Field Performance of Seedlings and Micropropagated Plantlets of Carob Tree

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Abstract

Micropropagated plantlets of cv. Mulata and cv. Galhosa growing in pots, after acclimatization in the glasshouse and growth for several months under natural conditions, were transferred to a field trial. Carob seedlings of 'Mulata' were also transferred under the same conditions. The field trial has been established with 100% of success in micropropagated plants and 97% in seedlings. Three months after transfer to the field, plants showed good growth but micropropagated plants of 'Mulata' exhibited more vigour than 'Galhosa' and than seedlings, with the greatest number of branches and a larger length increase in the main stem. Before transference to the field, net photosynthetic rates (P_N), water use efficiency (P_N/g_s) and quantum yield of PSII (ϕ_{PSII}) of the potted plants were higher in seedlings than in micropropagated plants. Three months after transplantation all gas exchange parameters were improved for all types of plants, particularly net photosynthetic rate, and no significant differences were observed between plants either micropropagated or seedlings. On the other hand, ϕ_{PSII} decreased significantly in seedlings. The results suggest that the pre-acclimation, in pots, to external environmental conditions might have improved field survival, plant vigour and enhanced the endurance to adverse conditions of micropropagated carob trees.

INTRODUCTION

Carob tree (*Ceratonia siliqua* L.) is a large (10-20 m) evergreen polygamo-trioecious tree of slow growth and great longevity. Due to its interesting agroecological features, such as resistance to drought and salinity, adaptation to poor soils, and minimal cultural requirements, it has been introduced into the whole Mediterranean basin and other Mediterranean-like regions (Battle and Tous, 1997). Traditional carob propagation has been achieved by grafting saplings with female buds of chosen productive trees. This traditional method of propagation has failed to meet the market demand for new, selected plant material, required to establish the new early-producing orchards on a large scale. Thus, the use of micropropagation techniques seems to be appropriate to fulfill the increased demand for propagating this tree (Romano *et al.* 2002). The transplantation of micropropagated plants into the field is usually a critical step and substantial numbers of plants dye after transfer. Although carob tree is well adapted to the Mediterranean climates, the performance of micropropagated plants can be affected when moved to the field, since they will be coping with substantial lower relative humidity, higher light and higher or lower temperature which are potential stressful conditions to them. It has been demonstrated that the combination of these factors, that predispose plants to photoinhibition or down-regulation process, contributes to the reduction in carbon assimilation that will further affect the young plants ability for growth and survival (Chaves *et al,* 2002). In particular, a CO_2 deprivation at the chloroplast level by stomatal closure could enhance the sensitivity of the photosynthetic apparatus to high irradiance (Flexas et al. 1998). Protection mechanisms against excess light are thus an important strategy under Mediterranean conditions and may be achieved by several mechanisms such as the regulated thermal dissipation at the light harvesting complexes (Demmig-Adams and Adams 1996). This photoprotective mechanism competes with photochemistry for the absorbed energy, leading to a decrease in quantum yield of PSII (Genty et al. 1989).

As it is still a matter of uncertainty how environmental constraint factors affect the field establishment of micropropagated plants of carob tree, the present research was undertaken in order to investigate how micropropagated plants of two Portuguese carob cultivars, Galhosa and Mulata, deal with the new environment in terms of survival, growth and photosynthetic performance. Differences between micropropagated plants and seedlings were also evaluated.

MATERIAL AND METHODS

Micropropagated plants of *Ceratonia siliqua* (L.) from cv Mulata and cv Galhosa growing in pots, after acclimatization in the glasshouse and growth for several months under natural conditions, were transferred to a field trial in the south of Portugal, where survival rate and physiological performance has been followed. Carob seedlings of ‘Mulata’ were also transferred under the same conditions. Before transplantation superphosphate and natural organic fertilizers were applied to the soil. Plants were irrigated twice a week during summer. Gas exchange and chlorophyll *a* fluorescence measurements were taken one week before and three months after transplantation. Gas exchange measurements were carried out in the youngest fully expanded leaf at the middle of the light period, using a portable Minicuvette System HCM 1000 (Walz, Effeltrich, Germany). Leaf net photosynthetic rate (P_N) and stomatal conductance (g_s) were calculated according to the equations of von Caemmerer and Farquhar (1981). Chlorophyll *a* fluorescence measurements were performed in the same leaves using a portable pulse amplitude modulation fluorometer (PAM-2000 system, Walz, Germany). The maximal photochemical efficiency of PSII (F_v/F_m) was estimated from F_o (basal fluorescence) and F_m (maximal fluorescence) which values were taken before dawn. The intrinsic efficiency of open PSII reaction centers (F'_v/F'_m) was calculated from basal and maximal fluorescence measured under natural irradiance (F'_o and F'_m) and the quantum yield of PSII in light-adapted leaves (ϕ_{PSII}) was evaluated by the $(F'_m-F_s)/F'_m$ ratio (Genty et al. 1989). The photochemical quenching (q_p), which was used as an estimate of the fraction of open centres, was calculated as: $q_p = 1-(F_s-F'_o)/(F'_m-F'_o)$ (Bilger and Schreiber 1986) and the thermal energy dissipation at the PSII as: NPQ = $(F_m/F'_m)-1$ (Cornic 1994). The electron transport rate (*ETR*) was estimated as described by Krall and Edwards (1992), by multiplying $\Delta F/F'_m \times$ PPFD $\times$ 0.5×0.84. Plant survival and some shoot growth parameters were also evaluated.

RESULTS AND DISCUSSION

Survival and Growth

Micropropagated plants survived transplanting in excellent conditions. The field trial was established with 100% of success in micropropagated plants and 97% in seedlings. In general, plants showed good growth three months after transfer to the field, but micropropagated plants of 'Mulata' exhibited more vigour than 'Galhosa' and than 'Mulata' seedlings, exhibiting the greatest number of branches. The shoot length increase was also higher in micropropagated 'Mulata' plants, although no statistical significant difference has been observed between plants (Table 1).

Gas Exchange and Chlorophyll Fluorescence

Before transplantation no significant differences were observed in net photosynthetic rate (P_N), stomatal conductance (g_s), and instantaneous water use efficiency (P_N/g_s), between 'Galhosa' and 'Mulata'. P_N and P_N/g_s were higher in seedlings than in micropropagated plants, but these differences were only significant relatively to 'Mulata' (Fig. 1A, B, C). Despite the reduction in P_N of micropropagated plants relative to seedlings, the maximal PSII photochemical efficiency (F_v/F_m) was similar and high (0.78-0.73) in the three types of plants, which indicates no PSII damage. No significant differences were observed in the intrinsic efficiency of open PSII reaction centers (F'_v/F'_m), the photochemical quenching (q_p) and non-photochemical quenching (NPQ) of chlorophyll fluorescence between the three types of plants (Fig. 2B, C, D). However, the quantum yield of PSII electron transport in the light (ϕ_{PSII}) was higher in seedlings (Fig. 2A), which agrees with the best photosynthetic capacity of these plants, and can be related with a greater concentration of chlorophyll.

After transplantation all gas exchange parameters were improved and no significant differences were observed between plants (Fig. 1D, E, F, G), despite ϕ_{PSII} has decreased in seedlings (Fig. 2 D). On the other hand, the (F_v/F_m) ratio declined significantly (0.57-0.63), which may indicate that some degree of photoinhibition occurred. The observed decrease on NPQ (Fig. 2H), also point out that regulated thermal dissipation in light harvesting complexes were not promoted suggesting that this mechanism could not be enough to avoid some photoinhibition. Carob tree has been considered the most sensitive species, among other Mediterranean sclerophyll species, to low temperatures (Larcher, 1981). Considering the low night temperatures that occurred in the week of the field measurements (about 6 ºC at predawn), the authors ascribe the photoinhibitory effect to this particular environmental condition and not to transplantation *per se*.

CONCLUSIONS

This study shows that micropropagated plants of carob tree have excellent field survival. Results suggest that the pre-acclimation to external environmental conditions in pots might have improved field survival and plant vigour. The enhanced photosynthetic carbon assimilation and growth three months after transplantation are presumably related with adequate fertilization (enough nutrient reserves), and with the absence of a volumetric limitation to the root system development.

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Tables

Table 1. Main shoot length increase and number of new leaves and lateral branches of micropropagated 'Mulata and 'Galhosa' and seedlings of carob tree three months after transplantation at field.

Plant Type	Survival (%)	Increase in shoot length (cm)	Nº new leaves on terminal shoot	Nº of new lateral branches
'Mulata'	100 a	6.4 ± 1.2 a	4.2 ± 0.5 ab	1.8 ± 0.3 a
'Galhosa'	100 a	3.7 ± 1.1 a	5.5 ± 0.4 a	0.1 ± 0.2 ab
Seedling	97 a	3.9 ± 1.5 a	3.3 ± 0.5 b	0.0 ± 0.0 b

The values shown are means ± SE from five samples. Values followed by the same letter are not significantly different at P≤0.05 (one-way ANOVA, Bonferroni test).

Figures

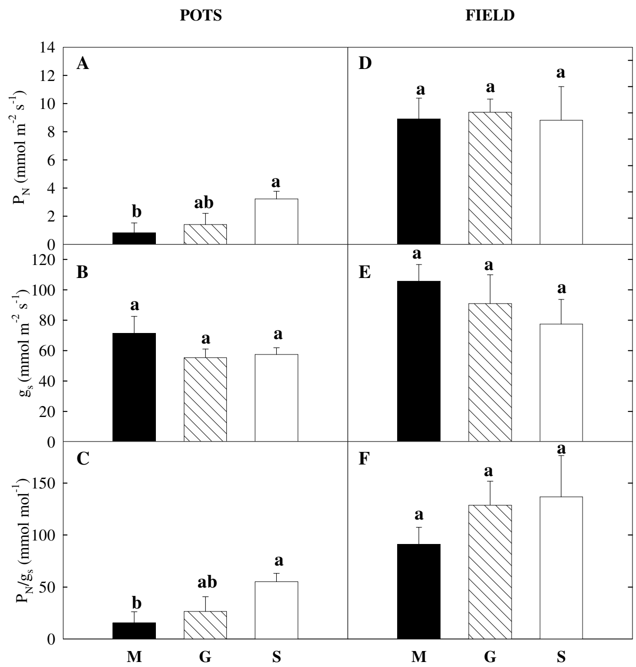

Figure 1. Net photosynthesis rate (P_N), stomatal conductance (g_s) and instantaneous water use efficiency P_N/g_s of micropropagated plants and seedlings of carob tree before (Pots) and after (Field) transplantation. The values shown are means ± SE from five samples. Values followed by the same letter are not significantly different at P≤0.05 (one-way ANOVA, Bonferroni test).

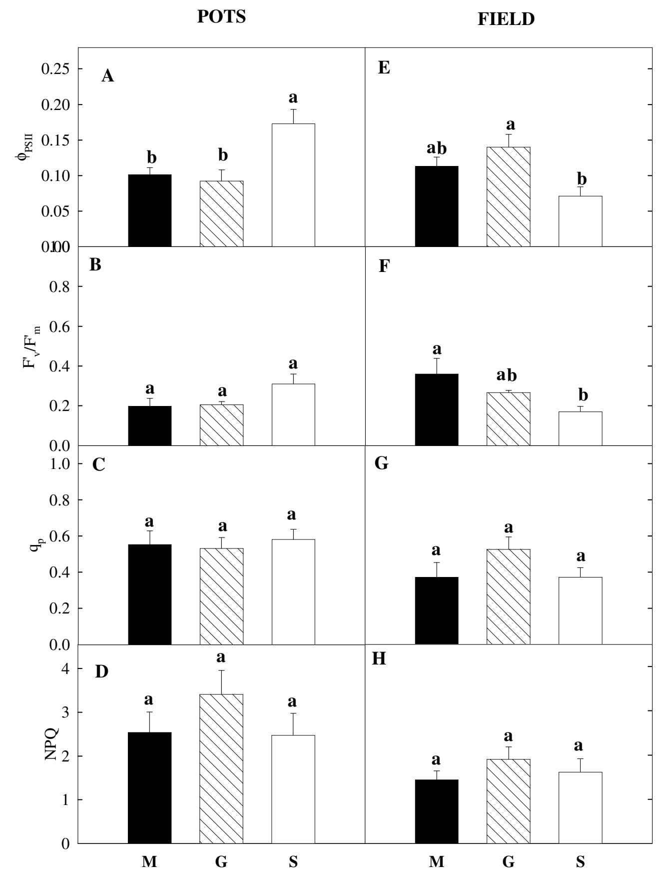

Figure 2. Quantum yield of PSII (ϕ_{PSII}), intrinsic efficiency of open PSII reaction centers (F'_v/F'_m), photochemical quenching (q_p) and non-photochemical quenching (*NPQ*) of chlorophyll fluorescence of micropropagated plants and seedlings of carob tree before (Pots) and after (Field) transplantation. The values shown are means ± SE from five samples. Values followed by the same letter are not significantly different at P≤0.05 (one-way ANOVA, Bonferroni test).