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Endophytic fungi of leaf of Atlas pistachio of Dayate Aiat (Laghouat, Algeria)

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Abstract. Atlas Pistachio, *Pistacia atlantica* Desf. is an Anacardiaceae, ubiquitous North Africa and the Middle East. In Algeria, this species is widespread in arid regions where difficult natural conditions the push to form symbioses to counter the water deficit. Its leaves are deciduous. They are present on the tree when the rains decrease and temperatures rise. To mitigate its constraints symbiotic associations with mutualistic fungal endophytes are possible. Our sampling was done at a daya of the wilaya of Laghouat. Harvested in April 2013 and ten normal individuals subjectively selected leaves are stained by the method of Hayman and Phillips (1970) and observed under an optical microscope. Observations have shown the presence of several differently colored structures. Different ranges of colors are distinct along the limbus at all compartments of the leaflets. Endophytic fungi are noted in stomata, glandular hairs and between certain epidermal cells within parenchymal cells, but also in the primary and secondary veins (xylem and phloem). A variety of fungal endophytes is noted in the leaves of pistachio Atlas. These are probably able to maintain the eco-physiological and adaptive balance of this species under the restrictive conditions of the natural environment in which it lives.

Keywords. *Pistacia atlantica* Desf. – Laghouat – Algeria – Foliar mycoendophytes.

Champignons endophytes foliaires du pistachier de l'Atlas de dayate Aïat (Laghouat, Algérie)

Résumé. *Le pistachier de l'Atlas,* Pistacia atlantica *L. est une Anacardiacée, ubiquiste du Nord de l'Afrique et du Proche Orient. En Algérie, cette essence est répandue dans les régions arides où les conditions naturelles difficiles la poussent à former des symbioses pour contrer le déficit hydrique. Ses feuilles sont caduques. Elles sont présentes sur l'arbre lorsque les pluies diminuent et les températures augmentent. Pour pallier à ses contraintes, des associations symbiotiques avec des champignons endophytes mutualistes sont possibles. Notre échantillonnage a été fait au niveau d'une daya de la wilaya de Laghouat. Les feuilles récoltées en avril 2013 sur dix individus sains et choisis de manière subjective, sont colorées selon la méthode de Phillips et Hayman (1970) et observées au microscope optique. Les observations ont montré la présence de plusieurs structures colorées différemment. Des plages de différentes couleurs sont bien distinctes tout le long du limbe, au niveau de tous les compartiments des folioles. Des champignons endophytes sont notés au niveau des stomates, des poils glandulaires et entre certaines cellules épidermiques, à l'intérieur de cellules parenchymateuses, mais aussi au niveau des nervures principale et secondaires (xylème et phloème). Une diversité de champignons endophytes est notée au niveau des feuilles du pistachier de l'Atlas. Ces derniers sont probablement capables de maintenir l'équilibre écophysiologique et adaptatif de cette espèce sous les conditions contraignantes du milieu naturel dans lequel elle vit.*

Mots-clés. Pistacia atlantica *Desf. – Laghouat – Algérie – Mycoendophytes foliaires.*

I – Introduction

The Atlas pistachio is the most ubiquitous tree in Northern Africa and the Middle East (Monjauze, 1980). It presents an ecological amplitude and a remarkable plasticity. It is found in the heart of the Sahara to the edges moist bioclimate (Quézel and Médail, 2003). These plants adapted to arid ecosystems have developed several mechanisms, such as symbiotic associations that reduce this stress, improve nutrition and survival (Barrow Aaltonen, 2001). These symbioses are often con-

tract with fungi and can concern the roots, but also the leaves. They are an integral part of plant microbiome. The mycoendophytes penetrate plant tissues without causing disease symptoms (Li *et al*., 2012). They confer benefits to their hosts through improved nutrient absorption (Mandaym and Jumpponen, 2005) and increased resistance to pathogens (Ghimire *et al*., 2010).

This mycoendophytes diversity is not known at the Atlas pistachio. We are interested in this study to its highlighted and its distribution in the different compartments of the leaves of this species.

II – Material and methods

The leaves of pistachio Atlas were collected in April 2013 from dayate Aïat (area of Timzerth, Laghouat), of ten subjects chosen subjectively. The subjects were in good health status. For each selected tree, ten leaves are harvested around the canopy. For the coloring of the leaves, it was made according to the protocol of Phillips and Hayman (1970). Observations were made by an optical microscope. Photos are taken at different magnifications.

III – Results and discussion

Observations under an optical microscope reveals the presence of hyphae at the inter– and intracellular spaces of epidermis of the leaf of *Pistacia atlantica* (Fig. 1).

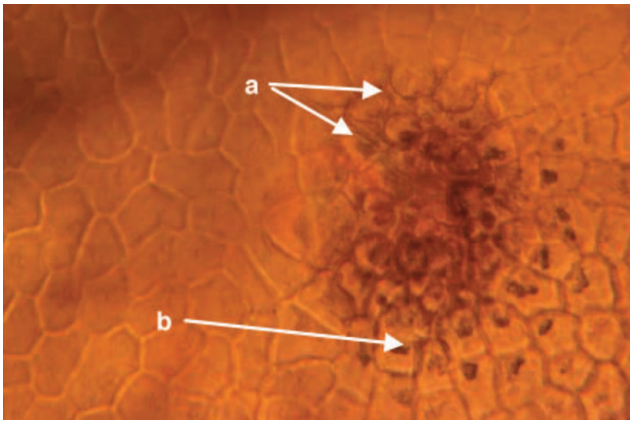

Fig. 1. Microscopic observation of inter (a) and intracellular (b) colonization of the epidermal cells of the leaf of Atlas pistachio (X 400).

These results are agree with those of Bernadi-Wenzel *et al*. (2010) and Orlandelli *et al*. (2012), which showed that endophytic fungi can form associations from an inter and intracellular colonization of the host plant tissue. It should be noted that the intercellular space is rich in substances needed to support the growth of fungal endophytes (Kuldan and Bacon, 2008).

The presence of mycoendophytes seems as important in stomata (ostioles, guard cells and the cells that surround the stomata) (Fig. 2). Fungal endophytes can alter hormone levels that control the stomatal behavior and osmotic adjustment (Mandyam and Jumpponen, 2005 ; Bezzerra *et al*., 2013).

Glandular hairs are also affected by the presence of endophytic fungi (Fig. 3). Indeed, they are responsible for a significant portion of the secondary chemistry of a plant (Glas *et al*., 2012).

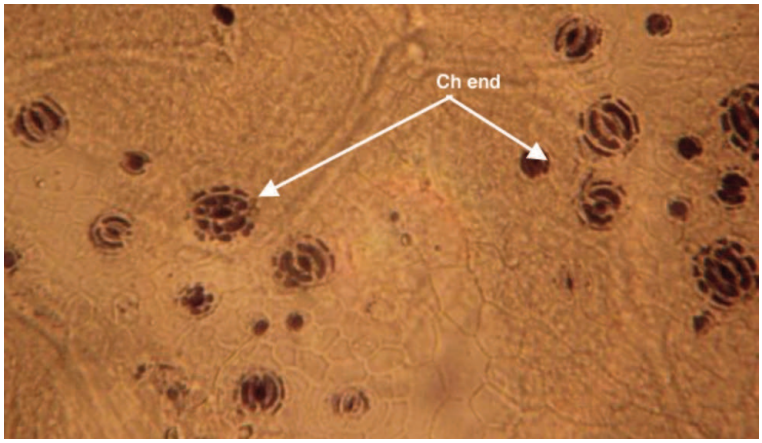

Fig. 2. Microscopic observation of endophytic fungi colored in blue (ch end) in the stomata of the leaf of Atlas pistachio (X 400).

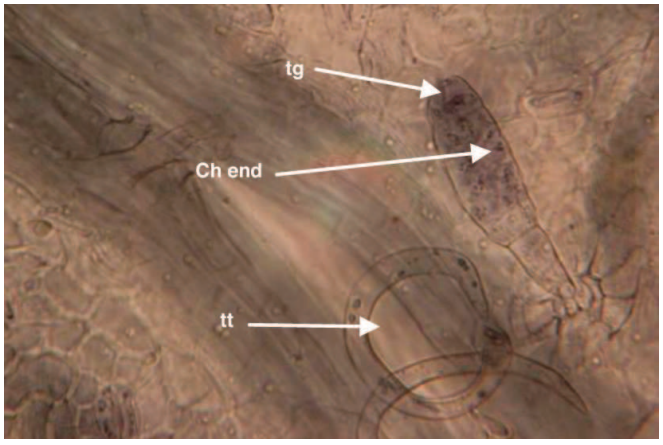

Fig. 3. Microscopic observation of a glandular hair (tg) colonized by mycoendophytes (Ch end) and a tector trichome (tt) not colonized by them at the leaf of Atlas pistachio (X 400).

It should be noted, however, the absence of endophytic fungi at the tector trichomes (Fig. 3). This can be explained by the absence of synthetic defense metabolites in these structures. In fact, the tector trichomes provide more mechanical role against abiotic and biotic factors (Szyndler *et al*., 2013).

Microscopic observations of leaves show the existence of endophytic fungi within and between the cells of the parenchyma, but also at the level of conductive tissues (xylem and phloem) (Fig. 4). These results confirm those of Sanchez-Azofeira *et al*. (2012).

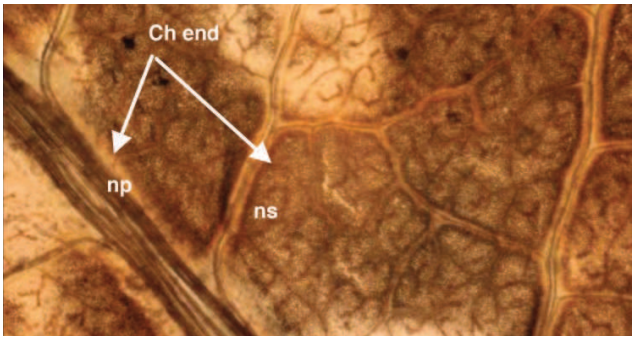

Fig. 4. Microscopic observation of endophytic fungi (Ch end) at the midrib (np) and secondary veins (ns) of the leaf of Atlas pistachio (X 40).

IV – Conclusion

Microscopic observation leaves *Pistacia atlantica* Desf. stained with trypan blue showed the presence of endophytic fungi in almost all compartments of the leaf (epidermal cells, stomata, glandular hairs, parenchyma, xylem and phloem) from all sampled subjects.

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