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Effectiveness of histological techniques for early identification of scion rootstock incompatibility

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Abstract. Traditionally almond was cultivated in Tunisia extensively under rainfed conditions with local almond seedlings as rootstock. The new tendency for modernization of almond orchards has implemented the use of some non native cultivars that are compatible with the existing rootstocks. In order to evaluate the behaviour of some local cultivars for high density orchard some trials were established using the cultivars 'Achaak' and 'Porto' with 'GF-677' and 'Garnem' as rootstocks. Early observations revealed a plausible incompatibility of the cultivar 'Achaak' with 'Garnem'. In order to contribute to the understanding of this phenomenon and to detect other cases of scion-rootstocks incompatibility histological techniques were used as an early detection practice. Preliminary results for the combination 'Achaak'/'Garnem' showed some discontinuity of cells in the intermediate zone between scion and rootstocks in addition to an accumulation of black points of starch. Moreover, observations in field showed low circumferences values and weak growth of both parts. Our work reveals for the first time this scion-rootstock incompatibility and underlines the necessity of biochemical tools for a better understanding of this phenomenon.

Keywords. Local cultivars – Intensification – Interface scion/rootstock – *Cambium* cells.

Efficience des Techniques histologiques pour la détection précoce de l'incompatibilité greffon/porte-greffe chez l'amandier

Résumé. Traditionnellement, l'amandier a été conduit en Tunisie en extensif avec les semis d'amande amer comme porte-greffes. La nouvelle tendance vers la modernisation des vergers d'amandier a entraîné l'utilisation de variétés introduites avec des porte-greffes bien adaptés à ce système de conduite. Dans le but d'étudier le comportement de quelques variétés locales d'amandier conduites en vergers à haute densité un essai a été réalisé pour la variété 'Achaak' greffée sur les deux porte-greffes 'GF-677' et 'Garnem'. Les premières observations ont montré l'existence de quelques symptômes d'incompatibilité avec ce dernier. Dans le but d'approfondir nos connaissances sur le sujet on s'est intéressé à l'application de quelques techniques histologiques pour une éventuelle détection précoce de cas semblables d'incompatibilité. Les premiers résultats ont montré chez la combinaison 'Achaak'/'Garnem' une discontinuité au niveau des cellules cambiales existantes dans la zone d'interface et une accumulation de polyphénols. Ces résultats viennent appuyer des observations sur terrain relatives à un rétrécissement de la circonférence des individus étudiés de part et d'autre du point de greffe et de confirmer l'incompatibilité de la variété 'Achaak' avec le porte-greffe 'Garnem'. Finalement ce travail souligne l'efficacité des techniques histologiques pour une détection précoce et approuve l'apport de l'outil biochimique.

Mots-clés. Variétés locales – Intensification – Interface greffon/porte – Cellules du cambium.

I – Introduction

In Tunisia, Almond [*Prunus dulcis* (Miller) D.A. Webb, syn. *Prunus amygdalus* Batsch] has been known since the Carthaginian era. About 90% of the almond production is in the semi-arid central and southern part of the country and conducted under rainfed conditions. Traditional almond plantations are dominated by old selected cultivars such as 'Achaak', 'Fekhfekh', 'Ksontini', 'Zahaaf',

selected by farmers for fruit quality, good production and high adaptation to specific agro-ecosystems (Gouta *et al*, 2012) and grafted on bitter almond seedlings.

Three rootstocks 'GF-677', 'Cadaman' and 'Garnem' are being used when almond trees are conducted under irrigation conditions. Each one has advantages and disadvantages associated with its use. Although 'GF-677' is not resistant to nematodes, it is still used by nurseries while it has not shown any compatibility problems with any of the local cultivars. 'Cadaman' was used for few years but due to many phytosanitary problems it was replaced by 'Garnem'. In fact, this last was supposed to be tolerant to irrigated soil conditions and resistant to root knot nematodes. With the recent tendency for almond planting under irrigation conditions and in order to overcome the excessive use of introduced almond cultivars in modern plantations, 'Garnem' was used as a rootstock for local cultivars such as 'Achaak', 'Fekhfekh', 'Ksantini' and 'Zahaaf'. Field observations and discussions with many responsible of nurseries have incited some researcher to evocate a dwarfing effect and an eventual incompatibility of this rootstock when used with the most important local cultivar 'Achaak'. For that, and in order to contribute to a deep study of this phenomenon an investigation was started since last year. The aim of this study was to prospect plantations where 'Garnem' was used as a main rootstock and to establish a preventive method based on histological techniques to an early detection of scion-rootstock incompatibility for local almond cultivars.

II – Materials and methods

Two rootstocks 'Garnem' and 'GF-677' showing different graft compatibility performance with almond cultivar 'Achaak' were used. The two rootstocks were propagated in a seed plot and transferred in a nursery. Budwood of the cultivar 'Achaak' were collected from the Experimental Station of Ettaous (Sfax, Tunisia) and grafted in situ on the two rootstocks on June 2013. Two types of combinations were carried out (GF-677/Achaak and Garnem/Achaak) for a deep understanding of the phenomena. Scion/rootstock interface zones were collected and fixed for further histological analyses, after five and eight months of grafting.

Three graft unions per combination were collected and each stem piece (3-5 mm in diameter) was fixed in 2.5% glutaraldehyde, containing 0.1% caffeine in 0.1 M phosphate buffer (pH 7.4), for 4 h at 4°C according to Ermel *et al.*, (1999). A free hand section is the simplest method of preparing sections for microscopic viewing. Stem pieces were cutting several sections at a time. Sections will certainly vary in thickness. The thinnest sections were selected and transferred onto a glass slide. Samples were swabbed in 50% (v/v) sodium hypochlorite for 20 min. and rinsed at least three-times with sterile distilled water. The last step two drops of acetic acid were added. Sections were stained with iodine green and observed under a bright field in a light microscope (Ernst Leitz, Wetzlar, Germany) equipped with a digital camera (Sony, Japan).

III – Results and discussion

Field observations for 'Achaak' grafted on the rootstock 'Garnem' showed no sign of incompatibility at the nursery level. In fact the scion is normally vigorous with green leaves. Nevertheless, after two to three years of plantation the first clear symptoms are a tendency for dwarfing. In fact both parts remain weak with small trunk diameters and no expanding branches and shoots with large cracklings at both sides of the grafting point (Fig. 1A, 1B, 1C and 1D). This corroborate with earlier observations of Kester and Hansen (1964) who reported that the incompatibility symptoms advance, trees show signs of ill-health even in the early spring: poor growth, dieback of shoots, small leaves and small trunk diameters and general decline sometimes results in death.

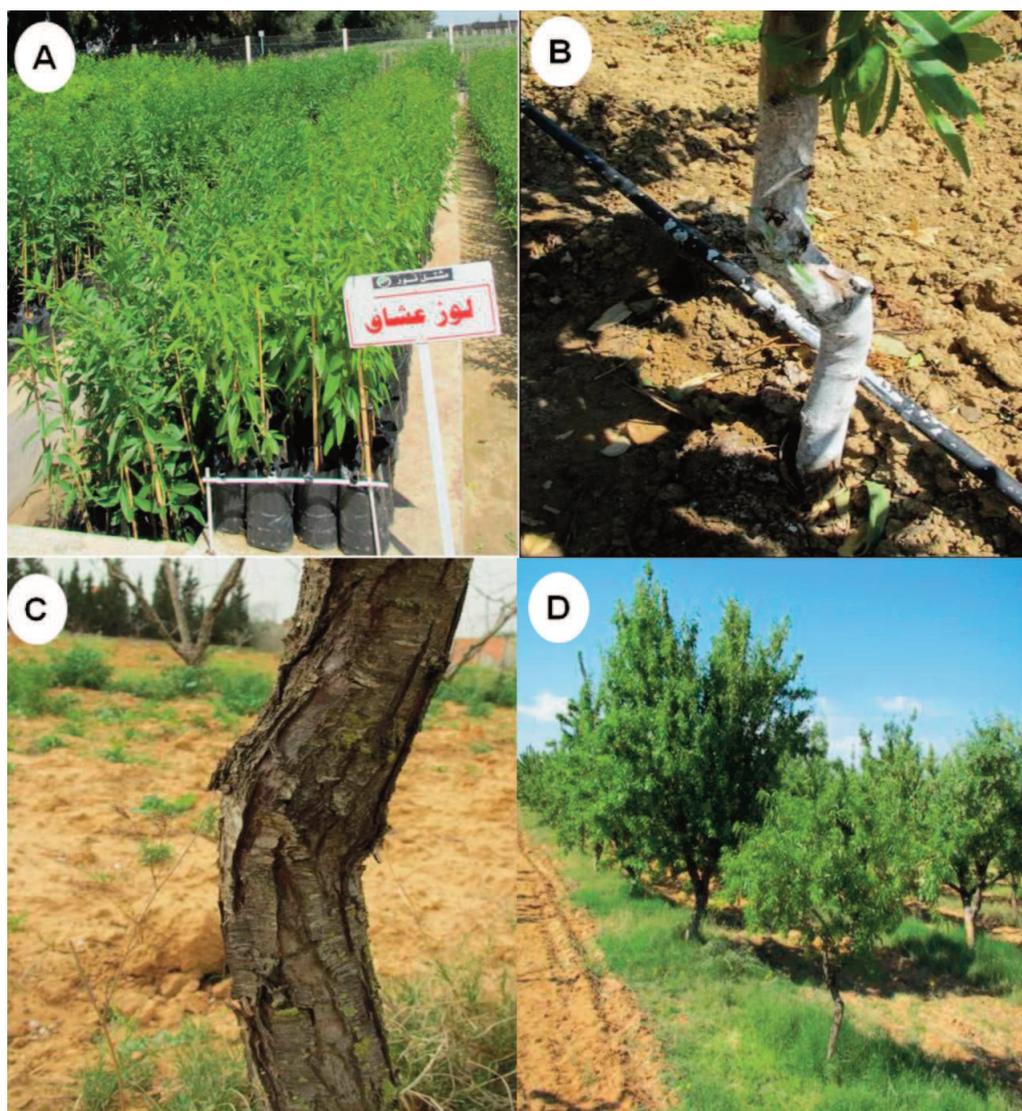


Fig. 1. Field observations of incompatibility between scion cv. 'Achaak' and rootstock 'Garnem'. (A) Nursery plants with normal behavior. (B) Three years old 'Achaak' tree grafted on 'Garnem' (C) Symptoms of large cracklings at both sides of the grafting. (D) Difference in vigor between 'Achaak' and 'Fekfekh' cultivars grafted on 'Garnem' rootstock.

Figure 2 shows the major differences found in the histological study at the interface level, related to cambium and vascular organization. In fact, the interface scion/stock showed a separation between the bark tissues of the two components at the graft union. Also, a distinct brown line at the union delimiting scion from rootstock tissues (Fig. 2B) was strongly stained with iodine green and was continuous for the incompatible combination 'Achaak'/'Garnem' (Fig. 2C). The compatible combination 'Achaak'/'GF-677' showed organized and homogenous arrangement of cambium cells in the contact interface with a complete differentiation of wood rays and scion phloem (Fig. 2D). In contrast, cambium cells in the incompatible interface showed a disorganized arrangement (Fig. 2A).

Differences exclusively located at the scion/rootstock interface mainly in cambium and vascular components, have already been mentioned by other authors (Zarrouk *et al.*, 2010) for some peach/plum cultivars and are in line with reports for woody (Ermel *et al.*, 1999) and herbaceous species (Wang and Kollmann, 1996). Moreover, the fact that the arrangement of the cambium cells was less organized in graft interface of the incompatible combinations corroborates previous reports regarding apricot (Errea *et al.*, 2001) and pear (Ermel *et al.*, 1997). Moreover, as it was already reported in pear (Espen *et al.*, 2005), incompatible grafts showed less tracheary elements (Fig. 2B). Finally, this supports, that cambium cell disorganization is an early indicator of graft incompatibility.

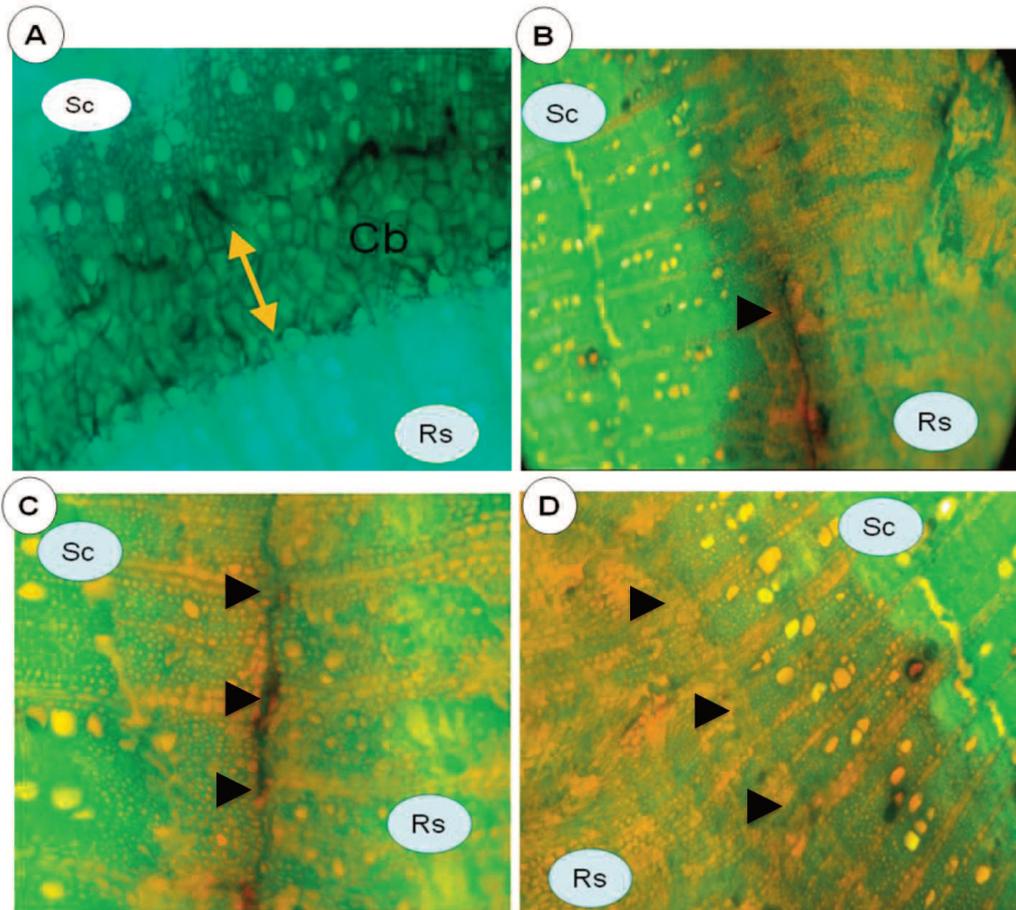


Fig. 2. Transversal sections of graft interface structure of ‘Achaak’/‘Garnem’ and ‘Achaak’/ ‘GF-677’ combinations. (A) Cell cambium disorganization. (B) Incompatible overstraining line delaminated part of stock from scion. (C) Continuous line of the interface zone. (D) Compatible line delimited stock ‘GF-677’ from scion ‘Achaak’.

IV – Conclusions

Field observations regarding the scion/rootstock incompatibility between 'Achaak' and 'Garnem' were confirmed by histological techniques. At a first level the heterogeneous cambium cell arrangement can be considered as a first indicator followed by the presence of the brown line at the union delimiting scion from rootstock tissues. Thus, our study confirmed the efficiency of this technique for early detection of scion/rootstock incompatibility for almond.

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