsma *et al.*, 1996)**. En effet, on assiste à un stress hydrique lorsque la demande en eau dépasse la quantité disponible pendant une certaine période ou lorsque sa mauvaise qualité en limite l'usage **(Madhava Rao *et al.*, 2006)**.

Le stress hydrique a lieu chaque fois que la vitesse de transpiration de la plante dépasse celle de l'absorption en eau; autrement dit, lorsque les besoins en eau de la plante ne peuvent être satisfaits par la réserve en eau du sol **(Laberche, 2004)**. Ce dernier se traduit généralement par une réduction de la croissance, mais aussi des échanges gazeux et du métabolisme général de la plante. Il agit différemment en fonction de son intensité et du stade physiologique **(Debaeke *et al.*, 1996)**

7.4. Effet du stress hydrique sur la morphologie et la physiologie de la plante

Les plantes perçoivent et réagissent rapidement aux modifications de la quantité d'eau dans les cellules à travers une série d'évenements paralléles d'ordre anatomique, physiologique, cellulaire et moléculaire **(Chaves *et al.*, 2009)**. L'effet du stress hydrique au niveau de la plante entiére se manifeste par la diminution de la croissance, de la photosynthèse et par l'altération du métabolisme carboné et azoté **(Debaeke *et al.*, 1996)**.

Le stress hydrique affecte pratiquement tous les aspects de la croissance de la plante en modifiant son anatomie et sa morphologie. La croissance est l'un des processus physiologiques le plus sensible au stress hydrique, à cause de la baisse du potentiel de turgescence **(Shao *et al.*, 2008)**. Le stress hydrique réduit considérablement le développement et la croissance des cellules **(Jalleel *et al.*, 2007 ; in Lazali , 2009)**. L'allongement cellulaire et la division cellulaire et donc la taille des feuilles et des entre –nœuds sont les premiers processus affectés par un déficit hydrique **(Wery et Turc, 1988)**. La croissance de la partie aérienne, et surtout celle des feuilles, est généralement plus sensible que celle des racines **(Mahajan et Tuteja, 2005)**. Chez le maïs la croissance des feuilles est fortement réduite lorsque le potentiel hydrique atteint – 0,45 MPa, et totalement inhibée, à –1,00 MPa. En même temps, la croissance des racines est normale, jusqu'à – 0,85 MPa, et n'est pas totalement inhibé à des valeurs de – 1,4 MPa **(Westgate et Boyer, 1985)**. Le stress hydrique réduit la hauteur et le diamètre de la tige, le nombre des feuilles ainsi

que la surface foliaire chez les plantes d'une manière générale. **Shao *et al.*, (2008)** sur *Abelmoschus esculentum* montrent que le stress hydrique diminue la longueur des racines et des tiges, la surface foliaire, le nombre de feuilles par plant, la longévité des feuilles et la biomasse verte et sèche. En revanche, **Pandey *et al.*, (1984)** indiquent que l'arachide soumis à un stress hydrique développe un système dense et pivotant par rapport aux autres légumineuses.

Plusieurs études montrent que, le stress hydrique a un effet défavorable sur la photosynthèse, la nutrition minérale, le métabolisme, la croissance et le rendement de l'arachide **(Reddy *et al.*, 2003)**. La teneur relative en eau, la résistance stomatique, le potentiel hydrique foliaire, le taux de transpiration, la température du couvert végétal sont des paramètres importants qui influencent le statut hydrique chez l'arachide **(Reddy *et al.*, 2003)**. Chez les plantes d'arachides soumises à un stress hydrique, la TRE est de l'ordre de 30 % par rapport aux plantes non stressées qui ont une TRE de l'ordre de 85-90 % **(Babu et Rao, 1983)**. **Black *et al.*, (1985)**, ont montré, que le stress hydrique diminue le potentiel hydrique foliaire, le potentiel de turgescence et la conductance stomatique.

Le potentiel hydrique foliaire, le taux de transpiration et le taux de photosynthèse diminuent progressivement avec l'augmentation de la durée du stress hydrique **(Subramaniam et Maheswari, 1990). Babu et Rao (1983)**, ont examiné l'effet de la durée du stress hydrique (35 jours) sur l'arachide entre le 20ème et le 55 ème jour après le semis ; sans restriction hydrique, le potentiel hydrique foliaire varie entre – 0,15 et – 1,15 MPa à 6,00 AM et 4,00 PM respectivement. La teneur relative en eau varie entre 100 % et 87 % pendant le premier jour du stress hydrique. A la fin de cette durée de la contrainte hydrique, le potentiel hydrique foliaire est de l'ordre de – 5 MPa, et la teneur relative en eau de 29,7 %. **Bhagsari *et al.*, (1976)**, ont observé une forte réduction de la photosynthèse et de la conductance stomatique quand la teneur relative en eau de l'arachide diminué de 80 à 75 %.

Le développement des feuilles est plus sensible au stress hydrique que la fermeture de stomates. Le stress hydrique diminue la surface foliaire par le ralentissement de développement des feuilles et par la réduction del'approvisionnement des carbohydrates. Un stress hydrique sévère diminue le niveau de chlorophylle a et b **(Yordanov *et al.*, 2000)** . La diminution de la teneur en chlorophylles est expliquée par l'inhibition de leur synthèse **(Reddy *et al.*, 2003)**. Le stress hydrique n'a pas un effet seulement sur la diminution de la teneur en chlorophylle, mais aussi sur la désorganisation des membranes des thylakoïdes **(Ladjal *et al.*, 2000)**.

Lors d'un déficit hydrique, l'activité physiologique de la feuille, et plus particulièrement la photosynthèse, est affectée. La réduction de la photosynthèse, est liée à la diminution de la conductance stomatique et de la surface foliaire. Le stress hydrique induit la fermeture des stomates, avec pour conséquence une diminution de la conductance à la diffusion du CO_2, qui se répercute négativement sur le taux de la photosynthèse **(Reddy *et al.*, 2003 ; Athar et Ashraf, 2005 ; Mahajan et Tuteja, 2005)**.

La diminution du taux de la photosynthèse foliaire est expliquée par : (1) la diminution de la teneur relative en eau et aussi du potentiel hydrique foliaire **(Lawlor et Cornic, 2002)** ; (2) la fermeture des stomates **(Cornic, 2000)** ; (3) l'affaiblissement métabolique **(Tezara *et al.*, 1999 ; Lawson *et al.*, 2003)** ; (4) le déséquilibre entre la lumière captée et son utilisation **(Foyer et Noctor, 2000)**. La fermeture des stomates est généralement la cause principale de la réduction de la photosynthèse sous l'effet d'un stress hydrique **(Cornic, 2000)**. Elle diminue la concentration interne en CO2, qui empêche le métabolisme photosynthétique. La fermeture des stomates est progressive en fonction de la sévérité du stress hydrique. Il est bien connu que la teneur en eau des feuilles est influencée toujours par la conductance stomatique et une bonne corrélation entre le potentiel hydrique des feuilles et la conductance stomatique a été établie sous l'effet du stress hydrique **(Ramachandra Reddy *et al.*, 2004)**.

La plante se comporte comme un système biologique dont les caractéristiques physiologiques changent avec l'état hydrique. L'effet dépressif du stress hydrique sur la plupart des processus physiologiques et métaboliques de la plante se répercute sur les composantes du rendement, le rendement lui-même, ainsi que sa qualité **(De Raissac, 1992)**. Le déficit hydrique est un des principaux facteurs limitant de la production des légumineuses **(Wery et Turc, 1990)**. Le plus souvent, un déficit hydrique pendant les étapes de la fructification abaisse largement la production des grains **(Cartter, 1962 ; Dusek et Coll, 1971; Doss et Coll, 1974 ; in Bouniois *et al.*, 1985)**.

L'effet d'un déficit hydrique sur le rendement en graines dépendra de sa position par rapport aux phases phénologiques (de croissance végétative, de floraison – fécondation et de remplissage des graines), ou de son intensité s'il est étalé sur l'ensemble de la période reproductrice **(Wery et Turc, 1990)**. Des études sur le tournesol montrent que, les composantes du rendement, tels que le nombre de graines, le nombre de fleures, sont fortement diminués sous l'effet d'un stress hydrique **(Shao *et al.*, 2008)**. **Shao *et al* (2008)** ont constaté également que l'influence du stress hydrique est plus marquée aux stades floraison et maturation où la quantité et la qualité du

rendement sont réduites. Comme le montrent les travaux de **Meisner et Karnok (1992)** effectués sur l'arachide, l'effet du stress hydrique réduit le taux de production des fleurs par contre le nombre de fleurs/plant n'est pas affecté, cela à cause de l'augmentation de la durée de floraison. L'élongation des gynophores est retardée sous l'effet de la contrainte hydrique pendant lequel le sol devient trop sec pour la pénétration des gynophores, ce qui retarde le développement des gousses et des graines. Le desséchement du sol au cours de développement des gynophores diminue le taux de croissance des gousses et des graines de l'ordre de 30 %. Le développement des gousses est progressivement inhibé par l'effet du stress hydrique à cause de l'insuffisance de la turgescence cellulaire et le manque de carbohydrates **(Reddy *et al.*, 2003)**.

7.5. Effet du stress hydrique sur la nutrition azotée

La plupart des travaux sur la sécheresse ont abordé le problème de l'effet du stress hydrique sur la fixation et l'assimilation de l'azote par l'étude des mécanismes qui interviennent lors de la réponse du végétal à la contrainte hydrique. La photosynthèse demeure le phénomène le plus étudié dans ces conditions et souvent, il a été proposé comme critère de sélection pour la résistance à la sécheresse des plantes. Il n'en demeure pas moins que d'autres mécanismes peuvent jouer un rôle important dans l'adaptation des plantes aux contraintes hydriques. Il est actuellement admis que le stress hydrique affecte l'ensemble des phénomènes biologiques qui peuvent conduire à la mort de la plante. Le métabolisme général des plantes est lui aussi concerné par cette réaction, notamment le métabolisme azoté qui présente la particularité d'être alimenté par deux voies, l'assimilation de l'azote combiné du sol et la fixation symbiotique de l'azote atmosphérique. Moins de travaux sont consacrés à l'effet du stress hydrique sur la nutrition azotée des légumineuses malgré leur importance économique et agronomique **(Ounane, 2004). Dubey et Pessarakli (2001)** ont montré que l'absorption et l'assimilation de l'azote diminuent chez des plantes soumises à un stress hydrique. La diminution de l'assimilation de l'azote est expliquée par la réduction de l'activité de la nitrate réductase **(Reddy *et al.*, 2003)**. Le stress hydrique diminue l'activité de la nitrate réductase à différents stades de développement chez la plupart des espèces cultivées, cependant cette activité reste élevée dans les plantes fortement fertilisées par rapport aux plantes faiblement fertilisées **(Dubey et Pessarakli, 2001)**. La fixation de l'azote est beaucoup plus sensible au déficit hydrique que l'assimilation du nitrate. En effet, la fixation de l'azote baisse très rapidement pour un potentiel hydrique foliaire de – 0,74MPa puis s'annule pour un potentiel de – 1,58 MPa, alors que l'activité de nitrate réductase baisse plus tardivement pour un potentiel hydrique foliaire voisin de – 1,6 MPa **(Obaton *et al.*, 1994)**.

7.6. Effet du stress hydrique sur la nodulation

Considérant l'importance des effets de la sécheresse sur la fixation de l'azote et son impact sur la production, il est surprenant de constater que seulement quelques études sont consacrées à la formation du nodule et sa croissance sous stress hydrique. La relation entre humidité du sol et nodulation est reconnue depuis longtemps **(Ounane, 2004)**.

Masefield (1961) a rapporté des baisses importantes dans le nombre et la masse de nodules pour quatre espèces de légumineuse soumises à une contrainte hydrique.

L'excès d'eau dans le sol aussi bien que son manque affecte sérieusement la nodulation et la fixation de l'azote en réduisant la croissance du nodule **(Sinha, 1981)**,

Lors d'un déficit hydrique dans le sol, la survie des bactéries (Rhizobium) est perturbée. On observe une grande mortalité ainsi qu'une entrave à la mobilité des bactéries qui provoquerait une baisse de l'infection des racines et par conséquent une faible production de nodules **(Chatel et Parker, 1973)**.

Chez la plupart des légumineuses, le nombre et la taille des nodules diminuent sous une contrainte hydrique **(Sprent, 1971 ; Bouniols, 1986 ; Kirda *et al.*, 1989)**. Comparant les effets de la sécheresse sur la nodulation, la fixation, et la croissance de la plante, **Kirda *et al.*, (1989)** ont montré que la fixation était le paramètre le plus sensible, bien que la croissance du nodule fût aussi fortement affectée. **Smith *et al.*, (1988)** ont rapporté des baisses substantielles dans la masse du nodule de la fève et du soja soumis à la sécheresse. Chez le soja, **Sinclair *et al.*, (1988)** ont trouvé que le nombre de nodules et le poids sec ont diminué seulement après une sécheresse sévère.

7.7. Effet du stress hydrique sur la fixation symbiotique de l'azote

La fixation symbiotique de l'azote mesurée par la réduction de l'acétylène *in situ* est extrêmement sensible au stress hydrique provoqué par un dessèchement du sol **(Weisz *et al.*, 1985)**. La fixation est plus sensible à la déshydratation du sol que les autres fonctions physiologiques tel que la photosynthèse **(Durand *et al.*, 1987**), la transpiration **(Sall et Sinclair, 1991)**, ou l'assimilation du nitrate **(Obaton *et al.*, 1982)**. La grande sensibilité de la fixation à la sécheresse, comparée avec les échanges gazeux de la feuille, a été observée sous des conditions contrôlées **(Sinclair, 1986; Durand *et al.*, 1987)** et sous des conditions de champs **(Sinclair *et al.*, 1987)**. La perte de l'activité de la fixation est précédée par une réduction des échanges gazeux de la feuille et une baisse du contenu relatif en eau, ce qui constitue une contrainte

importante pour l'accumulation de l'azote et le rendement potentiel des récoltes même dans un sol modérément desséché. En conditions de déficit hydrique prolongé et intense, la survie de la population bactérienne (rhizobiums) est affectée **(Van Gestel *et al.*, 1993)**. En outre, le mouvement de ces microorganismes est entravé, induisant de ce fait une baisse de l'infection des racines **(Dommergues et Mangenot, 1970)**. Le stress hydrique affecte l'initiation, le développement et le fonctionnement nodulaire ainsi que la capacité photosynthétique des feuilles **(Serraj et Drevon, 1994)**.

L'effet inhibiteur du stress hydrique sur la nodulation pourrait être attribué à l'altération de l'activité métabolique des nodules ou bien à des effets directs sur la perméabilité nodulaire à l'oxygène **(Gonzalez *et al.*, 1995)**. Par ailleurs, l'activité nitrate réductase est considérablement influencée par le stress hydrique. **(Silveira *et al.*, 2001)**. La diminution de l'ANR est associée à la diminution du flux des nitrates au limbe, à l'inhibition du flux transpiratoire qui transporte le nitrate vers les lieux de l'induction de l'enzyme, et à la baisse de la photosynthèse et de la respiration, sources d'énergie pour l'ANR.

8. Mécanismes d'adaptation d'une plante soumise au stress hydrique

La résistance d'une plante à une contrainte hydrique peut être définie, du point de vue physiologique, par sa capacité à survivre et à croître et du point de vue agronomique, par l'obtention d'un rendement plus élevé que celui des plantes sensibles **(Madhava Rao *et al.*, 2006)**. La résistance globale d'une plante au stress hydrique apparaît comme le résultat de nombreuses modifications phénologiques, anatomiques, morphologiques, physiologiques, biochimiques et moléculaires qui interagissent pour permettre le maintien de la croissance, du développement et de la production **(Hsissou, 1994)**. (Figure I.10.).

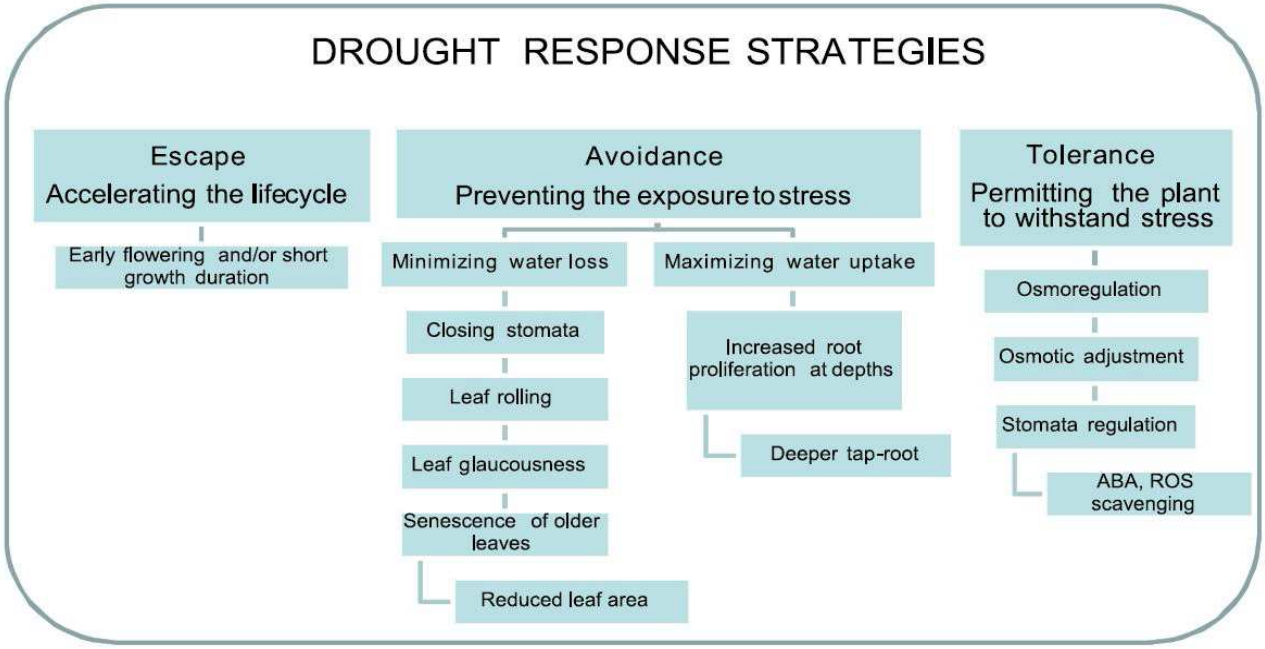


Figure I.10. *Mécanisme de réponse des plantes à la sécheresse et les caractères impliqués dans la réponse* ***(De Leonardis et al., 2010).***

L'adaptation des plantes à des environnements hostiles implique une multitude de réponses métaboliques et de modifications physiologiques qui peuvent permettre leur acclimatation et leur survie **(Lawlor, 2009)**.

Selon **De Raissac (1992)**, les plantes ont recours à des comportements qui leur permettent de survivre à des situations de déficit hydrique par des méthodes d'adaptation. Ces méthodes leur permettent de préserver l'intégrité de leurs fonctions physiologiques et de leur assurer un état productif et de survie. En effet, le caractère adaptatif d'une plante doit non seulement lui permettre de survivre, mais aussi de se reproduire et de se pérenniser dans son environnement.

Jones (1992), a défini et établi une classification des '***stratégies***' d'adaptation des plantes au stress hydrique. La premiére consiste à « ***éviter*** » toute déshydratation générée par l'environnement extérieur et inclut l'échappement ou l'esquive et l'évitement. La deuxiéme stratégie consiste à developper une capacité à « tolérer cette déshydratation ».

8.1. L'échappement et l'évitement

8.1.1. L'échappement ou esquive

La première façon d'éviter la sécheresse est l'esquive **(*Drought escape*)**. L'esquive permet à la

plante de réduire ou d'annuler les effets de la contrainte hydrique par une bonne adaptation de son cycle de culture à la longueur de la saison des pluies.

La variabilité génétique pour la longueur de cycle est généralement importante dans les plantes et plus particulièrement chez les espèces à floraison indéterminée comme l'arachide. Le développement phénologique rapide avec une floraison précoce, permet à la plante d'éviter les périodes sèches. Cette stratégie appliquée aux espèces cultivées amene à décaler la date de semis et/ou à sélectionner des variétés plus précoces permettant d'éviter les déficits hydriques de fin de cycle **(Fukai et Cooper, 1995 ; Monneveux, 1997 ; Hopkins, 2003 ; Tardieu, 2005)**.

Le rendement de nombreuses variétés a été amélioré grâce au raccourcissement des longueurs de cycle (précocité) et ceci chez pratiquement toutes les espèces annuelles cultivées **(Turner, 2001)**, sur les légumineuses **(Subbarao *et al*., 1995)**, comme sur les céréales **(Fukai et Cooper, 1995 ; Fukai *et al*., 1999)**.
Ce mécanisme est particulièrement efficace dans les environnements avec déficits hydriques fréquents en fin de cycle.

8.1.2. L'évitement

Deux grands types de réponses permettent à la plante d'éviter ou, plus exactement de suporter une sécheresse significative tout en limitant la déshydratation de ses tissus **(Tuner *et al*., 2001)**. Ce mécanisme est appelé « évitement » ou ***(Drought avoidance)*** ou parfois « déshydratation postponement ».

La stratégie de l'évitement est essentiellement liée, d'une part, à la réduction de la transpiration et d'autre part, à une optimisation de l'absorption d'eau par les racines. **(Monneveux et Belhassen, 1996 ; Liu *et al*., 2005)**. La diminution de la transpiration est principalement liée à la fermeture des stomates. Il s'ensuit une chute de l'assimilation de CO_2 donc une baisse de production de biomasse. Elle peut être due également à une diminution des surfaces évaporantes. Parmi les mécanismes permettant de réduire la transpiration, la réduction de la surface foliaire et la diminution de la conductance stomatique jouent un rôle déterminant **(Tardieu, 2005)**.

Lors de sécheresses précoces, la réduction de la surface foliaire est associée à une diminution de l'expansion foliaire plus qu'à une sénescence accélérée des feuilles **(Turner, 1997)**. La régulation de la conductance stomatique reste le mécanisme majeur intervenant à court terme pour limiter les pertes d'eau. Le potentiel hydrique foliaire sera maintenu d'autant plus

longtemps que la fermeture des stomates est précoce **(Turner, 1997)**. Les génotypes à faible conductance sont plus sensibles au déficit hydrique que les génotypes à forte conductance.

Une faible conductance est généralement proposée comme un critère de sélection pour la résistance des plantes à la sécheresse **(Jones, 1979, 1980, 1987 ; Turner, 1979, 1982, 1986, cités par Ludlow et Muchow, 1990).**

De nombreuses plantes adaptées aux zones arides ne contrôlent que très peu leurs pertes en eau par transpiration, mais possèdent un enracinement très profond capable d'extraire l'eau du sol. Un système racinaire capable d'extraire l'eau du sol est un critère essentiel pour la résistance à la sécheresse. Cette caractéristique revêt une importance particulière pour les cultures qui subissent régulièrement des déficits hydriques de fin de cycle **(Khalfaoui *et al.*, 1990 ; Subbarao *et al.*, 1995).** Son impact sur le rendement est particulièrement élevé car elle intervient directement dans l'efficience de l'utilisation de l'eau en conditions de stress.

La majorité des résultats montre une corrélation positive entre la profondeur d'enracinement et le rendement en grain notamment chez l'arachide **(Ketring, 1984 ; Wright *et al.*, 1991)**. Cependant deux types de raisons limitent beaucoup l'utilisation des critères racinaires par les sélectionneurs **(Turner *et al.*, 2001)** : L'impraticabilité du criblage au champ pour cette caractéristique sur une grande échelle et la difficulté de corréler des observations au champ à celles qui sont faites en pots. L'absence d'une compréhension précise du rôle exact des racines en conditions de ressources hydriques limitées est un autre facteur limitant à la mise en place d'un système de criblage économe et efficace **(Turner *et al.*, 2001)**.

8.2. La tolérance

La tolérance au déficit hydrique ou ***(Drought tolerance)*** est définie comme étant la capacité de la plante à survivre, croitre et produire de manière satisfaisante sous conditions limitantes en eau **(Tardieu, 2005)**. La tolérance au déficit hydrique est un caractère dont le phénotype et le contrôle génétique sont complexes **(Tardieu, 2005 ; Shinozaki et Yamagushi-shinozaki, 2007)**.

Le maintien de la turgescence lors d'un déficit hydrique permet de retarder la fermeture des stomates **(Mojayad et Planchon, 1994)**, de maintenir le volume chloroplastique **(Gupta et Berkowitz, 1987)**, et de réduire le flétrissement foliaire **(Jones et Turner, 1980)**. Cette aptitude confère à la plante une meilleure tolérance au déficit hydrique interne **(Ludlow *et al.*, 1983)**.

Cette tolérance au déficit hydrique interne permet un fonctionnement prolongé de la photosynthèse. Les produits carbonés peuvent alors être utilisés autant pour l'ajustement osmotique que la croissance racinaire.

Une autre conséquence du maintien du métabolisme carboné sera une diminution de la fréquence des épisodes de photo-inhibition. L'ajustement osmotique est l'un des mécanismes de tolérance au stress hydrique **(Bray, 1997 ; Babu et Henry, 2006)**. Dans ce processus, les plantes diminuent leur potentiel osmotique cellulaire par l'accumulation des solutés, cette diminution de potentiel osmotique permet la rentrée de l'eau à l'intérieur des cellules qui augmente le potentiel de turgescence **(Mahajan et Tuteja, 2005)**.

L'ajustement osmotique est un processus majeur de l'adaptation des plantes à la déshydratation. Il consiste en une accumulation active des solutés ou osmolytes dans le compartiment cellulaire, de façon à maintenir la pression de turgescence lorsque le potentiel hydrique décroit **(Turner, 1986).**

Lors de cet ajustement osmotique la plante accumule au niveau de cytoplasme certains solutés tels que les ions inorganiques (K+), les composés contenant l'azote (proline, acides aminés), les polyamines et les composés d'ammonium quaternaires (glycine bétaïne) **(Tamura *et al.*, 2003)**. D'autres osmolytes sont produits en réponse au stress hydrique tels que le sucrose, les polyols, le pinitol et les oligosaccharides. Les osmolytes jouent un rôle dans la stabilisation des membranes et le maintien de la conformation des protéines à des potentiels hydriques foliaires faibles **(Ramachandra Reddy *et al.*, 2004)**, comme ils jouent un rôle majeur dans la protection des cellules par l'élimination des molécules réactives d'oxygènes **(Pinhero *et al.*, 2001)**. La proline est également connue pour être impliquée dans la réduction des dommages au niveau des membranes de thylakoïdes par scavenging et/ou réduction de la production de l'oxygène singulier **(Ashraf et Foolad, 2007)**. La glycine bétaïne joue un rôle important dans la protection des enzymes et des membranes et aussi dans la stabilisation des complexes protéiques du photosystème II **(Papageorgiou et Morata, 1995)**.

Dans les conditions de stress hydrique, la synthèse de l'acide abscissique (ABA) joue un rôle majeur dans la tolérance des plantes à cette contrainte **(Shinozaki et Yamaguchi-Shinozaki, 1999)**. L'acide abscissique (ABA), qualifié « d'hormone de stress », est impliqué dans la communication entre des racines soumises à un stress hydrique et les feuilles. La synthèse et l'accumulation des osmolytes changent selon les espèces et même aussi chez la même espèce selon les variétés **(Ramachandra Reddy *et al.*, 2004)**.

9. Le stress oxydatif et les systémes antioxydants chez les plantes

Les stress environnementaux nés de la fluctuation des facteurs abioiques (sécheresse, salinité, basses température) affectent les conditions de croissance et le rendement végétal. Les végétaux perçoivent les signaux environnementaux et les transmettent à la machinerie cellulaire pour activer des mécanismes de réponses. La connaissance de ces réponses, basées sur la transduction des signaux de stress, est donc la base des études visant à améliorer la réponse des plantes cultivées aux différents stress **(Laurent *et al.*,1994)**.

La voie de transduction du signal commence par sa perception au niveau de la membrane cellulaire, suivie par la production de seconds messagers et de facteurs de transcription. Ces facteurs de transcription controlent alors l'expréssion des génes impliqués dans la réponse au stress, incluant des changements morphologiques, physiologiques et biochimiques.

9.1. Le stress oxydatif chez les plantes

9.1.1. Quelques notions sur la photosynthèse

Chez les végétaux, la photosynthèse se déroule dans les chloroplastes et plus particulièrement au niveau des thylakoïdes. Ceux-ci contiennent des unités photosynthétiques appelées photosystèmes (pigments couplés à des protéines) absorbant l'énergie lumineuse. Généralement, deux photosystèmes (PSII et PSI) entrent en jeu et opèrent en série dans l'oxydation de l'eau, la réduction du NADP+ et la production d'ATP **(Morishige et Dreyfuss, 1998)**. Le PSII et le PSI sont composés d'un centre réactionnel (RC) constitué respectivement d'une chlorophylle P680 et d'une P700 recevant l'énergie lumineuse, des antennes ou « Light-harvesting Complex (LHC) », qui les entourent. La photosynthèse réalisée par les plantes est dictée par la lumière visible (400-700 nm de longueur d'onde). La première étape de la conversion de l'énergie lumineuse en énergie chimique consiste en l'absorption de photons par le système antennaire (LHC). L'énergie d'excitation fait alors passer la chlorophylle P680 à l'état singulet excité, ensuite, celle-ci transfère un électron à la phéophytine et aux plastoquinones (PQ), QA et QB. QB2- compense sa charge négative en prenant deux protons du stroma. Les molécules de PQ protonées (QBH2), diffusent vers le cytochrome B6f où elles sont oxydées. Les électrons provenant de cette réaction sont cédés au Cyt f. L'oxydation de PQH2 s'accompagne de la libération de deux protons dans le lumen intervenant dans la constitution d'une énergie qui permettra la conversion d'ADP en ATP. Les électrons une fois sur le Cyt f, sont transférés à la plastocyanine (PC) qui réduit la Chl P700 du PSI. Finalement, les électrons passent successivement par divers accepteurs (A0, A1, FA,

FB), dont la Ferredoxine (Fd) réduite, qui agit comme réducteur du NADP+ en NADPH **(Raven, 1992)**.

9.1.2. Les causes du stress oxydatif

Chez les végétaux, les processus aérobiques tels que la respiration et la photosynthèse conduisent à la production continue des Espèces Réactives de l'Oxygène (ERO) ou (Reactive Oxygen Species (ROS)) principalement au niveau des mitochondries, des chloroplastes et des peroxysomes **(Appel et Hirt, 2004)**. Dans des conditions physiologiques normales ces molécules sont éliminées par les différents complexes enzymatiques et non-enzymatiques du système de défense anti-agents oxydants **(Alscher *et al.*, 1997)**. Cependant, l'équilibre entre la production et l'élimination de ces ROS peut être perturbé par des facteurs environnementaux pouvant être biotiques : imposés par d'autres organismes, ou abiotiques : résultant d'une altération de l'environnement physique ou chimique **(Appel et Hirt, 2004)**. Une irradiance importante, des basses ou des hautes températures auraient pour conséquence d'augmenter rapidement la concentration intracellulaire de ces composés et de dépasser les capacités de détoxification des cellules **(Prasad *et al.*, 1994)**. Le stress oxydatif chez les plantes fait l'objet de très nombreuses revues bibliographiques **(Bartosz, 1997; Bolwell et Wojtaszek, 1997; Van Breusegem *et al.*, 2001; Potters *et al.*, 2002**; **Schutzendubel et Polle, 2002; Blokhina *et al.*, 2003; Apel et Hirt, 2004; Foyer et Noctor, 2005a; Pitzschke *et al.*, 2006; Wormuth *et al.*, 2007)** et de plusieurs livres **(Inzé et Montagu, 2001; Smirnoff *et al.*, 2005)**.

9.1.3. Effets des Espèces Réactives de l'Oxygène (ERO)

L'oxygène singulet produit au sein des chloroplastes est hautement réactif, il peut déstabiliser la membrane thylakoïde par peroxydation lipidique **(Asada, 1996)** et entraver le système de renouvellement de la protéine D1 du RC du PSII **(Nishiyama *et al.*, 2004)**. Cependant, des travaux récents suggèrent que les dommages oxydatifs au niveau du PSII ne seraient qu'une étape secondaire qui découlerait des dégradations au niveau du cycle de Calvin **(Jones *et al.*, 1998)**. Les radicaux superoxydes, quant à eux, donnent naissance à des oxydants plus puissants tels que le peroxyde d'hydrogène (H_2O_2) **(Fridovich, 1986)**. Cette molécule non chargée, peut diffuser facilement à travers les membranes biologiques et causer des dommages significatifs. Le peroxinitrite (ONOO-) peut inactiver plusieurs étapes de la chaîne de transport mitochondriale et de la synthèse d'ATP, mais aussi accroître la production de ROS. Il est également capable d'inactiver les Mn-SOD (Super Oxyde Dismutase) et d'influencer l'homéostasie en augmentant

la perméabilité de la membrane mitochondriale ; ce qui libèrerait des molécules potentiellement pro-apoptotiques telles que le cytochrome c des mitochondries **(Radi *et al.*, 2000)**.

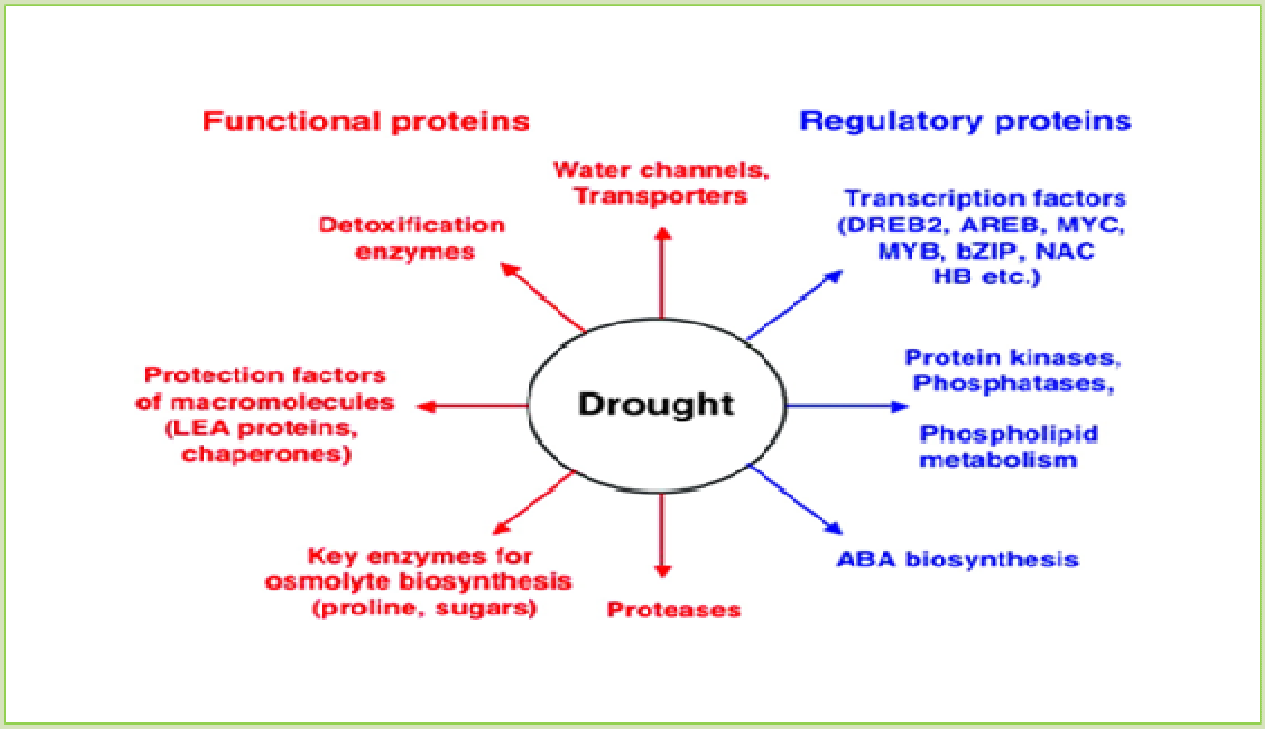
9.2. Les systémes antioxydants

9.2.1. Définition des antioxydants

Peut être considérée comme antioxydante une molécule qui, étant présente en une faible concentration par rapport à celle d'un substrat oxydable, retarde ou empêche significativement l'oxydation de ce substrat **(Halliwell et Whiteman, 2004)**. Selon **Servais (2004)**, un antioxydant peut etre défini comme toute substance qui est capable, à concentration relativement faibe, d'entrer en compétition avec d'autres substarts oxydables et ainsi retarder ou empécher l'oxydation de ces substrats. Les antioxydants cellulaires sont de nature enzymatique et non enzymatique.

Pour se protéger contre les intermédiaires oxygénés toxiques, les cellules végétales et leurs organites comme les chloroplastes, mitochondrie et peroxysomes sont pourvus de systémes de défense antioxydants. Les plantes possèdent des systémes et des mécanismes leur permettant de limiter la production d'ERO lors de leurs processus métaboliques. Certaines plantes se sont adaptées, au cours de l'évolution, à des conditions particulières du milieu, et ont développé des aptitudes métaboliques leur permettant de limiter la saturation des CTE. Citons par exemple les métabolismes C4 et CAM des plantes grasses, la possibilité de mettre en dormance l'appareil photosynthétique lors des saisons sèches par des régulations post-transcriptionnelles ou encore des adaptations morphologiques des feuilles **(Mittler *et al.*, 2001; Mittler, 2002)**. Les plantes possèdent également de nombreux composés et enzymes leur permettant d'empêcher la production d'ERO ou de la contrôler. Un grand nombre de travaux de recherche ont établi que l'induction et la régulation de ces systémes sont essentielles pour la protection contre les effets de diverses contraintes biotiques et abiotiques. Selon **Mittler *et al.*, 2004** ; **Chen et Dickman, 2005**, les composants antioxydants du systémes de défenses sont enzymatiques [(SOD), (CAT), (APX), (MDHAR), (DHAR), (GR)] ou non enzymatiques [(GSH) (ASC), les caroténoides tocophérols]. Des protéines spécifiquement [Dehydrines (Protéines LEA)] impliquées dans la tolérance à la déshydratation cellulaire sont également synthétisées **(Shinozaki *et al.*, 2003 Bartels et Sunkar,2005)** (Figure I.11.). Ainsi, des génes avec des fonctions diverses sont induits ou réprimés par le stress hydrique ou d'autres contraintes osmotique **(Bartels et Sunkar, 2005 ; Yamaguchi-Shinozaki et Shinozaki, 2005 ; Shinozaki et Yamaguchi-Shinozaki, 2007)** (Figure I.12.).

Figure I.11. *Fonctions des gènes inductibles par le déficit hydrique dans les mécanismes de tolérance d'après* ***Shinozaki et Yamaguchi-Shinozaki*** *(2007).*



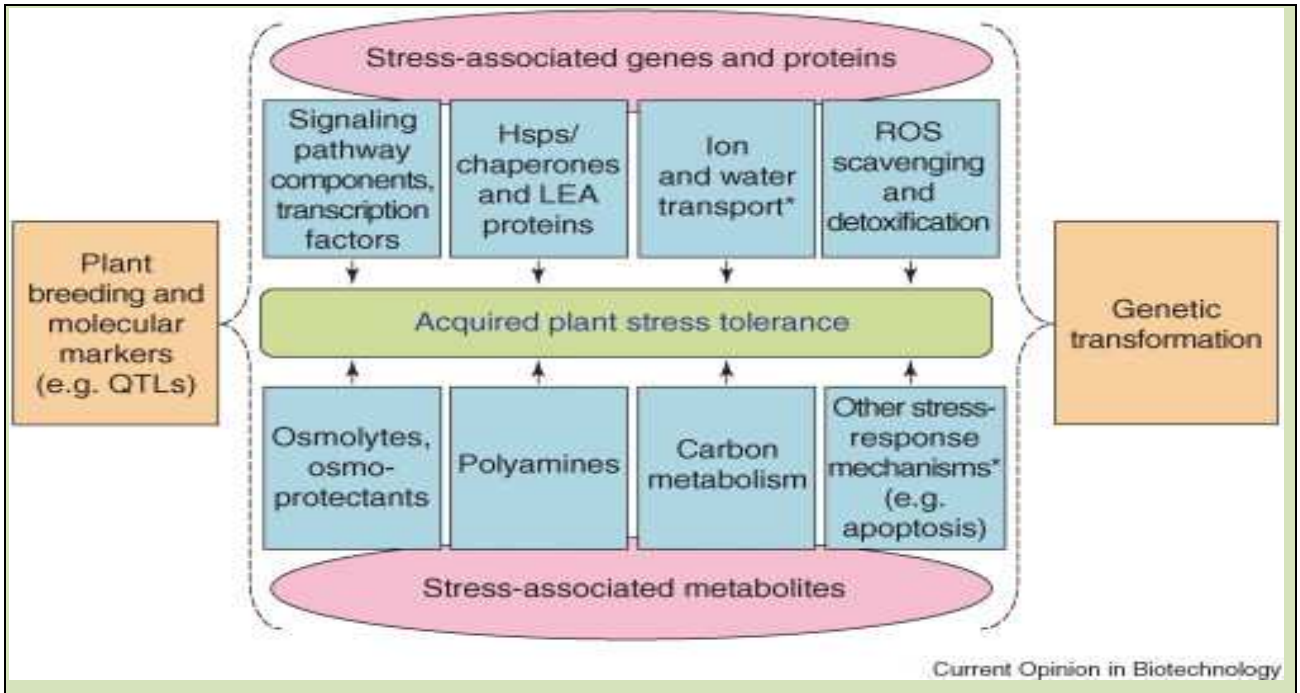


Figure I.12. *Role des protéines de stress et l'expression génique en reponse au stress abiotiques d'après* ***Vinocur et Altman (2005).***

9.2.2. Les systémes antioxydants non- enzymatiques de faible poids moléculaire

Il est important de noter qu'il existe des systèmes non-enzymatiques de dégradation des ROS. Il s'agit le plus souvent du glutathion, de l'ascorbate, de l'α -tocophérol, des flavonoïdes et des caroténoïdes. Ces antioxydants réagissent directement avec les agents oxydants et les désactivent **(Cillard *et al.*, 1982)**.

9.2.2.1. L'acide ascorbique (l'ascorbate ou vitamine C)

L'acide L ascorbique (ASC) est un des principaux acides faibles de la cellule végétale. Aux pH physiologiques, il se dissocie en anion ascorbate. L'ascorbate est essentiellement utilisé au niveau cellulaire comme un donneur d'électrons. Le premier produit de la réaction d'oxydation de l'ascorbate est le radical monodéhydroascorbate (MDHA ; Figure I.13.). Du fait de son électron libre très excentré, le MDHA n'est pas très réactif avec les autres molécules biologiques **(Navas *et al.*, 1994)**. De plus, étant relativement instable, il se transforme spontanément en ASC et déhydroascorbate (DHA) à une vitesse comprise entre 10^5 et $2{,}8.10^6$ $M^{-1}.s^{-1}$ à pH 7 **(Heber *et al.*, 1996)**. Le DHA est également une molécule instable et subit rapidement une hydrolyse conduisant à la formation d'acide 2,3-dikétogulonique (**Deutsch, 1997, 2000)**.

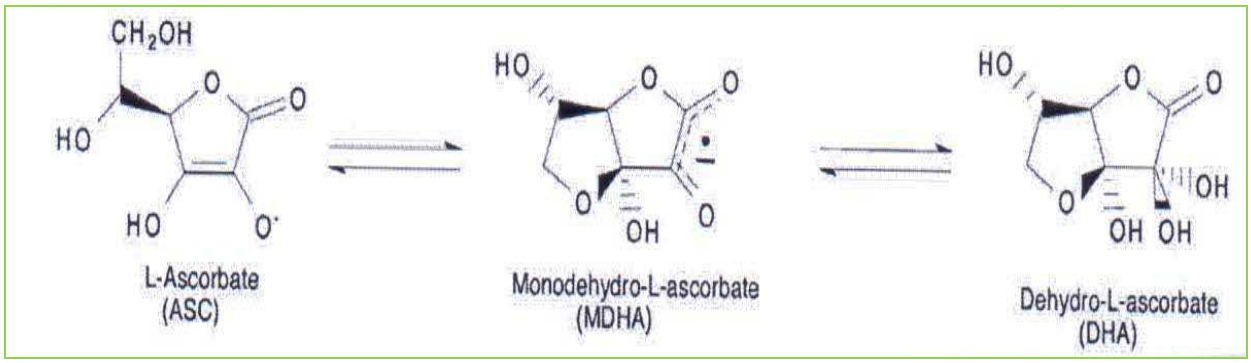


Figure I.13. *Formes redox de l'ascorbate d'après* ***Potters et al., (2002).***

L'ascorbate est présent dans tous les compartiments cellulaires, ainsi que dans la matrice extracellulaire. Contrairement aux cellules animales, la concentration en ASC est très élevée dans les cellules végétales (plusieurs millimolaires) ce qui en fait un composant incontournable chez les plantes. Il interviendrait notamment dans la régulation du cycle cellulaire et dans l'extension de la paroi **(Horemans *et al.*, 2000)**. L'ascorbate est toutefois beaucoup plus connu pour ses propriétés antioxydantes **(Navas *et al.*, 1994; Mehlhorn *et al.*, 1996; Horemans *et al.*, 2000; Turcsanyi *et al.*, 2000; Potters *et al.*, 2002; Pignocchi and Foyer, 2003; Chen and Gallie, 2004; Foyer et Noctor, 2005a)**. En effet, il réagit rapidement avec l'anion superoxyde et

l'oxygène singulet, ou encore avec le peroxyde d'hydrogène, mais cette dernière réaction est catalysée par l'ascorbate peroxydase (APX). L'ascorbate est indispensable par sa capacité à réduire d'autres antioxydants oxydés comme la vitamine E ou les caroténoïdes **(Asada, 1994)**.

9.2.2.2. L'alpha-tocophérol (Vitamine E)

Le terme de vitamine E désigne un groupe de composés lipophiles possédant l'activité biologique de l'α-tocophérol. Ce groupe comprend les α-, β-, γ-, et δ-tocophérols et les α-, β-, γ-, et δ-tocotrienols. Des huit molécules composant le groupe de la vitamine E, l'α-tocophérol possède la plus grande activité biologique **(Weiser et Vecchi, 1982)**. Au niveau cellulaire, ces molécules sont associées aux différentes membranes biologiques. La vitamine E, comme la vitamine C, est un antioxydant très efficace du fait de sa faible propension à être un donneur d'électrons. Elle agit principalement par le transfert direct d'atomes d'hydrogène **(Njus et Kelley, 1991)**. Cet antioxydant est considéré comme étant le plus important chez les animaux et les humains **(Bramley *et al.*, 2000)**, mais son rôle chez les plantes a été beaucoup moins étudié. Toutefois, l'α-tocophérol est un piégeur d'oxygène singulet et de radical hydroxyle **(Krieger-Liszkay et Trebst, 2006)**. Sa localisation, au niveau des membranes, en fait l'antioxydant le plus important dans la prévention de la peroxydation des lipides membranaires **(Fryer, 1992)**. Par ailleurs, il peut réduire les peroxydes lipidiques et bloquer la réaction en chaîne de peroxydation lipidique qui apparaissent après la peroxydation d'acides gras polyinsaturés **(Collin *et al.*, 2008)**. La vitamine E est donc un antioxydant clé dans la protection de l'intégrité des membranes, en particulier celles des chloroplastes. Il protège également les pigments photosynthétiques, participant ainsi à la protection de l'appareil photosynthétique **(Fryer, 1992)**.

L'utilisation de plantes mutantes ne possédant pas de vitamine E, a conduit à des résultats surprenants. En effet, ces plantes ne montraient pas un phénotype significativement différent de celui des plantes témoins, et surtout n'étaient pas plus sensibles que ces dernières à un stress lumineux **(Havaux *et al.*, 2005)**. Ces résultats s'expliquent par un phénomène de protection croisée entre les caroténoïdes et la vitamine E, les uns compensant l'absence de l'autre **(Havaux *et al.*, 2005)**. Cependant, les mutants déficients en vitamine E sont particulièrement sensibles aux métaux lourds, ce qui laisse penser à un mécanisme complexe de protection des cellules par cet antioxydant **(Collin *et al.*, 2008)**. En effet, l'absence de vitamine E peut être compensée, au niveau des chloroplastes, pour éviter la production d'ERO au niveau de la CTE. A contrario, cela ne semble pas être le cas lorsque la génération d'ERO se situe vraisemblablement à un autre endroit de la cellule.

9.2.2.3. Le glutathion

Le glutathion est un thiol très abondant se retrouvant de façon ubiquitaire chez les animaux et les végétaux. Il possède deux formes redox distinctes. La forme réduite (GSH) est un tripeptide (γ-Glu-Cys-Gly), stable, à fort pouvoir réducteur et très soluble dans l'eau. Toutes ces caractéristiques en font un donneur d'électrons adéquat dans les réactions physiologiques. L'oxydation du glutathion entraîne la formation d'un pont disulfure entre les cystéines de deux GSH. Cette forme oxydée du glutathion est appelée GSSG (Figure I.14.). En conditions non stressantes, les cellules maintiennent un ratio GSH/GSSG très important, supérieur à 100 dans le cytosol et la mitochondrie **(May *et al.*, 1998; Noctor *et al.*, 1998)**.

La concentration en GSH est très importante dans les cellules végétales, et en particulier dans les chloroplastes où elle peut atteindre 5 mM **(Noctor *et al.*, 1998)**.

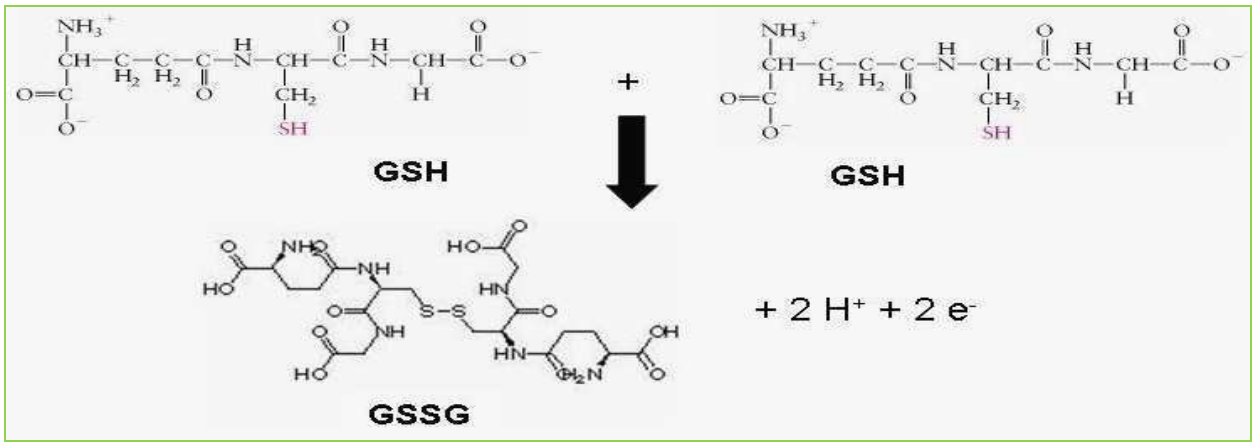


Figure I.14. *Oxydation de deux molécules de GSH conduisant à l'établissement d'un pont disulfure et la formation de GSS d'après* ***May et al., 1998.***

Noctor *et al.*, 1998). Il représente une forme importante de stockage de sulfure réduit, et participe à l'allocation en sulfure des différents compartiments cellulaires et des différents organes **(Herschbach et Rennenberg, 1991; Lappartient et Touraine, 1996)**. C'est également un régulateur de l'expression génique **(Wingate *et al.*, 1988; Baier et Dietz, 1997)** et le précurseur des phytochélatines (PC) jouant un rôle prédominant dans la séquestration des métaux chez les végétaux **(Grill *et al.*, 1989; Clemens, 2006b; Clemens, 2006a)**. Le GSH sert également de substrat pour la glutathion S-transférase (GST) qui catalyse sa conjugaison avec des xénobiotiques, participant ainsi à leur détoxication **(Marrs, 1996)**. Il est impliqué dans la régulation redox du cycle cellulaire **(Gyuris *et al.*, 1993; Noctor *et al.*, 1998)**.

Du fait de sa forte concentration dans les tissus végétaux et de son fort pouvoir réducteur, le GSH participe au statut redox cellulaire en jouant le rôle de tampon redox dans les cellules

(Noctor *et al.*, 1998; Foyer et Noctor, 2000; Foyer *et al.*, 2001; Foyer et Noctor, 2003; Foyer et Noctor, 2005b, a; Noctor, 2006). La synthèse de GSH est stimulée lors des différentes situations de stress et son accumulation est souvent concomitante avec celle des ERO **(May *et al.*, 1998; Noctor *et al.*, 1998; Potters *et al.*, 2002)**. Le GSH peut directement réduire l'H_2O_2 mais également d'autres ERO, des hydroperoxydes organiques et des peroxydes lipidiques :

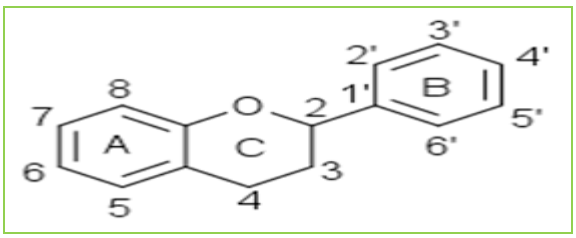
$$H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$$

$$ROOH + 2\ GSH \rightarrow ROH + GSSG + H_2O$$

Un autre rôle important du GSH dans la régulation des ERO est son implication dans la régénération de l'ascorbate via le cycle enzymatique dit ascorbate/glutathion.

9.2.2.4. Les flavonoïdes

Le terme flavonoïde désigne une très large gamme de composés naturels appartenant à la famille des polyphénols. Ils sont considérés comme des pigments quasi universels des végétaux. Structuralement, les flavonoïdes se répartissent en plusieurs classes de molécules, dont les plus importantes sont les flavones, les flavonols, les flavanones, les dihydroflavonols, les isoflavones, les isoflavanones, les chalcones, les aurones, les anthocyanes et les tanins. Ces diverses substances se rencontrent à la fois sous forme libre ou sous forme de glycosides. On les trouve, d'une manière très générale, dans toutes les plantes vasculaires, où ils peuvent être localisés dans divers organes : racines, tiges, bois, feuilles, fleurs et fruits **(Middleton, 1993)** et aussi dans le miel **(Grange, 1990)**. Les flavonoïdes sont des dérivés du noyau FLAVONE ou 2-PHENYL CHROMONE portant des fonctions phénols libres, éthers ou glycosides.



NOYAU FLAVONE

Les flavonoïdes sont donc des polyphénols complexes dont la structure est constituée de deux noyaux aromatiques (noyaux A et B) et d'un hétérocycle oxygéné (cycle C).

Les flavonoïdes sont supposés avoir de nombreuses fonctions : pigmentation des fleurs, fruits et graines, protection contre rayons UV, défense contre les agents phytopathogénes (micro-organismes pathogénes, insectes, animaux). Ils jouent également un role dans la fertilité des plantes et la germination du pollen **(Olsen *et al* ., 2010)**. Le role clé des flavonoïdes dans la

protection contre les rayonnements UV-B a été montré en examinant des mutants d'arabidopsis présentant une capacité de synthétiser les flavonoides réprimée **(Bieza et Lois, 2001)**.

De nombreux génes de biosynthése des flavonoïdes sont induits dans des conditions de stress. En effet, il a été constaté une forte augmentation des quantités de flavonoïdes à la suite de contraintes biotiques et abiotiques, telles que les blessures, la sécheresse, une exposition à des métaux et une carence en nutriments **(Winkel- Shirley, 2002)**. Les flavonoïdes servent de piégeurs d'ERO et peuvent neutraliser des radicaux avant qu'ils n'endommagent les structures cellulaires, ce qui est important pour les plantes dans des conditions environnementales défavorables **(Løvdal *et al.*, 2010)**.

9.2.2.5. Les caroténoïdes

Les caroténoïdes sont des pigments végétaux lipophiles formant une famille de plus de 600 molécules. Ils jouent le rôle de pigments accessoires de l'antenne collectrice des photosystèmes. En dehors de cette implication dans le processus photosynthétique, les caroténoïdes associés au PSII ou à l'antenne collectrice, participent à la protection de l'appareil photosynthétique contre les ERO **(Asada, 1994; Miller *et al.*, 1996)**. En effet, ces pigments possèdent la capacité de capter l'énergie de la chlorophylle triplet ou de l'oxygène singulet **(Telfer *et al.*, 1994; Mozzo *et al.*, 2008)**, ce qui les convertit en état triplet. La capacité de transfert d'énergie des caroténoïdes vers le dioxygène étant faible, ces pigments retrouvent leurs états initiaux en perdant leurs énergies sous forme de chaleur. Le mécanisme captage d'énergie/perte d'énergie par chaleur peut également directement s'effectuer à partir de la chlorophylle singulet **(Demmig-Adams et Adams, 1996).**

9.2.3. Les systémes antioxydants enzymatiques

Les enzymes du système antioxydant régulent différentes espèces de ROS. La superoxyde dismutase (SOD) est chargée de transformer les radicaux superoxydes en H_2O_2. L'élimination de ce dernier est alors l'œuvre d'autres enzymes telles que la catalase et les peroxydases **(Monk *et al.*, 1989; Becana *et al.*, 2000; Matamoros *et al.*, 2003).**

9.2.3.1. Les superoxyde dismutases (SOD)

Les superoxydes dismutases sont des métallo-enzymes se retrouvant dans l'ensemble du monde du vivant, mis à part dans quelques microorganismes **(Alscher *et al.*, 2002)**. Les plantes possèdent trois types de SOD contenant des groupements prosthétiques renfermant des métaux

différents : du fer (FeSOD), du manganèse (MnSOD) ou du cuivre et du zinc (CuZnSOD). Toutes catalysent la dismutation de deux anions superoxydes en dioxygène et peroxyde d'hydrogène **(Bowler *et al.*, 1994; Arora *et al.*, 2002)** :

$$SOD - M^{(n+1)+} + O_2^{\cdot -} \rightarrow SOD - M^{n+} + O_2$$
$$SOD - M^{n+} + O_2^{\cdot -} + 2\,H^+ \rightarrow SOD - M^{(n+1)+} + H_2O_2$$

$$2\,O_2^{\cdot -} + 2\,H^+ \rightarrow O_2 + H_2O_2$$

avec M = Cu (n=1) ; Mn (n=2) ; Fe (n=2).

Au niveau cellulaire, les SOD se retrouvent à proximité des sites de production d'$O_2^{\cdot -}$. Les MnSOD sont localisées principalement dans la matrice de la mitochondrie **(Bowler *et al.*, 1994; del Rio *et al.*, 2003)**, dans la matrice des glyoxysomes ou liées à la membrane des peroxysomes des cellules foliaires **(Sandalio et Del Rio, 1988; Hernandez *et al.*, 1999; del Rio *et al.*, 2003; Rodriguez-Serrano *et al.*, 2007)**. Les FeSOD se situent essentiellement dans le stroma des chloroplastes **(Salin, 1988; Bowler *et al.*, 1994; Asada, 2000)** et chez quelques espèces particulières, dans les matrices des peroxysomes et des mitochondries **(Salin, 1988; Droillard and Paulin, 1990)**, ou dans la fraction cytosolique des racines nodulées **(Becana *et al.*, 1989; Moran *et al.*, 2003)**. Les CuZnSOD se retrouvent essentiellement sous forme libre dans le cytosol **(Duke et Salin, 1983; Bowler *et al.*, 1994)**, dans les peroxysomes ou l'apoplaste **(Ogawa *et al.*, 1996; Sandalio *et al.*, 1997; Corpas *et al.*, 1998)**. Il existe, toutefois, une CuZnSOD liée à la membrane du thylakoïde au niveau du PSI, directement au contact des anions superoxydes produits par la CTE chloroplastique **(Salin, 1988; Bowler *et al.*, 1994; Ogawa *et al.*, 1995)**.

9.2.3.2. Les glutathion péroxydases (GPX)

Les glutathion peroxydases (GPX) sont des enzymes-clés des mécanismes de détoxication chez les plantes **(Navrot *et al.*, 2006)**. En effet, contrairement à leurs homologues animales, les GPX végétales ne possèdent plus de groupement prosthétique sélénium-cystéine dans leur site catalytique, mais seulement un résidu cystéine **(Navrot *et al.*, 2006)**.

Bien que les GPX possèdent encore la capacité d'utiliser le GSH pour réduire l'H_2O_2, des peroxydes lipidiques ou des hydroperoxydes organiques **(Ursini *et al.*, 1995; Mullineaux *et al.*, 1998)**, elles utilisent de façon préférentielle et plus efficiente les thioredoxines comme substrat (**Navrot *et al.*, 2006)**. Certaines GPX végétales sont même incapables d'utiliser le GSH.

9.2.3.3. Les catalases (CAT)

Les catalases sont des enzymes majoritairement peroxysomales catalysant la dismutation du peroxyde d'hydrogène **(Arora *et al.*, 2002)**. Elles sont formées de quatre chaînes polypeptidiques d'environ 500 acides aminés, comportant chacune un groupe hémique comprenant un atome de fer. Pour catalyser la réaction, l'atome de fer réalise une coupure hétérolytique de la liaison O-O du peroxyde d'hydrogène, créant de ce fait une molécule d'eau et un groupement Fe(IV)=O hautement oxydant. Ce dernier peut ensuite oxyder une autre molécule de peroxyde d'hydrogène pour donner du dioxygène et de l'eau **:**

$$H_2O_2 + \text{Fe (III)-CAT} \rightarrow H_2O + \text{O=Fe (IV)-CAT}$$

$$H_2O_2 + \text{O=Fe (IV)-CAT} \rightarrow O_2 + \text{Fe (III)-CAT} + H_2O$$

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Du fait de sa localisation et de sa faible affinité pour l'H_2O_2 le rôle principal de la CAT est de détoxiquer le peroxyde d'hydrogène produit à proximité par la CTE chloroplastique, et surtout par les processus peroxysomaux de β–oxydation et de photorespiration **(Smirnoff, 1998)**. Bien qu'elle ait été longtemps considérée comme une des enzymes antioxydantes les plus importantes, il semble désormais que son importance dans la détoxication de l'H_2O_2 soit réduite, en dehors de celui issu du métabolisme peroxysomal **(Arora *et al.*, 2002; Blokhina *et al.*, 2003; Foyer et Noctor, 2005a, b; Halliwell, 2006; Pitzschke *et al.*, 2006)**. Les peroxydases et peroxyredoxines sont maintenant considérées comme les enzymes clefs de la détoxication de cet ERO chez les plantes. La CAT ne serait qu'un « dévidoir » permettant de limiter les productions excessives d'ERO **(Willekens *et al.*, 1997)**. Cependant, cette vision pourrait être en partie erronée, puisque, bien que les résultats soient souvent contradictoires dans la littérature, l'activité catalasique est souvent affectée négativement lors de stress.

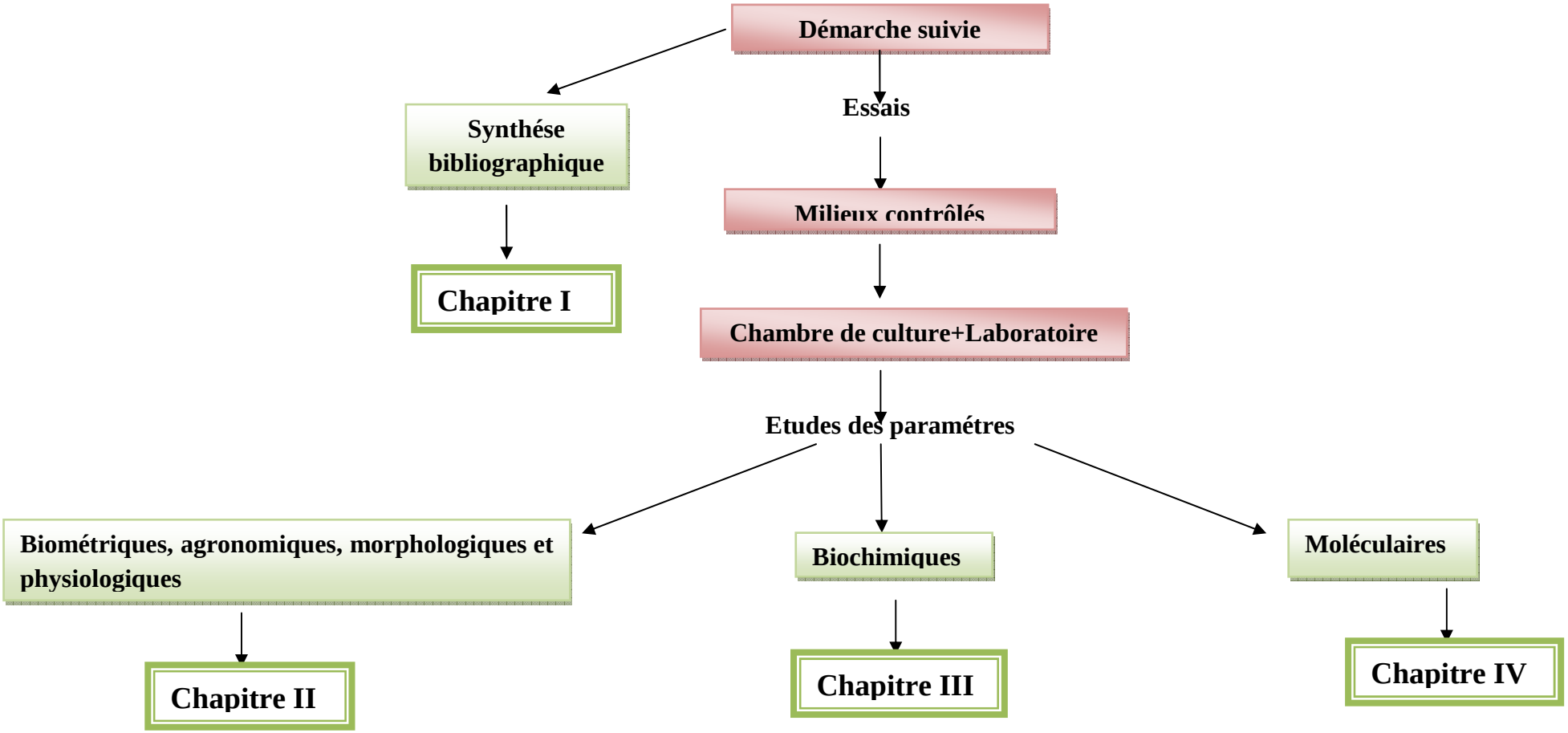


Figure I.13. *Plan de la thèse. Les résultats obtenus feront l'objet des chapitres de cette thèse.* ***Le chapitre I*** *étant consacré à la synthèse bibliographique alors que* ***le chapitre V*** *fera l'objet d'un chapitre intitulé: « Agricultural, socio-economic, and cultural relevance of crop wild relatives, in particular food legume landraces, in Northern Africa » qui sera publié dans le livre « Water Stress and Crop Plants: A Sustainable Approach, 2 » qui a été soumis en 2014 à l'édition John Wiley & Sons Ltd.*

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Chapitre II

Etude des caractères morpho-physiologiques impliqués dans la tolérance au stress hydrique chez quelques populations algériennes d'arachide (*Arachis hypogaea L.*).

Morpho-physiological traits contributing to water stress tolerance in a peanut (Arachis hypogaea L.) landraces collection from the Algerian Maghreb.

L'eau a un rôle fondamental dans la vie des plantes, dans la mesure où elle conditionne leurs activités physiologiques et métaboliques. Elle est le vecteur des éléments nutritifs de la plante **(Riou, 1993)**. Sa carence peut affecter la croissance, elle est, de ce fait, le principal facteur limitant de la production végétale dans les régions arides et semi-arides. Le manque d'eau induit, chez les plantes stressées, une diminution du contenu relatif en eau et une réduction significative de la production de biomasse totale (**Kramer, 1980** ; **Albouchi *et al.*, 2000**) concomitante à une réduction de la croissance en diamètre et en hauteur des tiges **(Van Hees,1997; Aussenac *et al.*,1984 ; Thomas *et al.*,2000)**. Le stress hydrique impose le plus souvent une diminution de la croissance accompagnée, au niveau cellulaire, d'une diminution du nombre de polysomes, d'un ajustement osmotique, d'une modification de l'extensibilité des parois et d'une altération des métabolismes carboné et azoté. Cette diminution est suivie par une réduction de la photosynthèse due à l'accumulation de l'ABA, à la fermeture des stomates et aux dommages causés aux thylakoïdes **(Wakabayashi *et al.*, 1997** ; **Tardieu *et al.*, 2000** ; **Lizana *et al.*, 2006** ; **Martinez *et al.*, 2007)**. La réduction de l'activité photosynthétique induit le plus souvent une baisse de la production économique **(Condon *et al.*, 2002)**. Cependant, l'importance de ces effets varie selon l'espèce et l'intensité du stress hydrique subi.

La capacité d'évaluer quantitativement les performances des plantes cultivées subissant un stress hydrique est très importante au niveau des programmes de recherche qui visent la réhabilitation et l'amélioration de la production en région semi aride **(INRA, 2000)**.

De ce fait l'amélioration de la résistance aux stress abiotiques a été depuis longtemps un des principaux objectifs de l'amélioration des plantes. De nombreuses études ont été conduites pour mieux comprendre les mécanismes impliqués et identifier les caractères qui peuvent être utilisés comme critères de sélection de la tolérance **(Sinclair et Venables, 1983** ; **Blum *et al.*, 1996** ; **Bajji *et al.*, 2001** ; **Richards *et al.*, 2010** ; **Fleury *et al.*, 2010)**.

L'arachide (*Arachis hypogaea* L.) est l'une des plus importantes oléagineuses cultivées dans le monde, notamment dans les régions arides et semi-arides de l'Afrique. Son succès réside dans le fait que c'est une oléo-protéagineuse aux nombreuses utilisations alimentaires et fourragères

(graine, huile, tourteaux, fanes et coques). C'est une légumineuse qui peut utiliser l'azote atmosphérique grâce à la symbiose avec des bactéries de la famille des rhizobiaceae pouvant fournir jusqu'à 30 % de l'azote nécessaire à la plante **(Wani *et al.*, 1995)**.

Chez l'arachide, le stress hydrique réduit la croissance des plantes **(Bontang *et al.*, 2010)** et la productivité **(Nigam *et al.*, 2005**; **Bontang *et al.*, 2010**; **Ratnakumar et Valdez, 2011**). Dans les régions arides et semi-arides, les pertes de rendement chez l'arachide peuvent être atténuées par des pratiques de gestion, mais aussi par l'amélioration génétique, basée sur la variabilité génétique pour la tolérance au stress hydrique qui peut être trouvée dans des collections de germoplasmes existants **(Boontang *et al.*, 2010**; **Hamidou *et al.*, 2011)** où obtenue à partir de prospections et de collections de populations végétales endémiques adaptées aux conditions climatique et édaphiques locales .

L'arachide s'adapte au deficit hydrique par une panoplie de mécanismes morpho – physiologiques dans lesquels, les parties aériennes et soutéraines de la plante peuvent jouer un rôle important. **Ratnakumar et Valdez (2011)** ont souligné que les variétés d'arachide locales tolérantes à la sécheresse mettent en place « une stratégie de conservation d 'eau », par la plus faible demande d'eau possible et cela par l'abaissement de la surface foliaire par rapport aux plantes témoins bien irriguées.

Selon **Bootang *et al.*, (2010)**, des variétés locales d'arachide tolérantes à la sécheresse réduisent leur surface foliaire spécifique (SFS, i.e. la surface foliaire par unité de poids) et accroîssent leur contenu foliaire en chlorophylle (mesurés par le SPAD chlorophyll meter readings, SCMR). **Pandey *et al.*, (1984)** indiquent que l'arachide soumis à un stress hydrique développe un système racinaire dense et pivotant par rapport aux autres légumineuses.

Des études récentes sur les mécanismes morphophysiologiques racinaires impliqués dans l'adaptation de l'arachide à la sécheresse ont pu montré que le poids sec des racines **(Puangbut *et al.*, 2009**;. **Jongrungklang *et al.*, 2013**), la localisation des racines en strates de sol humide **(Jongrungklang *et al.*, 2011; Jongrungklang *et al.*, 2012)**, constituent les principaux contributeurs à la tolérance au stress hydrique chez l' arachide. En outre, plusieurs études ont souligné les capacité fonctionnelle des différntes catégories de racines pour l'acquisition de l'eau **(Rewald *et al.*, 2011)** et leur role dans la symbiose fixatrice d'azote **(Tajima *et al.*, 2008)**, soulignant ainsi l'importance d'étudier la morphologie interne des racines pour obtenir de

nouvelles informations sur la relation qui existe entre la tolérance à la sécheresse et le système racinaire.

Dans ce cadre, les objectifs de ce travail sont I) d' évaluer la diversité biologique d'une collection de populations locales d'arachide en réponse au stress hydrique traditionnellement cultivées dans différentes régions du pays, II) d'explorer les relations entre les multiples traits morpho-physiologiques et la tolérance à la sécheresse, au moyen d'une approche statistique multivariée, et III) d'identifier, dans ces populations locales, des caractères d'intérêt agronomique qui pourraient être utilisés dans les programmes de sélection visant à accroître la tolérance au stress hydrique.

Le matériel végétal consacré à cette étude est composé de dix populations d'arachides (Figure I.14.) provenant de la collection de Mme Benmouhamed et Mme Ghalmi fournies par le département de Productions végétales « ENSA ».

Figure I.14. *Les populations d'arachide* (Arachis hypogaea L) *utilisées dans cette étude d'après* ***Tellah S, 2010.***

Les caractéristiques de ces dix populations d'arachide étudiées sont représentées dans le tableau I.5.

Tableau I.5. *Caractéristiques des dix populations d'arachide étudiées d'après* ***Benmohamed et al., 2004****.*

Critères	Berrihane (BER)	Oum-Tboul (OT)	Tonga (TO)	Timimoun (TIM)	Metlili (MET)	SebSeb (SEB)	Boumalek (BMK)	El Frin (FR)	Adrar (AD)	Oued souf (OS)
Cycle	90 – 120 jours	90 – 120 jours	90 – 120 jours	100 – 140 jours	100 – 140 jours	100 – 140 jours	90 – 120 jours	90 – 120 jours	100 – 140 jours	100 – 140 jours
Type	Spanich	Spanich	Spanich	Spanich	Virginia	Spanich	Spanich	Spanich	Spanish	Spanich
La taille de la gousse	Petite	Moyenne	Moyenne	Moyenne	Grande	Grande	Moyenne	Moyenne	Grande	Grande
Port	Erigé	Erigé	Erigé	Erigé	Rampant	Erigé	Erigé	Erigé	Rampant	Erigé
Ceinture	Absente	Légére	Moyenne	Prononcée	Prononcée	Prononcée	Absente	Absente	Prononcée	Prononcée
Nombre de cavité	1 à 2	1 à 2	1 à 2	1 à 3	1 à 3	1 à 3	1 à 2	1 à 2	1 à 3	1 à 3
Poids de cent grains (gr)	71,11	82,65	71,75	81,73	172,23	247,53	75,89	75,41	76,80	107,50
Coque	Très mince	Mince	Mince	Dure	Dure	Dure	Mince	Mince	Dure	Dure
Rendement	Très élevé	Moyen	Elevé	Moyen	Moyen	Elevé	Très élevé	Elevé	Moyen	Moyen
Origine	El-Kala	El-Kala	El-Kala	Timimoun	Ghardaia	Ghardaia	El-Kala	El-Kala	Adrar	Oued Souf

La dénomination de ces populations correspond au nom des localités d'où elles ont été prélevées (Tableau I.6.). Les sites de collecte sont géographiquement et climatiquement divisés en deux régions (Figure I.15.) suivant les données de l'Office national de météorologie (ONM):

(1) El Kala, (provenances du Nord-Est);

(2) Oasis du Sahara centrale et orientale (Adrar, Timimoun, Ghardaïa et Oued Souf).

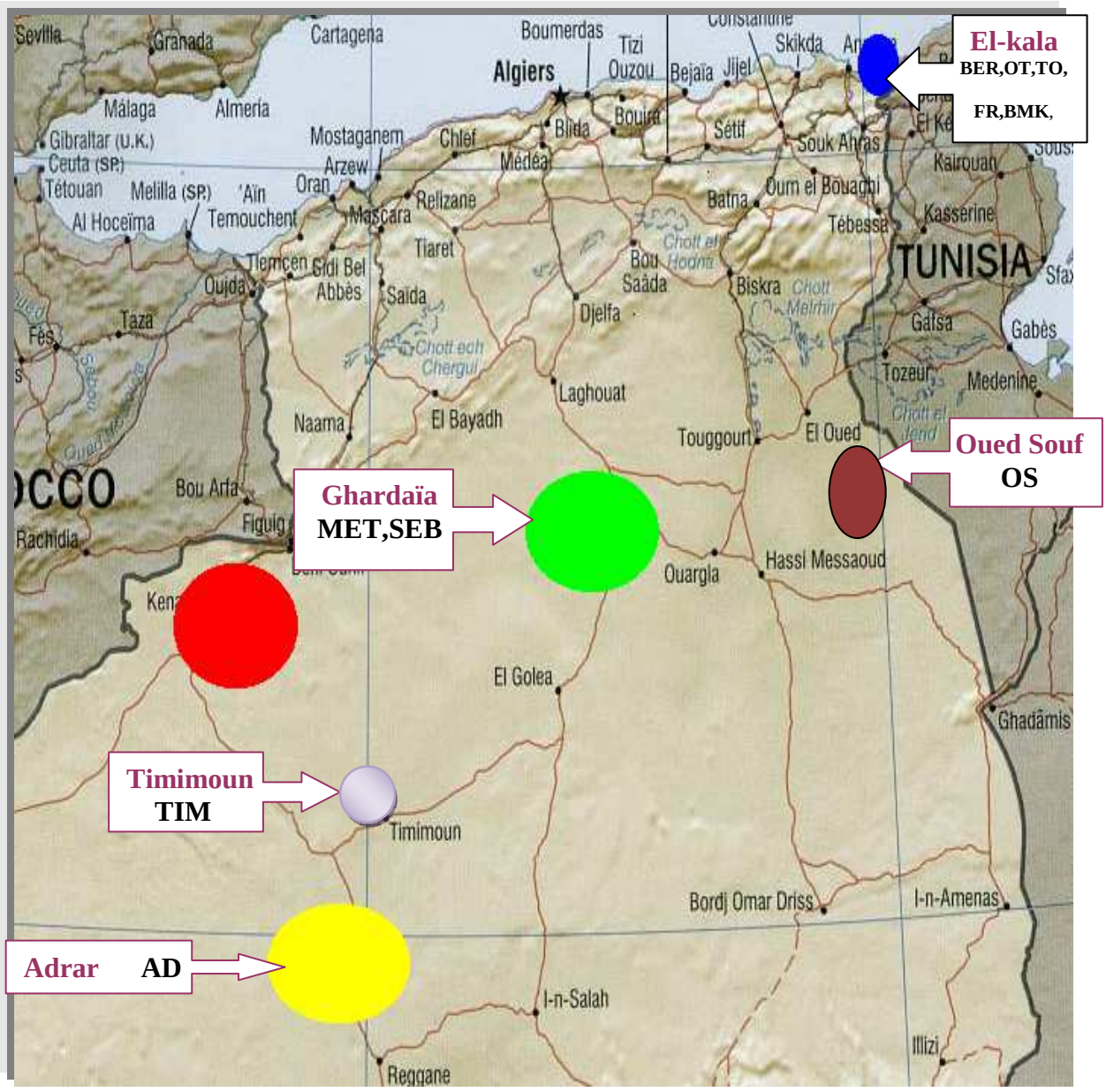

Figure I.15. *Provenance des populations d'arachide (Arachis hypogaea L.) étudiées d'après* ***Tellah S, 2010.***

Les coordonées géographiques des différentes populations étudiées sont reportées dans le tableau I.7.

***Tableau I.6.** Provenance des populations et leur dénomination d'après **Benmohamed et al., 2004**.*

Zone géographique	**Etage bioclimatique**	**Région de collecte**	**Lieu de collecte**	**Dénomination des populations**
Sahara central	**Saharien**	Adrar	Tsabit	Adrar (AD)
		Oued Souf	Oued souf	Oued souf (OS)
		Timimoun	Ouled Said	Timimoun (TIM)
Littoral est.	**Subhumide**	El-Kala	Au nord est du Lac Tonga	Oum Tboul (OT)
			À l'ouest du Lac tonga	Tonga south east (TO)
			À l'est du lac Mellah.	Boumalek (BMK)
			Au sud du lac des oiseaux.	Berrihane (BER)
			Au nord Est du lac obeira	El Frin (FR)
Sahara du Nord	**saharien**	Ghardaia	Metlili	Metlili (MET)
			SebSeb	SebSeb (SEB)

Tableau I.7. *La longitude, latitude et altitude des populatios locales d'arachide étudiées (Arachis hypogaea L.) d'après* ***Benmohamed et al., 2004****.*

Populations	Longitude (m)	Latitude (m)	Altitude (m)
Oum Tboul (OT)	08° 31E	36° 53N	2.2
El Frin (FR)	08° 42E	36°83N	28
Boumalek (BMK)	08 ° 33E	36°87N	3
Metlili (MET), SebSeb (SEB)	02°87E	30°57N	380
Adrar (AD)	00°25W	28°36N	256
Timimoun (TIM)	00°27E	29°30N	335
Tonga (TO)	8° 32'E	36° 49 N	2.2
Oued souf (OS)	06° 52E	33°22N	77
Berrihane (BER)	36°42 N	08°07 E	10

Un isolat rhizobien a été retenu au cours de cette étude BRE 1.2. Cet isolat est caractérisé par une croissance lente, il a été isolé à partir de nodules d'arachide (Annexes I). Les caractéristiques de cet isolat bactérien BRE 1.2. sont représentées dans le tableau I.8 et Figure I.17.

Tableau I.8. *Les caractéristiques morphologiques de la souche bactérienne BRE 1.2. utilisée dans notre étude d'après* ***Djebara et al., 2004.***

Souche	Taille des colonies			Couleur	Forme	Mucosité	Type de culture	Provenance
	48H	72H	7days					
BRE 1.2.	Absence de colonies	Punctifo-rmes	Stries pleines	Opaques blanchâtres	Circulaires à bords lisses	Mucoïde	Isolat à croissance lente	Berrihane (El kala)

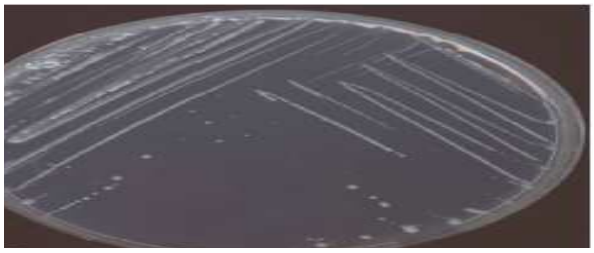

Figure I. 16. *Aspect cultural de l'isolat BR1.2. sur milieu YEMA : isolat à croissance lente (colonies opaques) d'après* ***Tellah S, 2011****.*

Résumé

Le travail présente un large éventail de mécanismes morpho-physiologiques, impliquant différentes parties de la plante, qui entrent en jeu dans le processus d'adaptation au stress hydrique. Pour cette raison, l'analyse unievariée est souvent insuffisante pour mettre en évidence les relations entre ces mécanismes et la tolérance au stress hydrique. Ainsi, l'application d'une approche multivariée, comme l'analyse des composantes principales (ACP), à l'ensemble des données présentées a été utilisée pour mettre en évidence cette relation.

Les résultats obtenus dans ce chapitre ont permis d'identifier les relations entre quelques caractères morpho-physiologiques et la tolérance au stress hydrique ; et ceci par une évaluation de la réponse au stress hydrique d'une collection locale d'arachide (*Arachis hypogaea* L.) traditionnellement cultivée dans différentes régions d'Algérie. Une approche statistique multivariée a été appliquée pour les données obtenues : deux composantes biologiquement significatives ont été identifiées: (i) la «*vigueur des racines* » associée à un système racinaire long et profond, présentant à la fois une forte distribution de racines latérales contenant plusieurs nodules, et une croissance prononcée de la tige; (ii) les «*mécanismes- physiologiques*», associés à la capacité de maintenir un bon état physiologique des feuilles et un abaissement de la température du couvert végétal, par l'intermédiaire des valeurs permissives du potentiel hydrique des feuilles qui permet un certain degré de perméabilité stomatique, même dans des conditions de stress hydrique sévère (25 jours de stress hydrique).

De ce fait, il est devenu possible de modéliser de façon fiable, la tolérance au stress hydrique et d'identifier un sous-groupe au niveau des populations locales d'arachide étudiées formé par **Berrihane (BER)**, **Tonga (TO)**, **Adrar (AD)**, **Oum Tboul (OT)** et **Boumalek (BMK)** qui ont donné les meilleurs performances pour les deux composantes étudiées à savoir la ***«vigueur des racines »*** et ***«mécanismes- physiologiques »***, tandis que **Metlili (MET), SebSeb (SEB), Oued Souf (OS), El Frin (FR)** et **Timimoun (TIM)** ont donné les valeurs les moins performantes.

Sur cette base, il est suggéré ici que ces populations locales d'arachide étudiées, présentant un système racinaire idéotype en mesure d'optimiser l'absorption de l'eau, accompagné par une bonne biomasse aérienne et une teneur chlorophyllienne élevée, même dans des conditions de stress hydrique sévère pourraient être utilisées comme des lignées parentales dans les programmes de sélection dans les zones de cultures potentielles caractérisées par des sécheresses au cours du cycle de l'arachide. Dans l'ensemble, et en raison de leur provenance et leur grande

variabilité inhérente, les populations d'arachide étudiées peuvent être considérées comme une collection utile pour l'étude des mécanismes morphologiques et physiologiques déployés par cette espèce.

L'ensemble des résultats obtenus a fait l'objet d'un premier article intitulé: «Morpho-physiological traits contributing to water stress tolerance in a peanut (*Arachis hypogaea* L.) landraces collection from the Algerian Maghreb».

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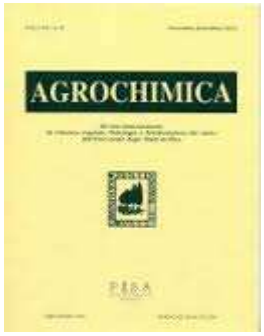

Morpho-physiological traits contributing to water stress tolerance in a peanut (*Arachis hypogaea* L.) landraces collection from the Algerian Maghreb.

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Keywords	drought, landraces, Maghreb, peanut, root morphology.
Abstract	To explore the relationships between morpho-physiological traits and drought tolerance, we evaluated here the response to water stress exhibited by a collection of peanut (Arachis hypogaea L.) landraces traditionally grown in different climatic regions of the Algerian Maghreb. Upon applying a multivariate statistical approach to the data obtained from water-stressed plants, two components were identified: 1) the 'root vigour', associated to a long root system, exhibiting both deep and laterally spreading rooting pattern, abundant N2-fixing nodules, and pronounced stem growth; 2) the 'above-ground physiological mechanisms', associated to the ability to maintain greener leaves and cooler canopy temperature, via permissive values of leaf water potential allowing a certain degree of stomatal perviousness, even under conditions of severe water stress. On such bases, it became possible to identify a subgroup of peanut landraces which could be considered as parental lines in breeding programs for those cultivation environments facing endemic water shortage.

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SUMMARY. – To explore the relationships between morpho-physiological traits and drought tolerance, we evaluated here the response to water stress exhibited by a collection of peanut (*Arachis hypogaea* L.) landraces traditionally grown in different climatic regions of the Algerian Maghreb. Upon applying a multivariate statistical approach to the data obtained from water-stressed plants, two components were identified: 1) the 'root vigour', associated to a long root system, exhibiting both deep and laterally spreading rooting pattern, abundant N2-fixing nodules, and pronounced stem growth; 2) the 'above-ground physiological mechanisms', associated to the ability to maintain greener leaves and cooler canopy temperature, via permissive values of leaf water potential allowing a certain degree of stomatal perviousness, even under conditions of severe water stress. On such bases, it became possible to identify a subgroup of peanut landraces which could be considered as parental lines in breeding programs for those cultivation environments facing endemic water shortage.

1. **INTRODUCTION**. **–** Drought is the worldwide environmental stressor with the highest negative impact on crop biological performance and marketable yield, both of which are expected to be challenged by the increase in frequency, intensity and duration of water stress episodes associated with global climate changes.

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Peanut (*Arachis hypogaea* L.) is an important food and cash legume, widely grown in arid and semiarid environments. Drought stress reduces peanut growth (Boontang *et al.,* 2010) and productivity (Nigam *et al.,* 2005; Boontang *et al.,* 2010; Ratnakumar and Vadez, 2011). Peanut yield losses in arid environments may be ameliorated by management practices, but major progress might be achieved by genetic improvement, based on the genetic vari-ability for drought stress tolerance which can be found in existing germplasm collections (Boontang P*et al.,* 2010; Hamidou *et al.,* 2011) or obtained from ad hoc prospections in search of crop landraces, which are cultivated plant populations adapted to local climatic and edaphic conditions.

Peanut adapts to water-limited environments by an array of morphophysiological mechanisms, in which both the above- and the belowground plant parts can play a role. Ratnakumar and Vadez (2011) pointed out that drought-tolerant peanut landraces put in place a 'conservative water strategy', achieving the lowest possible water demand by lowering their leaf area respect to well-watered controls. Furthermore, to support the highest possible photosynthetic rate under drought conditions, drought-tolerant peanut landraces reduce their specific leaf area, (SLA, i.e. leaf area per unit of weight) and increase their chlorophyll foliar content (measured as SPAD chlorophyll meter readings, SCMR; Bootang *et al.,* 2010).

A complex picture also emerged from recent studies on root morphophysiological mechanisms participating in peanut adaptation to drought. Indeed, root dry weight (Puangbut et *al.,* 2009; Jongrungklang *et al.,* 2013), or root localization into moist soil strata (Jongrungklang *et al.,* 2011; Jongrungklang *et al.,* 2012), have been indicated as being among the major contributors to water stress tolerance in peanut germplasm, although such results have been questioned (Ratnakumar and Vadez (2011).

Considering a larger number of "root phenes or traits", however, could help in reconciling among each other contradictory data sets, allowing a better understanding of the root strategies contributing to drought tolerance. In this respect, Lynch and Brown (2012) proposed that plants exhibit an array of root traits of importance for acquisition of soil resources, traits which could be exploited in breeding programs. Among them, the root length ratio (i.e. the root length per unit of the plant's dry mass; Ryser, 1998). the root mass ratio (the root mass per unit of the plant's dry mass), the root fineness (the root length per unit root volume; Rewald *et al.,* 2011; Tucker *et al.,* 2011), and the root tissue density (the root dry mass per unit root volume; Ryser,

1998; Romano *et al.,* 2013). Furthermore, several studies underlined the different functional ability of the diverse root orders, both towards water acquisition (Rewald *et al.,* 2011) and for hosting N2-fixing symbiosis (Tajiima *et al.,* 2008), thus highlighting the importance of studying within-root morphology for obtaining novel information on the relationship between drought tolerance and the root system.

Within this framework, the aims of the present work were 1) to evaluate the biological diversity in response to water stress exhibited by a collection of peanut landraces traditionally grown in different regions of the Algerian Maghreb, 2) to explore the relationships between multiple morpho-physiological traits and drought tolerance, by means of a multivariate statistical approach, and 3) to identify, in selected peanut landraces, useful traits which might be promising for breeding programs aiming at increasing drought stress tolerance in commercial lines.

2. MATERIALS AND METHODS.

2.1. Plant material. – Experiments were performed on 10 peanut Algerian landraces obtained from a prospection conducted by the Ecole Nationale Supérieure Agronomique, Algiers, Algeria, during the years 2003-2005. Based on the locations in which they were collected, the peanut landraces were assigned the following names: Berrihane (BER), Tonga (TO), Oum Tboul (OT), El Frin (FR), Seb Seb (SEB), Oued Souf (OS), Adrar (AD), Boumalek (BMK), Timimoun (TIM) and Metlili (MET). Botanical and agronomic features of peanut landraces, as well as their geographical and climatic distribution within the Algerian territory, are reported in Amri (2008) and Lazali (2009). To avoid confusion with the acronyms used for plant parameters, the full names of the peanut landraces are used in the text thereafter, whereas their acronyms are used in tables and figures.

2.2. Seeds inoculation. – Two days before sowing, healthy seeds of uniform size were selected for each peanut landrace. Selected seeds were first soaked into an aqueous solution of 40% (w/v) arabic gum and then evenly coated with peat inoculated with the rhizobial strain BRE 1.2 which was previously isolated from nodules of the same peanut populations (Benmohamed *et al.,* 2004). A trapping test conducted before seeds inoculation allowed to conclude that the soil mix used for plant growth did not contain any non-specific rhizobia able to infect the experimental plant material.

2.3. Plants' growth conditions. – Four seeds for each landraces were sown in each of six 6 L-pots, each filled with 4 kg of a 50/20/30 (v/v/v) agricultural soil/peat/washed quartz sand mixture. The soil physico-chemical characteristics are reported in Gelsomino *et al.* (2012). Peatmedium properties were (%, w/w dry weight): organic C 52%, organic N 0.8 %, organic matter 90%, pH_{H2O} 7,3. The particle size of quartz sand (Progettinelblu.com) was 0.5 mm. After the emergence (5 days after sowing, DAS), two seedlings of each landraces per pot was used for the experiment.

Planted pots were randomly distributed (Annex II) within a walk-in climatic chamber, where an air temperature of 26 ± 1°C, a relative air humidity of 70 ± 5 %, and a photoperiod of 12 h were set. Photosynthetic photons flux density at plants' height was 350 μmol m^{-2} s^{-1} (LI-190SA quantum sensor, Li-Cor, Lincoln, NE).

Since both agricultural soil and peat medium were reputed to contain adequate levels of N, P and organic matter, no further fertilizer was supplied during the plants' growth. Planted pots were given 200 mL of tap water every four days because preliminary trials indicated that such amount and frequency of water supply was required to compensate water losses by evapotranspiration.

2.4. Imposition of water stress. – After six weeks of growth (47 DAS), plant material was divided into two halves, each consisting of 3 pots for each peanut landrace. One half pots continued to receive adequate water supply (see above) and acted as the well-watered control (W-plants), whereas irrigation was withheld on the other half of pots, thus imposing a progressive water stress to plants (S-plants). Keeping in mind the results of Amri (2008), who observed that nodulation, plant height and dry matter production of Algerian peanut landraces were affected after 15 days of irrigation withdrawal, water deprivation on the present S-plants lasted for 25 days. During water stress treatment, S- and W-plants were kept together in the same controlled environment where they were previously grown (see above).

2.5. Above-ground growth, functional traits and drought tolerance index. – Drought effects on the above-ground growth rates of peanut landraces were estimated by measuring the following parameters immediately before (47 DAS) and at the end (72 DAS) of the water deprivation period: stem length (StL, cm), leaf area (LA, cm^2), number of branches (NB), and number of leaves (NL). This allowed the calculation of rates for stem elongation (SER, cm d^{-1}),

leaf area expansion (LAER, $cm^2\ d^{-1}$), stem branching (SBR, $n\ d^{-1}$) and foliation (FoR, $cm\ d^{-1}$). At the end of the water stress treatment, the seedlings were harvested and divided into leaves and stems, which were quickly weighed to obtain the leaf (LFW, g) and stem (StFW, g) fresh weights and then placed in an oven at 70°C for 2 days to determine the leaf dry weight (LDW, g). Finally, the leaf dry mass per area (LMA, g cm2) was calculated by the LDW/LA ratio (Matias *et al.*, 2012).

For each peanut landrace, the yield tolerance index (YTI; Ober *et al.*, 2004), based on the LDW, was calculated according to the following formula:

$$YTI = (mLDW_S/mLDW_W) / (ALDW_W)^2.$$

where $mLDW_S$ and $mLDW_W$ were, respectively, the mean LDW measured in S- and W-plants of that landraces, whereas $ALDW_W$ was the average value of LDW among all the landraces under well-watered conditions.

Chlorophyll content (Chlorophyll Meter Model SPAD-502 Plus, Minolta Camera Co. Ltd, Osaka, Japan) was indirectly measured in vivo on four half-eight fully expanded leaves for each landrace, always between 10:00 and 11:00 h (GMT).

2.6. Root and nodule morphological analysis. – At the end of the water stress treatment, the whole peanut seedlings were carefully excavated from pots and divided into shoot and roots. After gently removing the adhering soil substrate, the roots were washed in sequence with tap water and deionised water, paper-blotted and then divided into primary and laterals. The root material was then stained according to Romano *et al.* (2013) and finally scanned at a resolution of 300 dpi by a WinRhizo STD 1600 image acquisition system, equipped with a WinRhizo Pro v. 4.0 software package (Instruments Régent Inc., Canada). The following root parameters were measured: length (L, cm), surface area (SA, cm^2), volume (V, cm^3) of the primary (Pr) and lateral root (Lr), and number of lateral roots (N). Image analysis was also employed for measuring the diameter, number, and form of the root nodules. The dry weight of the shoot (ShDW, g) and total root dry weight (RDW, g) were measured after oven-drying at 70°C for 48 h and the plant dry weight (PDW, g) was calculated as the sum of ShDW and RDW.

Based on the above measurements, total length (RL, cm) and volume of root system (RV, cm^3), root length ratio (RLR = RL/PDW, $cm\ g^{-1}$), root mass ratio (RMR = RDW/PDW, $g\ g^{-1}$),

root fineness (RF = RL/RV, cm cm-3) and root tissue density (RTD = RDW/RV, g cm-3), Total length of lateral roots (LL, cm) and average lateral root length (ALL, cm) were calculated.

2.7. Plant water relations. – To evaluate water stress intensity, the predawn leaf water potential, which is assumed to equilibrate to the soil water potential (Richter, 1997), was measured immediately before the beginning of the growth chamber photoperiod on 3 leaves for each landrace, by using a portable pressure chamber (3005 Plant Water Status Console, Soilmoisture Equipment Corp., Santa Barbara, CA). By following such procedure, water potential in potted soil hosting W- and S-plants was estimated to be -0.05 ± 0.01 and -0.61 ± 0.1 MPa, respectively (n = 30).

To investigate plant water relations, the maximum diurnal values of leaf conductance to water vapour (g_{Lmax}), the minimum diurnal values of the leaf water potential (Ψ_{Lmin}) and the relative water content of the leaves (RWC) were measured, always between 12.00 and 14.00 h (GMT). (LiCor mod. LI-1600, Lincoln, NE). For each landrace and water stress regime, g_{Lmax} was measured on at least five leaves from different plants using a steady-state porometer Ψ_{Lmin} was measured with a portable pressure chamber (see above) on at least three leaves from different plants, following the procedure of Lo Gullo *et al.* (2003). The relative water content was measured on 3 leaves from different plants following the procedure of Lo Gullo *et al.* (2003) and calculated as reported therein. The values of leaf water potential at the turgor loss point (Ψ_{tlp}) and of osmotic potential at full turgor (Ψ_{π}) were obtained from pressure-volume curves of three leaves per landraces and per treatment (Tyree and Hammel 1972; Salleo 1983). Because no statistical differences were recorded between W- and S-plants, the mean Ψ_{tlp} and Ψ_{π} values calculated on both water regimes are reported.

2.8. Statistical analysis. – All data were tested for normality (Kolmogorov- Smirnoff test) and homogeneity of variance (Levene Median test) and, where required, the data were transformed.

Values obtained for shoot and root growth, root and nodule morphology, and g_{Lmax}, RWC and Ψ_{Lmin}, were statistically analyzed by a twoway ANOVA, with the peanut landrace and the water regime (W or S) as the main factors. Subsequently, the Tukey's test was applied to compare the

mean values among landraces and drought stress levels. One-way ANOVA, in combination with post-hoc pair-wise comparisons by using the Tukey's test, were adopted for the statistical analysis of Ψ_{tlp} and Ψ_{π}. The dataset obtained from the S-plants only was subjected to a principal components analysis (PCA), based on a correlation matrix of all the measured parameters (Afifi *et al.,* 2004). Finally, cluster analysis was carried out to measure the hierarchical similarity among peanut landraces.

In particular, from the PCA values, an Euclidean distance matrix was established to obtain a relative dendrogram. The entries were clustered using Ward's minimum-variance method (Afifi *et al.,* 2004). For statistical analysis, the SPSS Statistics v. 15.0 software (IBM Corp., Armonk, NY) was used. Graphics were prepared by using the SigmaPlot v. 8.0 software (Jandel Scientific, San Rafael, CA).

3. RESULTS AND DISCUSSION

3.1. Criteria for identifying droughttolerant and sensitive peanut landraces. – Biomass production was adopted here as the criterion for ranking the studied group of peanut landraces according to their capacity to withstand water deprivation. Indeed, biomass production is closely correlated to water use efficiency (Jongrungklang *et al.,* 2008), to yield components (Jongrungklang *et al.,* 2012) and to drought tolerance in peanut germplasm growing under conditions of water limitation (Nigam *et al.,* 2005).

In the present study, the LDW trait, assumed to be a reliable proxy of biomass production, was modified by the landrace factor only (L factor in Fig. 1a): at the end of the water deprivation period, the peanut landraces Berrihane, Tonga, Oum Tboul and El Frin showed the highest LDW values, whereas Seb Seb, Oued Souf and Metlili exhibited the lowest ones.

Following the indication of Ober *et al.* (2004), we calculated the yield tolerance index (YTI) values based on the LDW. The results obtained suggest that Berrihane (YTI = 3.46), Tonga (2.09) and Oum Tboul (1.18) can be regarded as the most drought-tolerant, whereas Seb Seb (0.23), Oued Souf (0.35) and Metlili (0.45) as the most droughtsensitive; Boumalek (1.13), Adrar (1.07), El Frin (0.56) and Timimoun (0.52) showed intermediate YTI values. On the whole, these results were found to correlate with those shown in Fig. 1a. Fig. 1b shows in a synoptical form, in terms of LDW of each landrace, the relationship among its adaptation potential (efficiency under drought) and its yield potential (responsiveness to adequate irrigation): Berrihane, Tonga

and Oum Tboul resulted to be drought-tolerant and high-yielding, El Frin drought-tolerant but low-yielding, Adrar and Boumalek high yielding but drought-sensitive, and, finally, Timimoun, Metlili, Oued Souf, and Seb Seb were both drought-sensitive and low-yielding.

3.2. Above-ground growth and functional analysis. – The above-ground plant performance under water stress was evaluated by measuring allocation (LFW), growth (SER and LAER), initiation processes (SBR and FoR) and leaf functional traits (LMA and SCMR; Table 1). A wide genotypic variability was noticed, which included both constitutive and drought-adaptive features. In particular, LFW was remarkably influenced by both the L and the T factors, with values ranging as much as from 1.9 to 5.7 g and from 1 to 2.9 under well watered and drought

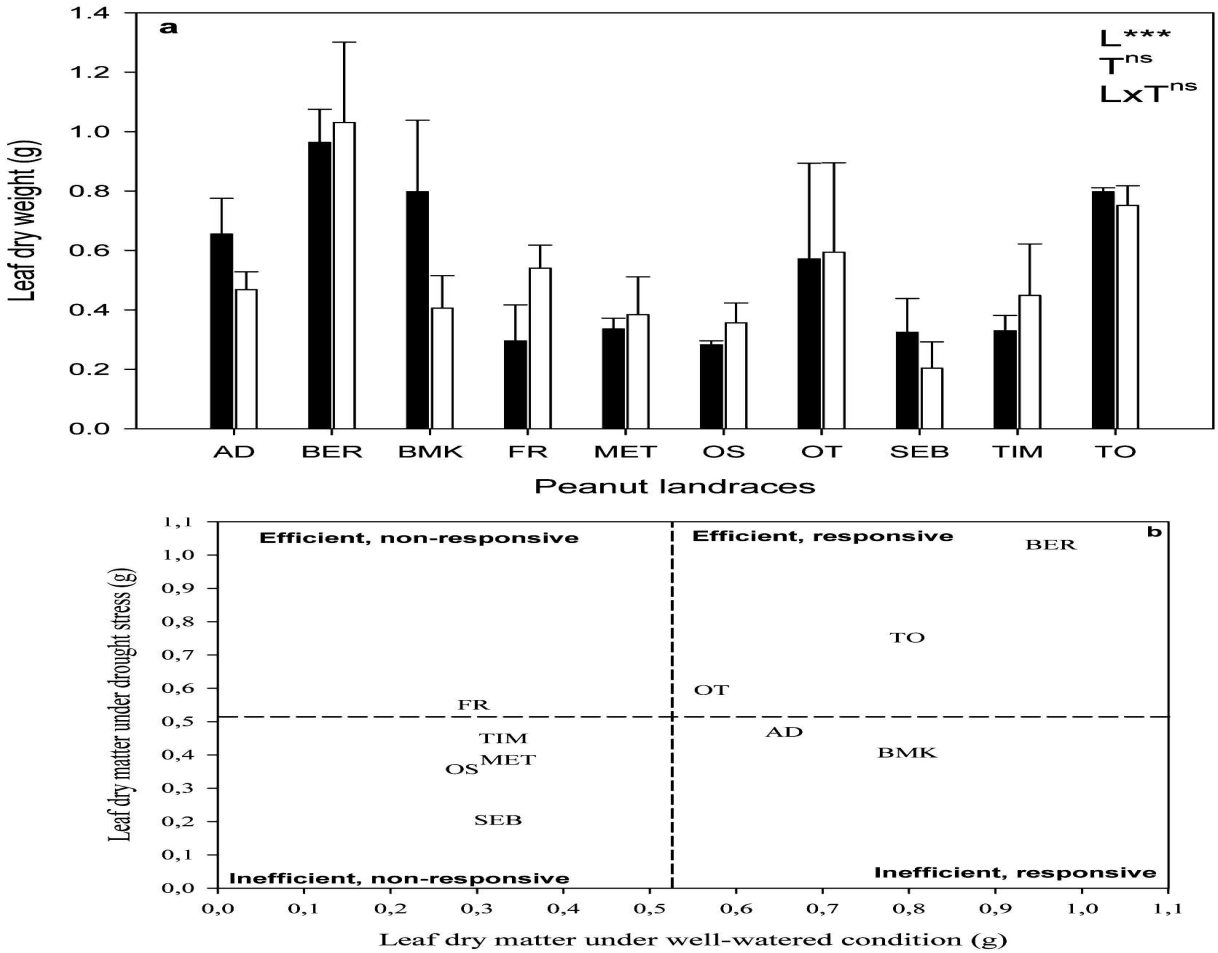

Fig. 1. – a) Leaf dry matter in Algerian peanut landraces after 25 days of water deprivation (□). Filled histograms (■) denote well-watered controls. Statistically significant effects of the landrace (L), the water regime (T) and of their interaction are reported (*P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant), as evaluated by a two-way ANOVA. For peanut landraces acronyms, see the text. b) Identification of Algerian peanut landraces as efficient under conditions of water stress (Y axis) or

responsive to optimal watering (X axis). Dashed lines denote the mean values from all landraces. For landraces acronyms, see the text.

conditions, respectively. Berrihane showed the highest LFW among the studied landraces, whereas Oued Souf and the Adrar showed the lowest ones. Furthermore, a twice reduction of LFW in presence of water stress was observed.

The stem elongation rate, as LFW did, was strongly influenced both by the L and by T factors

(Table 1), with variability among landraces spanning by a factor of three under well watered conditions and even by one order of magnitude under drought stress. Further, a statistically significant LxT interaction was also obtained: Timimoun, Tonga, and El Frin showed no reduction of SER under drought stress, whereas all the remaining peanut landraces did, being Boumalek the least and Oued Souf the most affected.

Similarly to SER, LAER was also affected by the L factor and a statistically significant LxT interaction was also evident (Table 1). In particular, water stress reduced the LAER only in the Oued Souf, Metlili, Oum Tboul and Adrar landraces.

Compared to SER and LAER, the SBR and FoR, both reflecting initiation processes, were less affected by water deprivation (Table 1). This confirms that, in peanut, drought stress inhibits more the intermodal length than node and branch numbers (Chung et al., 1997). Besides, both the L factor and the LxT interaction were found to significantly affect SBR. In particular, Berrihane and Adrar were the sole peanut landraces in which SBR was reduced by drought stress. Unlike SBR, FoR was not modified by the L factor, although a significant LxT interaction was observed. Indeed, Berrihane and Adrar landraces increased or reduced their FoR, respectively, in the presence of water stress. Apart from leaf morphological traits, we considered here certain leaf functional parameters strictly linked to adaptation to drought, i.e. LMA and SCMR. The former parameter, together with its reciprocal, i.e. SLA, plays a prominent role in plant adaptation to drought (Matias *et al.*, 2012), whereas the latter is assumed to reflect adaptation in terms of photoassimilation. Specific leaf area and SCMR were found to be negatively and positively correlated, respectively, with water use efficiency (Puangbut *et al.*, 2009). Besides, both of them were statistically correlated with pod yield and biomass in different peanut landraces under drought stress (Bootang *et al.*, 2010).

Variable	Statistics	Treatment (T factor)	Landraces (L factor) OS	SEB	MET	BER	BMK	TIM	TO	OT	AD	FR	*AT*
SER	L***	W	0.89^{AB}	1.05^{A}	0.65^{BC}	0.56^{BC}	0.69^{AB}	0.53^{BC}	0.54^{BC}	0.74^{AB}	0.91^{AB}	0.31^{C}	0.69^{a}
	T***	S	**0.03**^{B}	**0.13**^{AB}	**0.12**^{AB}	**0.15**^{AB}	**0.34**^{A}	0.34^{A}	0.33^{A}	**0.35**^{A}	**0.17**^{AB}	0.11^{AB}	0.21^{b}
	LxT***	*AL*	0.46^{AB}	0.59^{A}	0.38^{AB}	0.35^{AB}	0.52^{A}	0.44^{AB}	0.43^{AB}	0.54^{A}	0.54^{A}	0.21^{B}	
LAER	L***	W	0.090^{C}	0.090^{A}	0.080^{C}	0.111^{A}	0.015^{BC}	0.045^{ABC}	0.077^{AB}	0.105^{A}	0.106^{A}	0.077^{AB}	0.062^{a}
	T^{NS}	S	**0.048**^{A}	0.065^{A}	**0.039**^{A}	0.082^{A}	0.029^{A}	0.042^{A}	0.056^{A}	**0.062**^{A}	**0.034**^{A}	0.065^{A}	0.052^{b}
	LxT**	*AL*	0.021^{DE}	0.078^{AB}	0.017^{E}	0.097^{A}	0.022^{CDE}	0.044^{BCDE}	0.067^{ABCD}	0.084^{A}	0.070^{ABCD}	0.071^{ABC}	
FoR	L^{NS}	W	0.48^{AB}	0.59^{AB}	0.91^{A}	0.75^{A}	0.59^{AB}	0.53^{AB}	0.32^{AB}	1.01^{A}	0.21^{B}	0.69^{A}	*0.58*
	T^{NS}	S	0.64^{A}	0.75^{A}	0.52^{A}	**0.21**^{A}	0.32^{A}	0.53^{A}	0.37^{A}	0.59^{A}	**0.69**^{A}	0.53^{A}	*0.52*
	LxT*	*AL*	*0.56*	*0.67*	*0.71*	*0.48*	*0.45*	*0.53*	*0.35*	*0.80*	*0.29*	*0.61*	
SBR	L*	W	0.12^{AB}	0.15^{AB}	0.23^{AB}	0.19^{AB}	0.15^{AB}	0.20^{AB}	0.08^{AB}	0.25^{A}	0.05^{B}	0.11^{AB}	0.14^{a}
	T^{NS}	S	0.16^{A}	0.19^{A}	0.16^{A}	**0.05**^{A}	0.08^{A}	0.17^{A}	0.09^{A}	0.15^{A}	**0.17**^{A}	0.09^{A}	0.13^{b}
	LxT*	*AL*	0.14^{AB}	0.17^{AB}	0.19^{AB}	0.12^{AB}	0.11^{AB}	0.19^{AB}	0.09^{AB}	0.20^{A}	0.07^{B}	0.10^{AB}	
LFW	L***	W	1.9	4.2	2.4	5.6	2.5	4.7	5.0	4.2	2.7	5.7	3.9^{a}
	T***	S	1.8	2.0	2.0	2.9	1.5	2.6	2.7	2.0	1.0	2.7	2.1^{b}
	LxT^{NS}	*AL*	1.9^{B}	3.1^{AB}	2.2^{AB}	4.2^{A}	2.0^{B}	3.6^{AB}	3.8^{AB}	3.1^{AB}	1.9^{B}	4.2^{A}	
LMA	L***	W	0.012^{B}	0.010^{BC}	0.001^{D}	0.002^{D}	0.006^{BCD}	0.006^{BCD}	0.004^{CD}	0.011^{BC}	0.022^{A}	0.006^{BCD}	0.008^{b}
	T*	S	0.011^{B}	**0.029**^{A}	0.002^{C}	**0.008**^{BC}	0.008^{BC}	0.007^{BC}	0.006^{BC}	**0.005**^{BC}	**0.012**^{B}	0.008^{BC}	0.010^{a}
	LxT***	*AL*	0.011^{B}	0.019^{A}	0.001^{D}	0.005^{CD}	0.007^{BC}	0.007^{BC}	0.005^{CD}	0.008^{BC}	0.017^{A}	0.007^{BC}	
SCMR	L***	W	45.57	45.40	45.37	51.00	43.29	49.87	45.27	45.15	40.52	46.37	45.78^{a}
	T***	S	37.13	33.89	34.69	45.17	40.08	41.66	43.24	36.87	34.74	45.53	39.30^{b}
	LxT^{NS}	*AL*	41.35^{BCD}	39.64^{CD}	40.03^{BCD}	48.08^{A}	41.68^{BCD}	45.76^{ABC}	44.26^{ABC}	41.01^{BCD}	37.63^{D}	45.95^{AB}	

DROUGHT TOLERANCE IN PEANUT LANDRACES

In the present work, LMA was sharply modified both by the L factor and, to a lesser extent, by water stress. However, a highly statistically significant of LxT interaction was also observed. In response to water stress, Seb Seb and Berrihane increased their LMA, whereas a remarkable decrease was measured for Oum Tboul and Adrar, being this parameter not statistically modified in the remaining landraces (Table 1). Nevertheless, under water stress, the highest LMA values were observed in Seb Seb, Adrar and Oued Souf, whereas Metlili showed the lowest one. The SCMR was modified by both the L and the T factors. In particular, Berrihane, El Frin and Timimoun were the peanut landraces with the highest SCMR values, whereas the Adrar and Seb Seb landraces exhibited the lowest ones. Further, drought stress reduced the chlorophyll content per unit leaf area by 14%.

3.3. Root and nodule morphology. – The RLR, i.e. the root length in relative terms, was reported to be prominently involved in plant adaptation to nutritional deficiencies (Sorgonà *et al.*, 2007), but no experiment was conducted so far, to the best of our knowledge, to test this root parameter under conditions of water stress.

In the present study, RLR showed an ample variability among the different landraces, under both water regimes (Table 2). Under well watered conditions, Timimoun ranked first in terms of RLR, followed by Oum Tboul, Tonga, Metlili and Boumalek, whereas Oued Souf, El Frin and Adrar were the peanut landrace group with the lowest RLR values. Following water deprivation, RLR values were found to be distributed among a top-ranking group, including Oum Tboul, Timimoun, Boumalek and Tonga, an intermediate, including Seb Seb, Metlili, Oued Souf and Berrihane, and a bottom-ranking one, including Adrar and El Frin. Any change in RLR arises from interplay among different morphological components, i.e. RMR (the allocation component), RF and RTD, being the last two regarded as structural parameters. Therefore, plants may produce longer roots either by increasing biomass allocation to them (Sorgonà *et al.*, 2007) or by increasing RF and/or reducing RTD, leaving biomass allocation unchanged (Ryser, 1998). Evaluating the above parameters might be therefore helpful to understand how efficiently plants build their root systems in terms of “root length” in response to water stress. The results in Table 2 suggest that the morphological components RMR, RF, and RTD were responsible for the large RLR differences observed among the landraces exhibiting the longest root systems, namely Timimoun, Tonga and Oum Tboul, and those showing the shortest ones,

Variable	Statistics	Treatment (T factor)	Landraces (L factor)										
			OS	**SEB**	**MET**	**BER**	**BMK**	**TIM**	**TO**	**OT**	**AD**	**FR**	*AT*
RLR (cm g^{-1})	L***	W	107^{C}	171^{BC}	300^{ABC}	168^{BC}	260^{ABC}	502^{A}	303^{ABC}	345^{AB}	22^{D}	137^{BC}	*232^{a}*
	T^{NS}	S	159^{ABC}	152^{ABCD}	**152^{BCD}**	200^{ABC}	360^{AB}	365^{AB}	300^{AB}	429^{A}	**97^{CD}**	**50^{D}**	*226^{a}*
	LxT***	*AL*	*133^{DE}*	*161^{CDE}*	*226^{ABCD}*	*184^{BCD}*	*310^{ABC}*	*434^{A}*	*302^{ABCD}*	*388^{AB}*	*60^{F}*	*93^{EF}*	
RMR (g g^{-1})	L***	W	0.25^{A}	0.20^{AB}	0.17^{ABC}	0.06^{C}	0.07^{CD}	0.21^{AB}	0.15^{ABC}	0.03^{D}	0.10^{BCD}	0.12^{BCD}	*0.14^{a}*
	T***	S	**0.05^{BC}**	**0.05^{BC}**	**0.03^{BC}**	0.08^{ABC}	**0.19^{A}**	**0.08^{AB}**	0.09^{AB}	0.04^{B}	**0.02^{C}**	0.10^{AB}	*0.08^{b}*
	LxT***	*AL*	*0.15^{A}*	*0.12^{ABC}*	*0.10^{ABCD}*	*0.07^{BCD}*	*0.13^{ABC}*	*0.15^{AB}*	*0.13^{ABC}*	*0.04^{D}*	*0.06^{CD}*	*0.11^{ABCD}*	
RF (cm cm^{-3})	L***	W	90^{C}	140^{C}	244^{BC}	741^{ABC}	848^{ABC}	1131^{A}	1050^{AB}	1220^{A}	115^{C}	74^{C}	*565^{b}*
	T***	S	215^{B}	128^{B}	196^{B}	**2122^{A}**	**2091^{A}**	**2092^{A}**	**1603^{A}**	1469^{A}	139^{B}	122^{B}	*1018^{a}*
	LxT***	*AL*	*153^{B}*	*133^{B}*	*220^{B}*	*1431^{A}*	*1470^{A}*	*1612^{A}*	*1327^{A}*	*1344^{A}*	*127^{B}*	*98^{B}*	
RTD (g cm^{-3})	L***	W	0.21^{ABC}	0.17^{ABC}	0.13^{BC}	0.25^{AB}	0.28^{ABC}	0.46^{A}	0.47^{A}	0.11^{BC}	0.52^{A}	0.07^{C}	*0.27^{a}*
	T^{NS}	S	**0.07^{DE}**	**0.04^{D}**	**0.05^{DE}**	**0.87^{A}**	**1.44^{A}**	0.52^{AB}	0.49^{AB}	0.13^{CD}	**0.04^{E}**	**0.25^{BC}**	*0.39^{a}*
	LxT***	*AL*	*0.14^{B}*	*0.11^{B}*	*0.09^{B}*	*0.56^{AB}*	*0.86^{A}*	*0.49^{AB}*	*0.48^{AB}*	*0.12^{B}*	*0.28^{AB}*	*0.16^{B}*	
PrL (cm)	L***	W	19^{DE}	38^{CD}	35^{CDE}	55^{BC}	73^{AB}	63^{AB}	83^{A}	64^{AB}	12^{E}	24^{DE}	*47*
	T^{NS}	S	21^{B}	**15^{B}**	23^{B}	**72^{A}**	70^{A}	**78^{A}**	73^{A}	75^{A}	**27^{B}**	12^{B}	*47*
	LxT***	*AL*	*20^{B}*	*27^{B}*	*29^{B}*	*64^{A}*	*72^{A}*	*71^{A}*	*78^{A}*	*69^{A}*	*19^{B}*	*18^{B}*	
LL (cm)	L***	W	21^{BC}	27^{BC}	86^{AB}	115^{A}	137^{A}	143^{A}	123^{A}	125^{A}	4^{B}	13^{B}	*80*
	T^{NS}	S	37^{C}	13^{C}	30^{C}	141^{AB}	111^{AB}	81^{BC}	172^{A}	150^{AB}	18^{C}	17^{C}	*77*
	LxT***	*AL*	*29^{B}*	*20^{B}*	*58^{B}*	*128^{A}*	*124^{A}*	*112^{A}*	*147^{A}*	*138^{A}*	*12^{B}*	*15^{B}*	
BD (n.LL/PrL)	L***	W	2.0^{A}	1.2^{BCD}	1.5^{AB}	0.5^{DE}	0.5^{E}	0.8^{BCDE}	0.6^{CDE}	0.6^{CDE}	2.1^{A}	1.2^{BC}	*1.1*
	T^{NS}	S	1.9^{AB}	1.6^{AB}	1.4^{B}	0.6^{C}	0.5^{C}	0.5^{C}	0.6^{C}	0.3^{C}	1.7^{AB}	**2.2^{A}**	*1.1*
	LxT***	*AL*	*1.9^{A}*	*1.4^{A}*	*1.4^{A}*	*0.6^{B}*	*0.5^{B}*	*0.6^{B}*	*0.6^{B}*	*0.5^{B}*	*1.9^{A}*	*1.7^{A}*	
ALL (LL/n.LL)	L***	W	0.6^{C}	0.6^{C}	1.9^{BC}	4.0^{A}	3.9^{A}	2.9^{AB}	2.6^{AB}	3.5^{AB}	0.2^{C}	0.4^{C}	*2.1*
	T^{NS}	S	0.9^{DE}	0.5^{E}	0.9^{DE}	3.3^{BC}	3.2^{BC}	2.3^{CD}	**4.2^{B}**	**6.0^{A}**	0.4^{E}	0.7^{DE}	*2.1*
	LxT***	*AL*	*0.8^{D}*	*0.6^{D}*	*1.4^{CD}*	*3.7^{AB}*	*3.5^{B}*	*2.6^{BC}*	*3.4^{B}*	*4.8^{A}*	*0.3^{D}*	*0.5^{D}*	

namely Adrar, El Frin and Seb Seb. Indeed, the RLR differences among the two groups was explained for the most part by differences in terms of RF, since Timimoun, Tonga and Oum Tboul had a RF one order of magnitude higher than that of Adrar, El Frin and Seb Seb. The RF trait is important under water stress conditions because thinner roots increase the root-soil interface, and this results in a higher radial conductivity, due to a decreased resistance to the radial flow of water (Huang and Eissenstat 2000), and a higher root hydraulic conductance per stem cross-section area (Hernandez *et al.,* 2010).

Studying root adaptation to drought stress requires to consider the contribution of different root orders, which differently respond to environmental cues and influence the plant's competitive ability to acquire soil resources. Indeed, the higher root orders were found to be the main sites of water uptake (Rewald *et al.,* 2011). Consequently, we evaluated here the drought-induced morphological changes of the different root orders forming the root system of A. hypogaea, which is constituted by a taproot and by lateral roots of several different orders (Tajima *et al.,* 2008). The results in Table 2 suggest that the root systems of Boumalek, Timimoun, Tonga, Oum Tboul and Berrihane were characterized by a higher length of the primary root, with longer and fewer lateral roots. Such kind of root architecture can be regarded as highly suited for coping with drought stress, because it provides a potential maximum rooting depth (high LPr) together with a densely rooted soil layer (high ALL). Indeed, root distribution in deep soil strata was a plant trait which permitted the discrimination of drought tolerant peanut genotypes under drought stress (Jongrunkland *et al.,* 2011; Jongrunkland *et al.,* 2012). However, to the best of our knowledge, the present study suggests for the first time that a dimorphic root system, combining growth into deep soil with lateral spreading, confers to peanut landraces superior adaptive ability towards drought stress.

Biological N_2-fixation in A. hypogaea is decreased by drought stress (Sinclair *et al.,* 1995). Consistently, although estimated via indirect measurements, such as nodules weight, diamater and number, the present work confirms both that N_2-fixation machinery was affected in the studied peanut landraces exposed to water stress, and that wide differences for such trait exist among the landraces, under both water regimes (data not shown).

3.4. Plant water status. – Table 3 shows that both $\Psi_{\pi 0}$ and Ψ_{tlp}, reflecting constitutive capacity for water uptake, were remarkably variable within the landraces group studied here. The least

negative Ψπ0 values were measured in Seb Seb, Boumalek and Oum Tboul. The least negative

Table 3. – Osmotic potential at full turgor ($\Psi_{\pi 0}$) and leaf water potential at turgor loss point (Ψ_{tlp}) as measured during pressure-volume curves in the leaves of the peanut landraces in Fig. 1. Means are reported ± SD (n = 3). Different letters indicate significant differences after Tukey's pairwise comparisons ($p < 0.001$). For landraces acronyms, see the text.

Landrace	$-\Psi_{\pi 0}$ (MPa)	$-\Psi_{tlp}$ (MPa)
AD	0.94 ± 0.06a	1.26 ± 0.05a
MET	0.99 ± 0.09a	1.15 ± 0.12ab
FR	0.96 ± 0.10a	1.23 ± 0.05a
OS	0.94 ± 0.07a	1.14 ± 0.04ab
SEB	0.56 ± 0.07b	1.02 ± 0.07b
BER	0.96 ± 0.09a	1.40 ± 0.09a
TO	0.84 ± 0.09a	1.36 ± 0.07a
TIM	0.93 ± 0.09a	1.43 ± 0.04a
BMK	0.61 ± 0.05b	1.14 ± 0.06ab
OT	0.62 ± 0.10b	1.16 ± 0.07ab

Ψ_{tlp} value was that of Seb Seb, followed by those measured in Metlili, Oued Souf, Boumalek and Oum Tboul leaves, being the most negative ones measured in Adrar, El Frin, Tonga, and Timimoun landraces.

As far as g_{Lmax} is concerned, it was possible to gather the W plants into two subgroups, one of which, including Adrar, Metlili, Oued Souf, Berrihane, Tonga, and Oum Tboul, showing values around 80 mmol m^{-2} s^{-1}, and another, including El Frin, Seb Seb, Timimoun, and Boumalek, whose values were around 60 mmol m^{-2} s^{-1} (Fig. 2). Imposition of water stress induced stomatal closure in all the peanut landraces, although with different patterns among them. In particular, a severe water stressinduced stomatal closure near to cuticular values was observed for El Frin, Seb Seb, Berrihane, Tonga, Timimoun, and Boumalek starting from 10 days of water deprivation onwards (data not shown).

The Ψ_{Lmin} and RWC values tended to be rather uniform in well watered landraces, with the

former value around -0.4 MPa, and the latter one ranging between 93%, as in El Frin and Seb Seb, and 96%, for the remaining landraces (Fig. 2). Such uniformity of Ψ_{Lmin} and RWC among the different landraces was abolished upon imposition of water stress, in spite of the low g_{Lmax} measured in all the landraces. In particular, leaf RWC of S-plants was ~ 65% for Adrar, Metlili, El Frin, Seb Seb and Boumalek, ~75% for Berrihane, Tonga, Timimoun and Boumalek ones and only 58.53 % for Oued Souf. Under water stress, besides, El Frin, Seb Seb, Berrihane,

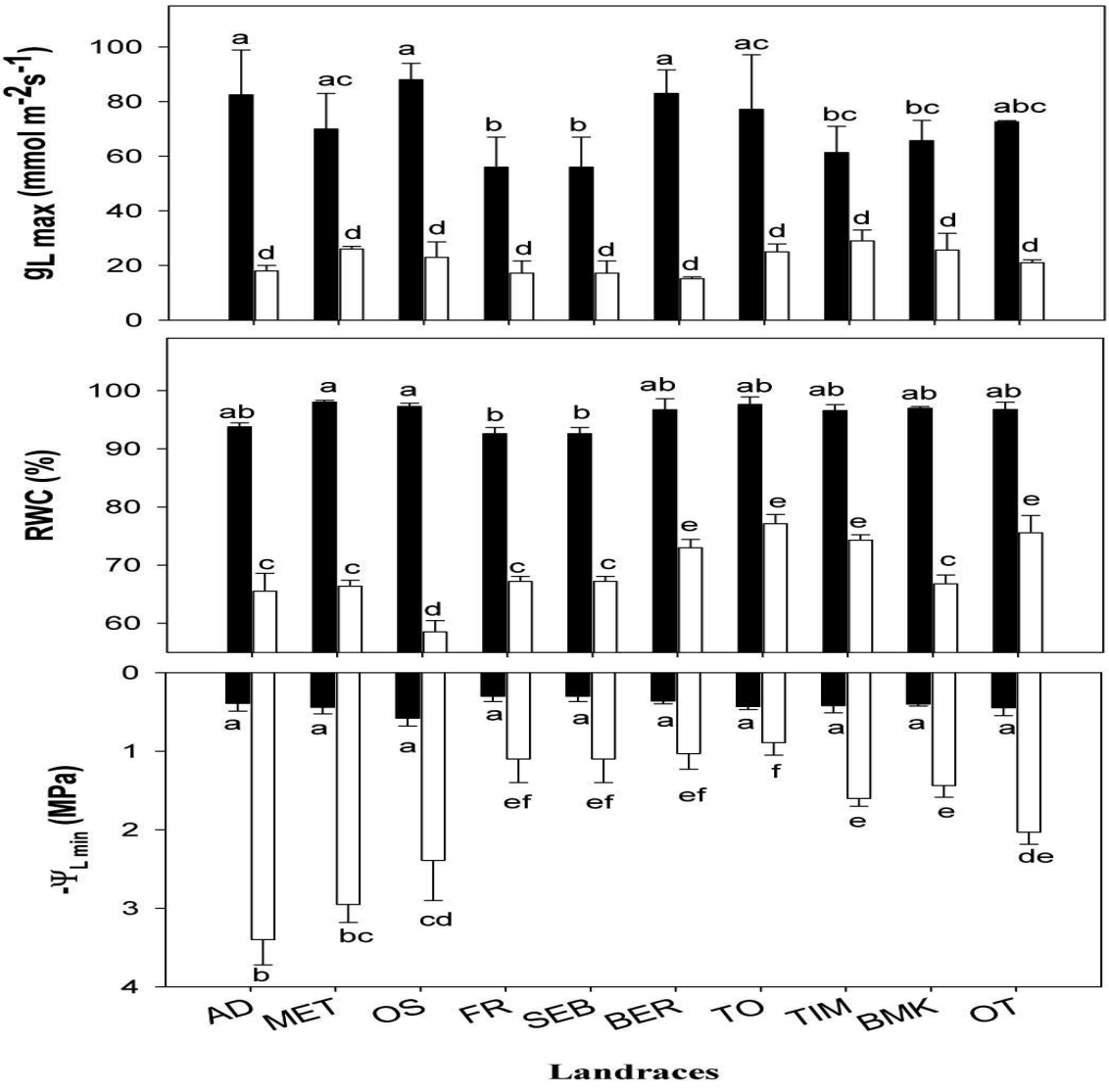

Fig. 2. – Maximum diurnal values of leaf conductance to water vapour (g_{Lmax}) (n = 5), relative water content (RWC) (n = 3) and minimum diurnal values of leaf water potential ($\Psi_{L\ min}$) (n = 3) as measured in the leaves of the peanut landraces in Fig. 1a. Open bars denote water stressed plants; filled bars denote well watered plants. Each value is the mean ± SD. Different letters indicate significant differences for Tukey's pairwise comparisons ($P < 0.001$). For landraces acronyms, see the text.

and Tonga showed Ψ_{Lmin} values above (i.e. less negative than) their respective turgor loss point region, whereas Ψ_{Lmin} was found to be much more negative than Ψ_{tlp} in Adrar, Metlili, Oued Souf and Oum Tboul (compare Fig. 2 and Table 3).

3.5. Principal component analysis. – In the present work, the principal component analysis, as a multivariate technique, was used to discriminate drought-tolerant and -susceptible peanut landraces via reduction and grouping into components of their above-ground growth and functional traits, root morphological and architectural features, rhizobium nodule morphological parameters, and indices describing their water status, expressed under conditions of water deprivation.

In the present work, the total variability of the three dimensional space was efficiently summarized by the two principal components, which individually accounted for 43% and 26% of the variability (Table 4). The first component (Prin1) consisted of high positive loadings for the root length ratio, the length of primary and lateral roots, the number of nodule in the lateral roots and the stem elongation rate, with high negative loadings for root branching density. Therefore, Prin1 can be assumed to mostly reflect the "root vigor" feature: positive values for this component indicate a long root system, resulting from deep and, at the same time, laterally spreading rooting pattern, with abundant root nodulation, accompanied by a pronounced growth of the stem.

By plotting the single peanut landraces by means of their component scores, Prin1 sharply separated the Oum Tboul, Tonga, Timimoun, Boumalek and Berrihane landraces, featuring a vigorous root system, with longer primary and lateral roots and abundant proliferation of nodule on the lateral roots (positive value), from El Frin, Oued Souf, Seb Seb, Metlili and Adrar landraces, exhibiting a comparatively lower vigor of the root system (negative values; Fig. 3). Hence, the former landrace group exhibits a root ideotype suited to face water scarcity by exploring both deep and shallow soil layers. Accordingly, an increase of the root length density (root length per soil volume) along with deepening in the soil profile was found to correlate with pod yield and harvest index (Jongrungklang *et al.,* 2011; Jongrungklang *et al.,* 2012) and water uptake (Vadez *et al.,* 2013) in peanut genotypes, although, Ratnakumar and Vadez (2011) suggested a scarce capacity to discriminate drought-tolerant genotypes by root morphological traits.

DROUGHT TOLERANCE IN PEANUT LANDRACES

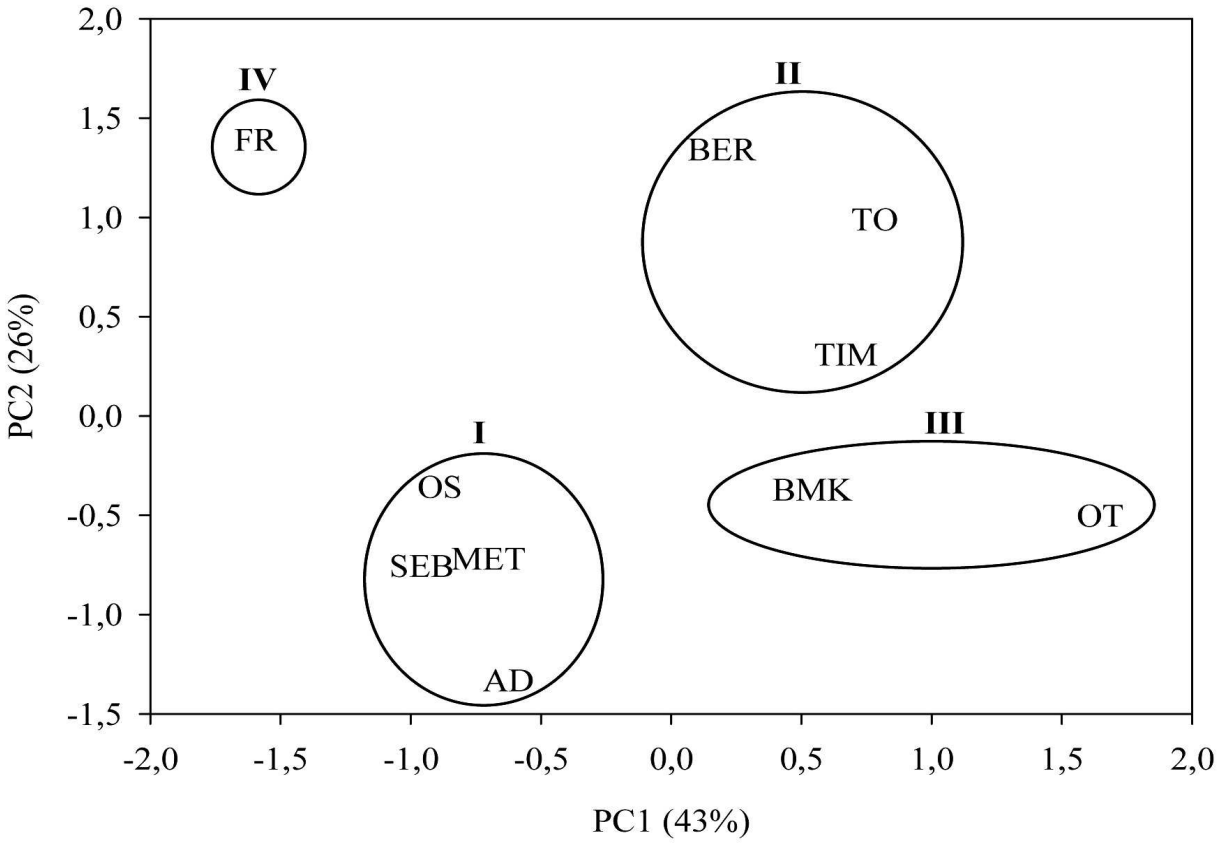

Fig. 3. – Biplot of the scores of the principal components (PC) 1 and 2 extracted from morphological and physiological parameters measured in peanut landraces exposed to 25 days of water deprivation. The arrows indicate the interpretation of the principal components. The proportion of variability explained by each PC is given within brackets. Circles and roman numbers denote the grouping of the peanut landraces after cluster analysis.

The second principal component (Prin2) had positive loadings for chlorophyll content, leaf fresh weight and positive differences between Ψ_{Lmin} and Ψ_{tlp}, and a negative loading for Ψ_{Lmin} (Table 4). Prin2 can be assumed to reflect the "aboveground" physiological responses to drought stress. In particular, positive values of Prin2 depict a peanut landrace with a comparatively higher canopy size (higher leaf fresh weight), with high chlorophyll content, and positive differences between Ψ_{Lmin} and Ψ_{tlp}. As shown in Fig. 3, Prin2 (positive vs. negative values) separated, under conditions of water stress, El frin, Berrihane, Tonga and Timimoun, exhibiting "superior" aboveground traits, from Oued Souf, Boumalek, Oum Tboul, Metlili, Seb Seb, and Adrar. The above analysis of the Prin2 component suggests that, under water stress, maintaining greener leaves and cooler canopy temperature, via permissive values of leaf water potential allowing a certain degree of stomatal perviousness, could contribute to preserve adequate

photosynthetic ability to sustain grain filling and to allow greater absorption and use of water.

Table 4. – Principal components of morpho-physiological traits of Arachis hypogaea landraces exposed to drought stress.

Statistics	Prin 1	Prin 2
Eigenvalues and variability		
Eigenvalue	6.42	2.26
Proportion of variability (%)	43.2	26.5
Variable	Attribute loading for	
	Prin 1	Prin 2
Eigenvectors		
Root length ratio	**0.810**	0.012
Length of the primary root	**0.902**	0.283
Total length of the lateral roots	**0.856**	0.381
Root branching density	**-0.904**	-0.115
Average length of the lateral roots	**0.906**	0.214
Rhizobium nodule number on lateral roots	**0.740**	0.262
SPAD chlorophyll meter recordings	0.088	**0.818**
Stem elongation rate	**0.728**	-0.047
Leaf fresh weight	0.020	**0.799**
Ψ_{Lmin}	0.351	**0.834**
Difference between Ψ_{Lmin} and Ψ_{tlp}	0.336	**0.832**

Accordingly, a close relationship was observed among SCMR and water use efficiency (Jongrungklang *et al.*, 2008), number of pod, pod yield and seed size (Boontang *et al.*, 2010) and total biomass (Boontang *et al.*, 2010) in different peanut genotypes exposed to water stress, although such relationships have been questioned (Hamidou *et al.*, 2011).

Cluster analysis performed with the Prin1 and Prin2 component scores (not shown) allowed

to classify the *A. hypogaea* landraces into four groups (Fig. 3). Cluster I included Metlili, Seb Seb, Oued Souf, and Adrar, having in common among them remarkably scarce PCA scores for all the root and above-ground traits. On the other hand, Cluster II, including Timimoun, Tonga, and Berrihane, grouped together those peanut landraces showing all of those favorable morpho-physiological traits concurring at defining a "plant ideotype" efficient in facing drought stress: a vigorous root system, with long primary and lateral roots, an abundant N_2-fixing nodule proliferation on the lateral roots, integrated with a good canopy size and efficient, chlorophyll-rich, leaf machinery, and the capacity to maintain leaf water potential as above as possible respect to that causing turgor loss, even under conditions of severe water stress.

Accordingly, Jongrungklang *et al.* (2013) suggested that the most efficient strategy for a plant to acquire drought tolerance is based on root localization in the moisture-elevated deep soil layers, allowing sustained leaf transpiration and hence sustained photosynthesis to take place. Clusters III and IV grouped together peanut landraces with variable drought-facing strategies: the former, including Boumalek and Oum Tboul, relying on root responses for adapting to a water-limited environment, and the latter, including only El Frin, rather adopting a "leaf-response" adaptation strategy.

By comparing the results from PCA (Fig. 3) with the YTI/LDWbased drought tolerance rankings reported above and in Fig. 1b, respectively, an acceptable degree of overall correlation can be observed. Indeed, Berrihane and Tonga, both showing a favorable combination of morpho-physiological traits against water stress (Fig. 3), were classified as efficient/responsive and drought-tolerant landraces (Fig 1b and see above). Furthermore, Oum Tboul/Boumalek and El Frin, deploying a "root-based" or a "leaf-based" drought tolerance strategy, respectively (Fig. 3 and see above), were either ranked as efficient (El Frin) or showed intermediate YTI level (Boumalek) or both (Oum Tboul; Fig. 1b and see above). Finally, Oued Souf, Seb Seb, Metlili, and Adrar, all exhibiting poor combinations of leaf and root traits in response to drought (Fig. 3), were predicted to be both inefficient and drought-sensitive (Oued Souf, Seb Seb and Metlili, Fig. 1b and see above) or inefficient (Adrar, Fig. 1b) landraces. Timimoun was a peanut landrace for which the PCA results did not fit the YTI criterion: indeed, although exhibiting the best combination of morpho-physiological traits (Fig. 3), it was predicted to behave as a drought-sensitive landrace (see above).

4. CONCLUSIONS. – On the whole, and because of its provenance and inherent ample variability, the peanut landrace collection studied here might be regarded as an useful inventory of the morpho-physiological strategies and mechanisms deployed by this economically and socially important legume species to withstand water stress.

The present work showed that a wide array of morpho-physiological mechanisms, involving both above- and below-ground plant parts, comes into play in the process of adaptation to water shortage. For this reason, univariate analysis is often inadequate to highlight clear links among morpho-physiological adaptation mechanisms and drought tolerance. Instead, applying a multivariate approach, such as PCA, to the data set presented here was helpful for putting into evidence relationships among drought tolerance and morpho-physiological response patterns. As a result, two biologically meaningful components were identified, i.e. the 'root vigor' and the 'above-ground physiological mechanisms', on the basis of which it became possible to reliably model drought tolerance within the studied group of peanut landraces. Berrihane and Tonga featured high scores for both the above components, while Metlili, Seb Seb, Oued Souf and Adrar showed the lowest ones. On such basis, it is suggested here that the former landraces, exhibiting a root ideotype able to optimize water uptake, accompanied by high canopy and chlorophyll densities even under conditions of severe water stress, could be used as parental lines in breeding programs for those cultivation environments facing endemic water shortage.

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Chapitre III

Implication des mécanismes antioxydants enzymatiques et non enzymatiques dans la tolérance au stress hydrique chez quelques populations algériennes d'arachide (Arachis hypogaea L).

Implication of enzymatic and no enzymatic antioxidants mechanisms to water stress tolerance in some Algerian populations of peanut (Arachis hypogaea L.).

Les plantes sont constamment soumises à des variations environnementales. Ces changements peuvent engendrer un stress qui modifie l'homéostasie cellulaire par la production de formes réactives de l'oxygène. L'accumulation phytotoxique de ces différents radicaux oxygénés peut entraîner la mort de la plante ; cependant, ils ont récemment été identifiés comme des acteurs essentiels de la réponse au stress et leur rôle comme messager secondaire est maintenant clairement établi. Leur implication dans la régulation de l'expression génique a aussi permis de démontrer leur rôle d'inducteurs de la mort cellulaire programmée, mort génétiquement contrôlée que l'on retrouve non seulement dans les processus développementaux, mais également typiquement observée dans la réponse au stress **(Parent *et al.*, 2008)**.

Pour se protéger contre les intermédiaires oxygénés toxiques, les cellules végétales et leurs organites comme les chloroplastes, mitochondrie et peroxysomes sont pourvus de systémes de défense antioxydants. Un grand nombre de travaux de recherche ont établi que l'induction et la régulation de ces systémes sont essentielles pour la protection contre les effets de diverses contraintes biotiques et abiotiques. Les composants du systémes de défenses antioxydantes sont non enzymatiques ou enzymatiques **(Noctor et Foyer, 1998 ; Havaux et Niyogi, 1999 ; Havaux *et al.*, 2005).**

Les systémes antioxydants non enzymatiques sont représentés principalement par des composés de bas poids moléculaire soit solubles comme la glutathion réduit (GSH) et l'acide ascorbique (ASC), soit lipophiles comme les caroténoides et les tocophérols **(Mitler *et al.*, 2004 ; Chen et Dickman, 2005)**.

Les systémes antioxydants enzymatiques comprennent les superoxydes dismutases (SOD), les catalases (CAT), les ascorbate peroxydases (APX), les monodéhydroascorbate réductases (MDHAR), les déhydroascorbate réductases (DHAR), les glutathion réductases (GR) et les réductases à thiols.

Dans notre travail nous nous somme intéréssés aux systémes antioxydants non enzymatiques de bas poids moléculaire tels que le glutathion (GSH) et l'acide ascorbique ou vitamine C. Alors que pour les systémes antioxydants enzymatiques, nous avons étudié la superoxyde dismutase (SOD). Ce troisième chapitre s'intéresse donc à la caractérisation des mécanismes de réponse au stress hydrique, ayant trait aux systémes antioxydants, chez dix populations locales d'arachide.

Résumé

Afin d'étudier la relation entre le stress hydrique, le stress oxydatif et l'implication des mécanismes antioxydants enzymatiques et non enzymatiques de l'arachide la mesure de l'activité enzymatique antioxydante de la superoxyde dismutase **(SOD)**, la teneur du glutathion **(GSH)** et de l'acide ascorbique **(AsA)** ont été mesurés sur des plants d'arachide soumis à différents niveaux de stress hydrique (**10,15** et **25** jours de stress).

Les résultats obtenus montrent que sous la contrainte hydrique, les différentes populations enregistrent une baisse non significative de l'activité superoxyde dismutase **(SOD)** sauf la population **Tonga (TO)** qui enregistre la valeur la plus élevée de cette enzyme, confirmant ainsi une meilleure tolérance au stress hydrique. Aucune différence significative n'a été observée, en ce qui concerne la teneur en **GSH** ; la teneur la plus élevée de cet antioxydant a été observée chez **Tonga (TO)** et **Adrar (AD)** sous les différents niveaux de stress hydrique.

En revanche, toutes les populations étudiées ont montré entre elles une nette différence de la teneur en acide ascorbique **(AsA)**. **Adrar (AD)**, **Tonga (TO)**, **Oum Tboul (OT)**, **El Frin (FR)** et **Boumalek (BMK)** ont présenté la teneur la plus élevée de de cet acide.

Les différents comportements des populations d'arachide étudiées impliquent des réponses distinctes et spécifiques concernant les mécanismes antioxydants. Ces résultats montrent implicitement que chaque population développe une stratégie propre vis-à-vis du stress hydrique. En effet, ils sont cohérents avec ceux obtenus par certains chercheurs, qui confirment que le stress hydrique entraîne un stress oxydatif chez l'arachide.

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Implication of enzymatic and no enzymatic antioxidants mechanisms to water stress tolerance in some Algerian populations of peanut (*Arachis hypogaea* L.).

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***Keywords**: antioxidative enzyme, reduced glutathione (GSH), ascorbic acid (AsA), superoxide dismutase (SOD), drought, landraces, peanut (Arachis hypogaea* L.*).*

SUMMARY. -

To study the relationship between drought and oxidative stress and the implication of enzymatic and no enzymatic antioxidants mechanisms in peanut (*Arachis hypogaea* L.), the mesure of the antioxidant enzyme activity of SOD, GSH and AsA content were measured in 6 weeks-old seedlings plants exposed to a different level of water deprivation (10,15 and 25 days). The results showed that under different levels of water stress, the different peanut landraces studied display lower levels of superoxide dismutase activity. Drought did not have any significant effect on Superoxide dismutase (SOD) antioxidant enzyme activities of shoots of the studied populations; but activity of these antioxidants in Tonga (TO) population was obviously higher than in the remaining studied populations. These results showed a higher water stress tolerance for Tonga (TO). No great difference was observed regarding GSH content,the higher content of this antioxidant was observed in two landraces (TO) and (AD) in different levels od water deprivation. In contrast, upon water stress, the studied landraces showed a clear different content of ascorbic acid (AsA) amoung them. Adrar (AD), Tonga (TO), Oum Tboul (OT), El Frin (FR) and Boumalek (BMK) presented a higher content of foliar ascorbic acid. Altogether, these data show that the differential behavior of the ten peanut landraces involves distinct responses regarding antioxidant mechanisms. This allows to better recognise that each landrace exhibits its own response to water stress. These results are consistent with the contentions that drought

causes oxidative stress in peanut plants, and that mechanisms of antioxidative protection contribute to withstand drought-induced oxidative stress in this arid plant species.

Abbreviations: GSH, reduced glutathione; AsA ascorbic acid; SOD, superoxide dismutase; ROS, reactive oxygen species; $O_2^{\bullet-}$ Superoxide anion radical ; $^1O_2$, Singlet oxygen ; H_2O_2 Hydrogen peroxide; APX, Ascorbate peroxidase; CAT, Catalase; DHA, Dehydroascorbate; DHAR, Dehydroascorbate reductase; GR, Glutathione reductase; MDA, Malondialdehyde; MDHAR, Monodehydroascorbate reductase; DNA, Deoxyribonucleic acid ; AD, Adrar; TO, Tonga; OT, Oum Tboul; FR, El Frin; BMK, Boumalek; MET, Metlili; OS, Oued Souf; TIM, Timimoun; BER, Berrihane; SEB, Sebseb.

1. INTRODUCTION

Drought is one of the most important abiotic stresses affecting plant growth and development. It affects plants at various levels of their organization. In fact, under prolonged drought, many plants will dehydrate and die. Oxidative stress, which frequently accompanies many abiotic stresses like high temperature, salinity, or drought stress, causes a serious secondary effect on cells. Oxidative stress is accompanied by the formation of ROS such as $O_2^{\bullet-}$, $^1O_2$, H_2O_2, and OH^- . ROSs damage membranes and macromolecules affect cellular metabolism and play a crucial role in causing cellular damage under drought stress.

Drought creates an imbalance between light capture and its utilization, which inhibits the photosynthesis in leaves. In this process imbalance between the generation and utilization of electrons is created. Dissipation of excess light energy in photosynthetic apparatus results in generation of ROS. Denaturation of functional and structural macromolecules is the well-known results of ROS production in cells. DNA nicking, amino acids, protein and photosynthetic pigments oxidation, and lipid peroxidation are the reported effects of ROS. As a consequence, cells activate some responses such as an increase in the expression of genes for antioxidant functions and production of stress proteins, up-regulation of anti-oxidants systems, including antioxidant enzymes and accumulation of compatible solutes. All these responses increase scavenging capacity against ROS **(Seyed *et al.*, 2012)**.

Food legumes, such as peanut (*Arachis hypogaea* L) are important crops in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, and Central and South America **(Singh *et al.*, 1997)**, and in some temperate areas, including the Mediterranean region and the southern states of the USA **(Pasquet, 2000)**. Peanut plants are exposed to a drought

stress; this abiotic stress may lead to an imbalance between antioxidant defenses and the amount of ROS resulting in oxidative stress **(Smirnoff, 1993)**. ROS are necessary for inter- and intracellular signaling **(Van Breusegem *et al.*, 2001)**, but at high concentrations can cause damage at various levels of organization, including chloroplasts **(Asada, 1999)**. Plants have therefore evolved several mechanisms of antioxidative protection to withstand drought-induced oxidative stress. Apart from the xanthophyll cycle, photorespiration and other changes in metabolic activity (**Demmig-Adams, 1992; Kosaki, 1996)**, a number of enzymatic and non-enzymatic antioxidants are present in chloroplasts that serve to prevent ROS accumulation **(Smirnoff, 1993; Asada, 1999; Dietz, 2003)**. Non-enzymatic antioxidants, such as carotenoïds, glutathione and ascorbate cooperate to maintain the integrity of the photosynthetic membranes under oxidative stress **(Asada, 1999; Havaux, 1998; Munné-Bosch *et al.*, 2002)**. Also enzymatic antioxidants, such as SOD induction by different environmental stresses has been reported **(Bowler, 1992)**, its role as part of a putative protection mechanism in stress situations is still being discussed **(Scandalios, 1997)**. Functional approaches using transgenic plants have shown that plants overexpressing SOD are more tolerant to different stressful conditions **(Van Breusegem, 1999)**. The dismutation reaction catalyzed by the superoxide dismutase (SOD, E.C 1.15.1.1) maintains intracellular $O_2^{\bullet-}$ within normal levels and various authors have implicated this enzyme in the protection of cells from hydric-oxidative stress **(Smirnoff, 1993).**

The purpose of the present work was to evaluate the antioxidant response system in leaves from peanut (*Arachis hypogaea* L.) subjected sequentially to drought and watering. Drought was imposed to 6 weeks-old seedlings of peanut landreaces by withholding irrigation at different level of water deprivation (10, 15 and 25 days). Enzymatic antioxidant (SOD) and content of (GSH) and (AsA) were mesured.

2. MATERIALS AND METHODS

2.1. Plant material

Experiments were performed on 10 peanut Algerian landraces obtained from a prospection conducted by the Ecole Nationale Supérieure Agronomique, Algiers, Algeria, during the years 2003-2005. Based on the locations in which they were collected, the peanut landraces were assigned the following names: Berrihane (BER), Tonga (TO), Oum Tboul (OT), El Frin (FR), Seb Seb (SEB), Oued Souf (OS), Adrar (AD), Boumalek (BMK), Timimoun (TIM) and Metlili (MET). Botanical and agronomic features of peanut landraces, as well as their geographical and

climatic distribution within the Algerian territory, are reported in **Amri (2008)** and **Lazali (2009)**. To avoid confusion with the acronyms used for plant parameters, the full names of the peanut landraces are used in the text thereafter, whereas their acronyms are used in figures.

2.2. Seeds inoculation

Two days before sowing, healthy seeds of uniform size were selected for each peanut landrace. Selected seeds were first soaked into an aqueous solution of 40% (w/v) arabic gum and then evenly coated with peat inoculated with the rhizobial strain BRE 1.2 which was previously isolated from nodules of the same peanut populations **(Benmohamed *et al*., 2004)**. A trapping test conducted before seeds inoculation allowed to conclude that the soil mix used for plant growth did not contain any non-specific rhizobia able to infect the experimental plant material.

2.3. Plants' growth conditions

Four seeds for each landraces were sown in each of six 6 L-pots, each filled with 4 kg of a 50/20/30 (v/v/v) agricultural soil/peat/washed quartz sand mixture. The soil physico-chemical characteristics are reported in **Gelsomino *et al*., (2012)**. Peat medium properties were (%, w/w dry weight): organic C 52%, organic N 0.8 %, organic matter 90%, pH_{H2O} 3. The particle size of quartz sand (Progettinelblu.com) was 0.5 mm. After the emergence (5 days after sowing, DAS), two seedlings of each landraces per pot was used for the experiment.

Planted pots were randomly distributed within a walk-in climatic chamber, where an air temperature of 26 ± 1°C, a relative air humidity of 70 ± 5 %, and a photoperiod of 12 h were set. Photosynthetic photons flux density at plants' height was 350 μmol m^{-2} s^{-1} (LI-190SA quantum sensor, Li-Cor, Lincoln, NE).

Since both agricultural soil and peat medium were reputed to contain adequate levels of N, P and organic matter, no further fertilizer was supplied during the plants' growth. Planted pots were given 200 mL of tap water every four days because preliminary trials indicated that such amount and frequency of water supply was required to compensate water losses by evapotranspiration.

2.4. Imposition of water stress.

After six weeks of growth (47 DAS), plant material was divided into two halves, each consisting of 3 pots for each peanut landrace. One half pots continued to receive adequate water supply (see above) and acted as the well-watered control (W-plants), whereas irrigation was withheld on the other half of pots, thus imposing a progressive water stress to plants (S-plants). Keeping in mind the results of **Amri (2008)**, who observed that nodulation, plant height and dry matter production of Algerian peanut landraces were

affected after 15 days of irrigation withdrawal, water deprivation on the present S-plants lasted for 25 days. During water stress treatment, S- and W-plants were kept together in the same controlled environment where they were previously grown (see above).

2.5. Biochemical analyses

Two plants in each pot were sampled. After being excavated as reported above, the shoots was separated from the roots and then subsamples of leaves were taken, wrapped in aluminum foils and finally immediately dipped into liquid N_2, until storage at – 80 °C. On these samples, antioxidant metabolites, including ascorbate (AsA) and reduced glutathione (GSH) pools, as well as antioxidant enzymes, such superoxide dismutase (SOD) were being determined.

For these biochemical analyses, leaves were first weighted while still frozen, then placed in a mortar containing liquid N_2 and crushed with a pestle to a fine powder. Each sample was divided into two separate aliquots: one, for the analyses of AsA and SOD, was extracted with 4 volumes of 50 mM phosphate buffer pH 7.00, made 5 mM with 2-mercaptoethanol, 2 mM with dithiothreitol,2mM with sodium-hydrogen-EDTA, and 1% (w/v) insoluble polyvinylpyrrolidone. The other extraction medium, for the analysis of GSH, consisted of 4 volumes of 0.1 N aqueous HCl, containing 1% (w/v) insoluble polyvinylpyrrolidone. After filtration by four layers of cheese-cloth, the extracts were centrifuged. In both cases, after centrifuging the extracts at 10,000 x *g* for 20 min, the supernatants were collected, their volume measured, then split into 1 mL-aliquots, dipped into liquid N_2 and finally stored at -80 °C. The supernatant was then assessed for antioxidant enzyme activity. Antioxidant metabolites and enzymes were measured by spectrophotometer (Model λ5, Perkin-Elmer) according to standardized assay methods, reported in details elsewhere **(Paolacci *et al.*, 1997)**. All the above analyses were performed at 4°C. All data obtained from the different sampling times have been pooled to obtain the overall data shown in figures and tables. Reagents used were of analytical grade and were obtained from Aldrich (Steinheim, Germany), BDH (Poole, U.K.), Merck (Darmstadt, Germany), Serva (Heidelberg, Germany) and Sigma (St. Louis, USA).

2.5.1. Measurement of Ascorbate

Ascorbate was determined using the method of **Arakawa *et al.*, (1981)** and **Nakagawara** and **Sagisaka (1984)**. This assay is based on the reduction of ferric ion to ferrous ion by the ascorbic acid solution followed by formation of a red chelate between ferrous ion and 4,7-diphenyl-l,l0-phenanthrolin (bathophenanthroline) that absorbs at 534 nm. A standard curve covering the range 0–10 nmol AsA was used.

2.5.2. Measurement of Glutathione

Glutathione content was determined by the spectrophotometric method of **Griffith (1980)**, using an assay based on the enzymatic recycling of GSH, where GSH was sequentially oxidized and reduced by 5,5'-dithiobis- (2-nitrobenzoic acid) (DTNB) and GR, respectively, the rate of formation DTNB was monitored at 412 nm.

2.5.3. Measurement of superoxide dismutase

The total activity of SOD was determined according to the method of **Elstner *et al.*, 1983**, the adopted assay method is based on the generation of a saturating concentration of superoxide radical anions arising from the autoxidation of reduced anthraquinone, followed by the superoxide-dependent oxidation of hydroxylamine to nitrite, and then by the colourimetric detection of the nitrite formed at 540 nm; by intercepting $O_2^{\bullet-}$,SOD is able to inhibit the final colourimetric reaction in a concentration-dependent manner. The units of the enzymatic activities were defined and calculated according to the respective assay methods. One unit of SOD activity was defined as the amount of enzymatic protein capable of inhibiting by 50% the rate of $O_2^{\bullet-}$ generation in the reference reaction.

2.5.4. Protein determination

Protein concentration of leaf extracts was determined on the same supernatants used for enzyme assays, protein concentration was determined according to the method of **Bradford (1976)**, with the Bio-Rad protein assay (Bio-Rad Italia, Milano, Italy) and using BSA as a standard.

2.6. Statistical analysis

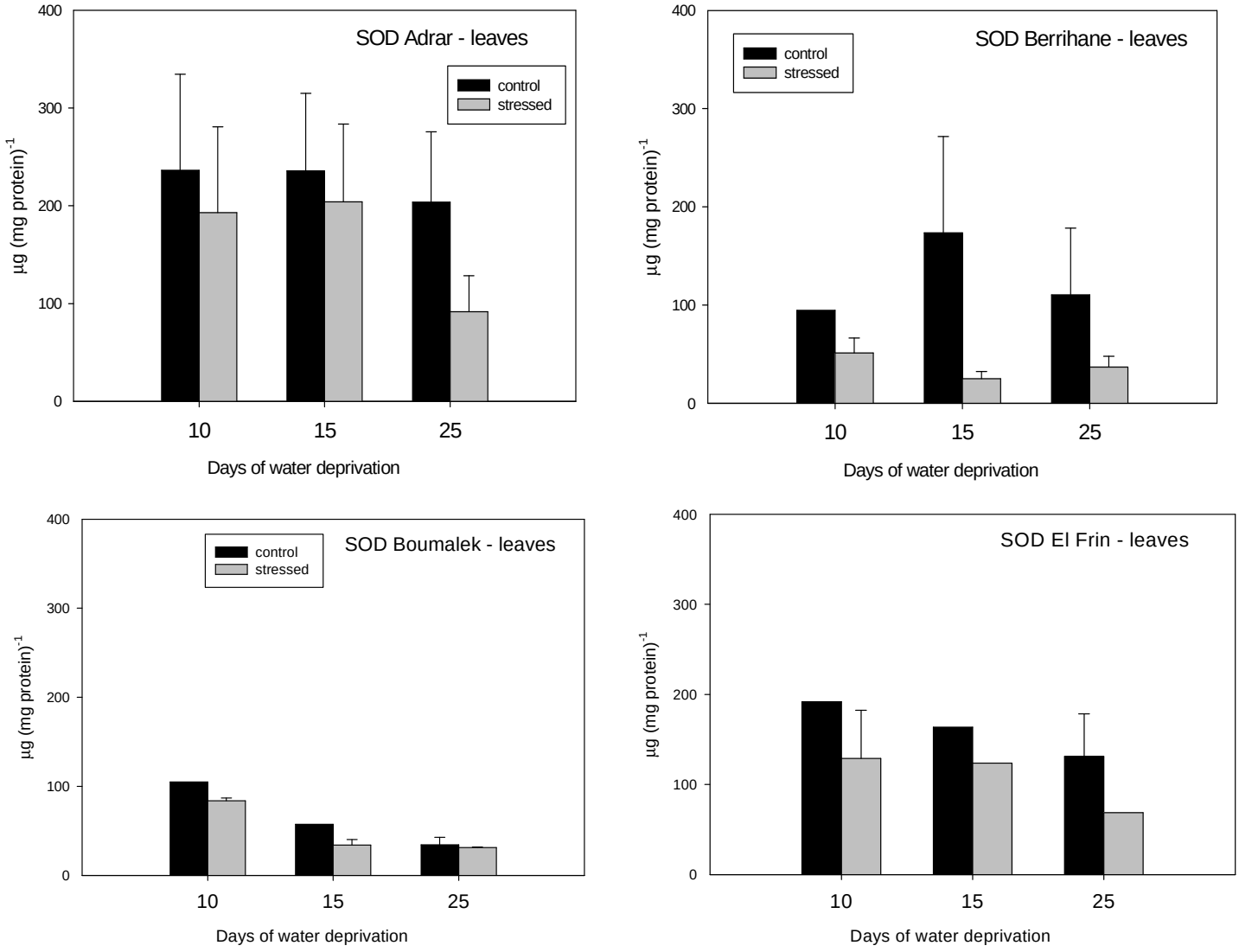
The entire experimental set consisted of 60 pots of plants of ten populations of peanut (30 well-watered control (W-plants) + 30 water stress treatment (S-plants) ones). All experiments were carried out on at least 6 replicates of leaves. All data were tested for normality (Kolmogorov- Smirnoff test) and homogeneity of variance (Levene Median test) and, where required, the data were transformed. Differences in enzyme activities, metabolite amounts were evaluated. Values obtained were statistically analyzed by a two way ANOVA, with the peanut landrace and the water regime (W or S) as the main factors. Subsequently, the Tukey's test was applied to compare the mean values among landraces and drought stress levels. For statistical analysis, the SPSS Statistics v. 15.0 software (IBM Corp., Armonk, NY) was used. Graphics were prepared by using the SigmaPlot v. 8.0 software (Jandel Scientific, San Rafael, CA).

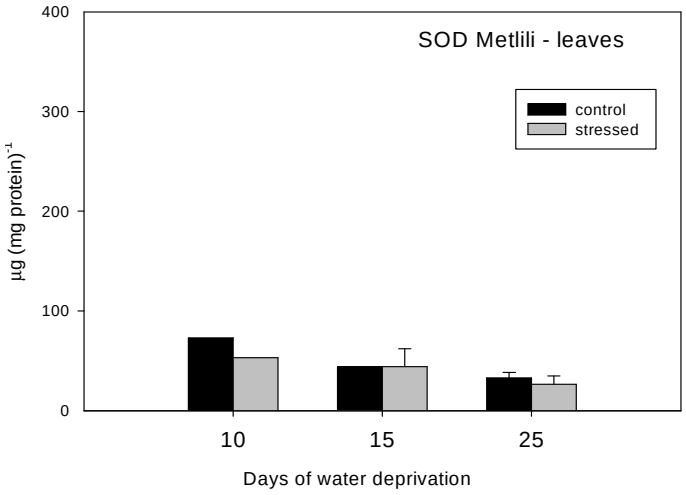
3. **RESULTS**

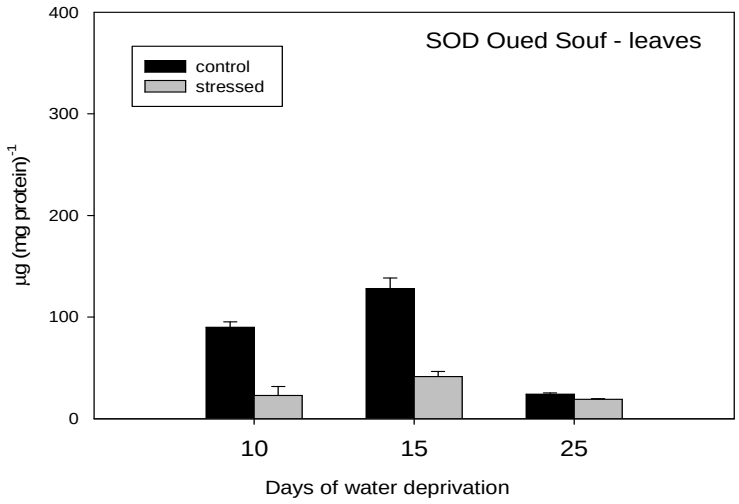
In peanut leaves, various antioxidant enzymes exhibited different responses during water deficit exposure. No differences in protein contents in leaves of these studied peanut populations were noticed through the entire duration of the experiments, either in controls or in stressed treatments at 10,15 and 25 days of water stress duration (data not shown).

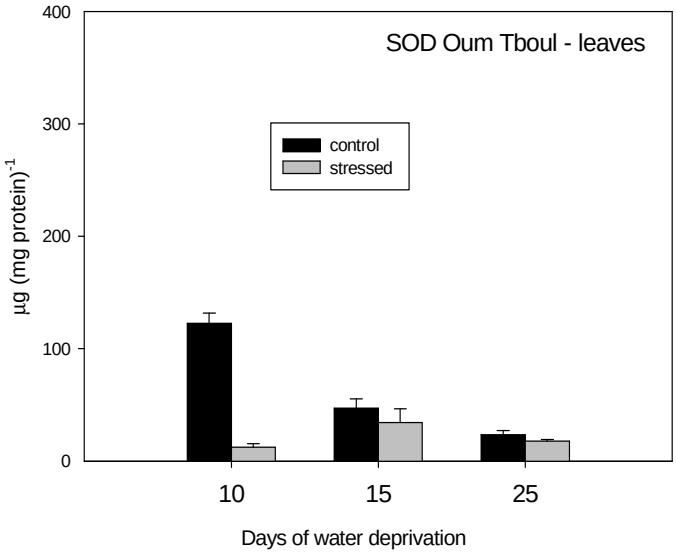
3.1. Effect of Drought on Antioxidant Enzymes (SOD)

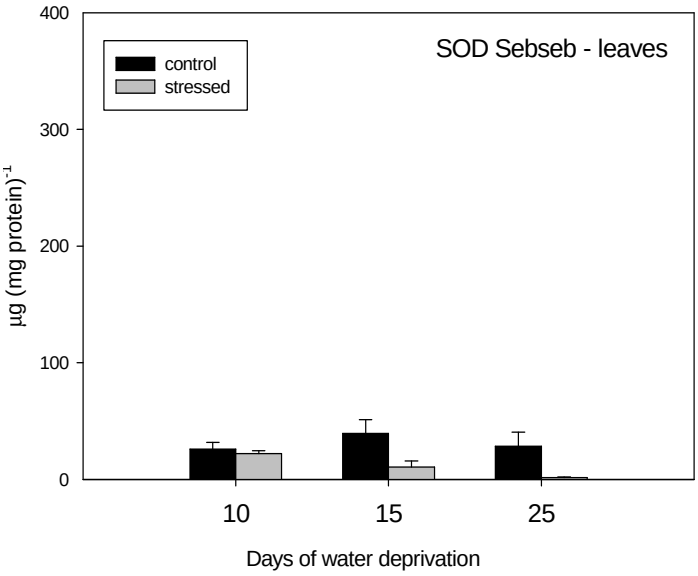
In nine peanut populations studied no significant differences in SOD activity were recorded between well-watered control (W-plants) and water stress treatment (S-plants) (data not shown), the different populations showed a downward trend of activity in the presence of different durations of water depriviation, but no actual significant differences. However, in Tonga (TO) population, SOD activity increased during the drought period (Fig. 1).

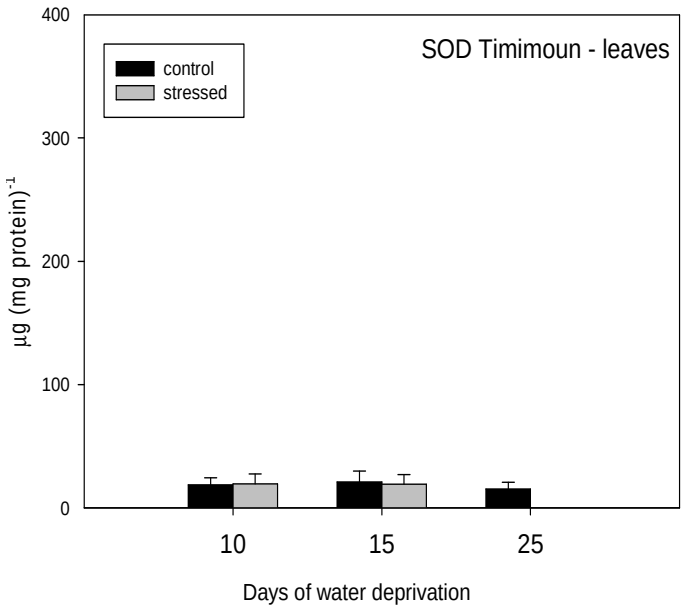



SOD Metlili - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation


SOD Oued Souf - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation


SOD Oum Tboul - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation


SOD Sebseb - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation


SOD Timimoun - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation

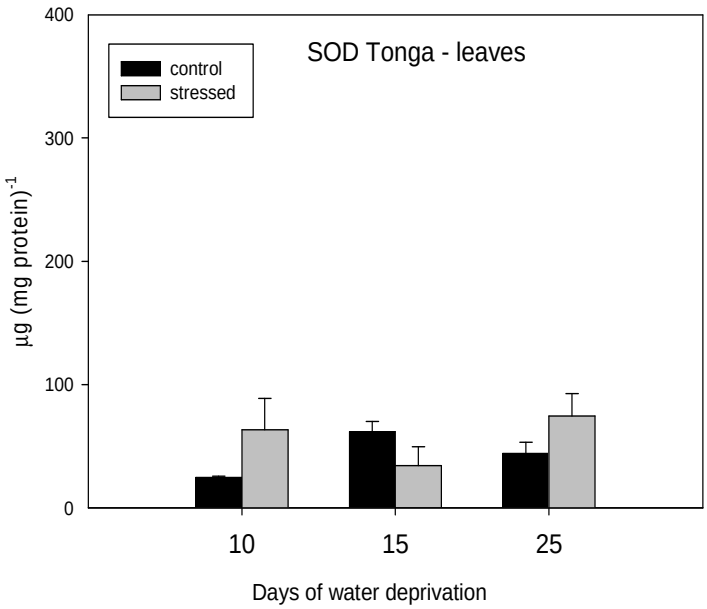

SOD Tonga - leaves
control
stressed
µg (mg protein)-1
400
300
200
100
0
10
15
25
Days of water deprivation

Fig. 1 Activity of superoxide dismutase (SOD) in Algerian peanut landraces after 10, 15and 25 days of water deprivation (□).Histograms (■) denote well-watered controls. Values are the mean (±S.E.) of six replicates n=6. Statistically significant effects of the landrace (L), the water regime (T) and of their interaction are reported (*P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant), as evaluated by a two-way ANOVA. For the antioxidant abbreviations, see Abbreviations used.

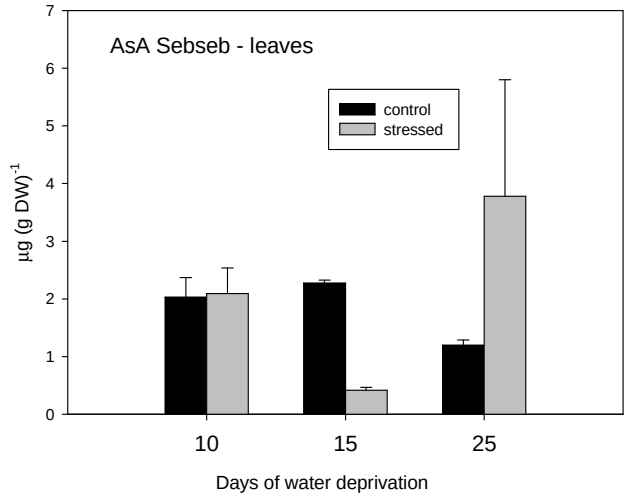
In these graphs, the scale of the Y axis has been made the same for all. This allows to notice the striking variability among the landraces in terms of SOD constitutive contents, i.e. levels in control plants. Again, therefore, and similarly of the results obtained for nearly all the biometric and physiological traits studied, each landrace seems to possess its own specific features. The same degree of remarkable variability is also apparent both in the time course of SOD activity in control plants, and in the response of SOD activity to water stress.

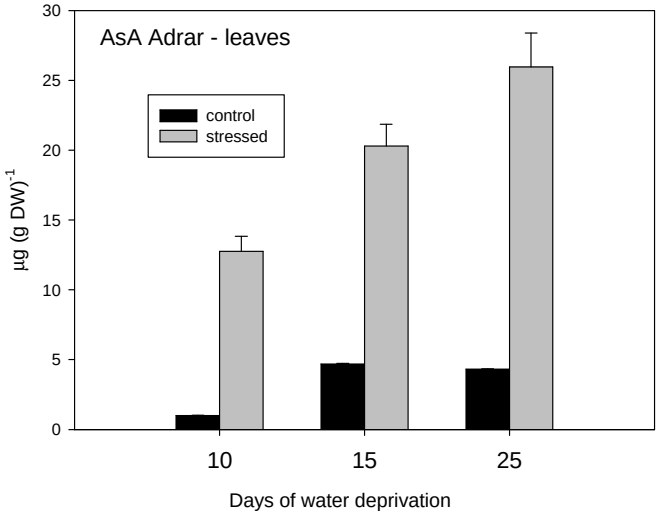
3.2. Effect of Drought on Antioxidant Metabolites

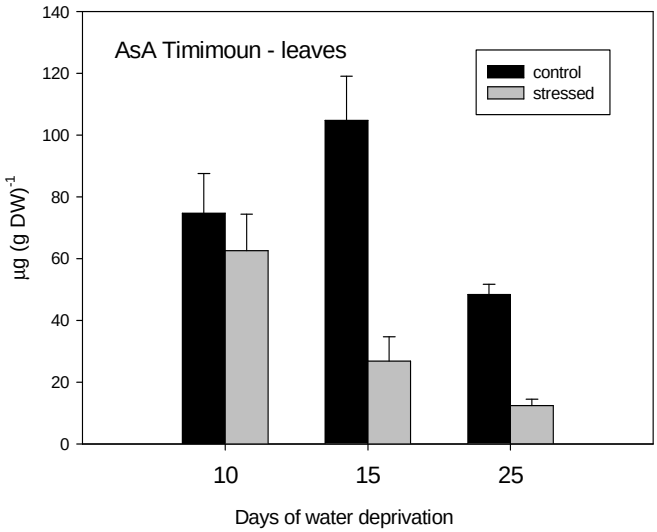
Levels of AsA and GSH were determined in shoots of peanut seedlings after six weeks of growth, plant material was divided into two halves, each consisting of 3 pots for each peanut landrace. One half pots continued to receive adequate water supply (see above) and acted as the well-watered control (W-plants), whereas irrigation was withheld on the other half of pots, thus imposing a progressive water stress to plants (S-plants).

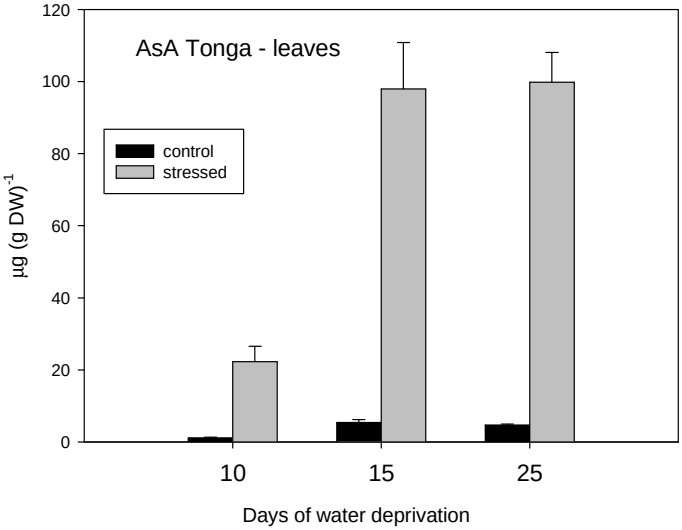
3.2.1. Effect of Drought on AsA

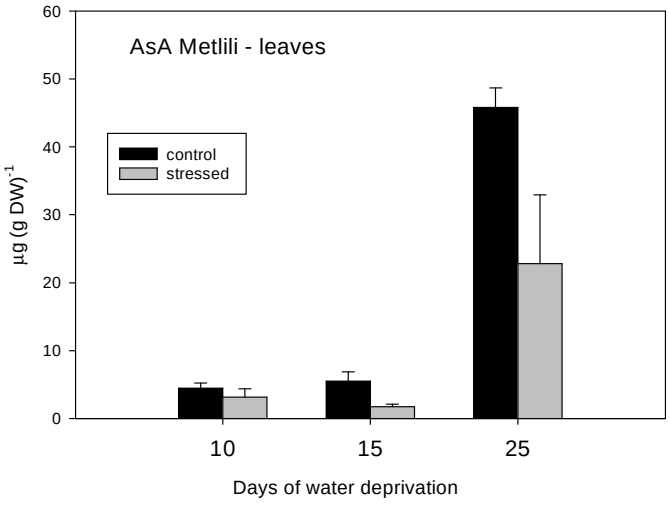
To put more into evidence the specific response pattern of each landrace to water stress, in the ascorbic acid graphs the Y axis has not been made uniform (Fig.2). This allows to better recognise that each landrace exhibits its own response to water stress: there are cases in which an increased duration of water stress provokes a clear decrease in foliar ascorbic acid [Timimoun (TIM), Metlili (MET), Oued Souf (OS) and Berrihane (BER)] and, at the opposite, landraces in which ascorbic acid remarkably increased [Tonga (TO), Oum Tboul (OT), El Frin (FR), Boumalek (BMK), Sebseb (SEB), Oum Tboul (OT) and Adrar (AD)] over time in stressed plants, but not in control ones.

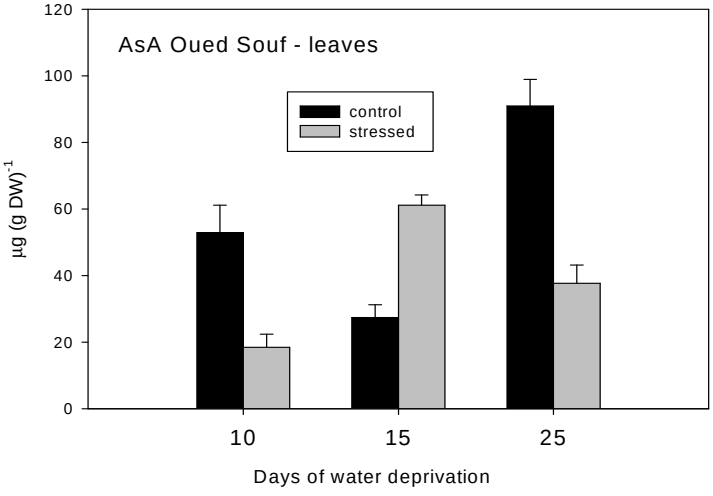

AsA Sebseb - leaves
control
stressed
µg (g DW)-1
0
1
2
3
4
5
6
7
10
15
25
Days of water deprivation


AsA Adrar - leaves
control
stressed
µg (g DW)-1
0
5
10
15
20
25
30
10
15
25
Days of water deprivation


AsA Timimoun - leaves
control
stressed
µg (g DW)-1
0
20
40
60
80
100
120
140
10
15
25
Days of water deprivation


AsA Tonga - leaves
control
stressed
µg (g DW)-1
0
20
40
60
80
100
120
10
15
25
Days of water deprivation


AsA Metlili - leaves
control
stressed
µg (g DW)-1
0
10
20
30
40
50
60
10
15
25
Days of water deprivation


AsA Oued Souf - leaves
control
stressed
µg (g DW)-1
0
20
40
60
80
100
120
10
15
25
Days of water deprivation

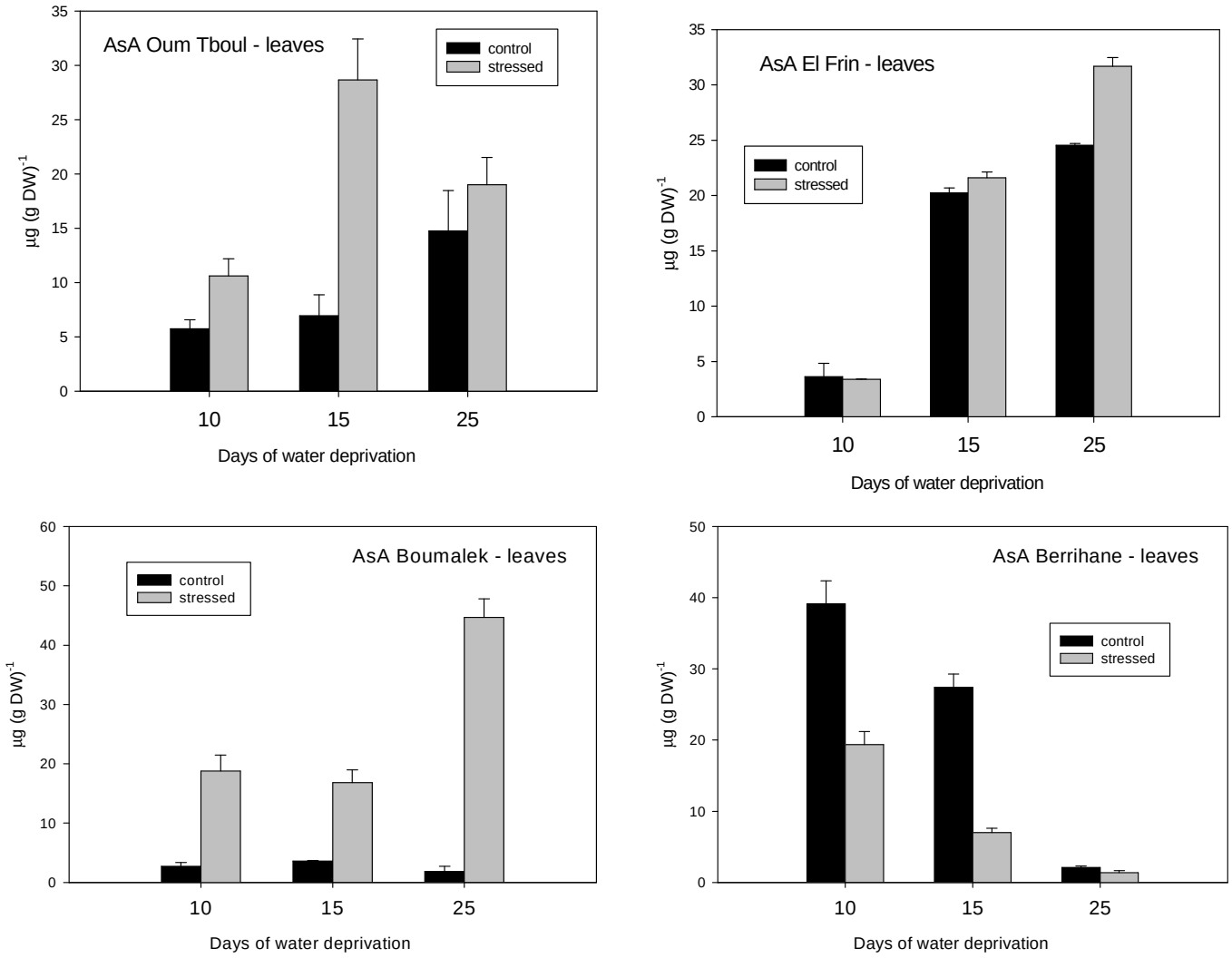


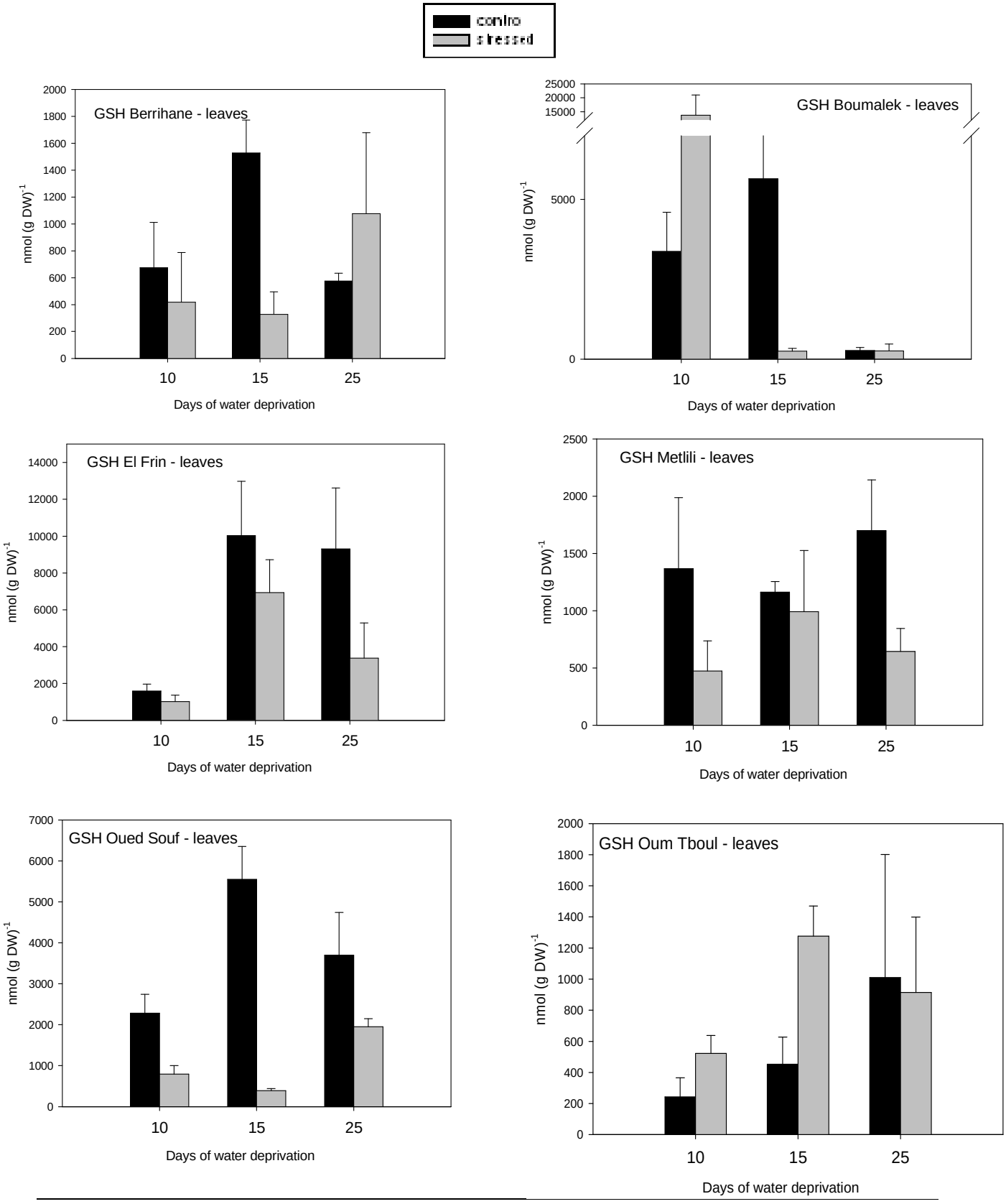
Fig. 2 Ascorbate (AsA) concentration µg (g DW)$^{-1}$ in Algerian peanut landraces after 10, 15and 25 days of water deprivation (□).Histograms (■) denote well-watered controls. Values are the mean (±S.E.) of six replicates n=6. Statistically significant effects of the landrace (L), the water regime (T) and of their interaction are reported (*P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant), as evaluated by a two-way ANOVA. For the antioxidant abbreviations, see Abbreviations used.

3.2.2. Effect of Drought on GSH

A third level of variability is found when the results of reduced glutathione are considered. In this case also to put more into evidence the specific response pattern of each landrace to water stress, in the glutathione graphs the Y axis has been made different.

Here, constitutive levels and remarkable differences responses to water stress among peanut popuations are highly variable. In fact, no obvious effect of water stress on reduced glutathione is seen for all the peanut populations studied. But on the other side a promotive effect of water stress on the levels of reduced GSH is clearly seen in Berrihane (BER), Oum Tboul (OT),

Boumalek (BMK), Sebseb (SEB), Tonga (TO) and Adrar (AD) populations. However, a noticeable downward trend of GSH content was recorded in water stress treatment of El Frin (FR), Metlili (MET), Oued Souf (OS) and Timimoun (TIM) (Fig.3).



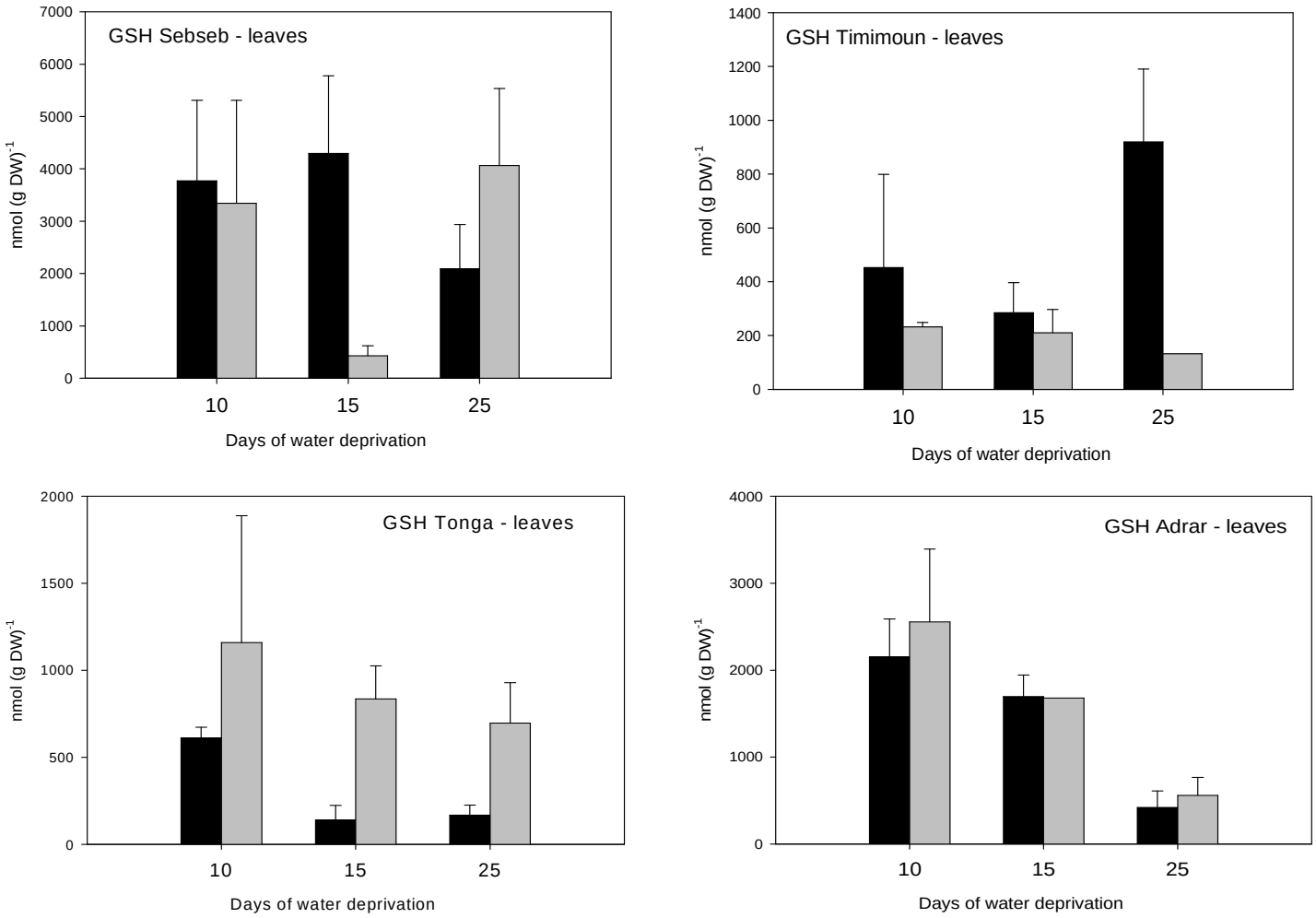


Fig. 3 Glutathione (GSH) concentration n mol (g DW)$^{-1}$ in Algerian peanut landraces after 10, 15and 25 days of water deprivation (□).Histograms (■) denote well-watered controls. Values are the mean (±S.E.) of six replicates n=6. Statistically significant effects of the landrace (L), the water regime (T) and of their interaction are reported (*P < 0.05; **P < 0.01; ***P < 0.001; ns = not significant), as evaluated by a two-way ANOVA. For the antioxidant abbreviations, see Abbreviations used.

4. DISCUSSION

Drought is the major limiting factor in many parts of the world, which seriously affects plant growth and yield **(Shao *et al.*, 2007)**. Plant experiences drought either when the water supply to roots becomes difficult or when the transpiration rate becomes very high **(Sankar *et al.*, 2007)**. Water stress adversely affects many physiological and biochemical process in plants **(Reddy *et al.*, 2004; Manivannan *et al.*, 2007a, b)**. The reactions of the plants to water stress differ significantly at various organizational levels depending on intensity and duration of stress as well

as plant species and its stage of development **(Chaves *et al.*, 2003)**. Understanding plant responses to water stress is of great importance and also fundamental parts for making the crops stress tolerant **(Reddy *et al.*, 2004)**.

Oxidative stress is regarded as a major detrimental factor in plants exposed to a variety of abiotic stresses including drought **(Sharma and Dubey, 2005)**. Plants have evolved a wide range of defense mechanisms to survive continuous assault by an arsenal of biotic attacks as well as constantly changing weather and other environmental conditions **(Shao *et al.*, 2006)**. Drought, due to its osmotic effect in natural and agricultural habitats can induce a wide number of responses such as growth inhibition and synthesis of some non-toxic compounds to enhance the osmotic potential of the cell and thus allow metabolic processes to continue to increase of some antioxidant enzyme activities **(Turkan *et al.*, 2005)**.

In the present study, ten populatins of peanut were subjected to three different levels of water stress (10, 15 and 25 days of water deprivation) to evaluate the effects of water stress on oxidative parameters and antioxidant defence mechanisms in these plants.

The mechanisms that confer protection to oxidative stress were investigated. For this purpose, AsA, GSH concentrations and the activity of antioxidant enzyme SOD were analysed. The principal results obtained in this present research, showed a significant difference in the AsA, GSH content and antioxidant enzyme activity of SOD amoung the ten peanut populations studied under water stress condition.

It is vital for plants to adjust the enzymatic and non enzymatic antioxidant systems to control the amounts of ROS to avoid oxidative stress **(Allen, 1995)**. The regulation of the activities of SOD, CAT and ascorbate-glutathione cycle enzymes is a rapid and efficient response to limit the excess of ROS generated by environmental stresses and it was observed in different studies **(Scebba *et al.*, 2001; Sofo *et al.*, 2004)**. An excess of reducing power, with the consequent increase in H_2O_2 and other ROS concentration, likely caused changes in the regulation of some antioxidant enzymes during the water stress period. The antioxidant enzymes play different and complementary roles in the concerted cell defence, such as direct scavenging of ROS **(Palatnik *et al.*, 1999)**. In plants, this enzymatic response could constitute an adaptive advantage in the protection from oxidative stress. The $O_2^{\bullet-}$ radical is the main source of oxidative injury in plants and several works have been carried out to establish the role of its terminator systems in relation to water stress tolerance **(Scandalios, 1997)**. The dismutation of $O_2^{\bullet-}$ into H_2O_2 reaction catalyzed by the SOD maintains intracellular $O_2^{\bullet-}$ within normal levels and various authors have implicated this enzyme in the protection of cells from oxidative stress **(Smirnoff, 1993)**.

To cope with detrimental effects of oxidative stresses under extremely adverse conditions, plants have developed an antioxidant defense system that includes the antioxidant enzymes SOD, APX, POD, and CAT. The levels of antioxidant enzymes are higher in tolerant species than in sensitive ones under various environmental stresses **(Wang *et al.*, 2009)**. It is also reported that composition of antioxidant varies with duration and nature of stress. Antioxidant enzymes and metabolites increases under various environmental stresses, with their comparatively higher activity in stress tolerant genotypes, suggesting that higher antioxidant activity impart tolerance **(Sairam et Saxena, 2000)**. Accordingly, we observed higher SOD activity in Tonga (TO) population compared to the other remaining populations under drought stresses (Fig. 3), which suggest that the drought-tolerant Tonga population possesses a better reactive oxygen species scavenging ability. These results are in agreement with prior reports revealing the increased SOD activity in drought-tolerant cultivar of bean **(Turkan *et al.*, 2005)**, sesame **(Fazeli *et al.*, 2007)**, alfalfa **(Wang *et al.*, 2009)**, and horsegram **(Bhardwaj and Yadav, 2012)**. In addition, **Abedi and Pakniyat (2010)** and **Tohidi- Moghadam *et al.*, (2009)** showed that SOD activity was increased in some other canola cultivars which might lead to their higher protection against drought stress. SOD converts the toxic $O_2^{\bullet-}$ radicals to H_2O_2 which must be scavenged to O_2 and water by the antioxidant enzymes such as CAT, POD, and APX **(Ozkur *et al.*, 2009)**. In this respect, the activation of SOD in stressed plants may be interpreted as a direct response to augmented $O_2^{\bullet-}$ generation. SOD induction by different environmental stresses has been reported **(Bowler *et al.*, 1992)**. It was also reported that the increase in SOD activity enhance plants water stress tolerance **(Manivannan *et al.*, 2007c**). In tomato plants, SOD activity was induced strongly by water stress **(Noctor *et al.*, 2000)**. In plants, SOD enzymes are major scavengers of $O_2^{\bullet-}$ free radicals and convert them into O_2 and H_2O_2. Hydrogen peroxide is then eliminated by various antioxidant enzymes such as catalases, guaiacol peroxidases and ascorbate peroxidases **(Apel et Hirt, 2004)**.

An overview of changes in SOD activity at different water stress treatments in the remaining peanut populations studied indicates that levels of SOD decrease in peanut plants; this could mean a decrease in SOD ability to scavenge ROS. The activity of the major ROS-scavenging enzyme, namely SOD, was either found to decrease, as observed by **Miszalski *et al.*, 1996** in *Rhizipogon roseulus*. A consequence of a decrease in SOD activity, is not clear, and deserves further study.

Efficient destruction of ROS in plant cells requires the concerted action of antioxidants. Among the non-enzymatic antioxidants, AsA is found to be one of the best characterized compounds, required for many key metabolic functions in plant cells **(Foyer, 1993)**. In addition to its ability to directly scavenge ROS, ascorbate together with APX, MDHAR and DHAR participates in one of the major $H_2O_2^-$scavenging path ways in plant cells the ascorbateglutathione cycle. In plant cells, the most important reducing substrate for H_2O_2 scavenging is AsA, a high level of AsA is essential effectively to maintain the antioxidant system that protects plants from oxidative damage due to the biotic and abiotic stresses **(Reddy *et al.*, 2004)**. The beneficial effects of AsA in mitigating partially or completely the adverse effects of drought stress may be one aspects of the role of these in the activation of some enzymatic reactions **(Kefeli, 1981)**. It is generally assumed that plant tolerance to environmental stresses is positively correlated with AsA content **(Knörzer *et al.*, 1996; Tambussi *et al.*, 2000)**. In our study, the degree to which the AsA concentration changed under water stress is extremely variable among different water stress treatments and different peanut populations studied (Fig. 2). Higher production of non-enzymatic antioxidants such as AsA in Tonga (TO), Oum Tboul (OT), El Frin (FR), Boumalek (BMK), Sebseb (SEB) and Adrar (AD) is in agreement with the results obtained by **Murshed *et al.*, 2008**, where water stress caused changes in AsA concentration in tomato fruits. These changes in AsA concentration in peanut populations under water stress treatments would occur because of either changes in AsA synthesis or changes in AsA regeneration from DHA, which can result from water stress-induced changes in MDHAR and DHAR activities **(Jiménez *et al.*, 2002)**. These populations of peanut have appreciably more amount of antioxidant content in its leaves facing severe drought stress at 25 days of water deficit.With increase in stress degree there was no significant changes in the antioxidant content of Timimoun (TIM), Metlili (MET), Oued Souf (OS) and Berrihane (BER). Water deficit may influence levels of several antioxidant substances such as ASC in plants **(Rao *et al.*, 1997; Dat *et al.*, 1998)**. Our data indicated that severe water stress elevated the levels of ASC. A high level of AsA is essential to maintain the antioxidant capacity that protects plants from oxidative stresses **(Zhou *et al.*, 2009)**. Increase in AsA content plays an important role in preserving APX activity. Ascorbate plays a major role as an antioxidant in chloroplasts and other cell compartments **(Asada, 1999; Smirnoff *et al.*, 2000)** and also functions as a co-substrate of plant peroxidases, such as the ascorbate peroxidase or oxidase system that produces dehydroascorbate **(Halliwell, 1982)**. Given that ascorbate can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via

the APX reaction **(Noctor et Foyer 1998)**, the increase of AsA concentration in peanut plants suggests an important role for this antioxidant against oxidative stress provoked by water stress.

Like GSH is a low molecular mass compound with recognized antioxidant functions **(Huang *et al.*, 2008)**. Glutathione is essential for the antioxidant defense system particularly in chloroplasts, because it protects the photosynthetic apparatus counteracting the damaging effects of ROS. The cellular concentration of GSH varies considerably under biotic stresses and strong evidences correlate the ability of plant to withstand metal induced oxidative stress with high GSH concentrations **(Pietrini *et al.*, 2003; Sun *et al.*, 2007)**. GSH reacts chemically with a range of ROS while enzyme-catalyzed reactions link GSH to detoxification of H_2O_2 in the ascorbate-glutathione cycle. Glutathione furthermore prevents denaturation of proteins caused by oxidation of thiol groups during stress. In our work, GSH content in water stress treatment (S-Plants) for Berrihane (BER), Oum Tboul (OT), Boumalek (BMK), Sebseb (SEB), Tonga (TO) and Adrar (AD) was higher than control ones. Even GSH has been occasionally found to strongly increase in response to severe water stress (25 days), as observed in Berrihane (BER), Sebseb (SEB) and Tonga (TO) as occurred in *S. cerevisiae* **(Vido *et al.*, 2001)**. These results may be relative to the changes of GR and DHAR. Water stress increased GR activity, resulting in an increase in GSH content compared to control plants. In addition, water stress may increase drought tolerance of Berrihane (BER), Oum Tboul (OT), Boumalek (BMK), Sebseb (SEB), Tonga (TO) and Adrar (AD) by promoting the ascorbate-glutathione cycle.

The results of this study show that some populations of peanut studied responded to water stress induced oxidative stress increasing enzymatic and non-enzymatic antioxidant defenses (Fig. 1, 2, 3). Importantly, the antioxidant response seemed to be proportional to the extent of the water stress imposed. Water stress tolerance has been correlated to an improved oxidative stress response in several crops **(Gosset *et al.*, 1994**, **Dionisio-Sese et Tobita 1998**, **Hernández *et al.*, 2000**, **Shalata *et al.*, 2001**, **Santos *et al.*, 2001)**. Under the conditions imposed in this study, only a moderate increase in the levels of SOD in leaves of Tonga (TO) population was observed after 25 days of water stress treatment. This seems to be as a result of an effective antioxidant protection. All the antioxidant enzyme activities increased in response to water stress although differences in the pattern of upregulation were found among them (Fig. 1). It has been reported that ROS up-regulate the enzymatic antioxidant system **(Foyer *et al.*, 1994)**. This seems to occur also in Tonga (TO) population although the specific regulation of the enzyme activities in

response to water stress and the interrelations among them is a complex problem that needs further studies to be elucidated. However, SOD did not further increase with time and even strongly decreased at the end of water stress treatment in the nine peanut populations studied.
ROS enzymatic scavengers, antioxidant-regenerating enzymes, AsA, GSH and other morpho-physiological response mechanisms rather than a single detoxification mechanism, may be effective in dealing with water stress stress in peanut populations.

5. CONCLUSION

From these results, it can be concluded that a relationship can exist between water stress and oxidative stress occurring in peanut populations, as has also been postulated for other plant species **(Iturbe-Ormaetxe *et al.*, 1998; Borsani *et al.*, 2001; Rahman *et al.*, 2002)**. We have found evidence for changes in ROS scavenging enzymes, ascorbate and glutathione pools in plants under water stress conditions. We can also confirm that different stress levels at the beginning of flowering stage play a key role in the regulation of ascorbate–glutathione cycle, causing different antioxidant responses in peanut species. The results obtained in this investigation underline the important role of some antioxidant enzymes and compounds in protecting cellular apparatus during water stress conditions and may be important for the selection for drought resistance in peanut.

In summary and as a whole data suggest that peanut populations are able to cope with the oxidative stress induced by water stress. The results obtained seem to confirm that water stress has a profound effect on the foliar antioxidant status. We do believe that the remarkable variability in response to water stress, among plant organ, among species and among landraces in each species has not to be seen as an embarrassment in interpreting the results, but as richness, instead. We are confident that adequate statistical analysis, albeit complex to run, will reveal important association among biochemical traits and the physiological and agronomic features of the legume landraces. This will allow to look at antioxidant enzymes and metabolites as useful traits to assist the selection of valuable plant material to be tested in the field, as well as markers for the selection of plant material better suited to withstand water stress. In conclusion, the amazing levels of biodiversity found in collections of peanut landraces from the Algerian territory might reveal to be a mostly valuable resource in a much wider context, in the present era of climate changes.

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Chapitre IV

Isolement de gènes et abondance des transcrits de l'enzyme superoxyde dismutase (SOD) chez l'arachide (Arachis hypogaea L.)

Gene isolation and transcripts abundance of the enzyme superoxide dismutase (SOD) in peanut (Arachis hypogaea L.)

Une meilleure compréhension des mécanismes génétiques, qui sont à la base de la tolérance à la sécheresse, chez les plantes, est nécessaire pour développer des variétés plus adaptées et performantes **(Rebetzke *et al.*, 2008)**.

La biologie moléculaire propose des outils pour l'étude de l'expression des gènes et des protéines. La génomique fonctionnelle s'intéresse à l'ensemble des transcrits et des protéines. Elle étudie les méthodes d'analyse de l'expression des gènes et des protéines. En comparant l'expression des gènes et /ou des protéines, au sein d'un même organisme et sous diverses conditions de croissance, il est possible de prédire le fonctionnement des gènes induits **(De Leonardis *et al.*, 2007)**. Ces informations sont fournies par l'analyse des variations d'expression entre différents états physiologiques, différents stades de développement et différents tissus **(Neffar *et al.*, 2011)**.

Deux approches sont utilisées pour comprendre les mécanismes moléculaires de la tolérance au stress. La première consiste à étudier les enzymes impliquées dans des voies métaboliques particulières alors que la seconde cherche à identifier des gènes exprimés de manière différentielle chez des plantes conduites sous stress et en l'absence du stress. Ces approches ont permis d'identifier de nombreux gènes de fonctions connues **(Mazzucotelli *et al.*, 2008)**.

La cascade d'événements moléculaires conduisant au développement de la tolérance au stress comporte :

1- La perception du signal de stress par les récepteurs de la membrane cellulaire qui captent les variations du milieu externe ;
2- la transduction du signal du stress du cytoplasme vers le noyau cellulaire, suivie d'une cascade d'évènements matérialisés par l'accumulation transitoire des ions Ca^{++}, l'activation des protéines de type kinases, l'accumulation de l'ABA et la modification de la structure de la membrane plasmique ;
3- l'activation, dans le noyau, des facteurs de transcription associés à l'expression des gènes de réponse au stress ; et
4- l'expression des gènes du stress codant des protéines qui gèrent la situation de stress en réparant ou en maintenant l'homéostasie cellulaire **(Yamaguchi-Shinozaki *et al.*, 2002)**.

Les changements de l'expression génique qui se produisent au niveau cellulaire sont intégrés dans la réponse de la plante entière. L'importance et la rapidité de la réponse cellulaire au stress dépendent de la durée et de l'intensité du stress **(Yamaguchi-Shinozaki *et al.*, 2002)**.
La dernière étape de la réponse moléculaire aux stress se traduit par l'expression des gènes spécifiques qui codent pour la synthèse des protéines impliquées directement dans la tolérance au stress. Ces gènes aux différentes fonctions sont induits ou réprimés par le stress **(Yamaguchi-Shinozaki et Shinozaki, 2005)**.

Les gènes, induits sous stress hydrique, assurent la protection de la cellule contre l'effet du déficit hydrique, en produisant des protéines fonctionnelles, et régulent le signal de transduction en réponse au stress hydrique **(Yamaguchi-Shinozaki *et al.*, 2002; Shinozaki et Yamaguchi-Shinozaki, 1997; 2007)**. Ces protéines fonctionnelles, comme les enzymes de la biosynthèse des osmoprotecteurs et les protéines *LEA* (Late Embryogenesis Abundant), protègent les cellules de la déshydratation.

Parmi ces protéines fonctionnelles figurent aussi les enzymes de détoxification comme le gluthatione S-transférase, le super-oxyde dismutase, l'ascorbate peroxidase et l'époxyde hydrolase qui protégent les cellules des effets négatifs des ROS induites par le stress hydrique **(Ingram et Bartels, 1996; Yamaguchi-Shinozaki *et al.*, 2002)**. Les gènes du stress, une fois induits, sont transcrits en mRNAs qui migrent du noyau vers le cytoplasme. Ces derniers transcrits sont traduits en protéines spécifiques au stress considéré dans le cytosol. La vitesse de la transcription et la quantité des protéines synthétisées sont déterminées par des facteurs dépendant des stimuli du milieu ambiant **(Miller *et al.*, 2001)**.

Le stress induit une diversité de réponses qui résultent de l'expression génique et des modifications du métabolisme cellulaire, se traduisant par une variation de la croissance et du développement de la plante **(Gilmour *et al.*, 1998)**. L'adaptation de la plante s'accompagne par une série complexe de réactions enzymatiques conduisant à l'évitement ou à la réduction des dommages susceptibles d'être causés, par le stress, à la cellule, et par là même à la plante entière **(Cattivelli *et al.*, 2002)**. La cascade de réactions moléculaires se déclenche dès que la cellule perçoit le stress **(Vinocur et Altman, 2005)**. Le signal de perception du stress diffuse dans le cytoplasme et le noyau pour activer l'expression des gènes et des métabolites spécifiques.
Les gènes impliqués dans la réponse à la contrainte abiotique, qu'ils soient induits ou réprimés,

codent pour une large gamme de protéines assurant diverses fonctions. Parmi ces fonctions, il y a la stimulation et la régulation du signal de transduction, la régulation de l'expression des gènes induits directement dans la réponse au stress, la protection et le maintient des fonctions et structures cellulaires. Toutes ces fonctions conduisent vers l'amélioration de la tolérance vis-à-vis des stress abiotiques **(Vinocur et Altman, 2005 ; De Leornadis *et al.*, 2010)**.

Sur le plan moléculaire, nous nous sommes intéressés à l'expression différentielle de gènes en réponse à la contrainte hydrique. Parallèlement, une recherche ciblée, consacrée au superoxide dismutase, a été menée. Donc, ce quatriéme chapitre s'intéresse à l'isolement, la caractérisation et l'analyse de l'expression des gènes codant pour l'enzyme antioxidante, la superoxide dismutase chez quelques populations Algériennes d'arachide (*Arachis hypogaea* L) sous les conditions du stress hydrique.

Résumé

Le stress hydrique est connu pour induire des espèces actives de l'oxygène **(EAO)** dans les plantes. L'accumulation de ces espèces nuisibles doit être empêchée par ces plantes aussi rapidement que possible pour maintenir la croissance et la productivité. Cette étude a été réalisée pour comprendre les mécanismes anti-oxydants de l'arachide dans des conditions de stress hydrique, pour cela quatre populations locales d'arachide (*Arachis hypogaea* L.): **EL FRIN (FR)**, **Tonga (TO)**, **Oued Souf (OS)** et **Sebseb (SEB)** ont été soumises à un stress hydrique pendant **25 jours**. L'expression des gènes de la superoxyde dismutase **(SOD)** est analysée, compte tenu de son rôle central dans les antioxydants de défense chez tous les organismes et de son implication dans les réponses anti-oxydantes sous contrainte hydrique. L'**ARN** total isolé à partir des plantes stressées et non stressées permet de déterminer le taux d'expression du gène de la superoxyde dismutase **(SOD)** par la technique **RT-PCR**.

Les résultats montrent que le stress hydrique régule la synthèse et l'activité de la superoxyde dismutase et la manière dont cette enzyme contribue à protéger contre les effets dommageables de radicaux superoxyde chez l'arachide. Les niveaux d'expression relative de la **SOD** augmentent après 25 jours de stress hydrique chez **Tonga (TO)**, par contre, ils diminuent chez **Sebseb (SEB)**. Les résultats révèlent de grandes différences "***constitutives***" dans l'expression du gène de la **SOD** chez les quatre populations d'arachide. En effet, **SOD** transcripts est plus abondant chez **Tonga (TO)** que chez **Sebseb (SEB)**.

En conclusion, bien qu'il soit difficile de comprendre tous les mécanismes antioxydants des plantes sous stress environnementaux, cette étude serait une étape préliminaire pour une étude moléculaire de certaines enzymes antioxydantes. Le profil d'expression génique de la catalase **(CAT)**, chloroplastique/mitochondrial **GR** et chloroplastique / stromale **APX** sera la prochaine étape de cette étude. Aussi, la comparaison des profils d'expression génique des différentes enzymes antioxydantes et des activités enzymatiques permettrait d'approfondir la connaissance des mécanismes de protection moléculaire chez l'arachide sous contrainte hydrique.

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Gene isolation and transcripts abundance of the enzyme superoxide dismutase (SOD) in peanut (*Arachis hypogaea* L.)

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SUMMARY. –

Water stress is known to induce active oxygen species in plants. The accumulation of these harmful species must be prevented by plants as rapidly as possible to maintain growth and productivity. This study was carried out for understanding of antioxidant mechanisms of peanut under water stress conditions. For this aim, after six weeks of growth (47 DAS), four peanut (*Arachis hypogaea* L.) landraces: EL FRIN (FR), TONGA (TO), OUED SOUF (OS) and SEBSEB (SEB) were subjected to drought stress for 25 days. Plants were submitted to water stress by withholding water supply and the attention was given to the expression analysis of the superoxide dismutase (SOD) gene, in view of its central role for antioxidant defence in all aerobic organisms and of its involvement in antioxidant responses to water shortage. PCR conditions for SOD antioxidant enzyme were optimized. Then, total RNA was isolated from stressed and non-stressed plant shoots. The gene expression levels SOD were examined by semiquantitative reverse transcription polymerase chain reaction (RT-PCR) technique. Results show how water stress regulates the synthesis and the activity of superoxide dismutase and how these enzymes contribute to protect against the damageable effects of superoxide radicals in peanut. Relative expression levels of SOD increased after 25 days of drough treatment in shoots of Tonga (TO) landrace. On the other hand, expression levels of SOD decreased on SEBSEB (SEB) landrace after 25 days of water stress in shoot tissues. Although in the presence of an equal sample loading, still the results reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Indeed, SOD transcripts appeared to be most abundant in TO and least

abundant in SEB. Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study.

1. INTRODUCTION

Plants respond to water deficit and adapt to drought conditions by various physiological changes including transition in gene expression during water deficit. The mechanisms of drought response have been investigated most extensively in a model plant, Arabidopsis **(Shinozaki et Yamaguchi-Shinozaki, 1997, 1999)**. The most feared and widespread plant stress agents are active oxygen species. These include redox intermediates in the reduction and oxidation between dioxygen and water; superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$), and the electronically- excited oxygen species, singlet excited oxygen ($^1O_2$). These species are able to react with DNA, lipids, proteins, and almost any other constituent of plant or animal cells **(Beauchamp et Fridovich, 1971; Demming et Björkman, 1987; Halliwell et Gutteridge, 1989)**. These reduced oxygen species are not only generated as by-products of endogenous biological reactions, but also their formation increases during biotic and abiotic stresses. Superoxide dismutase (SOD, EC 1.15.1.1) is a key enzyme which constitutes the first line of defence against oxygen toxicity and catalyses the dismutation of the superoxide anions to dioxygen and hydrogen peroxide **(Elstner, 1982; Foyer *et al.*, 1994)**. The three known types of this enzyme can be distinguished according to their metal cofactor made of manganese (MnSOD), iron (FeSOD) or copper and zinc (Cu/Zn SOD) **(Bannister *et al.*, 1987)**, and according to their behaviour towards specific inhibitors **(Bowler *et al.*, 1992)**. The identification of the three types of isozymes is based on the differential inhibition of SOD activity on polyacrylamide gels preincubated with KCN or H_2O_2 **(Bowler *et al.*, 1992; Rubio *et al.*, 2001)**. H_2O_2 generated from the activity of SOD is scavenged by catalase in peroxisomes and by ascorbate peroxidase in Halliwell- Asada cycle **(Elstner, 1982; Halliwell et Gutteridge, 1989)**. Identification of resistance mechanisms to water stress is often difficult because resistance to drought stress is a quantitative trait. Induction of antioxidative enzymes can be assumed to reflect a general strategy required to overcome increased oxidative stress induced by water stress. In this paper, we point out superoxide dismutase (SOD) in some peanut populations, and we study it activity during water stress induction in order to understand the level of implication of this enzyme in the physiology of resistance to water stress. We presented isolation, characterization and expression analysis of genes coding for superoxide dismutase in Algerian peanut (*Arachis hypogaea* L.) landraces under water stress conditions.

2. MATERIALS AND METHODS

2.1. Plant material

Peanut (*Arachis hypogaea* L.) is a leguminous crop that is widely cultivated all over the world, especially in the semi-arid tropical regions. This species presents a good resistance to drought **(Singh *et al.*, 1985)** but a large number of varieties and cultivars have been created that are adapted to varied local environmental conditions. In this study, experiments were performed on 04 peanut Algerian landraces obtained from a prospection conducted by the Ecole Nationale Supérieure Agronomique, Algiers, Algeria, during the years 2003-2005. Based on the locations in which they were collected, the peanut landraces were assigned the following names: Tonga (TO), Oum Tboul (OT), El Frin (FR) and SebSeb (SEB). Botanical and agronomic features of peanut landraces, as well as their geographical and climatic distribution within the Algerian territory, are reported in **Amri (2008)** and **Lazali (2009)**. To avoid confusion with the acronyms used for plant parameters, the full names of the peanut landraces are used in the text thereafter, whereas their acronyms are used in figures.

2.2. Seeds inoculation

Two days before sowing, healthy seeds of uniform size were selected for each peanut landrace. Selected seeds were first soaked into an aqueous solution of 40% (w/v) arabic gum and then evenly coated with peat inoculated with the rhizobial strain BRE 1.2 which was previously isolated from nodules of the same peanut populations **(Benmohamed *et al.*, 2004)**. A trapping test conducted before seeds inoculation allowed to conclude that the soil mix used for plant growth did not contain any non-specific rhizobia able to infect the experimental plant material.

2.3. Plants' growth conditions

Four seeds for each landraces were sown in each of six 6 L-pots, each filled with 4 kg of a 50/20/30 (v/v/v) agricultural soil/peat/washed quartz sand mixture. The soil physico-chemical characteristics are reported in **Gelsomino *et al.*, (2012)**. Peat medium properties were (%, w/w dry weight): organic C 52%, organic N 0.8 %, organic matter 90%, pH $_{H2O}$ 3. The particle size of quartz sand (Progettinelblu.com) was 0.5 mm. After the emergence (5 days after sowing, DAS), two seedlings of each landraces per pot was used for the experiment.

Planted pots were randomly distributed within a walk-in climatic chamber, where an air temperature of 26 ± 1°C, a relative air humidity of 70 ± 5 %, and a photoperiod of 12 h were set. Photosynthetic photons flux density at plants' height was 350 µmol m-2 s-1 (LI-190SA quantum sensor, Li-Cor, Lincoln, NE). Since both agricultural soil and peat medium were reputed to

contain adequate levels of N, P and organic matter, no further fertilizer was supplied during the plants' growth. Planted pots were given 200 mL of tap water every four days because preliminary trials indicated that such amount and frequency of water supply was required to compensate water losses by evapotranspiration.

2.4. Imposition of water stress.

After six weeks of growth (47 DAS), plant material was divided into two halves, each consisting of 3 pots for each peanut landrace. One half pots continued to receive adequate water supply (see above) and acted as the well-watered control (W-plants), whereas irrigation was withheld on the other half of pots, thus imposing a progressive water stress to plants (S-plants). Keeping in mind the results of **Amri (2008)**, who observed that nodulation, plant height and dry matter production of Algerian peanut landraces were affected after 15 days of irrigation withdrawal, water deprivation on the present S-plants lasted for 25 days. During water stress treatment, S- and W-plants were kept together in the same controlled environment where they were previously grown (see above).

2.5. Isolation, characterization and expression analysis of genes coding for superoxide dismutase in Algerian peanut (Arachis hypogaea L.) landraces under water stress conditions

Attention was given to the expression analysis of the superoxide dismutase (SOD) gene, in view of its central role for antioxidant defence in all aerobic organisms and of its involvement in antioxidant responses to water shortage **(Apel and Hirt, 2004; Gill et Tuteja, 2010)**. The expected complexity of the SOD gene family in *Arachis* spp was further exacerbated by finding that scanty previous work was carried out so far on the SOD genomic organisation in such species, except in seeds, which are of course the economically valued part of the plant.

2.5.1. Searching databases of complete sequences or EST (Expressed Sequence Tags) coding for the enzyme SOD in Peanut (Arachis hypogaea L,);

The first part of the work was focused on identification of sequences coding for SOD in databanks. Such database search included SOD-codifying sequences not only in peanut, but also in cowpea and in the model species *Arabidopsis*, in order to evaluate interspecific similarities and differences in the genomic organisation of the SOD gene. It was conducted by performing a research using the BLAST program **(Altschul *et al.*, 1997)** in different protein databases (GenBank, PDB, SwissProt, PIR and PRF), nucleotide (EMBL, Genbank, DDBJ and PDB) and EST (GenBank, EMBL and DDBJ) using EMB-BLAST (European Molecular Biology network - Swiss node -) available at the website: http://www.ch.embnet.org/software/aBLAST.html.

2.5.2. RNA extraction and cDNA synthesis

Leaves were rapidly cut off, immediately frozen in liquid nitrogen and stored at - 80°C until used. For RNA extraction, leaf samples were ground in liquid nitrogen with a pestle and mortar. From the W-25 leaves of the four peanut landraces selected for gene expression experiments, total RNA was extracted using the CTAB method **(Rogers *et al.*, 1991, Ciaffi *et al.*, 2001)**. The resulting RNA was treated with Rnase-free DNase I (Promega) according to the manufacturer's protocol. Following digestion, nucleotides were removed from RNA using a G50 sepharose buffer exchange column (Amersham). Absence of genomic DNA contamination in DNase I-treated samples was checked by PCR of 0.125 µg of RNA template using a primer pair (5'-GGTTCCCTGAGATCACAACT-3' and 5'-CCAAACGTTCCCATCTATGAGG- 3') designed to amplify an intron sequence of a gene encoding SOD. When a single DNase treatment did not completely remove interfering genomic DNA, a second DNase incubation was performed to eliminate any detectable DNA. RNA concentration and integrity were checked with a UV/VIS spectrophotometer Lambda 3B (Perkin Elmer) before and after DNase I digestion. Only RNA samples with 260/280 wavelength ratio between 1.9 and 2.1 and 260/230 wavelength ratio greater than 2.0 before and after DNase I digestion were used for cDNA synthesis. The quality of RNA samples was also assessed by electrophoresis on 1% formaldehyde agarose gels and stained with ethidium bromide. First-strand cDNA was synthesized from 3 µg of total RNA by Expand™ Reverse Transcriptase (RT) (Roche) and RACE (Rapid Amplification of cDNA Ends) for obtaining cDNA clones spanning the entire coding sequence (full-length) of SOD and diluted 1:5 before use in PCR assays.

2.5.3. Amplification by PCR using specific primers designed on database sequences

A search in the BLAST program found 13 sequences of *peanut* encoding SOD displaying a high degree of homology among each other. The primers used for PCR analysis (forward: 5'-GGTTCCCTGAGATCACAACT-3' and reverse: 5'-CCAAACGTTCCCATCTATGAGG- 3') wich were designed in conserved regions of the 13 transcripts.

First-strand cDNA was synthesized as described above and the PCR reactions were performed by the TripleMaster PCR system (Eppendorf) using 1 µl of the RT reaction. After initial denaturation at 94°C for 2 min, amplification conditions were 35 cycles each at 94°C for 30 s, 60°C for 1 min and 72°C for 3 min, followed by a final extension step at 72°C for 7 min. Samples of the amplification products (3 µl) were collected after 28, 32 and 35 PCR cycles and analysed by electrophoresis on 2% agarose gel. Each PCR experiment was independently

repeated twice to test amplification reproducibility. The specificity of the amplicons was checked by sequencing of the PCR products in order to confirm that its sequence corresponded to the target gene.

2.5.4. Cloning of the amplification products

The differential amplification products were inserted by ligase in the modified EcoRV restriction site of the plasmid vector pGEM-T (Promega) and sequenced. Plasmid purification was performed using the Quiagen "Mini Plasmid Prep Kit".

2.5.5. DNA sequencing

The nucleotide sequence was determined on one strand of denatured plasmid DNA using the dideoxy chain-termination method **(Sanger *et al.*, 1977)** with the Oncor sequencing kit, using the electrophoretic system of Kodak; the other strand was sequenced by ESGS (Paris, France).

3. RESULTS AND DISCUSSION

As a result, 13 transcripts (complete or partial) coding for SOD were identified in peanut sequences databases, displaying a high degree of homology among each other (Fig. 1). Aligning the sequences shown in Fig. 1 allowed to identify highly conserved regions, against which oligonucleotidic primer pairs (forward and reverse) were designed (Fig. 2). In particular, the primers F1 and R1 allowed to obtain the entire SOD transcript, expected to encompass in length about 500 base pairs (bp). In addition, the F4 primer was designed within the SOD-coding region (Fig. 2), in order to capture differences among the components of the SOD gene family and among the two genomes present in *Arachis*. Such differences are in fact expected to reside mostly in the 3' region, beyond the stop codon and before the polyA tail, which are transcribed but not translated. The F4 primer allowed to apply the RACE methodology to the 3' region, to obtain complete transcripts until the polyA tail (Fig. 2). The lower panel in Fig. 3 shows quality control and equal loading of total RNA extracted from the W-25 leaves of the four peanut landraces selected for gene expression experiments. To isolate the SOD gene(s), an aliquot of such total RNA was reverse-transcribed and PCR-amplified by using the F1-R1 primer pairs shown in Fig. 2. Electrophoretic separation of amplification products gave the banding pattern shown in the upper panel of Fig. 3. Although they are at the very beginning of the work leading to SOD gene characterisation and expression analysis in the peanut landraces at study, the results reported in Fig. 3 (upper panel) might deserve some comments.

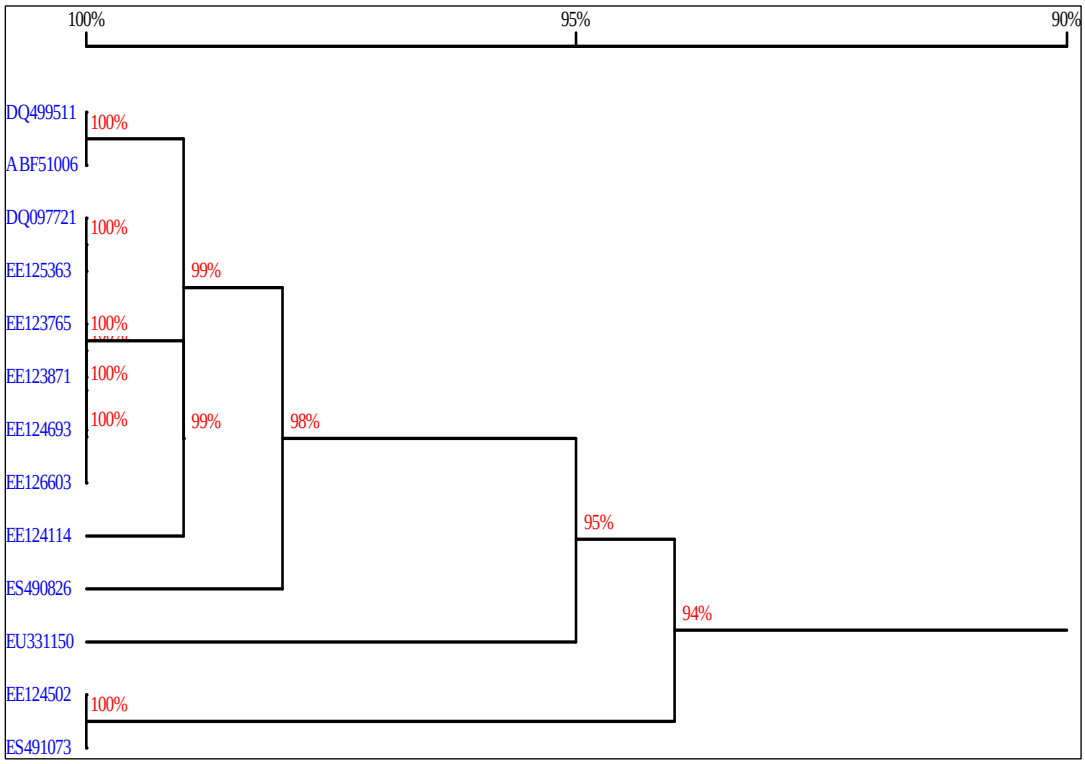


Figure 1 - Dendrogram of the 13 SOD-coding sequences identified in *Arachis* spp by data base. search

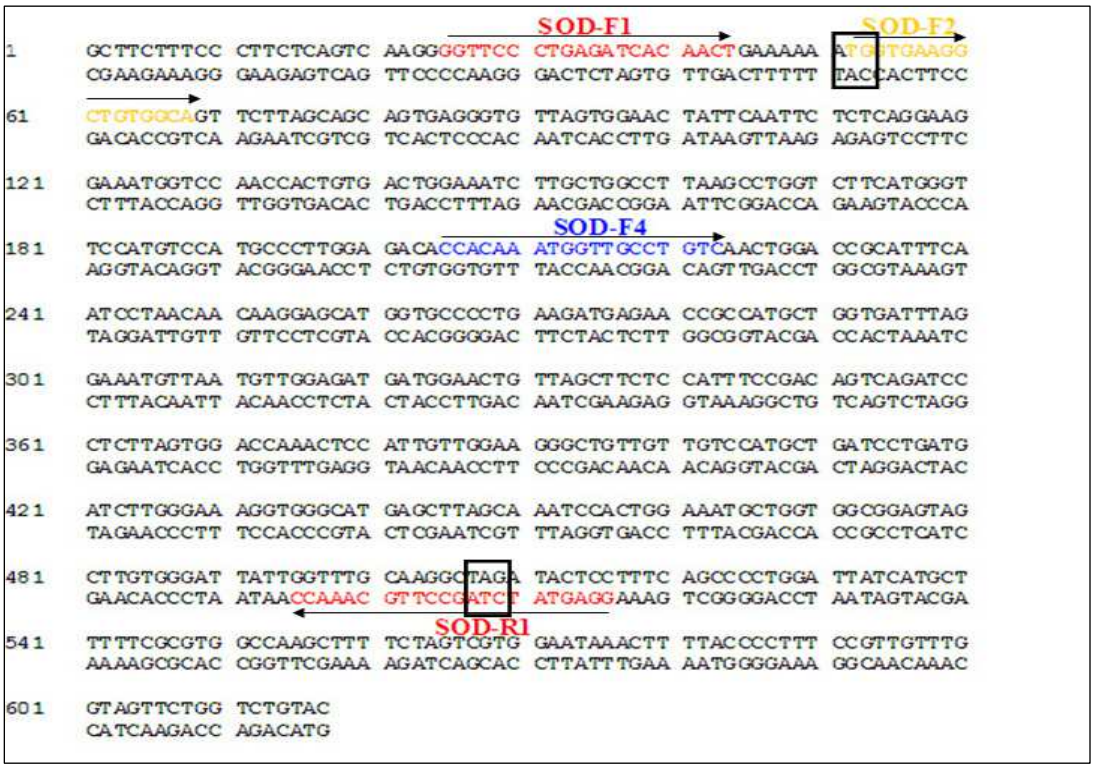


Figure 2 - Sequence alignment and oligonucleotidic primers designed for delimiting highly conserved regions within SOD-coding DNA sequences.

Firstly, it is noteworthy that, i) although not intended to put into evidence differences among SOD transcripts abundance, because of the high number of PCR amplification cycles, ii) although carried out on well watered plants, thus not taking into account any effect of water stress, and iii) although in the presence of an equal sample loading, still the results in Fig. 3 reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Interestingly enough, SOD expression in the four landraces seemed to be roughly in accordance with the ranking of their overall tolerance to water stress, put into evidence by PCA. Indeed, SOD transcripts appeared to be most abundant in TO and least abundant in SEB (Fig. 3). Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study.

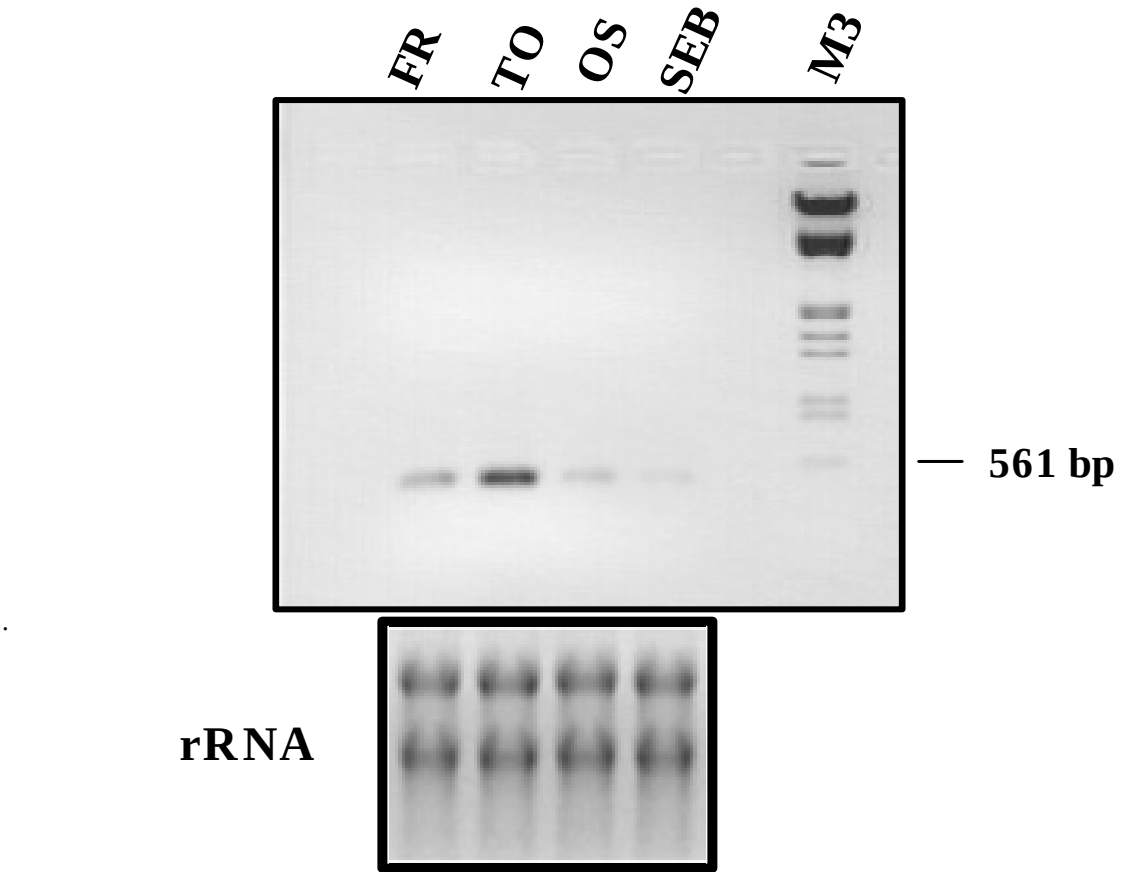
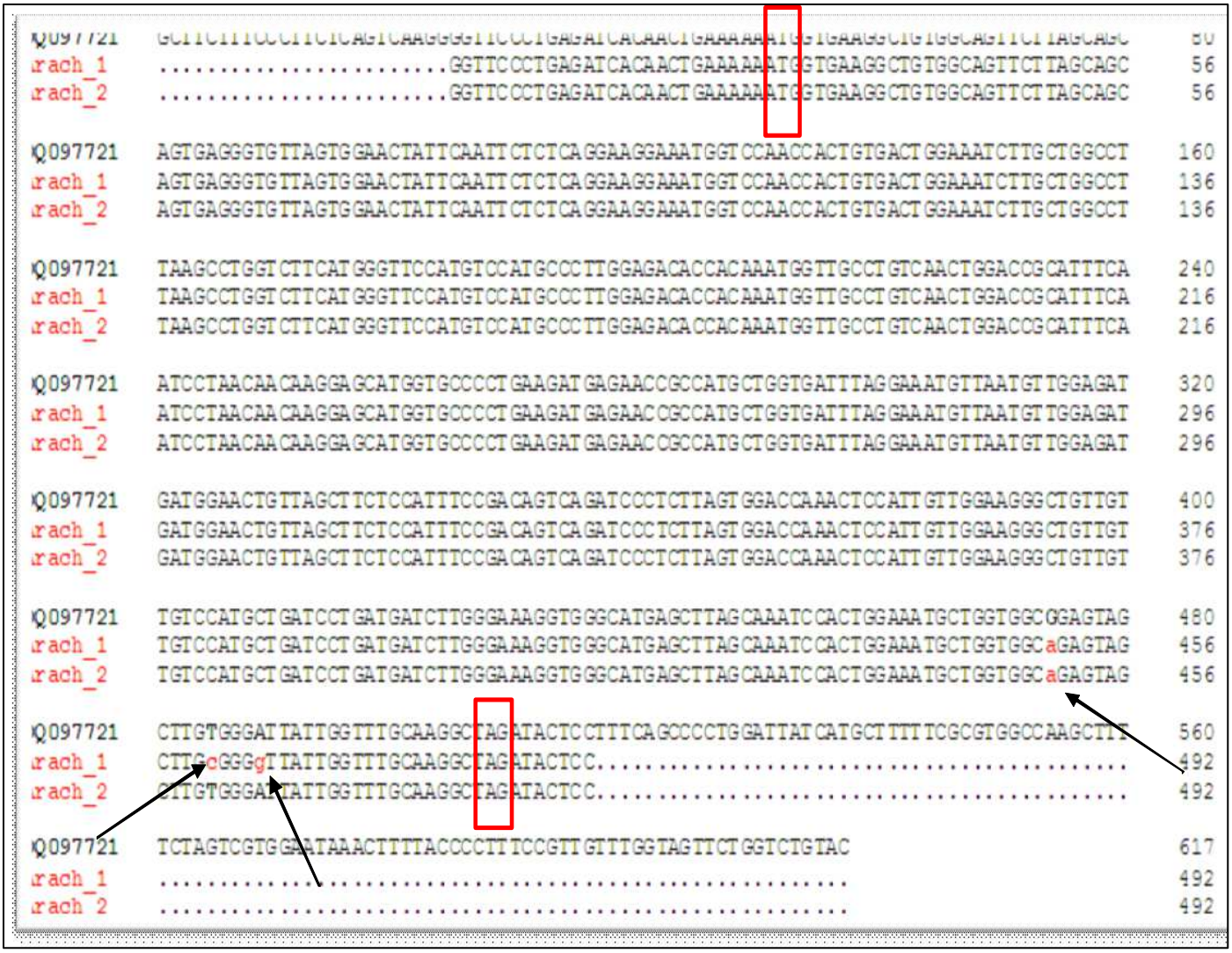


Figure 3 – Lower panel: gel electrophoregram showing quality control and equal loading of total RNA (3 µg for each lane) extracted from the leaves of W-25 plants belonging to four peanut landraces (for landraces acronyms, see the text). Upper panel: gel electrophoregram of cDNA fragments coding for superoxide dismutase obtained after the reverse-transcription of total RNAs (2 µL each) and their PCR-amplification (35 cycles) by using the F1-R1 primers pair shown in Fig. 5. M3, DNA marker.

Secondly, it should be emphasised that each of the cDNA amplification products shown in Fig. 3 represents the global expression of all the SOD genes present in the peanut genome, with no possible discrimination at the genomic, transcriptional and translational levels (see above). Cloning of individual SOD-coding sequences isolated by means of the RACE approach will be

helpful in deciphering the genomic architecture of SOD in Arachis, in revealing which components of its gene family are possibly involved in the response to water stress and in evaluating whether differences exist in SOD expression levels among the peanut landraces. In such context, and as an example of the work to be carried out, Fig. 4, upper panel, shows the alignment among a SOD-coding sequence obtained from data banks (Fig. 1) and the sequences obtained from two of the cloned SOD fragments from the landrace TO. As expected, a high overall homology between the three sequences was found, although differences emerged at the level of single bases. As single bases substitutions may lead to mere cases of triplet synonymy, or instead may give raise to translational changes into the aminoacidic sequence, the three nucleotidic sequences of Fig. 4 were translated in silico and then aligned again (Fig. 4, lower panel). This revealed that indeed single bases substitutions had consequences in terms of protein secondary structure, causing substitutions in aminoacids. It remains to be seen whether such changes may have in turn consequences in terms of tertiary structure and of catalytic properties of the SOD enzyme.



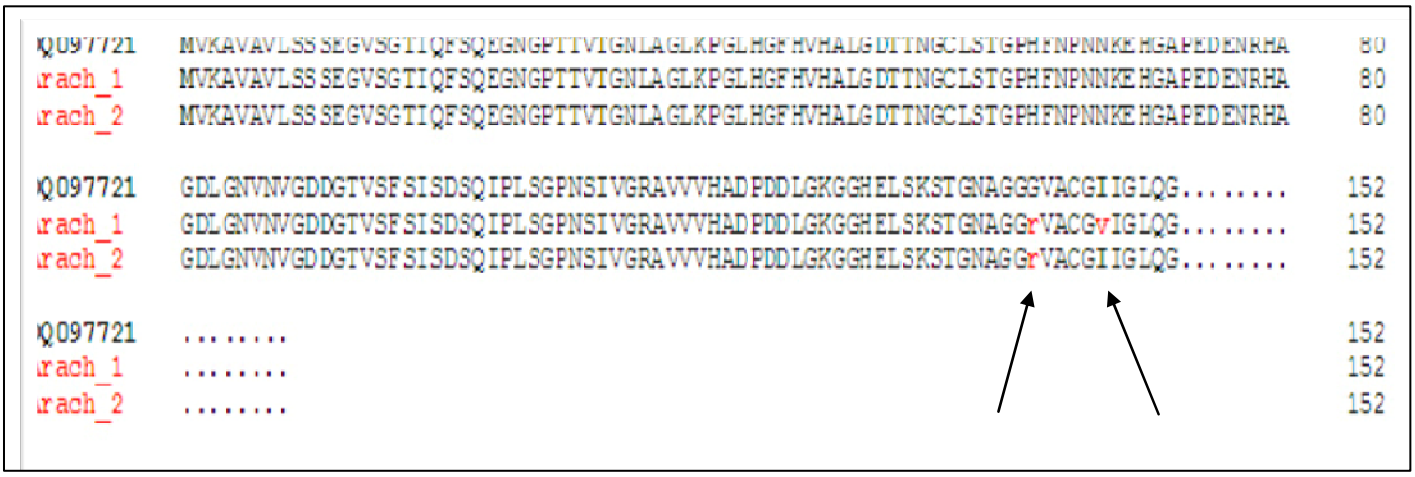


Figure 4 – Upper panel: aligning two cloned SOD-coding sequences isolated from the peanut landrace Tonga with a database peanut sequence; boxes denote start and stop translation codons. Arrows and red letters denote single base substitutions. Lower panel: alignment of the in silico translation products of the nucleotidic sequences above. Symbols as above .

4. CONCLUSION

The results pertaining to the effect of drought stress on SOD indicated how water stress regulates the synthesis and the activity of superoxide dismutase and how these enzymes contribute to protect against the damageable effects of superoxide radicals in peanut. Relative expression levels of SOD increased after 25 days of drough treatment in shoots of Tonga (TO) landrace. On the other hand, expression levels of SOD decreased on SEBSEB (SEB) landrace after 25 days of water stress in shoot tissues. Although in the presence of an equal sample loading, still the results reveal ample "constitutive" differences in SOD gene expression among the four peanut landraces. Indeed, SOD transcripts appeared to be most abundant in TO and least abundant in SEB. Further work will reveal whether contrasting SOD expression maybe associated to varying responsiveness to water stress in the peanut landraces at study. In conlusion, learning from biodiversity…

- With an equal loading of total RNA…
- Saturating amplification conditions (35 PCR cycles)….
-…But still strong differences in SOD transcripts abundance among WELL-WATERED peanut landraces….
- …So that interesting lessons expected from constitutive and stress-inducible antioxidant status in food legume landraces. Although it is nearly impossible to understand the whole antioxidant mechanism of plants under environmental stresses, this study was a step to learn about molecular background of some antioxidant enzymes. Gene expression profiles of CAT, chloroplast/mitochondrial GR and chloroplast/stromal APX will be the next step of this study. By this way, the comparison of gene expression profiles of different antioxidant enzymes with each other and also with enzyme activities will improve our knowledge of molecular protection mechanisms in peanut against drought stress.

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Chapitre V

Intérêt agronomique, socio-économique des légumineuses alimentaires, en Algérie.

Agricultural, socio-economic, and cultural relevance of crop wild relatives, in particular food legume landraces, in Northern Africa.

Au Maghreb, les légumineuses alimentaires sont essentiellement cultivées pour leurs rôles dans l'alimentation humaine et dans l'amélioration de la fertilité du sol. La production des légumineuses alimentaires dans les trois pays du Maghreb (Maroc, Algérie et Tunisie) demeure largement un secteur traditionnel où la majorité des producteurs sont de petits exploitants agricoles disposant d'une main d'œuvre familiale abondante. Mais l'augmentation de la demande intérieure et la stagnation des rendements font que la production ne satisfait plus les besins et le Maghreb est devenu un net importateur depuis 1979.

Les principales légumineuses alimentaires cultivées au Maghreb (Maroc, Algérie et Tunisie) sont la fève, le pois chiche, le pois sec et la lentille. Ces quatre espèces couvrent plus de 80% des superficies destinées aux légumineuses alimentaires. D'autres espèces d'importance mineure sont aussi cultivées (haricot, niébé, soja, arachide, lupin ...). La part qu'occupent les légumineuses alimentaires dans l'assolement est d'environ 3% en Algérie et en Tunisie et d'environ 6% au Maroc. La majorité de ces légumineuses sont conduites en agriculture pluviale, en rotation avec les céréales, particulièrement dans les zones Nord du Maghreb où la pluviométrie annuelle dépasse 350 mm. Dans ces régions, les légumineuses alimentaires occupent environ 25% des terres cultivables.

La production de légumineuses alimentaires au Maghreb demeure encore un secteur traditionnel où la majorité de producteurs sont de petits exploitants agricoles. Les techniques actuelles de production sont caractérisées par une forte utilisation de la main d'œuvre et de la traction animale même au niveau des exploitants ayant largement amélioré et mécanisé la production des céréales. Avec l'augmentation des coûts de la main d'œuvre, la stagnation des rendements à des niveaux inférieurs à 1 T/ha et la forte variabilité interannuelle des rendements, la production des légumineuses alimentaires est devenue de moins en moins rentable en comparaison avec les céréales cultures qui bénéficient de subventions de l'Etat.

L'opportunité d'augmenter les superficies emblavées en légumineuses alimentaires, particulièrement aux dépens de la jachère, demeure encore une option pour augmenter la production à court terme.

Dans le Maghreb, la jachère occupe encore entre 10 à 40 % des terres cultivables. En Turquie, l'augmentation des superficies de la lentille et du pois chiche, à travers une politique de remplacement de la jachère, a été une expérience réussie. L'adoption à grande échelle de la technologie du pois chiche d'hiver est un autre moyen d'étendre la culture des légumineuses alimentaires aux régions où le système de culture dominant est basé sur la rotation céréale-jachère. L'augmentation de la productivité par unité de surface est une autre voie sure pour augmenter la production. Cette option implique cependant un bond technologique important et des efforts conjugués et soutenus des chercheurs, des vulgarisateurs, des agriculteurs et des décideurs **(Bamoh, 1995)**.

En Algérie, la plupart des terres cultivées sont situées sous des climats arides ou semi-arides, où les contraintes climatiques contribuent à la fragilisation du milieu et constituent de ce fait une contrainte majeure à la mise en place et la conduite des cultures. Le choix des espèces et des techniques culturales doit impérativement tenir compte de cette sensibilité particulière et promouvoir les pratiques adaptées qui préservent l'intégrité des agrosystèmes et favorisent le développement durable des productions végétales. Le déficit hydrique, la salinité et la déficience en éléments minéreux des sols sont parmi les principaux facteurs limitants la production agricole en Algérie.

Pour contournée ces contraines, des solutions sont proposées à savoir:

1- La valorisation et l'exploitation des associations symbiotiques fixatrices d'azote dans les zones arides qui sont soumises encore plus aux changements climatiques;
2- Indiquation d'une approche intégrative associant un ensemble de disciplines (biologie, physiologie, écophysiologie, agronomie, sciences économiques et sociales) et participative avec les agriculteurs en zones de référence afin de cerner tous les facteurs qui seront mis en jeu ;
3- La proposition des rotations ou associations culturales qui exploitent au mieux les benefices ecologiques des légumineuses. Cette démarche obéit à quatre hypothèses: i) l'azote fixé par les légumineuses contribue à enrichir le sol en azote ; ii) il augmente la nutrition azotée et les rendements des cultures succédant aux légumineuses ; iii) il réduit l'utilisation de l'engrais azoté par les cultures succédant aux légumineuses ; iv) les

légumineuses améliorent à long terme les propriétés physico-chimiques et biologiques des sols ;

4- L'intensification écologique des agro-systèmes offre plus de garanties pour preserver l'environnement et contribuer à la durabilité des agrosystèmes. L'introduction des légumineuses fixatrices d'azote dans les systèmes de cultures céréalieres peut dans une certaine mesure améliorer durablement la productivité de ce système tout en réduisant l'utilisation des engrais azotés et phosphatés responsables des pollutions du milieu.

Donc, il convient en premier lieu de choisir les espèces ou les variétés cultivées qui s'adaptent le mieux aux conditions écologiques locales. Ce choix se base sur l'exploitation des ressources génétiques disponible à partir d'une collection variétale représentative qui constitue dés lors un préalable indispensable à la sélection variétale.Il s'agira ensuite de séléctionner les especes et les varietés qui s'adaptent au mieux aux conditions climatiques et edaphiques propres à chaque région.

Dans ce chapitre, nous avons jugé utile et intéressant de montrer le rôle important que présentent les deux espèces de légumineuses alimentaires à savoir l'arachide (*Arachis hypogaea* L.) et le niébé (*Vigna unguiculata subsp. unguiculata* (L.) *Walp*.) dans le développement agricole durable, en particulier dans les pays du Maghreb et les régions sub-sahariennes, où la sécheresse et la salinité limitent souvent leur production.

Les projets en cours en Algérie ont pour objectifs de définir une stratégie dynamique de conservation et une exploitation raisonnée de la diversité génétique à travers les populations locales de légumineuses alimentaires collectées à l'échelle nationale. Les principaux résultats présentés concernent la prospection, la collecte et la conservation des populations locales et des écotypes de légumineuses alimentaires, fèves, pois chiches, niébé, arachide et les souches rhizobiennes qui leur sont associées. Le comportement de l'association plante-rhizobium sous stress abiotiques tels que le stress hydrique et le stress salin est abordé dans ce chapitre.

Malgré les efforts des chercheurs, l'arachide et le niébé restent très peu étudiés en Algérie. La conservation et la valorisation de ces deux espèces devient donc urgente. L'identification des sites de culture traditionnelle de cette espèce suivie par des missions de prospections et de collectes semblent indispensables pour l'établissement de collections des populations d'arachide

et de niébé cultivées en Algérie, ainsi que les savoirs faires populaires qui leur sont associés , avant leur complète disparition. La préservation de ce patrimoine local nécessite aussi une connaissance approfondie de sa diversité et de ses caractéristiques. L'évaluation de ces collections permettra l'établissement d'une base de données et l'identification de populations présentant un intérêt certain pour le développement direct de ces espèces et leur utilisation future dans des programmes de création variétale.

L'ensemble des résultats obtenus fait l'objet de la rédaction d'un chapitre intitulé: « Agricultural, socio-economic, and cultural relevance of crop wild relatives, in particular food legume landraces, in Northern Africa » publié dans le livre « Water Stress and Crop Plants: A Sustainable Approach, 2 » qui est accepté en 2015 par l'édition John Wiley & Sons Ltd.

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Agricultural, socio-economic, and cultural relevance of crop wild relatives, in particular food legume landraces, in Northern Africa.

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Agricultural, socio-economic, and cultural relevance of crop wild relatives, in particular food legume landraces, in Northern Africa.

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Keywords

Food legumes; cowpea; *Vigna unguiculata subsp. unguiculata* (L.) *Walp*.; peanut; *Arachis hypogaea* L.; drought; salinity; landraces; sustainable agriculture.

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Abstract

Food legumes, such as cowpea [*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.], and peanut (*Arachis hypogaea* L.) are important crops in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, and Central and South America, and in some temperate areas, including the Mediterranean region and the southern states of the USA. Cowpea and peanut seeds possess high nutritive value. The plants are well adapted to grow under high temperature, drought and low soil fertility owing to their nitrogen fixation ability and their potential to form effective symbiotic association. Therefore, cowpea and peanut can play an important role in sustainable agricultural development, particularly in the Maghreb and sub-Saharan regions, where drought and salinity frequently limit crop production. Here, ongoing projects launched in recent years are described, whose aims are to define a dynamic conservation strategy and a reasoned exploitation of the genetic diversity embedded in food legume landraces collected across the Algerian territory. The main results are presented concerning the prospection, collection and conservation of local populations and ecotypes of food legumes, broad bean, chickpea, cowpea and peanut - and rhizobacterial strains associated with them, the behaviour of plant-rhizobia systems in front of water and salt stress, and the development of screening test to identify drought/salinity tolerant associations.

Keywords

Food legumes; cowpea; *Vigna unguiculata* subsp. *unguiculata* (L.) Walp.; peanut; *Arachis hypogaea* L.; drought; salinity; landraces; sustainable agriculture.

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1. INTRODUCTION

Legumes, apart from serving as food, offer a variety of other uses including their ability to harbour nitrogen fixing bacteria and serve as green manure crops to improve soil fertility and soil organic matter content.

Nitrogen is an essential element for plant growth and development and a major constraint for productive agriculture. Most studies indicate that nitrogen fertilizers contribute to resolving the challenge the world is facing, i.e. feeding the human population. The Green Revolution was accompanied by an enormous increase in the application of nitrogen fertilizer. Nitrogen fixation into living organisms across the world is estimated at 250 million Mg per year, of which 60% comes from BNF, 10% from the atmospheric events and the remaining 30% enters the biota in the form of chemical fertilizers.

Biological Nitrogen Fixation (BNF) is regarded as main factor for sustainable agriculture and indispensable for maintaining soil fertility. Research on microorganisms and plants able to fix nitrogen contribute largely to the production of bio-fertilizers. Thus, it is important to ensure that BNF research and development will take into account the needs of farmers in the developing countries mainly.

On agronomic plan, the N_2-fixing symbiosis legume-rhizobia are a major challenge for agriculture **(*Commissariat Général au Développement Durable*, CGDD, Point No. 40, January 2010)**. The CGDD believes that the increasing legume cultivation from 4% to 7% of the arable land in France would save about 10% of mineral fertilizers and reduce greenhouse gases emissions by about 1.8 Tg CO_2 eq. **(CGDD, Studies and Documents No. 15, December 2009)** The nitrogen-fixing symbiotic associations "legume-rhizobia" are major objective for sustainable agriculture, paving way towards the decreased application of synthetic nitrogen fertilizer inputs and consequently resulting in a reduction of environmental pollution and greenhouse gas emission associated with them. This symbiosis is especially important as it concurs to all development strategies and leads to sustainable environment. In legumes, the nitrogen reduction occurs in nodules, root organs where rhizobia, nitrogen-fixing bacteria live. These bacteria live in symbiosis with legumes and are able to reduce N_2 to NH_3 by an enzyme called nitrogenase. As nitrogen fixation leads to a decrease in soil pH, the resulting acidification increases phosphorus availability **(Oldroyd *et al.*, 2005)**. Nitrogen fixation requires ample energy surplus and is found susceptible to external environmental factors.

The diverse kinds of stress like drought, salinity and mineral deficiencies affect its mechanism at the level of nodules in the host plant.

In Algeria, the most cultivated land are located in arid and semi-arid areas where climatic constraints contribute to the fragility of the environment and thus constitute a major constraint to the development and conduct of cultures. The choice of species and cultivation techniques must take into account the particular sensitivity and promote appropriate practices that maintain the integrity of agro ecosystems and promote sustainable crop production. The answer to this requirement can be provided by the development and operation of the nitrogen-fixing symbiotic associations in these arid areas, which are still subjected to climate change.

From year to year, reports of the Intergovernmental Panel on Climate Change **(IPCC)**, in fact, forecast additional increases of drought and flood episodes, which could disrupt production systems **(www.ipcc.ch / home_langage_main_french.shtml)**. The Institutes of the Consultative Group on International Agricultural Research **(CGIAR)** are currently working on methods to make crops more resistant to changes in their environment. **(http://www.scidev.net)**.

Algeria is characterized by enormous contrasts, both in geographical and ecological farming practices. Water deficits, salinity, deficiency of mineral elements in soils, are among the main factors limiting agricultural production. Circumvention of these factors ensures ecological intensification of agricultural systems and offers more promise to preserve the environment and contribute to sustainable agro-system. The introduction of nitrogen fixing legumes in cereal cropping systems can to some extent improve the sustainable productivity of the system while reducing the use of nitrogen and phosphate fertilizers responsible for pollution of the environment.

As a general strategy, it is first necessary to select species or varieties that are best adapted to local ecological conditions. This choice is based on the utilization of genetic resources available from a representative varietal collection which is indispensable for varietal selection. This could assist in the selection of species and varieties that better adapt to the edaphic and climatic conditions of each region. For this, an integrative approach an integrative approach combining a range of disciplines (biology, physiology, eco-physiology, agronomy, economics and social sciences), along with active participation of farmers in reference areas, could facilitate the identification of all the factors that will be involved. The aim is to provide cultural rotations or associations that maximize the ecological profits of legumes. This approach is based on four assumptions: i) the nitrogen fixed by legumes helps to enrich the soil with nitrogen, ii) it

increases the nitrogen nutrition and crop yields of the cultures which succeed the legumes iii) it reduces the use of nitrogen fertilizer by crops succeeding the legume; iv) legumes acts a long-term improvement of physico-chemical and biological properties of soils.

Legume crop faces several constraints including limited soil availability of phosphorus. Such an intricacy is complicated further when legumes perform symbiotic fixation of nitrogen **(Hernandez *et al*., 2007)**. Phosphorus content of the nodules is also much higher than in other plant organs **(Vadez, 1996)**. Symbiotic fixation of nitrogen, which is usually accompanied by acidification of the rhizosphere, normally results in a significant increase in the bioavailability of phosphorus **(Behi, 2003)**. This effect is explained by the major role played by nitrogen in the balance of cations and anions taken up by the plant and, ultimately, in changing the pH of the rhizosphere following extrusion of protons to compensate for the excess of cations in the balance sheet **(Hisinger *et al*., 2003)**. By using N_2 instead of NO_3^-, legumes can acidify their rhizosphere further, resulting in an increase in the bioavailability of P **(Alkama *et al*., 2009)**. Indeed there is a close link between phosphate nutrition and nitrogen fixation in the energy-intensive metabolism of the nodules.

2. BIOLOGICAL NITROGEN FIXATION AND ITS IMPORTANCE FOR SUSTAINABLE AGRICULTURE

2.1 Biological nitrogen fixation

Nitrogen was discovered by **Daniel Rutherford in 1772**. It was found to be so inert that Antoine Lavoisier named it "*azote*", meaning "without life". Dinitrogen (N_2) has a triple bond and does not readily accept or donate electrons. As a gas or liquid, nitrogen is colourless and odourless. Two allotropic forms of solid nitrogen exist, with the transition from the α to the β form taking place at −237 ◦C. Similarly to many other elements, the N cycle can be summarized as a transfer among its inorganic forms. Certain microorganisms have the ability to use a renewable source of energy to fix atmospheric nitrogen (constituting 78% of air) under mild conditions, such as normal temperature and pressure. Nitrogen fixation is a key process in which molecular nitrogen is reduced to form ammonia, which is then used by autotrophic organisms for the synthesis of many bioorganic compounds. Biologically-fixed nitrogen could be directly "absorbed" by plants and keep the environment almost "untouched". Crop rotation with legumes has been recognized to increase soil fertility and agricultural productivity since ancient China and Rome.

However, the science behind such practice was not revealed until Boussingault experimented with N_2-fixing leguminous crops in **1838; Hellriegel and Wilfarth** brought conclusive evidence for N_2 fixation by microbes in legumes in 1886. Annually, approximately 250 Tg NH_3 are fixed from the atmosphere by BNF (by legumes and cyanobacteria) and approximately 80 Tg NH_3 are manufactured by ammonia industry. All plants need relatively large amounts of N for proper growth and development. Only a selected group of plants is able to obtain N this way, with the help of soil microorganisms. Among forage plants, the group of plants known as legumes (plants in the botanical family Fabaceae) are well known for being able to obtain N from atmospheric N_2.

In agricultural settings, perhaps 79% of this biologically fixed N_2 comes from symbioses involving leguminous plants and bacteria of the family Rhizobiaceae. The family Rhizobiaceae currently includes six genera: *Rhizobium*, *Ensifer* (*Sinorhizobium*), *Mesorhizobium*, *Allorhizobium*, *Azorhizobium*, and *Bradyrhizobium*, which are collectively referred to as rhizobia **(Van Berkum et Eardly 1998)**. In recent years, however other α-proteobacteria have been shown to produce nodules in legumes **(Moullin *et al*., 2002)**: *Methylobacterium* **(Sy *et al*., 2001)**; *Blastobacter* **(Van Berkun et Eardly, 2002)**, and *Devosia* **(Rivas *et al*., 2002)** as well as β- proteobacteria such as *Ralstonia* **(Chen *et al*., 2001)** and *Burkholderia* **(Chen *et al*., 2003)**. Nodulation is a highly host-specific interaction in which, with few exceptions, specific rhizobacterial strains infect a limited range of plant hosts. Plants secrete flavonoids that are recognized by the compatible bacteria, resulting in the induction of nodulation genes. These nodulation genes encode enzymes that synthesize a specific lipochitin nodulation signal (Nod signal), which activates many of the early events in the root hair infection process **(Oldroyd *et al*., 2005)**.

Nitrogen fixation by legumes can also maintain soil fertility and can be of benefit to the following crop. Legumes have considerable potential in crop rotations in sustainable agricultural systems in maintaining soil fertility and thus reducing the need for nitrogen fertilizer. Reduced use of nitrogen fertilizer can decrease nitrate leaching from soils and so can reduce eutrophication, which is a major environmental problem worldwide **(Date, 1996)**. The agronomic implications of this symbiosis have promoted research on biological nitrogen fixation and on the characterization of rhizobia **(Fernández-Pascual *et al*. 2007)** as well as success in constructing better inoculants. For inoculantion, strains need to be improved in order to compete successfully with indigenous strains for root nodulation of legumes. Several loci have been

identified to date that affect competitiveness for strains nodule habitation. Usually, mutations in these loci affect the ability of a strain to form nodules rapidly and efficiently. Other loci, such as those that confer antibiotic production, can be added to strains to enhance nodulation competitiveness when co-inoculated with antibiotic-sensitive strains. Second, the inoculum strains must be improved with respect to symbiotic nitrogen fixation **(Maier et Triplett 1996)**. Several environmental conditions are limiting factors to the growth and activity of the N_2-fixing plants. A principle of limiting factors states that "the level of crop production cannot be higher

than that allowed by the maximum limiting factor" **(Brockwell *et al*., 1995)**. In the *Rhizobium*-legume symbiosis, the process of N_2 fixation is strongly related to the physiological state of the host plant **(Haldar et Sengupta, 2015)**. Therefore, a competitive and persistent rhizobacterial strain is not expected to express its full capacity for N_2 fixation if limiting factors (e.g., salinity, unfavourable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigor of the host legume **(Brockwell *et al*., 1995; Thies *et al*., 1995)**.

2.2 The issue of sustainability in agriculture

Sustainability is defined as `the successful management of resources to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving resources' **(TAC-CGIAR, 1988)**. Economists measure sustainability as the ratio of output to input, taking into account stock depletion. Stocks in agriculture include soil, water, non-renewable energy resources, and environmental quality. Modern agriculture is based on maximum output in the short term, with inadequate concern for input efficiency or stock maintenance. Nitrogen fertilizer ranks first among the external inputs to maximize output in agriculture. Input efficiency of N fertilizer is one of the lowest among the plant nutrients and, in turn, contributes substantially to environmental pollution. The continued and unabated use of N fertilizers would further accelerate depletion of stocks of non-renewable energy resources used in fertilizer production. The removal of large quantities of crop produce from the land additionally depletes soil of its native N reserves. On the other hand, nitrogen input through BNF can help maintain soil N reserves as well as substitute for N fertilizer to attain large crop yields **(Peoples et Craswell, 1992)**.

2.3 Production sustainability and environmental quality

The external costs of environmental degradation and human health far exceed economic concerns. Nitrate in groundwater is a major health concern in the corn belt of the U.S., and other intensively cultivated areas. Nitrogen in run- off and surface waters has led to extensive pollution and eutrophication of rivers and lakes. The gaseous oxides of nitrogen, derived from N fertilizers, are highly reactive and pose a threat to the stability of the ozone layer.

Long-term sustainability of agricultural systems must rely, as much as possible, on use and effective management of internal resources. Nitrogen-fixing plants offer an economically attractive and ecologically sound mean for reducing external inputs and improving the quality and quantity of internal resources. Biological N fixation can be a major source of N in agriculture when symbiotic N_2-fixing systems are used; the amount of N input is reported to be as high as 360 kg N ha^{-1}. On the other hand, the nitrogen contributions from no symbiotic (associative and free-living) microorganisms are relatively minor, thus requiring fertilizer N supplementation. Among symbiotic N_2 -fixing systems, nodulated legumes have been used in cropping systems for centuries. They can serve multitude of purposes in sustainable agriculture.

They are used as primary sources of food, fuel, fiber and fertilizer, or, secondarily, to enrich the soil, preserve moisture and prevent soil erosion. They can also be used for windbreak, ground cover, trellis, hedgerow and shade, or as a source of resins, gums, dyes and oils. Some of the most ornamental flowering plants in the tropics are legumes. Some of the nodulated non-legumes, notably species of *Casuarina,* are hardy nitrogen-fixing plants, which produce high-quality fuel wood in marginal lands, and have also been used for stabilizing sand dunes and eroding hillsides, as well as for reclaiming marshlands affected by fluctuating brackish/fresh water. The trees are useful for shade, windbreaks and hedges.

In the humid uplands, where Ultisols and Oxisols predominate on all three tropical continents, severe acidity and P deficiency are characteristic problems **(Sanchez, 1980; Li *et al*., 2015)**. Unless they can be alleviated through soil amelioration with external nutrient sources, production of conventional grain legumes is not favoured. The contribution of BNF on these soils is also hampered by the fact that nitrogen is often not the primary limiting nutrient.

The approach of developing unconventional BNF systems for the domain of strongly acidic soils is now receiving remarkable attention **(Arunachalam et Kannan, 2013)**. For sustaining intensive production, particular emphasis is directed to BNF in alley-cropping systems using leguminous trees or forages **(Kang, 1990)**. For fallow-rotation systems, which occupy vast areas,

managed fallows of leguminous cover crops are being developed. Until recently, little research attention has been devoted to these practices. In the wetland rice production systems of the humid tropics, both leguminous green manures and grain legumes have traditionally played a critically important BNF role **(King, 1911)**. The contribution of both declined in recent decades, as fertilizer-N use became widespread.

Research is developing new species to better exploit the available cropping-system niches. One striking example is the introduction of stem nodulating green manure species **(Ladha *et al*., 1992)** that are better adapted to waterlogged conditions. However, the typical agronomic constraints common to all green manures remain as actual obstructions to implementation **(Garrity, 1990)**. Although *Azolla* is a potential BNF system for wetlands, the technology suffers from many farm-level constraints which limit any widespread use by farmers. Major constraints are: difficulty in maintaining and distributing inocula round the year and susceptibility to insects and diseases. In the semi-arid tropics legumes occupy a more important role than in the humid tropics **(Willey, 1979)**. They are commonly intercropped with cereals **(Fujita *et al*., 1992)**: pigeon peas with sorghum in India, cowpeas with sorghum or maize in West Africa. The superiority of legume cereal intercropping is well established for these long-duration annual crop associations. However, the long-term viability of these systems is still uncertain, unless they can be more effectively subjected to sustained improvements in agronomic efficiency.

In the temperate zone, legume oilseeds, particularly soybeans **(Keyser et Li, 1992)** and forages **(Ledgard et Steele, 1992)** are of major importance. Intensive agronomic research has assured their products as valuable commodities. However, their indirect role as BNF suppliers to subsequent crops in rotations, although readily perceived by farmers, is reduced in practice. This occurs because of the lack of dependable quantitative measures of the actual N contribution, season by season, at the field level. Consequently, farmers usually ignore the contribution of the prior legume N when they calculate N fertilizer applications to the subsequent cereal. Unless better tools to estimate legume N contributions are established, legumes may be found to inadvertently accelerate nitrate leaching into the groundwater, thus creating negative environmental consequences.

2.4 Socio-cultural constraints

It is important to emphasize that the constraints to fuller adoption of BNF technologies are not exclusively scientific but include cultural, educational, economic and political factors. A successful BNF-based program should be inculcated in addition to scientific research, efforts in

training, education, outreach and technical assistance. Evaluations of socio–economical constraints are needed to publicize the benefits of BNF technology and provide caution about potential difficulties, facilitating their removal or circumvention. Most farmers in developing countries do not know that legumes fix nitrogen in their root nodules. Yet traditional and modern farming systems of the tropics almost invariably include legumes.

The legume cultivation results from recognition by farmers over many centuries that legumes are valuable components in farming systems, rather than from intentional exploitation of biological nitrogen fixation per se. The task of training and educating persons to deliver BNF information and material has also been made difficult by the critical shortage of specialists with knowledge and interest in practical and applied aspects of the technology. The mass evacuation of microbiologists and plant biologists from applied BNF to molecular biology and genetics has created a serious void in programs addressing BNF needs of sustainable agriculture systems in developing countries.

National and international subsidies for nitrogen fertilizers, in both developed and developing countries have been a disincentive for farmers to use BNF products. However, as national debt rises and subsidies are phased out, BNF becomes more attractive and acceptable. Donor agencies should exercise more to educate and train in-country personnel to be able to respond to increasing demands for BNF, as sustainability and environmental issues demand biological alternatives to the use of industrial fertilizers. Crop subsidies and support programs are, in some countries, limited to cereal crops. The United States is a major example. This situation distorts the entire agricultural system. It provides a de-facto incentive to abandon legume-cereal crop rotations in favour of cereal mono-cropping. In countries where virtually no differential crop support is practiced, for example in Australia, exploitation of cereal-legume systems is a dominant feature of agriculture.

3. RELEVANCE OF FOOD LEGUMES IN NORTHERN AFRICA

Food legume crops play an increasing role in farming systems for North Africa, with the emphasis on development of sustainable agricultural production. This requires an increased use of legumes in cereal farming systems because: (a) replacement of a fallow phase in rotation by legumes reduces the hazard of soil erosion by providing protective ground cover and indispensable farm income during critical periods. A cereal crop in a legume rotation develops a deeper root system than with continuous cereals because of better soil physical conditions and is

thus better able to exploit soil water and nutrients within the soil profile **(Jongrungklang *et al*., 2012; Lynch et Brown, 2012)**; (b) the total influx of N into the system is higher with a legume incorporated in the rotation as compared to cereal-fallow rotation. Considerable soil nitrogen savings are achieved compared to continuous cereal; and (c) rotation with legumes will reduce the build-up of noxious weeds, insects, and pathogens of cereals. This increased emphasis on sustainable agricultural production systems will create an expanding demand on genetic resources of existing legume species; and generate a requirement for additional legume species to be brought into agricultural production **(Acikgoz, 1988; Browning, 1962; Durutan *et al*., 1988; Stinner *et al*., 1992)**.The important food legume crops of the North Africa region are faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum L*.), and lentil (*Lens culinaris* Medik.). The majority of the production of faba bean is in Egypt and Morocco. The International Center for Agricultural Research in the Dry Areas (ICARDA) was authorized to maintain germplasm collections for lentil, faba bean, vetch (*Vicia spp*.) and chickling vetch (*Lathyrus spp*.). Furthermore, ICARDA allocates the authorization for maintaining a germplasm collection of chickpea with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),

India, and ICARDA, maintaining a large collection of kabuli chickpea germplasm. For lentil and chickpea, collections are also maintained of the wild relatives and progenitor species. For crop improvement, ICARDA has a global authority for lentil, a regional authorization for forage legumes, and an allocated authorizer with ICRISAT for improvement of kabuli chickpea. Abiotic stresses are a major constraint to the production of food legumes in North Africa. These crops are subjected to extremes of temperature, both cold and heat, and moisture supply and to deficiency or toxicity of mineral nutrients in the soil **(Saxena, 1993)**. The other major set of constraints to production of these species are biotic stresses, including those caused by parasitic *Orobanche* spp. weeds, ascochyta blight, botrytis for faba bean, wilts for lentil and chickpea, and nematodes **(Singh et Saxena, 1993)**. Constraints to productivity also include agronomic problems of pod shedding, pod shattering, lodging, and spreading growth habit with lentil and the inability to set seed without bee pollination in the case of faba bean. Other constraints to the use of these crops include nutritional concerns for protein content.

4. FOOD LEGUMES OF THE ALGERIAN MAGHREB

In order of importance, food legumes grown in the Algerian Maghreb are mainly faba bean and chickpea, representing respectively 51.68% and 33.42% of the total. The rest is divided in

order of importance by the peas, lentils and beans. Aside from the chickpea, which is grown for its dry grain, the other legumes are widely used. Food legumes are very little cultivated in Algeria, despite their agronomic benefit. They represent only 1% of the total arable land and 2% of the cultivated area for cereals. Depending on the region, they are either integrated into the culture grain, or they are grown in kitchen garden for daily food requirements and for the sale in the local market. They are an important source of protein, as an alternative to animal protein, and thus occupy a place in significant food for a large segment of the Algerian population, whose diet is based exclusively on cereals. In the agronomic plan, they share the ground with fallow, as they help to improve the structure and fertility of soils. Despite its advantages, domestic production covers annually from 20 to 40% of needs, depending on the years, so that Algeria relies on imports to compensate for the massive deficit, whose bill exceeds an annual average of 100 million dollars. Therefore, the recovery of legume crops and food is required to be undertaken to improve the performance of these cultures and respond to the socio economic food security **(Abraham *et al.*, 2014)**.

Significant variations characterize the area of food legumes in Algeria, which passed from 77,510 ha in 1998 to 261,490 ha in 2000 and decreased until 62,166 ha in 2002. Since then, a slight inflation reached 87,286 ha in 2011. However, the western region is characterized by a relatively stable area from 43,500 ha in 1998 to 49,580 ha in 2011 **(INRAA, 2012)**. For the west, it seems that the trend is less pronounced and acreage has remained fairly stable ranging from 43,540 ha in 1998 to 38,177 ha in 2008. Since then, it followed the same trend as the national production, with a slow but steady increase to reach as 49,580 ha in 2011. This stability was explained by anchoring important legume crop in the traditions of the western region of the country. However, it should be noted that the annual change in the same area to the west is a function of several factors, the most significant being rainfall and the availability of seeds.

Changes in domestic production are saw tooth-shaped, and varies from 450,000 Mg in 1998, to fall by half (218,000 Mg in 2000) and back to 788,710 Mg in 2011. The Western Region follows the trend, with nearly 190,000 Mg produced in 1998, cut down by half in 2000 and rising again to 398,344 Mg in 2011. This is also illustrated by the observed variation in yield over the past 13 years, both nationally and across the western region of the Country **(INRAA, 2012)**.

Yields in the West for all species by district ("*wilaya*") vary from 0.2 to 8 Mg ha^{-1}. The *wilayates* were the highest yields are recorded are: Chlef, Sidi Belabbes and Relizane; lowest yields are recorded in Saida, Tissemssilt and Tiaret. **(INRAA,2012)**. Yields vary from 0.7 Mg

ha^{-1} for Sidi Bel Abbes to only 0.15 Mg ha^{-1} for the province of Tiaret. The average yield of the species is 0.44 Mg ha^{-1} for the region and 0.56 Mg ha^{-1} for the Country. Pea is the cultivable crop most widely practiced in the Western areas. The most productive areas are Tlemecen, Ain Temouchent, Mostaganem and Mascara. The performance achieved for each *wilaya* for peas varies from 0.59 to 0.13 Mg ha^{-1}. The best yields are obtained in Chlef and Mascara, with 0.59 and 0.55 Mg ha-1, respectively.

5. PROSPECTION, COLLECTION AND TAXONOMY OF FOOD LEGUMES BIODIVERSITY (PLANT POPULATION AND RHIZOBACTERIAL STRAINS) IN NORTHERN AFRICA

5.1 Aims and criteria

Food legumes, such as cowpea [*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.], and peanut (*Arachis hypogaea* L) are important crops in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, and Central and South America **(Singh *et al*., 1997)**, and in some temperate areas, including the Mediterranean region and the southern states of the USA **(Pasquet, 2000)**. Cowpea and peanut seeds possess high nutritive value **(Ehlers et Hall, 1997; Marques *et al*., 2015))**. The plants are well adapted to grow under high temperature and drought **(Hall et Patel, 1985)** and tolerate low soil fertility due to their high rate of BNF **(Eloward et Hall, 1987)** and ability to form effective symbiotic mycorrhizae **(Kwapata et Hall, 1985; Ilyas et Sopian, 2013; Hameed *et al*., 2014)**. Therefore, cowpea and peanut can play an important role in agricultural development, particularly in the Maghreb and sub-Saharan regions, where drought and salinity frequently limit crop production.

In Afro-Mediterranean Countries, despite of the economic and cultural importance of the above legumes, which are consumed not only as a source of food, but often used for their therapeutic properties, both plant material and cultivation practices are neither uniform nor standardized. Instead, their cultivation is often based on landraces, each of which occupies relatively few hectares. Traditional farmers in many areas, whether by choice or by need, conserve and develop local phytogenetic resources by preserving landraces and associated local knowledge. Although not desirable in terms of yield and yield stability over time, and hence of food availability and farmers' income, such a richness in biodiversity, arisen long since under the constant adaptive pressure of a challenging environment, can be regarded as a formidable genetic reservoir for the isolation and multidisciplinary characterization of useful traits to improve, both on a local basis and on a wider scale, e.g. Mediterranean, drought and salt stress tolerance in the

present era of climate changes **(Bruning et Rozema, 2013; Kudapa *et al*., 2013; Lucas *et al*., 2013; Singh *et al*., 2014)**.

An ongoing project launched in recent years by the Algerian *Ecole Nationale Supérieure Agronomique* (ENSA), "*Amélioration des légumineuses alimentaires pour la tolérance au stress hydrique*", aims at defining a dynamic conservation strategy and a reasoned exploitation of the genetic diversity embedded in food legume landraces collected across the Algerian territory **(Ghalmi *et al*., 2010)**.

ENSA Project activities are:

►Prospection, collection and conservation of local populations and ecotypes of food legumes broad bean, chickpea, cowpea and peanut - and rhizobacterial strains associated with them;

►Studying the behaviour of plant-rhizobia systems in front of water stress, under controlled conditions;

► Developing a screening test and identifying drought tolerant associations **(Pungulani *et al*., 2012)**;

► cultivating the plant-rhizobia selections in a simplified production system (legume-wheat) in the reference area.

5.2 Collecting seeds of local ecotypes of cowpea cultivated in Algeria

In Algeria, ecotypes of cowpea were maintained in remote areas (mountains of Kabylia, oasis of the Sahara, the coast of El Kala). This species is used in traditional cooking in some regions, where it is sometimes credited with therapeutic properties. It is cultivated on small scales, often under gardening which can cause long-term extinction. Local ecotypes of cowpea, probably introduced from the East Africa (the centre of origin of this species), were maintained for several centuries in spatially diversified ecological conditions, which allowed them to accumulate a large genetic diversity still poorly studied. Despite the efforts of researchers, such a resource remains scarcely studied in Algeria and is still under-exploited by local populations. Conservation and recovery of this species has become urgent.

The present study is a modest contribution to previous work **(e.g. Grönemeyer *et al*., 2014; Pandey *et al*., 2014)** by expanding the prospection in cowpea cultivation zones not yet explored

i.e. Northern Algeria (Kabylia, El Kala) and Oasis of the South, by collecting a maximum of existing variability for building a collection of ecotypes grown in Algeria. From the information gained during preliminary investigations, established with agricultural authorities in different regions, the sites of cowpea cultivation in Algeria have been identified, and several sites of traditional culture of this species have been located throughout the Country (Figure 1).

To collect the existing variability, several exploratory missions and collections took place throughout the above regions, characterized by a high agro-ecological diversity. A total of 67 sites were prospected and 122 cowpea accessions collected. Collecting missions were programmed on August and October 2003 in El Kala region in October 2003 in the region of Touat, in April 2004 in the region of Gourara and the valley of M'zab, in June 2004 in the region of Bejaia, in September 2004 the region of Tizi-Ouzou, in February 2005 in the region of Bechar, in April 2005 in the region of Djanet and in March 2006 in the region of Tidikelt.

Farms, whatever their size, usually have a vegetable garden and an attic or storage area of the seeds. Seed lots were collected from the garden or the attic by assigning a record of information for each batch. Samples were taken wherever the presence of local ecotypes of cowpea was indicated, avoiding the border zones. The sample included 40 plants per ecotype. In storage bags, the choice of 40 cloves or 400 seeds per sample was made at random. Each sample was followed by an interview with the farmer who supplied the samples. Information on the names of the farmer, the variety, the location of the village, the origin of the seeds, growing techniques, pest attacks and usual practices related to cowpea were collected for each sample. Collected seeds were dried and stored in a cold room (4 ° C).

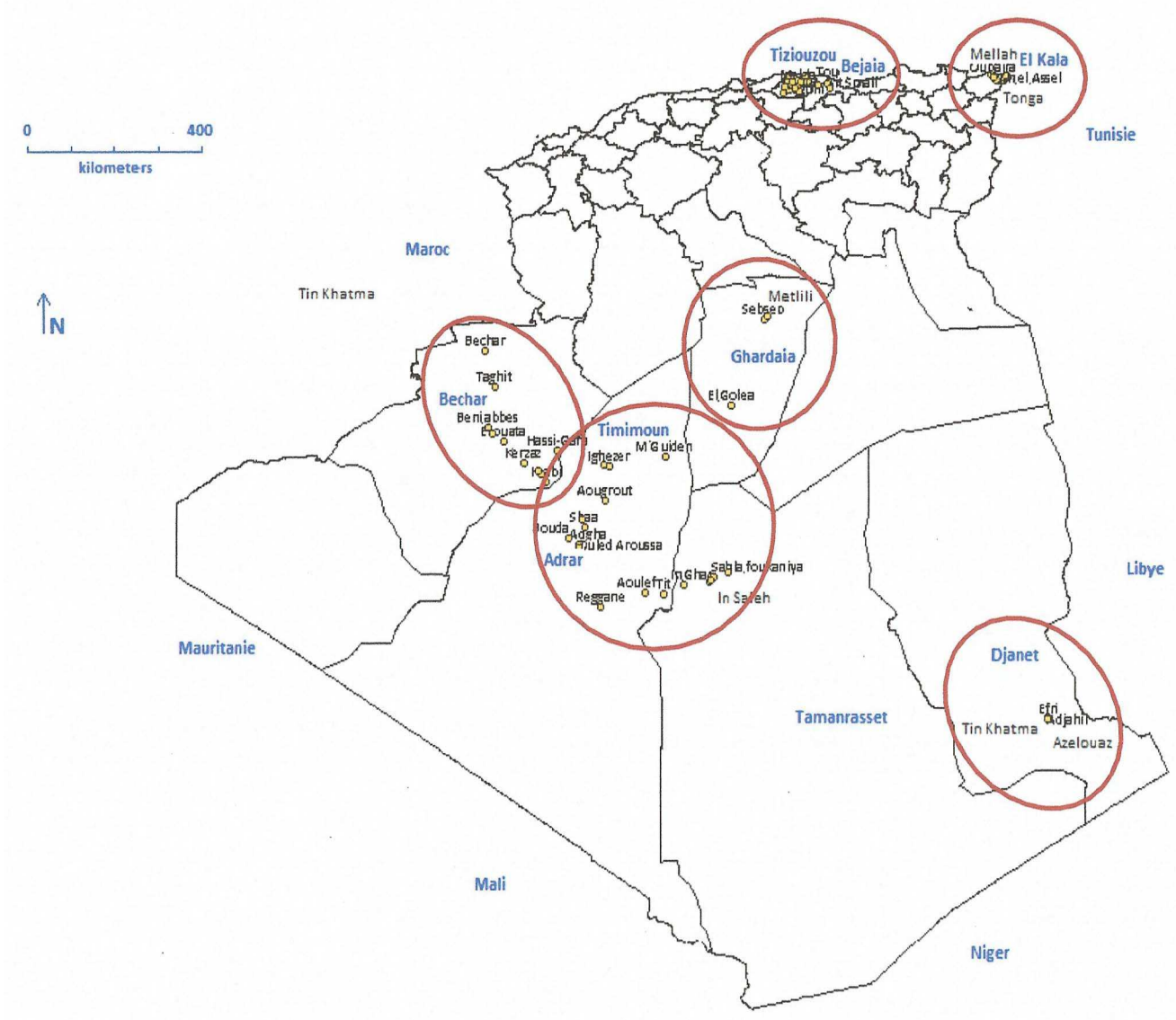

Figure 1 - Map showing traditional areas of the culture of cowpea in Algeria.

a. For a preliminary characterization of accessions collected, qualitative and quantitative characters of the seed, commonly used to describe cultivars and cultigroups of cowpea, were selected. They were the colour, shape, texture and length of the seed, as well as the colour of the eye (which is the area most often colourful, located around the hilum), seed, and weight of hundred seeds (Table 1).

The measurements were conducted with 50 seeds per accession.The data were processed by different methods of statistical analysis. Multivariate analyzes were performed with SAS software **(SAS 8 for Windows, 2001)**.

Table 1- Main morphological characteristics in seeds of Algerian cowpea landraces.

Descriptors
Seed color (SC): *1 white, 2 cream, beige, brown 3, 4 brown 5 brown-ocher, olive-brown 6, 7Black and white Holstein, 8 black, 9 red, 10 speckled beige.*
Eye color (EC): *0 absent, 1 brown, 2 brown wide, 3 black.*
Form (FS): *1 kidney, 2 Egg, 3 Round, 4 globular Rhomboid 5.*
Texture (TS): *1 smooth, 2 smooth to rough, 3 rough to wrinkled, 4 wrinkled.*
Weight of hundred of seeds (mg) (WHS*): weight of hundreds of seeds in g.*
Class 1: *<7.92 g, Class 2 :7,92-9, 21 g, Class 3 :9,21-10, 68 g, :10,68 Class 4-17, 55 g,*
Class 5: *17.55 to 25.66 g, Class 6 :> 25.66 g.*
Length of the seed (cm) (LS*): measure of mature seeds.*
Class 1: *<7 mm, Class 2:7, 1-8, 6 mm, and class3:8, 6-9, 9 mm, Classe4 :> 9.9 mm.*

Each cowpea accession bore information about its geographical coordinates (altitude, latitude and longitude). This information was used to develop maps of geographical distribution of samples, by using the software DIVA-GIS 2.4 **(Hijmans *et al.*, 2002)**. DIVA software allowed to visualize the spatial distribution of the 122 accessions of cowpea analyzed in this study (Figure 2). Accessions are distributed in the north and in the south of Algeria. In Fig. 2, each point represents where an accession of cowpea was found and sampled for banking of *ex situ* conservation.

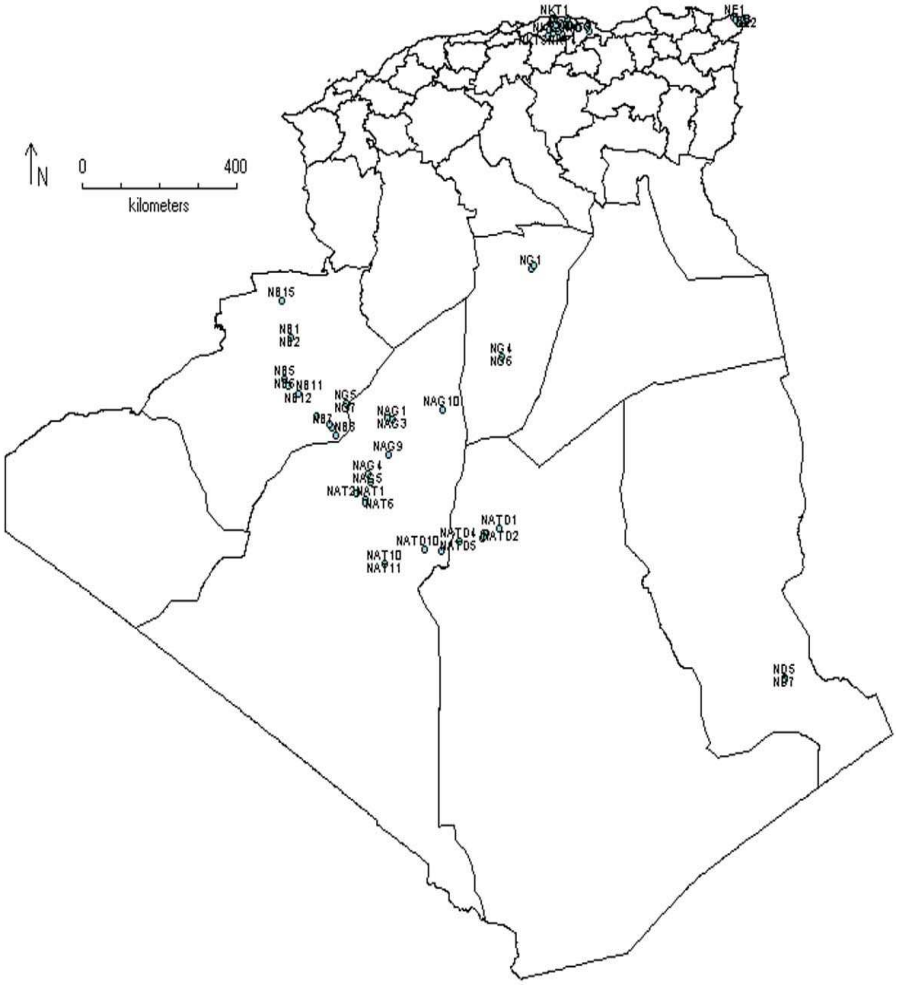

Figure 2 - Geographical distribution of different ecotypes of cowpea cultivated in Algeria.

Multiple Correspondence Analysis (MCA) yielded a graphical view of the structure of cowpea accessions collected in Algeria (Figure 3). Only the first three axes accounted for nearly 65.26% of the variability expressed. Axis 1 opposed the original seed forms found in the oasis of the Sahara to those found in the North of the Country, with some exceptions: indeed, "black-eye/cream-colour" seeds collected in the South (NAG5, NAT9 and NB13) were found to group with the northern forms and shapes collected in the region of Djanet (ND1, ND6, ND3 and ND7), exhibiting features which can be compared to other seed forms found in Sahara. By looking at the distribution of the variables along the same axis, it is found that Axis 1 opposes the "black-eye/cream-colour" seeds to seed exhibiting black, brown or beige-brown colours.

Axis 2 isolates seed accessions "black-eye/cream-colour" from the Kabylia region from the accessions bearing these same features collected in the El Kala region. It also isolates "black-eye/cream-colour" seeds, identified mainly in El Kala, from "brown-eye/cream-colour"seeds, collected in this same region.

By looking at the distribution of the variables on this same axis, an opposition can be seen among the seed characters “brown-eye and wide around the hilum” and “black-eye”.

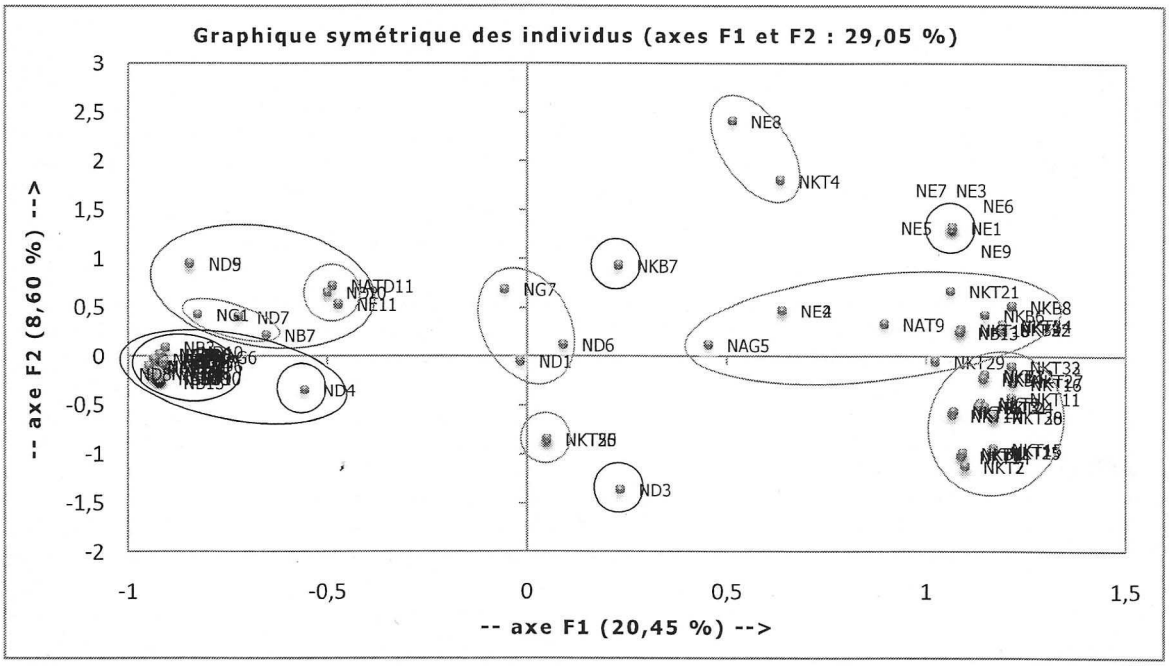

Figure 3 - Multiple Correspondence Analysis of 122 accessions of cowpea landraces collected in Algeria.

In the north of the Country, cowpea was identified only in Kabylia and in the far North, i.e. in the El Kala region. In the district of Tizi-Ouzou in Kabylia, it was commonplace to collect several accessions within the same village, whereas in the region of Bejaia few farmers cultivating cowpeas were identified. In the valley of M'zab, cowpea is not known at all. It was only down to the Metlili region, in the southern *wilaya* of Ghardaia, that cowpea accessions were identified; these might have been introduced there by seasonal farm workers provening from the Gourara and the Touat oases. Consistently, during surveys carried out in Algeria between 1993 and 1995, **Echikh *et al.*, (1997)** identified the northern boundary of the area of the *Tadelaght* culture in the oasis of El Golea, where cowpea is grown in gardens belonging to families coming from the Touat region, and particularly from Timimoun. This was explained by the transfer of seeds along with the temporary migration of seasonal workers. Cowpea was also found in the oasis of Gourara, Touat, Tidikelt and Saura (South-Western Algeria) and in the oases of Tassili and Hoggar N'ajjer (South-East). Among the areas identified as locations in which cowpea can be found; only the Hoggar region was not prospected.

In each of the sites surveyed, several different cowpea types were identified. Characteristics of seeds and pods were found to be immensely diverse in cultivated forms of cowpea.

Such features are widely used to describe cowpea cultivars **(Piper, 1912; Pasquet et Fotso, 1994)** and to identify its cultigroups **(Pasquet, 1996)**.

The most common morphotype in Kabylia, locally known as *Loubia Kabyle,* is characterized by cream-coloured seeds, black eye, smooth-to-rough or rough-to-wrinkled texture, and reniform or rhomboid form **(Ghalmi *et al.*, 2005)**. In the El Kala region, a morphotypes locally known as *Loubia arebi* is present, showing cream-coloured seeds, brown or brown-wide eye, and smooth to rough texture. In the Sahara oases, the *Tadelaght* morphotype is most common, showing a smooth coat, black, brown or beige-brown colours, and no eye surrounding the hilum. Rare ecotypes were also identified in different regions of the Country, as in the Djanet region, where specific morphotypes occur not found in any other region. In general, a greater diversity in seeds morphotypes was observed in the Sahara oases, respect to the Kabylia region. This is probably the result of a differential degree of genetic erosion, significant in the North but less pronounced in the South, as also observed by **Echikh (2000)**.

Prospection missions and seed collection allowed to delineate the range of cowpea landraces present in Algeria. Such species was identified in several distinct areas: the North (Kabylia, El Kala), the South-West, the oasis (El Golea, Adrar and Bechar) and the South-East (Djanet and Tamanrasset). In these regions, richness exists in terms of plant and animal biodiversity, as well as of plant breeding heritage, associated with diverse cultural traditions. and traditional techniques of plant management and conservation. Local ecotypes of cowpea maintained for several centuries in diverse ecological conditions were able to accumulate a large genetic diversity, which deserves more intensive research. As the number of farmers cultivating and maintaining local ecotypes of cowpea decreased steadily in Algeria, there is an urgent need to identify them and to take actions aimed at supervising and helping them to maintain and increase the inherent richness in plant biodiversity.

After determining the ecological limits of extension of cowpea ecotypes cultivated in Algeria, collected plant material has been conserved at the Institute of Technical and Industrial Vegetable Crops (ITCMI, Staouali, Algiers) and Advanced National Agricultural School (ENSA, El Harrach, Algiers) of Algiers (*ex-situ* conservation). Since it was reputed to be essential to characterize and identify the seed samples collected, studies were undertaken aiming at agro-morphological characterization in the field, as well as molecular characterization in the laboratory, to identify the different ecotypes and classify them within cultigroups of *Vigna unguiculata.*

5.3 Rehabilitation of the culture of cowpea and peanut in Algeria.

Seed of cowpea and peanut were collected from several areas across Algeria (Table 2; Figure 4) during years 2003, 2004, and 2005. The seeds were conserved *ex situ* (seeds dried and stored at 4°C and multiplied in a field collection) at ITCM and ENSA. The collection sites were geographically and climatically divided into three regions following the data of the Algerian National Office of Meteorology) (Table 2):

1. the region of Kabylia (Tizi Ouzou) in the North of Algeria.
2. the region of El Kala, eastern north of Algeria.
3. the oases of Sahara (Oued Souf, Ghardaia, Bechar, Timimoun, Adrar, Ain salah and Djanet), central and eastern south of the Sahara.

Table 2- Geographic location of Algerian landraces of V. unguiculata and A.hypogaea

Région de provenance	Latitude	Longitude	Altitude	Type de climat
El Kala	36°43 N à 36°57 N	7°43 E à 8° 37 E	15 m	Mediterranean climate with mild winter, rainy and hottest and dried summer. (Humid climate).
Adrar	28° 13 N	00° 10 O	275 m	Desert climate with cold winter and hottest and dried summer.
Timimoun	29° 15 N	00° 17 O	312 m	Very hot and dry summer and very cold in winter.
Bechar	31° 01 N	02° 42 O	769 m	Arid
Oued Souf	33°22N	06° 52E	77m	Hot and dry climate
Ghardaia	30°32 N	00° 47 E	402 m	Arid
Tizi Ouzou (Kabylia)	36°43N	04° 02 E	270 m	Mediterranean climate with mild winter, rainy and hottest and dried summer.(Sub Humid climate).
In Salah	27°14N	02°30E	279m	Desert climate with summer temperatures are consistently high - with heat indices reaching extreme levels for four months of the year - but temperatures at night fall low enough to be quite tolerable. Winter nights can be chilly and frost is by no means unknown but the days are warm and sunny.
Djanet	24°33N	09° 29 E	1094 m	Desert climate characterized by seasonal rains without rhythm and long dry periods with cold winters and hot summers.

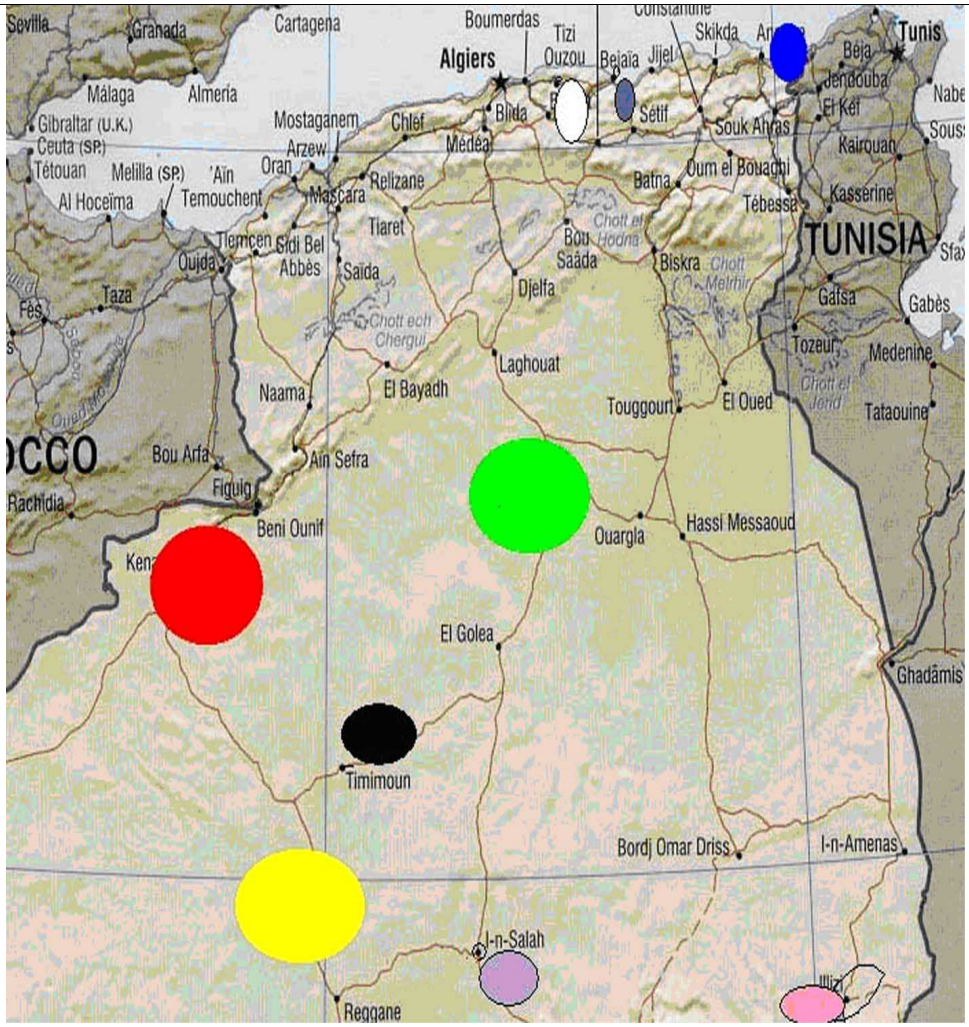

Figure 4 - Map of Algeria showing locations of cowpea and groundnut landraces of collections.

Kabylia is a region in the North of Algeria, part of the Tell Atlas, located at the edge of the Mediterranean Sea. Its climate is sub-humid with a temperate winter and rainfall (700 mm per year). Local soils, which are composed of clay-sand to marl, are often degraded on the slopes. Family gardens are the predominant form of agricultural production. The most common species are olive, fig and almond trees. Other vegetable species are cultivated in small gardens. El Kala is located in the extreme northeast of Algeria, in a triangle of coastal plains and hills bounded by the Mediterranean Sea and the Tunisian border. This region includes a national natural park which is a reservoir of both plant and animal biodiversity. It was recognized as a biosphere reserve by the UNESCO. The climate of El Kala is humid to sub-humid, with mild winters and annual average precipitation of 910–1,300 mm. Several soil types are present: marshes, boggy soil, dunes, and wet meadows, which become dry in the summer. Agriculture is dominated by peanut and vegetable crops.

The oases of the Sahara are characterized by a hyper-arid climate, with cold winters and hot, dry summers. Seasonal variation may be huge, from over 50°C during summer days, to temperatures below freezing during winter nights. Not only does the Sahara receive meagre rain, but precipitation events are also extremely irregular, ranging from 1 to 180 mm per year with a high degree of evapotranspiration. Local soils are mainly comprised of sand. Oasis culture is based on multi-storey cropping systems with palms, fruit trees, vegetables, and cereals. In the South, the plateau of Djanet rises up from the desert, receives more rainfall and presents slightly cooler summer temperatures. These highlands support dry woodlands and shrub-lands distinct from the hot, dry desert lowlands. In general, soils of this region include sands and sandy alluvial soils with low salinity. The Djanet oasis is relatively rich in water, supporting garden culture, palm groves, vegetables and fruit trees.

5.4 Morphological characterization

The study was carried out during the summer 2005 at the experimental fields of the ITCM and ENSA, located in the northern region of Algeria, close to Algiers, at an elevation of 32 m, 02_5300800 E longitude and 36_4502400 N latitude. This zone is characterized by a sub-humid climate, with a mild winter and a hot, dry summer. Annual precipitation ranges from 600 to 700 mm. Half of the precipitation occurs during winter, with a maximum in December. The soil type is sandy loam.

Table 3 - Morphological descriptors used in the seed analysis of Algerian cowpea landraces

Descriptors
Seed colour: 1 cream, 2 cream brown, 3 brown, 4 brown ochre, 5 brown olive, 6 holstein white and black, 7 black
Eye colour: 0 absent, 1 brown, 2 brown large, 3 black
Shape: 1 kidney, 2 ovoid, 3 crowder, 4 globose, 5 rhomboids
Texture: 1 smooth, 2 smooth to rough, 3 Rough
Flower colour: 1 white, 2 pink, 3 purple-pink, 4 purple
Weight of hundred seeds (mg): weight of 100 seeds with moisture content of 12%
Seed number per pod: measured on the 10 mature pods from 5 randomly selected plants
Seed length (cm): measured on the 10 mature seeds from 5 randomly selected plants
Pod purple pigmentation: 0 absence, 1 presence
Pod length (cm): measured on the 10 mature pods from 5 randomly selected plants
Number of ovules per pod: measured on the 10 mature pods from 5 randomly selected plants
Duration (days): from sowing to 50% of flowering

Table 4 - Morphological descriptors used in the seed analysis of Algerian peanut landraces

Descriptors
Type: Spanish or Virginia
Pod length (cm): measured on the 10 mature pods from 5 randomly selected plants
Port of the Pod: Erected or Rampant
Belt of the Pod: Absent, Slight, Medium and Pronounced
Texture of the Hull: Very thin, Thin and Hard
Number of cavity: 1 to 2 or 1 to 3
Weight of hundred seeds (mg): weight of 100 seeds with moisture content of 12%
Seed number per pod: measured on the 10 mature pods from 5 randomly selected plants
Seed length (cm): measured on the 10 mature seeds from 5 randomly selected plants
Duration (days): from sowing to 50% of flowering

Source: Adapted from IBPGR (1983)

Cowpea and peanut landraces were evaluated for their morphological and agronomic traits **(Matías *et al*., 2012; Razakou *et al*., 2013; Romano *et al*., 2013)**, and samples were collected from three replications of 5 plants or 15 plants per landrace. Features analyzed for each sample (Tables 3 and 4) included qualitative characters (purple pod pigmentation, flower colour, seed shape, eye colour, seed colour, and seed texture) and quantitative plant and seed traits (duration from sowing to 50% flowering, pod length, number of seeds per pod, number of ovules per pod, seed length and 100-seeds weight), chosen from cowpea descriptors of the International Board for Plant Genetic Resources **(IBPGR, 1983)**.

5.5 Molecular characterization

DNA extraction: seedlings for DNA extraction were raised in pots in a glasshouse. Leaf samples were pooled from ten 20-day-old individuals of each landrace used in the morphological study. From each plant, 100 mg of young leaf tissue was taken and frozen in liquid nitrogen for DNA extraction by using DNase TM Plant Mini extraction kit **(Qiagen, Germany)**. Each sample DNA concentration was quantified on 0.8% agarose gel and then diluted to approximately 20 ng μL^{-1} for RAPD and ISSR analysis. All diluted DNA samples were stored at -20 °C until used.

RAPD analysis: Twenty primers were tested for PCR amplification. Primers were selected from those chosen for previous studies of cowpea genetic diversity **(Menendez *et al*., 1997; Ba *et al*., 2004)**. After preliminary testing on a few samples, eleven primers that gave clear polymorphic and reproducible banding patterns were selected to assess the genetic variability of the landraces. Each amplification was repeated at least twice to identify repeatable polymorphisms. RAPD patterns were scored as the presence or absence of clearly reproducible bands in duplicate PCR reactions. RAPD were identified by the primer used and the size, in base pairs, of the amplified product.

ISSR analysis: Twenty ISSR primers were chosen based on their performances to distinguish taxa within the genus **(Ajibade *et al*., 2000; Souframanien et Gopalakrishna, 2004)**. Out of the 20 chosen primers, 12 were selected for further analysis based on clarity, scorability, reproducibility of banding patterns.

Data analysis: RAPD and ISSR repeatable bands were scored as present (1) or absent (0), and each characteristic state was treated independently. For each primer, the number of different bands and the frequency of polymorphic bands were calculated. Genetic similarity and cluster analysis were performed by calculating Jaccard's similarity index and the resulting genetic

distance matrix **(Treecon software, Van de Peer et de Wachter, 1994)** and by generating dendrograms (Unweighted Pair Group Mean with Arithmetic, UPGMA) using Statistica 7.0 software (Statsoft, Groningen, The Netherlands). For quantitative morphological characteristics, an ANOVA analysis was performed, homogeneous groups (Newman and Keuls) were determined and intra-landraces Cv were calculated by using Statistica 7.0 software. Distance matrices for morphological characteristics based on Gower's distance between pairs of landraces were calculated by using SAS 9.1 (SAS Institute Inc., Cary, USA), and dendrograms were constructed from these matrices with Statistica 7.0 software using the UPGMA clustering algorithm. Analysis of molecular variance (AMOVA) was performed using Arlequin ver. 2 **(Schneider *et al.*, 2000)**. ANOVA was done first to analyse the variability intra and inter-landraces and second among and within geographical regions. Correlations between molecular, morphological and geographical Euclidean distance matrices, and correlation between each similarity matrix (i.e., RAPD and ISSR), were performed by Mantel test using Passage software 1.1. **(Mantel, 1967; Rosenberg, 2001)**.

The study revealed for the first time how Algerian cowpea landraces can be classified in relation to the major described cultivar groups and their geographic origins through both molecular and morphological characterization. Genetic intra-accession variability was not found, and inter-accession variability is low within clusters and between major clusters, except for three clearly different landraces. Three different cultigroups were located in Algeria: Biflora that was dominant in the Sahara, Melanophtalmus in the North and Unguiculata including one landrace in Kabylia and two in Sahara. Finally, our results demonstrate that RAPD and ISSR techniques can both be used for quantifying genetic distances among cowpea landraces. Breeders can maximize the use of genetic resources by keeping in mind genetic differences among Algerian landraces.

In conclusion, conservation of Algerian traditional landraces is urgently required before they are mislaid. Future investigations should consider developing Algerian landraces for their drought-resistance potential and other unique traits, especially in the case of Saharian ones, which are especially well adapted to arid environments.

6. DIVERSITY OF RHIZOBIA NODULATING PEANUT AND RESPONSE TO INOCULATION

Isolates of *Rhizobium* nodulating groundnut populations provening from different soils of areas classified as highly-producing in Algeria, were characterized in terms of phenotype. The analysis of tolerance to salinity, extreme temperatures, acid and alkaline pH, as well as the use of

different carbohydrates, conducted on a range of peanut *Rhizobium* strains, allowed to select characters satisfying different requirements. With the majority of strains tested, a positive response to inoculation was obtained, with an increase in seed and pod yields.

Apart from tolerance characters (see above), the bacterial strains were also selected on the basis of their capacity for nitrogen fixation, nodulation and dry matter yield.

Groundnut is one of the most important oilseed crops in the world, particularly in arid and semi-arid regions of Africa. Its success lies in the fact that it is both an oily and a protein culture which can be employed for many foods and feed uses (seed, oil, animal foods). Its cultivation is taking an important place in Algeria, where it is found in several provinces (El Tarf, Adrar, Ghardaia, El Oued and Skikda). However, national production falls short of the requirements, because yields are low and highly variable. Indeed, the average yield is 12.5 q ha^{-1} with a maximum of 18 q ha^{-1} recorded in Ghardaia in 2010 **(MADR, 2010)**, while the yield potential of peanut is 60 to 70 q ha^{-1} **(Aune, 2007)**.

Being a legume, peanuts can use atmospheric nitrogen through symbiosis with the bacterial family Rhizobiaceae. Thus, peanut can fix from 22 to 92% of its nitrogen from the atmosphere. Interest in symbiotic nitrogen fixation is currently amplified by the ongoing requirement for sustainable agriculture and environmental ethics. From the perspective of using,the peanut-*Rhizobium* symbiosis, the potential of Algerian soils in which groundnut is grown needs to be evaluated, to build up a collection of rhizobacterial strains able to nodulate peanut, to characterize the phenotypic level and to evaluate the influence of these strains on plant growth and yield.

Soil samples used in the present study were collected (30 cm depth) in groundnut farms located mainly in the North-East of Algeria. The sampling sites were

- **El Tarf**:
- Berrihane (Berrihane, Sebaà, Réghia et Gréat)
- El Kala.
- Souarekh.
- **Guelma** : 3 Sites.
- **Adrar** : 1 Site.
- **Alger** : 1 Site

The assessment of nodulating potential in soil is routinely carried out by the technique of selective trapping. Three seeds of peanuts (Spanish type, short cycle, non-dormant cv) are grown in soil samples previously dried in open air, sieved to 2 mm and then distributed into 0,5 L plastic pots (3 pots per soil sample). These are then placed in a glasshouse at a temperature of 18-22 ° C and watered every four days.

After 8 weeks, the seedlings are dug with their root systems and the presence or absence of nodule is noted. For the isolation and purification of strains of rhizobia, we adopted the methods recommended by the International Biological Program (IBP) **(Vincent, 1970)**. The incubation is carried out at 28 ° C for 3 to 7 days.

For the microbiological selection of bacterial strains, peanut seeds (Spanish type) are inoculated before planting for three hours by immersion in a liquid inoculum of a bacterial culture in exponential growth phase, mixed with sterile peat. They are then transferred to pots containing soil with no nodulating power towards peanut, with three replicates for each isolate.

Bacterial isolates able to nodulate their plant were subjected to specific tests, related to the characterization of rhizobia:

- **Bacterial growth of isolates** is followed by measuring the turbidity (absorbance at 620 nm) of the culture suspension.
- **Acidifying or alkalizing power** of isolates is determined by inoculation of isolates in test tubes containing medium YEMB added with pH indicator bromotymol blue.
- **Temperature tolerance** is tested at 5, 10, 15, 35, and 40 ° C.
- **Tolerance to acidity and alkalinity:** bacterial growth is estimated on YEM medium with a pH adjusted to 4, 4.5, 5, 6, 7, 8, 9, 9.5 and 10.
- **Salinity tolerance** is estimated on YEMA medium containing different concentrations of NaCl, i.e. 0.2, 0.4, 0.6, 0.8 and 1 M.
- **The use of different carbohydrate is estimated by** culturing isolates on YEMA medium supplemented with bromotymol blue in which mannitol is replaced by another carbohydrate. Carbohydrates tested were: galactose, sorbose, cellobiose, maltose, sucrose, dulcitol, raffinose, inositol, mannitol, dextrin, methyl mannoside. The breakdown of the tested sugars by bacteria causes a change in the growth medium pH, which is revealed by a colour change of the pH indicator.

To establish relationships among the bacterial isolates, a numerical analysis was performed on the basis of a data matrix which included phenotypic characters. To develop dendrograms, the UPGMA method was adopted and the Multi-Variate Statistical Package software (v. 3.12, Kovach Computing Services) was used. The test was conducted in pots with a completely randomized experimental design with three blocks. The nodulating isolates were denoted as follows: BA1, BA2, BA11, BA15, BA8, G1A1, G1A2, G1A3, G1A5, G3A2, G3A3, G3A4, GRA4, RGA1, RGA3, RGA6, SA1, SA2, and SKA6. For evaluating the effects of bacterial isolates on the plant performance, peanut seeds (local Spanish type, see above)) were inoculated as described above and then sown in 6 L pots containing soil free from groundnut-nodulating rhizobia (3 seeds per pot and 3 pots per treatment). Irrigation was carried out every four days. After emergence, thinning was carried out leaving one plant per pot.A non-inoculated control (T) and a control with added nitrogen (100 N units ha^{-1}; TN) were also set.

The measurements carried out included:

- infectivity (number of nodules),
- efficiency of N_2 fixation, estimated at full bloom stage, by measuring *in situ* the reducing activity of nitrogenase on acetylene **(Balandreau et Dommergues (1971))**.
- dry matter yield,
- pod yield and seed yield per plant.

The statistical analysis (ANOVA, classification averages and the calculation of correlation coefficients) was performed using the software STATITCF.

All the soils provening from the North East of Algeria were able to nodulate peanut. Nodulation rates ranged from a minimum of 4.66 (El Kala region) to a maximum of 27.33 (Berrihane) nodules $plant^{-1}$, followed by Great (26) and Guelma (25 for G3 and 15.33 for G1). Rates observed for groundnut nodulation were close to those obtained in temperate zones of Algeria, thus indicating that the sites selected are representative of areas where peanut is well established. The morphological features of the nineteen bacterial isolates studied here allowed to assign them to three main groups: those whose colonies did not exceed 1 mm, denoted as punctiform; those forming colonies of intermediate size (2-4 mm); and those forming large colonies (4-6 mm), exhibiting a mucoid appearance

Evaluating generation time of isolates yielded again three groups: a first one, , consisting of two isolates, whose generation time was found to be ≤ 3 h, referred to as “rapid growth” strains;

a group with "intermediate growth" (3-6 h; ten isolates); and a "slow growth" group (<6 h: seven isolates). The majority of isolates had therefore a generation time between 3 and 6 hours (intermediate growth). Although peanut is listed as a species being nodulated by the slow-growing *Bradyrhizobium* **(Zhang *et al*., 1999)**, the presence of fast growing strains is also reported **(Zablotowicz et Focht, 1981; Sprent, 2001; Taurian *et al*., 2006; Barbosa *et al*., 2013)**.

Isolates BA1, BA2, G3A3, G1A1, G1A2, G1A3, G3A3 and G3A4 were found to alkalize the culture medium, whereas isolates BA11, BA15, BA8, G1A1, G3A2, GRA4, RGA1, RGA3, RGA6, SA1, SA2 and SKA6 behaved as acidifying. Such acidification, which is due to the excretion of protons and organic acids, may be increased by production of extracellular polysaccharides.Indeed, rhizobia producing high amounts of extracellular gum often acidify the culture medium, unlike rhizobia producing little gum, which instead tend to alkalize the culture medium **(Ayanaba *et al*., 1983; Graham, 1992; Zakhia et Lajudie, 2001)**. All isolates tested were able to grow in the range 20-35 ° C. Eight isolates tolerated 40 °C, whereas others were able to withstand suboptimal growth temperatures, such as 15 (13 isolates), 10 (3 isolates) or even 5 °C (1 isolate, namely BA11). Temperature is a key factor for survival and persistence of rhizobia in soil **(Lowendorf 1980; Pueppke 1986; Hamdi, 1986)**. For most rhizobia, the optimum range for growth is between 28 and 31 °C, and many cannot grow beyond 37 °C **(Graham, 1992; Gouffi *et al*., 1999)**.

All the tested isolates were able to grow in the pH range 6-9. Almost all of them (18 out of 19) were also grew at pH 5; 9 of them at pH 4.5 and 7 at pH 4. At the other extreme, eleven isolates proliferated at pH 9.5 and pH 10. Isolates with rapid growth, such as GRA4 and SKA6, were sensitive to pH below 5; those showing intermediate growth were able to tolerate pH 4.5, whereas all the slow growing ones tolerated acidity as well as alkalinity in their culture medium. **Berraho (1984) et Hamdi (1986)** reported that the majority of rhizobacterial species grow well at a pH between 5.5 and 7 sensitivity to acidic pH is marked for most species with a fast-growing habitus, such as *Rhizobium trifolii* and *Rhizobium meliloti.* . Cowpea rhizobia group are much more tolerant to acidic pH **(Graham *et al*., 1994)** and fast growing strains of *Vigna unguiculata* able to tolerate even a pH below 4 have been described **(Mpepereki *et al*., 1997)**.

All the rhizobacterial isolates studied tolerate up to 0,4 M NaCl; 14 of them tolerated up to 0,6 M, 6 up to 0,8 M and 2, namely BA15 and RGA6, were tolerant enough to withstand up to 1 M NaCl. The most tolerant isolates (0.8 - 1 M NaCl) were mucoid, while those not tolerating

more than 0.6 M produced less polysaccharide. Rhizobacterial isolates studied here exhibited high tolerance to salinity, which offers a choice to bypass such limiting constraint which affects peanut production areas. It is established that rhizobia are relatively more tolerant to salinity than their corresponding host plants **(Singleton *et al.*, 1982; Egamberdieva *et al.*, 2014)** and it is precisely nodulation and nitrogen fixation which are the most concerned **(Singleton et Bohlool 1983; Serraj et Drevon, 1994)**. Consistently, the present results confirm the findings of **Upchurch et Elkan (1977)** who showed a direct relationship between expolysaccharides production and salt tolerance in *Rhizobium* strains. **Singleton *et al.*, (1982)** and **Yelton *et al.*, (1983)** further documented relationship between rapid growth and salt resistance.

According to **Beunard (1995)**, alkalinization of the medium culture brought about by *B. japonicum* strains, and acidification by *R. tropici* ones, definitely plays a role in the different behavior of the two types of strains in front of salinity, with the former being much more sensitive than the latter **(Tu, 1980; Beunard, 1995)**. The results relating to the use of carbohydrates showed that all the rhizobacterial isolates were able to metabolize cellobiose. Among them, 17, 16, 14 and 12 also used galactose, maltose, sucrose/inositol, and dulcitol/dextrin, respectively. Nine isolates used sorbose, 8 used raffinose, and 7 only used methyl mannoside. According to **Stowers (1985) et Van Rossum *et al.*, (1995)**, fast growing rhizobacterial strains are able to use a wide range of carbohydrates, while the slow-growing ones require simple sugars for growth **(Vincent, 1981)**. The intermediate-growth isolates, such as BA8 and RGA6, utilised all the tested sugars, except dulcitol for BA8, and sorbose and methyl mannoside for RGA6. Description of new genera of *Rhizobium* was accompanied by the revelation of wide profile diversity in the use of carbon sources **(Stowers et Elkan, 1984; Zhang *et al.*, 1991, Lajudie *et al.*, 1994; Odee *et al.*, 1997; Khalid *et al.*, 2015)**. In the present work, the numerical analysis of phenotypic profiles yielded the dendrogram in Fig. 5, which summarizes the degree of similarity and phenotypic homology among the 19 bacterial isolates studied.

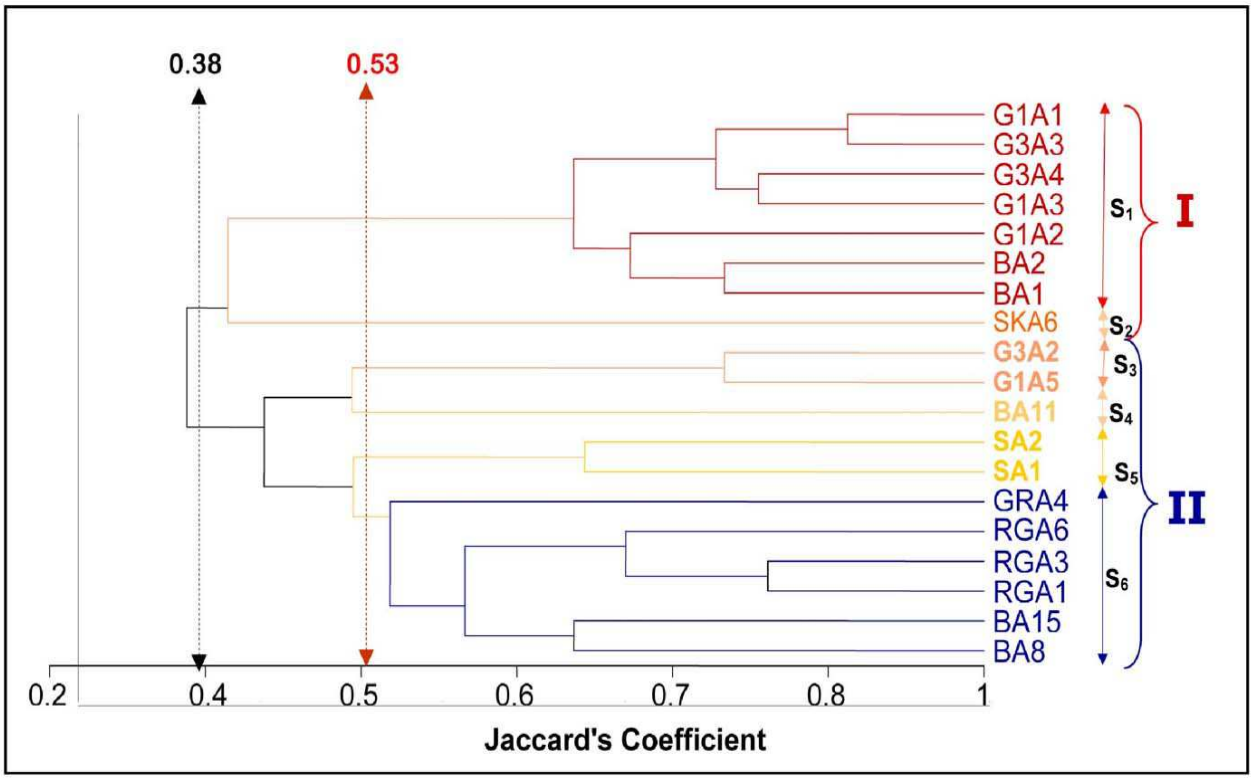

Figure 5 - Dendrogram showing the distribution of strains depending on the degree of similarity of their phenotypic profiles.

At a level of 38% similarity, the rhizobacterial isolates were divided into two main groups, namely I and II, with the former including slow-growth isolates and the latter rapid- and intermediate- growth ones. Isolates in group I (G1A1, G3A3, G3A4, G1A3, G1A2, BHA2 and BA1) are similar among each other in terms of colony size, generation time, and alkalizing effect on medium culture. Such features are shared with *Bradyrhizobium*. .The two groups branch at 53% of similarity, to form six distinct phenotypic groups.

In terms of nodulation, strains SA1, SKA6, G3A4, and SA2 were shown to be the most infective (167.33, 165, 127.67 and 101 nodules plant^{-1}, respectively), being the average value for all strains 62.24 nodules plant^{-1}. ANOVA for this parameter showed highly significant differences and the Newman-Keuls test revealed six homogeneous groups. After plant inoculation, all the host-strain symbiotic couples showed N_2-fixing activity, whose extent variied among the different strains. Such variability was found to be highly significant after ANOVA. The Newman-Keuls test at 5% revealed nine homogenous groups. The strains SKA6, G1A1, G1A2, and G3A4 were proved to be the most efficient, with 59.67, 57.33, 52.67, and 52 µmol C_2H_4 h^{-1} plant^{-1}, being the average value for all strains 41.58 µmol C_2H_4 h^{-1} plant^{-1}.

Indeed, the ability for BNF in legumes depends on the plant genotype and includes *Rhizobium* strain and host-strain interactions **(Sadiki, 1992)**. However, the degree of nitrogenase activity may vary from one rhizobacterial strain to another **(Hartwig *et al*., 1987)**. Moreover, it seems that the level BNF carried out by a symbiotic couple reflects a compromise between infection incidence and nodules activity **(Djordjevic *et al*., 1987)**. Indeed, the lower the number of the nodules, the higher is the amount of photosynthates each of them receives from the plant, allowing in the bacteroids a higher expression of their potential for BNF **(Gall *et al*., 1987)**.

In the present study, accumulation of dry matter (DW) in the aerial plant parts varied depending on the strain used for inoculation (data not shown), and highly significant differences were revealed by ANOVA. The highest DW value was obtained for the TN control. Inoculation with rhizobia permitted an important increase in DW, especially in plants inoculated with strains GRA4, BA15 and BA8, with an increase above 30% compared to T control, and more than 20% in plants inoculated with strains G1A5, SA2 and SA1. However, only a slight increase was noticed in plants inoculated with strains G1A1, G3A2, and RGA1. With the rest of the strains collection, including the majority of slow-growing ones, namely BA1, BA2, G1A2, G1A3, G3A3 and G3A4, DW values below the T control were obtained. An increase in DW in plants following inoculation with specific *Rhizobium* strains was emphasized by many researchers, such as **Patel et Thakur, (1997)** for peanut and **Berraho (1984) et Maatallah *et al*., (2002)** for chickpea.

As far as pod yield is concerned, although ANOVA showed no significant difference between the strains for this parameter, the following findings are worth noting:

- Plants inoculated with strains BA15 and SA1 yielded, respectively, 21.13 g and 19.74 g pods plant $^{-1}$, showing a 20.08% and a 20.59% increase over the T control, respectively, and even a 10.40% and a 3.13% increase, respectively, when compared to the TN control.
- Plants inoculated with strains G3A2, BA1, BA11 and BA8 yielded 18.27, 18.16, 17.53, and 17.21 g pods plant $^{-1}$, also showing an increase respect to the T control whose extent was 11.61%, 10.93%, 7.09% and 5.13%, respectively.

Similarly, **Hiep *et al*., (2002)** observed a significant increase in pod yield in inoculated peanut plants, compared both to non-inoculated controls and to a peanut culture receiving 100 U N ha^{-1}. **Thakur et Patel (1998)** also reported a significant difference between inoculated and non-inoculated peanut crops, with an 8.20% increase in pod yield in favour of the former.

An increase in pod yield in groundnut inoculated with different strains of rhizobia was observed by several authors, e.g. **Diatloff et Langford (1975), Joshi et Kulkarni (1983), Nambiar *et al.*, (1984), Ndiaye (1986), Pal *et al.*, (2000), Rao *et al.*, (2004)** and **Azad *et al.*, (2014)**. A positive response to inoculation was also found in bean, in which a 66% increase in pod yield compared to non-inoculated control was observed **(Guene *et al.*, 2004; see also Diouf *et al.*, 1999).**

In the present work, although ANOVA showed no significant effect between treatments for the parameter, rhizobacterial inoculation affected the average weight of seeds per plant. Indeed, inoculation with the SA1, BA15, G3A2, BHA1.1 and BA11 strains caused 71.79%, 59.92%, 45 , 91%, 32.49% and 21.01% increases, respectively, compared to T control, and 47.17%, 37%, 25%, 13.50% and 3.67%, increases, respectively, compared to TN control. An increase in seed yield compared to T control was also observed in plants inoculated with the RGA3, GRA4, G1A3, G1A1, BA8, G3A3, and G1A2 strains. The above results are consistent with those obtained by The **Dem Ngo *et al.*, (2000)** cited by **Van Toan (2002)**, who reported that inoculated peanut plantations had a higher seed yield compared to non-inoculated ones. Similarly, **Boujghagh (1993)** observed a 37% increase in grain yield in inoculated, not fertilised, soybean plants, compared to non-inoculated treatment receiving 100 kg N ha^{-1}.

In conclusion, the assessment of rhizobacterial potential in soils from groundnut areas in Algeria indicated the presence of bacterial strains able to establish a symbiosis with the legume. The phenotypic characterization of the 19 bacterial isolates allowed to put into evidence a remarkable diversity among them. Numerical analysis showed two major groups at 38% ofsimilarity: a group including isolates with fast and intermediate growth and a group including slow-growth isolates, probably belonging to *Bradyrhizobium* sp.

On the basis of their potential for BNF, strains SKA6, G1A1, G1A2, G3A4, BA15, BA2, RGA3, G3A2, G1A3, SA1, and G3A3 were found to be the most efficient, in terms of symbiotic nodulation and N_2 -fixing capacity, outcompeting the performance of BA2, G3A4 and SKA6 strains. For dry matter yield, an increase of over 20% compared to non-inoculated control was obtained with strains GRA4, BA15, BA8, G1A5, SA2 and SA1. Other strains, such as GRA4, BH15, and BA8, allowed their plants partners to achieve performances comparable to those obtained by supplying 100 units of mineral nitrogen. Inoculation with these strains, therefore, confer to peanut plants partial or almost complete cover of their N requirement, thus limiting the widespread use of nitrogen fertilizers.

Upon inoculation on the host plants, the majority of strains (over 63%) resulted in an increase in seed yield, whose highest extent was more than 71% compared to non-inoculated control and more than 47% compared to non-inoulated control supplied with N fertilisers. A less prominent gain was also recorded for pod yield. However, it is necessary to confirm the performance of these symbiotic strains by inoculation tests in the field and by using peanut varieties or other food legume species, such as cowpea, to select the best symbiotic partners able to adapt to climatic conditions occurring in the Algerian Maghreb.

In a subsequent study, rhizobacterial strains were extracted from nodules of peanut plants and used to inoculate a range of peanut populations collected during the prospection exercise cited above, after which the effects of inoculation on the yield components were evaluated. The slow-growing bacterial strain R1 induced nodulation in three of the peanut populations studied, with a high number of nodules being found in the peanut populations P1 from Boumalek and P3 from Guelma 2. The effect of inoculation on the increase in the number of leaves, fresh and dry weight of the aerial part, weight of the whole plant and all yield components, except weight of 100 pods, was significant. However, inoculation effect on plant height, number of primary branches, number of flowers, fresh and dry weight of root, weight of 100 pods, harvest index, fruiting rate and rate of peeling was found to be not statistically significant.

7. CHALLENGING THE LEGUME-RHIZOBIA SYMBIONTS WITH WATER STRESS AND SALINITY UNDER CONTROLLED CONDITIONS

In the following, a selection of results is reported obtained during the last decade from combining Algerian food legume landraces and indigenous rhizobacterial strains and studying the responses of the resulting symbionts to water deprivation and excess salinity

7.1 Water stress

7.1.1 Case studies

a) A first study in a series was conducted to evaluate the responses to water stress in Algerian cowpea landraces inoculated with 11 indigenous rhizobacterial strains.Phenological traits were in general influenced by water stress which caused a shortening of the developmental cycle in the legume species The superiority of the RSV6 strain for the production of dry matter of leaves and stems was noticed.

The symbiotic characterization of five rhizobacterial strains - isolated from cowpea plants and belonging to the slow-growing group (*Bradyrhizobium* sp., see above) - showed that the

RG_1V_1 and RG_1V_3 strains were the most efficient. A notable result was that *V. unguiculata* plants had a higher nodulation rate at nine weeks than at five weeks. Nitrate reductase activity was stable during the vegetative stage and then decreased in plants inoculated with certain strains, regardless of the nodulating power of strains at pods formation stage.

As far as BNF is concerned, the superiority of the $RB_rV_{1.2}$ strain under both normal and water stress conditions was shown. Such an advantage was best expressed at the flowering stage, during which *V. unguiculata* fixes the maximum of nitrogen, and was indicated by a peak in nitrogenase activity. A remarkable accumulation of proline and soluble sugars occurred in the experimental plant material, although showing a large variability among the different symbionts. Plants inoculated with strains RSV_6 or $RBrV_{17}$ ranked first in terms of proline accumulation.

Seed yield was expectedly influenced by water stress and the plants inoculated with strain RSV_6 were the most able to maintain seed production under such condition.Water stress decreased nodulation, the height and dry weight of the plants, as well as their nitrate reductase and nitrogenase activities **(De Abreu *et al.*, 2014)**. As the above parameters contribute to yield formation and therefore determine the productivity of cowpea, it was concluded that the above strains may be of interest for inoculating cowpea plants likely to be grown in areas where water stress is a limiting factor to crop production. It is believed that, in the Algerian territory, studying genetic diversity of indigenous Rhizobiaceae can still reveal many characters which are linked to the adaptation of the strains to water deficit; this could possibly contribute to improve the yield and the production in cowpea

b) In a second work, responses to water stress, from mild to severe, were studied in two Algerian populations of cowpea, namely P1 from Tizi Ouzou and P2 from Djanet, inoculated with four indigenous rhizobacterial strains.

Water stress had a significant depressive effect on all the measured vegetative characters, including growth parameters, whereas the root-to-shoot DW ratio tended to increase.

Water deficit had a negative effect on the contents of foliar pigments, whereas it significantly increased the foliar contents of proline and soluble sugars. As for the latter, there was a significant difference between the two cowpea populations, the highest value (23.99 mg g^{-1}) being measured in the P1 population **(Meftah, 2012)**.

Water deficit (moderate or severe) negatively and dramatically affected all the yield components, such as the number of pods, the number of seeds, the weight of pods and the seed weight. Among the two cowpea landraces compared, P1 appeared more productive than P2, both

under water stress and under optimal water supply. Inoculation did not reveal any significant effect on all the measured characters. However, a slight improvement in the fresh and dry weight of both root and shoot, as well as in the collar diameter and the number of leaves per plant was recorded in inoculated plant compared to non-inoculated controls.

c) Despite the sizable resistance level developed by groundnut **(Negi *et al.*, 2015; Rustagi *et al.*, 2015)**, production in the dry ecosystems is affected by periods of water deficit occurring during the growth cycle. Therefore, in the third work in a series, the responses to water deficit, mild, moderate or severe, were studied in 5 peanut populations, either Algerian landraces or of foreign origin, inoculated by three indigenous rhizobacterial strains, to identify criteria for selecting symbiotic interactions better adapted to drought. Work was focused on morpho-physiological features **(Jeyaramraja *et al.*, 2013)**, such as height of the stem, leaf area, accumulation of proline and soluble sugar, chlorophyll content; biochemical aspects, such as BNF; plant water status, such as relative water content, leaf water potential, stomatal resistance, leaf temperature and water use efficiency **(Pimratch *et al.*, 2013)**; and agronomic aspects, such as yield and its components.

Water stress decreased the relative water content (RWC) as well as water potential of leaves, and increased stomatal resistance and leaf temperature. All these effects impact negatively vegetative growth, nitrogen assimilation and fixation, as well as yield and its components. The studied plant-rhizoboum symbionts behaved differently depending on the severity of water stress. Symbionts Symbionts SA3-Berrihane, SA3-Oum Tboul, SA3-Tonga, SA3-Timimoun, SA1- Berrihane, SA1- Tonga and SA1- Oum Tboul proved to be the most tolerant to the different degrees of water stress applied. On the other hand, the symbiotic interactions SA2-Timimoun and SA2-NIG showed a significant sensitivity toward the lack of water. The behaviour of other symbiotic interactions varied depending on the severity of stress applied. Variability in response to water stress did not appear to be related to the geographical origin of peanut populations, but rather to their phenology and morphology. This study could be of help in defining criteria for resistance to drought to be used in breeding **(Krishna *et al.*, 2015)**.

7.1.2 Adaptive responses to water stress

Leaf proline content - Water stress causes an increase in proline content which sometimes exceeds 100% compared to control. In the case of severe stress, the Algerian cowpea landraces

used in the above studies can accumulate up 30 mg g^{-1} proline in the stress-tolerant populations **(Meftah, 2012)**. **Sarker *et al.*, (2005)** showed that severe stress induces a strong increase, up to 115%, in proline content in cowpea and a slight reduction in the rate of recovery after irrigation.

Lobato *et al*., (2008), who studied the effect of water stress on proline content, soluble sugar and protein in cowpea leaf tissue during the vegetative phase, reported that the free proline content in stressed tissues increased up to 97.3% compared to control. **Da Costa *et al*., (2008)**, however, observed less prominent increases after exposing two cowpea varieties to water stress, i.e. 45.32% in the *semper verde* variety and 57.57% in the *pituiba* one. The osmoprotectant proline is the best known and the most frequently measured in the studies on water stress. Its content in plant tissues reflects the level of sensitivity or tolerance to drought of a species or a variety. The accumulation of proline strongly correlates with the accumulation of the stress hormone abscisic acid, which is involved in the signalling chain against this constraint **(Sanchez-Diaz *et al.*, 2008; Da Costa *et al.*, 2011; Li *et al*., 2014)**.

Leaf soluble sugars - Water stress significantly increased the foliar soluble sugars in the Algerian cowpea landraces, being such an increase correlated with the stress intensity. Soluble sugars play an important role in the mechanism of drought tolerance by "osmotic adjustment". Accumulation in leaf tissues of the plant has been found in several species due to water stress. It is accompanied by a reduction in the amount of starch stored in the tissues. This action is performed by activation of amylase, which increases with the intensity of the stress **(Lobato *et al.*, 2008; Hamidou *et al.*, 2007)**. The depletion of starch in the tissues by the effect of stress is important for providing the energy needed to maintain the proper functioning of plant metabolism, so compensating the stress-induced decrease in photosynthesis, and to provide soluble sugars necessary for osmo-protection. It should be noted that the biosynthesis and accumulation of organic solutes such as proline and soluble sugars requires more energy than the absorption of inorganic solutes used in osmotic adjustment, such as potassium and nitrate **(Yousfi *et al*., 2010)**.

Lobato *et al*. (2008) reported that the levels of soluble sugars increased in the tissues of cowpea plants subjected to water stress by stopping irrigation for 9 days. **Da Costa *et al*., (2008)** reported that withholding irrigation for 5 days increased the levels of soluble sugars in two cowpea varieties, by 57.81% in the *semper verde* variety and by 72.15% in the *pituiba* one.

Prasad (2011) summarized the different plant responses to stress in terms of enzymatic (superoxide dismutase, catalase, peroxidase guaicol, ascorbate peroxidase and glutathione reductase), as well as non-enzymatic (glutathione, ascorbic acid, alpha-tocopherol, flavonoids and carotenoids) antioxidants. During the stress, certain metabolites, such as polyphenols, polysaccharides, proline, glycinebetaine and (probably) trehalose, are accumulated, and several volatile organic compounds may be released, such as terpenes, oxylipins, methanol, ethanol, formaldehyde, acetaldehyde, and others. Excessive stress can also trigger cellular detoxification processes as cascades of reactions. For example, recent research has confirmed the major role of mitogen-activated protein kinase (MAPK) cascades in response to both biotic and abiotic stresses in plants. Certainly, the identification of origin of eukaryotic species and speciation can be obtained through the evaluation for other atypical forms of MAPKs known as classical sense in plants (MAPK-A, MAPK-B, MAPK-C and MAPK-D) **(Kazuya *et al*., 2002)**. In addition, and in consideration of the common role of MAPKs in the development, growth and multiplication of eukaryotic cells, cellular inhibitors of MAPKs cascades have recently been exploited as anti-neoplastic substances **(Kim *et al*., 2012)**.

Leaf pigments - Photosynthesis is easily disturbed by water stress, which causes a decrease in leaf pigment content **(Jaleel *et al*., 2009)**. In the Algerian cowpea landraces studied here, chlorophyll (Chl) *a* and *b* decreased by 40% under severe stress, while carotenoid content decreases by 50%, and these decreases were greater in the population P1 from Tizi Ouzou **(Meftah, 2012)**. This confirms previous results on the reduction of Chl content in different legume species subjected to water stress, such as soybean **(Atti, 2002)**, bean **(Nemeskeri *et al*., 2010)**, chickpea **(Labidi *et al*., 2009; Mafakheri *et al*., 2010; Matos *et al*., 2010)** and cowpea **(Sawadogo, 2009)**. Some authors have attributed stress-induced decrease in carotenoids to their conversion to zeaxanthin, which is involved in protection against photo-inhibition **(Cowan *et al*., 1995; Sultana *et al*., 1999)**. In addition, under stress, glutamate, a precursor of Chl, is diverted to the synthesis of proline **(Tahri *et al*., 1998)**.

Relative water content - A highly significant reduction of RWC up to 23% occurred in the Algerian cowpea landraces exposed to severe water stress **(Meftah, 2012)**.

Significant positive correlations were found among the decrease in RWC and water stress effects on all traits related to growth, accumulation of dry matter and yield **(Meftah, 2012)**.

The correlation was negative when comparing RWC with proline content and soluble sugars. These results are in agreement with those obtained on groundnut **(Clavel *et al*., 2004)**, *Medicago truncatula* and *Medicago lacinata* **(Yousfi *et al*., 2010)**, chickpea **(Derya *et al*., 2010; Matos *et al*., 2010)** and cowpea **(Lobato *et al*., 2009)**.

Lobato *et al*,. (2008) studied the effect of water deficit on cowpea by withholding irrigation for 9 days. They noted that the RWC of leaf undergoes a significant reduction of 25.7%, while the control value remained stable between 88% and 91%. In another study, **Anyia et Herzog (2004)** tested the effect of the stress applied by maintaining the soil water potential at a value of -75 kPa on 10 cultivars of *Vigna unguiculata*. They measured an average reduction of 12.2% of the leaf RWC of stressed plants compared to control, although the extent of such reduction significantly depended on the cultivar considered. **Lobato *et al*., (2009)** observed a greater decrease, up to 31%, in the RWC of two cowpea varieties after 5 days of water deprivation. **Matos *et al*., (2010)** found a significant reduction in the RWC, greater than 55%, by cultivating chickpea under water stress.

Growth and yield components - Water stress reduces the growth and development of legumes It also leads to an obstruction in the production of flowers and reproductive organs, due to poor translocation of dry matter during the pod filling stage **(Jongrungklang *et al*., 2013)**. The reduction of seed filling is attributable to a decrease in the distribution of photosynthates, and decline of the enzymatic activity involved in sucrose and starch synthesis in seeds **(Farooq *et al*., 2009; Karunaratne et Azam-Ali, 2013; Karunaratne *et al*., 2013)**. This always results in a decrease of seed yield, which can reach up to 40% or 70% under moderate or severe stress, respectively **(Meftah, 2012)**. In the present work, the P1 population from Tizi Ouzou was still more productive than P2 from Djanet, regardless of the water regime applied **(Meftah, 2012)**.

Following water stress, several authors observed a reduction in the plant performance mainly due to a decrease in the number of seeds. **Suliman et Ahmed (2010)** found a significant reduction in seed yield by 50% under water deficit in cowpea, just as they have a significant reduction greater than 40% in three culture cycles of the species. Similar results were also obtained by **Dadson *et al*., (2005)** in ten cowpea cultivars under water stress. **Hamidou *et al*., (2007)** reported that water stress did not significantly reduce the number of seeds per pod in cowpea, but instead the total number of pods and seeds per plant.

Ogbonnaya *et al*., (2003) reported reduction of pod and seed yield by 37.05% and 43.03%, respectively, in three cowpea varieties grown under water stress.

7.2 Salt stress

7.2.1 Case studies

a) Algerian populations of peanut were exposed to salt stress, to identify physiological evaluation criteria to assess salt tolerance in such species**.** The effect of salt stress was highly significant for almost all the physiological traits studied. For proline, juvenile peanut plants showed a capacity for synthesis which varied depending on salt concentration and the population studied. Indeed, the higher the level of the applied salt stresses, the higher the level of proline accumulation. This confirms that such amino acid may be one of the components of plant metabolic adaptation to salinity.

However, the populations Mellah (South West), Obeira (North East) and Tonga (South) showed the highest capacity for proline synthesis, thus the strongest capacity for osmo-regulation by means of such amino acid. In addition, the level of salt stress applied increased the levels of soluble sugars, which alone are believed to account for more than 50 % of the osmotic potential in glycophytes subjected to salt stress. Certainly, as the salt stress became severe, the young peanut plants showed high sugar content, whatever the population. The results obtained for sugars and proline thus confirmed the osmo-regulatory capacity of the peanut populations studied in coping with salt stress.

Sodium cation accumulation in leaves and roots increased progressively along with the intensity of salt stress, with a similar behaviour for all the populations studied. They therefore appeared to belong to the group of the salt-sensitive glycophytes. Certainly, the above Na^+ accumulation did not reflect an efficient cellular compartmentation in roots and leaves, but instead the disability to lower the cytoplasmic level on toxic salts. However, within a species built on this model, varietal comparison pointed out that tolerance was shown by those genotypes able to limit Na^+ export from roots to shoot. This reveals the sensitivity of the studied peanut populations in front of salt stress.

Along with the accumulation of Na^+ in leaves and roots, increasing salt stress induced a significant and proportional decrease in the K^+ content of leaves, with a similar behaviour for all the populations. As a high selectivity of K^+ over Na^+ can be considered as an important criterion for selecting salinity tolerance in plants **(Ashraf, 1994a; Gorham *et al.*, 1997. Ashraf, 2002; Wenxue *et al.*, 2003**), the young peanut plants studied here appeared to lack effective mechanisms to maintain an adequate K^+/Na^+ ratio, necessary to preserve physiological functioning. Na^+ accumulation also generated a decrease in Ca^{2+} concentration in leaves, with

the levels of the latter ion gradually decreasing as salt stress increased. As the maintenance of calcium uptake and transport under salt stress is an important indicator of salt tolerance of plants **(Snoussi *et al.*, 2001; Abdel Latef et Chaoxing, 2014)**, the peanut populations studied here seemed seemed to be unable to maintain an adequate absorption of Ca^{2+} compared to Na^{+} under salt stress.

In summary, the peanut populations studied here showed a significant capacity for osmoregulation, being able to accumulate large amounts of proline and soluble sugars in the presence of salt stress. However, their apparent inability to protect their photosynthetic apparatus from the undesirable effects of Na^{+} accumulation (leading to direct toxicity as well as nutritional imbalances) caused them to fall into the category of glycophytic plants sensitive to salt stress. Additional physiological and biochemical parameters, closely correlated with those studied here, need to be studied, to establish the degree of tolerance of Algerian peanut populations to salinity and understand the related adapation mechanisms In such respect, comparative studies including peanut germplasm already known for its tolerance to salinity would be of help, for a better evaluation of salinity tolerance exhibited by Algeria peanut populations.

b) Further studies were carried out to assess the effect of salinity on the germination, growth, biochemical parameters, water potential and yield of five *Vigna unguiculata* populations from different regions of Algeria. For the first experiment, the seeds of 5 populations were put to germinate in the presence of six NaCl concentrations ranging from 0 to 20 dS m^{-1} for 8 days. The maximum germination was achieved in distilled water for all populations. Populations P1, P7 and P23, native of El Kala, Tizi Ouzou and Bechar, respectively, were found to be the most tolerant. In fact, increasing salinity up to 12 (P7) or 16 dS m^{-1} (P1 and P23), although delaying germination, did not reduce its final percentage. Instead, germination was reduced significantly at 16 (P7) or 20 dS m^{-1} (P1 and P23). The P17 population (Adrar region) was found to be moderately tolerant, being its final germination percentage significantly reduced from 12 dS m^{-1} onwards. The P13 population (In Salah region) was the most sensitive to salinity, with a reduction of its final germination percentage appearing from 4 dS m^{-1} onwards the highest NaCl concentration (20 dS m^{-1}) completely inhibited the germination of P13 and P17.

All the tested cowpea populations exhibited common optimal conditions for germination in the absence of NaCl in the medium **(Nabi, 2009)**, which confirms the quasi-general rule on the germination of halophytes **(Grouzis *et al*., 1976; Belkhodja et Bidai, 2004, El-Keblawy et Al-Rawai, 2005)** and glycophytes **(Meloni *et al*., 2008)**.

Other studies also reported that the seeds of most species reach their maximum germination in distilled water **(Partridje et Wilson, 1987; Khan et Gulzar, 2003; Kift et Naidoo, 2006; Vicente *et al*., 2007; Wei *et al*., 2008)**.

In general, low NaCl concentrations tended to delay germination, without reducing germinating ability to a significant extent when compared to unstressed control. Moderate or high salinity, instead, impacted germination rate, which became totally inhibited in the latter case **(Nabi, 2009; Nabi, 2010)**.

Previous studies reported that low salt concentrations can delay germination without reducing germinating ability, whereas both effects occur at the same time in the presence of high salt stress **(Khan *et al*., 2000; Ghoulam et Fares, 2001; Thakur et Sharma, 2005; Al-Khateeb, 2006; Naidoo et Kift, 2006)**. It appears that seeds of halophytes and glycophytes respond in a similar way to salt stress by reducing the total number of germinating seeds and causing a delay in the initiation of the germination process **(Khan, 2003; Rubio-casal *et al*., 2003; Jamil *et al*., 2006; Yildirim et Guvenc, 2006; Jaleel, 2007a; Igra *et al*., 2008; Wei *et al*., 2008)**. Salinity affects germination by decreasing the water potential of the medium, thus reducing water absorption in the seed **(Tlig *et al*., 2008)**. In addition, salinity facilitates the absorption of toxic ions, which can cause changes in enzyme and hormonal activities in the germinating seed **(Yildirim et Guvenc (2006)**. Salinity-induced oxidative stress might also contribute to the depressive effect on germination **(Amor *et al*., 2005; Demiral et Turkan, 2005; Liu *et al*., 2007; Wahid *et al*., 2007; Yazici *et al*., 2007)**. Indeed, protective antioxidant systems become ineffective in seeds exposed to salt stress, thus leading to inhibition of germination **(Khan *et al*., 2006)**. These authors suggested an antioxidant treatment with ascorbic acid to improve salt stress tolerance during germination in some halophytes **(see also Maia *et al*., 2013)**.

It has been suggested that increasing salinity causes two effects on seed germination in *V. unguiculata*: at NaCl concentrations up to 12 dS m^{-1}, germination is delayed, but no reduction in germinating ability occurs. However, germinating ability is significantly reduced at higher NaCl concentrations (16 or 20 dS m^{-1}; **West et Francis, 1982)**. Delay in seed germination might be the consequence of osmotic effects, whereas reduction in germination percentage might be due to salt-induced toxicity, alone or in combination with osmotic effects **(Francois et West, 1982)**. Similar results were obtained by **Dantas *et al*., (2005)**, who showed that the germination of seeds in three cultivars of *V. unguiculata* is significantly reduced to 25% when treated with 100 mM NaCl, which, in terms of conductance, is equivalent to 10.2 dS m^{-1}.

Several authors found that differences in salinity tolerance exist not only between different species, but also within species, among cultivars and populations **(Grouzis *et al*., 1976; Alonso *et al*., 1999; Sy *et al*., 2001; Murillo-Amador *et al*., 2002; Raccuia *et al*., 2004; Ye *et al*., 2005)**. Differences in germination between populations are most important under salt stress. **Yildirim et Guvenc (2006)** emphasized that these differences can be attributed to genotypic variability which is expressed at a high level under salt stress, and therefore mightbe regarded as a valuable tool for studying tolerance mechanisms. One of these mechanisms depends on the capacity for osmotic adjustment, which allows the growth to be continued under salt stress conditions **(Murillo-Amador *et al*., 2002; Misra et Dwivedi, 2004)**.

Apart from the effects of salt stress on seed germination, responses in terms of plant growth were also examined in the cowpea landraces collected during the prospection of the Algerian territory. To such aim, potted cowpea plants were irrigated with saline solutions (4 salinity levels: 1.93, 3.8, 4.7 and 6.0. dS m^{-1}) starting 20 days after sowing and during the subsequent 2 months. Biochemical and water potential analysis were made immediately after salt treatmen t. Salinity significantly reduced plant height, number of leaves and leaf area, leaf water potential, levels of Chl *a*, Chl *b* and carotenoids, whereas the Chl *a*/*b* ratio was increased. Leaf proline and sugar contents were increased significantly as a result of increasing salt concentration. At maturity, yield components, such as number of pods $plant^{-1}$, length of pods $plant^{-1}$, weight of 100 seeds and number of seeds pod^{-1}, were significantly affected by salt. The most affected was the number of pods $plant^{-1}$. However, the effects varied depending on the salt level and the population considered. On the basis of growth and yield parameters, P1, P7 and P23 were more tolerant than P13 and P17. Nevertheless, P13 and P17 had most abundant foliage, which makes them valuable as fodder for livestock. Such genetic variability during germination until maturity can be used with success in selection programs. In conclusion, the *Vigna unguiculata* landraces studied here showed a moderate level of salt tolerance.

c) Whether and how salt stress directly affects the growth of indigenous rhizobacterial strains, as well as the responses of inoculated cowpea landraces to salinity, were the objects of a dedicated series of experiment. In a first experiment, four rhizobacterial strains, i.e. S1 and S2, slow growing and able to develop up to 0.2 M NaCl, and S3 and S4, fast growing and tolerating up 0.4 M NaCl (see above), were exposed to increasing NaCl concentrations in their culture medium, to assess their *in vitro* tolerance to salinity **(Abed, 2010)**.

The growth curve of the slow-growing strain S2 under 0.05 M NaCl was slightly depressed, whereas that of the S1 strain was accelerated. Beyond 0.15 M, the growth of both slow-growing strains was strongly inhibited. With strains S3 and S4, growth curves started to decline from 0.3 M NaCl onwards, and growth was stopped at 0.4 M.

Thus, the fast-growing strains were more halotolerant than the slow-growing ones. Similar results were also reported by several authors **(Jordan, 1982; Elsheikh et Wood, 1989)**. **Serraj *et al* (2004)** showed that the sensitivity of *Rhizobium* strains to salinity varies with the strain considered. It seems that halotolerance of fast growing strains is due to their acidifying power (unlike the slow growing strains, which are alkalizing) and their ability to accumulate osmoprotectant solutes faster than the slow-growing strains, in addition to their strong mucoid nature. **(Beunard, 1995)**.

Low or medium NaCl concentration in the culture medium retards bacterial growth **(Faituri *et al.*, 2001)**, resulting in an increase in the generation time of bacterial strains **(Singleton *et al.*, 1982)**. At high NaCl concentrations, growth is inhibited **(Abdelwahab et Zahran, 1979; Beunard, 1995)**. **Elsheikh et Wood (1989)** observed a reduction in the multiplication rate of a fast-growing rhizobacterial strain isolated from chickpea upon exposure to 0.34 M NaCl, and inhibition at 0.51 M. **Talibart *et al* (1994)** reported growth inhibition by 50% and 95% at 0.5 M and 0.7 M NaCl, respectively, expressed through a lengthening in the latency phase and a generation time 4 to 15 times longer than in control.

Graham et Parker (1964) reported that concentrations above 0.350 M and below 0.0030 M NaCl are lethal for bacterial growth. **Breedveld *et al* (2006)** found that when strains are transferred from a culture medium without salt to one containing 0.6 M NaCl, the maximum growth occurs in four days, instead of two days in the control medium. This delay is due to a lengthening in the latency phase, which may be due to an adaptation to a saline environment **(Merabet, 2007)**. **Elsheikh (1998)** explains the strategy beyond this as an attempt from the bacterial cell to counteract a reduction in the external water potential, by diverting metabolic resources towards osmotic adjustment, at the expense of growth and duplication. Instead, **Dominguez-Ferras *et al* (2006)** explained the delay of bacterial growth under salt stress as due to a slowing of metabolism caused by a general repression of ribosomal genes and of those encoding the cofactors involved in the tricarboxylic acid cycle and the respiratory chain.

Rhizobacterial strains respond to salt stress by increasing their cellular levels of compatible solutes acting as osmoprotectants **(Madkour *et al.*, 1990; Vriezen *et al.*, 2007)**, such as ectoine

(Talibart *et al.*, 1994), glycine betaine, mannosucrose (Essendoubi *et al.*, 2006), trehalose **(Elsheikh et Wood, 1990)**, the dipepetide N-acetylglutaminylglutamine **(Smith et Smith, 1989)**, poly-β-hydroxybutyrate **(Arora *et al.*, 2006)**, proline betaine **(Gloux et Le Rudulier, 1989)** and the K^+ ion **(Bernard *et al.*, 1986)**, the accumulation of which runs in parallel with an intracellular increase of anionic amino acids, such as glutamate or aspartate **(Berkia *et al.*, 2003)**. According to **Hua *et al* (1982)**, in the presence of NaCl the intracellular concentration of glutamate reaches as much as 88% of the entire pool of amino acids. However, the accumulation of these solutes at too high concentrations can inhibit enzyme activities **(Elsheikh et Wood, 1989)**.

The osmotic potential of the growth medium influences the metabolism of the above compatible solutes. For example, *R. meliloti* uses proline betaine as a source of C and N under normal conditions, but accumulates it as an osmoprotectant in the presence of salt stress **(Gloux et Le Rudelier, 1989)**. The nature of the culture medium and the growth phase which is subjected to stress can influence the type and amount of synthesized osmolytes. In a C-rich environment, high concentrations of carbohydrates are accumulated in response to salt stress, whereas in a N-rich one different osmoregulatory mechanisms take place, such as the accumulation of glycine and proline betaine **(Breedveld *et al.*, 2006)**. The chemical nature of solutes accumulated by rhizobia in response to salt stress also differs from one strain to another. In *Ensifer meliloti*, glutamate remains the most abundant intracellular osmolyte. Also, glycine betaine does not seem to be common to all rhizobia, since an improvement in the growth of *Bradyrhizobium japonicum* at 0.08 M NaCl in the presence of this osmolyte has been reported, whereas it is absent in other species of same genus exposed to salt stress **(Elsheikh et Wood, 1989)**.

The carbon source may affect the tolerance of rhizobia to salt. A given strain may respond differently to the same salt stress also depending on the composition of the culture medium. It has been reported that strains of *R. fredii* tolerate up to 0.34 M NaCl in the YMA medium, whereas the same strains, in the presence of the same amount of NaCl, does not grow on YMA medium amended with 1.0 g L^{-1} of yeast extract **(Elsheikh et Wood, 1989)**. In addition to this, fast-growing rhizobia nodulating peanut accumulate trehalose in the presence of various carbon sources (mannitol, sucrose or lactose), but the slow-growing strains do accumulate it only in the presence of mannitol **(Zahran, 1999; Bhauso *et al.*, 2014)**. Some authors assume that the osmotolerance of certain strains is at the genomic level.

According to **Dominguez-Ferreras *et al* (2006)**, in *E. meliloti*, genes encoding proteins whose functions are still unknown and located at the symbiotic plasmid B (*pSymB*), are involved in the osmotolerance of this strain. These authors, as well as Shamseldin (2008), argue that the *pSymB* plays a major role in the saprophytic competence of *E. meliloti* to grow in saline soils. **Talibart *et al* (1994)** speculate that since the osmoprotectant ectoine shows an effect in *E. meliloti* without being accumulated in the cells, the mechanism of osmoprotection must be located at the gene expression level. In this case, ectoine might trigger the expression of a group of *osm* genes, coding for enzymes involved in the biosynthesis of endogenous osmolytes.

This is consistent with the fact that the transcription of several genes is activated by environmental signals. On the other side, **Wood *et al* (2001)** identified proteins acting as osmosensers and osmoregulators at the membrane level of certain Gram-negative bacteria, so that osmoregulatory responses against salt stress might be orchestrated post-transcriptionally.

Other studies have demonstrated the involvement of lipopolysaccharide (LPS) in the osmotolerance of rhizobia against salt stress. According to **Breedveld *et al* (2006)**, LPS biosynthesis, occurring mainly during the stationary phase, is amplified when NaCl is added to the medium. Thus, the amount of LPS excreted by strains is proportional to the intensity of salt stress in the growth medium, probably as an osmoregulatory response. **Tao *et al,* 1992** have shown that changes in osmotic pressure induce changes in LPS structure in response to salt stress.

Lioret *et al* (1999) observed structural changes in rhizobia induced by ionic and osmotic stress, which may represent adaptive mechanisms to withstand environmental challenges impacting rhizobia either in their free state or as bacteroids. These changes generally affect the O antigen, are specifically induced by salt stress, and are complementary to the accumulation of compatible solutes. The same authors concluded that changes in LPS should be considered as an adaptive mechanism of rhizobia to the environment in which they are isolated. Similar changes observed during nodule development have been described as mechanisms of adaptation to microenvironments surrounding the rhizobia. Since rhizobia are more halotolerant than legumes **(Singleton *et al.,* 1982)**, inoculation of these species with halotolerant strains could be an option for agriculture in arid and semi-arid areas where soils are often saline.

d) A complementary experiment was then set up in which the indigenous S1-S4 rhizobacterial strains were used to inoculate a cowpea landrace then grown in the presence of salt

stress (NaCl), in order to evaluate the effects of co-inoculation on plant growth, development and physiology **(Abed, 2010)**.

Salt stress affected growth, development and nodulation in cowpea. A significant reduction in the number of nodules, in fresh and dry weight of the aerial parts, in the leaf area, and in stem height was observed **(Abed, 2010)**. Salt stress also induced a decrease in photosynthetic pigments (total chlorophyll and carotenoids), as well as protein level, and leaf relative water content. Simultaneously, it caused appreciably increased levels of soluble sugars and proline in leaves **(Abed, 2010)**. Grain yield and pods were also affected, but an improvement was observed with the studied rhizobacterial strains, except with strain S4, which was less efficient despite its *in vitro* salt tolerance **(see above; Abed, 2010)**.

The above results confirm those of **Maas et Hoffman (1977)**, who reported a moderate

tolerance of cowpea to salinity. Rhizobacterial co-inoculation, i.e. inoculating plant roots with more than one rhizobacterial strain, can largely reduce the depressive effects of salt stress. The inoculation with a mixture of rhizobacterial strains S1 and S3, in particular, resulted in a significant improvement of the parameters studied **(Abed, 2010)**. The co-inoculated plants showed superior growth and yield, as well as higher levels of photosynthetic pigments, protein, leaf RWC, and compatible solutes, and the largest number of root nodules, not only relative to non-inoculated controls, but also to mono-inoculated plants **(Abed, 2010)**.

Nitrogen fixation was also improved in the case of co-inoculation **(Abed, 2010)**. Similar results were obtained by **Paau (1989)** and **Lesueur *et al* (2009)**, who observed a significant increase in growth and yield due to co-inoculation. **May et Bohlool (1983)**, after co-inoculating two *Rhizobium* strains, obtained an increased plant DW and number of nodules. **Bordeleau et Antoun (1977)** also obtained an increased number of nodules in *Medicago sativa* after co-inoculation with two strains of *Sinorhizobium meliloti*. **Tham et Tham (2007)** noticed in *Parasponia andersonii* and *Acacia mangium* better performances and increases ranging from 14 to 25% in nodule number after a co-inoculation *Rhizobium-Bradyrhizobium.* Accordingly, **Svenning *et al* (2001)** and **Lowther *et al* (2002)** reported that the co-inoculation of two strains of *Rhizobium leguminosarum* biovar *trifolii* had quadrupled the performance of white clover plants compared to non-inoculated control, and doubled it compared to mono-inoculated plants.

Van Der Heijden *et al.*, (2006) found in forage legumes inoculated with several strains of rhizobia a better yield of 35% and nitrogen content greater than 85%, compared to non-inoculated controls. **Qureshi *et al.*, (2009)** showed that the association *Mesorhizobium*-

Azotobacter allowed better growth and higher yield compared to the mono-inoculation. This association also doubled the biomass, grain yield, and improved significantly the nodular biomass, as well as the P and N contents of the legume. According to **Selvakumar *et al.*, (2009)** and **Qureshi *et al.*, (2009)**, co-inoculation might boost the action of rhizobia on the host plant tolerance against salt stress. The above authors explained co-inoculation synergism in terms of mutual stimulation between strains, their growth and their ability to colonize the rhizosphere niches. Such synergism among different strains would imply: a) an improved MBF, a better water status and mineral nutrition, thereby curbing the harmful effects of abiotic stress; b) an increased exudation of flavonoids (Nod factors) by the roots, with a higher production of LPS, improving the exchange of signals between rhizobia and the host plant; c) an increased production of plant growth hormones and related enzymes, such as cytokines, gibberellic acid, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, lowering plant ethylene levels

(Glick, 2014), which co-operatively stimulate the growth of lateral roots, allowing more sites of infection; d) an increased production of auxins, promoting colonization and rhizobia proliferation in the rhizosphere, thereby reducing the negative impact of salt stress on nodulation and nitrogen fixation **(Bajgiran *et al.*, 2008; Dardanelli *et al.*, 2008, Mehry *et al.*, 2008; Cassan *et al.*, 2009; Qureshi *et al.*, 2009** and **Selvakumar *et al.*, 2009).**

7.2.2 Adaptive responses to salt stress

Growth - Salt stress caused reduction in stem height, leaf area and number of leaves of plants in five populations of Algerian cowpea used in the experiments reported above **(Nabi, 2010)**. Several authors reported that a general response of plants to salinity is growth reduction **(Rogers et Noble, 1992; Croser *et al.*, 2001; Romero-aranda *et al.*, 2001; Hameed et Ashraf, 2008, Jaleel *et al.*, 2008; Perica *et al.*, 2008)**. **Xiong et Zhu (2002)** considered that reduction in growth is an adaptive capacity necessary for the survival of a plant exposed to abiotic stress.

Reduction in leaf area was also reported by **Wang et Nil (2000)**. These authors emphasized that an immediate response to salt stress is a reduction in the expansion rate of leaf surface, until its cessation with increasing salt concentrations. Regarding the number of leaves, **Neumann (1997)** considered that salt-induced inhibition of leaf expansion reduces the volume of leaf tissue and, therefore, limits the production of new leaves. Therefore, a decreased number of leaves is strongly linked to the reduction of the leaf surface. **Lovato *et al.*, (1999)** observed in *Stylosanthes humilis* (*Fabaceae*) a reduction in leaf number in all the populations studied.

Salt stress induces a significant reduction in leaf number and in the fresh and dry weight of leaves, stems and roots **(Kurban *et al.*, 1999; Mehari *et al.*, 2005; Silva *et al.*, 2008)**. The depressive effect of salinity on plant growth may be related to imbalances in the soil water status caused by the osmotic effects of salts **(Das et Parida, 2005; Flowers et Flowers, 2005)**. Physiological drought occurs when osmotic adjustment is not sufficient, making acquisition of water and nutrients difficult by the plant, as well as maintaining the turgidity of the leaves **(Hopkins, 2003)**. Therefore, leaf expansion is slowed down, hence reducing photosynthetic leaf area **(Shannon, 1997)**. In addition, **Slama (2004)** reported that the effect of NaCl on photosynthesis is carried out by a decrease in Chl content, and a decrease in the leaf surface area. Among other causes of reduced growth in response to salt stress, toxic effects of salts, ionic, nutritional and hormonal imbalances, **(Khan *et al.*, 1999; Khan, 2001; Jimenez bayuelo *et al.*, 2003; Haoula *et al.*, 2007)** or a combination of these factors **(Shannon, 1992; Hayashi et Murata, 1998; Yeo, 1998)** can be considered. Munns & **Termaat (1986)** illustrated the processes which reduce plant growth. They distinguished between short-term and long-term salt stress.

In the short term, physiological drought is not the cause of the reduced growth of stressed plants. Rather, metabolic energy required for growth is insufficient, because it is expended to accumulate ions and solutes for osmotic adjustment. The reduction in growth is due, however, to the presence of toxic concentrations of Na^+ and Cl^- ions in the leaves of plants subjected to severe salt stress. In the long term, moderate levels of salinity, even for long periods, can cause significant reductions in growth and yield of most crops without symptoms of toxicity and leaf damage. In this context, the growth reduction observed following the addition of NaCl to the watering solution for a long time is due to the osmotic effects of salts.

Consistently, **Francois et West (1982)** showed that growth in *V. unguiculata* was significantly reduced by 9% per unit of increase in electrical conductivity in soil, starting from the threshold of 1.6 dS m^{-1}. **Maas et Poss (1989a)** reported a significant reduction in the growth of *V. unguiculata* when salt stress was applied at the vegetative stage. Similar results were reported by **Ashraf et Rasul (1988)** and **Zayed et Zeid (1998)** for *V. radiate*. Furthermore, **Silva *et al.*, (2003)** demonstrated that salinity (75 mM NaCl) reduces the growth of *V. unguiculata*. In addition, **Silveira *et al.*, (2001)** reported a reduction in the growth of *V. unguiculata* when subjected to salt stress (50 and 100 mM NaCl). The same authors confirmed that growth reduction was due to a restriction or a decrease of nitrate uptake from the nutritive solution,

resulting in a decreased nitrate reductase activity in the leaves. **Rejili *et al.*, (2008)** reported that 9 g L^{-1} NaCl in the irrigation solution affected significantly growth in height of two populations of *Medicago sativa*.

Contradictory results have been reported in the literature on halophytes, indicating a stimulatory effect of growth in a saltwater environment. So, **Patel et Pandey (2007)** reported stimulation of growth under salt stress (up to 10 dS m^{-1}) in *Cassia montana* (Fabaceae) due to osmotic adjustment and increased leaf surface. **Radhouane (2008)** reported a stimulation of growth resulting in increased leaf area with increasing salinity (4 and 7 g L^{-1}) in some ecotypes of Tunisian mil. Furthermore, **Al-Khateeb (2006)** emphasized that NaCl concentrations ranging from 25 to 50 mM stimulate the growth of *Panicum turgidum.*

In the experiments reported here, salt stress caused reduction in height, leaf area and number of leaves per plant in all the populations of cowpea used **(Nabi, 2010)**. However, there was variability in these measures in relation to the genotype and parameters studied. Certainly, at the end of salt stress application, P13 was the most sensitive landrace in terms of number of leaves, whereas P17 was the most sensitive in terms of leaf surface **(Nabi, 2010)**. Numerous studies showed that reduction in growth occurs in all plants **(Ahmed *et al.*, 2008)**, but their tolerance level and the rate of reduction in the presence of lethal salt concentrations vary widely within and between the different plant species **(Maas, 1996; Das et Parida, 2005; Yildirim et Guvenc, 2006)**, the cultivar or biotype **(Ashraf, 2001; Ghulam *et al.*, 2002)**, the stage of development at which salinity is applied **(Maas, 1996; Vicente *et al.*, 2004; Hamidou *et al.*, 2012)** and the interactions with the environment **(Maas et Hoffman, 1977)**. Genotypic variation was also reported by **Murillo-Amador *et al.*, (2002)** in two cultivars of *V. unguiculata*. The same group of authors **(Murillo-Amador *et al.*, 2006)** showed that 85 and 170 mM NaCl significantly affected the biomass of 25 genotypes of *Vigna unguiculata*. Genotypic variants have been identified, with tolerant- and moderately tolerant genotypes having a higher biomass than susceptible ones.

Chlorophyll content – In the present plant material, the increase in salinity led to significant reductions in the levels of Chl *a*, Chl *b*, total chlorophylls, and carotenoids. Certainly, the levels of total chlorophyll and carotenoids generally decrease under salt stress **(Rajesh *et al.*, 1998; Dajic, 2006; Manivannan *et al.*, 2008; Pinheiro *et al.*, 2008)**. Reductions in concentrations of photosynthetic pigments observed in salt-stressed plants have been attributed to the inhibitory effect of the accumulation of Na^+ and Cl^- on the structure of chloroplasts and hence on the

biosynthesis of various pigment **(Mohammed, 2007)**. The salt-induced increase in chlorophyllase activity may also cause a decrease in chlorophyll content **(Ali dinar 1999; Sultana *et al*., 1999)**.

Levitt (1980) postulated that reductions in pigment levels are due to protein instability caused by salinity, which affects the bonding forces among pigment and proteins in the light harvesting complexes (LHC). Accordingly, **Jaleel *et al*., (2007b)** reported that such reduction in pigment contents are due to the interference of salt ions on protein turnover within the LHC, rather than to degradation of chlorophylls. About decreases in carotenoids levels under salt stress, **Sultana *et al*., (1999)** emphasized that this reduction leads to the degradation of β-carotene and zeaxanthin formation, involved in protection against photoinhibition.

Significant reductions in the levels of Chl *a*, Chl *b*, total chlorophylls and carotenoids appear to be commonplace in the genus *Vigna* exposed to salt stress, having been reported for *V. unguiculata* **(Tawfik, 2008)**, *V. radiata* **(Ashraf et Rasul, 1988; Zayed et Zeid, 1998)**, and *V. mungo* **(Ashraf, 1989)**. However, contradictory results were obtained by **Lee *et al*., (2004)**, who illustrated an increase in chlorophyll content with increasing salinity (up to 49.7 dS m^{-1}) in *Paspalum vaginatum* (halophyte), which led to hypothesize that tolerance strategy against salt stress might be associated to an increase in the photosynthetic capacity and chlorophyll content.

Leaf soluble sugars – In the experiments reported above, the effect of salt stress was associated to a significant increase in the foliar levels of proline and soluble sugars, which occurred in all the tested cowpea landraces except in P17 **(Nabi, 2010)**. Populations irrigated with saline solutions at high concentrations accumulate more proline and sugars than those subject to low or to moderate salt concentrations **(Nabi, 2010).**

Tawfik (2008) demonstrated in salt-stressed *V. unguiculata* a significant accumulation of soluble sugars and leaf proline. **Silva *et al*., (2003)** also observed an increase in soluble sugars content of *V. unguiculata*. **Mohammed (2007)** reported that increasing salinity is associated with elevated contents of leaf proline in *V. radiata*. However, conflicting findings were reported concerning proline accumulation under salt stress: thus, **Silveira *et al*., (2001)** demonstrated a slight increase in *V. unguiculata*, whereas no change in proline levels was reported by **Silva *et al*., (2003)** for this same species. **Franco *et al*., (1999)** found lower levels of sugars and proline in *V. unguiculata* in relation to salinity. Besides, **Ashraf et Rasul (1988)** observed a reduction in soluble sugar contents in two cultivars of *V. radiata* under salt stress. These authors suggested that such depressive effects were due to the negative impact of salinity on carbohydrate

metabolism, mediated by the inhibitory effect of Cl^- ions on photosynthesis. These same results were obtained by **Mohammed (2007)**. This may explain the reduced levels of soluble sugars with increasing salinity in the cowpea population P17 studied here.

Data from the literature report that the accumulation of proline and soluble sugars in the leaves of stressed plants is a mechanism of salt tolerance. It plays an important role in cell osmotic adjustment in case of water deficit **(Jaleel *et al.*, 2007b; Turk *et al.*, 2008)**. This reinforces the idea that the accumulation of proline and soluble sugars is the result of an attempt of the plant to withstand stress-associated damage, rather than being associated to stress tolerance **(Lacerda *et al.*, 2003, 2005; Naidoo *et al.*, 2008)**. **Naidoo *et al.*, (2008)** suggested that proline is a compatible solute which ensures the stabilization of certain macromolecules and organelles such as proteins, protein complexes and membranes and prevents the denaturation of enzymes following inactivation by hydroxyl radicals and other reactive oxygen species. Similar findings were reported for *Vigna unguiculata* by other authors. **Silva *et al.*, (2003)** suggested that the increase in proline content in response to salt stress is not sufficient to explain its contribution to osmotic adjustment. Indeed, an increase in proline content was observed in plants exhibiting the strongest reduction in growth. Besides, **Ashraf (1989)** showed an increased content of proline, but not sufficient for osmotic adjustment in two cultivars of *V. mungo*. This author proposed that the accumulation of proline is not related to the salt tolerance of cultivars and it must be combined with other compatible solutes to provide osmotic adjustment.

Ghoulam *et al.*, (2002) came to the same conclusion after studying five sugar beet cultivars. **Patel & Pandey (2007)** showed that the accumulation of proline in *Cassia montana* plays a positive role as a compatible solute to balance the accumulation of Na^+ and Cl^-ions induced by salt stress. The accumulation of compatible solutes can also be a storage form of nitrogen or carbon used in times of stress **(Hare *et al.*, 1998; Parvaiz et Satyawati, 2008).**

8. CONCLUSION AND PROSPECTS

Food legume crops play an increasing role in farming systems for North Africa with the emphasis on development of sustainable agricultural production. Drought and excess salinity in soil are worldwide environmental stressor with the highest negative impact on crop biological performance and marketable yield, both of which are expected to be challenged by the increase in frequency, intensity and duration of water stress occurrence associated with global climate changes.

Within this framework, major directions for future research are: 1) to evaluate the biological diversity in response to water and salt stress exhibited by collection of crop wild relatives and landraces traditionally grown in arid and semi-arid environment, 2) to explore the relationships between multiple morpho-physiological traits and drought/salinity tolerance, and 3) to identify useful traits which might be promising for breeding programs targeted to augment drought and salt stress tolerance in commercial lines. This will be achieved by an integrative approach, combining a range of disciplines (biology, physiology, eco-physiology, agronomy, economics and social sciences), and active contribution of farmers from reference areas, to identify all the factors which should be implicated.

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Conclusion générale

Conclusion générale

Ce travail qui porte sur l'étude des mécanismes agro morphologiques, physiologiques, biochimiques et moléculaires a permis d'identifier plusieurs réponses au stress hydrique de quelques populations d'arachide. Elles peuvent être de diverse nature et se déclencher séparément ou ensemble, pour contrecarrer les effets de cette contrainte. Cette diversité constitue un atout majeur pouvant être exploité pour déterminer les mécanismes les plus efficients de la tolérance au stress hydrique. L'arachide déploie plusieurs stratégies adaptatives en fonction de la durée de cette contrainte : *(i)* ajustement osmotique par l'accumulation de la proline et des sucres solubles ; *(ii)* modifications morphologiques (*évitement*) par la diminution de la hauteur de la tige, la réduction de la surface foliaire et l'augmentation de la résistance stomatique ; *(iii)* baisse de l'état hydrique de la plante par la diminution de la teneur relative en eau, du potentiel hydrique foliaire et la diminution de la température du couvert végétal.

Les principaux résultats obtenus permettent d'identifier des relations entre quelques caractères morpho-physiologiques et la tolérance au stress hydrique. Une approche statistique multivariée permet d'observer deux composantes biologiquement significatives : *(i)* la ***«vigueur des racines »*** associée à un système racinaire long et profond, présentant à la fois une forte distribution de racines latérales contenant plusieurs nodules, et une croissance prononcée de la tige; *(ii)* les ***«mécanismes- physiologiques»***, associés à la capacité de maintenir un bon état physiologique des feuilles et un abaissement de la température du couvert végétal, par l'intermédiaire des valeurs permissives du potentiel hydrique des feuilles qui permet un certain degré de perméabilité stomatique, même dans des conditions de stress hydrique sévère **(25 jours de stress hydrique)**.

De ce fait, il est devenu possible de modéliser de façon fiable, la tolérance au stress hydrique et d'identifier un sous-groupe au niveau des populations locales d'arachide étudiées formé par **Berrihane (BER)**, **Tonga (TO)**, **Adrar (AD)**, **Oum Tboul (OT)** et **Boumalek (BMK)** qui ont donné les meilleurs performances pour les deux composantes étudiées à savoir la ***«vigueur des racines »*** et ***«mécanismes- physiologiques ».***

Les résultats obtenus montrent également que sous la contrainte hydrique, les différentes populations enregistrent une baisse non significative de l'activité superoxyde dismutase **(SOD)** sauf la population **Tonga (TO)** qui enregistre la valeur la plus élevée de cette enzyme, confirmant ainsi une meilleure tolérance au stress hydrique. Aucune différence significative n'a

été observée, en ce qui concerne la teneur en **GSH** ; la teneur la plus élevée de cet antioxydant a été observée chez **Tonga (TO)** et **Adrar (AD)** sous les différents niveaux de stress hydrique.

En revanche, toutes les populations étudiées ont montré entre elles une nette différence de la teneur en acide ascorbique **(AsA)**. **Adrar (AD)**, **Tonga (TO)**, **Oum Tboul (OT)**, **El Frin (FR)** et **Boumalek (BMK)** ont présenté la teneur la plus élevée de de cet acide.

Les différents comportements des populations d'arachide étudiées impliquent des réponses distinctes et spécifiques concernant les mécanismes antioxydants. Ces résultats montrent implicitement que chaque population développe une stratégie propre vis-à-vis du stress hydrique. En effet, ils sont cohérents avec ceux obtenus par certains chercheurs, qui confirment que le stress hydrique entraîne un stress oxydatif chez l'arachide.

L'expression des gènes de la superoxyde dismutase **(SOD)** est analysée, compte tenu de son rôle central dans les antioxydants de défense chez tous les organismes et de son implication dans les réponses anti-oxydantes sous contrainte hydrique. L'**ARN** total isolé à partir des plantes stressées et non stressées permet de déterminer le taux d'expression du gène de la superoxyde dismutase **(SOD)** par la technique **RT-PCR**.

Les résultats montrent que le stress hydrique régule la synthèse et l'activité de la superoxyde dismutase et la manière dont cette enzyme contribue à protéger contre les effets dommageables de radicaux superoxyde chez l'arachide. Les niveaux d'expression relative de la **SOD** augmentent après 25 jours de stress hydrique chez **Tonga (TO)**, par contre, ils diminuent chez **Sebseb (SEB)**. Les résultats révèlent de grandes différences "***constitutives***" dans l'expression du gène de la **SOD** chez les quatre populations d'arachide. En effet, **SOD** transcripts est plus abondant chez **Tonga (TO)** que chez **Sebseb (SEB)**.

Bien qu'il soit difficile de comprendre tous les mécanismes antioxydants des plantes sous stress environnementaux, cette étude serait une étape préliminaire pour une étude moléculaire de certaines enzymes antioxydantes. Le profil d'expression génique de la catalase **(CAT)**, chloroplastique/mitochondrial **GR** et chloroplastique / stromale **APX** sera la prochaine étape de cette étude. Aussi, la comparaison des profils d'expression génique des différentes enzymes antioxydantes et des activités enzymatiques permettrait d'approfondir la connaissance des mécanismes de protection moléculaire chez l'arachide sous contrainte hydrique.

Enfin, dans l'ensemble, et en raison de leur provenance et leur grande variabilité inhérente, les populations d'arachide étudiées peuvent être considérées comme une collection utile pour l'étude des mécanismes morpho-physiologiques, biochimiques et moléculaires déployés par cette espèce.

Perspectives

A partir de tous les résultats obtenus, des recommandations et projections peuvent être proposées pour continuer ce travail :

- Il serait intéressant de poursuivre cette étude par le comportement des populations d'arachide attestées tolérantes à la sécheresse en conditions de plein champ.
- Il serait plus important de vérifier les résultats obtenus par des études complémentaires, avec d'autres populations locales afin d'élargir la variabilité génotypique.
- D'autres travaux sont nécessaires pour valider les rôles distincts des divers systèmes antioxydants, et ce en intégrant les antioxydants de bas poids moléculaire comme ligne secondaire de défense.
- Notre travail suggère que les systèmes enzymatiques de type **SOD** jouent un rôle essentiel dans la tolérance au stress hydrique chez la population **Tonga (TO)**. Ils ouvrent ainsi une voie de recherche dans le but de :

    * Sélectionner des populations d'arachide plus tolérantes à cette contrainte par différentes méthodes comme la modification de l'expression des gènes codant pour cette enzyme antioxydante (par des méthodes biotechnologiques de type transgénèse).

    * La sélection de populations présentant naturellement des niveaux élevés d'expression pour cette enzyme (par des méthodes génétiques plus classiques et des croisements).

- Une étude approfondie sur les mécanismes de tolérance au stress hydrique ne peut être envisagée sans une approche moléculaire ***(identification d'un ou des gènes de résistance)*** et biochimique ***(recherche de protéines synthétisées ou inhibées par le stress)*** et serait indispensable pour améliorer rapidement la productivité de cette espèce.
- Une analyse de l'expression d'un nombre plus grand de gènes **(*array*)**, et pour différents scénarios de stress hydrique mérite d'être conduite afin d'effectuer une analyse plus exhaustive des gènes impliqués dans la tolérance de l'arachide à la sécheresse.
- Enfin, ces populations pourraient être utilisées comme des lignées parentales dans les programmes de sélection pour les zones arides ou semi arides.

Annexes

Annexes

Annexe I : Composition de milieu de culture YEMA minimum

Mannitol	2g
K_2HPO_4	0,5g
$MgSO_4, 7H_2O$	0,2g
Extrait de levure	0,1g
NaCl	0,1g
Eau distillée	1000ml
Agar-agar	15g

Annexe II Dispositif expérimental (Randomisation totale) au niveau de la chambre de culture.

1,80 m

4,80 m

BER R2 Stressed	**TO R2 Controlled**	**TIM R2 Stressed**
OT R3 Stressed	**TIM R1 Controlled**	**BMK R2 Stressed**
TO R1 stressed	**BER R1 Stressed**	**OT R2 Stressed**
BMK R1 Controlled	**BER R1 Controlled**	**TO R3 Controlled**
BER R2 Controlled	**OT R3 Controlled**	**TIM R1 Stressed**
TO R1 Controlled	**TIM R2 Controlled**	**BMK R3 Stressed**
OT R1 Controlled	**BER R3 Controlled**	**TO R2 stressed**
BMK R2 Controlled	**TIM R3 Stressed**	**OM R1 Stressed**
OT R2 Controlled	**BMK R1 Stressed**	**TIM R3 Controlled**
TO R3 Stressed	**BER R3 Stressed**	**BMK R3 Controlled**
MET R2 Stressed	**OS R1 Stressed**	**FR R2 Stressed**
SEB R3 Stressed	**FR R1 Controlled**	**OS R2 Stressed**
AD R1 Stressed	**MET R1 Stressed**	**SEB R2 Stressed**
OS R1 Controlled	**MET R1 Controlled**	**AD R3 Controlled**
MET R2 Controlled	**SB R3 Controlled**	**FR R1 Stressed**
AD R1 Controlled	**FR R2 Controlled**	**OS R3 Stressed**
SEB R2 Controlled	**MET R3 Controlled**	**AD R2 Stressed**
OS R2 Controlled	**FR R3 Stressed**	**SEB R1 Stressed**
AD R3 Stressed	**MET R3 Stressed**	**OS R3 Controlled**
SEB R1 Controlled	**AD R2 Controlled**	**FR R2 Stressed**

1.80 m