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Rate of endomycorrhizal colonization in *Pistacia vera* L.

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Abstract. In arid areas, plants develop several strategies to adapt to bad conditions of survival especially drought. To resist, they develop associations with symbiotic fungi: mycorrhizae. The object of our study is to know the influence of seasonal variation (spring and winter) on root colonization rates by these fungi. For this purpose we chose *Pistacia vera* L., a fruit species cultivated in the orchard of Ross-Loayone located in the province of Djelfa in Algeria. The soil analysis have revealed that the soil is not salty, has high total calcium and is rich in organic matter. Microscopic observations showed the presence of endomycorrhizae-type fungal structures belonging to the class of Glomyromycètes. These fungal structures are vesicles, arbuscules and siphoned hyphae. T-test shows that there is a highly significant difference at $p < 0.001$ between two seasons for the number of vesicles and arbuscules per fragment, that they are higher in the spring and that there is a good positive correlation between them. The hyphae are siphoned intra and extra –roots, and they are present at high rate in the winter.

Keywords. Arid areas – Endomycorrhizae – *Pistacia vera* L. – Djelfa.

Évaluation du taux de colonisation endomycorhizienne chez *Pistacia vera* L.

Résumé. En milieu aride, les végétaux développent plusieurs stratégies pour s'adapter aux conditions difficiles de survie surtout pour la sécheresse. Pour résister, elles développent des associations avec les champignons symbiotiques : la mycorhize. L'objectif de notre étude est de connaître l'influence de la variation saisonnière (printemps et hiver) sur le taux de colonisation par ces champignons. Pour cela on a choisi le *Pistacia vera* qui est une espèce fruitière cultivé dans le verger de Ross-Loyoune situé dans la province de Djelfa en Algérie. Les résultats obtenus ont révélé que le sol est non salé, a un taux élevé de calcaire total et est riche en matière organique. Les observations microscopiques ont montré la présence des structures fongiques de type endomycorhize appartenant à la classe des Glomyromycètes. Ces structures fongiques sont les vésicules, les arbuscules et les hyphes siphonnés. Le test- t montre qu'il y a une différence hautement significative à $p < 0.001$ entre les deux saisons pour le nombre de vésicules et le nombre des arbuscules par fragment. Ils sont élevés au printemps et ont une corrélation positive élevée entre eux. Les hyphes siphonnés se trouvent au niveau intra et extra-racinaire et, sont plus élevés en hiver.

Mots-clés. Milieu aride – Endomycorhize – *Pistacia vera* L. – Djelfa.

I – Introduction

Terrestrial plants have developed numerous strategies to cope with diverse edapho-climatic conditions. One of the most successful strategies is the ability of root systems to establish symbiotic relationships with mycorrhizal fungi (Gianinazzi-Pearson, 1996). The plant typically provides fungus carbohydrates while the latter provides a water intake or elements by increasing the absorption surface (Dalpé, 1997).

This study was interested to determine the type of mycorrhizal association and to know the effects of seasonal variations (spring and winter) on arbuscular mycorrhizae (AM) colonization, for un-

derstanding more about this dynamic life process. For this we chose *Pistacia vera* L., a fruit species belonging to the family of Anacardiaceae. The development of this culture is interesting for many arid and semi-arid regions in Algeria while the study of these symbiotic fungi is the one of the best solutions to improve its quantitative and qualitative production.

II – Materials and methods

The study area is located in Ross-Loyoune the province of Djelfa which is 300 km south of Algiers, at latitude 34°38' North and longitude 3°15' East. This site is located at 1153 m in altitude, it is characterized by a semi-arid climate with cool winters, average temperatures of 16.78°C and rainfall of 312.76 mm/year. Soils under *Pistacia vera* trees are alkaline (pH: 8.90) and unsalty (Electrical conductivity: 0.163 mmhos/cm) with 43.04% in humidity. The organic matter content is about 4.35%. These soils are highly calcareous (TC \geq 34.05) with a high amount of active calcareous (AC = 12.5).

Sampling was conducted in 2014. The roots were collected simultaneously with soils under 10 trees of *Pistacia vera* L., at one level (20 cm) and in two seasons (spring and winter). Roots with diameter less 0.5 mm were selected from the soil samples, cut (\approx 1 cm) and preserved in ethanol at 70°.

The observation and detection of AM fungi and their structures (vesicles, hyphae and arbuscules) is only possible after the treatment of the samples adopting the Phillips and Hayman staining technique (1970). Roots fragments are mounted between microscope slides and cover slips then observed under light microscopy. A total of 1000 observations are performed. Brundrett *et al.* (1996) method is used to count the number of vesicles, arbuscules and hyphae per fragment, where an observer simply provide a visual estimate of the degree of mycorrhizal colonisation.

Descriptive statistics, correlation matrix and t-test of student were performed using Excel software.

III – Results and discussion

Our observation about colorful roots of *Pistacia vera* shows colonization by AM in both spring and winter seasons. These AM are characterized by the presence of three structures: the vesicles, the arbuscules and the siphoned hyphae (Table 1). The vesicles are present in a large number, having different sizes and shapes (oval or round, large and small). These structures are formed in the intracellular cell wall where they appear much larger than in the intracellular spaces of the cortical parenchyma. Arbuscules were observed within some root cells (structure of exchange between partners). Siphoned hyphae are intracellular or intercellular. The involved fungi belong to the monophyletic group of Glomeromycetes (Balzergue, 2012).

The number of vesicles and arbuscules per fragment is important in spring (exceeds 2) (Table 1). While for hyphae, their number was higher in winter (0.6 hyphae per fragment). The important number of symbiotic structures (arbuscules and vesicles) in spring time may be due to the increase in symbiotic exchange between plants and AM. The arbuscules are the functional organ between the two partners (plant and fungus), while the vesicles are the preserved organ in root cells (Harly and Smith, 1983).

The t-test shows a highly significant difference ($p < 0.001$) between the two seasons regarding the number of vesicles and arbuscules per fragment, while no significant difference is registered for the number of hyphae (Table 1). In spring time, fungus activity is increased, showing higher values for the vesicles and arbuscules; this can be explained by the symbiotic relationship between the two partners, as the leaves of this species synthesized sugars (source of energy for the fungus) in spring time through photosynthesis. Correlations between the fungal structures are not significant except between vesicles and arbuscules, the value is moderately significant ($r = 0.62$). After Hampp and Winkler (1997) sugars (sucrose and glucose), are transformed by fungus into specific sugars (trehalose or mannitol) and consumed for growth and respiration. This flow of carbon is also carried out in the form of amino acids and vitamins (biotin and thiamine) (Lassac, 1992).

Table 1. Number of vesicles, arbuscules and hyphae per 1 cm root fragment of *Pistacia vera* in spring and winter time

	Spring Mean \pm S.D (min-max)	Winter Mean \pm S.D (min-max)	T test
Number of vesicles (cm ⁻¹ root)	2.16 \pm 2.53 (0-9)	0.78 \pm 1.34 (0-6)	***
Number of arbuscules (cm ⁻¹ root)	2.78 \pm 2.53 (0-7)	0.92 \pm 1.55 (0-7)	***
Number of hyphae (cm ⁻¹ root)	0.38 \pm 0.95 (0-4)	0.6 \pm 0.93 (0-4)	NS

S.D.: Standard deviation; *** P < 0.001; NS, not significant.

IV – Conclusions

According to our results, there is a close relationship between the two partners, translated by the number of the fungal structures which may change depending on the vegetative state of the plant, the latter being influenced by the climatic conditions. In spring time (which matches with higher arbuscules and vesicles rates in the rooting system), in presence of solar radiation, plants photosynthesize better, this requires water which is extracted by fungus from the soil particles and solution. In return, the fungus get photosynthesized sugars which are stored or used for their development. In general, the use of the mycorrhizae in agriculture must be regarded as a biological strategy for the increase and the improvement of the production. Hence, the tolerance and adaptation of *Pistacia vera* to a large range of soil types is due to symbiotic fungi such as endomycorrhizae which play several roles such as resistance to drought, protection against disease and an increase in production. Studying symbiotic fungi is one of the solutions to improve pistachio culture. It has been suggested that AM symbiotic association can be used as a biofertilizer to improve nutrient cycling and crop productivity by reducing the fertilizer inputs, thereby conserving soil fertility and reducing production and environmental costs. Mycorrhizal colonization, by helping plants to become established in eroded and degraded habitats, for example in soils with high calcareous rate in arid and semi-arid areas, can be regarded as an important alternative strategy for a more rational and sustainable agriculture. This work is a first contribution to the knowledge of behavioral response of *Pistacia vera* and its corresponding mycorrhizal fungus. Further trials must be conducted in different sites and during different seasons in order to improve our knowledge in this field.

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