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THÈME

Réponses Physiologiques à une contrainte hydrique de trois espèces forestières
(*Quercus suber* L., *Quercus ilex* L. et *Pinus halepensis* M.)
Influences de la provenance et de la mycorhization.

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A la mémoire de mon père

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LISTE DES ABREVIATIONS :

APX : Ascorbate Peroxydase

Cat : Catalase

Ch: Chlorophylle

Ch T: Chlorophylles totales

Car T: Caroténoïdes totaux

DSP: Chloroplastic-localized Dehydration Stress Protein

ECM: Ectomycorrhizae

GlyBet: Glycine Betaïne

GR: Glutathion Reductase

LEA: Late Embryogenesis Abundant

MDHA: Monodehydrase Ascorbate

MDHAR: Monodehydra Ascorbate Reductase

NW: None watered (Non arrosé)

POD: Peroxydase

Pro: Proline

RAB: Response Abscissic Acid

RWC: Relative Water Content

SLA: Specific Leaf Area (Surface foliaire spécifique)

SOD: Superoxydase

SWC: Soil Water Content

δ 13C: 13 C Content

W: Watered (Arrosé)

WI: Watered inoculated (Arrosé inoculé)

WNI: Watered none inoculated (Arrosé non inoculé)

WUE: Water Use Efficiency

Introduction Générale

Le bassin méditerranéen constitue un « hot spot » de biodiversité parmi les 34 identifiés au plan mondial (Quezel et Médail, 2003). La forêt méditerranéenne est caractérisée par une richesse et une diversité génétique importantes grâce à sa forte concentration en espèces endémiques. En effet, sur environ 25 000 espèces de plantes présentes dans la région (Myers *et al.*, 2000), environ 60% sont endémiques (Thompson *et al.*, 2005).

La superficie du domaine forestier méditerranéen était estimée en 2010 à 85 millions d'hectares environ soit 2% de la surface forestière mondiale (FAO, 2013). Cependant, ce dernier subit une régression alarmante dans certaines régions dont l'Algérie, qui met en danger sa biodiversité (Borsali *et al.*, 2014 ; Zine El Abidine, 2003). En effet, environ 18% des espèces d'arbres méditerranéennes sont menacées d'extinction. Parmi elles, on dénombre cinq espèces de chênes endémiques (FAO, 2013).

Les causes de cette régression sont multiples et résultent de l'interaction de facteurs biotiques et abiotiques :

- les causes biotiques sont essentiellement des maladies fongiques, des insectes ravageurs, des maladies bactériennes, le surpâturage, les espèces ligneuses invasives, la surexploitation par l'Homme (coupes, surpâturage etc.), les plantes parasites etc.
- les causes abiotiques sont liées à des facteurs climatiques tels que, les tempêtes, les incendies (souvent d'origine anthropique), la pollution, la sécheresse etc.

La sécheresse est la principale contrainte environnementale, réduisant la croissance et la productivité subie par la végétation méditerranéenne (Cherubini *et al.*, 2003). En effet, le climat méditerranéen se caractérise par une sécheresse estivale souvent longue s'étalant quelques fois sur cinq mois (de mai à octobre) et parfois sur plusieurs années, exposant ainsi, les espèces végétales à de fortes radiations solaires, et par voie de conséquences aux stress hydrique et thermique (Aussenac, 2002 ; Faria *et al.*, 1996). La sécheresse peut affecter les forêts de façon très variable : mortalité accrue (Klein, 2015), baisse de la productivité, dépérissement et susceptibilité accrue aux insectes ravageurs et autres agents pathogènes (Desprez-Loustau *et al.*, 2006).

Le réchauffement climatique, lié au phénomène d'effet de serre, entraînerait une augmentation significative des températures et une diminution des précipitations dans le bassin méditerranéen (IPCC, 2007). En effet, le bassin méditerranéen est actuellement

considéré comme l'une des régions les plus vulnérables au changement climatique tant aux niveaux écosystémique que socio-économique (Schröter *et al.*, 2005). Les périodes de sécheresse en Méditerranée débuteraient plus tôt dans l'année, dureraient plus longtemps et seraient plus intenses, ce qui allongerait les périodes de contraintes hydriques d'ici la fin du 21ème siècle (Polemio et Casarano, 2004). Ainsi, les espèces méditerranéennes seraient exposées à un stress hydrique plus intense (Aussenac, 2002 ; Miranda *et al.*, 2002) qui induirait des modifications de leur croissance et de leur taux de survie (Ogaya et Peñuelas, 2007) ainsi que de leur distribution et de leur abondance (Schröter *et al.*, 2005). Selon ce scénario de réchauffement climatique, l'aire potentielle des essences forestières subirait une contraction dans la partie sud et à basse altitude et une extension vers le nord et en altitude (Aussenac, 2002).

Cependant, les effets de l'augmentation de la sécheresse pourraient varier selon les espèces, notamment en fonction de leurs caractéristiques (Ogaya *et al.*, 2003 ; Quero *et al.*, 2008). Selon Schröter *et al.* (2005), l'aire de répartition de certaines espèces végétales méditerranéennes telles que le chêne liège (*Quercus suber*), le chêne vert (*Quercus ilex*), le pin d'Alep (*Pinus halepensis*) et le pin maritime (*Pinus pinaster*) pourrait diminuer (Resco De Dios *et al.*, 2007). Les conséquences du réchauffement climatique dans cette région seraient des modifications des caractéristiques morphologiques et physiologiques des espèces (Dreesen *et al.*, 2012 ; Peñuelas *et al.*, 1996), une augmentation des taux de mortalité des arbres (Martínez-Vilalta et Piñol, 2002) et une diminution de la productivité (Chaves *et al.*, 2002 ; Dreesen *et al.*, 2012 ; Faria *et al.*, 1998 ; Pereira *et al.*, 2007).

En Afrique du Nord, le capital forestier régresse en superficie de 2 à 3% par an (Quézel et Barbero, 1990). La forêt algérienne est l'une des forêts méditerranéenne les plus concernée par cette régression. En effet, les superficies forestières, en Algérie, estimées à 4 321 956 ha en 2015, n'ont jamais cessé de diminuer ; elles constituent approximativement la moitié de la superficie primitive, se répartissant entre une superficie de 1.428.000 ha de forêts naturelles et une superficie de 1 245 000 ha de reboisements (DGF, 2015). Les incendies constituent la principale cause de régression de cette forêt (Bouhraoua, 2015) car ils détruisent en moyenne près de 2% de la surface forestière nationale annuellement (Borsali *et al.*, 2014) aggravée par une succession de périodes de sécheresse notamment à l'Ouest, dont la plus marquée reste celle des années 1980 (Khaldi, 2005).

Les mycorhizes sont des associations symbiotiques entre les racines et les champignons qui jouent un rôle important dans l'alimentation hydrique des plantes (Strullu,

1991). Les ectomycorhizes, type mycorhizien le plus fréquent chez les espèces ligneuses (Strullu, 1991), ont de nombreuses applications, de part leur action positive sur la croissance des arbres et leur protection contre les stress biotiques et abiotiques (Le Tacon, 1978).

Certaines études ont montré que l'ectomycorhization des plants en pépinières peut avoir des effets bénéfiques sur leur comportement face à un stress hydrique. Certains champignons ectomycorhiziens peuvent aider les plantes à tolérer le stress hydrique par augmentation de la surface d'absorption et la capacité du champignon à pénétrer des pores très fins dans les sols identiques à ceux explorés par les poils absorbants (Parke *et al.*, 1983). Selon Mohan *et al.* (2014), les associations mycorhiziennes pourraient réduire les effets du stress hydrique imposé par le changement climatique. D'autres études ont montré que la mycorhization contrôlée, en pépinières, n'améliore pas la croissance mais procure plusieurs bénéfices physiologiques aux plantules de *Quercus robur* (Fini *et al.*, 2011). Cependant, selon certains auteurs, l'effet bénéfique des mycorhizes dépendraient de plusieurs facteurs dont le type de champignon symbiotique (Kennedy et Peay, 2007 ; Ding *et al.*, 2011).

La compréhension du comportement des espèces forestières vis-à-vis de la sécheresse s'impose, non seulement d'un point de vue théorique mais également pour des buts de pratiques sylvicoles. De nombreux reboisements effectués pour restaurer la forêt méditerranéenne, sont voués à l'échec (Hamrouni *et al.*, 2011 ; Bouhraoua, 2015). Pour une meilleure réussite de ces opérations de reboisements, il serait intéressant de choisir les espèces ainsi que les populations les plus adaptées à la sécheresse actuelle et à la sécheresse future annoncée par le réchauffement climatique dans la région méditerranéenne.

De nombreuses études, ont, en effet, montré, ces dernières années, l'existence, en plus de la variabilité entre espèces (inter-spécifiques), l'existence d'une variabilité intra-populationnelle (intra-spécifiques), montrée par des différences considérables des caractéristiques morphologiques stipulant des différences physiologiques notamment au niveau des réponses au stress hydrique chez le chêne liège (Abdessamad *et al.*, 2014 ; Gandour *et al.*, 2007; Tlili *et al.*, 2015), le chêne vert (Aguadé *et al.*, 2015; Camarero *et al.*, 2015) et le pin d'Alep (Calamassi *et al.*, 2001).

Ce présent travail s'inscrit dans le cadre de la recherche d'espèces et de populations ligneuses qui seraient les plus adaptées à la sécheresse estivale actuelle (et future?).

Notre problématique est l'étude du comportement de trois espèces ligneuses présentant des intérêts économique et écologique : *Quercus suber*, *Q. ilex* subsp *maghrebina* et *Pinus halepensis*, vis-à-vis de la sécheresse. En effet, même si ces espèces sont très étudiées dans de nombreux pays méditerranéens (Nardini *et al.*, 1999; Nardini et Tyree, 1999; Calamassia *et al.*, 2001 ; Otieno *et al.*, 2006 ; Otieno *et al.*, 2007 ; Alexou, 2013, Camarero *et al.*, 2015, Aguadé *et al.*, 2015 ; Klein, 2015), très peu d'études existent sur leurs comportements en Algérie (Acherar *et al.*, 1991).

Ce présent travail est divisé en trois parties :

- la compréhension du comportement de jeunes plants de *Quercus suber*, puis sa comparaison avec celui des jeunes plants de *Quercus ilex*, face à une sécheresse estivale simulée par un arrêt d'arrosage
- l'exploration de la variabilité des réponses entre différentes populations algériennes de *Q. suber* face à la sécheresse estivale
- et enfin, l'apport d'une inoculation ectomycorhizienne aux jeunes plants de *Pinus halepensis* exposés à une sécheresse estivale.

Synthèse Bibliographique

1-La sécheresse :

Le concept de sécheresse est complexe à définir. Mais, la sécheresse se réfère généralement à la sécheresse météorologique. L'organisation météorologique mondiale (OMM) définit, en 1966, la sécheresse comme «l'absence ou le déficit marqué prolongé des précipitations».

La sécheresse est le facteur climatique responsable de la diminution de la croissance et de la productivité des espèces ligneuses dans la région méditerranéenne. En effet, elle expose ces dernières à différents types de stress : hydrique, thermique et radiatif.

2- Définition du stress hydrique :

Le terme stress est un concept flou malgré toutes les nombreuses définitions proposées (De Oliveira, 2013). Selon Levitt (1980), le terme stress désigne toute pression exercée par un paramètre de l'environnement qui perturbe le fonctionnement habituel de la plante diminuant ou modifiant défavorablement la croissance ou le développement de la plante. Selon Grime (1977), le stress est une contrainte externe abiotique (température, eau, salinité) ou biotique (herbivore) qui limite l'intensité photosynthétique et réduit la capacité de la plante à convertir l'énergie en biomasse.

Le stress hydrique peut être provoqué par des températures basses, des températures élevées, des concentrations élevées de sels dans les sols, d'une insuffisance de la quantité d'eau dans le sol....etc. Le stress hydrique provoqué par un déficit hydrique est plus fréquent ; il résulte d'un déficit hydrique qui se développe lorsque les pertes d'eau par transpiration dépassent son absorption par les racines (Hopkins, 2003).

3- Effets du stress hydrique sur la végétation :

Les modifications du métabolisme, en réponse à un déficit hydrique ne sont pas normatives et sont liées à de nombreux facteurs tels que l'intensité et la durée du stress, la vitesse avec laquelle le stress s'impose, de l'environnement et de l'espèce (génotype, phase de développement, organe, tissu...) (Farooq *et al.*, 2009). Selon Larcher (1995), la sécheresse méditerranéenne s'installe lentement et augmente d'intensité avec le temps.

Les effets directs de la sécheresse sont constatés à différents niveaux : morphologique (organe), tissulaire, physiologique et moléculaire (Farooq *et al.*, 2009). Le stress hydrique provoque une réduction de la teneur en eau des plantes qui inhibe l'expansion cellulaire et donc l'expansion des feuilles d'où une réduction de la surface foliaire également provoquée

par chute des feuilles (McDowell *et al.*, 2008) et diminution de la croissance (De Oliveira *et al.*, 2013). A l'échelle tissulaire, le stress hydrique peut induire une cavitation des vaisseaux du xylème (Cochard *et al.*, 2009). De nombreux effets physiologiques sont également notés tels que la diminution de l'activité photosynthétique par fermeture des stomates ou diminution des activités des enzymes intervenant dans les réactions photosynthétiques telle que la Rubisco dont la synthèse ainsi que l'activité peuvent être inhibées (Hsiao *et al.*, 1976 ; Farooq *et al.*, 2009) ou encore diminution de la synthèse d'ATP (Farooq *et al.*, 2009), diminution (ou augmentation) de la respiration (Flexas *et al.*, 2005), perturbation de certaines voies de biosynthèse (protéines, chlorophylles,...) (Farooq *et al.*, 2009). Le stress hydrique peut également conduire à une absorption d'énergie lumineuse supérieure à celle requise pour la fixation du carbone qui peut engendrer des dommages dans les photosystèmes et la formation de radicaux libres et des formes réactives de l'oxygène (ROS), engendrant un stress oxydant chez la plante (Faria *et al.*, 1996). Les ROS s'attaquent et endommagent les macromolécules cellulaires (protéines, lipides et ADN) conduisant à une peroxydation des lipides membranaires qui provoque une perte de l'intégrité membranaire, une dégradation de l'ADN et une atteinte des protéines structurales et fonctionnelles. La perte de l'intégrité membranaire serait due à de multiples causes qui ne sont pas toutes connues (Farooq *et al.*, 2009). Une sécheresse sévère peut diminuer la reproduction et même tuer une plante ce qui explique la diminution de la productivité des écosystèmes (Bacelar, 2004). La Fig. 1 énumère différents effets du stress hydrique selon Hsiao *et al.* (1976).

En plus de ces effets directs, la sécheresse est responsable d'effets indirects par fragilisation des plantes devenant plus sensibles aux maladies (Zine El Abidine, 2003 ; McDowell *et al.*, 2008). Le stress hydrique associé à des températures élevées constitue généralement des conditions favorables au développement de certains ravageurs. En effet, la sécheresse affaiblit la plante hôte qui devient ainsi plus réceptive aux attaques des ravageurs, alors que les températures élevées accélèrent le développement des insectes leur permettant de réaliser, dans certains cas, plusieurs générations dans la même année (Mirault et Regard, 1992).

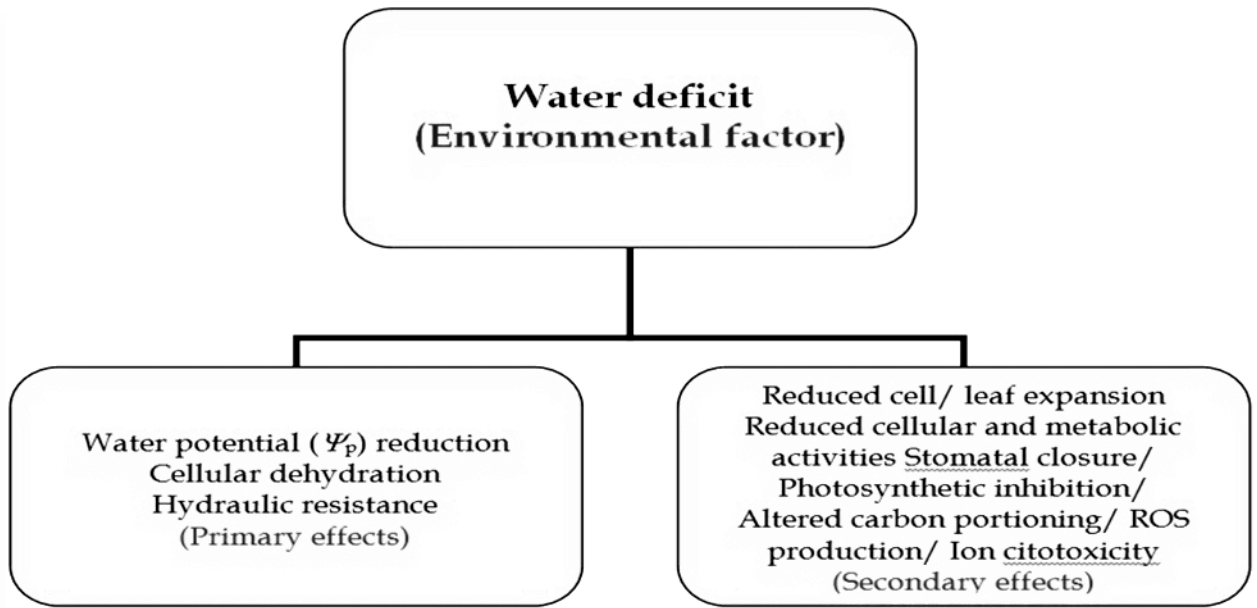


Fig. 1. Quelques perturbations physiologiques et biochimiques provoquées par un stress environnemental (déficit hydrique) chez les plantes (Taiz et Zeiger, 2009).

4- Adaptations de la végétation à la sécheresse :

Face au stress hydrique estival, de nombreuses espèces végétales ont développé différentes stratégies d'adaptation de nature morphologique, anatomique, physiologique et moléculaire (Belhassen *et al.*, 1995 ; Caritat *et al.*, 2006 ; Farooq *et al.*, 2009).

4- 1- Stratégie d'échappement :

Elle permet aux plantes éphémérophytes telles que les plantes désertiques et les plantes annuelles de réduire à quelques jours leur cycle de vie qui se déroule pendant les épisodes pluvieux ; les plantes ont ainsi une durée de vie courte et ne sont pas confrontées au stress hydrique.

4- 2- Stratégie d'évitement :

Certaines espèces tolérantes peuvent échapper au stress hydrique par maintien d'une teneur élevée en eau en optimisant l'absorption de l'eau et en minimisant les pertes d'eau.

- L'absorption de l'eau est augmentée par un important développement des systèmes racinaires, ces derniers sont très ramifiés, denses, longs et profonds. Généralement, le rapport biomasse système racinaire/biomasse système aérien est élevée.
- La diminution des pertes en eau est assurée par des feuilles présentant certaines caractéristiques morphologiques et anatomiques : épaisses, enroulées, de petite taille et

recouvertes de poils, diminution de la surface foliaire, cuticule épaisse recouverte de cires épicuticulaires quelques fois, stomates petits, nombreux et profonds ou par sénescence et abscission des feuilles âgées (Abrams, 1990).

4- 3- Stratégie de tolérance :

Elle se réfère à des adaptations permettant aux plantes de vivre avec le stress avec diminution ou non de ses performances de croissance et/ou de reproduction (Farooq *et al.*, 2009). En effet, la réduction de la durée de croissance, donc arrêt de la croissance durant la sécheresse estivale méditerranéenne, est considérée comme une stratégie d'adaptation (Kurze-Besson *et al.*, 2006 ; Farooq *et al.*, 2009) (Fig. 2). Les mécanismes de tolérance sont très variés ; ils peuvent être physiologiques, biochimiques et moléculaires.

4 -3- 1- Ajustement osmotique :

C'est un processus permettant un abaissement du potentiel hydrique foliaire par accumulation de solutés dans les cellules qui maintient la turgescence cellulaire. Les solutés cellulaires, aussi appelés osmolytes ou solutés compatibles, les plus impliqués dans ce mécanisme sont les sucres solubles (associés à une diminution du taux d'amidon) (Picon *et al.*, 1997 , Yancey, 2005), les protéines solubles de faible poids moléculaires (Bacelar *et al.* 2004), les acides organiques (Gebre *et al.*, 1994), les acides aminés dont le plus important est la proline, les polyols (mannitol, glycérol, sorbitol) (Yancey, 2005), les ions (K^+ , nitrate NO_3^-) (Gebre *et al.*, 1994). Certains osmolytes permettent également une détoxification des espèces réactives de l'oxygène, une stabilisation des membranes et une protection des protéines de structure et des enzymes telle que la proline (Farooq *et al.*, 2009 ; Yancey, 2005).

4-3-2- Système anti-oxydant :

Deux types de mécanismes anti-oxydant existent chez les plantes : enzymatique (catalase, peroxydase, glutathion reductase...) et non enzymatique (acide ascorbique, cystéine...). De même différents mécanismes physiologiques de régulation sont utilisés par les espèces méditerranéennes pour tolérer de fortes irradiations et dissiper les excès d'énergie, comme la diminution de la photosynthèse via une diminution de l'efficacité photochimique (Damesin et Rambal, 1995), la diminution des teneurs en chlorophylles (Kyparissis *et al.*, 1995), un changement dans les composants du cycle des xanthophylles dont le rôle est la dissipation de l'énergie captée par les antennes collectrices du PSII et une stimulation des processus visant à éliminer les espèces réactives d'oxygène (Faria *et al.*, 1998), l'apparition de protéines chaperonnes visant à maintenir la conformation des structures protéiques

perturbées par les modifications des charges électriques dans leur environnement (suite à l'augmentation de la concentration en ions, consécutive à la diminution de la quantité d'eau dans les cellules) (Farooq *et al.*, 2009). De nombreux composés peuvent empêcher les interactions moléculaires avec les constituants membranaires ; ce sont la proline, le glutamate, la glycine bêtaïne, la carnitine, le mannitol, le sorbitol, les fructanes, les polyols, le tréhalose, le sucre et les oligosaccharides. Ces molécules maintiendraient la stabilité membranaire (Farooq *et al.*, 2009).

4-3-3-Régulation hormonale :

Les phytohormones jouent un rôle vital dans la tolérance à la sécheresse chez les plantes. Le stress hydrique provoque, généralement, une diminution des teneurs en auxines, gibbérellines et cytokinines et une augmentation des teneurs en acide abscissique et en éthylène (Taiz et Zeiger, 2009). Plusieurs stress environnementaux telle que la sécheresse induisent, chez les plantes, une accumulation de l'acide abscissique, de ce fait considéré comme une hormone de stress, qui régule l'expression des gènes et sert de signal à l'initiation des processus d'adaptation à la sécheresse et à d'autres stress environnementaux. Il a été proposé que l'acide abscissique et les cytokinines jouent des rôles opposés dans le stress dû à la sécheresse. L'accumulation de l'acide abscissique et la diminution des taux de cytokinines favorisent la fermeture stomatique et limitent la perte d'eau due à la transpiration sous stress hydrique par déclenchement d'une série complexe d'événements (Hopkins, 2003). L'acide abscissique entraîne, également, de nombreux changements dans le développement, la physiologie et la croissance ; il augmente le rapport pondéral racine-tiges par inhibition de la croissance foliaire et par production de racines profondes. L'éthylène régulerait la croissance et la sénescence foliaires. D'autres facteurs de régulation de la croissance sous stress hydrique tels que l'acide salicylique et les polyamines seraient impliqués (Farooq *et al.*, 2009)

4-3-4-Mécanismes moléculaires :

Ils sont l'expression de gènes impliqués dans la tolérance au stress hydrique. En effet, de nombreux gènes et des facteurs de transcription impliqués dans la réponse des plantes à la sécheresse ont été identifiés. Chez les plantes exposées à un stress hydrique, les gènes codant pour l'acide abscissique montrent une surexpression, qui augmente sa quantité. L'acide abscissique produit dans les racines, en plus de réguler la croissance, joue le rôle de signal de fermeture des stomates dans les feuilles où il induit également sa synthèse. L'acide abscissique, par une série de réactions en cascade pas totalement élucidées, induirait l'expression d'autres gènes impliqués dans les mécanismes de tolérance : les gènes codant

pour les aquaporines qui sont des protéines permettant le flux d'eau qui augmentent la conductance hydraulique et les gènes codant pour la biosynthèse de la proline, des protéines de protection etc. (Santos *et al.*, 2009) (Fig. 2).

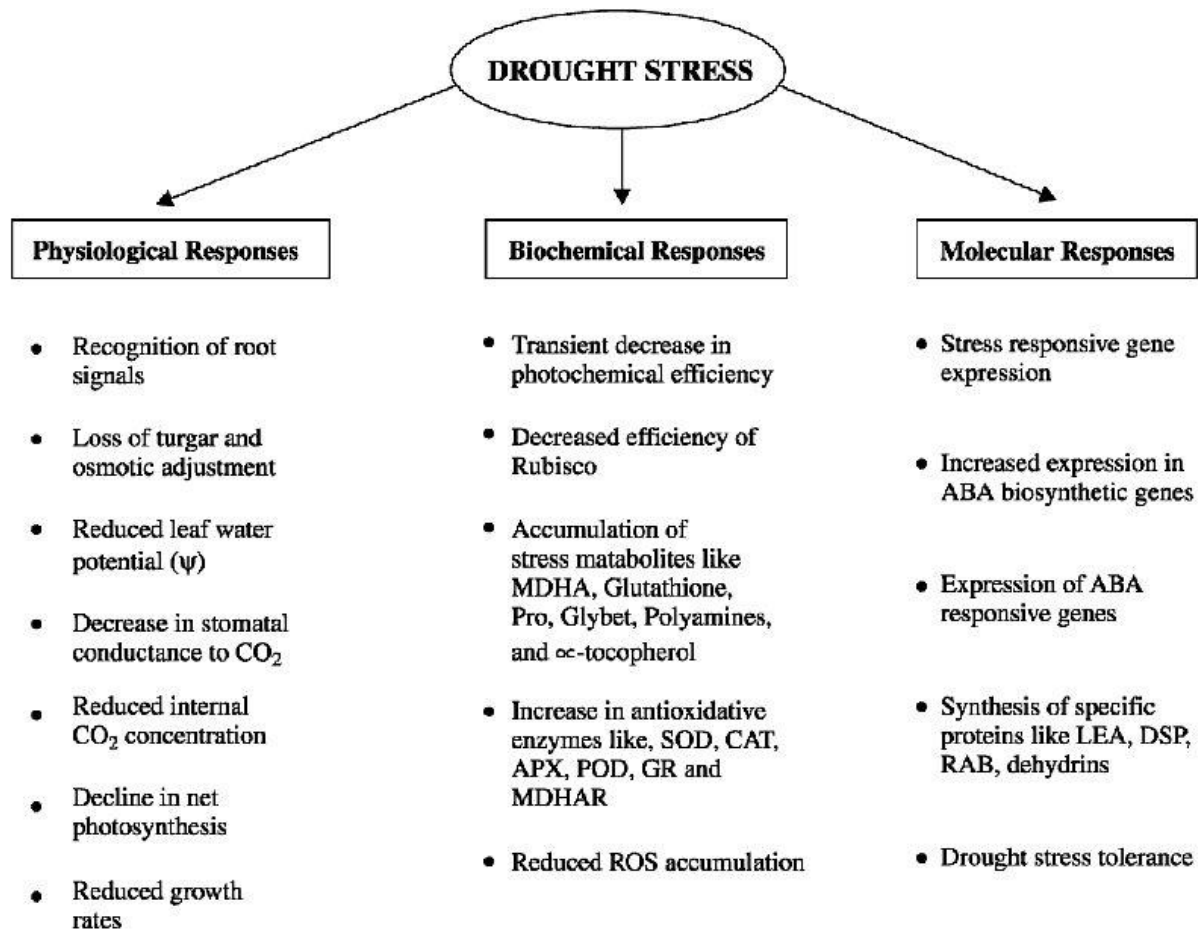


Fig. 2. Bases physiologiques et moléculaires de la tolérance au stress hydrique
(Santos *et al.*, 2009)

5-Présentation des espèces étudiées :

De nombreuses espèces appartenant à la famille des Fagaceae et au genre *Quercus* sont présentes dans la région méditerranéenne, parmi elles *Q. suber* (chêne liège) et *Q. ilex* (chêne vert) qui font partie des cinq espèces du genre *Quercus* endémiques dans la région (Quezel et Médail, 2003). Ce sont deux espèces sclérophylles sempervirentes présentant des intérêts économique et social et montrant une régression de leurs superficies en Algérie. *Pinus halepensis* est l'espèce la plus fréquente dans la région méditerranéenne appartenant à la famille des Pinaceae (Quezel et Médail, 2003).

5-1-Le chêne liège :

La classification des espèces appartenant au genre *Quercus*, famille des Fagaceae, comporte de nombreuses difficultés ; ce genre a été divisé en plusieurs sous genres ou sections et le nombre ainsi que les noms des sous genres ou sections diffèrent d'un auteur à un autre. Ainsi, selon Bussotti et Grossoni (1998), le chêne liège, *Quercus suber* L. appartient au sous genre Cerris (Schwarz, 1993) et à la section Suber (Krüssmann, 1986).

C'est une espèce typiquement ouest méditerranéenne, qui ne se rencontre nulle part ailleurs dans le monde (Fig. 3). Il constitue, avec une superficie de 227 000 ha, la 2^{ème} espèce importante en Algérie et la première dans la wilaya de Tizi-Ouzou (DGF, 2015). Près des 4/5 de la subéraie algérienne sont localisées dans le tell Oriental au Nord Est de l'Algérie jusqu'à la frontière tunisienne (Yessad et Andre, 2000). Il s'étend d'une manière assez continue le long de la zone littorale en une ligne passant approximativement par Tizi-Ouzou, Guelma et Kherrata, et il est disséminé sous formes d'îlot de moindre importance dans la partie Ouest (Tlemcen, Oran, Mascara).

Q. suber pousse entre 0 et 2000m d'altitude dans les étages bioclimatiques subhumide et humide mais peut atteindre 2400m d'altitude (Quezel et Médail, 2003). Du point de vue étages de végétation, le chêne-liège apparaît au méso-méditerranéen et au thermo-méditerranéen mais il peut aussi se développer au supra-méditerranéen et au méditerranéen supérieur sans qu'il soit l'essence principale de ces deux étages de végétation (Benabid, 2000). C'est une espèce héliophile, exigeant de forts ensoleillements, et thermophile poussant entre 13 et 16°C, craignant les gelées. Elle est strictement calcifuge colonisant surtout les sols siliceux (Quezel et Médail, 2003). Les racines superficielles peuvent être mycorhizées par des champignons tels que: *Boletus*, *Russula* et *Lactarius* (Azul et al., 2010). La surface forestière du chêne liège en Algérie est en constante régression. Les causes sont nombreuses (régénération naturelle faible, enrésinement, mauvaise exploitation du liège, attaques parasitaires...) mais les incendies restent la principale cause de cette régression (Bouhraoua, 2015). Les conséquences de la réduction des subéraies sont la disparition de cet écosystème typique de la méditerranée mais aussi la réduction de la production en liège. Afin de reconstituer le couvert forestier, de nombreuses opérations de reboisements ont été entreprises. En effet, depuis 2000, le chêne liège occupe la première place des espèces reboisées (24% des superficies boisées). Malheureusement, les taux de réussite de ces reboisements sont souvent faibles et insatisfaisants et les causes de cet échec sont multiples

(technique, organisationnel et écologique). Le taux de survie des plants passe de 80-90% à 20-50% après la saison estivale (Messaoudène *et al.*, 2011 ; Bouhraoua, 2015).

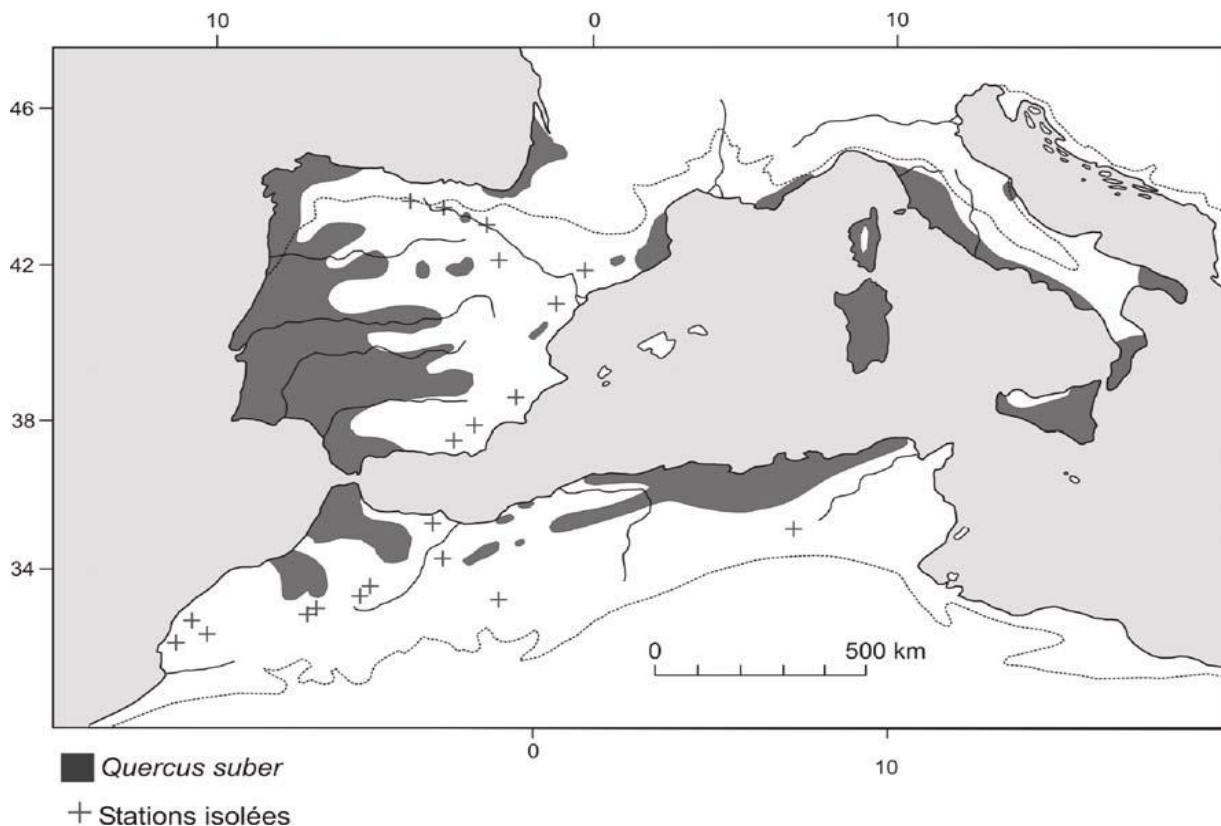


Fig.3. Aire de répartition naturelle du chêne liège dans le monde
(Quezel et Médail, 2003)

C'est une espèce à feuillage persistant, monoïque, anémophile et allogame stricte (Quezel et Médail, 2003). Il s'hybride facilement avec d'autres espèces de chêne surtout *Quercus afares* (donnant des hybrides : *Q. kabylica* Trabut et *Q. numidica* Trabut) et *Quercus canariensis* (Bussotti et Grossoni, 1998).

Le chêne liège est considérée comme une espèce tolérante à la sécheresse : **a drought-avoiding species**, qui, en été, continue à extraire la quantité d'eau nécessaire au maintien de l'hydratation de ses feuilles (Nardini *et al.*, 1999) et, sous contrainte hydrique, continue à absorber l'eau du sol grâce à un système racinaire très développé et un ajustement osmotique dans les feuilles par accumulation de certaines molécules organiques tels que les sucres solubles, les protéines solubles et la proline (Kwak *et al.*, 2011, Otieno *et al.* 2006, Pardos *et al.*, 2005). Il réduit ses pertes en eau par une diminution de sa surface foliaire et de sa conductance

stomatique (Ksontini *et al.*, 1998). Il possède également des systèmes anti-oxydants contre les dommages du stress oxydant généré par le stress hydrique (Faria *et al.*, 1996).

5-2-Le chêne vert:

Le chêne vert, *Quercus ilex* L., appartient au sous genre Sclerophyllodrys de Schwarz (1993) et à la section Ilex de Krüssmann (1986) (Bussotti et Grossoni, 1998). Il est scindé en deux sous espèces : *Q. ilex sensu stricto* s'étendant des Alpes maritime jusqu'en Turquie et *Q. ilex* subsp *rotundifolia* (Lam) (incluant *Q. ballota* Desf) présent de la péninsule ibérique jusqu'à l'Afrique du Nord (Quezel et Médail, 2003). Lebreton *et al.* (2001), sur la base de critères morphologiques et biochimiques, classent les populations nord-africaines comme une sous espèce particulière : subsp *maghrebiana* Lebreton, Barbero et Quezel. Ces différentes sous espèces sont largement présentes en Méditerranée occidentale et centrale (Quezel et Médail, 2003) (Fig. 4).

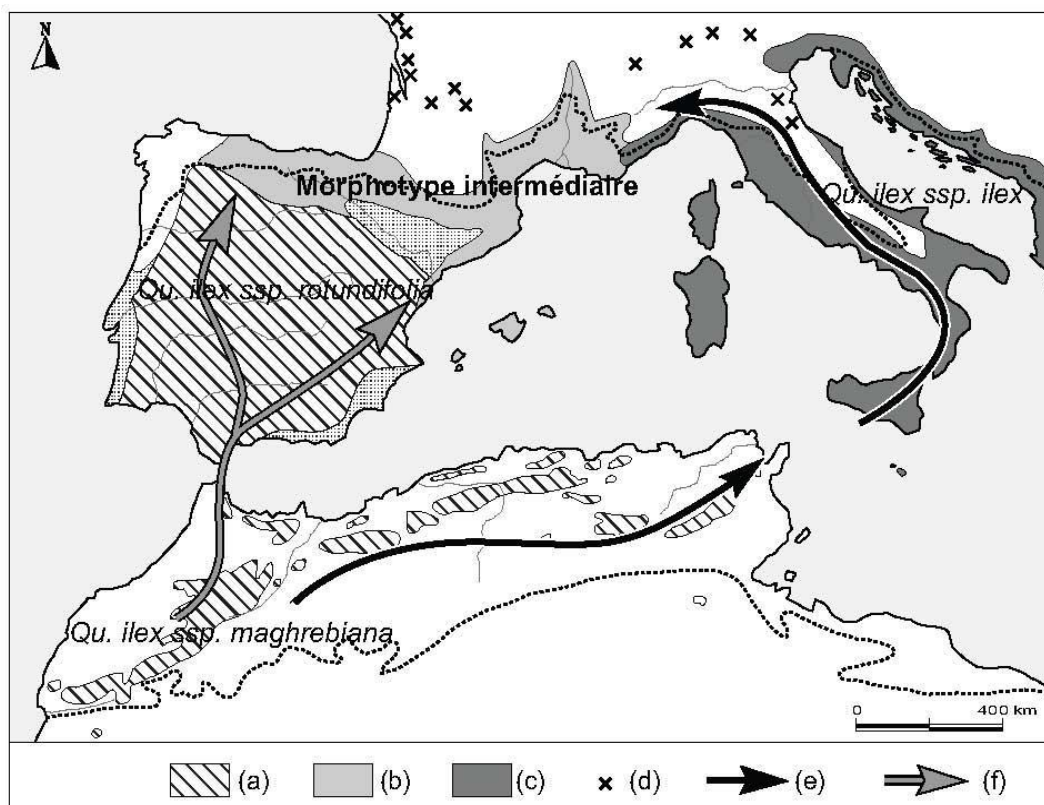


Fig. 4. Aire de répartition naturelle du chêne vert dans le monde
(Quezel et Médail, 2003)

Le chêne vert se concentre essentiellement entre 400 et 1200 m d'altitude même si on le rencontre entre 0 et 2000m d'altitude. Il présente une large amplitude écologique vis à vis

de l'eau qui le rattache aux étages de végétation thermo et méso-méditerranéens, et à l'étage supra- méditerranéen (Dahmani, 1984) et poussant dans les étages bioclimatiques semi-aride, sub-humide et humide, dans leurs variantes froide, fraîche et tempérée et même très froide. C'est une espèce sciaphile, tolérant un intervalle de températures allant de -17°C à 42°C et peu exigeant sur le plan édaphique puisqu'il peut pousser sur différents types de substrats : calcaires, siliceux et grès (Quezel et Médail, 2003). Le chêne vert occupe la troisième place des espèces les plus abondantes en Algérie avec 149 419 ha (FAO, 2013).

C'est une espèce sempervirente, monoïque, anémophile et allogame stricte (Yacine et Lumaret, 1988) qui peut se croiser avec d'autres espèces du genre *Quercus* telles que *Q. petraea*, *Q. pubescens*, *Q. coccifera* et *Q. faginea* (Bussotti et Grossoni, 1998).

Des études génétiques ont montré que le chêne liège et le chêne vert peuvent s'hybrider occasionnellement (le chêne vert étant souvent l'espèce maternelle) et surtout s'introgresser par croisement en retour des hybrides avec les formes parentales (Yacine et Lumaret, 1988).

Son comportement vis-à-vis du stress hydrique est très étudié en Europe. Sa tolérance au stress hydrique est plus élevée que celle du chêne liège. Il est considéré comme a **tolerant drought species** expliquant sa présence dans le semi-aride et sa large distribution géographique (David *et al.*, 2007). Il se caractérise par un système racinaire très développé (Ksontini *et al.*, 1998). Il possède un système d'ajustement osmotique par accumulation de certaines molécules tels que les sucres solubles (Rivas-Ubach *et al.*, 2014), les protéines et la proline (Villagrosa *et al.*, 2010).

5-3- Le Pin d'Alep:

Selon Gaussen (1960), le groupe "*halepensis*" du genre *Pinus* représente un des trois groupes de la section "*Halepensis*", caractérisé par des feuilles à deux aiguilles et des cônes caducs où plusieurs espèces ont été décrites, mais deux seulement sont considérées comme de véritables espèces par la majorité des systématiciens : *Pinus halepensis* Mill. et *Pinus brutia* Ten. Ces deux espèces se croisent souvent (Quezel et Médail, 2003).

Le Pin d'Alep est très présent au sein de la région méditerranéenne en peuplements spontanés, subspontanés ou plantés (Nahal, 1962) (Fig. 5). En Algérie, il constitue avec une superficie de 1 108 711ha la première espèce forestière (DGF, 2015). Il est l'une des espèces les plus utilisées dans les opérations de reboisements dans les conditions sèches ; il représente la plus importante espèce du barrage vert.

Sa répartition altitudinale varie en fonction de la latitude. Il se développe essentiellement aux étages thermo et méso-méditerranéens mais pénètre largement dans le supra-méditerranéen pour atteindre localement des altitudes élevées (Quezel et Barbero, 1990). En Europe, il est présent entre 0 et 800m d'altitude et en Afrique du Nord, il est très répandu entre 0 et 1400m mais il s'élève jusqu'à 2000m (versant saharien des Aurès en Algérie) et même jusqu'à 2800m d'altitude sous forme d'individus épars (Haut Atlas central au Maroc) (Quezel et Médail, 2003).

C'est une espèce de l'étage bioclimatique semi-aride même si on le rencontre également dans l'aride supérieur, le sub-humide et l'humide (Nahal, 1962). Toutefois, il présente son développement maximal dans le semi-aride et le subhumide soit entre 350 et 700mm de pluviométrie annuelle même si on peut le rencontrer entre 200 et 1500mm (Quezel et Barbero, 1990).

Le Pin d'Alep est indifférent à la nature physique ou chimique de la roche-mère (présent sur roches calcaires ou siliceuses, argileuse ou sableuse) mais lors de la régénération, il exige une certaine friabilité des matériaux (Quezel et Médail, 2003 ; Mañas *et al.*, 2010).

C'est une espèce héliophile et thermophile supportant des températures allant -2°C jusqu'à 42°C, tolérante à la sécheresse, et il est probablement l'un des pins les plus tolérants aux températures et sécheresses élevées (Scarascia-Mugnozza, 1986) ce qui explique sa large distribution géographique. L'effet de la sécheresse chez cette espèce dépend plus de sa durée que de son intensité et du stade de développement des plantules, et c'est la température et non la teneur en eau du sol qui est plus déterminante (Alexou, 2013). Face à la sécheresse, il limite les pertes d'eau en réduisant la surface foliaire (Lin *et al.*, 2010) et par fermeture précoce et rapide des stomates (Borghetti *et al.*, 1998) comme il développe aussi un système racinaire profond et ramifié pour une meilleure absorption de l'eau. Il possède également des mécanismes de protection anti-oxydants (Müller *et al.*, 2001). C'est une espèce qui peut s'associer à de nombreux champignons ectomycorhiziens (El Karkouri *et al.*, 2005).

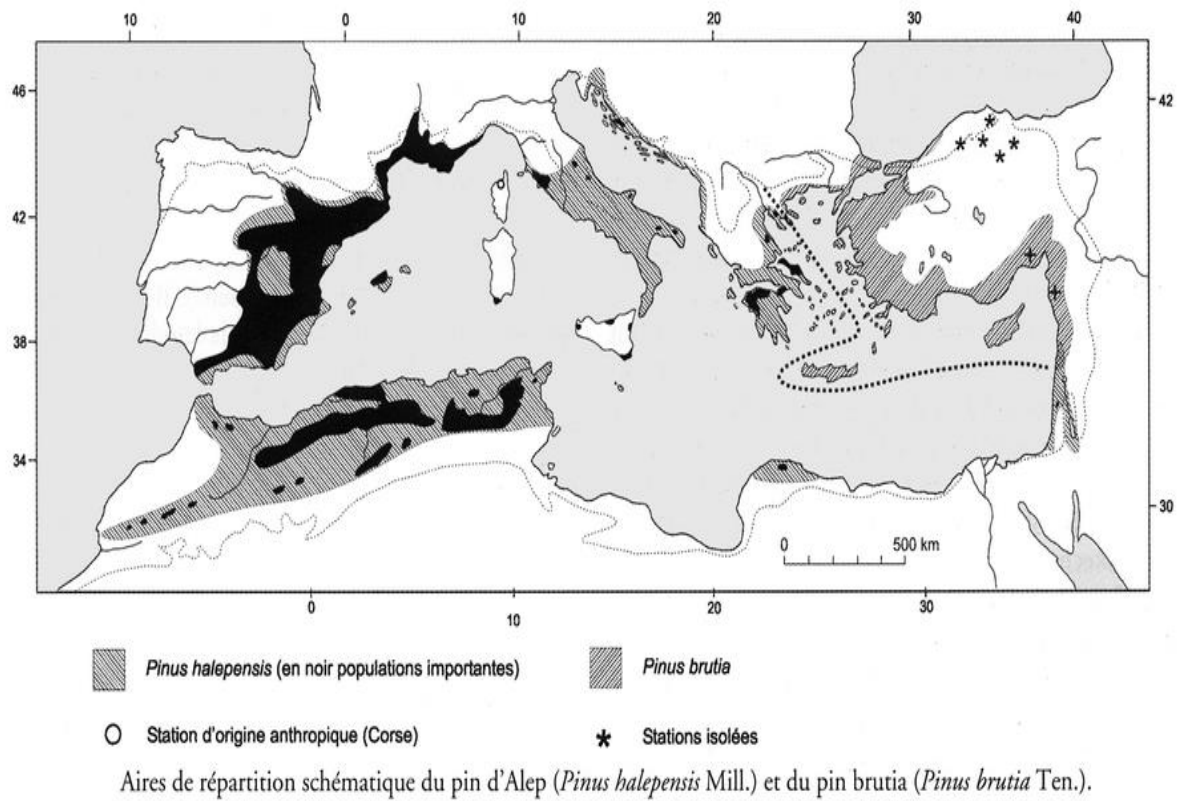


Fig. 5. Aire de répartition géographique de *Pinus halepensis* et de *Pinus brutia* (Quezel et Médail, 2003)

Matériel et Méthodes

1-Etude de l'influence d'une contrainte hydrique sur différentes provenances de chêne liège et du chêne vert:

1-1-Provenances et récolte des glands:

Les glands des deux espèces de chêne ont été récoltés de différentes stations, au pied de 10 arbres d'apparence saine, fin novembre (date de maturité des glands).

1-1-1-Le Chêne liège:

Des glands provenant de la forêt de Beni Ghobri, située à Azazga (wilaya de Tizi-Ouzou) ont été utilisés pour l'étude du comportement du chêne liège et pour l'étude comparative avec le chêne vert face à une contrainte hydrique.

Pour l'étude de l'influence des provenances des glands sur le comportement de ce chêne, face à la contrainte hydrique, nous avons récolté les glands de trois forêts : Beni-Ghobri (Azazga), Aghzar (Jijel) et M'Sila (Oran).

1-1-1-1-Azazga (Forêt de Beni Ghobri):

La station de récolte des glands est située dans la forêt de Beni Ghobri localisée dans la daïra d'Azazga, dans la wilaya de Tizi-Ouzou, à une altitude de 530m. Les caractéristiques géographiques, pédologiques et climatiques de cette station sont notées dans le tab. I. La station est située dans l'étage bioclimatique subhumide et où la sécheresse estivale dure en moyenne presque quatre mois, du début juin à mi-septembre et quelques fois plus (Fig. 6). Les données climatiques utilisées pour caractériser la station sont celles de la ville la plus proche, Azazga située à 5km

Les dates de récolte de cette forêt sont :

- fin Novembre 2010 pour l'étude de son comportement face à la contrainte hydrique,
- fin Novembre 2011 pour l'étude comparative de son comportement face à la contrainte hydrique avec celui du chêne vert.
- fin novembre 2012 pour l'étude de l'influence des provenances de chêne liège sur son comportement face à la contrainte hydrique.

1-1-1-2-Oran (Forêt de M'Sila):

La forêt de M'Sila est située dans la wilaya d'Oran à une altitude de 350m. Les caractéristiques de cette station sont notées dans le tab.I. Ce sont les données climatiques de la ville, la plus proche, Misserghine, qui sont utilisées pour la caractérisation de la station. La forêt de M'Sila constitue la provenance la plus aride et est située dans l'étage bioclimatique

semi-aride. La durée de de la saison estivale sèche est de presque cinq mois, de mi-mai à mi-octobre et quelques fois même plus (jusqu'à sept mois) (Fig. 7). Les glands ont été récoltés de cette forêt fin novembre 2012.

1-1-1-3-Jijel (Forêt d'Aghzer):

La forêt Kissir d'Aghzer est localisée dans la wilaya de Jijel à 20m d'altitude, face au siège de l'INRF, à 10km de la ville de Jijel d'où l'utilisation des données de températures et de précipitations de cette ville pour la caractérisation climatique de cette station (Tab.I). Cette forêt est la station la plus humide et se trouve dans l'étage bioclimatique humide. La sécheresse estivale dure en moyenne trois mois de juin à septembre (Fig. 8).

Les glands ont été récoltés de cette forêt fin novembre 2012.

Tab. I Localisation et caractéristiques géographiques, climatiques et pédologiques des stations de récolte des glands de chêne liège (Beni Ghobri, M'Sila et Aghzer)

Localisation	Jijel	Oran	Azazga (Tizi-Ouzou)
Forêt	Aghzer	M'Sila	BeniGhobri
Altitude	20m	350 m	530m
Longitude	36°49'0.259''E	0° 50' 19.7" W	4°22' to 4°27' W
Latitude	5° 44' 56.7''N	35°38' 22.6"N	36°42'to 6°47'N
Température annulle moyenne	18	18.1	16.7
Précipitation annuelle moyenne	1022 mm	397mm	944mm
Etage Bioclimatique	Humide (Q2=123)	Semi- aride (Q2= 55,2)	Sub-Humide (Q2=94,7)
Substrat	(Grès numidiens)	Schiste jurassique	Grès numidien
Texture Sol (Horizon superficiel)	Limoneux-sableuse	Argilo-limoneuse	Limoneux-argileuse
Matière organique	2,65	2,75	4,595
pH	6,525	6,7	5,8

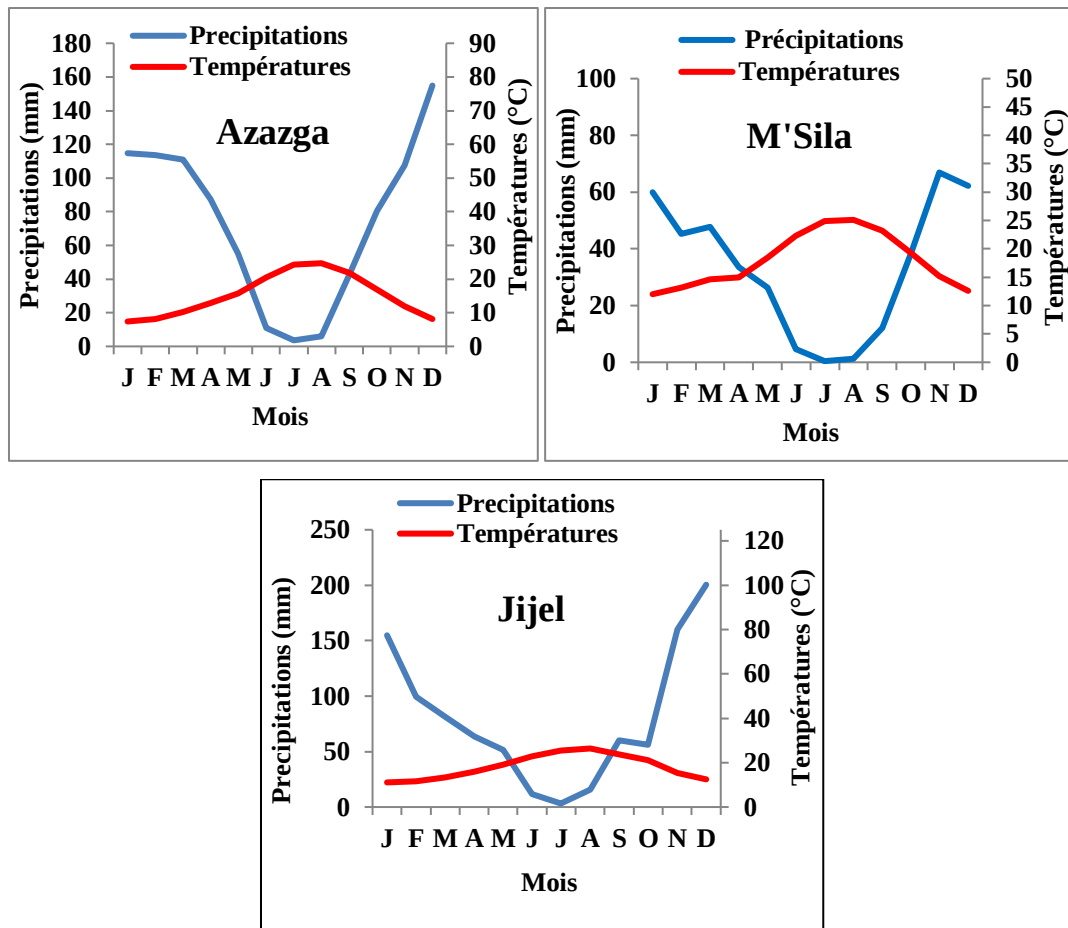


Fig.6. Diagrammes ombrothermiques de Bagnouls et Gaussen (1953) des différentes stations de récoltes des glands de *Q. suber* (Azazga, M'Sila et Jijel) (Données climatiques 2001-2011)

1-1-2-Le Chêne vert :

Les glands de *Quercus ilex* subsp *maghrebiana* ont été récoltés, en même temps que les glands de chêne liège (de la forêt de Beni Ghobri), fin novembre 2011, d'Ait Mahmoud, appartenant à la commune de Mekla dans la wilaya de Tizi-Ouzou à une altitude de 927m, dans l'étage bioclimatique sub-humide (Tab. II) afin de comparer leurs comportements face à la contrainte hydrique appliquée. Le manque de données climatiques de cette station nous a poussé à utiliser les données de la ville de Larbaa Nath Irathen située à 942m d'altitude.

Tab.II Données géographiques et caractéristiques climatiques de la station de récolte des glands de chêne vert (Mekla)

Altitude	Latitude	Longitude	Précipitation annuelle moyenne (mm)	Température annuelle moyenne (°C)	Etage bioclimatique
927m	36° 30' 30. 22'' N	3° 59' 35. 4729'' E	900	17,2	Sub-Humide (Q ₂ =104,551)

Le diagramme ombrothermique de Bagnouls et Gaussen (1953) montre l'existence d'une sécheresse estivale s'étalant sur quatre mois, de mi-mai à mi-septembre (Fig. 7).

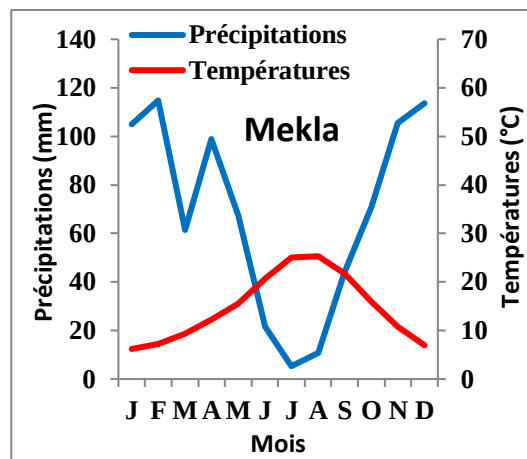


Fig.7. Diagramme ombrothermique de Bagnouls et Gaussen (1953) de la station de récolte des glands de *Q. ilex*

1-2-Germination des graines :

Avant la germination, nous avons soumis les glands à une stratification (4°C pendant 30 jours) pour la levée de dormance. La germination des glands a ensuite été effectuée dans des boîtes de Pétri à une température de $20 \pm 2^\circ\text{C}$ à raison de 10 glands par boîte.

1-3-Transplantation des plantules :

Les glands germés ayant une racicule de 1cm de longueur ont été transplantés, fin janvier :

- pour l'étude du comportement du chêne liège seul, sur sol forestier de la forêt de Beni Ghobri mélangé avec du sable (2mm de diamètre approximativement) dans une proportion de 2/3 et 1/3 respectivement,

- pour l'étude comparative du comportement du chêne liège et du chêne vert ainsi que de l'étude de l'influence des trois provenances de chêne liège face à la contrainte hydrique, sur un même substrat composé de 2/3 de terreau (N 110-250 mg/l, P₂O₅ 60-140 mg/l, K₂O 120-280mg/l, structure fine et pH de 6,2) et de 1/3 de sable afin de ne pas favoriser une espèce ou une provenance donnée (Tab. III).

Des sachets en matière plastique, de 20cm de diamètre et de 30cm de profondeur, sont remplis avec la même quantité de substrats. Les plantules ont été cultivées dans une serre non contrôlée, située à l'intérieur de la faculté des Sciences Biologiques et des Sciences Agronomiques, à Bastos, à l'UMMTO (coordonnées géographiques : 36° 43' 464 N et 4° 1' 531 E, altitude de 140 m, étage bioclimatique subhumide, Température annuelle moyenne 19,2°C, Précipitation annuelle moyenne 705mm). L'arrosage a été pratiqué régulièrement à raison de 50ml d'eau (trois fois par semaine) pendant cinq mois (février à juin).

1-4-Application de la contrainte hydrique en serre :

La contrainte hydrique a été appliquée par arrêt d'arrosage, pour simuler la sécheresse estivale méditerranéenne, sur des plantules âgées approximativement de six mois, pendant 10 semaines, de fin juin à mi-septembre pour les deux chênes (liège et vert) et les trois provenances de chêne liège. Parallèlement aux plants stressés non arrosés, des plants ont été arrosés pour servir de témoins. Les températures de la serre variaient entre 25 et 41°C (Tab. III).

Deux lots de plantules, avec des hauteurs moyennes des tiges principales identiques (hauteur des plantules d'un lot = moyenne $\pm$ 1cm), sont constitués par espèce : *Q. suber* arrosé (*Q. suber* W) et non arrosé (*Q. suber* NW) et *Q. ilex* arrosé (*Q. ilex* W) et non arrosé (*Q. ilex* NW) et par provenance : Jijel arrosé (J W), Jijel non arrosé (J NW), M'Sila arrosé (M W), M'Sila non arrosé (M NW), Azazga arrosé (A W) et Azazga non arrosé (A NW).

Nous avons réalisé trois répétitions par étude :

- à raison de 30 plants/répétition et chaque répétition était constituée de 15 plants/ traitement : arrosé (*Q. suber* W) et non arrosé (*Q. suber* NW) pour l'étude de l'influence de la contrainte hydrique par arrêt d'arrosage sur le chêne liège.
- à raison de 40 plants/répétition, chaque répétition était constituée de 10 plants/traitement/espèce : *Q. suber* arrosé (*Q. suber* W), *Q. suber* non arrosé (*Q. suber* NW), *Q. ilex* arrosé (*Q. ilex* W) et *Q. ilex* non arrosé (*Q. ilex* NW) pour l'étude comparative de l'influence de l'arrêt d'arrosage sur le chêne liège et le chêne vert.

- à raison de 60 plants par répétition, chaque répétition était constituée de 10 plants/traitement/provenance : Jijel arrosé (J W), Jijel non arrosé (J NW), M'Sila arrosé (M W), M'Sila non arrosé (M NW), Azazga arrosé (A W) et Azazga non arrosé (A NW) pour l'étude de l'influence de la provenance sur la réponse du chêne liège à l'arrêt d'arrosage.

Le protocole expérimental est résumé dans le tab.III.

Tab. III. Récapitulatif des expériences réalisées

Espèce/Provenance	Date Récolte	Substrat	T° Serre (°C)	Nombre de répétitions	Nombre Plants/Répétition
<i>Q. suber</i> Azazga	Fin Novembre 2010	Sol Beni Ghobri 2/3 + Sable 1/3	25-41	3	30 à raison de 15 Plants/Traitement (Q. s W, Q. s NW)
<i>Q. suber</i> Azazga	Fin Novembre 2011	Terreau 2/3 + Sable 1/3	25-38	3	40 à raison de 10 Plants/Traitement/Espèce (Q. s W, Q. s NW, Q.i W, Q. i NW)
<i>Q. ilex</i> Mekla					
<i>Q. suber</i> Azazga	Fin Novembre 2012	Terreau 2/3 + Sable 1/3	25-38	3	60 à raison de 10 Plant/Traitement/Provenance (JW, J NW, M W, M NW, A W, A NW)
<i>Q. suber</i> M'Sila					
<i>Q. suber</i> Jijel					

1-5-Evaluation de l'influence de la contrainte hydrique sur le statut hydrique:

Après 10 semaines d'arrêt d'arrosage, soit mi-septembre, nous avons évalué l'influence de la contrainte hydrique par arrêt d'arrosage sur le statut hydrique, la croissance et des paramètres biochimiques des jeunes plants âgés approximativement de 9 mois.

1-5-1-Evaluation de la teneur en eau du sol (SWC) :

La teneur en eau du sol (SWC) a été déterminée sur des échantillons de sol prélevés à une profondeur de 10 cm pour tous les lots (Q.s W, Q.s NW, Q.i W et Q.i NW) à raison de 10 répétitions/lot. Elle a été calculée par la formule de Mathieu et Pielain (2003):

$$PF-PS/PF \times 100$$

PF correspond au poids frais et PS au poids sec (105°C pendant 72h) d'un gramme de sol.

1-5-2-Evaluation de la teneur relative en eau (RWC):

La teneur relative en eau (ou RWC) a été déterminée selon la méthode de Nardini *et al.*, (1999) pour tous les lots non arrosés et témoins à raison de cinq plants/lot par la formule :

$RWC (\%) = \frac{PF - PS}{PT - PS} \times 100$

PF : poids frais d'une feuille

PS : poids sec de la feuille (72h à 75°C)

PT : poids turgescant de la feuille

1-5-3- Evaluation du pourcentage de l'ectomycorhization :

Nous avons déterminé le taux de l'infection ectomycorhizienne des plante de chêne liège seulement transplantés sur sol forestier de Beni Ghobri en observant les systèmes racinaires entiers nettoyés à l'eau, à raison de 10 plants/traitement : arrosé (W) et non arrosé (NW). Les critères permettant d'identifier une ectomycorhize sont :

- sous loupe binoculaire : absence de poils absorbants, présence ou non de mycélium extramatriciel
- au microscope optique : présence d'un manteau fongique

Le pourcentage de l'infection ectomycorhizienne a été calculé selon la formule de Parke *et al.*, (1983) :

$$\% \text{Ectomycorrhization} = \frac{\text{Nombre d'apex ectomycorhizés}}{\text{Nombre total d'apex (ectomycorhizés et non ectomycorhizés)}} \times 100$$

1-6-Evaluation de l'influence de la contrainte hydrique sur la croissance des plants:

Après dix semaines d'arrêt d'arrosage, nous avons mesuré sur les lots témoins et stressés (10 plants/lot), plusieurs paramètres morphologiques de croissance : hauteur de la tige principale (cm), le poids de la biomasse foliaire et les poids secs des deux systèmes aérien et racinaire (après 72h à 65°C). Nous avons ensuite calculé le rapport poids sec du système racinaire/poids sec du système aérien (R/S). La surface foliaire spécifique (SLA) a été déterminée à l'aide d'un scanner AM350 Portable Leaf Area Meter selon la méthode de *Faria et al.* (1996).

1-7-Evaluation de l'influence de la contrainte hydrique sur les paramètres biochimiques:

L'effet de la contrainte hydrique (arrêt d'arrosage) sur plusieurs paramètres biochimiques a été évalué, par des méthodes spectrophotométriques, chez deux lots de plants témoins (arrosés) et stressés (non arrosés), à raison de cinq plants par lot : Nous avons ainsi mesuré :

- La teneur en chlorophylles et caroténoïdes (Lichtenthaler et Buschmann 2001).

- Les quantités de sucres solubles selon la méthode de Cerning-Berorard (1975).
- Les teneurs en protéines solubles selon la méthode de Bradford (1976).
- Les quantités de proline par la méthode de Monneveux et Nemmar (1986)
- Les taux de polyphénols totaux uniquement chez le chêne liège en 2010 (Peñuelas *et al.*, 1996).
- Les teneurs en $\delta^{13}\text{C}$, %C et %N foliaires selon la méthode de Warren et Adams (2000).

1-8-Détermination du taux de mortalité :

Les taux de mortalité ont été déterminés, après 10 semaines de contrainte hydrique (arrêt d'arrosage), dans tous les lots non arrosés des deux espèces chêne liège et vert et des trois provenances de chêne liège (Aghzer à Jijel, M'Sila à Oran et Beni Ghobri à Tizi-Ouzou), par la formule suivante :

$$\text{Taux de mortalité} = \frac{\text{Nombre de plants morts}}{\text{Nombre total de plants}} \times 100$$

2- Etude de l'influence d'une inoculation ectomycorhizienne sur la réponse à une contrainte hydrique des plantules de Pin d'Alep :

2-1-Provenance des graines :

Les graines de *Pinus halepensis* M. ont été récoltées de la forêt domaniale de Tlemcen, située dans le parc national de Tlemcen. Cette forêt est constituée par un boisement artificiel à base de Pin d'Alep réalisé en 1890. Les caractéristiques géographiques et climatiques de cette station sont notées dans le tab.IV.

Tab.IV Données géographiques et caractéristiques climatiques de la station de récolte des graines de Pin d'Alep (Tlemcen)

Altitude (m)	Latitude	Longitude	Précipitation annuelle moyenne (mm)	Température annuelle moyenne (°C)	Etage bioclimatique
994	34°51' 573	1°.355 101	433,6	17,5	Semi-aride (Q ₂ =93,823)

La saison sèche est longue, elle s'étale des mois d'Avril à Octobre quelques fois (Fig. 8).

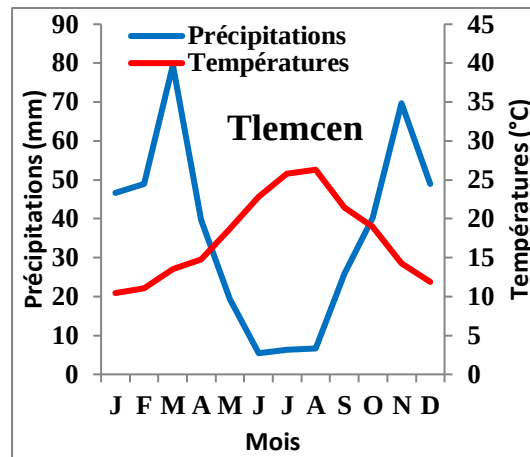


Fig.8. Diagramme ombrothermique de Bagnouls et Gaussen (1953) de la station de récolte (Tlemcen) des graines de *Pinus halepensis*

2-2-Germination des graines :

Les graines de Pin d'Alep ont été désinfectées dans du H₂O₂ 30% pendant 30mn (Fini *et al.*, 2011). Après plusieurs rinçages à l'eau distillée stérile, les graines sont mises à stratifier, dans des boîtes de Pétri à raison de 20 graines/boîte, à 4°C pendant le mois de janvier 2015 puis mises à germer à 20 ±2°C, au début du mois de février 2015.

2-3-Transplantation des plantules:

Les graines germées ayant des racines de 2cm de longueur ont été transplantées, fin février, dans des sachets en matière plastique de 20cm de diamètre et de 30cm de profondeur, remplis avec la même quantité de substrat. Le substrat utilisé est un mélange de 2/3 de terreau (N 110-250 mg/l, P₂O₅ 60-140 mg/l, K₂O 120-280 mg/l, pH of 6.2) et de 1/3 de sable stérilisé deux fois à 200°C pendant une heure à intervalle de 48h (Fini *et al.*, 2011). Les transplantations sont réalisées dans des conditions d'asepsie rigoureuses à raison d'un plant/sachet.

2-4-Inoculation ectomycorhizienne :

Deux lots de 90 plants chacun, ont été constitués après transplantation : un lot inoculé avec *Boletus edulis* et un lot non inoculé, considéré comme témoin. Les carpophores de *B. edulis* ont été récoltés de la forêt de Bois de Rejouit à Bordeaux (France) et déterminés par l'association mycologique locale.

L'inoculation des plantules a été effectuée deux fois : au moment de la transplantation et 15 jours après transplantation à l'aide de 50ml d'une suspension sporale, préparée dans de l'eau distillée stérile. Chaque plant inoculé a reçu une quantité finale approximative de 10⁶ spores (détermination à l'aide de la cellule de Malassez). Les plantules inoculées et témoins

ont été cultivées en serre en conditions non contrôlées, localisée à l'intérieur de la faculté des Sciences Biologiques et des Sciences Agronomiques, à Bastos (UMMTO). Tous les plants, inoculés ou non, ont été arrosés d'une manière régulière (trois fois par semaine) jusqu'à fin juillet.

2-5-Application de la contrainte hydrique :

Avant l'application de la contrainte hydrique, nous avons constitué deux lots de 90 plants chacun : un lot inoculé et un lot non inoculé. La contrainte hydrique a été appliquée à 45 plants/lot, en arrêtant l'arrosage, pendant six semaines à partir de fin juillet jusqu'à mi-septembre 2015. Nous avons effectué trois répétitions inoculées (30 plants/répétition dont 15 plants étaient arrosés et 15 plants n'étaient pas arrosés) et trois répétitions non inoculées (30 plants/répétition dont 15 plants étaient arrosés et 15 plants n'étaient pas arrosés). Durant cette période la température de la serre variait entre 25 et 41°C.

Après six semaines d'arrêt d'arrosage, mi-septembre 2015, nous avons évalué l'effet de l'arrêt d'arrosage sur le statut hydrique, l'ectomycorhization, la croissance et le taux de survie des plantules inoculées et non inoculées.

2-6-Evaluation de l'infection ectomycorhizienne :

Le pourcentage de l'infection ectomycorhizienne a été calculé selon la formule de Parke *et al.*, (1983), comme pour le chêne liège (§1-5-3) en observant les systèmes racinaires entiers nettoyés à l'eau, à raison de 10 plants/traitement : inoculé arrosé (IW), inoculé non arrosé (I NW), non inoculé arrosé (NI W) et non inoculé non arrosé (NI NW).

2-7-Etude de l'influence de la contrainte hydrique sur le statut hydrique :

Nous avons déterminé, de la même manière que pour les chênes étudiés, la SWC (10 répétitions/traitement) et la RWC (cinq répétitions/traitement).

2-8- Etude de l'influence de la contrainte hydrique sur la croissance :

Nous avons évalué l'effet de l'arrêt d'arrosage sur la croissance des plantules, âgées approximativement de six mois, par la mesure de certains paramètres morphologiques:

- Hauteur de la tige principale (en cm)
- Diamètre de la base du collet (en mm)
- Poids de la biomasse foliaire (en g)
- Rapport poids sec système racinaire/poids sec système aérien
- Poids moyen d'une feuille (en g)

Le nombre de répétitions est de 10 plants/traitement. Pour le poids moyen d'une feuille, le nombre de répétition est de cinq feuilles/plant et de 10 plants/traitement.

2-9- Etude de l'influence de la contrainte hydrique sur le taux de survie :

Le taux de survie a été déterminé chez les lots non arrosés seulement chaque semaine, pendant huit semaines par la formule :

$$\text{Taux de survie} = \frac{\text{Nombre de plants vivants}}{\text{Nombre total de plants}} \times 100$$

3- Analyse statistique :

Tous les résultats obtenus ont été soumis à une analyse statistique à l'aide du logiciel Statistica :

-par le test de Student quand les conditions de normalité et d'homogénéité des variances sont remplies sinon test de Mann-Whitney dans le cas contraire pour l'étude du comportement du chêne liège seul

-par l'ANOVA, quand les conditions des tests de normalité et d'homogénéité des variances sont remplies, dans le cas contraire, le test de Kruskal-Wallis est réalisé. Une différence est considérée significative quand $p < 0,05$.

Résultats et Discussion

1-Growth, ectomycorrhization and biochemical parameters of *Quercus suber* L. seedlings under drought conditions

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Abstract

Drought conditions are the major constraint for the early establishment *Quercus suber* species. However, drought responses of this species depend on provenances. The objective of this study was to obtain more comprehensive knowledge about the influence of drought conditions on the response of *Q. suber* L. seedlings originating from Algeria. Soil water status in soil (SWC) and relative water content in leaves (RWC), morphological parameters for growth evaluating and physiological parameters and ectomycorrhization were evaluated in two plots of seedlings: watered (W) and none watered (NW). Ten weeks of water starvation induced a reduction in relative water content (RWC), ectomycorrhization rate and length and diameter growth of none watered seedlings. Under drought conditions, both soluble sugars and proteins enhanced while polyphenols and %N decreased. The reduction of soil water content was negatively correlated to Carbone isotope discrimination. The survival of the seedlings under these drought conditions is due to two strategies: osmotic adjustment through soluble sugars and proteins accumulations in leaves and an increase in Carbone discrimination which enhances the water use efficiency (WUE).

Key words: *Quercus suber*, growth, drought, ectomycorrhization, pigments, sugars, starch, polyphenols, $\delta^{13}\text{C}$.

1-1-Introduction:

Summer drought in the Mediterranean region is characterized by four months dry period with little or no precipitations, high temperatures and high irradiance consider the major constraints for vegetation (Aussenac, 2000; Faria *et al.*, 1996). Mediterranean species are thus, often exposed to water stress during summer (Pardos *et al.*, 2005). Mediterranean oak species have developed mechanisms to avoid excessive loss of cell water and to maintain growth (Caritat *et al.*, 2006). Increased drought severity is expected in the Mediterranean basin over the twenty-

first century (Aussenac 2002; Ramirez-Valentine *et al.*, 2011) and the increase in the length of the dry season may lead to severe water deficit and tree mortality (Kurze-Besson *et al.*, 2006). Priority should be given to ecophysiological research for the study of the adaptative plants Mediterranean behavior under these predicted summer conditions.

The cork oak (*Quercus suber*) is a western Mediterranean species, widely distributed in Mediterranean forest, thus presenting significant ecological and economic interests. It is an evergreen and sclerophyllous species growing from the sea level up to 700 m in altitude (Quezel and Médail, 2003). This plant is well adapted to summer conditions because it maintained a favorable ratio between water loss and uptake during the dry period. Also, *Q. suber* is considered as a drought tolerant species (Nardini *et al.*, 1999; Nardini and Tyree 1999). Maintaining a favorable water status in tissues, with a high relative water content (RWC) during summer drought, is ensured by deep roots and/or osmotic adjustment through accumulation of molecules such as proteins, sugars and proline (Kwak *et al.*, 2011; Otieno *et al.* 2006; Pardos *et al.* 2005). A decrease in shoots and leaves biomass by reducing growth and root drop is also noted (Kurze-Besson *et al.*, 2006; Ksontini *et al.*, 1998).

Cork oak is an ectomycorrhizal species. Molecular and morphological approaches analysis led to the identification of fifty-five taxa of fungi forming wide diversity of ectmycorrhizas (Azul *et al.*, 2010). Under drought conditions, controlled ectomycorrhization increases the performance of *Quercus suber* L. (cork oak) nursery and field seedlings (Sebastiana *et al.* 2013) but little is known about the effect of drought on ectomycorrhization (Richard *et al.*, 2011; Shahin, 2012).

The carbon isotope discrimination ($\delta^{13}\text{C}$) of foliage integrates signals resulting from environmental constraints like water availability (Warren and Adams, 2000). Therefore, $\delta^{13}\text{C}$ may be a useful indicator of drought stress in seasonally dry climates (Warren *et al.*, 2001) because water supply affects the stomatal conductance and photosynthesis of plants, which changes $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates (Du *et al.*, 2015).

Although *Q. suber* is well adapted to dry conditions, it remains that its natural regeneration is low. This seedlings recruitment limitation probably will be amplified with the predicted global changes in Mediterranean region. Muhamed *et al.* (2013) showed that spatial patterns of association between understory shrubs and oak seedlings are very sensitive to increasing drought under climate change, while it is known that plants neighbors can promote oak regeneration.

Many studies on *Q. suber* trees and seedlings behavior to water stress on the northern Mediterranean shore exist (Nardini and Tyree, 1999; Nardini *et al.*, 1999; Otieno *et al.*, 2006), however few studies on the responses of this oak species face water stress were performed in Algeria (Acherar *et al.*, 1991).

Understanding the responses of cork oak to actual and predicted summer conditions is essential to determine the future sustainability of cork oak woodlands. In this context, the effects of water scarcity on *Q. suber* were studied through growth, water relations, ectomycorrhization, photosynthetic pigments, sugars, starch, protein, polyphenols, $\delta^{13}\text{C}$ and % N contents.

1-2-Materials and methods:

1-2-1-The experimental setting:

Acorns of cork oak (*Quercus suber*) were collected in November 2010, from Beni Ghobri forest located in Azazga (Tizi-Ouzou, Algeria) (36°42' to 36°47' N; 4°22' to 4°27' W longitude, 620 m altitude). After one month stratification at 4°C, seeds germinated at 20°C. To limit the effect of acorn variation in the seedlings, the seedlings with similar shoot height were transplanted to plastic bags (30 cm x 15 cm) filled with 1.5 kg of soil substrate. Soil substrate consisted of a mixture of natural soil from Beni Ghobri forest (2/3) as source of ectomycorrhizal inoculums and washed sand (1/3). The plants were grown in a greenhouse, localized at Tizi-Ouzou (140 m altitude, 36°42' 12886" N and 4°2' 53 3339" E) characterized by a Mediterranean climate, and were watered regularly (three fold a week) until mid-July. Then we constituted two plots (30 seedlings and 3 replicates/ plot) of seedlings: watered (W) and none watered (NW). Ten (10) weeks after mid-July (end of September), seedlings of the two treatments (W, NW) were harvested for determination of morphological and physiological parameters.

1-2-2-Measurement of substrate moisture contents:

Measurements of substrate moisture volumetric content were made for the plots. Soil samples of 1 g are taken from the plastic bags at a depth of 10 cm and dried at 105°C for 72 h and then water content was calculated using the formula: $(FW - DW) / FW$ (Mathieu and Pieltain, 2003) where FW and DW are the fresh and dry weight, respectively.

1-2-3-Measurement of relative water content (RWC):

The RWC was measured on fully expanded leaves as described by Nardini *et al.*, (1999). Five plants and three leaves per plants were examined. Fresh weight (FW) of the leaves was determined immediately after harvested and then were allowed to float on distilled water during 24 h at 4°C, then turgid weight (TW) of the leaves was determined. The dry weight (DW) of the leaves was determined after drying at 75°C during 72 h. The RWC were calculated as: $RWC = (FW - DW) / (TW - DW) \times 100$

1-2-4-Morphological parameters:

Morphological parameters were determined on 10 seedlings per treatment. Stem length and collar diameter were measured. Shoots, roots and leaves biomass were determined after drying at 75°C during 72 h. Then the root to shoot ratio was estimated.

1-2-5-Physiological parameters:

Chlorophylls, sugar, starch, proteins and total polyphenols contents were determined on five seedlings per treatment and two to three leaves/seedling. Chlorophylls contents of fresh leaf discs (0.1 g) were determined spectrophotometrically after extraction in acetone 80% in the dark and the chlorophylls contents were calculated as proposed by Lichtenthaler and Buschmann (2001) : $Chl\ a+b = 7.15 \times A_{663} - 18.71 \times A_{647}$

Soluble sugars were extracted from fresh leaves in ethanol (70%), the residues was incubated in HCl (1. 1% v/v) for 30 min at 95°C for starch extraction. Then, soluble sugars and starch were quantified colorimetrically at 625 nm with anthrone reagent following Cerning-Berorard (1975) method. Soluble proteins contents were determined following Bradford (1976) method. They were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595 nm after colorimetric reaction with Bradford reagent.

Total polyphenols were determined spectrophotometrically following the method described by Peñuelas *et al.* (1996). 2.5 g powder per sample of dry leaves were extracted with 20 ml of 70% aqueous methanol (v/v) acidified with some concentrated HCl drops. The samples were left at ambient temperature in the dark for an hour and a half. The extracts were filtered and the total polyphenols were quantified after colorimetric reaction using Folin-Ciocalteu reagent during one hour in dark at 765nm. The total polyphenols content was calculated as gallic acid equivalent from the calibration curve of gallic standard solutions and expressed as mg gallic acid equivalent / g of dry weight.

Foliage samples for analysis of carbone isotopic discrimination $\delta^{13}\text{C}$, %C and %N were dried at 70°C for 72 h and ground to a fine powder. The abundance in combusted samples was performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) in CNRS UMR 7266 LIENSS with a precision of 0.1‰. We calculated $\delta^{13}\text{C}$ (‰) with respect to the PDB Pee Dee Belemnite standard : $\delta^{13}\text{C} = (R_{\text{sample}} / R_{\text{standard}} - 1)1000$

Where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in a sample and the standard (Pee Dee Belemnite), respectively (Warren and Adams, 2000).

1-2-6-Ectomycorrhizal colonization assessment:

Percentage of ectomycorrhization was determined with a binocular. Percentage ectomycorrhization was calculated as mycorrhizal root apex / total root apex (mycorrhizal and non mycorrhizal) of the all root system (Parke *et al.*, 1983).

1-2-7-Statistics analysis:

Statistical analysis was performed using STATISTICA software (Version 7.1; StatSoft Inc.). The differences between the two lots (watered and none watered) for all recorded data were compared by the student test where the conditions of normality and equality of variances are checked. Otherwise, a Mann Whitney none parametric test was achieved. The significance level for all the tests was $P < 0.05$.

1-3-Results:

1-3-1-Water status:

The seedlings of *Q. suber* were grown in irrigated and water starvation conditions for 10 weeks in greenhouse prior to morphological and physiological data measurements. At the end of the culture, significant changes were shown from the two lots in terms of substrate water contents (Fig. 9a). The reduction of water availability was accompanied by a significant decrease in leaf relative water status (RWC), the RWC values were 85.43 and 70.78% in watered and none watered seedlings respectively (Fig. 9b).

1-3-2-Ectomycorrhization rate:

The soil of Beni Ghobri forest was rich in ectomycorrhizal inoculum. The approximatively eight month's cork oak seedlings were colonized by ectomycorrhizal fungi. The difference in extracellular matrix (ECM) rate between the two treatments was significant. ECM decreased in drought conditions (<9%) while the well watered seedlings showed higher ECM (>39%)

(Fig. 9c). So, the ectomycorrhization was approximatively 5-fold higher in well watered seedlings.

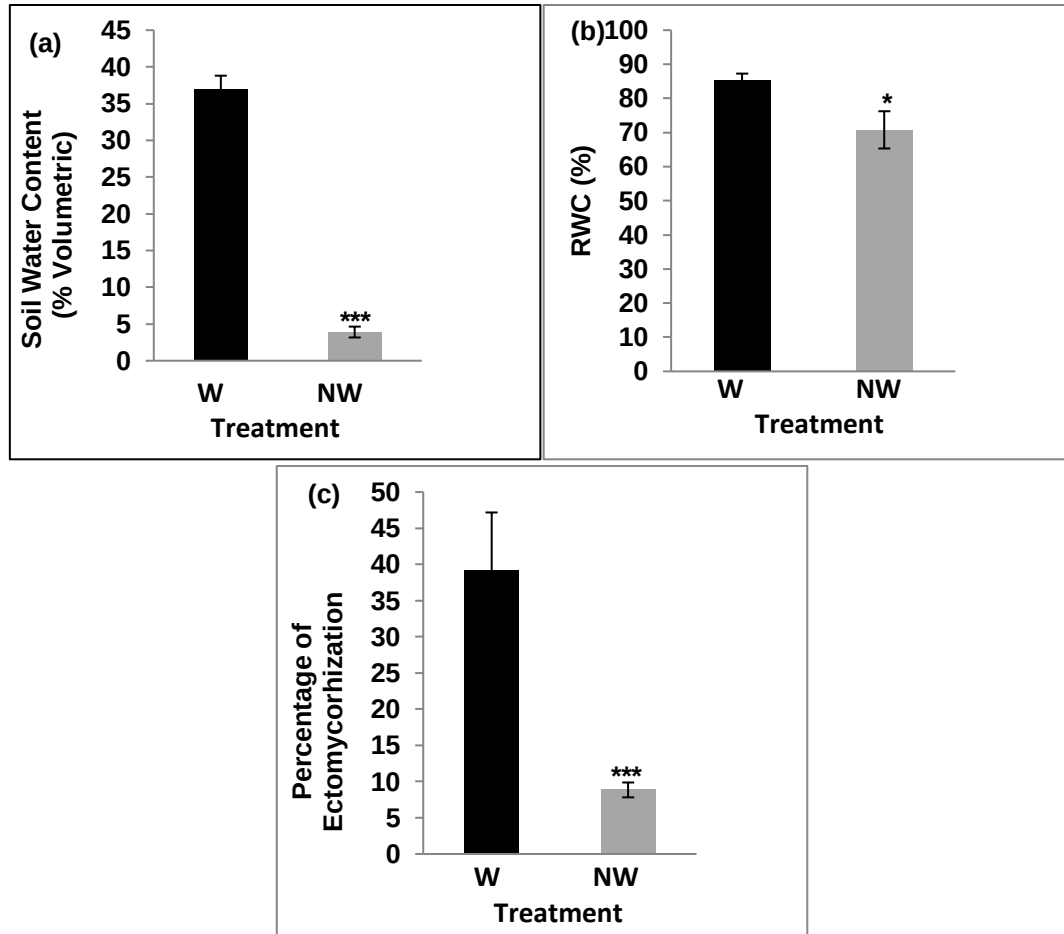


Fig. 9. Effects of drought conditions on water status and ectomycorrhization of *Quercus suber* seedlings: **(a)** Soil water content (SWC), **(b)** leaf relative water content (RWC) and **(c)** ectomycorrhization rate (ER) in watered (W) and non-watered (NW) seedlings. Means $\pm$ SE for RWC n=5, SWC n=10, and ER n=10. (*: p<0.05, ***: p<0.001).

1-3-3-Seedlings growth:

Length of the main stem was reduced in none irrigated lot (Fig. 10a). The leaf biomass and root/shoot ratios did not differ significantly between the two batches studied (W and NW) (Fig. 10b and c). The statistical analysis showed that the diameter of the stem at the base was significantly higher in control watered plants compare to none watered ones (Fig. 10d).

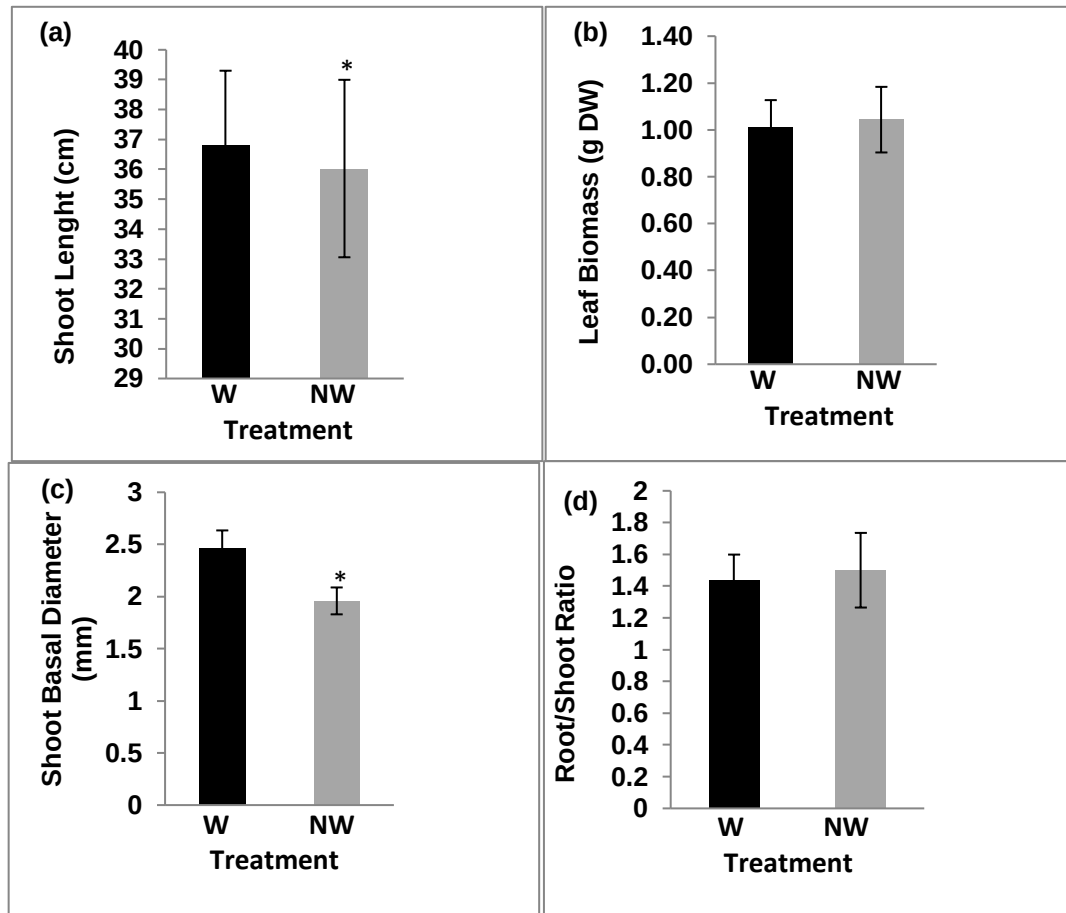


Fig. 10. The effects of drought conditions on morphological traits of *Quercus suber* seedlings: (a) shoot length (b) leaf biomass, (c) shoot basal diameter and (d) root/shoot ratio in watered (W) and non-watered (NW) seedlings. Means $\pm$ SE for shoot length n=20; leaf biomass n=12; shoot basal diameter n=13 and shoot/root ratio n=10. (*: $p < 0.05$).

1-3-4-Physiological parameters:

The majority of physiological variables investigated were statistically different (Fig. 11 and 12) between the two lots (W and NW). Total chlorophyll contents showed no significant difference between watered and non-watered seedlings (Fig. 11a). Proteins leaves contents recorded in none watered samples were higher than in well watered seedlings (Fig. 11b). The levels of soluble sugars tend to increase with drought conditions. Especially, this drop was approximately 3-fold in none watered conditions compare to watered conditions (Fig. 11c). The starch content showed a little increase in none watered seedlings (1.026 fold higher) (Fig. 11d). On the contrary, total polyphenols synthesis and/or accumulation globally decrease. A significant decrease was observed (1.5-fold) in none watered seedlings when compared to the control individuals (Fig. 11e).

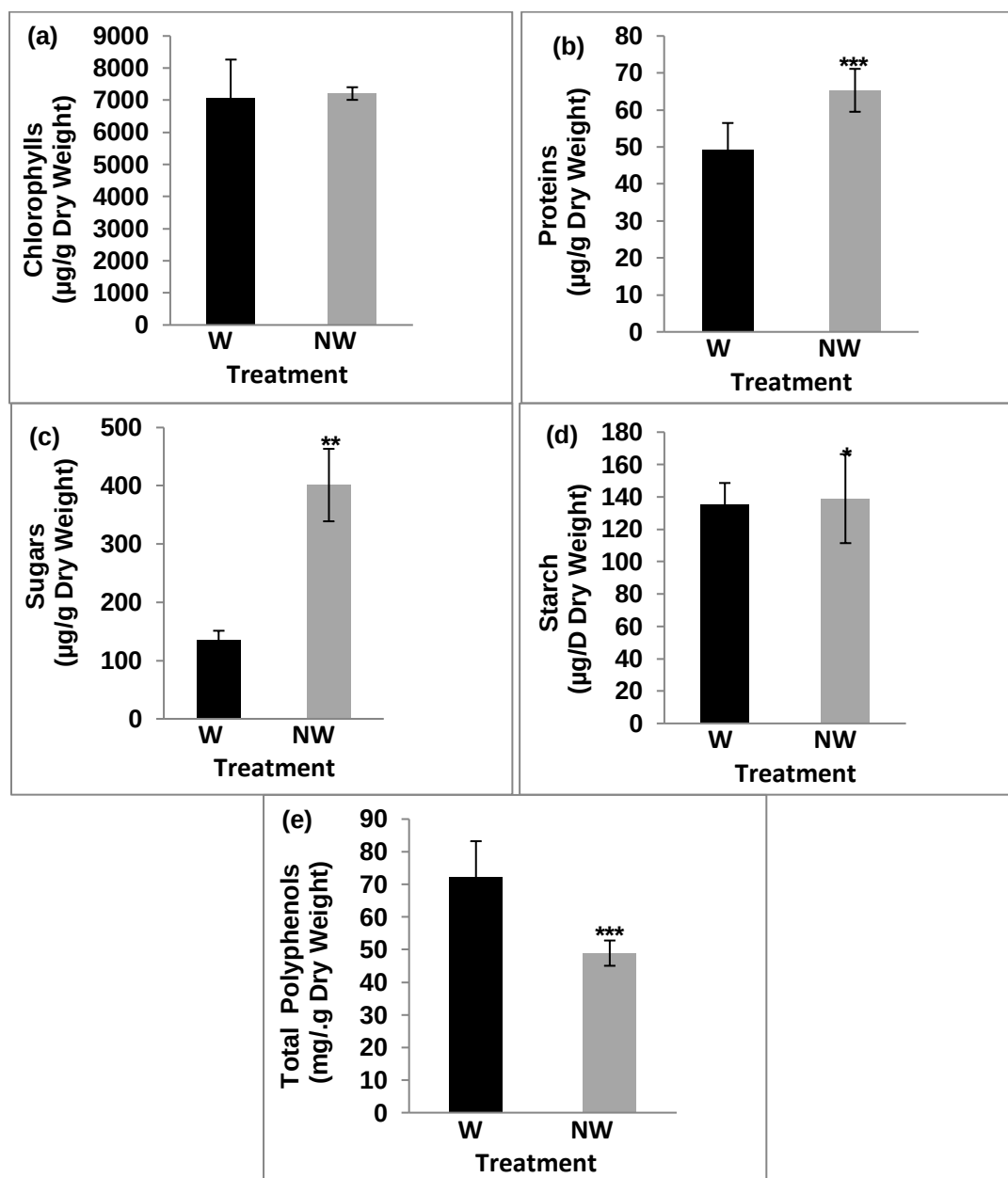


Fig. 11. The effects of drought conditions on biochemical traits of *Quercus suber* seedlings: (a) Chlorophylls, (b) proteins, (c) sugars, (d) starch and (e) total polyphenols leaves contents in watered (W) and non-watered (NW) seedlings. Means $\pm$ SE for all variables n=5. (*: $p < 0.05$, ***: $p < 0.001$).

Non watering induced a significant increase of $\delta^{13}\text{C}$ contents in leaves; the $\delta^{13}\text{C}$ was much higher in none watered (-32.04) than in watered (-32.79) *Q. suber* seedlings (Fig. 12a).

The total nitrogen concentrations (%N) in leaves were different between the two treatments; N content was reduced in drought conditions (Fig. 12b). The %C in leaves were similar in watered conditions, thus, drought had no negative effect on this parameter (Fig. 12c).

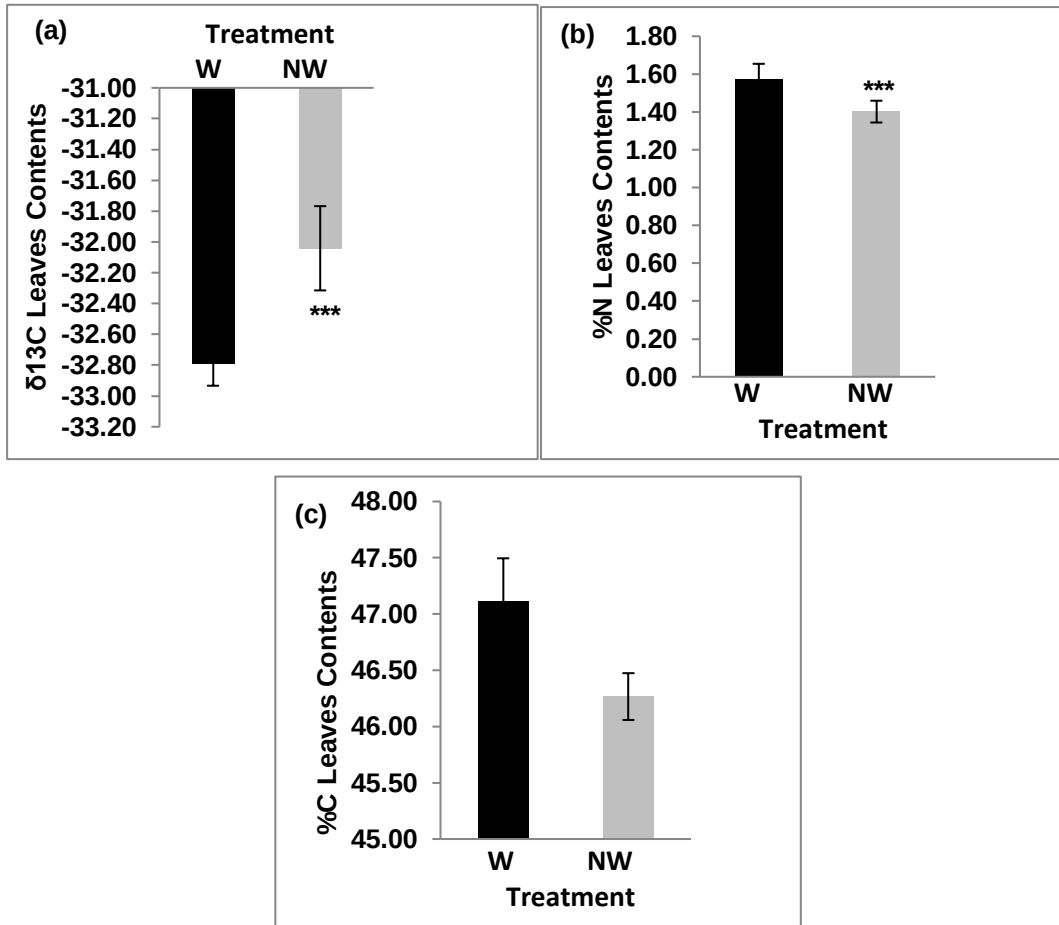


Fig. 12. The effects of drought conditions on $\delta^{13}\text{C}$, %C and %N leaves contents of *Quercus suber* seedlings: **(a)** $\delta^{13}\text{C}$, **(b)** %N and **(c)** %C in watered (W) and non-watered (NW) seedlings. Means $\pm$ SE. For all variables $n=5$. (***: $p<0.001$).

The study evaluated the correlations between SWC and ectomycorhization, $\delta^{13}\text{C}$, %N and %C parameters (Fig. 13). The $\delta^{13}\text{C}$ leaves contents showed a significant negative correlation with SWC; the reduction of SWC enhanced $\delta^{13}\text{C}$ leaves contents ($r^2 = 0.5829$; $r = -0.7635$; $p = 0.0006$; $y = -31.918 - 0.0293 \cdot x$) (Fig. 5a) while the total nitrogen (%N) and %C leaves concentration did not show correlation with the SWC ($p > 0.05$) (Fig. 13b and c). A positive correlation exists between SWC and ECM rate ($r^2 = 0.3109$; $r = 0.5576$; $p = 0.0162$; $y = 8.245 + 0.7775 \cdot x$), ECM is decreasing with SWC (Fig. 13d).

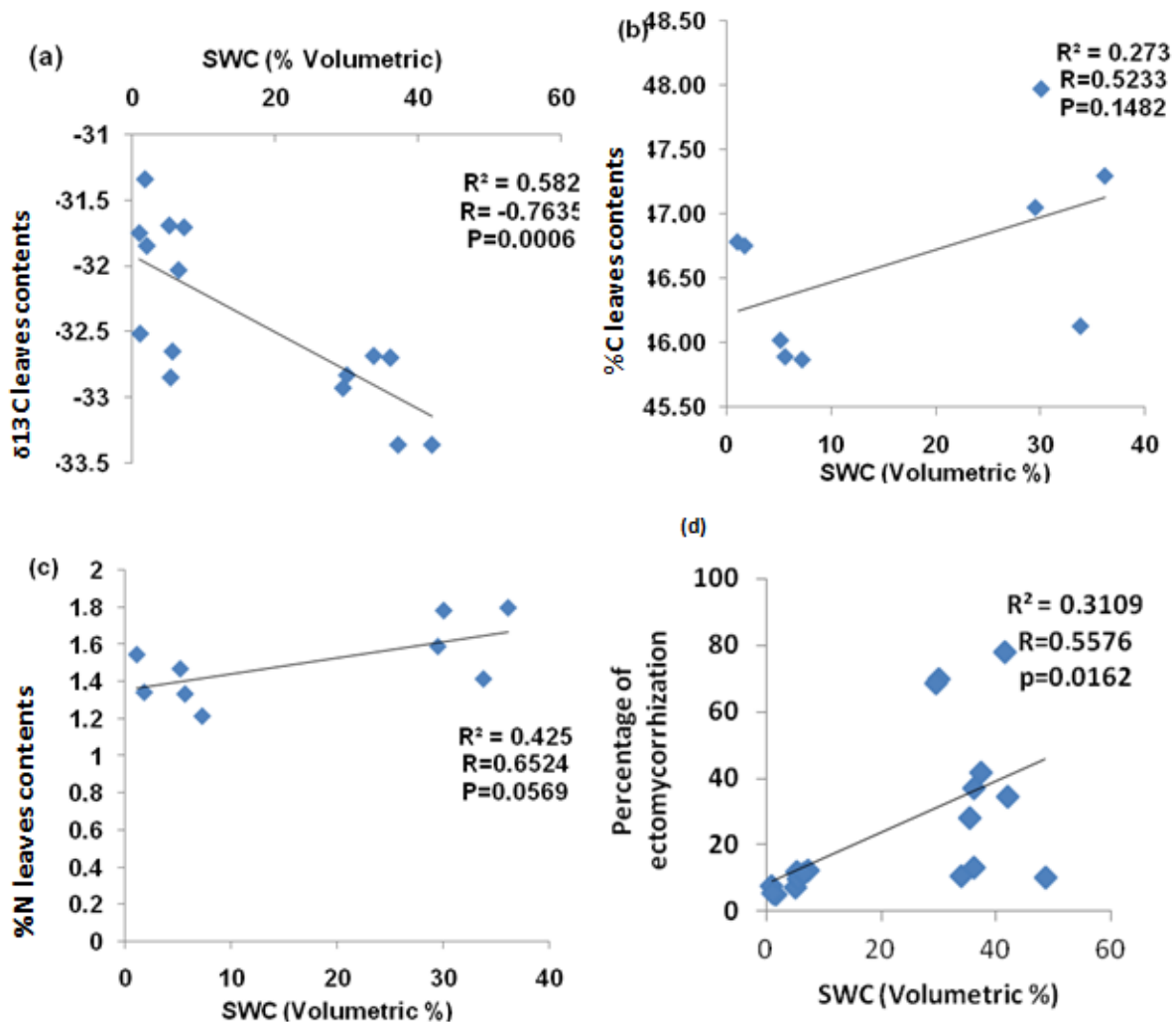


Fig. 13. Correlations between SWC and $\delta^{13}\text{C}$, %C, %N and ectomycorrhization parameters of *Quercus suber* seedlings: **(a)** $\delta^{13}\text{C}$, **(b)** %C, **(c)** %N leaves contents and **(d)** percentage ectomycorrhization.

1-4-Discussion:

When comparing the two treatments, soil water status in none watered pots was about 9.5-fold lower compare to watered substrates (Fig. 9). This decrease was accompanied by a significant reduction in leaf relative water content (RWC) of the eight-month old seedlings (from 85.43 to 70.78%). In summer, the soil moisture volumetric content dropped to 5 to 7% along the upper 0.6 m explored and leaf RWC decreased to a nearly constant level of about 82% that were only 8% less than those recorded in the spring (Nardini *et al.*, 1999). The low values of RWC recorded in this study may be due to more arid climatic conditions. Favorable tissue

water status in *Q. suber* during summer drought is achieved through deep rooting which facilitate soil water uptake (Otieno *et al.*, 2006).

The lower ECM colonization in *Q. suber* seedlings in drought conditions was previously obtained by Shahin (2012) in *Quercus ilex* and *Quercus pubescens* seedlings. The lower ECM colonization in dry conditions can be explained by the fact that photosynthates quantities are insufficient to both plant and fungal, thus they preferentially allocated to plant growth or ectomycorrhizal inoculum was less abundant in none watered lot (Shahin, 2012). Some studies reported that inoculated seedlings with ectomycorrhizal fungi enhanced plant tolerance to water stress due to their less resistance to water flow from soil to roots, by increasing the absorbing surface and the ability of the fungus to penetrate finer pores as those operated by hairy roots (Parke *et al.*, 1983).

Length and diameter growth were reduced by drought conditions (Fig. 2). While primary growth is sensitive to winter-spring warming, secondary growth is sensitive to summer-autumn warming (Camarero *et al.*, 2015). Cambium activity is known to be negatively influenced by drought (Caritat *et al.*, 2000). Interruption of shoot growth in the dry summer is a significant adaptation trait (Kurze-Besson *et al.*, 2006) and thus, water stress tends to decrease the ratio of shoot biomass / root biomass (Ksontini *et al.*, 1998). Regarding the physiological parameters investigated, differences were shown (Fig. 3 and 4).

Drought generally causes decrease in photosynthetic pigments (Rajasekar and Manivannan, 2015). Decrease in chlorophyll contents was due to decrease in chlorophyll biosynthesis rather than its degradation (Jain *et al.*, 2013). Vaz *et al.* (2010) showed no difference in *Q. suber* tree leaves from summer to autumn.

Osmotic adjustment is found to help cork oak seedlings to maintain turgor during moderate stress. To maintain water uptake, plants increased the water potential gradient between the plant cells and soil by increasing solute concentrations in the root cells. Like in this study results, in *Quercus* seedlings species, soluble sugars increased in drought-treated plants relative to control well watered plants and the opposite pattern was found in starch (Rodríguez-Calcerrada *et al.*, 2011). Higher soluble sugars concentrations in cork oak seedlings grown under moderate water stress provoked a decrease of the osmotic potential and are at least partly responsible of osmotic adjustment which maintain RWC >80% (Pardos *et al.*, 2005). Soluble proteins also contribute to osmotic adjustment (Kwak *et al.*, 2011).

Polyphenols contents decreased in non-watered seedlings of *Q. suber*. Moderate experimental drought increased the concentrations of polyphenolic compounds with antioxidant function in the leaves of *Q. ilex* trees (Rivas-Ubach *et al.*, 2014). Polyphenols are synthesized in large quantities during water stress, high temperatures or exposure to solar radiation, which are conditions that characterize the Mediterranean summer (Hernandez *et al.*, 2009). Sometimes, the quantities were higher in watered plants like in loblolly pine needles (Booker and Maier, 2001).

Secondary metabolism in plants remains unclear; different regulation responses in the polyphenols pathway probably exist varying with species, development stage of the plant, intensity, duration and rate of progression of the stress (Liu *et al.*, 2011).

The increased carbon discrimination recorded in drought conditions was previously reported in *Q. suber* trees by Gouveia and Freitas (2009) which observed that trees subjected to greater water stress had $\delta^{13}\text{C}$ enriched leaves reflecting the trade-off between assimilation rate and water loss. The increase of carbon isotope discrimination with drought induces an increase in water-use efficiency (WUE) (Richard *et al.*, 2011).

The study results show that drought conditions reduced %N leaves contents while previous results have reported that leaf total nitrogen did not show a significant change in water stressed treatments (Kwak *et al.*, 2011). In *Q. suber* trees, significant correlations were not found between total N and rainfall (Gouveia and Freitas, 2009).

The study carried out in semi-controlled conditions, concludes that *Q. suber* seedlings are affected by 10 weeks of summer drought. The survival of the seedlings under these conditions is due to two strategies: osmotic adjustment through soluble sugars and proteins accumulations in leaves and an increase in Carbon discrimination which enhances the water use efficiency (WUE).

Some responses (stability of chlorophylls contents and R/S ratios, decrease in polyphenols contents) obtained in this study with Algerian *Q. suber*, are contrary to other *Q. suber* provenances responses (Europe and Tunisia).

Further studies could involve the responses of different provenances face actual and future water stress, this will permit to understand the mechanisms of *Q. suber* tolerance to drought conditions. The success of reforestation in the perspective of Mediterranean climate aridity which is scheduled for the next decades needs provenances well adapted to these future conditions.

1-5-Conclusion :

L'étude réalisée dans les conditions semi-contrôlée a montré que la sécheresse estivale de 10 semaines a eu des effets négatifs sur les plantules de chêne liège. Pour leur survie, les plantules ont adopté deux stratégies : un ajustement osmotique par accumulation de sucres et des protéines solubles dans les feuilles et une augmentation des teneurs des feuilles en $\delta^{13}\text{C}$ pour une meilleure efficacité de l'utilisation de l'eau (WUE).

Cependant, certains effets observés avec cette provenance algérienne (stabilité des teneurs en chlorophylles et du rapport R/S et la diminution des teneurs en polyphénols) sont contraires aux résultats obtenus avec les provenances tunisiennes et européennes.

Pour une meilleure compréhension des mécanismes de tolérance du chêne liège à la sécheresse actuelle et future, d'autres études sur l'impact de la sécheresse sur différentes provenances sont nécessaires. En effet, la réussite des opérations de reboisements dans la région méditerranéenne, plus aride selon les prévisions des climatologues dans le futur, dépend des provenances utilisées qui doivent être plus adaptées à cette sécheresse future.

2-Comparative drought responses of *Quercus suber* L. and *Quercus ilex* L. seedlings under greenhouse conditions.

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Article soumis

Abstract

This study investigated the cork and holm oak seedlings responses to drought conditions. The drought conditions were achieved by not watering the seedlings during July and August. The water status and different morphological and physiological parameters were measured in watered and non watered seedlings of both species (controls and stressed) after ten weeks. The results showed that the drought conditions induced for both species a decrease of the relative water content (RWC), of the height and the thickness growths and of the specific leaf area (SLA). The roots/shoots ratios were enhanced in both species to permit a better water substrate exploration. In both species, the accumulation of soluble proteins and proline under drought conditions assured an osmotic adjustment which is considered as a drought tolerance mechanism. The effect of the drought on pigments and sugars concentrations differs between the two oak species. Not watering did not affect the %C and %N leaves contents in the two oaks. An improvement of the Carbone isotope discrimination in the two species was induced by the drought conditions. Survival rate was significantly higher in holm oak than in cork oak seedlings.

Keys words: *Quercus suber*; *Quercus ilex*; Drought; RWC; Growth; Physiology.

2-1-Introduction:

Drought is the main environmental stress for the Mediterranean vegetation (Ramírez-Valiente *et al.*, 2010). Indeed, the Mediterranean climate is characterized by a long summer drought often spanning over five months (May to October) which receive little or no precipitation (Faria *et al.*, 1996). The summer drought leads to water deficits that affect many physiological processes and can have consequences for survival and plant growth (Larcher, 2003; Lo Gullo *et al.*, 2003). Strategy adaptation is especially important at the seedling stage since seedling establishment and growth in field conditions strongly depend on soil water availability. The capacity of the different species to avoid damaging effects determines their chances of survival and growth (Vilagrosa *et al.*, 2010).

Given this water stress, mediterranean plant species have developed different strategies of adaptation: morphological (deep rooting, smaller leaf size), anatomical (increased leaf and cuticle thicknesses, increased stomatal density and decreased stomatal size) and physiological like osmotic adjustment (Abrams, 1990). A variety of compounds can contribute to osmotic adjustment, but soluble carbohydrates (Picon *et al.*, 1997), inorganic ions and amino acids predominate in most species (Gebre *et al.*, 1994).

The isotopic composition of carbon ($\delta^{13}\text{C}$) in leaves has been correlated with many factors such as altitude (Warren *et al.*, 2001), leaf specific area and nitrogen concentration (Ramírez-Valiente *et al.*, 2010), mean annual precipitation (Du *et al.*, 2015), water deficit and water use efficiency (WUE) (Roussel *et al.* 2009). However, water availability is a predominant determinant of the changes in the carbon-13 isotope leaves content ok at regional and global scales, this is due to the fact that water supply affects the stomatal conductance and photosynthesis of plants, which changes the $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates (Du *et al.*, 2015). The stable C isotope composition ($\delta^{13}\text{C}$) of leaf tissue has been used as an index of water availability (Warren *et al.*, 2001).

Holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.) are the main sclerophyllous evergreen oak species in the Western Mediterranean basin (Methyl *et al.*, 1996; Villar-Salvador *et al.*, 2004). *Q. ilex* grows in humid, sub-humid and semi-arid bioclimates, from sea level up to 2800 m elevation, although altitudes decreasing at higher latitudes. *Q. ilex* is even present in the Saharian Atlas in Algeria, where the summer drought lasts 6-7 months. *Quercus suber* grows between 0 and 2400 m elevation under humid and sub-humid bioclimates (Quezel and Médail, 2003). Therefore, these species face a long summer drought.

Oak species exhibit two drought-response strategies to water stress: drought avoidance mechanism like deep root system and smaller leaf area and drought tolerance mechanism like osmotic adjustment (Dickson and Tomlinson, 1996).

Many studies on *Q. ilex* (Camarero *et al.*, 2015 ; Aguadé *et al.*, 2015) and *Q. suber* (Nardini and Tyree, 1999 ; Nardini *et al.*, 1999 ; Otieno *et al.*, 2006) behavior under water stress on the northern Mediterranean shore exist but only few studies have made a direct comparison of the their drought tolerance features (Tyree and Cochard, 1996 ; Chaves *et al.*, 2002). *Q. ilex* seems to have more effective drought avoidance and drought tolerance mechanisms than *Q. suber* which is in accordance with the geographical distribution and leaf phenology of these species (David *et al.*, 2007). So, *Q. ilex* occupies dryer inland areas and has a leaf lifespan of

more than 18 month, whereas *Q. suber* has a shorter leaf longevity (about 12 months), dominates in the wetter western areas and can be considered as transitional between evergreen and deciduous oaks (Pereira *et al.*, 1987).

Global warming announced in the Mediterranean region should lead to a significant decrease in rainfall and an increase in the frequency, the duration and the intensity of summer droughts which would submit these species to a more intense water stress (Aussenac, 2002). Therefore additionnal research is needed for the prediction of the stress responsiveness of Mediterranean species in order to anticipate and prevent the effects of global warming in this region and to carry out a judicious choice of species for afforestation programs.

Understanding the responses of cork and holm oaks to actual and predicted summer conditions is indeed essential to determine the future sustainability of oaks in Mediterranean forests. Few studies on the responses of these two oak species face water stress were performed in Algeria (Acherar *et al.*, 1991; Daoudi *et al.*, 2016). Thus, the objective of this work was to measure the morphological and physiological behaviors of these species in a summer drought simulated by a water starvation.

2-2-Materils and methods:

2-2-1-Plants materials and experimental description:

Acorns of cork oak (*Quercus suber*) and holm oak (*Quercus ilex*) were collected, at the end of autumn 2011, respectively from Beni Ghobri and Mekla forests located at Tizi-Ouzou (Algeria). Tab. IV shows the characteristics of these two stations.

Tab. V Characteristics of *Q. suber* and *Q. ilex* harvest stations of acorns

Station	Latitude	Longitude	Altitude	Climate	Precipitation Annual mean (mm)	Temperature annual mean (°C)
Beni- Ghobri	36°42'to 6°47'N	4°22' to 4°27' W	620m	Mediterranean Sub-Humid	944	16,7
Mekla	36° 37' 370'' N	4° 17' 329'' E	927m	Mediterranean Humid	900	17,2

After one month stratification at 4°C, acorns germinated at 20°C in Petri dishes. One cm root length seedlings were transplanted into plastic bags (30cm x15cm) filled with 1.5 kg

of soil substrate consisting of a mixture of loam (2/3) and washed sand. All the plants were grown in a non controlled greenhouse, located next to the university of Tizi-Ouzou in Algeria (36° 43' 464 N and 4° 1' 531 E, 140 m elevation), a city which is characterised by a sub-humid Mediterranean climate. The seedlings were watered regularly (twice a week) until mid-June. Then four plots were constituted: *Q. ilex* watered (*Q.i* W) and non-watered (*Q.i* NW), *Q. suber* Watered (*Q.s* W) and non-watered (*Q.s* NW). The greenhouse temperatures varied between 25 °C to 38°C during the experiment. Ten weeks after mid-June, plants of the four plots were harvested in September to determine their morphological and physiological parameters.

2-2-2-Substrate water content (SWC) measurement:

Substrate samples of 1g were taken from the plastic bags of the four lots at a depth of 10 cm and dried at 105 ° C for 72 h. Their water content (SWC) was calculated using the equation 1 where FW and DW are the fresh and dry weights, respectively (Mathieu and Pieltain, 2003).

$$SWC = \frac{(FW - DW)}{FW} \times 100 \quad (\text{eq. 1})$$

2-2-3-Relative water content (RWC) measurement:

Relative water content (RWC) was measured in three young leaves of five plants per lot as described by Nardini *et al.* (1999). Leaves were detached and immediately weighted to get their fresh weight (FW). Leaves were then restaurated with water to near full turgor by immersing their petioles in water, covering the leaf blade with plastic film and leaving them in the dark for 12 h. Leaves were then reweighted to obtain their turgid weight (TW) and put into oven at 75°C for 3 days to obtain their dry weight (DW). Finally, RWC was calculated according to equation 2:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (\text{eq. 2})$$

2-2-4-Morphological parameters measurements:

To evaluate the seedling growth, different morphological parameters were measured for 10 plants per plot: stem heights, collar diameters and shoot, root and leave biomasses were determined after drying at 75°C during 72h. These measures allowed the estimation of root to shoot ratios.

The specific leaf area (SLA) was determined on three mature leaves per plant and for ten plants per plot as the ratio of Leaf Area (determined with AM350 Portable Leaf Area Meter) to dry mass of individual leaves, dry mass (DM) measured after oven-drying at 75 °C to a constant weight (Faria *et al.*, 1996).

2-2-5-Physiological parameter measurements:

Pigments, Sugar, Starch, Proteins, Proline, %C, %N and $\delta^{13}\text{C}$ leaves contents were determined on four seedlings per lot.

Total Chlorophylls and Carotenoids contents of 0,1g fresh leaf discs were determined spectrophotometrically after extraction in acetone 80% in dark and the Pigments contents were calculated according to equations 3 and 4 as proposed by (Lichtenthaler and Buschmann, 2001):

$$\text{Chl T} = (7.15 \times A_{663}) - (18.71 \times A_{647}) \quad (\text{eq. 3})$$

$$\text{Car T} = (1000 \times A_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b) \quad (\text{eq. 4})$$

After the extraction of the soluble sugars from fresh leaves in ethanol (70%), the solid fraction was used for starch analysis. Starch was incubated in HCl (1. 1% v/v) for 30 min at 95°C for starch extraction. Then, soluble sugars and starch concentrations were determined colorimetrically at 625 nm with anthrone reagent following method cited by (Cerning-Berorard, 1975). Glucose was used as a standard for both soluble sugars and starch.

Soluble Proteins were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595nm after colorimetric reaction with Bradford reagent following (Bradford, 1976). BSA was used as a standard.

Proline content was quantified by the ninhydrin-colorimetric method at 515nm after extraction in methanol 70% (Monneveux and Nemmar, 1986). Proline was used as a standard.

Foliage samples for $\delta^{13}\text{C}$, %C and %N analysis were dried at 75 °C for 72 h and ground to a fine powder. The abundance in combusted samples were performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) in CNRS UMR 7266 LIENSS with a precision of 0.1‰. We calculated $\delta^{13}\text{C}$ (‰) with respect to the PDB Pee Dee Belemnite standard (equation 5) where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in a sample and the standard (Pee Dee Belemnite), respectively (Warren and Adams, 2000).

$$\delta^{13}\text{C} = \frac{R_{\text{sample}}}{(R_{\text{standard}} - 1)} \times 100 \quad (\text{eq. 5})$$

2-2-6-Statistical analysis:

Statistical analysis was performed using STATISTICA software (Version 7.1; StatSoft Inc.). The differences among the plots (*Q.i* W, *Q.i* NW, *Q.s* W and *Q.s* NW) for all recorded data were compared by the one-way analysis of variance (ANOVA) where the conditions of normality and equality of variances are checked following an LSD test. Otherwise, a Kruskal-Wallis ANOVA was achieved. The significance level for all the tests was $P < 0.05$.

2-3-Results:

2-3-1-Water status:

The obtained results showed some differences between the two *Quercus* species behaviors in well watered and drought conditions.

Contrary to expectation, leaf water status was better in *Q. suber* than in *Q. ilex* seedlings as shown by the RWC recorded in watered and non-watered conditions. Water withholding conditions for 10 weeks, accompanied with an important reduction of water substrate content (Fig. 14a), had more negative effect on *Q. ilex* seedlings for which the RWC was significantly lower ($< 75\%$) than for *Q. suber* ($> 82\%$) (Fig. 14b).

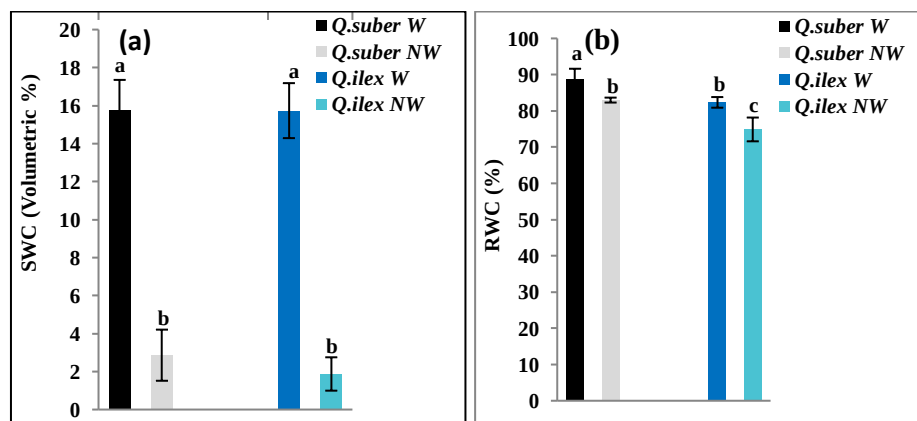


Fig.14. Water status measurements: **(a)** soil water contents and **(b)** relative water contents (RWC) in *Q. suber* and *Q. ilex* watered (W) and none watered (NW) seedlings. ($P < 0.05$). Means $\pm$ SE.

2-3-2-Seedlings growth:

In watered conditions, the height growth is better in cork oak while the thickness growth and the leaf biomass were higher in holm oak. Non-watering had, however, similar effects on the growth morphological parameters measured for both species; a significant reduction of stem

height (Fig. 15a), stem basal diameter (Fig. 15b) and leaf biomass is observed (Fig. 15c). Roots/ shoots ratios were significantly increased by the reduction of substrate water contents. In these conditions, deeper root systems were observed in the holm oak (Fig. 15d).

In watered seedlings, SLA was higher in the cork oak compared to holm oak. The reduction of the water substrate content negatively affected both oak SLA. The cork oak is however more affected than the holm oak (Fig. 15e).

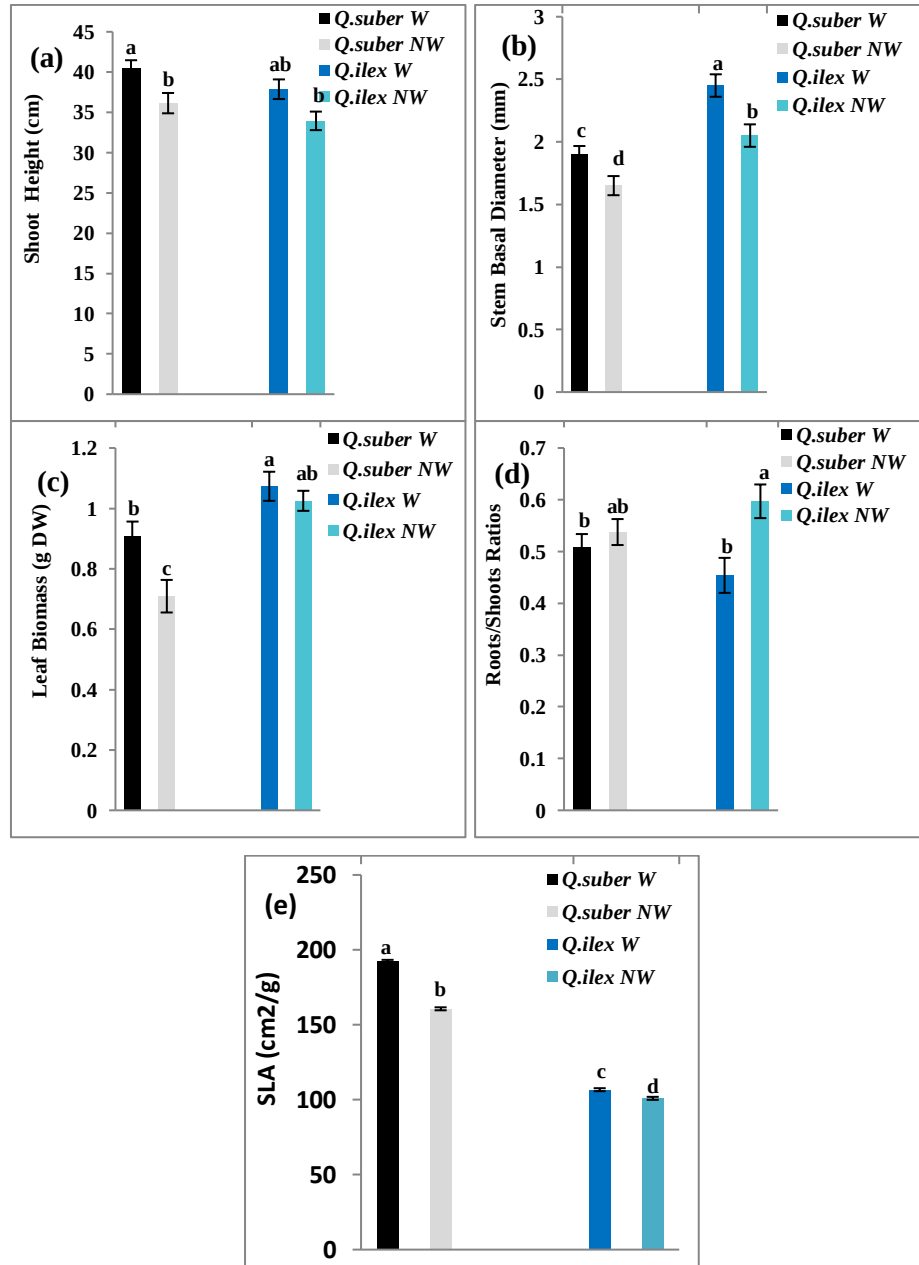


Fig. 15. Morphological parameters measurements: (a) Shoot height, (b) Stem basal diameter, (c) leaf biomass, (d) shoots/roots ratio and (e) SLA in *Q. suber* and *Q. ilex* watered (W) and non watered (NW) seedlings. (P<0.05). Means±SE.

2-3-3-Pigments contents:

The leaf biochemical responses of the two species are sometimes similar, sometimes different in drought conditions.

In watered conditions, pigments contents (Chlorophylls and Carotenoids) are higher in cork oak than in holm oak leaves. The drought conditions significantly reduced Carotenoids and total Chlorophyll leaf contents in holm oak while in cork oak carotenoids enhanced and Chlorophylls did not vary (Fig. 16a and b). The reduction of Chlorophyll and Carotenoids contents in holm oak, the increase of Carotenoids and the stability of total Chlorophyll in cork oak can be correlated to their observed growths.

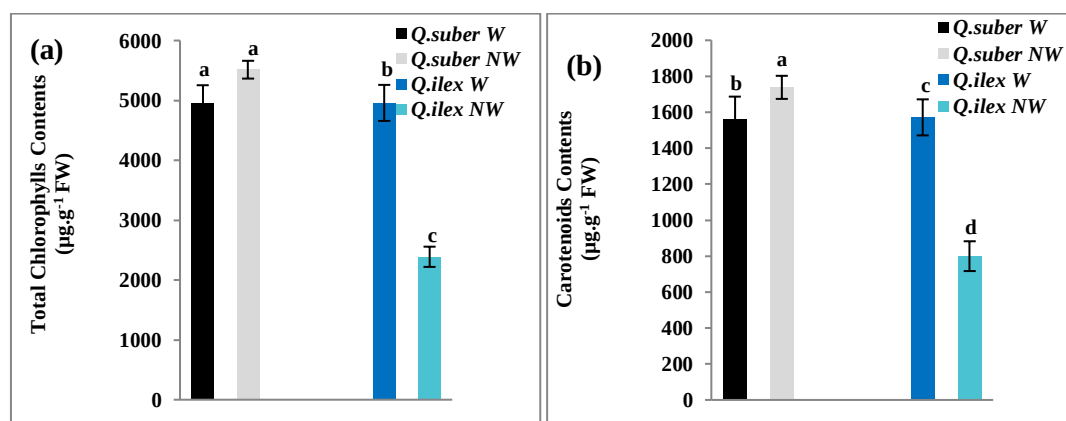


Fig. 16. Pigments measurements: **(a)** Chlorophyll contents and **(b)** Carotenoids Contents in *Q. suber* and *Q. ilex* watered (W) and non watered (NW) seedlings. ($P < 0.05$). Means $\pm$ SE.

2-3-4-Osmoregulation under drought conditions:

In watered seedlings, Sugars and Starch contents are higher in cork oak leaves; this richness can be a consequence of higher pigment contents. The drought conditions reduced starch contents in both species (Fig. 17a) and enhanced sugars contents in cork oak only (Fig. 17b).

Soluble proteins contents were enhanced in the two oaks non-watered seedlings and the augmentation was higher in holm oak (Fig. 17c).

A similar significant increase in leaf free proline concentration in the two oaks non-watered seedlings is noted. So, free proline quantities were 1,38 fold higher in non- watered leaves of the two oaks (Fig. 17d).

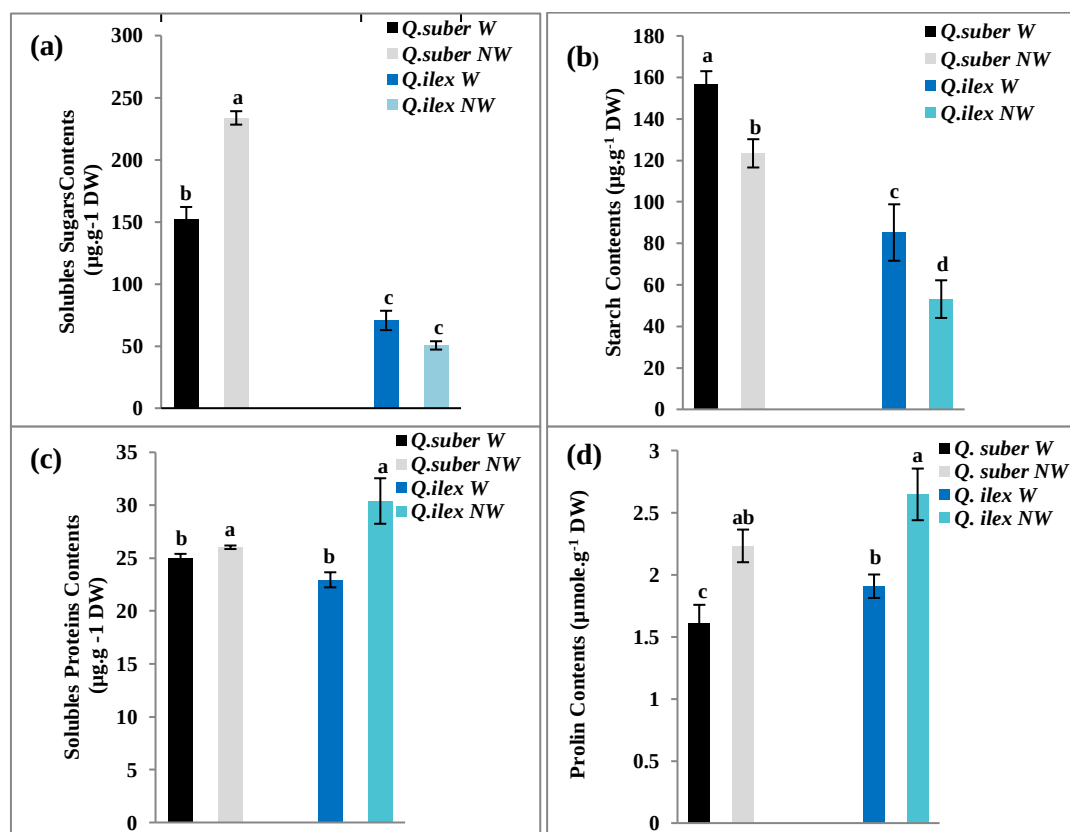


Fig. 17. Biochemical parameters measurements: **(a)** Solubles Sugars, **(b)** Starch Contents, **(c)** Solubles Proteins Contents and **(d)** Proline Contents in *Q. suber* and *Q. ilex* watered (W) and none watered (NW) seedlings. ($P < 0.05$). Means $\pm$ SE.

2-3-5-Carbone isotope discrimination:

The $\delta^{13}\text{C}$ in leaves were lower in *Q. suber* (-32.800) than in *Q. ilex* (-31.342) watered seedlings. The increase of $\delta^{13}\text{C}$ induced by the not-watering was significantly more important in cork oak (-31.390) than in holm oak (-31.164) (Fig. 18b). $\delta^{13}\text{C}$ leaf contents in the two oaks showed a significant negative correlation with soil water content; the reduction of soil water content enhanced $\delta^{13}\text{C}$ leaf contents. The negative correlations was higher in *Q. ilex* ($R^2=0.9429$, $R=-0.971$, $p\text{-value}=0.000003$) than in *Q. suber* ($R^2=0.4294$, $R=-0.6553$, $p=0.0397$) (Fig. 19 a and b).

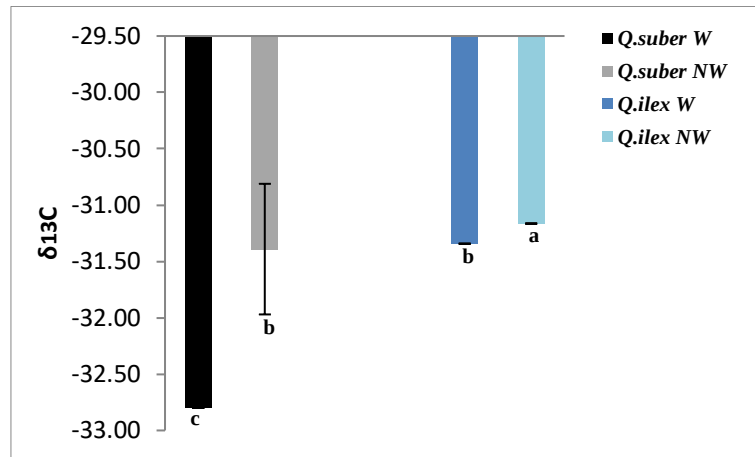


Fig. 18. $\delta^{13}\text{C}$ leaves contents measurements in *Q. suber* and *Q. ilex* watered (W) and none watered (NW) seedlings. ($P < 0.05$). Means $\pm$ SE

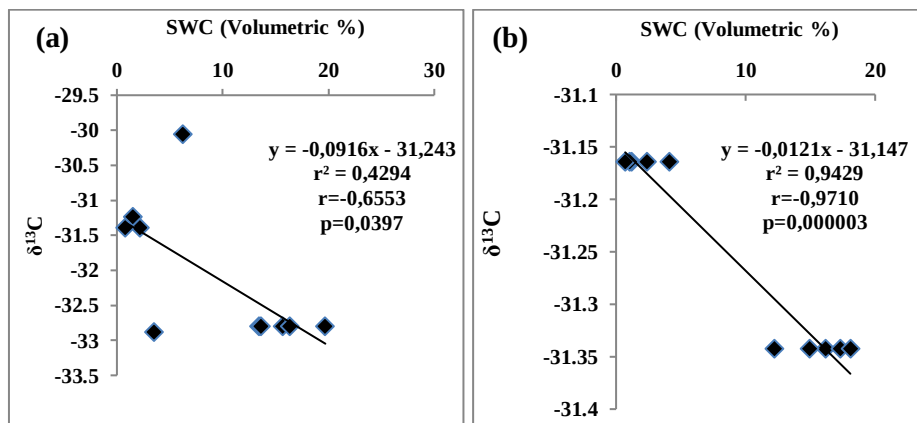


Fig. 19. Correlation between Soil Water Content (SWC) and $\delta^{13}\text{C}$ in (a) *Q. suber* and (b) *Q. ilex* leaves

2-3-6-C and N leaves contents:

The %C did not significantly differ between the two specie seedlings in watered or non-watered conditions (Fig. 20a). The total nitrogen concentrations (%N) in leaves were different between the two oak species; N content was higher in *Q. ilex* leaves but drought conditions had no significant effect on this parameter (Fig. 20b).

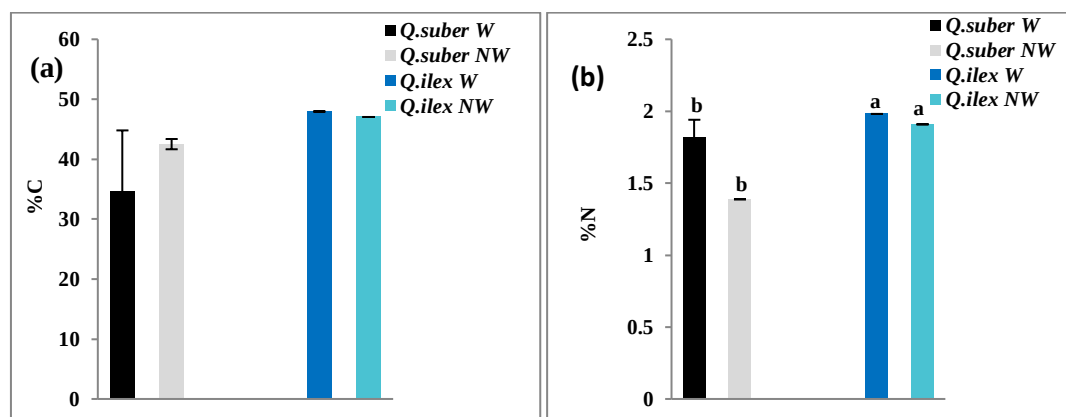


Fig. 20. C and N measurements: (a) %C and (b) %N Contents in *Q. suber* and *Q. ilex* watered (W) and none watered (NW) seedlings. ($P < 0.05$). Means $\pm$ SE

2-3-7-Survival rate:

After 10 weeks of no watering, seedling survival was statistically different between the two *Quercus* species. Our results showed higher sensitivity to drought but lower mortality in *Q. ilex* seedlings. Survival rate was higher in holm oak (68.88%) than in cork oak (57.77%) seedlings (Fig. 21).

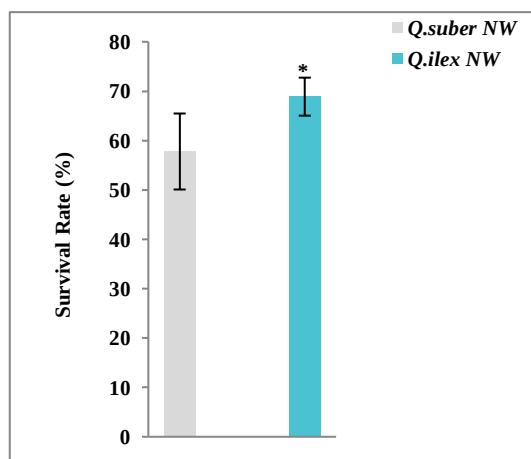


Fig. 21. Survival rates of *Q. suber* and *Q. ilex* seedlings in drought conditions ($P < 0.05$). Means $\pm$ SE.

2-4-Discussion:

The greenhouse conditions tested exposed the seedlings to a lack of water, an excessive irradiance and a high temperature, and higher light is more appropriate to cork oak. So, ecophysiological investigations demonstrated that *Q. ilex* is shade tolerant and more

xerophilous by having more effective drought avoidance and drought tolerance mechanisms than *Q. suber* (David *et al.*, 2007) but cork oak is drought-tolerant more heliophilous than holm oak, especially in young stages (Petroselli *et al.*, 2013). According to Grant *et al.* (2010), *Q. suber* is a very resilient tree since it was only severely affected in the third year of a severe drought. *Q. ilex* seedlings from five localities are characterized by different acorn sizes and morphological and physiological seedling features probably driven by the climate of the localities they originate from (Bonito *et al.*, 2011).

Under dry conditions, an important reduction in growth rates was noted in the Mediterranean region (Mooney, 1982). The most influential climatic variables upon holm and cork oak growth were late spring and early summer rainfall which enhanced growth and high temperatures in July and August, which negatively affected growth (Gea-Izquierdo *et al.*, 2009). While primary growth is sensitive to winter-spring warming, secondary growth is sensitive to summer-autumn warming (Camarero *et al.*, 2015). A great influence of water availability on stem radial growth has also been observed in the Mediterranean regions (Caritat *et al.*, 2000). Irrigation affected stem diameter growth of *Q. ilex* (Rey Benayas, 1998) and soil water deficit during summer months decreased diameter increment of this species of about 77% (Ogaya *et al.*, 2003). Cambium activity is negatively influenced by drought and high temperatures in *Q. suber* (Caritat *et al.*, 2000). Water stress tends to decrease the ratio of area biomass/ root biomass (Ksontini *et al.*, 1998). Interruption of shoot growth in the dry summer is a significant adaptation traits (Kurz-Besson *et al.*, 2006).

The leaves of *Q. ilex* had a lower SLA than those of *Q. suber* and the reduction of SLA showed by our results has fitness benefits in dry environment since more sclerophyllous leaves allow a more conservative water use and the lower SLA in *Q. ilex* is related to its higher degree of sclerophylly and tissue density (Ramírez-Valiente *et al.*, 2010). The differences in SLA between summer and autumn were not significant for both oak species (Vaz *et al.*, 2010).

The differences between the two oaks seedlings growth can be due to acorns sizes (which were not evaluated in this study). Seed size is one of the most important traits influencing the early stages of the life cycle of the plant, larger seeds help seedlings to endure drought (Timbal and Aussenac, 1996; McDowell *et al.*, 2008).

Leaf structure and chemical composition changed under drought stress (Penas-Roja *et al.*, 2005).

Generally, *Q. suber* water stressed seedlings showed a decrease in total chlorophylls and an increase in Chlorophyll b and carotenoids to cope with oxidative stress and a decrease of chlorophyll a to avoid excessive absorption of light energy (Faria *et al.*, 1996). However, other authors reported that severe drought did not modify the photosynthetic pigment composition of the both oak species (Vaz *et al.*, 2010; Vilagrosa *et al.*, 2010).

The enhancement of soluble sugars and proteins in *Q. suber* and proteins in *Q. ilex* in drought conditions may be involved in the osmotic adjustment phenomenon. Drought induced an increase of hexose (Picon *et al.*, 1997) and soluble proteins (Kwak *et al.*, 2011) leaves concentrations which may contribute to increase osmoregulation (Picon *et al.* 1997). Accumulation of compatible solutes in the cytoplasm is a well-established ecophysiological mechanism which allows to lower the osmotic potential whereby many plants adjust to low soil water availability (Morgan, 1984). Total soluble sugars and starch leaf concentrations were higher in *Q. ilex* than in *Q. suber* and were lower in summer than in autumn in both species (Aguadé *et al.*, 2015; Vaz *et al.*, 2010). Severe hardening reduced shoot soluble sugar concentration and increased shoot starch concentration (Villar-Salvador *et al.* 2004). Although mature holm oak trees in natural populations enhance their dehydration tolerance by osmotic adjustment in response to summer drought (Kyriakopoulos and Richter, 1991).

Our results agree with other studies reporting that these species increase the proline content which is considered as osmoregulator and osmoprotectant under intense stress conditions (Vilagrosa *et al.*, 2010 ; Kwak *et al.*, 2011).

The $\delta^{13}\text{C}$ leaf contents reflect a better water use efficiency (WUE) in *Q. ilex* than in *Q. suber* seedlings. *Q. suber* leaf $\delta^{13}\text{C}$ was higher in Mediterranean Portugal (from -28, 5‰ to -24, 58‰) (Gouveia and Freitas, 2009). A significant negative correlation is found between leaf $\delta^{13}\text{C}$ and mean annual rainfall (Ferrio *et al.*, 2003). *Q. ilex* exhibited a large spatial dependence of isotopic signals on the temperature regime (Shestakova *et al.*, 2014). Increases in WUE under drought, are expected to be accompanied by an increase in Carbon isotope discrimination (Du *et al.*, 2015).

N and C concentrations in leaves were not affected by water withholding. In evergreen species, N concentration increased with water supply, suggesting that higher water supply

induces N luxury consumption (Sanz-Pérez *et al.*, 2007). In *Q. ilex* seedlings N concentration was not affected by the soil water availability (Estiarte *et al.*, 2007).

Summer drought is the main cause of seedling mortality and seedlings can have more chances to survive but they can find growth restrictions (Marañóni *et al.*, 2003). The higher survival rate of *Q. ilex* can be attributed to its deeper roots. The difference in root-system length associated with the availability of water could have implications for seedling survival under natural conditions (Mancilla-Leytón *et al.*, 2016).

Our results confirmed that cork oak and holm oak are tolerant to drought conditions and contrary to expectations *Q. suber* seedlings showed better water status. However *Q. ilex* showed higher survival rate after 10 weeks of drought conditions. The Algerian sites of origin of these species probably play an important role. Cork oak (Gandour *et al.*, 2007) and holm oak (Ramirez-Valiente *et al.*, 2010) populations differ in survival, growth and functional traits related to drought stress tolerance. Inter-population differences in leaf size, SLA and $\delta^{13}\text{C}$ were associated with rainfall and temperature at the sites of origin (Ramirez-Valiente *et al.*, 2010).

Physiological knowledge concerning populations of *Q. ilex* and *Q. suber* seedlings originating from different climate are important to forecast the potential productivity to increasing drought stress expected in Mediterranean area.

2-5-Conclusion :

L'étude comparative de l'influence de la sécheresse estivale, simulée par un arrêt d'arrosage durant 10 semaines, de fin juin à mi-septembre, sur de jeunes plantules âgées approximativement de six mois, de deux espèces méditerranéennes *Q. suber* et *Q. ilex* a révélé des réponses souvent analogues:

- une modification similaire du statut hydrique foliaire par diminution de la RWC chez les deux espèces.
- des réponses morphologiques similaires chez les deux espèces :
 - une diminution de croissance (en hauteur et en épaisseur) considérée à la fois comme étant un effet négatif et une stratégie d'adaptation à la sécheresse dans la région méditerranéenne
 - une diminution de la biomasse foliaire et de la SLA pour éviter une importante perte d'eau par transpiration

→ une augmentation du rapport poids systèmes racinaires/ poids systèmes aériens grâce à un important développement du système racinaire afin de permettre de meilleures exploration et exploitation du sol

- des réponses physiologiques similaires :

→ une diminution des teneurs foliaires en amidon

→ une augmentation des teneurs foliaires en protéines solubles et proline jouant probablement un rôle important dans et l'osmorégulation et l'osmoprotection

→ une augmentation des teneurs foliaires en $\delta^{13}\text{C}$ reflétant probablement une meilleure utilisation de l'eau et une corrélation entre les teneurs en eau du sol et le $\delta^{13}\text{C}$ qui est plus importante chez le chêne liège

→ une diminution des teneurs foliaires en Azote (plus importante chez le chêne liège)

→ une stabilité des teneurs foliaires en Carbone total

- des réponses physiologiques différentes :

→ une importante augmentation des teneurs en sucres solubles chez le chêne liège et une légère diminution chez le chêne vert

→ une augmentation des teneurs foliaires en caroténoïdes chez le chêne liège et une diminution des teneurs en Chlorophylles totales et Caroténoïdes chez le chêne vert.

D'après ces résultats le chêne liège s'avère plus tolérant à la sécheresse même si les taux de survie sont meilleurs chez le chêne vert, ce qui est contraire aux résultats signalés par la bibliographie. L'origine ainsi que la taille des glands utilisés pourraient expliquer ces différences.

Cependant, l'influence de la sécheresse estivale sur le chêne liège a été étudiée plusieurs fois (plusieurs années successives) et les résultats enregistrés diffèrent d'une année à une autre. En effet, certains paramètres mesurés ne montrent pas les mêmes effets d'une année à une autre (teneurs en amidon, rapport Systèmes Racinaires/ Systèmes Aériens). Ces différences de réponses peuvent être dues :

- à une différence des conditions estivales car toutes les études ont été réalisées en serre non contrôlée,

- et /ou à l'existence d'une variabilité intra-spécifique lié au phénomène d'introgession existant chez cette espèce ou au facteur stationnel.

La non-conformité des résultats, obtenus chez le Chêne liège, comparés aux nombreuses études publiées sur cette espèce et l'obtention de résultats différents d'une année à une autre nous ont poussé à entamer une étude sur l'influence de la provenance des glands sur la réponse des plantules à la sécheresse estivale.

3-Comparative drought responses of *Quercus suber* seedlings of three Algerian provenances under greenhouse conditions

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Article accepté

Abstract

Cork oak (*Quercus suber* L.) is one of the most representative Mediterranean forest species well studied in Europe and known to be drought tolerant. However many studies showed that differences exist in drought tolerance mechanisms among provenances. Few reports exist on drought responses of Algerian *Q. suber* seedlings. The present study investigates summer drought behavior of seedlings originating from three Algerian provenances: from humid (Jijel), sub-humid (Azazga) and semi arid (M'Sila) Mediterranean areas. The summer conditions were simulated by stopping irrigation of the seedlings grown homogenously in greenhouse during 10 weeks from last June to mi-September. Water status, morphological and biochemical parameters were evaluated in watered (control) and non-watered seedlings and survival rate of non watered seedlings was determined for the three provenances. The results showed differences between seedlings behavior of the three provenances in watered and non-watered conditions. In watered conditions, M'Sila seedlings showed the highest Relative Water Content and the highest growth. In non watered conditions, the reduction of soil water content had negative effect on the Relative Water Content and growth (height and shoot diameter) of the seedlings of Jijel and Azazga provenances while only the stem basal diameter was reduced in the seedlings of M'Sila provenance. Leaves of M'Sila non irrigated seedlings, originating from the drier site (semi arid), showed morphological and physiological modifications that are known to be adaptative strategy to drought: low Specific Leaf Area, decrease in Chlorophyll a contents to avoid excessive absorption of light energy, a decrease in starch content, an accumulation of proteins, sugars and proline for the occurrence of an osmotic adjustment and an increase in $\delta^{13}\text{C}$ to enhance water use efficiency. Azazga seedlings showed an intermediate behavior between M'Sila and Jijel seedlings in drought conditions. However, contrary to expectation, survival rate was lower for M'Sila and higher for Azazga seedlings. The ecotypes studied in this work exhibited different functional traits related to the environmental conditions of the original provenance. Despite the low survival rate of provenances from the most arid environment, they are thought to be suitable candidates in long-term for reforestation in the context of global climate change.

Key words: *Quercus suber*. Algerian Provenances. Mediterranean forest. Seedlings. Drought. Growth. Physiology.

Résumé

Le Chêne liège est une espèce typiquement méditerranéenne présentant un intérêt économique et écologique. L'existence de mécanismes de tolérance à la sécheresse estivale méditerranéenne chez cette espèce a été démontrée par de nombreuses études réalisées, essentiellement en Europe. Cependant, ces dernières années, d'autres études ont montré que les mécanismes de tolérance diffèrent entre les provenances. Le comportement des

provenances algériennes de Chêne liège a été le sujet de très peu d'études. L'Algérie, étant un pays plus aride que les pays européens, ses provenances pourraient montrer des différences de comportement par rapport aux provenances européennes. L'objectif de ce travail est l'étude de la réponse des plantules originaires de trois provenances algériennes caractérisées par des étages bioclimatiques différents: Azazga (sub-humide), Jijel (humide) et M'Sila (semi-aride) à une sécheresse estivale. Un arrêt d'arrosage a été appliqué aux plantules, cultivées de façon homogène en serre, pendant 10 semaines, de fin-juin à mi-septembre, pour simuler la sécheresse estivale méditerranéenne. Le statut hydrique et des paramètres morphologiques et biochimiques ont été évalués chez les plantules arrosées (témoins) et non arrosées (stressées) et les taux de survie des plantules non arrosées ont été déterminés pour les trois provenances. Les résultats ont révélé des différences de comportement entre les trois provenances dans les deux conditions d'arrosage et de non arrosage. Dans les conditions d'arrosage, les plantules originaires de M'Sila ont montré la teneur relative en eau la plus élevée ainsi que la meilleure croissance. L'arrêt d'arrosage, ayant provoqué une diminution significative des teneurs en eau du sol, a provoqué une réduction de la teneur relative en eau et de la croissance (hauteur et diamètre de la tige) des plantules originaires de Jijel et d'Azazga alors que seul le diamètre des tiges est réduit chez les plantules originaire de M'Sila. Les feuilles des plantules originaires du site le plus aride, M'Sila, montrent des modifications morphologiques et physiologiques considérées comme étant des stratégies d'adaptation à la sécheresse : une faible surface foliaire spécifique, une diminution des teneurs en Chlorophylles a, pour éviter une absorption excessive de l'énergie lumineuse, une diminution des teneurs en amidon, une accumulation des protéines, des sucres et de la proline afin de permettre un ajustement osmotique et une augmentation du $\delta^{13}\text{C}$ pour une meilleure utilisation de l'eau. Dans les conditions de non arrosage, les plantules d'Azazga ont montré un comportement intermédiaire entre les plantules originaires de Jijel et de M'Sila. Cependant, contre toute attente, les plantules de M'Sila ont montré le plus faible taux de survie et le meilleur taux de survie est enregistré pour les plantules d'Azazga. Les écotypes étudiés dans ce travail montrent des traits fonctionnels différents pouvant être liés ou non aux conditions environnementales des provenances. Malgré le faible taux de survie des provenances du milieu le plus aride on peut penser qu'elles constituent de bons candidats, sur le long terme, en matière de reboisement dans le contexte du changement climatique global.

Mots Clés : *Quercus suber*. Provenances algériennes. Forêts méditerranéennes. Plantules. Sécheresse. Croissance. Physiologie

3-1-Introduction:

Cork oak (*Quercus suber* L.), a Fagaceae species, is one of the most widely distributed among Mediterranean forest trees (Quezel and Médail 2003) presenting ecological (Carbon sequestration, soil protection against erosion, hydrological cycle regulation) and economic (production of cork) interests in Mediterranean region (Pausas *et al.*, 2009). It's an evergreen and sclerophyllous species growing from the sea level up to 700m in altitude (Quezel and Médail, 2003). Cork oak, with an area of 227 000 ha, is the second most important species, after the Aleppo pine in Algeria and Algerian suberia constitutes 14% of the world suberia (FAO, 2013). Nearly 4/5 of the cork oak area are located essentially in the north east of

Algeria from Tizi-Ouzou to the Tunisian border and it is disseminated in the form of a smaller island in the western part (Bouhraoua, 2015).

It is a heliophilic and thermophilic species growing between 13 and 16 ° C and it is strictly calcifuge colonizing siliceous soils (Quezel and Médail, 2003).

- It's response to drought conditions which characterized summer in Mediterranean areas is well studied essentially in Europe (Faria *et al.*, 1996; Nardini *et al.*, 1999; Nardini and Tyree, 1999; Kurze-Besson *et al.*, 2006; Otieno *et al.*, 2006; Pardos *et al.*, 2005; Kwak *et al.*, 2011). Cork oak is well known to be drought tolerant species (Nardini *et al.*, 1999; Nardini et Tyree, 1999). There are many mechanisms by which it resists to drought periods: deep rooting, osmotic adjustment (Otieno *et al.*, 2006; Pardos *et al.*, 2006; Kwak *et al.*, 2011) and anti-oxydant system (Faria *et al.*, 1996). Ecophysiological investigations demonstrated that *Q. suber* is well adapted to summer conditions because it maintained a favorable ratio between water loss and uptake during the dry period. Maintaining a favorable water status in tissues, with a high relative water content (RWC) during summer drought, is ensured by deep roots and/or osmotic adjustment through accumulation of molecules such as proteins, sugars and proline (Otieno *et al.*, 2006; Pardos *et al.*, 2005; Oufir *et al.*, 2009; Kwak *et al.*, 2011). A decrease in shoots and leaves biomass by reduced growth and root drop is also noted (Ksontini *et al.*, 1998; Kurze-Besson *et al.*, 2006). A reduction of specific leaf area (SLA) was also observed (Ramirez-Valiente *et al.*, 2010). The presence and the nature of the cork in which wall cells are suberin impregnated contributes to protection against fire and also resistance to tissues desiccation (Pausas *et al.*, 2009). Under drought conditions, an increase of $\delta^{13}\text{C}$ leaves contents reflects a better water use efficiency (WUE) in *Q. suber* seedlings (Gouveia and Freitas, 2009).

Although the cork oak seems well adapted to the dry conditions of the Mediterranean climate, the mechanisms involved in this adaptation are still scare (Almeida *et al.*, 2013) and it remains that its natural regeneration is low and poorly understood (Gonzalez-Rodriguez *et al.*, 2011) because it is particularly high sensitive to drought in the early stages of development (Aranda *et al.*, 2005) due to the quasi-absence of cork (Pereira *et al.*, 2009). In Algeria, facing the decline of the cork oak forest due to many factors like fires (the most important factor), grazing and diseases, many reforestation operations have been undertaken (the cork oak occupies the first place of the reforested species with 24% of the wooded areas). However, the success rates of these reforestations are often low and unsatisfactory; the survival rate of

plants decreased from 80-90% to 20-50% after the summer season (Messaoudène *et al.*, 2011; Bouhraoua, 2015).

This seedlings recruitment limitation probably will be amplified with the predicted global changes in Mediterranean region (Caldeira *et al.*, 2014). Hence, climate change is expected to lead to longer dry spells, higher evaporative demand and more intense droughts in the coming decades in several regions of the world, including the Mediterranean basin (IPCC, 2007).

Since cork oak has a large distribution area with large variation in environmental conditions; it is present in areas with a mean annual rainfall of 400–1500 mm and a mean annual temperature of 13–20 °C (Díaz-Fernández *et al.*, 1995), it is expected large differentiation among populations in traits of adaptive significance such as ability to tolerate extended periods of drought (Varela *et al.*, 2014).

Many studies showed a high level of differentiation among the populations of *Q. suber* species. Differences among cork oak plants originated from different populations in phenotypic traits were mainly due to divergent selection imposed by temperature and rainfall variation and to neutral evolutionary processes such as founder effect or genetic drift (Ramirez-Valiente *et al.*, 2010). Genetic diversity parameters determined for different geographic areas of the entire *Q. suber* range, showed variation where paleogeography, hybridization, adaptation, fragmentation, and human impact play an important role in the evolutionary history of this species (Simeone *et al.*, 2010). Ennajah *et al.* (2013) showed a high phenotypic variability among and within Moroccan cork oak tree populations which was significantly correlated with rainfall; large differences between populations from highest and coldest sites as well as those of lowest and warm sites were detected and adaptive responses specific to some populations were founded. Some differences among populations have been observed concerning their ability for adaptation and production under drought conditions (Gandour *et al.*, 2007).

So, a better understanding of the effects of drought on plants originating from different provenances is essential for early selection of provenances for afforestation.

Based on the concept of plant-climate-coevolution, our working hypothesis was that differences exist in drought tolerance mechanisms among provenances; provenances native to dry regions have more capability to acclimate to drought conditions than provenances originated from a more temperate climate region. Thus ecophysiological comparison may prove useful for the choice of *Q. suber* provenances to afforestations.

Few reports exist on drought responses of Algerian *Q. suber* seedlings (Acherar *et al.*, 1991; Daoudi *et al.*, 2016). The present study investigates summer drought effects on seedlings originating from three Algerian provenances. The summer conditions were simulated by stopping irrigation of the seedlings grown in none controlled greenhouse during two months, July and august. Then, we evaluated different parameters: water status of the seedlings (RWC), morphological parameters (height and thickness growth and specific leaf area) and physiological parameters (Chlorophylls, Carotenoids, proteins, sugars, starch, $\delta^{13}\text{C}$, %C and %N) in leaves.

3-2-Materials and methods:

Cork oak acorns were collected in the end of November 2012 from three Algerian provenances: Jijel (Aghzer forest), M'Sila forest (Oran) and Azazga (Beni Ghobri forest). The forests of the three provenances showed foliar damages attributed to xylophages and phyllophages insect pests (the most important being *Platypus cylindrus*) and cryptogamic diseases (the most important is coal caused by *Hypoxylon mediterraneum*) (Bouchaour-Djabeur, 2013; Rouibah *et al.*, 2011). The decline of Algerian suberies is slow but chronic (Bouhraoua, 2015).

M'Sila is the drier provenance and Jijel is the higher humid provenance. The dry period is longer in M'Sila (Mid-April to Mid-September) and shorter in Jijel (May to September).

Acorns of $2.954 \pm 0.155\text{g}$, $2.458 \pm 0.093\text{g}$ and $2.195 \pm 0.256\text{g}$ weights for Jijel, M'Sila and Azazga provenances respectively germinated at 20°C in Petri dishes. The seedlings with 2 cm root length in mi-April were transplanted to plastic bags (15cm diameter and 30cm deep) filled soil substrate witch consisted of a mixture of loam (2/3) and washed sand (1/3). The loam (N 110-250 mg/l, P2O5 60-140 mg/l, K2O 120-280 mg/l) had a fine structure and a pH of 6.2.

All the *Q. Suber* seedlings of the three provenances were grown homogeneously in greenhouse (5x3 m dimension), located next to the university of Tizi-Ouzou in Algeria ($36^\circ 43' 464 \text{ N}$ and $4^\circ 1' 531 \text{ E}$, 140 m elevation), which is characterised by Subhumid Mediterranean climate (Temperature annual mean 19.2°C , Precipitation annual mean 705mm). The seedlings were watered regularly (three times a week) until the end of June. Then we constituted three plots of seedlings (60 seedlings/plot). Each plot was constituted with a mixture of 10 seedlings/Provenance (Jijel, M'Sila, Azazga)/Treatment (Watered and none watered): Jijel watered (JW) and none watered (JNW), M'Sila Watered (MW) and none

watered (MNW) and Azazga watered (AW) and none watered (ANW). Water stress treatment was given by withholding the water supply for 10 weeks, from end of June until Mid of September, to simulate Mediterranean summer drought conditions. According to IPCC (2007) dry days are projected to increase markedly in Mediterranean basin. The greenhouse temperatures varied between 25°C to 38°C during the experiments. Ten weeks after stop irrigation (Mid of September), plants of JW, JNW, MW, MNW, AW, ANW were harvested for determination of morphological and physiological parameters.

3-2-1-Substrate water contents measurement:

Substrate water contents (SWC) was determined as described by Mathieu and Pielain (2003). Substrate samples of 1 g were taken from the plastic bags of the six lots at a depth of 10 cm (10 repetitions/Provenance/Treatment) and dried at 105 ° C for 72 h and then water content was calculated using the formula:

$$SWC = \frac{FW - DW}{FW} \times 100$$

where FW and DW are the fresh and dry weight, respectively.

3-2-2-Relative water contents (RWC) measurement:

Relative water content (RWC) was measured in three young leaves of five plants per Provenance/Treatment using Nardini *et al.* (1999) method. Leaves were detached and immediately weighed to get their fresh weight (FW). Then the leaves were restored with distilled water to near full turgor by immersing their petioles in water, covering the leaf blade with plastic film and leaving them in the dark for 12 h. Leaves were then reweighed to obtain their turgid weight (TW) and put into oven at 75°C for 72h to obtain their dry weight (DW). Finally, RWC was calculated as:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

3-2-3-Morphological parameters measurements:

To evaluate seedlings growth in the both conditions watered and non-watered, different morphological parameters of 10 plants per Provenance/Treatment were measured: stem height, basal stem diameter and Shoots (stems and leaves), roots (total roots washed with distilled water) and leaves (with petioles) biomasses were determined after drying at 75°C during 72h. Then we estimated the root to shoot ratio.

The specific leaf area (SLA) was determined on three mature leaves per plant and ten plants per Provenance/Treatment as the ratio of Leaf Area determined with AM350 Portable Leaf Area Meter to Dry Mass of individual leaves dry mass (DM), measured after oven-drying at 75°C to a constant weight (Faria *et al.*, 1996).

3-2-4-Physiological parameters measurements:

Pigments, Sugar, Starch, Proteins, Proline, %C, %N and $\delta^{13}\text{C}$ contents were determined on five seedlings per Provenance/Treatment.

Chlorophylls (a and b) and determined spectrophotometrically after extraction in acetone 80% in dark and the Pigments contents were calculated as proposed by Lichtenthaler and Buschmann (2001):

$$\text{Chla} = 12.25 \times A_{663} - 2.79 \times A_{647}$$

$$\text{Chlb} = 21.50 \times A_{647} - 5.10 \times A_{663}$$

$$\text{ChlT} = 7.15 \times A_{663} - 18.71 \times A_{647}$$

$$\text{CarT} = 1000 \times A_{470} - 1.82 \times \text{Chla} - 85.02 \times \text{Chlb}$$

After the extraction of the soluble sugars from fresh leaves in ethanol (70%), the solid fraction was used for starch analysis. Starch was incubated in HCl (1.1% v/v) for 30 min at 95°C for hydrolysis into simple sugar. Then, soluble sugars and starch concentrations were determined colorimetrically at 625 nm with anthrone reagent following Cerning-Berorard (1975) method. Glucose was used as a standard for both soluble sugars and starch.

Soluble Proteins were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595nm after colorimetric reaction with Bradford reagent following Bradford (1976) method. BSA was used as standard.

Proline content was quantified by the ninhydrin-colorimetric method at 515nm after extraction in methanol 70% as described by Monneveux and Nemmar (1986). Proline was used as standard.

Foliage samples for analysis of $\delta^{13}\text{C}$, %C and %N were dried at 70 °C for 72 h and ground to a fine powder. The abundance in combusted samples were performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) at UMR-CNRS7266 LIENSS with a

precision of 0.1‰. We calculated $\delta^{13}\text{C}$ (‰) with respect to the PDB Pee Dee Belemnite standard:

$$\delta^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} \times 1000$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in a sample and the standard (Pee Dee Belemnite), respectively (Warren and Adams, 2000).

3-2-5-Statistics analysis:

Statistical analysis was performed using STATISTICA software (Version 7.1; StatSoft Inc.). The differences among the plots (JW, JNW, JW,JNW, AW and ANW) for all recorded data were compared by the one-way analysis of variance (ANOVA) where the conditions of normality and equality of variances are checked followed by LSD test. Otherwise, a Kruskal-Wallis test was achieved. The significant level for all the tests was $P < 0.05$.

3-3-Results:

In this study, we evaluated the response to drought conditions of seedlings of three *Q. suber* provenances originating from Algeria. The results showed morphological and physiological differences between Jijel (humid), M'Sila (semi-arid) and Azazga (sub-humid) provenances in watered and non watered conditions.

The irrigation cessation after ten weeks (in September), significantly reduced the SWC of the three provenances (Fig. 22a). In watered conditions, the seedlings of M'Sila provenances showed better water status with higher RWC (86.40%) than Jijel (84.96%) and Azazga (82.08%) provenances seedlings, but the reduction of the SWC decreased the RWC of the seedlings of the three provenances (Fig. 22b). The RWC of no irrigated plants of M'Sila decreased from 86.40 % to 72.41. Azazga showed the lowest decrease of the RWC (from 82.08% to 76.09%).

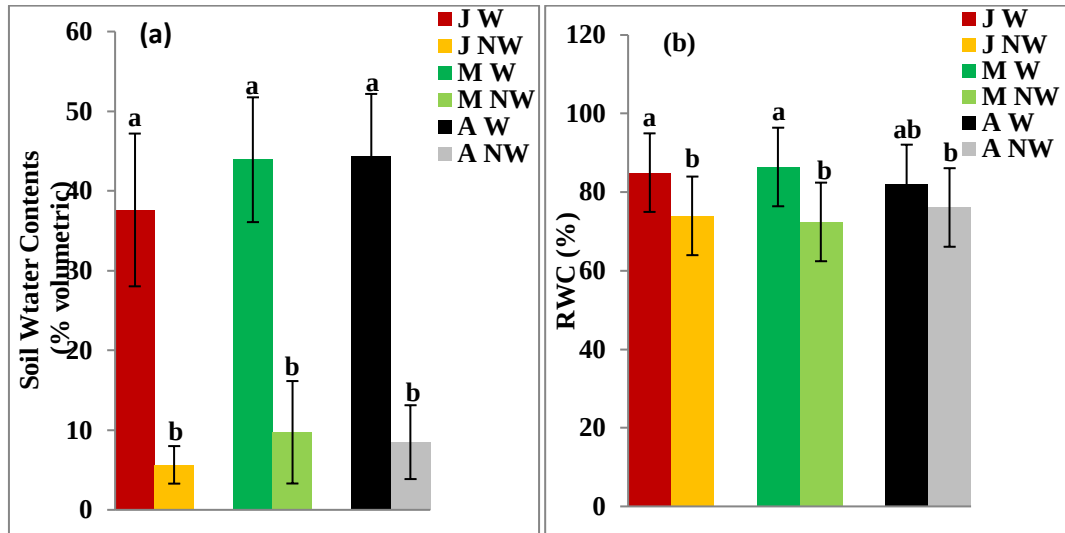


Fig. 22. Water status measurements: **(a)** soil water contents and **(b)** relative water contents (RWC) in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

The reduction of SWC had also negative effect on the seedlings growth of Jijel and Azazga provenances by reducing slightly their stem height while the seedlings of M'Sila provenance were not affected; their stem height were similar in watered and non watered conditions (Fig. 23a).

In watered conditions, the diameter growth of the seedlings was higher for Jijel provenance and lower for Azazga provenance. The lack of irrigation reduced the stem basal diameter of the seedlings of the three provenances. However, the stem basal diameter of non watered seedlings of Jijel provenance were similar to those of the watered seedlings of M'Sila provenance and the stem basal diameter of non watered seedlings of this provenance were similar to those of watered seedlings of Azazga provenance (Fig. 23b).

The leaf biomass and the roots/shoots ratios did not significantly differ between the three provenances and drought conditions did not affect this parameter (Fig. 23c and 23d).

The SLA did not differ significantly between the three provenances in irrigated conditions. The seedlings growing under conditions of water deficiency showed leaves with low SLA in the three provenances. The M'Sila seedlings showed the lowest value of the SLA (Fig. 23e).

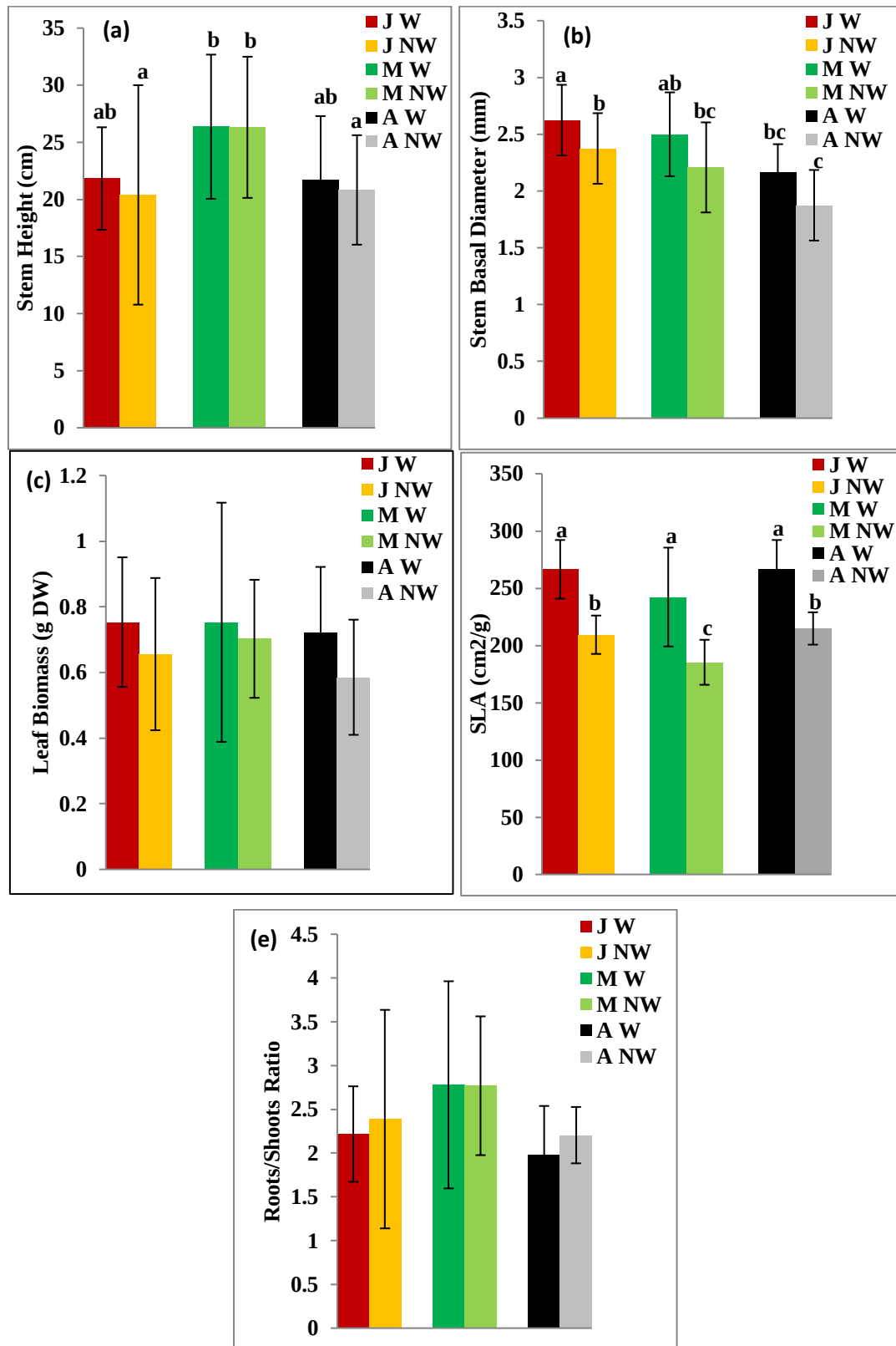


Fig. 23. Morphological parameters measurements: **(a)** Shoot height, **(b)** Stem basal diameter, **(c)** leaf biomass, **(d)** SLA and **(e)** roots/shoots ratio in *Quercus suber* seedlings from humid (J: Jijel), semi-arid(M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

Significant differences in Chla+b concentrations were observed among the three provenances. Higher values of Chla+b were observed in the leaves of Jijel provenance seedlings and the lowest values were noted for Azazga seedlings. The reduction of the SWC decreased the total leaves chlorophyll contents only in M'Sila provenance (Fig. 4a). The concentration of Chlorophyll a was higher in Jijel and M'Sila seedlings compared to Azazga seedlings but drought conditions reduced chlorophyll a contents in the Jijel and M'Sila provenances while for Azazga seedlings this biochemical parameter increased (Fig. 24b). Leaves contents of chlorophyll b were higher in Jijel and M'Sila than in Azazga seedlings. Under drought conditions, the leaves of M'Sila and Jijel seedlings showed an increase in Chlorophyll b concentration while Azazga seedlings were not affected (Fig. 24c). Carotenoids concentrations in leaves were higher in Jijel and M'Sila than in Azazga watered seedlings and in non-watered seedlings they significantly decreased in M'Sila and Azazga seedlings (Fig. 24 d).

Drought conditions induced an accumulation of soluble sugars and proteins only in leaves of M'Sila and Azazga seedlings (Fig. 25a and 25b); especially in M'Sila seedlings for sugars, this increase was approximately 1.5-fold in none watered conditions compare to watered conditions (Fig. 25a). The starch content showed an important decrease in none watered seedlings of the three provenances, the reduction was 2.7, 2.1 and 1.3fold in Jijel, M'Sila and Azazga provenances, respectively (Fig. 25c). The drought conditions also induced an increase in leaves proline content in the seedlings originating from M'Sila and Azazga provenances (Fig. 25d).

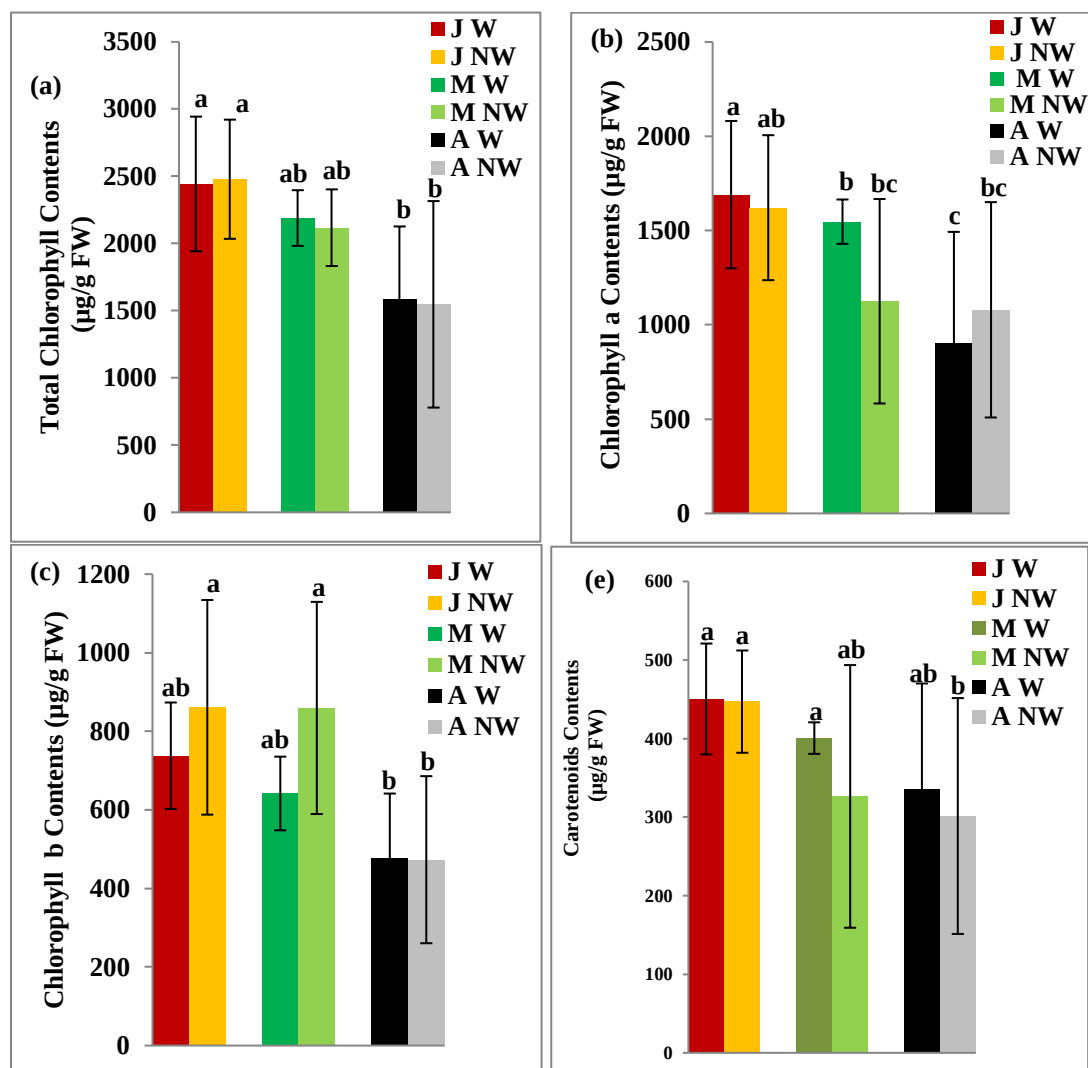


Fig. 24. Pigments leaves contents: **(a)** Total Chlorophyll, **(b)** Chlorophyll a, **(c)** Chlorophyll b, **(d)** Carotenoids in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE

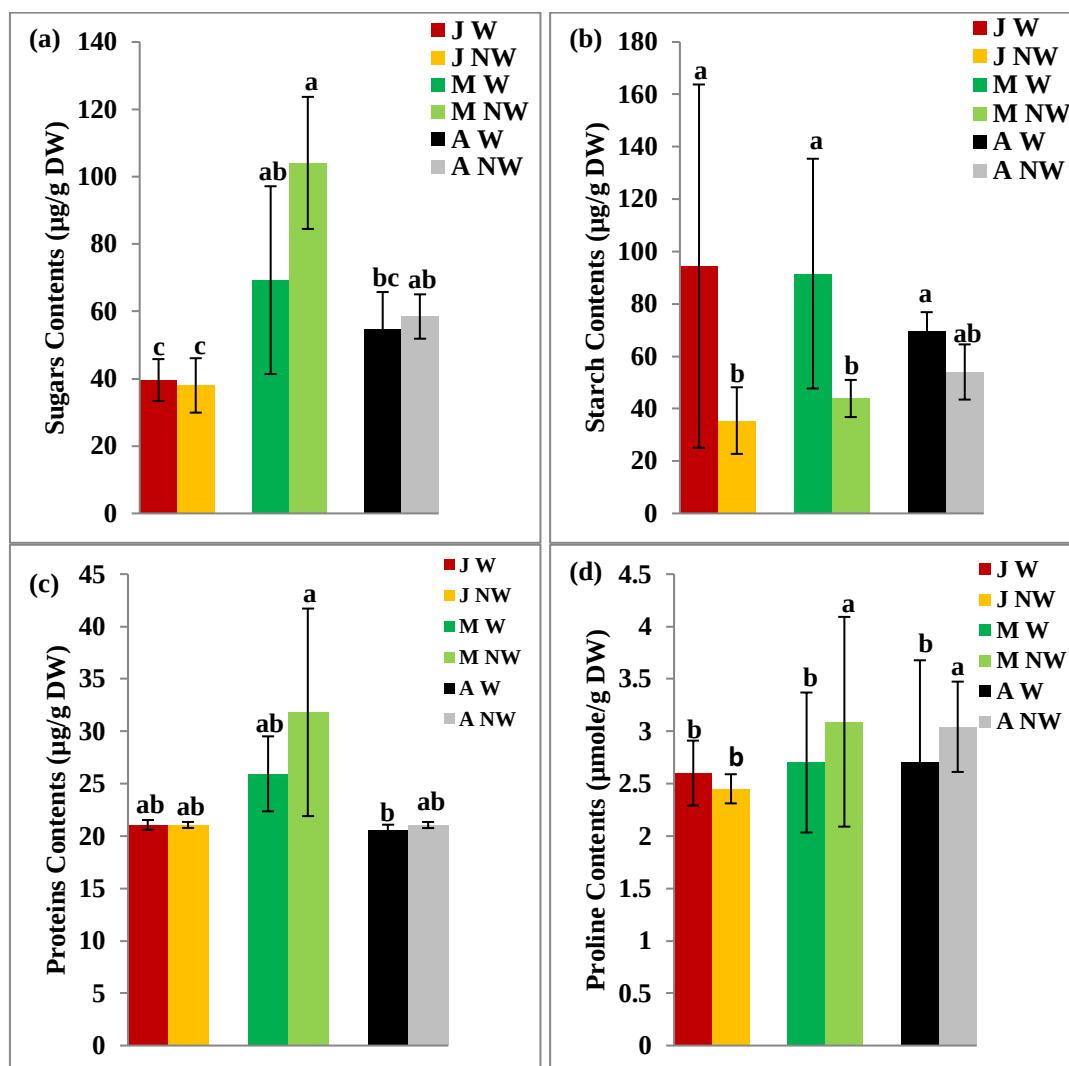


Fig.25. Biochemical parameters measurements: **(a)** Solubles Sugars, **(b)** Starch Contents, **(c)** Solubles Proteins Contents and **(d)** Proline Contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid(M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE

The three provenances studied showed differences in leaves $\delta^{13}\text{C}$. In watered conditions, the lower value was obtained in Azazga seedlings provenance (-31.72). The reduction of the SWC enhanced significantly the $\delta^{13}\text{C}$ leaves contents in Azazga and M'Sila provenances. The higher value of $\delta^{13}\text{C}$ (30.54‰) was recorded in M'Sila non watered seedlings (Fig. 26a). The $\delta^{13}\text{C}$ in Jijel seedlings was not affected by the drought.

In watered seedlings, the carbon rate was higher for Jijel provenance. The drought conditions reduced the level of %C in Jijel and enhanced it in M'Sila and Azazga provenances (Fig. 26b).

The nitrogen rate in watered seedlings did not differ between Jijel and M'Sila provenances and Azazga provenances showed the higher value. The reduction of the SWC induced a decrease of %N in the provenances studied (Fig. 26c).

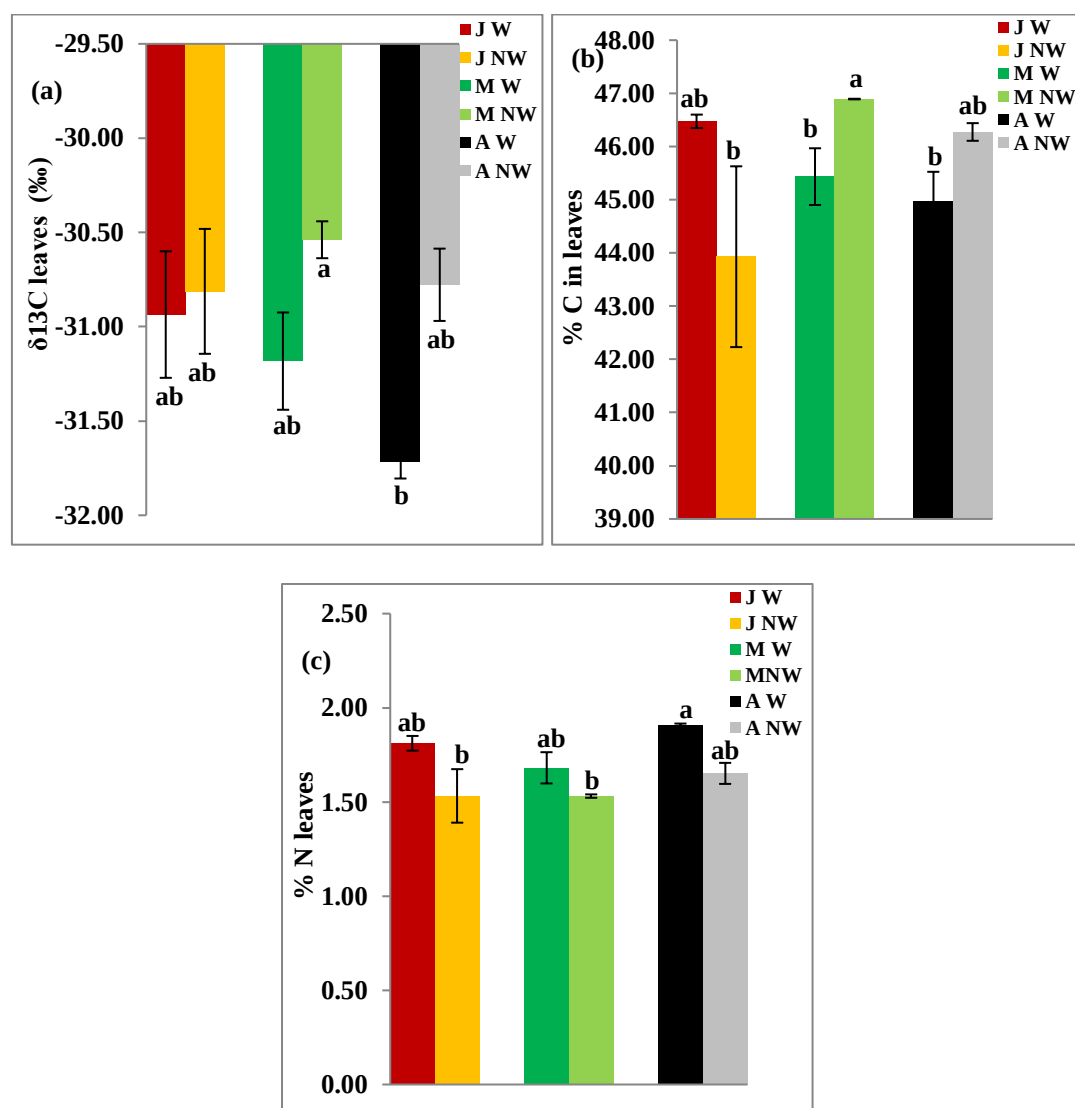


Fig.26. $\delta^{13}\text{C}$, C and N measurements: **(a)** $\delta^{13}\text{C}$, **(b)** %C and **(c)** %N leaves Contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid(M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0,05$). Means $\pm$ SE Mortality rate showed a significant difference among provenances ($P = 0.01$). It was 17.64%, 29.41% and 35.29% for Azazga, Jijel and M'Sila provenances respectively. M'Sila seedlings showed the lower survival rate (Fig. 27).

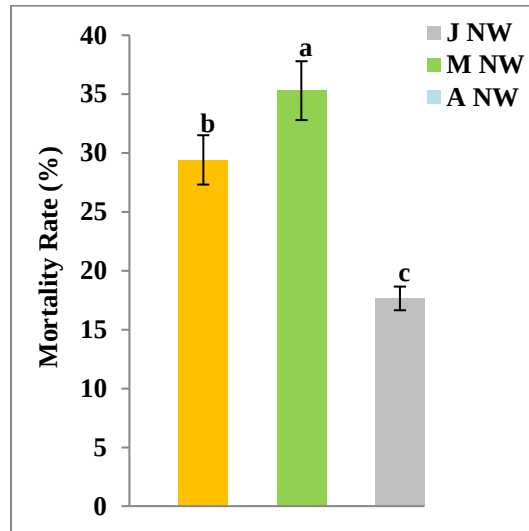


Fig. 27. Survival rates of *Q. suber* Jijel, M'Sila and Azazga seedlings provenances in drought conditions ($P < 0.05$). Means $\pm$ SE.

3-4-Discussion:

This study showed differences between the three provenances Jijel (humid), M'Sila (semi-arid) and Azazga (sub-humid) elevated homogeneously in greenhouse from watered and water stress conditions.

In watered conditions of all the morphological traits measured, only height and diameter growth exhibited significant population divergence. Previous studies had showed large differences between populations originating from different sites for morphological parameters. Total height was the most discriminate variable between 26 populations originating from six Mediterranean countries (Portugal, Spain, Tunisia, Morocco, Italia and Algeria) and contrary to our results where M'Sila showed the higher height growth, seedlings originating from high temperature sites displayed the lowest growth traits (Gandour *et al.*, 2007). Differences among seedlings provenances can be related to the climate of the seedling sources (Gandour *et al.*, 2007; Ramirez-Valiente *et al.*, 2010; Ennejah *et al.*, 2013) and to the seed mass (Quero *et al.*, 2007).

Drought conditions induced various morphological and physiological responses in the three provenances studied. So, *Q. suber* is particularly high sensitive to drought in the early stages of development (Aranda *et al.*, 2007)

The reduction of diameter growth and the SLA recorded in all seedlings provenances and the reduction of shoot growth was previously obtained by several authors (Ksontini *et al.*, 1998; Ramirez-Valiente *et al.*, 2010; Daoudi *et al.*, 2016). Interruption of shoot growth in the dry summer is a significant adaptation trait (Kurze-Besson *et al.*, 2006). SLA has often been observed to be reduced under drought conditions. In dry conditions, low SLA allow a more conservative water use maintaining photosynthetic activity and carbon gain over a longer period of time (Dudley, 1996).

The significant difference in diameter growth between Jijel, M'Sila and Azazga cork oaks populations, facing to water deficit were previously shown for Morocco populations; the early stages of oak population's seedlings development are affected differently by changes in soil water reserves and temperatures (Ennejah *et al.*, 2013; Ennejah *et al.*, 2014).

These intra-specific differences of traits appeared to be linked to interspecific differences in seed size in other hand to the confounding effect of seed size (Sanchez-Gomez *et al.*, 2008).

Water stress decreased the ratio of shoot biomass / root biomass of *Q. suber* seedlings (Ksontini *et al.*, 1998). The similar leaves biomass and root to shoot ratio traits in well-watered and water stressed plants maybe due to a similar degree of osmotic adjustment in root and leaf cells (Sobrado and Turner, 1986).

The reduction of starch and the accumulation of soluble sugars, soluble proteins and Proline in M'Sila and Azazga seedlings suggest the occurrence of an osmotic adjustment at leaf level which decrease leaf osmotic potential; this mechanism enhances seedlings potential to extract water from the drying soil by increasing the soil-plant water potential gradient (Kurze-Besson *et al.*, 2014). In Jijel provenance, osmotic adjustment can be accomplished by the accumulation of other metabolites. So, the compounds involved in osmotic adjustment differ widely among plant species and perhaps among populations (Patakas *et al.*, 2002).

Pigments leaves contents of the three provenances were not similarly affected by the drought. Only M'Sila seedlings showed a decrease in total chlorophylls, chlorophyll a, and carotenoids contents while Azazga seedlings showed a decrease only in carotenoids contents. Jijel seedlings were not affected for total chlorophylls and carotenoids contents. Generally, *Q. suber* water stressed seedlings showed a decrease of total chlorophyll with an increase in Chlorophyll b and carotenoids to cope with oxidative stress and a decrease of chlorophyll a to avoid excessive absorption of light energy (Faria *et al.*, 1996). Daoudi *et al.* (2016) found no

difference in total chlorophyll contents of seedlings originating from Azazga provenance and Vaz *et al.* (2010) reported that summer dry conditions did not affect Chlorophyll concentrations of *Q. suber* trees. Contrary to expectation, seedlings of M'Sila provenance which showed the better length growth had the lower chlorophyll leaves contents. A negative correlation between chlorophyll contents and growth was previously recorded by Ramirez-Valiente *et al.* (2010); plants exhibiting lower leaf chlorophyll content had larger annual shoot growth.

Large variations of leaf $\delta^{13}\text{C}$ have been observed for many species across their distribution range like *Q. Suber* (Gouveia and Freitas, 2009). The decrease of $\delta^{13}\text{C}$ leaves contents in Jijel and Azazga seedlings were similar to the results of Gouveia and Freitas (2009) who reported that trees subjected to greater water stress showed an increase of carbon isotope discrimination. This is due to the fact that water supply affects the stomatal conductance and photosynthesis of plants, which changes $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates (Du *et al.*, 2015). The reduction of %N in leaves of the three provenances seedlings was previously recorded by Daoudi *et al.* (2016) but was not observed by Kwak *et al.* (2011) for Tunisian provenance.

Summer drought is the main cause of seedling mortality in Mediterranean-climate areas. The significant difference in mortality rate under dry conditions between these Algerian provenances was not found by Gandour *et al.* (2007) for 26 provenances studied originating from six countries. However, differences in sapling survival across *Q. suber* provenances were recorded and positively related to the height of planted seedlings, and seedling size was closely related to acorn size, which was bigger in populations from warm and drier locations (Ramírez-Valiente *et al.*, 2009) in contrary to our results where M'Sila showed the better height but the lowest survival rate. Navarro *et al.* (2006) concluded that positive relationships between survival and seedling size were three times more frequent than cases showing negative relationships. Variation in seed mass is an important trait which may have consequence for growth and survival of seedlings because seed mass represents the reserves available for growth in the first stage of plant establishment (González-Rodríguez *et al.*, 2011). An increase in seedling size can result in higher transpiration, which increases plant vulnerability to drought on the short-term and this is the main argument for using small seedlings in dry sites (Villar-Salvador *et al.*, 2012).

In our experiments water stress did not act alone but was associated with high temperatures and high light stresses. Therefore, seedlings response to drought involves adjustment to stresses associated to drought.

This study showed differences in watered and non-watered conditions for morphological and physiological traits of three Algerian provenances (Jijel, M'Sila and Azazga) originating from humid, sub-humide and semi-arid floor at seedling stage. In terms of survival rate and water status, Azazga provenance seem to be the better provenance adapted to summer drought conditions of the Mediterranean area while survival rate of M'Sila provenance which is located in semi-arid floor was more affected by the drought. However, the growth of the seedlings was less affected for M'Sila than Azazga provenances. Indeed specimens from the site of M'Sila (semi-arid) when cultivated in water privation conditions, showed morphological and physiological changes that could be related to drought tolerance. They exhibited the lowest SLA, which means a reduction of the leaf evaporative surface. A decrease of Chlorophyll a contents was also noticed suggesting a potential reduction of the number of PSII for an efficient regulation of solar radiation absorbed in excess. A substantial enhancement of the water use efficiency (WUE) was obtained by $\delta^{13}\text{C}$ measurements. While starch content decreases, soluble sugars and proline increase, probably as potential compatible osmolytes and electron donor in response to drought. The intense metabolic activity related to plant tolerance to drought seems to be related to an increase accumulation and/or synthesis of total soluble proteins.

These results showed probably the existence of an inter-populations variability that may be linked to the specificity of plant-climate-coevolution. Hence, semi-arid sites of *Q. suber* may be considered as potential germplasm banks for reforestation in response to global climate change.

3-5-Conclusion :

Le comportement du Chêne liège, face à la sécheresse méditerranéenne, a été l'objet de nombreuses études essentiellement en Europe. Les provenances européennes et tunisiennes ont été largement étudiées alors que très peu d'études existent sur le comportement des provenances algériennes. L'Algérie, étant un pays plus aride que de nombreux pays européens, ses provenances pourraient montrer des différences de comportement par rapport aux provenances européennes.

L'étude de la réponse des plantules obtenues de glands originaires de trois provenances algériennes caractérisées par des étages bioclimatiques différents: Azazga (sub-humide), Jijel (humide) et M'Sila (semi-aride) à une sécheresse estivale simulée par un arrêt d'arrosage, pendant 10 semaines, de fin-juin à mi-septembre, a été réalisée dans une serre non contrôlée. Le statut hydrique et des paramètres morphologiques et biochimiques ont été évalués chez les plantules arrosées (témoins) et non arrosées (stressées) et les taux de survie des plantules non arrosées ont été déterminés pour les trois provenances.

Les résultats ont révélé des différences de comportement entre les trois provenances dans les deux conditions d'arrosage et de non arrosage.

Dans les conditions d'arrosage, les plantules originaires de M'Sila ont montré la teneur relative en eau (RWC) la plus élevée ainsi que la meilleure croissance.

L'arrêt d'arrosage, ayant provoqué une diminution significative des teneurs en eau du sol (SWC) et de la RWC des trois provenances, a réduit la croissance (hauteur et diamètre de la tige) des plantules originaires de Jijel et d'Azazga alors que seul le diamètre des tiges est réduit chez les plantules originaire de M'Sila.

Les feuilles des plantules originaires du site le plus aride, M'Sila, montrent des modifications morphologiques et physiologiques considérées comme étant des stratégies d'adaptation à la sécheresse : une faible SLA, une diminution des teneurs en Chlorophylles a, pour éviter une absorption excessive de l'énergie lumineuse, une diminution des teneurs en amidon, une accumulation des protéines, des sucres et de la proline afin de permettre un ajustement osmotique et une augmentation du $\delta^{13}\text{C}$ pour une meilleure utilisation de l'eau (WUE). Cependant, contre toute attente, les plantules de M'Sila ont montré le plus faible taux de survie.

Dans les conditions de non arrosage, les plantules d'Azazga ont montré un comportement intermédiaire entre les plantules originaires de Jijel et de M'Sila ainsi que le meilleur taux de survie.

Les écotypes étudiés dans ce travail montrent des traits fonctionnels différents pouvant être liés ou non aux conditions environnementales des provenances et suggérant l'existence d'une variabilité inter- et intra- populationnelle nécessitant des études plus approfondies.

4-Effect of sporal inoculation with *Boletus edulis* ectomycorrhizal fungi on growth and water status of *Pinus halepensis* M. seedlings growing under drought conditions.

Abstract

The main objective of this study was to obtain more comprehensive knowledge about the influence of sporal ectomycorrhizal inoculation with *Boletus edulis* on drought response of *Pinus halepensis* seedlings under greenhouse conditions. Relative water status in soil (SWC) and leaves (RWC), morphological parameters and mortality rate were evaluated in four plots of seedlings: watered, none watered, watered inoculated and none watered inoculated. Our results show that *B. edulis* ectomycorrhization did not improve, after six weeks of water starvation, soil and leaf relative water content, stem height, leaf biomass, shoot/root ratio. After eight weeks (mi-September), mortality rate was significantly higher in *B. edulis* inoculated (94,44%) than in non inoculated seedlings (83,26%). Under watered conditions *B. edulis* increased leaves biomass of the six months old seedlings.

4-1-Introduction:

Aleppo pine (*Pinus halepensis* Miller) is a native coniferous species in the Mediterranean region. It is the most widely distributed and abundant among the Mediterranean pines, covering nearly 6.8 million ha of this region (FAO, 2013). It widely covers the Mediterranean coasts concentrating in the western side of the basin. It is found at lower altitudes, occurring mostly in the thermo- and meso-Mediterranean zone, although it is also present at higher altitudes (more than 2 000 m in Morocco). Its habitat ranges from the lower arid or semi-arid to humid bioclimates favouring absolute minimum temperatures of between -2 and 10 °C and precipitation between 350 and 700 mm on marly limestones and marls (Chambel *et al.*, 2007). It grows very well in the hotter parts of the Mediterranean where forest fires are frequent. So, it is a thermophilous and heliophilous species well adapted to dry summer conditions of Mediterranean areas. It is one of the most drought-tolerant of all pines and this may have contributed to its widespread invasion of semi-arid. It's strategy towards drought is to avoid water loss by reducing its surface (lower needle growth rate, less amount of needles per branch and lower branching rate) which entails a lower leaf area index (LAI) (Lin *et al.*, 2010) and by closing precociously and rapidly its stomata (Borghetti *et al.*, 1998). Drought increases protective mechanism in *P. halepensis* (Müller *et al.*, 2001; Lin *et al.*, 2010). To protect from effect of high light intensity, *P. halepensis* posses a non-photochemical quenching process by reducing light harvesting pigments and increasing the reflectance and the excess thermal energy is dissipated by de-epoxidation in the xanthophylls cycle (Lin *et al.*, 2010).

Ectomycorrhizas (ECM) are symbiotic structures formed between plant roots and fungi that act as an extension of absorption system, where the fungal partner obtains photosynthetic

sugars from the host plant while, in return, the plant receives mineral nutrients from the fungus (Smith and Read, 1997). Many fungi build an ectomycorrhiza symbiosis with *Pinus halepensis* (El Karkouri *et al.*, 2005). Mycorrhizal inoculation of conifer roots is a key strategy to optimize establishment and performance of forest tree species under both natural and cultivated conditions and also to mitigate transplantation shock (Sanchez-Zabala *et al.*, 2013). It is hypothesized that ECM fungi may play a crucial role for tree regeneration, especially under adverse climatic conditions like drought (Kipfer *et al.*, 2012). There are many reports of positive mycorrhizal effects on seedling water uptake and seedling water relations during drought. So, some ectomycorrhizal fungi can help plants to tolerate water stress due to their less resistance to water flow from soil to roots, by increasing the absorbing surface and the ability of the fungus to penetrate finer pores as those operated by hairy roots (Parke *et al.*, 1983) and the most obvious indirect way for mycorrhizas to improve water relations is through efficient nutrient uptake which increase the efficiency of the photosynthetic machinery which will increase the water use efficiency (Lehto and Zwiazek, 2011). In terms of ecosystems response to global changes, mycorrhizal associations may reduce plants water stress (Mohan *et al.*, 2014). However, there are also many publications on those with no effects or negative effects of mycorrhizal colonization (Fini *et al.*, 2011). Inoculation of *P. halepensis* seedlings with *Pisolithus tinctorius* mycorrhizal fungi can improve the morphological and physiological qualities of plants, especially those used for regeneration of arid areas under drought conditions (Domínguez-Núñez *et al.*, 2013).

The effects of water scarcity on *P. halepensis* seedlings inoculated with *Boletus edulis* (Bull.: Fr.) were studied through water status and growth. Also, this work aims to provide management guideline in reducing seedling mortality and improving the productivity of Aleppo pine forests.

4-2-Materials and methods:

4-2-1-Experimental setting:

Seeds of *Pinus halepensis* Mill were collected, at the end of autumn, from national parc of Tlemcen located in Algeria (Longitude 1°35'51" 101, Latitude 34°8'51" 573, 994m altitude, semi-arid bioclimate) in October 2015. After one month stratification at 4°C, seeds were disinfected in 30% H₂O₂ for 20 min, rinsed in sterile distilled water and then germinated at 20°C. The seedlings were transplanted to plastic bags (30cm x15cm) filled with 1.5 kg of soil

substrate autoclaved twice in 24 h at 121°C, 1 h each time (Augé *et al.*, 2001; Chirino *et al.*, 2008). Soil substrate consisted of a mixture of loam (2/3) and washed sand (1/3) inoculated (I) or none inoculated (NI) with ectomycorrhizal fungi.

4-2-2-Ectomycorrhizal inoculation:

Spores suspension of *Boletus edulis* (Bull.: Fr.) collected from Bois de Rejouit forest (Cestas, Bordeaux, France) was used as mycorrhizal inoculum. The suspension was prepared in sterilised water. Two inoculations were made at 15 days intervals following seed germination. The final quantity of basidiospores per seedling was 10^6 . Control seedlings received no inoculation.

All the plants were grown in greenhouse, localized at Tizi-Ouzou characterised by a Mediterranean climate with a natural temperature and light cycle, and were watered regularly (twice a week) until end of July. Then we constituted two plots of 90 seedlings/plot: *B. edulis* inoculated and none inoculated. In each plot 45 seedlings were watered and 45 seedlings were not watered. Then we constituted three replicates/plot inoculated and none inoculated with 30 seedlings/replicate (15 seedlings watered and 15 seedlings not watered. Six weeks after end of July (mi-September), plants of the four treatments (W, NW, WI, NW I) were harvested for determination of water status and morphological parameters.

4-2-3-Ectomycorrhizal colonization assessment:

The percentage of ectomycorrhizal short roots was assessed under the stereomicroscope by counting all single root tips in the four treatments (WI, W NI, NW I, NW NI). Ectomycorrhizal root tips were detected according to the lack of root hairs and to the presence of fungal mantle and mycelium. Percentage ectomycorrhization was calculated as mycorrhizal root apex / total root apex (mycorrhizal and non mycorrhizal) of the root system (Parke *et al.*, 1983).

4-2-4-Measurement of substrate moisture contents (SWC):

Measurements of substrate moisture volumetric content (SWC) were made for all the treatments. Soil samples of 1 g are taken from the plastic bags at a depth of 10 cm and dried at 105°C for 72h and then water content was calculated using the formula: $(FW - DW) / FW$ (Mathieu and Pieltain 2003) where FW and DW are the fresh and dry weight, respectively.

4-2-5-Measurement of relative water content (RWC):

The RWC were measured on fully expanded leaves as described by Nardini *et al.* (1999). Five plants and three leaves per plants were examined. Fresh weight (FW) of the leaves was determined immediately after harvested and then were allowed to float on distilled water during 24 hours at 4°C, then turgid weight (TW) of the leaves were determined. The dry weight (DW) of the leaves was determined after drying at 75°C during 72h. The RWC were calculated as:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

The RWC was determined weekly during six weeks from start of august until mi-september.

4-2-6-Morphological parameters:

Morphological parameters were determined on ten seedlings per treatment. Shoot height and shoot basal diameter were measured. Shoots, roots, leaves biomass and mean leaf biomass (five leaves/seedlings and ten seedlings) were determined after drying at 75°C during 72h. Then we estimated the root to shoot ratio.

4-2-7-Mortality Rate:

Mortality rate in the four plots (WNI, NWN, WI, NWI) were determined every week during six weeks from start of August to mi-september.

4-3-Results:

4-3-1-Ectomycorrhizal rate:

Our results showed that the spore suspension used in this experiment was able to infect the roots of *P. halepensis* seedlings growing in plastic bags on loam and sand substrate. The results confirmed successful mycorrhizal synthesis between *P. halepensis* and *B. edulis*. The ectomycorrhizae formed have a dichotomous distribution (Fig. 28a). The cross sections of these ectomycorrhizae revealed the presence of a thick but discontinuous prosenchymatous fungal mantle and a well developed Hartig net over two to three layers of cortical cells (Fig. 28 b, c and d).

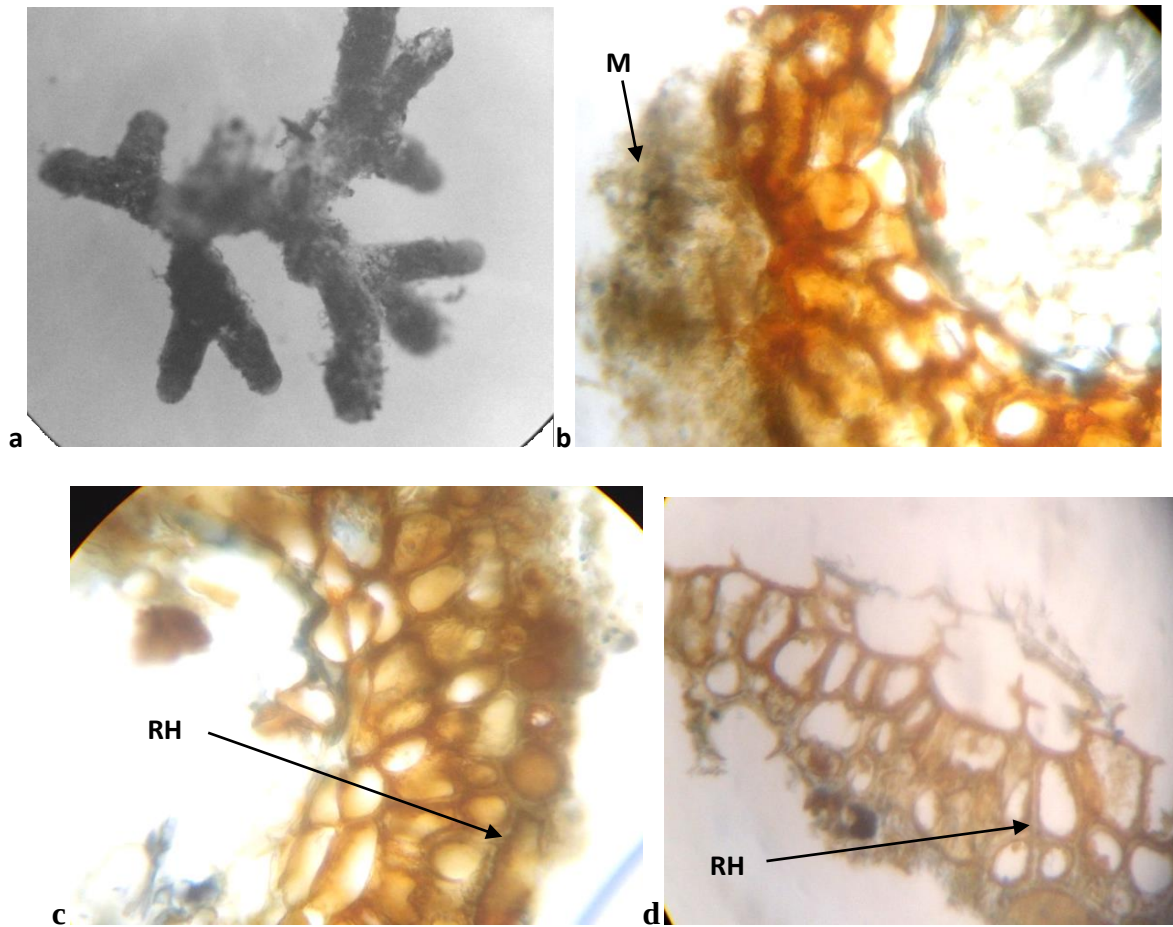


Fig. 28. Ectomycorrhiza formed between *Boletus edulis* and *Pinus halepensis* seedlings: **a** morphological aspect (X10 X20), **(b)**, **(c)** and **(d)** microscopical aspect showing **(b)** a discontinuous prosenchymatous mantle (M), **(c)** and **(d)** Hartig net well developed between cells of two to three cortical layers (RH) (X10X40).

The mycorrhization rate varies significantly according to the substrate water availability. Because the study was carried out in non-sterile conditions, a certain degree of colonization occurred in control seedlings. Four ectomycorrhizal morphotypes were identified in the four plots inoculated or not inoculated (W NI, NW NI, WI, NW I). Water starvation significantly reduces root tips ectomycorrhization. Ectomycorrhizal infection was 2.06 -fold higher in watered than in none watered seedlings and the lowest ectomycorrhization rate (9.119%) was recorded in unsprayed and none inoculated plants (Fig. 29a).

4-3-2-Water status:

The SWC is reduced by water starvation in inoculated and non inoculated plots. The occurrence of mycorrhizas did not affect significantly soil water status because it remains constant in inoculated and non inoculated water starvation conditions (Fig. 29b). The presence of the ectomycorrhizal fungi did not improve water status in plants since the RWC values in drought conditions were similar in inoculated and non inoculated seedlings (Tab. IV). During four weeks, seedlings of the four plots showed no significative difference in the RWC. After seven weeks, the drought conditions decreased significantly the RWC in non watered inoculated and in non watered non inoculated seedlings (Fig. 29c).

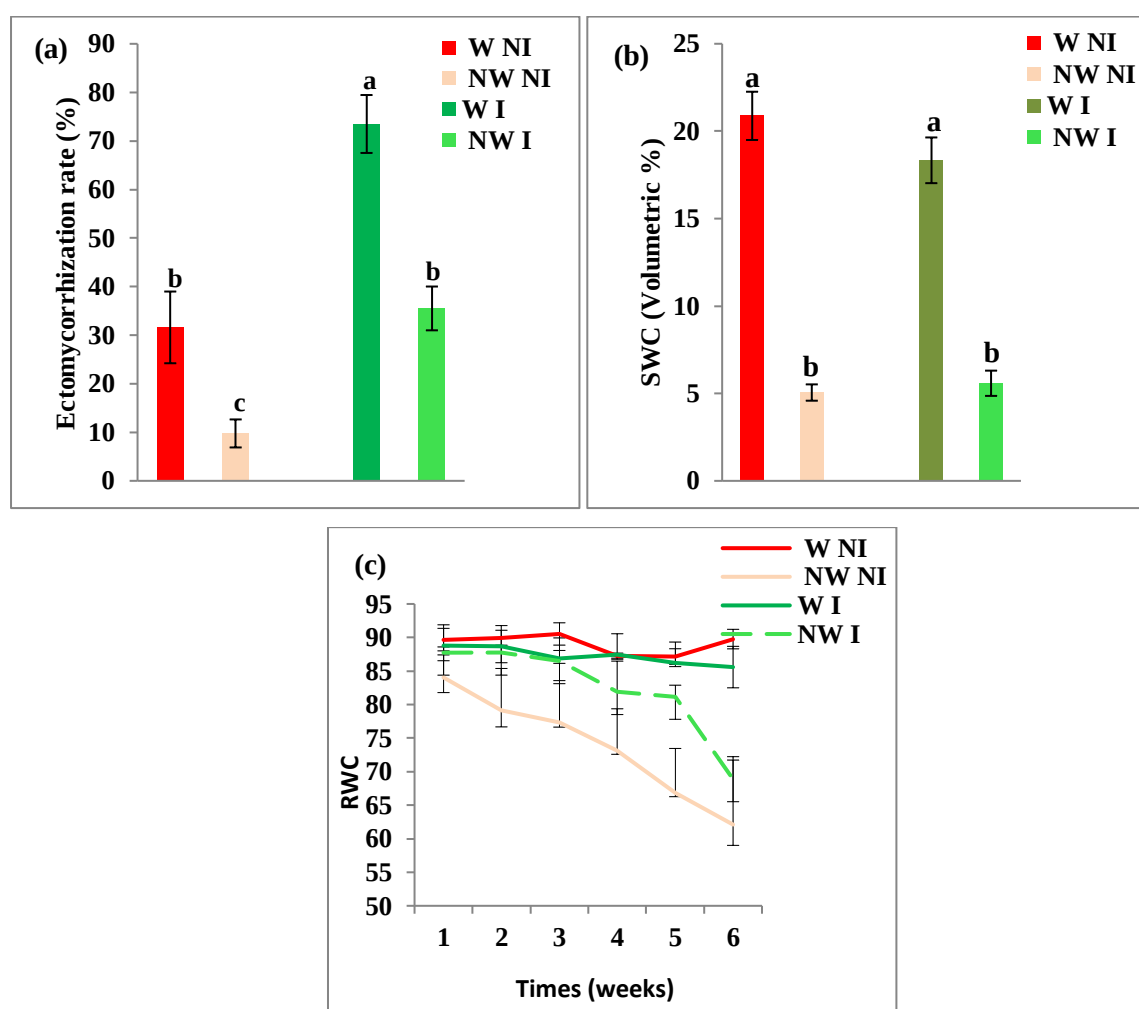


Fig. 29. Water status measurements and ectomycorrhization rate: **(a)** ectomycorrhization rate, **(b)** soil water contents and **(c)** RWC in *Pinus halepensis* seedlings watered non inoculated (W NI), non watered non inoculated (NW NI), watered inoculated (WI) and non watered inoculated (NW I). For ER n=10; RWC n=5 and SWC n=10. Letters indicate significant differences according to LSD test: a<b<c (P<0.05). Means±SE.

Tab. VI: evolution of the RWC in *Pinus halepensis* seedlings watered non inoculated (W NI), non watered non inoculated (NW NI), inoculated watered (I W), inoculated non watered (I NW) with time (mean±SE, p<0.05).

Times (Weeks)	W NI	NW NI	I W	I NW
1	89.682±1.683	84.043±3.349	88.879±2.259	87.956±0.834
2	89.969±1.109	79.111±6.269	88.679±2.447	87.756±0.937
3	90.527±1.656	77.327±6.241	86.830±0.683	86.471±1.594
4	87.218±0.457	73.123±6.241	87.457±0.535	81.864±4.618
5	87.157±1.158a	66.835±9.622b	86.229±0.571a	79.165±1.726b
6	89.761±1.458a	62.108±9.625b	85.585±3.096a	75.871±3.359b

4-3-3-Seedlings growth:

Comparing growth between watered inoculated and non inoculated seedlings, the results showed that ectomycorrhizal fungi increases leaves biomass while it did not affect the other growth parameters (Fig. 30). Shoot height (Fig. 30a), Leaf biomass (Fig. 30d) and R/S ratios (Figure 30e) were not affected by the drought conditions while shoot basal diameter (Fig. 30b) was reduced in inoculated and non inoculated seedlings after six weeks of growth. So, it appears that under drought conditions, the ectomycorrhizal inoculation did not improve the growth of *P. halepensis* seedlings because leaves biomass parameter decreased only in inoculated seedlings (Fig. 30c).

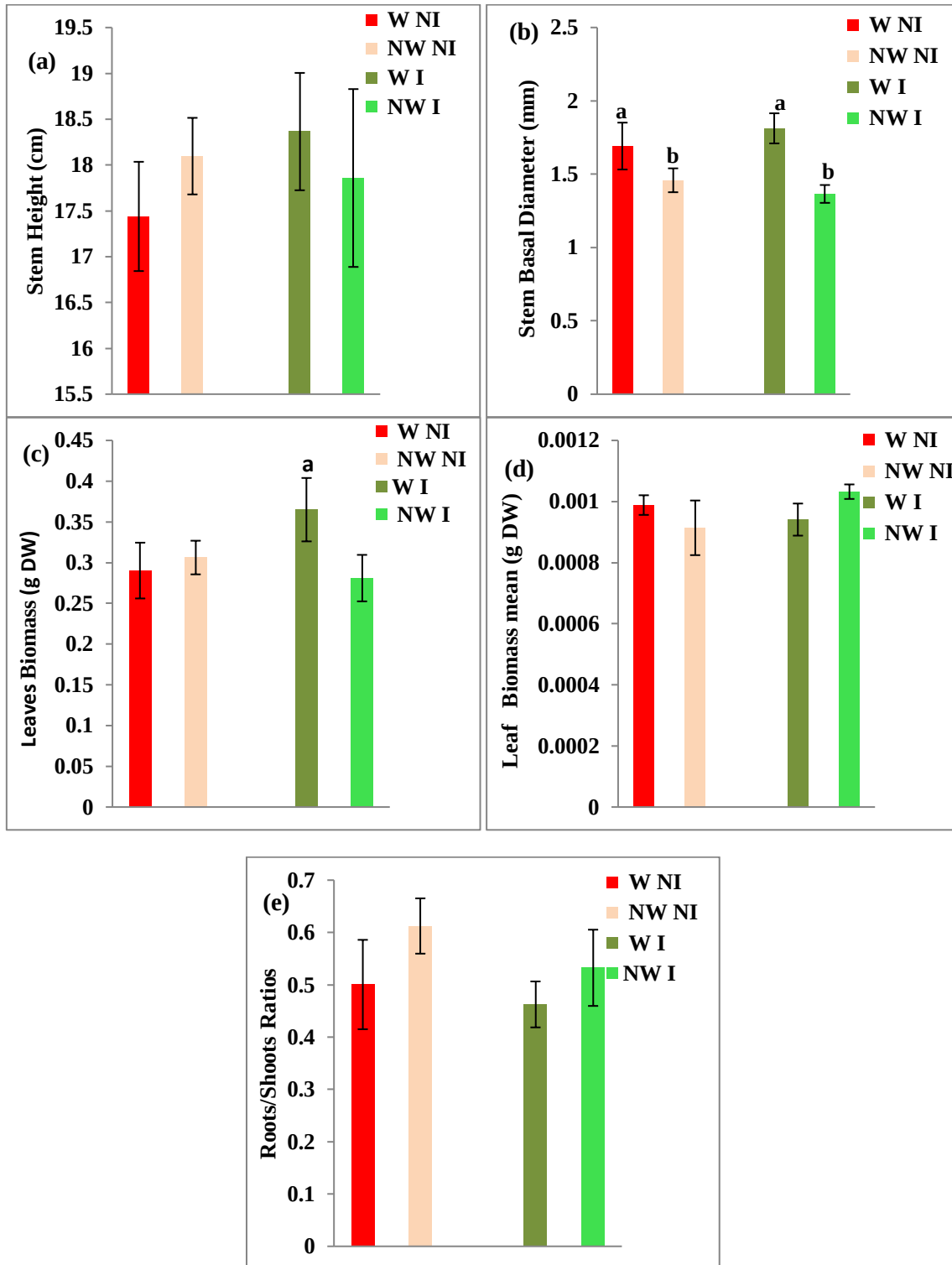


Fig. 30. Morphological parameters measurements: (a) Shoot height, (b) shoot basal diameter, (c) leaves biomass, (d) leaf biomass mean and (e) shoots/roots ratio in *Pinus halepensis* seedlings watered non inoculated (W NI), non watered non inoculated (NW NI), watered inoculated (W I) and non watered inoculated (NW I). (P<0,05). Means±SE.

4-3-4-Mortality Rate:

Seedlings mortality started the fourth week in both inoculated and non inoculated seedlings. Under drought conditions, after eight weeks (mi-September), mortality rate was significantly higher in *B. edulis* inoculated (94.44%) than in non inoculated seedlings (83.26%) (Fig. 31).

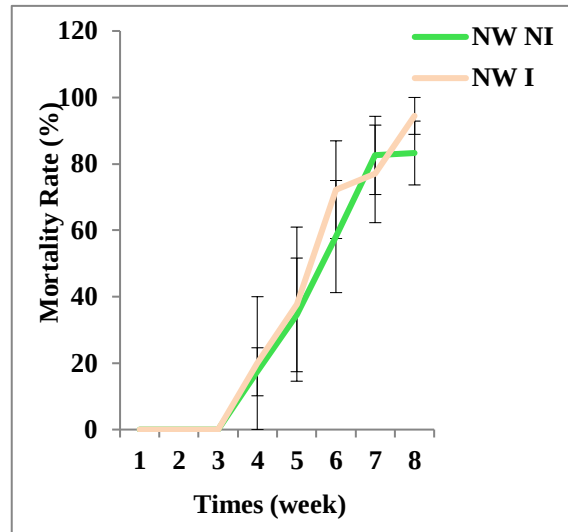


Fig. 31. Evolution of mortality rate during eight weeks in non watered non inoculated (NW NI) and non watered inoculated (NW I) *Pinus halepensis* seedlings.

4-4-Discussion:

The experiment of sterilized substrates inoculation with *Boletus edulis* led to infect seedlings of *Pinus halepensis*. So, the ectomycorrhiza formed is similar to the ectomycorrhiza formed between *B. edulis* 409 strain and *P. pinaster* (De la Verga Pastor, 2013). Torres and Honrubia (1994) had previously shown that the inoculation of soil with basidiospores suspension of three ectomycorrhizal fungi is effective for the development of ectomycorrhizas in *P. halepensis* six months old seedlings. The control seedlings were also colonized by mycorrhizal fungi. Mycorrhizal infection obtained in none inoculated seedlings was also found by other researchers working in greenhouse conditions (Fini *et al.*, 2011). We observed a higher ectomycorrhization rate in watered seedlings. Many studies found that watering significantly increased ECM colonization. For example, ECM rate was higher in *Quercus pubescens* and *Q. ilex* (Shahin, 2012) seedlings grown on adequate watered substrate. Water-stressed plants may be unable to support high levels of ECM colonization due to stomatal closure, which reduces the total amount of carbon available to the plant (Dosskey *et al.*,

1991). The presence of other ectomycorrhizal morphotypes in inoculated and non inoculated seedlings was often observed in greenhouse conditions (Fini *et al.*, 2011).

Inoculation with *B. edulis* ectomycorrhizal fungi did not improve SWC in irrigated and none irrigated substrates. Sometimes ECM induced changes in soil water retention properties derived from hyphal and exudates characteristics which affect soil structure and not from differential rooting patterns (Augé *et al.*, 2001).

The decrease in RWC in both inoculated and non-inoculated seedlings showed that *B. edulis* ectomycorrhizal fungi used did not improve the water status of the seedlings under drought conditions. Seedling water relations measured by water use efficiency and $\delta^{13}\text{C}$ did not differ by ECM status, but did differ by soil moisture treatment in *P. muricata* seedlings inoculated with *Rhizopogon* species (Kennedy and Peay, 2007).

Large variation in response to inoculation arises from factors such as the degree of host–fungus compatibility, mycorrhizal dependency of the host, fungal effectiveness in relation to biotic and abiotic site conditions, and the abundance and effectiveness of indigenous fungi (Ortega *et al.*, 2004). Seedlings height varied according to fungi species and the inoculum type used (spore suspension and mycelium inoculum).

Under drought conditions inoculation of *P. radiata* with two ECM fungi (*Rhizopogon roseolus* and *Scleroderma citrinum*) resulted in increased water uptake that was sufficient to permit the stomata to remain open for most of the day and the ability to maintain open stomata and photosynthesis during the early stages of a drought could increase the supply of carbon for growth and osmotic adjustment (Ortega *et al.*, 2004) while in *P. sylvestris* seedlings, four ECM fungi (*Cenococcum geophilum*, *Paxillus involutus*, *Rhizopogon roseolus* and *Suillus granulatus*) did not provide additional support during drought stress (Kipfer *et al.*, 2012).

Inoculation with different fungi generally did not stimulate and even reduced seedlings growth. Height growth and dry mass of leaves, shoots and roots were significantly reduced during an imposed drought period in *P. halepensis* stressed plants with high population divergence in phenotypic responses (Chambel *et al.*, 2007). Low growth rates in mycorrhizal plants have been often attributed to the high demand for carbohydrates required by the fungus. In nursery, the height of *P. halepensis* seedlings was significantly enhanced by inoculation with many ECM fungi (Rincón *et al.*, 2007). Kipfer *et al.* (2012) showed that shoot growth of *P. sylvestris* seedlings was enhanced only in the presence of *S. granulatus*, but not by three

other ECM fungal species investigated, but this positive effect on growth was more pronounced under moist (three fold increase) than under dry conditions (two fold increase) indicating that ECM fungi did not provide additional support during drought stress. *Rhizopogon* species had benefit effect on shoot growth of *P. muricata* seedlings under moist (13% volumetric water content) but not dry (7% volumetric water content) conditions (Kennedy and Peay, 2007). So, the ectomycorrhizal fungi were affected by water shortage and therefore had limited capacity to provide resources to their host (Kennedy and Peay 2007) or the bag size might have limited the growth of an extensive mycelium, and therefore constrained the beneficial effect of ECM fungi on water acquisition and uptake because under natural conditions some ECM fungi can form rhizomorphs (Kipfer *et al.*, 2012).

In mediterranean environments, tree-ring growth is mainly limited by soil water availability, and precipitation plays a major role in determining cambial activity. Cambial activity in *Pinus halepensis* can slow down, or even stop, during summer drought triggered by high temperatures and lack of precipitation (Cherubini *et al.*, 2003).

The increase of leaves biomass in inoculated seedlings permits the production of higher quantities of carbohydrates which can transfer to the mycorrhizal fungi.

B. edulis increased significantly rate mortality of *P. halepensis* six months old seedlings. Rincón *et al.* (2007) had previously showed that one and 2 years after planting of *P. halepensis* seedlings in the field, the survival was significantly improved by inoculation with *Suillus collinitus*, but *Amanita ovoidea* had no significant effect on seedling survival and *Rhizopogon roseolus* caused a significant mortality of seedlings. *R. luteolus* inoculation increased *P. radiata* seedling survival, but reduced plant size (Atala *et al.*, 2012).

Ectomycorrhizal inoculation with *Boletus edulis* did not improve water status and growth of *Pinus halepensis* seedlings under drought conditions. Many publications showed no effects or negative effects of mycorrhizal colonization on seedlings under drought conditions like Fini *et al.* (2011). Perhaps other ectomycorrhizal fungi can help seedlings in these stressed conditions.

4-5-Conclusion :

Des ectomycorhizes entre les plantules de *Pinus halepensis* Mill. et le champignon *Boletus edulis* ont été obtenues, par inoculation sporale, en serre non contrôlée, après stérilisation du substrat. Ces ectomycorhizes présentent les mêmes caractéristiques morphologiques et anatomiques que celles publiées par de nombreux auteurs chez les pins.

La réussite de cette ectomycorhization nous a permis d'étudier l'influence des ectomycorhizes formées par *B. edulis* sur la réponse des plantules de *P. halepensis* Mill., âgées approximativement de six mois, à une sécheresse estivale simulée par un arrêt d'arrosage pendant six semaines, de mi-juillet à fin août, dans une serre non contrôlée. Le statut hydrique, par mesure de la RWC, et différents paramètres morphologiques pour l'évaluation de la croissance ont été mesurés chez quatre lots de plants de Pin d'Alep : arrosé non inoculé, non arrosé non inoculé, arrosé inoculé et non arrosé inoculé.

Les résultats obtenus montrent que l'ectomycorhization avec *B. edulis* a un effet bénéfique sur la biomasse foliaire du Pin d'Alep en conditions d'arrosage seulement. Ainsi, d'importantes quantités d'Hydrates de Carbone seraient produites par les plants afin de satisfaire les besoins en hydrates de carbone du champignon symbiotique.

Les plants inoculés non arrosés montrent le même comportement que les plants non inoculés non arrosés : réduction de la RWC et réduction de la croissance en hauteur.

Le taux de survie est statistiquement inférieur chez les plants inoculés non arrosés que chez les plants non inoculés non arrosés.

De tels résultats sont de plus en plus signalés par de nombreux auteurs. En effet, l'ectomycorhization peut avoir :

- un effet neutre (sans effet) ;
- un effet positif ;
- un effet négatif essentiellement chez les plantules âgées de moins de deux ans.

Conclusion Générale

La sécheresse constitue la principale contrainte environnementale subie par la végétation méditerranéenne. Le stress hydrique est l'un des stress engendré par cette sécheresse. Ce stress hydrique induit de nombreux effets négatifs qui, généralement, quand la sécheresse est intense et récurrente, provoque une diminution de la productivité des forêts. Ce stress hydrique risque d'être amplifié par le réchauffement climatique annoncé dans la région méditerranéenne.

Cette étude a été consacrée à l'évaluation du comportement de jeunes plants de trois espèces ligneuses chêne liège, chêne vert et pin d'Alep, très importantes en Algérie, où elles sont très peu étudiées, et dans d'autres pays de la région méditerranéenne où elles sont très étudiées (Espagne, Portugal, Tunisie...), face au stress hydrique induit par un arrêt d'arrosage pendant l'été. Cette étude présente un double intérêt :

- théorique pour une meilleure compréhension des réponses de ces espèces face au stress hydrique
- théorique et pratique à la fois par la réalisation d'une comparaison des comportements de ces espèces originaires d'Algérie et de ces mêmes espèces originaires d'autres pays méditerranéens car des études récentes ont révélé l'existence de variabilité inter et/ou intra-spécifiques face au stress hydrique afin d'identifier l'espèce ou la provenance la plus tolérante et la privilégier dans les opérations de reboisement.

L'étude du comportement des plantules de chêne liège, espèce choisie pour son importance économique et écologique face à une sécheresse estivale, simulée par un arrêt d'arrosage, pendant 10 semaines (de fin juin à mi-septembre) dans une serre non contrôlée, a révélé que cette espèce montre une réduction significative de la RWC, de l'ectomycorhization et des croissances en hauteur et en épaisseur. L'arrêt d'arrosage a également provoqué une augmentation des teneurs en sucres et protéines solubles qui permettent un ajustement osmotique et une diminution des taux de polyphénols et de %N dans les feuilles. La réduction de la teneur en eau du sol (SWC) est négativement corrélée à la discrimination isotopique du carbone qui assure une augmentation de l'efficacité d'utilisation de l'eau (WUE).

Ces différentes réponses révèlent l'existence de plasticités morphologique et physiologique qui permettent de classer le chêne liège parmi les espèces tolérantes à la sécheresse.

L'étude comparative du comportement du chêne liège et du chêne vert, deux espèces ligneuses méditerranéennes très importantes, face à une sécheresse estivale, simulée par un arrêt d'arrosage pendant 10 semaines (de juillet à mi-septembre) a montré :

→ l'existence de plasticités morphologique et physiologique chez les deux espèces :

- morphologique par réduction de la SLA, des croissances en hauteur et en épaisseur et une augmentation du rapport R/S considérée comme des mécanismes de **drought avoiding tolerance**.

- physiologiques : grâce à un ajustement osmotique par accumulation de sucres, protéines et proline et une augmentation des teneurs en ^{13}C dans les feuilles montrant une augmentation de l'efficacité de l'utilisation de l'eau (WUE) permettant de les classer dans les **drought tolérante species**.

→ Contrairement aux résultats publiés sur ces deux espèces originaires d'Europe et de Tunisie, le chêne liège, originaire d'Algérie, montre une meilleure résistance au stress hydrique que le chêne vert également originaire d'Algérie.

→ Les provenances, ou l'origine des glands, paraissent avoir une influence sur la tolérance au stress hydrique. En effet, les caractéristiques des stations (climatiques, pédologique) provoquent une sélection d'espèces adaptées à la sécheresse.

L'étude du comportement des plantules obtenues à partir de glands originaires de trois provenances de chêne liège situées dans trois différents étages bioclimatiques humide (Jijel), sub-humide (Azazga) et semi-aride (M'Sila) face à une sécheresse estivale de 10 semaines par arrêt d'arrosage (de juillet à mi-septembre) a montré que :

- Le statut hydrique, la croissance (en hauteur et en épaisseur) et les paramètres biochimiques des plantules des trois provenances sont différents dans les conditions d'arrosage. Les plantules de M'Sila montrent un meilleur statut hydrique (RWC élevée) et la croissance la plus importante.

- L'arrêt d'arrosage, ayant induit une réduction de la teneur en eau du sol (SWC), a eu des effets négatifs similaires sur les plantules de Jijel et Azazga : une diminution de la RWC, de la croissance, de la SLA des teneurs foliaires en amidon, $\delta^{13}\text{C}$ et N.

- Les plantules dont les glands proviennent de M'Sila, qui est le site le plus sec (semi-aride), montrent des modifications foliaires morphologiques et physiologiques connues comme étant des stratégies d'adaptation à la sécheresse : une faible SLA, une diminution de la teneur en chlorophylle totales pour éviter l'absorption excessive de l'énergie lumineuse, une réduction de la teneur en amidon, une accumulation des protéines solubles, des sucres et de la proline permettant un ajustement osmotique et une augmentation des teneurs en $\delta^{13}\text{C}$ reflétant une meilleure efficacité de l'utilisation de l'eau (WUE).

- l'existence d'une variabilité inter-populations (et intra-populations ?) chez le chêne liège.

L'étude de l'effet d'une inoculation ectomycorhizienne avec *Boletus edulis*, champignon ectomycorhizien commun, sur le comportement des plantules de *Pinus halepensis* soumises à une sécheresse estivale simulée par un arrêt d'arrosage pendant six semaines, du début août à la mi-septembre, a montré que :

- l'inoculation des plantules avec une suspension sporale a permis la formation d'ectomycorhizes morphologiquement et anatomiquement identiques à l'ectomycorhize formée par *B. edulis* chez d'autres espèces de pins. Cependant, la concentration de spores de la suspension sporale utilisée est peut être insuffisante car les taux de mycorhization sont inférieurs à 100%. Quelques morphotypes ectomycorhiziens, souvent observés en pépinières forestières, ont été observés chez les plantules.
- dans les conditions d'arrosage, *B. edulis* a augmenté le poids de la biomasse foliaire des plantules
- dans les conditions de non arrosage, *B. edulis* n'a pas amélioré le statut hydrique, évalué par la teneur relative en eau (RWC) et la croissance des plantules, au contraire, il a significativement diminué la biomasse foliaire et le taux de survie des plantules.

Nos résultats rejoignent ceux de plusieurs auteurs, ces dernières années, qui ont montré que l'ectomycorhization n'a pas d'effet bénéfique chez les plantes stressées.

Perspectives :

Des études supplémentaires sur la compréhension du comportement des espèces forestières méditerranéennes face à la sécheresse sont nécessaires dans un but théorique, identification et compréhension des mécanismes impliqués dans la réponse, mais aussi dans un but pratique pour effectuer des choix plus judicieux des espèces destinées aux reboisements dans la région qui serait l'une des régions les affectées par le réchauffement climatique. De même l'application d'une contrainte hydrique en dehors de la saison estivale (espèces méditerranéennes souvent adaptées à cette sécheresse), par exemple en hiver, s'avère nécessaire car le réchauffement climatique annoncé provoquera une modification du régime de pluies dans la région.

Des études ultérieures sont nécessaires voire indispensables pour l'exploration de la variabilité inter ou intra-populationnelle des chênes et du pin d'Alep en Algérie ceci par utilisation de graines de différentes provenances. En effet, nos résultats rejoignent ceux de nombreux travaux récents montrant l'existence de différences morpho-anatomique et physiologique entre différentes provenances.

La recherche d'autres champignons ectomycorhiziens qui seraient tolérants au stress hydrique donc capables d'aider les plantules à supporter le choc de transplantation et la sécheresse estivale souvent longue dans la région méditerranéenne est importante. De tels champignons seraient sélectionnés et utilisés en mycorhization contrôlée en pépinières pour une meilleure réussite des reboisements qui seraient confrontés à des sécheresses intensifiées par le réchauffement climatique.

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Articles

Full Length Research Paper

Growth, ectomycorrhization and biochemical parameters of *Quercus suber* L. seedlings under drought conditions

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Drought conditions are the major constraint to the early establishment of *Quercus suber* species. However, drought responses of this species depend on provenances. The objective of this study was to obtain more comprehensive knowledge on the influence of drought conditions on the response of *Q. suber* L. seedlings originating from Algeria. Soil water status in soil (SWC) and relative water content (RWC) in leaves, morphological parameters for growth evaluation and physiological parameters, and ectomycorrhization were evaluated in two plots of seedlings: watered (W) and none watered (NW). Ten weeks of water starvation induced a reduction in RWC, ectomycorrhization rate and height and diameter growth of none watered seedlings. Under drought conditions, both soluble sugars and proteins were enhanced while polyphenols and %N decreased. The reduction of soil water content was negatively correlated with carbon isotope discrimination. The survival of the seedlings under these drought conditions was due to two strategies: osmotic adjustment through soluble sugars and proteins accumulations in leaves and an increase in carbon discrimination which enhances the water use efficiency (WUE).

Key words: *Quercus suber*, growth, drought, ectomycorrhization, pigments, sugars, starch, polyphenols, $\delta^{13}C$.

INTRODUCTION

Summer drought in the Mediterranean region is characterized by 4 months dry period with little or no precipitations, high temperatures and high irradiance, considering the major constraints for vegetation

(Aussenac, 2000; Faria et al., 1999). Mediterranean species are thus, often exposed to water stress during summer (Pardos et al., 2005). Mediterranean oak species have developed mechanisms to avoid excessive loss of

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cell water and to maintain growth (Caritat et al., 2006). Increased drought severity is expected in the Mediterranean basin over the twenty-first century (Aussenac, 2002; Ramirez-Valentine, 2011) and the increase in the length of the dry season may lead to severe water deficit and tree mortality (Kurze-Besson et al., 2006). Priority should be given to ecophysiological research for the study of the adaptative plants Mediterranean behavior under these predicted summer conditions.

The cork oak (*Quercus suber*) is a western Mediterranean species, widely distributed in Mediterranean forest, thus presenting significant ecological and economic interests. It is an evergreen and sclerophyllous species growing from the sea level up to 700 m in altitude (Quezel and Médail, 2003). This plant is well adapted to summer conditions because it maintains a favorable ratio between water loss and uptake during the dry period. Also, *Q. suber* is considered as a drought tolerant species (Nardini et al., 1999; Nardini and Tyree, 1999). Maintaining a favorable water status in tissues, with a high relative water content (RWC) during summer drought, is ensured by deep roots and/or osmotic adjustment through accumulation of molecules such as proteins, sugars and proline (Kwak et al., 2011; Otieno et al., 2006; Pardos et al., 2005). A decrease in shoots and leaves biomass by reducing growth and root drop is also noted (Kurze-Besson et al., 2006; Ksontini et al., 1998).

Cork oak is an ectomycorrhizal species. Molecular and morphological approaches analysis led to the identification of fifty-five taxa of fungi forming wide diversity of ectmycorrhizas (Azul et al., 2010). Under drought conditions, controlled ectomycorrhization increases the performance of *Q. suber* L. (cork oak) nursery and field seedlings (Sebastiana et al., 2013) but little is known about the effect of drought on ectomycorrhization (Richard, 2011; Shahin, 2012).

The carbon isotope discrimination ($\delta^{13}\text{C}$) of foliage integrates signals resulting from environmental constraints like water availability (Warren and Adams, 2000). Therefore, $\delta^{13}\text{C}$ may be a useful indicator of drought stress in seasonally dry climates (Warren and McGrath, 2001) because water supply affects the stomatal conductance and photosynthesis of plants, which changes $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates (Du et al., 2015).

Although, *Q. suber* is well adapted to dry conditions, it remains that its natural regeneration is low. This seedlings recruitment limitation probably will be amplified with the predicted global changes in Mediterranean region. Muhamed et al. (2013) showed that spatial patterns of association between understory shrubs and oak seedlings are very sensitive to increasing drought under climate change, while it is known that plants neighbors can promote oak regeneration.

Many studies on *Q. suber* trees and seedlings behavior

to water stress on the northern Mediterranean shore exist (Nardini and Tyree, 1999; Nardini et al., 1999; Otieno et al., 2006); however, few studies on the responses of this oak species to water stress were performed in Algeria (Acherar et al., 1992).

Understanding the responses of cork oak to actual and predicted summer conditions is essential to determine the future sustainability of cork oak woodlands. In this context, the effects of water scarcity on *Q. suber* were studied through growth, water relations, ectomycorrhization, photosynthetic pigments, sugars, starch, protein, polyphenols, $\delta^{13}\text{C}$ and % N contents.

MATERIALS AND METHODS

The experimental setting

Acorns of cork oak (*Quercus suber*) were collected, in November 2010, from Beni Ghobri forest located in Azazga (Tizi-Ouzou, Algeria) ($36^{\circ}42'$ to $36^{\circ}47'$ N; $4^{\circ}22'$ to $4^{\circ}27'$ W longitude, 620 m altitude). After one month stratification at 4°C , seeds germinated at 20°C . To limit the effect of acorn variation in the seedlings, the seedlings with similar shoot height were transplanted to plastic bags (30 x15 cm) filled with 1.5 kg of soil substrate. Soil substrate consisted of a mixture of natural soil from Beni Ghobri forest (2/3) as source of ectomycorrhizal inoculums and washed sand (1/3). The plants were grown in a greenhouse, localized at Tizi-Ouzou (140 m altitude, $36^{\circ} 42' 12886''$ N and $4^{\circ} 2' 53 3339''$ E) characterized by a Mediterranean climate, and were watered regularly (three fold a week) until mid-July. Then, two plots (30 seedlings and 3 replicates/ plot) of seedlings were constituted: watered (W) and none watered (NW). Ten weeks after end of September, seedlings of the two treatments (W, NW) were harvested for determination of morphological and physiological parameters.

Measurement of substrate moisture contents

Measurements of substrate moisture volumetric content were made for the plots. Soil samples of 1 g were taken from the plastic bags at a depth of 10 cm and dried at 105°C for 72 h and then water content was calculated using the formula: $(\text{FW} - \text{DW})/\text{FW}$ (Mathieu and Pieltain, 2003) where FW and DW are the fresh and dry weight, respectively.

Measurement of relative water content (RWC)

The RWC was measured on fully expanded leaves as described by Nardini et al. (1999). Five plants and three leaves per plants were examined. Fresh weight (FW) of the leaves was determined immediately after harvesting and then allowed to float on distilled water during 24 h at 4°C , then turgid weight (TW) of the leaves was determined. The dry weight (DW) of the leaves was determined after drying at 75°C during 72 h. The RWC were calculated as:

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Morphological parameters

Morphological parameters were determined on ten seedlings per

treatment. Stem length and collar diameter were measured. Shoots, roots and leaves biomass were determined after drying at 75°C for 72 h. Then, the root to shoot ratio was estimated.

Physiological parameters

Chlorophylls, sugar, starch, proteins and total polyphenols contents were determined on five seedlings per treatment and 2 to 3 leaves/seedling.

Chlorophylls contents of fresh leaf discs (0.1 g) were determined spectrophotometrically after extraction in acetone 80% in the dark and the chlorophyll contents were calculated as proposed by Lichtenthaler and Buschmann (2001):

$$\text{Chl} + \text{b} = 7.15 \times \text{A}_{663} - 18.71 \times \text{A}_{647}$$

Soluble sugars were extracted from fresh leaves in ethanol (70%), the residues were incubated in HCl (1.1% v/v) for 30 min at 95°C for starch extraction. Then, soluble sugars and starch were quantified colorimetrically at 625 nm with anthrone reagent following Cerning-Berard (1975) method.

Soluble proteins contents were determined following Bradford (1976) method. They were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595 nm after colorimetric reaction with Bioard reagent.

Total polyphenols were determined spectrophotometrically following the method described by Peñuelas et al. (1996). 2.5 g powder per sample of dry leaves were extracted with 20 ml of 70% aqueous methanol (v/v) acidified with some concentrated HCl drops. The samples were left at ambient temperature in the dark for an hour and a half. The extracts were filtered and the total polyphenols were quantified after colorimetric reaction using Folin-Ciocalteu reagent during one hour in dark at 765 nm. The total polyphenols content was calculated as gallic acid equivalent from the calibration curve of gallic standard solutions and expressed as mg gallic acid equivalent/g of dry weight.

Foliage samples for analysis of carbon isotopic discrimination $\delta^{13}\text{C}$, C% and N% were dried at 70°C for 72 h and ground to a fine powder. The abundance in combusted samples was performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) in CNRS UMR 7266 LIENSS with a precision of 0.1‰. $\delta^{13}\text{C}$ (‰) was calculated with respect to the PDB Pee Dee Belemnite standard:

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1)1000$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in a sample and the standard (Pee Dee Belemnite), respectively (Warren and Adams, 2000).

Ectomycorrhizal colonization assessment

Percentage of ectomycorrhization was determined with a binocular and calculated as mycorrhizal root apex/total root apex (mycorrhizal and non mycorrhizal) of all the root systems (Parke et al., 1983).

Statistics analysis

Statistical analysis was performed using STATISTICA software (Version 7.1; StatSoft Inc.). The differences between the two lots (watered and none watered) for all recorded data were compared by the student test where the conditions of normality and equality of variances are checked. Otherwise, a Mann Whitney none

parametric test was achieved. The significance level for all the tests was $P < 0.05$.

RESULTS

The seedlings of *Q. suber* were grown in irrigated and water starvation conditions for 10 weeks in greenhouse prior to morphological and physiological data measurements. At the end of the culture, significant changes were shown from the two lots in terms of substrate water contents (Figure 1a). The reduction of water availability was accompanied by a significant decrease in leaf relative water status (RWC), the RWC values were 85.43 and 70.78% in watered and none watered seedlings, respectively (Figure 1b).

The soil of Beni Ghobri forest was rich in ectomycorrhizal inoculum. Approximately eight month's cork oak seedlings were colonized by ectomycorrhizal fungi. The difference in ECM rate between the two treatments was significant. ECM decreased in drought conditions (<9%) while the well watered seedlings showed higher ECM (>39%) (Figure 1c). So, the ectomycorrhization was approximately 5-fold higher in well watered seedlings.

Length of the main stem was reduced in none irrigated lot (Figure 2a). The leaf biomass and root/shoot ratios did not differ significantly between the two batches studied (W and NW) (Figures 2b and c). The statistic analysis showed that the diameter of the stem at the base was significantly higher in the control watered plants as compared to the none watered ones (Figure 2d).

Majority of the physiological variables investigated were statistically different (Figures 3 and 4) between the two lots (W and NW). Total chlorophyll contents showed no significant difference between watered and non-watered seedlings (Figure 3a). Leaves protein content recorded in none watered samples were higher than in the well watered seedlings (Figure 3b). The levels of soluble sugars tend to increase with drought conditions. Especially, this drop was approximately 3-fold in none watered conditions as compared to the watered conditions (Figure 3c). The starch content showed a little increase in the none watered seedlings (1.026 fold higher) (Figure 3d).

On the contrary, total polyphenols synthesis and/or accumulation generally decrease. A significant decrease was observed (1.5-fold) in none watered seedlings as compared to the control individuals (Figure 3e). Non watering induced a significant increase of $\delta^{13}\text{C}$ contents in leaves; the $\delta^{13}\text{C}$ were higher in none watered (-32.04) than in watered (-32.79) *Q. suber* seedlings (Figure 4a). The total nitrogen concentrations (N%) in leaves were different between the two treatments; N content was reduced in drought conditions (Figure 4b).

The C% in leaves were similar in watered conditions,

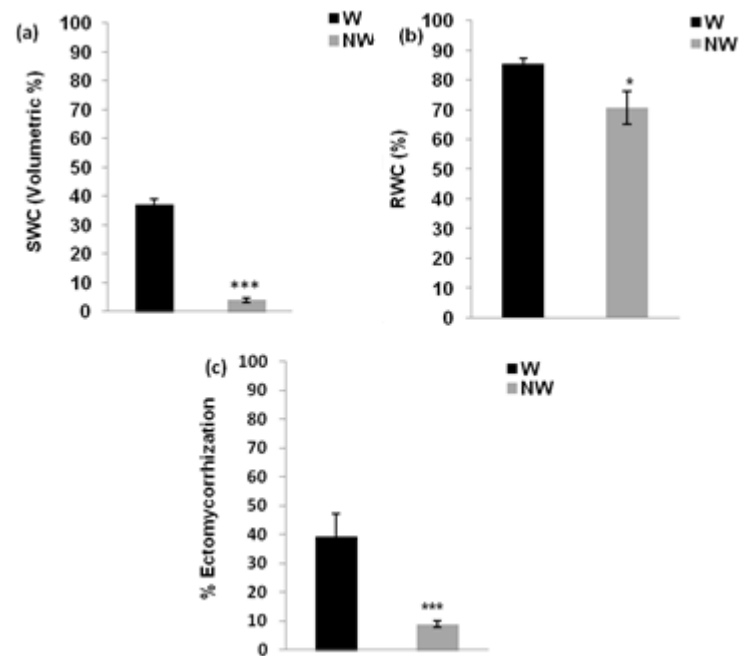


Figure 1. Effects of drought conditions on water status and ectomycorrhization of *Quercus suber* seedlings: (a) soil water content (SWC) and (b) leaf relative water content (RWC) (c) ectomycorrhization rate (ER). Means $\pm$ SE. for RWC n=5, SWC n=10, and ER n=10. (*: p<0.05, ***: p<0.001).

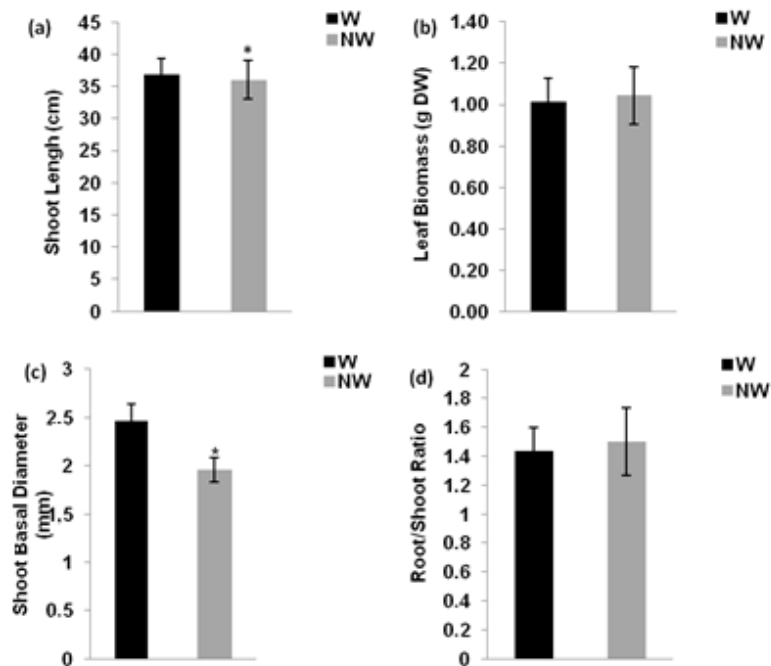


Figure 2. The effects of drought conditions on morphological traits of *Quercus suber* seedlings. (a) Shoot height, (b) Leaf biomass, (c) shoot basal diameter and (d) root/shoot ratio. Means $\pm$ SE. for shoot height n=20; leaf biomass n=12; shoot basal diameter n=13 and shoot/root ratio n=10. (*: p<0.05).

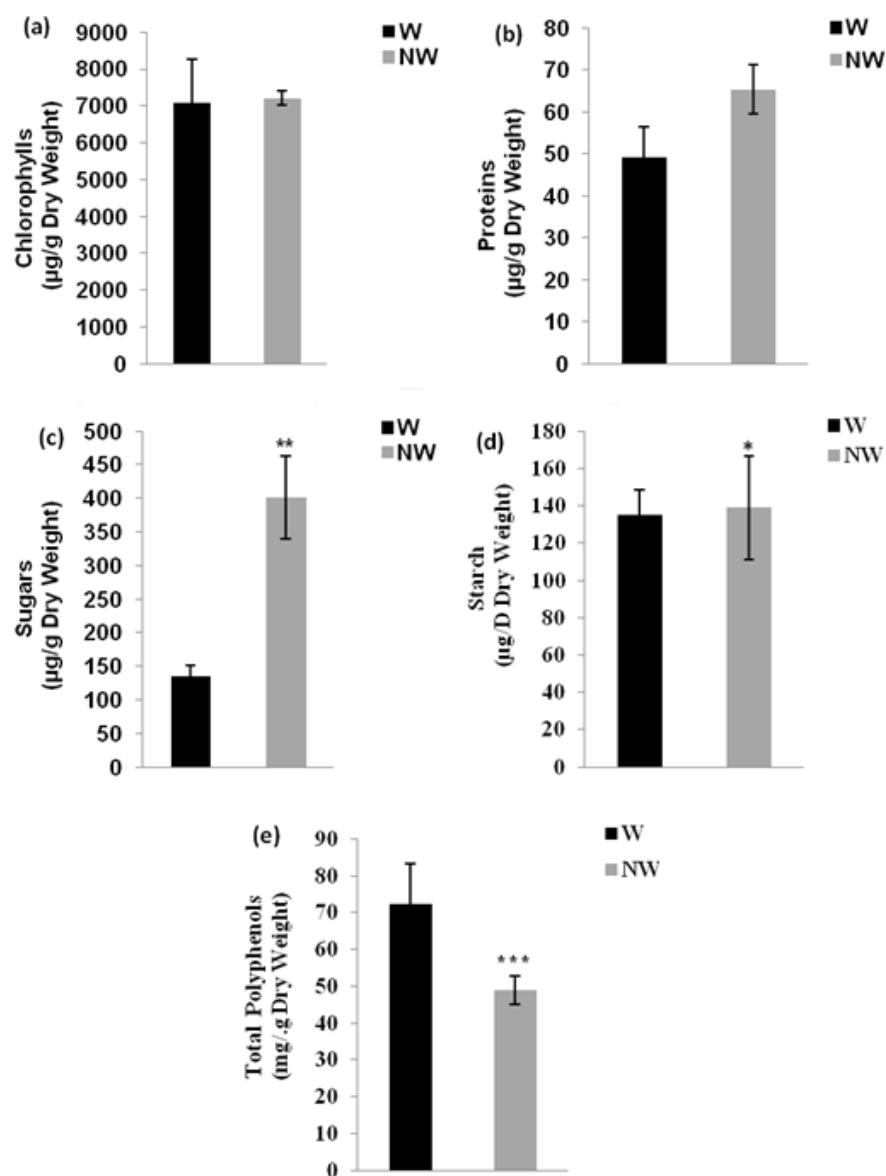


Figure 3. The effects of drought conditions on biochemical traits of *Quercus suber* seedlings. (a) Chlorophylls, (b) proteins, (c) sugars, (d) starch and (d) total polyphenols leaves contents. Means $\pm$ SE. for all variables $n=5$. (*: $p < 0.05$, ***: $p < 0.001$).

thus, drought had no negative effect on this parameter (Figure 4c). The correlations between SWC and ectomycorhization, $\delta^{13}\text{C}$, N% and C% parameters were evaluated (Figure 5). The $\delta^{13}\text{C}$ leaves contents showed a significant negative correlation with SWC; the reduction of SWC enhanced $\delta^{13}\text{C}$ leaves contents ($r^2 = 0.5829$; $r = -0.7635$; $p = 0.0006$; $y = -31.918 - 0.0293 \times x$) (Figure 5a), while the total nitrogen (N%) and C% leaves concentration did not show correlation with the SWC ($p > 0.05$) (Figures 5b and c). A positive correlation exists

between SWC and ECM rate ($r^2 = 0.3109$; $r = 0.5576$; $p = 0.0162$; $y = 8.245 + 0.7775 \times x$) and ECM decreases with SWC (Figure 5d).

DISCUSSION

When comparing the two treatments, soil water status in none watered pots was about 9.5-fold lower as compared to the watered substrates (Figure 1). This decrease was

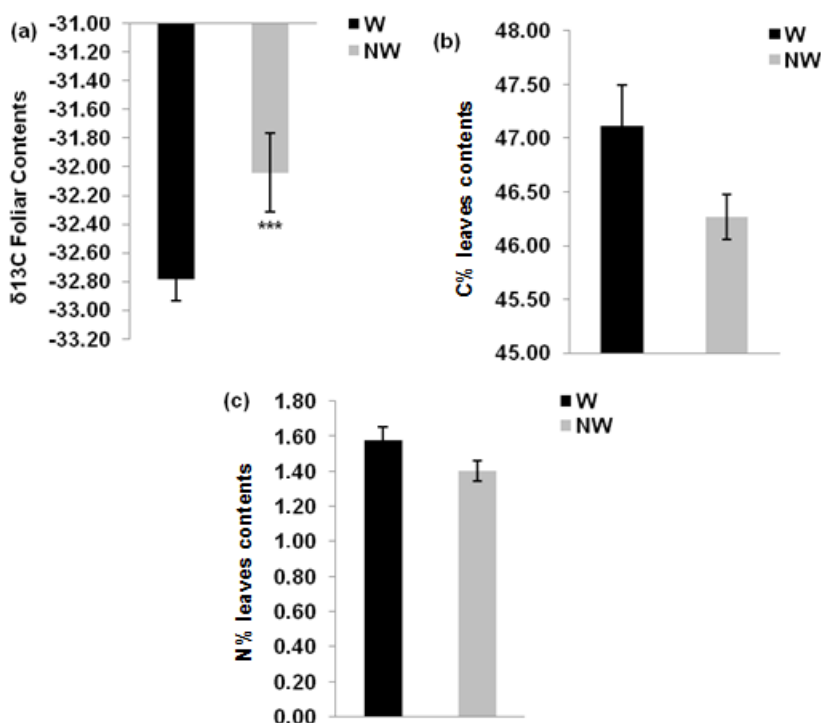


Figure 4. The effects of drought conditions on $\delta^{13}\text{C}$, C% and N% leaves contents of *Quercus suber* seedlings. (a) $\delta^{13}\text{C}$, (b) C% and N%. Means $\pm$ SE. for all variables n=5 (***: p<0.001).

accompanied by a significant reduction in leaf relative water content (RWC) of the eight-month old seedlings (from 85.43 to 70.78%). In summer, the soil moisture volumetric content dropped to 5-7 % along the upper 0.6 m explored and leaf RWC decreased to nearly a constant level of about 82% that were only 8% less than those recorded in the spring (Nardini et al., 1999). The low values of RWC recorded in the current study may be due to more arid climatic conditions. Favorable tissue water status in *Q. suber* during summer drought was achieved through deep rooting which facilitate soil water uptake (Otieno et al., 2006).

The lower ECM colonization in *Q. suber* seedlings in drought conditions was previously obtained by Shahin (2012) in *Q. ilex* and *Q. pubescens* seedlings. The lower ECM colonization in dry conditions can be explained by the fact that photosynthates quantities are insufficient to both plant and fungal, thus they are preferentially allocated to plant growth or ectomycorrhizal inoculum was less abundant in none watered lot (Shahin et al., 2012). Some studies reported that seedlings inoculated with ectomycorrhizal fungi enhanced plant tolerance to water stress due to their less resistance to water flow from soil to roots, by increasing the absorbing surface and the ability of the fungus to penetrate finer pores as those operated by hairy roots (Parke et al., 1983).

Length and Diameter growth were reduced by drought conditions (Figure 2). While primary growth is sensitive to winter-spring warming, secondary growth is sensitive to summer-autumn warming (Camarero et al., 2015). Cambium activity is known to be negatively influenced by drought (Caritat et al., 2000). Interruption of shoot growth in the dry summer is a significant adaptation trait (Kurze-Besson et al., 2006) and thus, water stress tends to decrease the ratio of shoot biomass/root biomass (Ksontini et al., 1998).

Regarding the physiological parameters investigated, differences were shown (Figures 3 and 4). Drought generally causes decrease in photosynthetic pigments (Rajasekar and Manivannan, 2015). Decrease in chlorophyll contents was due to decrease in chlorophyll biosynthesis rather than its degradation (Jain et al., 2013). Vaz et al. (2010) showed no difference in *Q. suber* tree leaves from summer to autumn.

Osmotic adjustment is found to help cork oak seedlings to maintain turgor during moderate stress. To maintain water uptake, plants increased the water potential gradient between the plant cells and soil by increasing solute concentrations in the root cells. Like the present study results, in *Quercus* seedlings species, soluble sugars increased in drought-treated plants relative to control well watered plants and the opposite pattern was

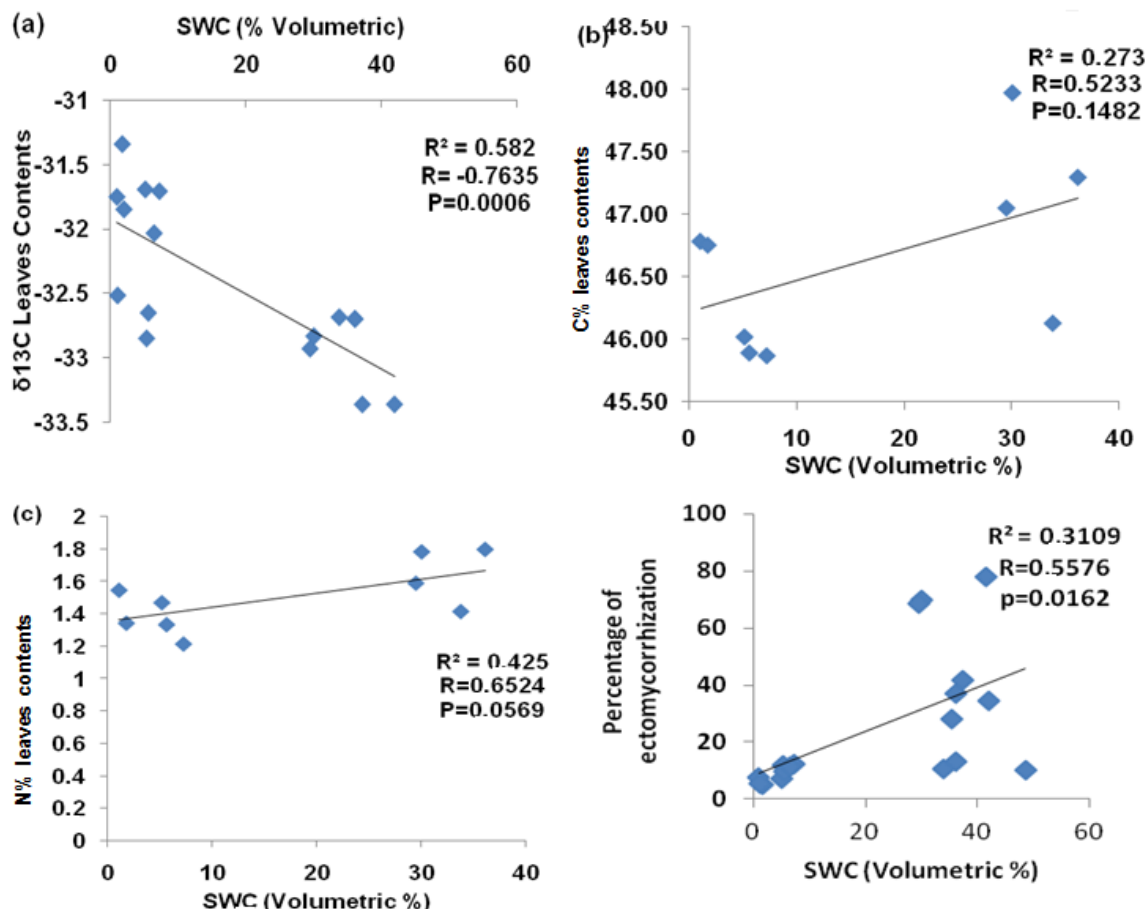


Figure 5. Correlations between SWC and $\delta^{13}\text{C}$, C%, N% and ectomycorrhization parameters of *Quercus suber* seedlings. (a) $\delta^{13}\text{C}$, (b) C%, (c) N% leaves contents and (d) percentage ectomycorrhization.

found in starch (Gonzalez-Rodríguez et al., 2011). Higher soluble sugars concentrations in cork oak seedlings grown under moderate water stress provoked a decrease of the osmotic potential and are at least partly responsible for osmotic adjustment which maintain RWC >80% (Pardos et al., 2005). Soluble proteins also contribute to osmotic adjustment (Kwak et al., 2011).

Polyphenols contents decreased in non-watered seedlings of *Q. suber*. Moderate experimental drought increased the concentrations of polyphenolic compounds with antioxidant function in the leaves of *Q. ilex* trees (Rivas-Ubach et al., 2014). Polyphenols are synthesized in large quantities during water stress, high temperatures or exposure to solar radiation, which are conditions that characterize the Mediterranean summer (Hernandez et al., 2009). Sometimes, the quantities were higher in watered plants like in loblolly pine needles (Booker and Maier, 2001).

Secondary metabolism in plants remains unclear; different regulation responses in the polyphenols pathway probably exist varying with species, development stage of the plant, intensity, duration and rate of progression of the stress (Liu et al., 2011). The increased carbon discrimination recorded in drought conditions was previously reported in *Q. suber* trees by Gouveia and Fortas (2009) which shows that trees subjected to greater water stress had $\delta^{13}\text{C}$ enriched leaves, reflecting the trade-off between assimilation rate and water loss. The increase of carbon isotope discrimination with drought induces an increase in water-use efficiency (WUE) (Shahin et al., 2011).

These results showed that drought conditions reduced N% leaves contents while previous results have reported that leaf total nitrogen did not show a significant change in water stressed treatments (Kwak et al., 2011). In *Q. suber* trees, significant correlations were not found

between total N and rainfall (Gouveia and Fortas, 2009).

Conclusion

This study carried out in semi-controlled conditions, shows that *Q. suber* seedlings are affected by 10 weeks of summer drought. The survival of the seedlings under these conditions is due to two strategies: osmotic adjustment through soluble sugars and proteins accumulations in leaves and an increase in carbon discrimination which enhances the water use efficiency (WUE). Some responses (stability of chlorophylls contents and R/S ratios and decrease in polyphenols contents) obtained in this study with Algerian *Q. suber*, are contrary to other *Q. suber* provenances responses (Europe and Tunisia).

Further studies could involve the responses of different provenances to actual and future water stress; this will permit understanding the mechanisms of *Q. suber* tolerance to drought conditions. The success of reforestation in the perspective of Mediterranean climate aridity which is scheduled for the next decades needs provenances well adapted to these future conditions.

Conflict of interests

The authors have not declared any conflict of interests.

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COMPARATIVE DROUGHT RESPONSES OF *QUERCUS SUBER* SEEDLINGS OF THREE ALGERIAN PROVENANCES UNDER GREENHOUSE CONDITIONS

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RÉSUMÉ.— *Réponses comparatives à la sécheresse en condition de serre des plantules de Quercus suber de trois provenances algériennes.*— Le Chêne-liège est une espèce typiquement méditerranéenne présentant un intérêt économique et écologique. L'existence de mécanismes de tolérance à la sécheresse estivale méditerranéenne chez cette espèce a été démontrée par de nombreuses études réalisées, essentiellement en Europe. Cependant, ces dernières années, d'autres études ont montré que les mécanismes de tolérance diffèrent entre les provenances. Le comportement des provenances algériennes de Chêne-liège a été le sujet de très peu d'études. L'Algérie, étant un pays plus aride que les pays européens, ses provenances pourraient montrer des différences de comportement par rapport aux provenances européennes. L'objectif de ce travail est l'étude de la réponse à une sécheresse estivale des plantules originaires de trois provenances algériennes caractérisées par des étages bioclimatiques différents : Azazga (subhumide), Jijel (humide) et M'Sila (semi-aride). Un arrêt d'arrosage a été appliqué aux plantules, cultivées de façon homogène en serre, pendant 10 semaines, de fin-juin à mi-septembre, pour simuler la sécheresse estivale méditerranéenne. Le statut hydrique et des paramètres morphologiques et biochimiques ont été évalués chez les plantules arrosées (témoins) et non arrosées (stressées) et les taux de survie des plantules non arrosées ont été déterminés pour les trois provenances. Les résultats ont révélé des différences de comportement entre les trois provenances dans les deux conditions d'arrosage et de non arrosage. Dans les conditions d'arrosage, les plantules originaires de M'Sila ont montré la teneur relative en eau la plus élevée ainsi que la meilleure croissance. L'arrêt d'arrosage, ayant provoqué une diminution significative des teneurs en eau du sol, a provoqué une réduction de la teneur relative en eau et de la croissance (hauteur et diamètre de la tige) des plantules originaires de Jijel et d'Azazga alors que seul le diamètre des tiges était réduit chez les plantules originaire de M'Sila. Les feuilles des plantules originaires du site le plus aride, M'Sila, ont montré des modifications morphologiques et physiologiques considérées comme étant des stratégies d'adaptation à la sécheresse : une faible surface foliaire spécifique, une diminution des teneurs en Chlorophylle a, pour éviter une absorption excessive de l'énergie lumineuse, une diminution des teneurs en amidon, une accumulation des protéines, des sucres et de la proline afin de permettre un ajustement osmotique et une augmentation du $\delta^{13}\text{C}$ pour une meilleure utilisation de l'eau. Dans les conditions de non arrosage, les plantules d'Azazga ont montré un comportement intermédiaire entre les plantules originaires de Jijel et de M'Sila. Cependant, contre toute attente, les plantules de M'Sila ont montré le plus faible taux de survie, le meilleur taux de survie étant enregistré pour les plantules d'Azazga. Les écotypes étudiés dans ce travail montrent donc des traits fonctionnels différents pouvant être liés aux conditions environnementales des provenances. Malgré le faible taux de survie des provenances du milieu le plus aride on peut penser qu'elles constituent de bons candidats, sur le long terme, en matière de reboisement dans le contexte du changement climatique global.

SUMMARY.— Cork oak (*Quercus suber* L.) is one of the most representative Mediterranean forest species that is well studied in Europe and recognized as drought tolerant. While many studies showed that differences exist in drought tolerance mechanisms among provenances, few reports exist on drought responses of Algerian *Q. suber* seedlings. The present study investigates summer drought behaviour of seedlings originating from three Algerian provenances: from humid (Jijel), sub humid (Azazga) and semi-arid (M'Sila) Mediterranean areas. The summer conditions were simulated by stopping irrigation of the seedlings grown homogenously in greenhouse during 10 weeks from last June to mid-September. Water status, morphological and biochemical parameters were evaluated in watered (control) and non-watered seedlings and survival rate of non-watered seedlings was determined for the three provenances. The results showed differences between seedlings behaviour of the three provenances in watered and non-watered conditions. In watered conditions, M'Sila seedlings showed the highest Relative Water Content and the highest growth. In non-watered conditions, the reduction of soil water content had negative effect on the Relative Water Content and growth (height and shoot diameter) of the seedlings of Jijel and Azazga provenances. Only the stem basal diameter was reduced in the seedlings of M'Sila provenance. Leaves of M'Sila non irrigated seedlings, originating from the drier site (semi-arid), showed morphological and physiological modifications that

are known as drought adaptive strategy: low Specific Leaf Area, decrease in Chlorophyll a contents thus avoiding excessive absorption of light energy, a decrease in starch content, an increased accumulation of proteins, sugars and proline. An increase in leaves $\delta^{13}\text{C}$ was also obtained suggesting a more efficient water use. Azazga seedlings showed an intermediate behaviour between M'Sila and Jijel seedlings in drought conditions. However, contrary to expectation, survival rate was lowest for M'Sila and highest for Azazga seedlings. The ecotypes studied in this work exhibited different functional traits related to the environmental conditions of the original provenance. Despite the low survival rate of provenances from the most arid environment, they are thought to be suitable candidates in long-term for reforestation in the context of global climate change.

Cork oak (*Quercus suber* L., Fagaceae), is a species, widely distributed among Mediterranean forest trees (Quézel & Médail, 2003) of great ecological (carbon sequestration, soil protection, hydrological cycle regulation) and economic (production of cork) importance (Pausas *et al.*, 2009). It is an evergreen and sclerophyllous species growing from the sea level up to 700 m in altitude between 13 and 16°C and is strictly calcifuge colonizing siliceous soils (Quézel & Médail, 2003). Cork oak stands in Algeria (227 000 ha) constitute the second largest stand after the Aleppo pine. Moreover, Algerian stands represent 14 % of the world cork oak forests (FAO 2013). Nearly 4/5 of the cork oak areas are located essentially in the north-east, from Tizi-Ouzou to the Tunisian border but, in the western part of Algeria the stands are scattered in the form of small islands (Bouhraoua, 2015).

Q. suber response to drought conditions which characterize summer in Mediterranean areas has been well studied, essentially in Europe (Faria *et al.*, 1999; Nardini *et al.*, 1999; Nardini & Tyree, 1999; Kurze-Besson *et al.*, 2006; Otieno *et al.*, 2006; Pardos *et al.*, 2006; Kwak *et al.*, 2011). Cork oak is well known as drought tolerant species (Nardini *et al.*, 1999; Nardini & Tyree, 1999). There are many mechanisms by which it resists to drought periods: deep rooting, osmotic adjustment (Otieno *et al.*, 2006; Pardos *et al.*, 2006; Kwak *et al.*, 2011) and anti-oxidant system (Faria *et al.*, 1999). Ecophysiological investigations demonstrated that *Q. suber* is well adapted to summer conditions because it maintains a favourable ratio between water loss and uptake during the dry period. Maintaining a favourable water status in tissues, with a high relative water content (RWC) during summer drought, is ensured by deep roots and/or osmotic adjustment through accumulation of molecules such as proteins, sugars and proline (Otieno *et al.*, 2006; Pardos *et al.*, 2006; Oufir *et al.*, 2009; Kwak *et al.*, 2011). A decrease in shoots and leaves biomass by reduced growth and root drop is also noted (Ksontini *et al.*, 1998; Kurze-Besson *et al.*, 2006). A reduction of specific leaf area (SLA) was also observed (Ramirez-Valiente *et al.*, 2010). The presence and the nature of the cork in which cell walls are suberin impregnated, contributes to protection against fire and also resistance to tissues desiccation (Pausas *et al.*, 2009). Under drought conditions, an increase of leaves $\delta^{13}\text{C}$ contents reflects a better water use efficiency (WUE) in *Q. suber* seedlings (Gouveia & Freitas, 2009).

Although the cork oak seems well adapted to the dry conditions of the Mediterranean climate, the mechanisms involved in this adaptation are still scarce (Almeida *et al.*, 2013). Also, the natural regeneration is low and poorly understood (Gonzalez-Rodriguez *et al.*, 2010) because *Q. suber* is particularly more sensitive to drought in the early stages of development (Aranda *et al.*, 2005) due to the quasi-absence of cork (Pereira *et al.*, 2009). In Algeria, facing the decline of the cork oak forest due to many factors like fires (the most important factor), grazing and diseases, many reforestation operations have been undertaken (the cork oak occupies the first place of the reforested species with 24 % of the wooded areas). However, the success rates of these reforestations are often low and unsatisfactory; the survival rate of plants decreased from 80-90 % to 20-50 % after the summer season (Messaoudène *et al.*, 2011; Bouhraoua, 2015). This seedlings recruitment limitation probably will be amplified with the predicted global changes in Mediterranean region (Caldeira *et al.*, 2014). Hence, climate change is expected to lead to longer dry spells, higher evaporative demand

and more intense droughts in the coming decades in several regions of the world, including the Mediterranean basin (IPCC, 2007).

Since cork oak has a large distribution area with large variation in environmental conditions [areas with a mean annual rainfall of 400–1500 mm and a mean annual temperature of 13–20°C (Díaz-Fernández *et al.*, 1995)], a large differentiation is expected among populations for significant adaptive traits such as the ability to tolerate extended periods of drought (Varela *et al.*, 2014).

Many studies showed a high level of differentiation among the populations of *Q. suber* species. Differences among cork oak plants originated from different populations in phenotypic traits were mainly due to divergent selection imposed by temperature and rainfall variation and to neutral evolutionary processes such as founder effect or genetic drift (Ramirez-Valiente *et al.*, 2010). Genetic diversity parameters determined for different geographic areas of the entire *Q. suber* range, showed variation where paleogeography, hybridization, adaptation, fragmentation, and human impact play an important role in the evolutionary history of this species (Simeone *et al.*, 2010). Ennajah *et al.* (2013) showed a high phenotypic variability among and within Moroccan cork oak tree populations which was significantly correlated with rainfall; large differences between populations from highest and coldest sites as well as those of lowest and warm sites were detected and adaptive responses specific to some populations were founded. Some differences among populations have been observed concerning their ability for adaptation and production under drought conditions (Gandour *et al.*, 2007).

Hence, a better understanding of the effects of drought on plants originating from different provenances is essential for early selection of provenances for afforestation.

Based on the concept of plant-climate-coevolution, our working hypothesis was that differences exist in drought tolerance mechanisms among provenances: provenances native to dry regions would have more capability to acclimate to drought conditions than provenances originated from a more temperate climate region. Thus, ecophysiological comparisons may prove useful for the choice of *Q. suber* provenances for afforestation.

Few reports exist on drought responses of Algerian *Q. suber* seedlings (Acherar *et al.*, 1991; Daoudi *et al.*, 2016). The present study investigates summer drought effects on seedlings originating from three Algerian provenances. The summer conditions were simulated by stopping irrigation of the seedlings grown in greenhouse during two months, July and August. Then, different parameters were evaluated: water status of the seedlings (RWC), morphological traits (height and thickness growths and specific leaf area) and physiological parameters (Chlorophylls, Carotenoids, proteins, sugars, starch, $\delta^{13}\text{C}$, % C and % N) in leaves.

MATERIALS AND METHODS

Cork oak acorns were collected in the end of November 2011 from three Algerian provenances: Jijel (Aghzer forest), M'Sila forest (Oran) and Azazga (Beni Ghobri forest). The forests of the three provenances showed foliar damages attributed to xylophages and phyllophages insect pests (the most important being *Platypus cylindrus*) and cryptogamic diseases (the most important is coal caused by *Hypoxylon mediterraneum*) (Bouchaour-Djabeur, 2013; Rouibah *et al.*, 2011). The decline of Algerian cork oak forests is slow but chronic (Bouhraoua *et al.*, 2010). The characteristics of Jijel, M'Sila and Azazga forests are shown in Tab. I.

Thus, M'Sila is the drier provenance and Jijel is the most humid provenance. The dry period is longer in M'Sila (mid-April to mid-September) and shorter in Jijel (May to September) as shown by the ombrothermic diagrams (Fig. 1).

The acorns of the different provenances were weighed: 2.954 ± 0.155 g, 2.458 ± 0.093 g and 2.195 ± 0.256 g for Jijel, M'Sila and Azazga respectively. After germination at 20°C in Petri dishes, the seedlings with 2 cm root length (mid-April) were transplanted to plastic bags (15 cm diameter and 30 cm deep) filled with substrate consisting of a mixture of loam (2/3) and washed sand (1/3). The loam (N 110-250 mg/l, P2O5 60-140 mg/l, K2O 120-280 mg/l) had a fine structure and a pH of 6.2.

All the *Q. Suber* seedlings of the three provenances were grown homogeneously in greenhouse (5 x 3 m dimension), located next to the university of Tizi-Ouzou in Algeria which is characterised by Sub-humid Mediterranean climate (mean annual temperature of 19.2°C, mean annual precipitation of 705 mm). The seedlings were watered regularly (three times a week) until the end of June. Then we constituted three plots of seedlings (60 seedlings/plot). Each plot was constituted by a

mixture of 10 seedlings/provenance (Jijel, M'Sila, Azazga)/treatment (watered and none watered): Jijel watered (JW) and non-watered (JNW), M'Sila watered (MW) and non-watered (MNW) and Azazga watered (AW) and non-watered (ANW). Water stress treatment was given by withholding the water supply for 10 weeks, from end of June until mid of September, to simulate Mediterranean summer drought conditions. According to ICCP (2007) dry days are projected to increase markedly in the Mediterranean basin. The greenhouse temperatures varied from 25°C to 38°C during the experiments. Ten weeks after irrigation was stopped (mid-September), plants of JW, JNW, MW, MNW, AW, ANW were harvested for determination of morphological and physiological parameters.

TABLE I

Location, soil characteristics and climatic data of acorns provenances (Jijel, M'Sila and Azazga)

	Jijel	M'Sila	Azazga
Altitude	20 m	350 m	530m
Longitude	36°49'0.259''E	0° 50' 19.7" W	4°22' to 4°27' W
Latitude	5° 44' 56.7''N	35°38' 22.6"N	36°42'to 6°47'N
Annual mean temperature	18	18.1	16.7
Annual mean precipitations	1022 mm/year	397mm/year	944mm/year
Mediterranean Bioclimatic Level	Humid	Semi- arid	Sub-Humid
Substrat	Numidian sandstone	Jurassic schist	Numidian sandstone
Soil Texture (superficial Horizon)	Sandy loam	Clay and silt	Clay loam
Organic Matter	2,65	2,75	4,595
pH	6,525	6,7	5,8
Exposition Dominance	NW	NW	N

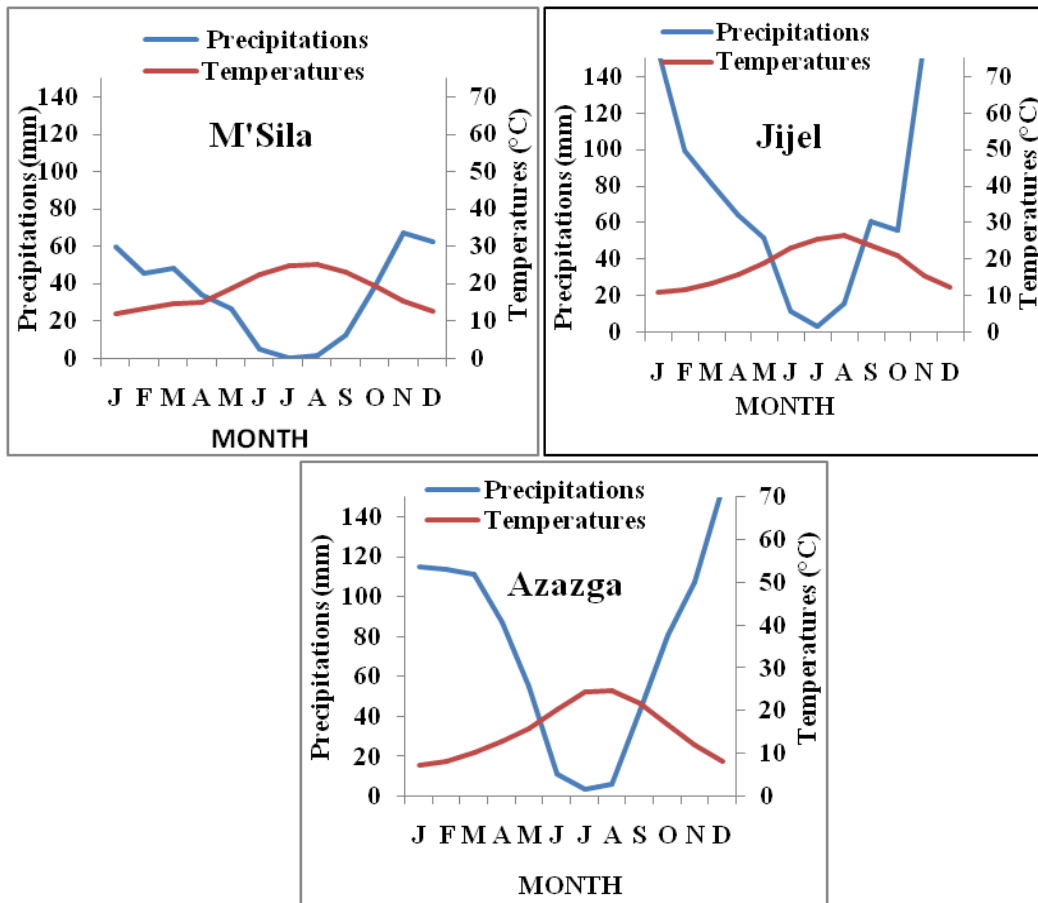


Figure 1.— Ombrothermic diagrams of M'Sila, Jijel and Azaga Forest provenances.

SUBSTRATE WATER CONTENTS MEASUREMENT

Substrate water contents (SWC) was determined as described by Mathieu & Pielain (2003). Substrate samples of 1 g were taken from the plastic bags of the six lots at a depth of 10 cm (10 repetitions/provenance/treatment) and dried at 105°C for 72 h and then water content was calculated using the formula:

$$SWC = \frac{FW - DW}{FW} \times 100$$

where FW and DW are the fresh and dry weight, respectively.

RELATIVE WATER CONTENTS (RWC) MEASUREMENT

Relative water content (RWC) was measured in three young leaves of five plants per provenance/treatment using Nardini *et al.* (2000) method. Leaves were detached and immediately weighed to get their fresh weight (FW). Then, the leaves were restored with distilled water to near full turgor by immersing their petioles in water, covering the leaf blade with plastic film and leaving them in the dark for 12 h. Leaves were then reweighed to obtain their turgid weight (TW) and put into oven at 70°C for 72 h to obtain their dry weight (DW). Finally, RWC was calculated as:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

MORPHOLOGICAL PARAMETERS MEASUREMENTS

To evaluate seedlings growth in both conditions, watered and non-watered, different morphological parameters of 10 plants per provenance/treatment were measured: stem height, basal stem diameter and shoots (stems and leaves), roots (total roots washed with distilled water) and leaves (with petioles) biomass were determined after drying at 75°C during 72 h. Then the root to shoot ratio was estimated.

The specific leaf area (SLA) was determined on three mature leaves per plant and ten plants per provenance/treatment as the ratio of leaf area (determined with AM350 Portable Leaf Area Meter) to individual leaves dry mass (DM), measured after oven-drying at 70°C to a constant weight (Faria *et al.*, 1999).

PHYSIOLOGICAL PARAMETERS MEASUREMENTS:

Pigments, sugar, starch, proteins, proline, %C, %N and $\delta^{13}\text{C}$ contents were determined on five seedlings per provenance/treatment.

After extraction in 80 % acetone in dark, the photosynthetic pigments absorbances were determined spectrophotometrically and their contents were calculated as proposed by Lichtenthaler & Buschmann (2001):

$$\text{Chlorophyll a as: } Chla = 12.25 \times A663 - 2.79 \times A647$$

$$\text{Chlorophyll b as: } Chlb = 21.50 \times A647 - 5.10 \times A663$$

$$\text{Total chlorophylls as: } ChlT = 7.15 \times A663 - 18.71 \times A647$$

$$\text{Total carotenoids as: } CarT = 1000 \times A470 - 1.82 \times Chla - 85.02 \times Chlb$$

After the extraction of the soluble sugars from fresh leaves in ethanol (70 %), the solid fraction was used for starch analysis. Starch was incubated in HCl (1.1 % v/v) for 30 min at 95°C for hydrolysis into simple sugar. Then, soluble sugars and starch concentrations were determined colorimetrically at 625 nm with anthrone reagent following Cerning-Berorard (1975) method. Glucose was used as standard for both soluble sugars and starch.

Soluble proteins were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595 nm after colorimetric reaction with Bioard reagent following Bradford (1976) method. BSA was used as standard.

Proline content was quantified by the ninhydrin-colorimetric method at 515 nm after extraction in methanol (70 %) as described by Monneveux & Nemmar (1986). Proline was used as standard.

Foliage samples for analysis of $\delta^{13}\text{C}$, %C and %N were dried at 70°C for 72 h and ground to a fine powder. The abundance in combusted samples were performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) at UMR-CNRS7266 LIENSS with a precision of 0.1 ‰. We calculated $\delta^{13}\text{C}$ (‰) with respect to the PDB Pee Dee Belemnite standard:

$$\delta^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} \times 1000$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios in a sample and the standard (Pee Dee Belemnite) respectively (Warren & Adams, 2000).

STATISTICAL ANALYSIS

Statistical analysis was performed using STATISTICA software (Version 7.1; Stat Soft Inc.). The differences among the plots (JW, JNW, JW, JNW, AW and ANW) for all recorded data were compared by the one-way analysis of variance (ANOVA) where the conditions of normality and equality of variances are checked followed by LSD test. Otherwise, a Kruskal-Wallis test was achieved. The significant level for all the tests was $P < 0.05$.

RESULTS

This study investigated the response to drought of *Q. suber* seedlings from three Algerian provenances. Results showed some morphological and physiological differences between humid (Jijel), semi-arid (M'Sila) and sub-humid (Azazga) provenances in watered and non-watered conditions.

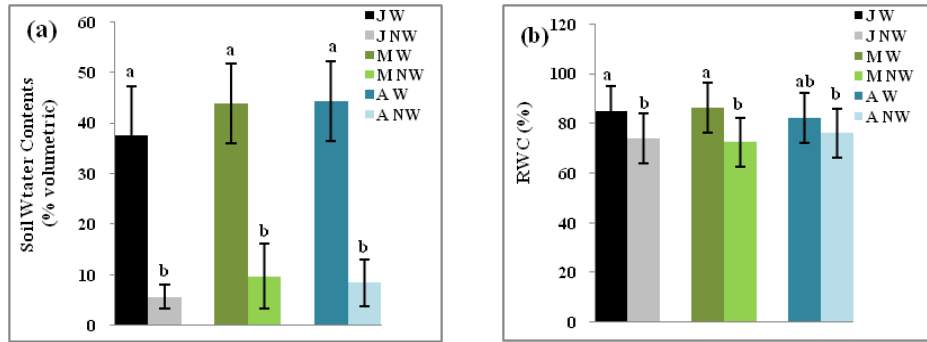


Figure 2.— Water status measurements (a) soil water contents and (b) relative water contents (RWC) in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

The irrigation cessation after ten weeks (mid-September), significantly reduced the SWC of the three provenances (Fig. 2a). In watered conditions, the seedlings of M'Sila provenances showed a better water status with a higher RWC (86.40 %) compare to Jijel (84.96 %) and Azazga (82.08 %) provenances but the reduction of the SWC decreased the RWC of the seedlings of the three provenances (Fig. 2b). The RWC of non-irrigated plants of M'Sila decreased from 86.40 to 72.41 %. Azazga showed the lowest decrease of the RWC (from 82.08 to 76.09 %).

The reduction of SWC had also negative effect on the seedlings growth of Jijel and Azazga provenances by reducing slightly their stem height while the seedlings of M'Sila provenance were not affected; their stem height were similar in watered and non-watered conditions (Fig. 3a).

In watered conditions, the diameter growth of the seedlings was higher for Jijel provenance and lower for Azazga provenance. The lack of irrigation reduced the stem basal diameter of the seedlings of the three provenances. However, the stem basal diameter of non-watered seedlings of Jijel provenance were similar to those of the watered seedlings of M'Sila provenance and the stem basal diameter of non-watered seedlings of this provenance were similar to those of watered seedlings of Azazga provenance (Fig. 3b).

The leaf biomass and the roots/shoots ratios did not significantly differ between the three provenances and drought conditions did not affect this parameter (Fig. 3c, d).

The SLA did not differ significantly between the three provenances in irrigated conditions. The seedlings growing under conditions of water deficiency showed leaves with low SLA in the three provenances. The M'Sila seedlings showed the lowest value of the SLA (Fig. 3e).

Significant differences in Chla+b concentrations were observed among the three provenances. Higher values of Chla+b were observed in the leaves of Jijel provenance seedlings and the lowest values were noted for Azazga seedlings. The reduction of the SWC did not decrease the total leaves chlorophyll contents in the three provenances (Fig. 4b). The concentration of chlorophyll a was higher in Jijel and M'Sila seedlings compared to Azazga seedlings but drought conditions reduced chlorophyll a contents in Jijel and M'Sila provenances while for Azazga seedlings this biochemical parameter increased (Fig. 4b). Leaves contents of chlorophyll b were higher in Jijel and M'Sila than in Azazga seedlings. Under drought conditions, the leaves of M'Sila and Jijel seedlings showed an

increase in chlorophyll b concentration while Azazga seedlings were not affected (Fig. 4c). Chlorophyll a/ chlorophyll b ratios did not significantly differ between the three provenances in watered and non-watered conditions (Fig. 4d). Carotenoids concentrations in leaves were higher in Jijel and M'Sila than in Azazga watered seedlings and in non-watered seedlings they significantly decreased in M'Sila and Azazga seedlings (Fig. 4e).

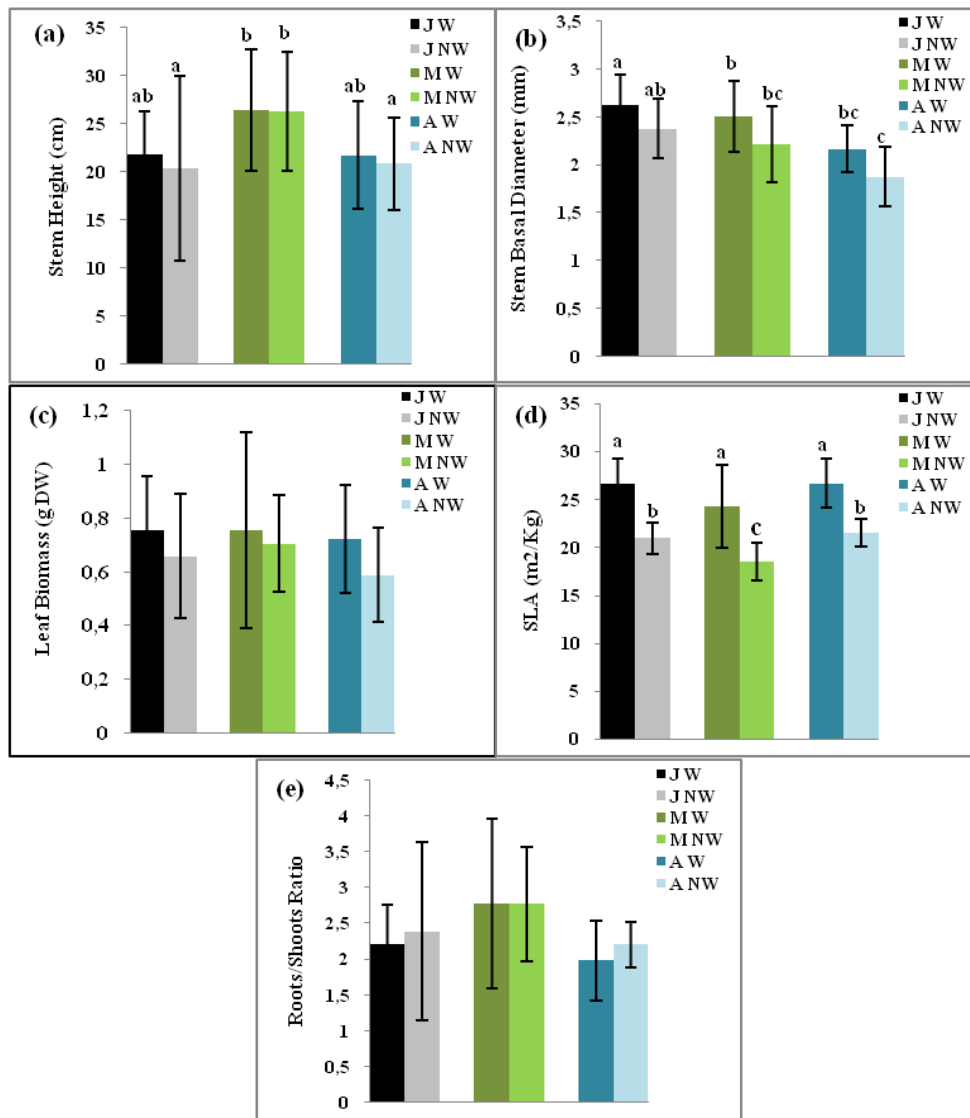


Figure 3.— Morphological parameters measurements (a) shoot height, (b) stem basal diameter, (c) leaf biomass, (e) SLA and (d) roots/shoots ratio in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

Drought conditions induced an accumulation of soluble sugars and proteins only in leaves of M'Sila and Azazga seedlings (Fig. 5a, b); especially in M'Sila seedlings for sugars, this increase was approximately 1.5-fold in non-watered compared to watered conditions (Fig. 5a). The starch content showed an important decrease in non-watered seedlings of the three provenances, the reduction was 2.7, 2.1 and 1.3-fold in Jijel, M'Sila and Azazga provenances, respectively (Fig.

5c). The drought conditions also induced an increase in leaves proline content in the seedlings originating from M'Sila and Azazga provenances (Fig. 5d).

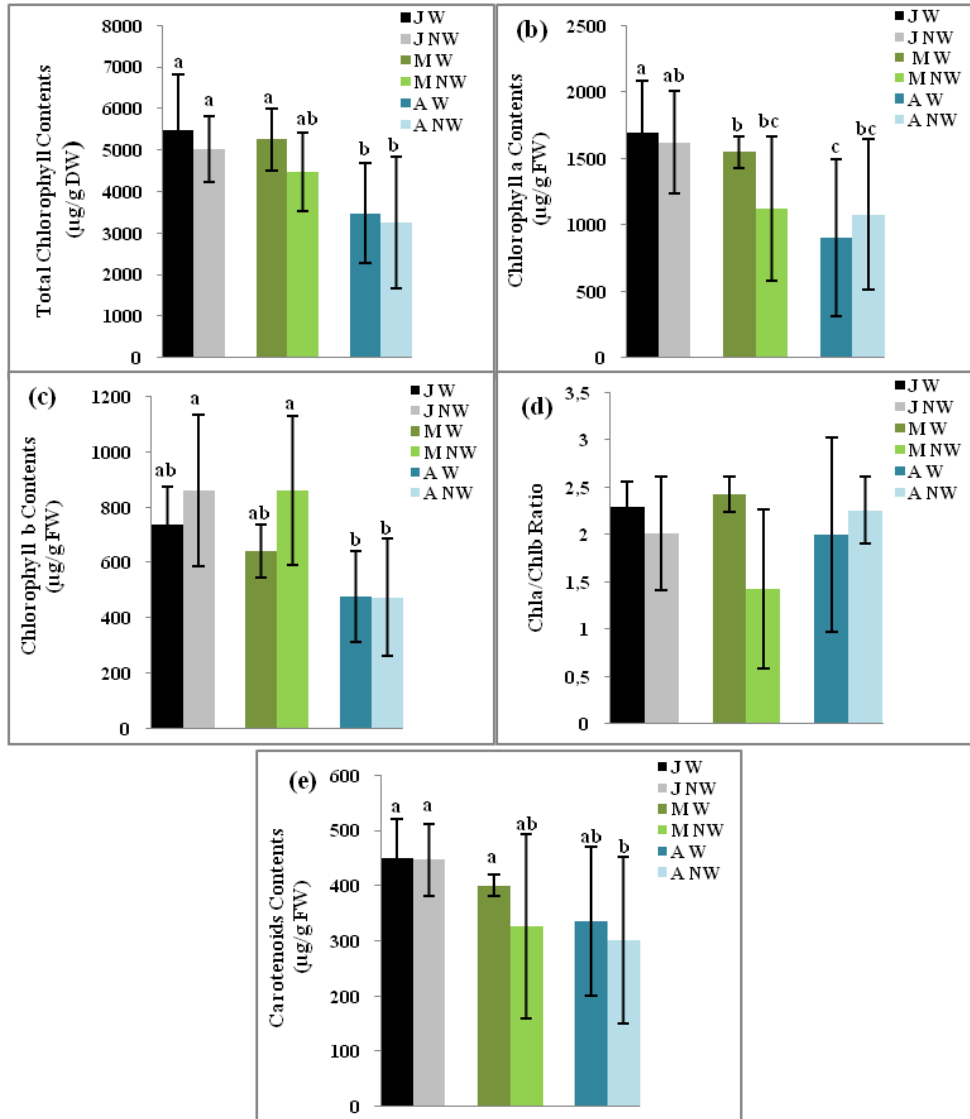


Figure 4.— Pigments leaves contents (a) Total Chlorophyll, (b) Chlorophyll a, (c) Chlorophyll b, (e) Carotenoids in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

The three provenances studied showed differences in leaves $\delta^{13}\text{C}$. In watered conditions, the lower value was obtained in Azazga seedlings provenance (-31.72‰). The reduction of the SWC enhanced significantly the $\delta^{13}\text{C}$ leaves content in Azazga and M'Sila provenances. The higher value of $\delta^{13}\text{C}$ (30.54‰) was recorded in M'Sila non-watered seedlings (Fig. 6a). The $\delta^{13}\text{C}$ from Jijel seedlings was not affected by drought.

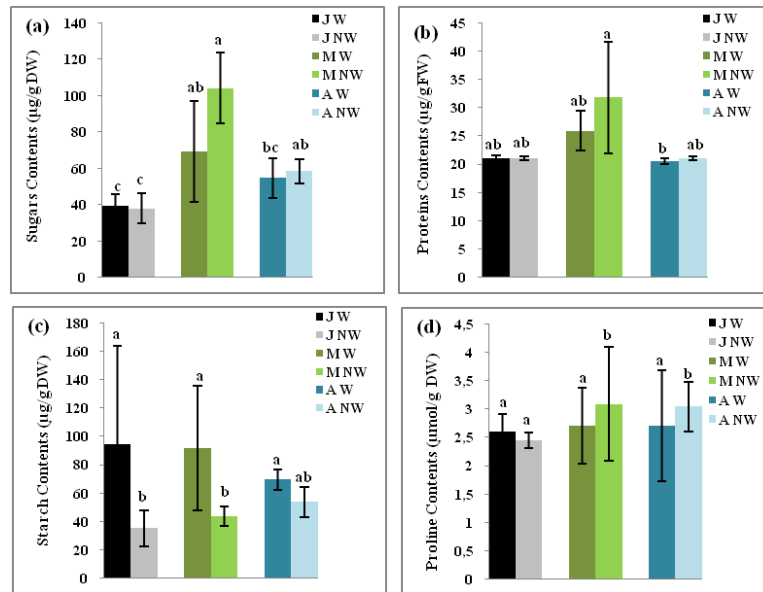


Figure 5.— Biochemical parameters measurements (a) solubles sugars, (b) starch contents, (c) solubles proteins contents and (d) proline contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

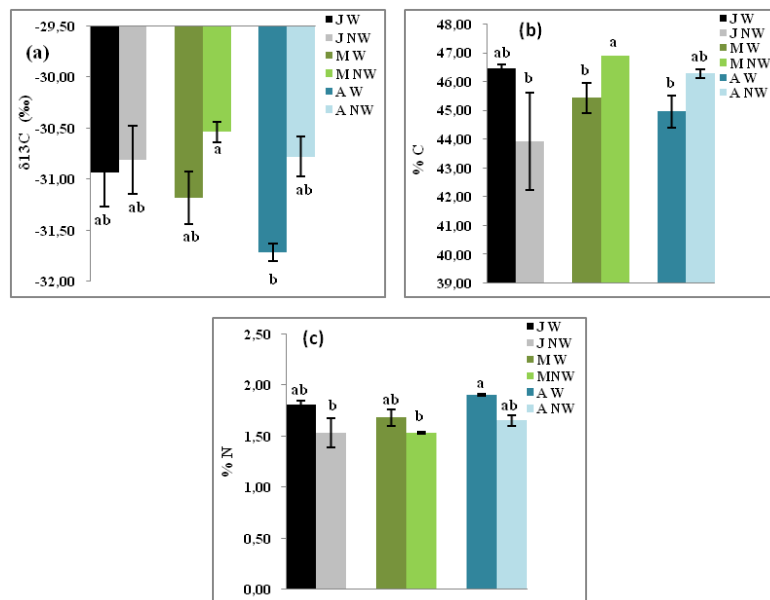


Figure 6.— $\delta^{13}\text{C}$, C and N measurements (a) $\delta^{13}\text{C}$, (b) %C and (c) %N leaves contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means $\pm$ SE.

In watered seedlings, the carbon rate was higher for Jijel provenance. The drought conditions reduced the level of %C in Jijel and enhanced it in M'Sila and Azazga provenances (Fig. 6b).

The nitrogen rate in watered seedlings did not differ between Jijel and M'Sila provenances and Azazga provenances showed the highest value. The reduction of the SWC induced a decrease of %N in the provenances studied (Fig. 6c).

Mortality rate showed a significant difference among provenances ($P = 0.01$). It was 17.64 %, 29.41 % and 35.29 % for Azazga, Jijel and M'Sila provenances respectively. M'Sila seedlings showed the lower survival rate (Fig. 7).

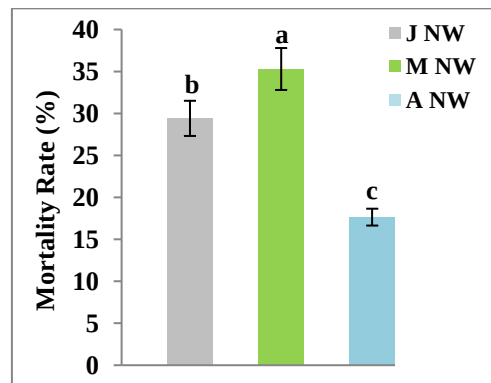


Figure 7.— Survival rates of *Q. suber* Jijel, M'Sila and Azazga seedlings provenances in drought conditions ($P < 0.05$). Means $\pm$ SE.

DISCUSSION

This study showed differences between seedlings of the three provenances: Jijel (humid), M'Sila (semi-arid) and Azazga (sub-humid) elevated homogeneously in greenhouse watered and non-watered conditions.

In watered treatments of all the morphological traits measured, only height, acorn weight and diameter growth exhibited significant population divergence. Previous studies have shown large differences between populations originating from different sites for morphological parameters. Total height was the most discriminating variable between 26 populations originating from six Mediterranean countries (Portugal, Spain, Tunisia, Morocco, Italia and Algeria). In our study, samples of the semi-arid area (M'Sila) exhibited the highest height growth while, in others report, seedlings originating from high temperature sites displayed the lowest growth traits (Gandour *et al.*, 2007). This discrepancy may be explained by the local environmental conditions and/or seeds mass. Among the three provenances investigated, the lightest acorns were from the sub-humid population (Azazga). Seed mass represents the reserves available for growth in the first stages of plant establishment and variation in seed mass is an important trait which may have consequences for growth and survival of seedlings (González-Rodríguez *et al.*, 2011). Our results are in accordance with several authors who reported that differences among seedlings provenances may be related to the climate of the seedling sources (Gandour *et al.*, 2007; Ramirez-Valiente *et al.*, 2010; Ennejah *et al.*, 2013) and to the seed mass (Quero *et al.*, 2007).

Drought conditions also induced various morphological and physiological responses in the three provenances studied. This is in agreement with Aranda *et al.* (2007), who have shown that *Q. suber* is more sensitive to drought in the early stages of development. In particular, the reduction of diameter growth and the SLA recorded from all the seedlings and the reduction of shoot growth are in agreement with previously obtained data (Ksontini *et al.*, 1998; Ramirez-Valiente *et al.*, 2010;

Daoudi *et al.*, 2016). Interruption of shoot growth in the dry summer is a significant adaptation trait (Kurze-Besson *et al.*, 2006). SLA has often been observed to be reduced under drought conditions. In dry conditions, low SLA allows a more conservative water use maintaining photosynthetic activity and carbon gain over a longer period of time (Dudley, 1996).

The significant difference in diameter growth between Jijel, M'Sila and Azazga cork oaks, facing to water deficit were previously shown for Morocco populations; the early stages of oak population's seedlings development are affected differently by changes in soil water reserves and temperatures (Ennejah *et al.*, 2013, 2014).

These intra-specific differences of traits appeared to be linked to interspecific differences in seed size and its confounding effect (Sanchez-Gomez *et al.*, 2007).

Water stress decreased the ratio of shoot biomass / root biomass of *Q. suber* seedlings (Ksontini *et al.*, 1998). The similar leaves biomass and root to shoot ratio traits in well-watered and water stressed plants may be due to a similar degree of osmotic adjustment in root and leaf cells (Sobrado & Turner, 1986). The reduction of starch and the accumulation of soluble sugars, soluble proteins and proline in M'Sila and Azazga seedlings suggest the occurrence of an osmotic adjustment at leaf level which decreases leaf osmotic potential; this mechanism enhances seedlings potential to extract water from the drying soil by increasing the soil-plant water potential gradient (Kurze-Besson *et al.*, 2014). In Jijel provenance, osmotic adjustment can be accomplished by the accumulation of other metabolites. So, the compounds involved in osmotic adjustment differ widely among plant species and perhaps among populations (Patakas *et al.*, 2002).

Leaves pigment contents of the three provenances were not similarly affected by drought. Only M'Sila seedlings showed a decrease in total chlorophylls, chlorophyll a, chlorophyll b and carotenoids contents while Azazga seedlings showed a decrease only in carotenoids contents. Jijel seedlings were not affected. Generally, *Q. suber* water stressed seedlings showed a decrease of total chlorophylls with an increase in chlorophyll b and carotenoids to cope with oxidative stress and a decrease of chlorophyll a to avoid excessive absorption of light energy (Faria *et al.*, 1999). Daoudi *et al.* (2016) did not find any difference in total chlorophyll contents of seedlings originating from Azazga provenance and Vaz *et al.* (2010) reported that summer dry conditions did not affect chlorophyll concentrations of *Q. suber* trees. Contrary to expectation, seedlings of M'Sila provenance which showed the better length growth had the lower chlorophyll contents. A negative correlation between chlorophyll contents and growth was previously recorded by Ramirez-Valiente *et al.* (2010); plants exhibiting lower leaf chlorophyll content had larger annual shoot growth.

Important variations of leaf $\delta^{13}\text{C}$ have been observed for many species across their distribution range like *Q. suber* (Gouveia & Freitas, 2009). The decrease of $\delta^{13}\text{C}$ leaves content in M'Sila and Azazga seedlings were similar to the results of Gouveia & Fortas (2009) who reported that trees subjected to greater water stress showed an increase of carbon isotope discrimination. This is due to the fact that water supply affects the stomatal conductance and photosynthesis of plants, which changes $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates (Du *et al.*, 2015). The reduction of %N in leaves of the three provenances was previously recorded by Daoudi *et al.* (2016) but this was not observed in Tunisian samples by Kwak *et al.* (2011).

Summer drought is the main cause of seedling mortality in Mediterranean-climate areas. The significant difference in mortality rate under dry conditions between these Algerian provenances was not found by Gandour *et al.* (2007) for 26 provenances studied from six countries. However, differences in sapling survival across *Q. suber* provenances were recorded and positively related to the height of planted seedlings, and seedling size was closely related to acorn size, which was bigger in populations from warm and drier locations (Ramírez-Valiente *et al.*, 2009) in contrary to our results where M'Sila showed the better height but the lowest survival rate. Navarro *et al.* (2006) concluded that positive relationships between survival and seedling size were three times more frequent than the cases showing negative relationships. Variation in seed mass is an important trait which may have consequence for growth and survival of seedlings because seed mass represents the

reserves available for growth in the first stage of plant establishment (González-Rodríguez *et al.*, 2011). An increase in seedling size can result in higher transpiration, which increases plant vulnerability to drought on the short-term and this is the main argument for using small seedlings in dry sites (Villar-Salvador *et al.*, 2012).

CONCLUSION

In our experiments water stress did not act alone but was associated with high temperatures and high light stresses. Therefore, seedlings response to drought involves adjustment to stresses associated to drought.

This study showed differences in watered and non-watered conditions for morphological and physiological traits of three Algerian provenances (Jijel, M'Sila and Azazga) originating from humid, sub-humid and semi-arid areas at seedling stage. In terms of survival rate and water status, Azazga provenance seems to be the better provenance adapted to summer drought conditions of the Mediterranean area while survival rate of M'Sila provenance which is located in semi-arid climate was more affected by drought. However, the growth of the seedlings was less affected for M'Sila than Azazga provenances. Indeed specimens from the site of M'Sila (semi-arid) when cultivated in water privation conditions, showed morphological and physiological changes that could be related to drought tolerance. They exhibited the lowest SLA, which means a reduction of the leaf evaporative surface. A decrease of chlorophyll a contents was also noticed suggesting a potential reduction of the number of PSII for an efficient regulation of solar radiation absorbed in excess. A substantial enhancement of the water use efficiency (WUE) was obtained by $\delta^{13}\text{C}$ measurements. While starch content decreases, soluble sugars and proline increase, probably as potential compatible osmolytes and electron donor in response to drought. The intense metabolic activity related to plant tolerance to drought seems to be related to an increased accumulation and/or synthesis of total soluble proteins.

These results showed probably the existence of an inter-populations variability that may be linked to the specificity of plant-climate-coevolution. Hence, semi-arid sites of *Q. suber* may be considered as potential germplasm banks for reforestation in response to global climate change. However, further studies are needed to deepen the physiological knowledge of *Q. suber* seedlings. The photosynthesis machinery should be explored both in terms of gas exchange and quantum yield as well as field measurements.

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In this study, we investigated the physiological and morphological responses of seedlings of three mediterranean species: *Q. suber*, *Q. ilex* and *Pinus halepensis*, grown in non controlled greenhouse subjected to drought conditions (no watering).

Q. suber and *Q. ilex* seedlings showed usually similar behavior face drought conditions: RWC and growth reduction and an increase of sugars, proteins and proline leaves contents. These responses are known to be adaptative strategy of Mediterranean species to summer drought. Little differences are noted which concern starch and pigments leaves contents.

Three Algerian provenances seedlings (Azazga, Jijel and M'Sila) subjected to drought conditions in non controlled greenhouse showed differences in their behavior. Seedlings originating from M'Sila, the drier site (semi-aride), showed morphological and physiological characteristics known to be adaptative strategy to drought but survival rate was lower for this provenance after eight weeks of no watering.

The ectomycorhization of *Pinus halepensis* seedlings with *Boletus edulis* did not improve water status and growth under drought conditions in non controlled greenhouse. Under watered conditions, this ectomycorrhizal fungi increased leaves biomass.

Keys words: *Quercus suber*, *Quercus ilex*, *Pinus halepensis*, sécheresse, RWC,

Croissance, Physiologie, Provenances, Ectomycorhization

Les réponses morphologiques et physiologiques des plantules de trois espèces forestières méditerranéennes : *Quercus suber* L, *Quercus ilex* L. et *Pinus halepensis* M. face à une contrainte hydrique par arrêt d'arrosage ont été étudiées en serre non contrôlée.

Après arrêt d'arrosage, *Q. suber* et *Q. ilex* ont montré pratiquement les mêmes réponses : une réduction de la RWC et de la croissance et une accumulation des sucres, des protéines solubles et de la proline dans les feuilles. Ces réponses sont considérées comme étant des stratégies d'adaptation à la sécheresse estivale méditerranéenne. Seules les teneurs des feuilles en pigments et en amidon diffèrent entre les deux espèces.

Les plantules originaires de trois provenances algériennes (Azazga, Jijel et M'Sila) ont montré des comportements différents face à l'arrêt d'arrosage. Les plantules provenant de M'Sila, site le plus aride (semi-aride), ont montré des caractéristiques morphologiques et physiologiques considérées comme étant des stratégies d'adaptation à la sécheresse. Cependant, le taux de survie était plus faible pour M'Sila.

L'ectomycorhization de *Pinus halepensis* avec *Boletus edulis* n'a pas eu d'effet positif en conditions d'arrêt d'arrosage sur le statut hydrique et la croissance des plantules. En conditions d'arrosage, *B. edulis* a provoqué une augmentation de la biomasse foliaire.

Mots clés : *Quercus suber*, *Quercus ilex*, *Pinus halepensis*, sécheresse, RWC,

Croissance, Physiologie, Provenances, Ectomycorhization