In Vitro Micrografting of Mature Carob Tree (*Ceratonia siliqua L.*)

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Abstract: A micrografting technique was developed for *Ceratonia Siliqua* using *in vitro* germinated seedlings as rootstocks and apex or shoot cultures (established from mature female tree source) as microscions. *In vitro* germinated seedlings were decapitated and used as rootstock. Mature explants were initiated on Murashige and Skoog medium containing 30 g/l sucrose, and 0.5 mg/l 6-benzylaminopurine, then transferred on the multiplication medium (Murashige and Skoog + 0.5 mg/l 6-benzylaminopurine + 0.1 mg/l gibberellic acid + 0.1 mg/l indol-3- butyric acid). Micrografts could be easily cultured on the same medium and developed after one month. Grafting success has been dependent on the method of grafting. The micrografting on hypocotyl (central grafting) method was significantly more successful (92, 50%) than the other grafts methods. The compatible graft union was observed one month after grafting. The histological cuttings showed that the graft union formation was initiated by the development of the callus bridge at the interface between scion and rootstock, after that, new vascular elements including vessels and tracheids were seen across the interface zone.

Key Words: *Ceratonia siliqua*, micrografting, female trees, *in vitro* culture.

INTRODUCTION

The carob tree (*Ceratonia siliqua* L) is an important species environmentally and economically prevailing calcareous soils of the Mediterranean region. Traditionally, grafted carob trees have been interplanted with olives, grapes, almonds and barley in low-intensity farming systems in most producing countries. In some areas, such as in Mediterranean Turkey, south Spain and Morocco, pods from un grafted spontaneous trees are collected [1-3]. Carob trees may be male, female and hermaphrodite or polygamous inflorescences, showing high plasticity in inflorescences and flower characteristics [4, 5]. Female plants always have been selected in preference to the hermaphrodite ones, as they are better pod bearers. The most common cultivars in commercial orchards are female, only a few hermaphrodite having sufficiently desirable attributes [6]. The female cultivars are the most important trees commercial groves of Mediterranean countries. Males and hermaphrodites are normally used as pollinators. Commercial world production of carob pods is estimated currently around 310000 t, and is mainly concentrated in Spain, Italy, Morocco and other countries. Carob production in Morocco has increased over the last 15 years and it is estimated to be about 26000 t [7].

The global receipt due to carobs would reach 7, 94% of plants-production value in the province of Chefchaouen (regions of northern Morocco) [8].

Traditional carob propagation has been achieved by grafting saplings with female buds chosen in productive trees (Battle and Tous 1997), this traditional carob propagation methods failed to meet the market request, the use of micropropagation seems to be appropriate in order to fulfil the increasing demand for propagating this tree [9].

The micropropagation of the carob tree was realized by certain researchers [10-12]. In the traditional grafting, the optimal leaf development is observed in trees of grafted female aged from 10 to 20 years [13].

In vitro micrografting may provide several advantages such as elimination of viruses, rejuvenation of mature tissues, year round plant production, enhance compatibility studies and correlative relation between rootstocks and scions, breeding for specific genotypic combinations to increase plan productivity, and extention of ecological limits of a particular plant species or cultivar to tolerate edaphic conditions [14, 15]

To optimize the production of carob, a right proposition between male and female plants is needed [16], with this objective; a micrografting of carob between seedling and explants from mature female tree was tested in our study.

MATERIALS AND METHODS

In Vitro Seed Germination and Establishment Of Rootstock

The seeds and explants were collected from the region of Chefchaoun (the North of Morocco). Mature seeds were softened by immersion in analytical sulphuric acid (95%) for 20 min, and surface sterilized with 7% calcium hypochlorite solution by agitation for one hour with the addition of one or two drops of Tween- 80, or in 0.01% mercury chloride ($HgCl_2$) for 30 min. After rinsing three times in sterile distilled water (SDW), the seeds were cultured in tubes containing DW solidified with 0.7% agar and autoclaved at 1 bar and 120 °C for 20 min. The cultures were incubated in the light in a growth chamber (25 °C) for germination and the seedlings obtained were used as rootstock.

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1874-8406/09

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Received: April 28, 2008 Revised: August 8, 2008 Accepted: March 3, 2009