Improved method for vegetative propagation of mature *Pinus halepensis* and its hybrids by cuttings

Joseph Riov^a, Hagar Fox^{a,b}, Rotem Attias^{a,b}, Galina Shklar^b, Lilach Farkash-Haim^b, Robert Sitbon^c, Yosef Moshe^b, Mohamad Abu-Abied, Einat Sadot^b and Rakefet David-Schwartz^b

^aInstitute of Plant Sciences and Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel; ^bInstitute of Plant Sciences, Agricultural Research Organization, The Volcani Center, Rishon Le-Zion 7505101, Israel; ^cSouth District, Forest Management, KKL-JNF, Israel

ABSTRACT

Forest trees possess high genetic diversity and high heterozygosity which allow adaptation to changing environmental conditions. There is a tendency to propagate successful and unique genotypes, which are identified at their mature stage in the forests, for future improvement programs and conservation purposes. However, vegetative propagation of mature forest trees is still a challenge in many conifers. In this study, we focused on improving the rooting of cuttings of mature and old *Pinus halepensis* and its hybrids. We observed that storage of cuttings before rooting at 4°C for 4 weeks and prolong immersion of cuttings in a solution containing 400 mg/l of indole-3-butyric acid, 5 mg/l of the auxin conjugate 2-(2,4-dichlorophenoxy)propanoic acid-glycine methyl ester, and 0.01% of Amistar fungicide significantly improved rooting of mature cuttings. The active ingredient in Amistar is azoxystrobin, an uncoupler of respiration, which seems to directly promote rooting. Rooted cuttings of selected clones demonstrated unique and uniform growth performance, most likely delivering the intrinsic growth parameters of the mother trees. It was also observed that trees growing under drought stress possess improved rooting ability. By using rooted cuttings, it will be possible to study the relationship between growth rate and adaptation to semi-arid climate conditions. The ability to clonal propagate mature and old *P. halepensis* trees not only enables vegetative propagation of elite trees for improvement programs, but also provides an opportunity to preserve unique naturally occurring old *P. halepensis* genotypes.

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Introduction

The global warming and the frequent drought events that occur in the recent years have led to an increase in forest tree mortality in different regions of the world (Allen et al. 2010). *Pinus halepensis* Miller (Aleppo pine) is a widespread native forest tree species in the Mediterranean basin, which has also been widely used for afforestation, particularly in semi-arid regions, due to its well-known drought resistance (Ne'eman et al. 2000; Quezel 2000; Chambel et al. 2013; Dorman et al. 2013; Klein et al. 2013; David-Schwartz et al. 2016; Voltas et al. 2018). The decrease in annual precipitation that is expected to occur in the Eastern Mediterranean (Giorgi and Lionello 2008) poses a threat to the existence of *P. halepensis* populations in semi-arid regions (Alfaro et al. 2014; Fady et al. 2016; Voltas et al. 2018). The

populations of *P. halepensis* in the Mediterranean basin contain genotypes which exhibit exceptional growth performance under sub-optimal conditions compared to other pine species. Other genotypes of interest are hybrids of *P. brutia* x *P. halepensis*, which also exhibit exceptional growth performance compared to their parents, particularly under adverse environmental conditions (Panetsos 1986). In order to maintain sustainable forests under the expected climate changes, it is necessary to artificially select these adapted genotypes for improvement and preservation programs (Alfaro et al. 2014; Fady et al. 2016; Serra-Varela et al. 2017). As pine trees are open-pollinated, each individual possesses a unique genetic makeup that can only be maintained by means of vegetative propagation. Since the potentially selected trees for improvement

and preservation are usually mature, there is a need to improve the rooting of cuttings from mature pine trees.

Studies on rooting of various pine species have been reported since the 50s of the past century, most of which used fascicular shoots (bracyblasts) collected from mother plants kept juvenile by continuous hedging as cuttings (Mitchell et al. 2004; Ragonezi et al. 2010). Pine cuttings originated from fascicular shoots usually rooted better than cuttings originated from normal shoots (Donald 1987). The methods developed in the above studies have been used for mass propagation of various pine species. Nevertheless, the data obtained in these studies, which sometimes also examined cuttings originated from normal shoots, might also be helpful for developing rooting methods for mature cuttings originating from normal shoots.

Several factors were found to affect the rooting ability of pine cuttings: (a) *Genetic background* – several studies on rooting pines demonstrated that the genetic background of the mother plants had a significant effect on the rooting ability (Foster 1990; Greenwood and Weir 1995). Similar results were obtained in rooting experiments of two clones of hybrids of *P. brutia* x *P. halepensis*, of which one clone had a rooting rate close to 100%, while the rooting rate of the second clone was only 25% (Panetsos et al. 1994). Madmony (2000) also found a significant variation in the rooting ability of mature cuttings between various clones of *P. brutia* x *P. halepensis* hybrids. (b) *Developmental stage of the mother plants* – it is well documented that the rooting ability of various pine species, similar to other plant species, decreases significantly upon transition of the mother plants from the juvenile to the mature phase, but the rate of decrease of the rooting ability with the increase in age differs greatly between species (Mitchell et al. 2004; Majada et al. 2011). Most pine species have a high rooting ability before they reach the age of 10 years and sometimes even later, but the rooting rate in the following years decreases rapidly. Studies on the rooting of *P. halepensis* cuttings demonstrated a rapid decrease in the rooting ability upon maturation of the mother plants, so that the rooting ability at the age of 4 years was already very low (Hamburger 1988; J. Riov, personal knowledge). (c) *Cutting collection season* – studies performed with different pine species demonstrated that the rooting ability is relatively high during the dormant season (Foster et al. 2000; Ragonezi et al. 2010), although rooted cutting

were also reported for shoots collected at the end of the summer from one-year-old *Pinus sylvestris* plants (Högberg 2005). In accordance with these observations, artificially induced dormancy by exposing the mother plants to short day and low temperature treatment significantly improved the rooting ability (Whitehill and Schwabe 1975). Similarly, cuttings of mature *P. halepensis* rooted much better in the winter than in the summer (Atzmon, N. and Riov, J., unpub. data). Hamburger (1988), however, reported that cuttings from two-year-old seedlings of *P. halepensis* exhibited relatively high rooting rates in the fall, winter, and late spring. (d) *Cold storage of cuttings before rooting* – pine cuttings stored at 4°C for several weeks exhibited significantly higher rooting rates than cuttings that were rooted immediately after collection (Whitehill and Schwabe 1975). (e) *Auxin treatment* – there is a large number of studies showing improvement of rooting by auxin application in various pine species (Whitehill and Schwabe 1975; Ragonezi et al. 2010; Sedaghat-hoor et al. 2016). These studies included various auxins, such as indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), and 1-naphthaleneacetic acid (NAA), different application methods, such as powder and solution, and for the latter treatment combinations of treatment duration and hormone concentration. Hunt et al. (2011) reported that application of IBA at the beginning of the rooting procedure yielded a higher rooting rate in pine hybrids than its application later during the rooting process. The possible use of auxin conjugates to promote rooting of various plant species has been examined in several studies. Haissig (1979) reported that aryl esters of IAA and IBA were more active than the free auxins in inducing adventitious root formation and development. Other studies reported improved rooting by application of IAA and IBA conjugates, mostly with amino acids. These studies included the examination of the rooting potential of various IBA conjugates in *Prosopis* species (Felker and Clark 1981), IBA-alanine in highbush blueberries (Mihaljevic and Salopek-Sondi 2012) and olive (Epstein and Wiesman 1987), and IBA-aspartate in mung bean (Wiesman et al. 1988, 1989). Van der Krieken et al. (1997) reported that various IAA and IBA conjugates, mostly amide-linked, proved to be highly active in in vitro root induction in various herbaceous and perennial species compared to the free auxins. Early studies reported that phenoxy acids also promoted rooting at relatively low concentrations, whereas at high concentrations they were

phytotoxic (Hitchcock and Zimmerman 1942; Weaver 1972). Therefore, phenoxy acids have not generally been used to improve rooting of cuttings because of their phytotoxicity. The observations that plants are capable of hydrolyzing auxin conjugates and the assumption that phenoxy acid conjugates might be less phytotoxic than the free acids led to the development of phenoxy acid conjugates to improve rooting (Riov 1993). Tel-Zur (1991) and Madmony (2000) demonstrated that the conjugate of 2-(2,4-dichlorophenoxy) propanoic acid with glycine methyl ester (2-DP-Gly) significantly improved rooting of cuttings of various plant species, including *P. pinea* and hybrids of *P. brutia* x *P. halepensis* compared to IBA.

The present study demonstrates the development of an effective rooting procedure, which enabled to obtain high rooting rates of cuttings originated from normal shoots of mature and old pine trees. This rooting procedure is being successfully implemented in improvement and preservation programs of *P. halepensis* and its hybrids.

Materials and methods

Plant material and rooting procedure

Pine cuttings

Cuttings of *P. halepensis* and hybrids of *P. brutia* x *P. halepensis* were prepared from upper shoots, which exhibited vigorous growth, collected during January from mature and old trees of different ages grown in experimental plots or planted forests (as indicated for each experiment in the results). In experiments in which cuttings were collected from several trees, each treatment contained the same number of cuttings from each tree. The shoots were sprayed with 0.2% Daconil fungicide (a.i. 720 g/kg chlorothalonil, Syngenta Crop Protection AG, Basel, Switzerland), and after partial drying were placed in polyethylene bags and stored at 4°C for 4 weeks. Apical cuttings, 15–18 cm long, were excised from the shoots, and the needle fascicles were removed from the basal 5- to 6-cm section of the cuttings. Except when otherwise noted, the base of the cuttings was soaked in a solution containing 5 mg/l of auxin conjugate 2-DP-Gly and 400 mg/l of indole-3-butyric acid potassium salt (K-IBA, Sigma) for 4 h (pulse method). 2-DP-Gly was synthesized as described by Tel-Zur (1991). A stock solution of 2-DP-Gly at a concentration of 10,000 mg/l in *N,N*-dimethyl formamide served for preparation of the

auxin rooting solution. The concentrations of Amistar fungicide (a.i. 250 g/l azoxystrobin, Syngenta) included in the auxin rooting solution in some experiments is related to the commercial product. In one experiment, the cuttings were treated with high concentrations of K-IBA for 10 sec (quick-deep method). Rooting was performed in a cooled and shaded (70% shade) greenhouse. The cuttings were rooted on rooting beds containing vermiculite No. 3:crushed polyethylene (1:1, v/v) medium heated to 25°C, with intermittent mist applied every 8 or 12 min for 10 sec. Each treatment included 16 to 40 cuttings. The rooting rate was recorded 2.5 to 3 months after the initiation of the experiment. Cuttings having several adventitious roots longer than 2 cm were defined as rooted.

Mung bean cuttings

Seeds of mung bean (*Vigna radiata* L.) were imbibed in aerated water for 24 h, and then sowed in vermiculite No. 3 and germinated at 25°C and 16-h photoperiod. Nine-day-old cuttings, each consisted of a terminal bud, two primary leaves, epicotyl, and 4 cm of the hypocotyl, were used. Four cuttings were placed in a 25-ml vial containing tap water or test solution for 24 h, and then replaced with tap water which was renewed every 24 h. Rooting was performed under the environmental conditions mentioned above. The number of adventitious roots longer than 2 mm was determined 9 days after the initiation of the experiment. The data reported are means of four vials, each containing four cuttings.

Growth conditions of rooted cuttings

Rooted cuttings were transplanted into QuickPot trays of 340 cm³ per cell containing Klasmann 602 medium:perlite (80:20, v/v) growing medium plus 2 g/l Osmocote controlled release fertilizer. Following establishment under mist for 14 days, the rooted cuttings were transferred to a greenhouse and grown under 16-h photoperiod to allow a continuous growth by preventing dormancy. About 2 months after transplanting, the rooted cuttings were continuously fertilized through the irrigation system with 7-3-7 NPK fertilizer (Shaphir, Gat Fertilizers Ltd. Kiryat Gat, Israel) at a concentration yielding 120 mg/l pure N in the irrigation water.

Statistical analysis

Results in this study were analyzed using JMP software (SAS Institute, Inc., Cary, North Carolina). Differences

Table 1. Effect of low temperature (4°C) storage duration on the rooting rate of cuttings from 16-year-old *P. halepensis* trees. Cuttings were collected from eight trees growing in a planted plot in Ben Shemen forest located in central Israel. Each treatment included 24 cuttings. The cuttings were treated with the standard rooting solution without Amistar.

Cold storage duration (weeks)	Rooting (%)
0	22
2	62
4	71

between means (three or more) were evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (Tukey–Kramer HSD) test. Comparison between two means was done using Student's *t*-test.

Results

Low temperature storage of cuttings

In order to test the effect of low temperature storage on the rooting ability, cuttings collected from 7-year-old *P. halepensis* trees were stored at 4°C for 0, 2, or 4 weeks prior to rooting. Cold storage for 2 and 4 weeks significantly increased the rooting rate to 62% and 71%, respectively, compared to 22% rooting rate in cuttings that were rooted immediately after collection (Table 1).

Auxin treatment

The study of the effect of auxin treatment on the rooting of mature *P. halepensis* cuttings focused on application of the auxin conjugate 2-DP-Gly. The effect of 2-DP-Gly on the rooting rate with or without IBA was examined by the pulse method. The reason for using a dilute solution for examining the effect of 2-DP-Gly was its low solubility in water. The effect of the above treatments was also compared to that obtained by application of different concentrations of IBA applied by the pulse and the quick-dip methods.

Although application of IBA or the auxin conjugate alone by the pulse method increased the rooting rate, the combination of both auxins yielded the highest rooting rate (Table 2). The rooting rate obtained with a mixture of 5 mg/l conjugate and 400 mg/l IBA was significantly higher than that obtained by the other auxin treatments applied by this method, and this treatment was used as the standard method of auxin application in the following experiments. Application of IBA alone by the quick-dip method also resulted in a relatively

Table 2. Effect of different auxin treatments on rooting of cuttings from 16-year-old *P. halepensis* trees. Cuttings were collected from 12 trees growing in a planted plot in Ben Shemen forest located in central Israel. Each treatment included 24 cuttings. The pulse method was used for all hormone treatments, except for the two treatments with high IBA concentrations, in which the quick-dip method was used. Different letters indicate significant differences between treatments at $P < 0.05$.

Auxin	Concentration (mg/l)	Rooting (%)
Control	0	4 d
2-DP-Gly	5	9 d
2-DP-Gly	10	11 cd
IBA	200	11 cd
IBA	400	19 bcd
2-DP-Gly + IBA	5 + 200	34 ab
2-DP-Gly + IBA	5 + 400	45 a
IBA	10,000	33 ab
IBA	20,000	25 abc

high rooting rate, but it was lower than that obtained with the above standard rooting method.

A similar trend of results was obtained in a similar experiment performed in another year, although the overall rooting rate in this experiment was relatively low (data not presented).

Amistar fungicide application

Rotted cutting bases are often observed during rooting, which might be caused by several factors, such as auxin phytotoxicity and pathogenic agents, namely fungi. This phenomenon was also observed in the experiments performed during the present study. To test the possible involvement of pathogenic fungi, we examined the effect of Amistar, a systemic fungicide, added to the standard rooting solution on the rooting of *P. halepensis* cuttings. The data presented in Figure 1 show that the two concentrations of Amistar examined significantly increased the rooting rate compared to the control. The concentration of 0.01% was used as the standard concentration of Amistar in the following experiments performed with pine cuttings.

The observations that Amistar significantly increased the rooting rate motivated us to examine whether Amistar affects rooting directly, independent of preventing infection by pathogenic fungi. To test this possibility, we examined the effect of a wide range of Amistar concentrations alone on rooting of mung bean cuttings, a common model system for studying rooting of cuttings. Amistar significantly increased the number of roots per cuttings at all concentrations applied, demonstrating an optimum

Figure 1. Effect of Amistar concentration applied with the standard auxin rooting solution on the rooting of *P. halepensis* cuttings from 7-year-old trees. Cuttings were collected from eight trees growing in an experimental plot planted in Volcani Center located in central Israel. Each treatment included 24 cuttings. Different letters indicate significant differences between treatments at $P < 0.05$.

Figure 2. Effect of different concentrations of Amistar alone on rooting of mung bean cuttings, defined as the number of roots per cutting. Data are means $\pm$ SE ($n = 4$).

response curve (Fig. 2), which is typical to biological effects. The highest effect, about 2.5-fold increase in the number of adventitious roots compared to the control, was obtained with 0.01% concentration, which was also effective in rooting of *P. halepensis* cuttings (Fig. 1).

The stimulatory effect of Amistar on rooting was further demonstrated in rooting of hybrids of *P. brutia* $\times$ *P. halepensis* (Madmony et al. 2003). Amistar, at 0.01% concentration added to the standard auxin solution, increased the rooting rate of all hybrids, in four of which

Table 3. Effect of 0.01% Amistar on rooting of cuttings from mature natural hybrids of *P. brutia* $\times$ *P. halepensis*. Cuttings were collected from the original hybrid trees, i.e. one tree of each hybrid, which were identified by genetic markers. The hybrids are growing in various planted forests located in the semi-arid region of Israel, except for Bakoa 1 which is growing in a forest located in central Israel. Each treatment included 16 cuttings. Amistar was applied with the standard auxin rooting solution. Different letters indicate a significant difference between treatments for each hybrid separately at $P < 0.05$.

Hybrid	Tree age (years)	Rooting (%)	
		– Amistar	+ Amistar
Gilat 1	25	41.9 b	58.7 a
Shacharia 1	18	21.7 b	67.4 a
Bakoa 7	25	20.2 b	46.8 a
Yatir 5	22	43.6 b	62.5 a
Yatir 10	22	24.7 a	36.7 a

Table 4. Variations in size and rooting ability of selected genotypes from a 20-year-old even-aged stand of *P. halepensis* in the semi-arid Yatir forest. Trees were selected based on their phenotype. The data for each tree size are of the three largest and the three smallest trees in the plot. Different lower case letters indicate significant differences between means at $P < 0.05$.

	Mother plant			Clones	
	Phenotype	Height (m)	DBH (cm)	Rooting rate (%)	DBH (mm) Height (cm)
Large		8 $\pm$ 0.0 a	20.3 $\pm$ 2.7 a	13.3 $\pm$ 0.8 a	9.67 $\pm$ 0.7 a 59.8 $\pm$ 5.0 a
Small		3 $\pm$ 0.6 b	4.60 $\pm$ 0.4 b	35.0 $\pm$ 3.8 b	11.64 $\pm$ 0.4 b 73.8 $\pm$ 3.7 b

the differences in the rooting rate compared to the control were significant (Table 3). In Bekoa 7 and Shacharia 1 clones, Amistar increased the rooting rate by about 2- and 3-fold, respectively, compared to the control.

Rooting selected genotypes from a semi-arid forest

The rooting method developed was utilized for vegetative propagation of superior forest trees with resilience against drought stress. For this purpose, we selected various genotypes from a 20-year-old even-aged stand of planted *P. halepensis* in the semi-arid Yatir forest in Israel (31°31'N, 35°06'E). The high phenotypic variation in the stand allowed selecting genotypes based on tree size. The largest three genotypes and the smallest three genotypes, which were selected for further examination, differed greatly in their height and diameter at breast height (DBH) (Table 4). Following cold treatment for 4 weeks, 40 cuttings from each genotype were rooted by the standard rooting method.

Cuttings from the small trees exhibited a significantly higher rooting rate than those from the large trees (Table 4). These rooted cutting were grown in the greenhouse for 2 years, and then their height and DBH were measured. There was a uniform growth pattern within genotypes. Prominently, all rooted cuttings

Figure 3. Intra-clonal growth pattern uniformity of 2-year-old clones. Clones from the same genotype exhibited a similar growth pattern. Lines T16, T10, and T1 are from large trees, and lines T6, T22, and T3 are from small trees. Each panel represents 5-6 clones from one genotype. Bars = 10 cm.

originated from the same genotype exhibited a similar growth pattern (Fig. 3). There were significant differences between genotypes in rooted cuttings height and DBH (Fig. 4A-B). Significant differences were also observed for the two mother tree types. Rooted cuttings originated from large trees exhibited significant smaller dimensions than those originated from small trees (Fig. 4C-D, Table 4).

Rooting old genotypes from peripheral population of *P. halepensis*

To examine whether the rooting method developed is effective for vegetative propagation for an old tree preservation program, cuttings were collected from

old trees of a native peripheral population on Mount Carmel in Israel (32°46'N, 34°59'E). The trees in this population are more than 100 years old, as estimated based on old maps (Conder and Kitchener 1880). It has been shown that these populations are under genetic homogenization process due to massive afforestation activities in the area (Steinitz et al. 2012). In efforts to preserve some of these natural unique trees, 40 cuttings from each of the 19 largest trees in this population were rooted by the standard method. Rooted cuttings were obtained in 15 of the 19 genotypes examined, with an average of 3.2 ± 0.5 rooted cuttings per genotype. Following acclimation and 2 years growth in a greenhouse, the rooted cuttings were planted in a preservation plot.

Figure 4. Variation of growth parameters among clones of the two size-type trees originated from selected 20-year-old genotypes growing in the semi-arid Yatir forest. A, Stem diameter variation among genotypes (inter-clonal). B, Stem height variation among genotypes. Bars represent the mean value of 4–12 clones from each genotype ($\pm$ SE). Different letters above bars represent significant differences between means ($P < 0.05$, one-way ANOVA followed by Tukey–Kramer HSD). Lines T16, T10, and T1 are from large trees, and lines T6, T22, and T3 are from small trees. C, Stem diameter variation between tree types. D, Tree height variation between tree types. Bars represent the mean value of 17 clones originated from large genotypes and 31 clones originated from small genotypes ($\pm$ SE). Different letters represent significant differences between means at $P < 0.05$ according to student *t*-test.

Discussion

The present research led to the development of an effective procedure to root mature cuttings of *P. halepensis* and hybrids of *P. brutia* $\times$ *P. halepensis*. This method allows to carrying out improvement programs, which are based on selection of mature successful phenotypes, and preservation of unique trees or populations. To the best of our knowledge, the current study is the first to report on successful rooting of cuttings from mature and old pine trees. Previous studies, which examined rooting capability in pine cuttings, demonstrated success of rooting mostly of cuttings originated from seedlings or from young trees aged up to 4–5 years (Whitehill and Schwabe 1975; Hamburger 1988; Hamann 1998; Mitchell et al. 2004; Majada et al. 2011). The rooting ability of mature *P. halepensis* was

observed to be very low (Hamburger 1988; J. Riov, personal knowledge). The rooting procedure developed in the present study is based on previous knowledge on rooting pine cuttings, including *P. halepensis*, and the application of two new rooting promoting substances, which have not been tested before with *P. halepensis* cuttings.

In order to obtain a reasonable rooting rate of mature *P. halepensis* cuttings and its hybrids, the following previously known conditions have to be implemented: 1. Cutting collection season - mature cuttings have to be collected only during the dormant season (N, Atzmon and J. Riov, unpubl. data). The cutting collection season has been demonstrated to be an important factor in rooting of cuttings, and plant species differ significantly in the preferable season for cutting collection

(Hartman et al. 2014); 2. Cutting type – cuttings have to be prepared from normal shoots collected at the upper part of the tree. There are two reasons for using upper shoots for cuttings, although developmentally they are more mature than lower shoots. Firstly, upper shoots exhibit a much higher annual growth rate than lower shoots. Besides the transition from the juvenile phase to the mature phase with increasing age, trees undergo a second process named aging (Kozłowski 1971). This process is expressed in a reduced annual shoot growth rate that starts at the base of the tree and continues upward. Therefore, the upper shoots exhibit a relatively vigorous growth, which ensures a vigorous orthotropic growth of the rooted cuttings. Secondly, male cones, which often rot during rooting and later on might inhibit the development of rooted cuttings by damaging the apical bud, do not appear on the upper shoots. Upper shoots might have female cones, but they do not interfere with rooting nor with the development of the rooted cuttings; 3. Cold storage of the cuttings before rooting – cold storage of *P. halepensis* cuttings significantly increased the rooting rate (Table 1), similarly to the observations in other pine species (Whitehill and Schwabe 1975). A study with chestnut cuttings suggests that cold storage reduces the level of rooting inhibitors (Gesto et al. 1981), but additional research is required to determine whether this is the common mechanism of the stimulatory effect of cold storage.

The successful development of an efficient rooting procedure for mature cuttings of *P. halepensis* and its hybrids can be also related to the use of two promoting substances, 2-DP-Gly and Amistar, combined with the above factors. As an auxin source, 2-DP-Gly has two advantages. Firstly, being a conjugate it acts as a slow release of auxin. This means that the cuttings are continuously exposed to active auxin during rooting. There are data indicating that prolonged exposure to auxin during rooting is beneficial (Blythe et al. 2007). For this reason various auxin conjugates, as described in the introduction, were more active than the free auxins in inducing rooting. Secondly, phenoxy acids, and particularly 2-DP, were shown to have a higher promoting effect in inducing rooting than IBA, the common auxin used to promote rooting (Hitchcock and Zimmerman 1942; Weaver 1972). This was also demonstrated in the present study by the observations that relatively very low concentrations of 2-DP-Gly were required to stimulate rooting. A combination of IBA, which provides the auxin required for the initial

stages of rooting, and 2-DP-Gly, which provides the auxin required for the later stages of rooting, proved to be more effective than each auxin alone (Table 2), as also reported for combinations of various other auxins applied to promote rooting (Blythe et al. 2007).

Adding the fungicide Amistar to the standard rooting solution significantly increased the rooting rate (Fig. 1, Table 3), an effect which was not reported before for this substance. The active ingredient in Amistar is azoxystrobin, which controls fungi by its uncoupling activity. The ability of uncouplers to stimulate rooting was demonstrated long ago. Nanda and Dhawan (1976) and Nanda et al. (1978) reported that 2,4-dinitrophenol (DNP) stimulated rooting of *Phaseolus mungo* cuttings, with maximum activity obtained in the presence of IAA and sucrose. Since various phenols, the chemical group to which DNP belongs, inhibit IAA metabolism (Lee 1980 and literature cited therein), it was assumed that DNP stimulated rooting by keeping a higher level of IAA in the treated tissues. However, the above authors demonstrated that non-phenolic uncouplers also stimulated rooting, ruling out this possibility. These results were later approved by Riov and Yang (1989), who reported that DNP alone stimulated rooting of mung bean cuttings. The data obtained in these studies indicate that uncouplers stimulate rooting by the increased respiration occurring in the treated plants as a result of their inhibitory effect on ATP biosynthesis, but the mechanism involved has not yet been investigated. Based on the data of Nithyameenakshi et al. (2006), it can be concluded that the concentrations of Amistar used in the present study to stimulate rooting are not phytotoxic, which agrees with our observations that no phytotoxic symptoms were observed in the treated cuttings during the present study.

The clones that were produced from the Yatir forest in this study displayed significant inter-clonal variation, mainly for stem diameter (Figs. 3 and 4A-B). In agreement with previous studies (Frampton et al. 2000; Isik et al. 2004; Emhart et al. 2007), growth uniformity was evident within clones (intra-clonal) originating from the same genetic stock. Emhart et al. (2007) showed growth differences in crown architectural traits between various genotypes of slash pine that were selected due to their superior growth potential, however the intra-clonal variation was low. Similarly, clones of loblolly pine demonstrated similar growth parameters to seedlings from the same full-sib crosses in the field (Frampton et al. 2000). These results suggest that vegetative propagation using rooted cuttings delivers

intrinsic above-ground, and most likely also below-ground growth parameters of the mother trees, indicating that this propagation approach might be suitable for forest plantings. This view is supported by studies conducted with various pine species, which demonstrated that rooted cuttings had a similar (Frampton et al. 2000) or improved (Lu et al. 2012) field performance compared to seedlings. Growth rate is genetically determined, but in trees growing under suboptimal environmental conditions, tree growth is significantly affected by the environment. In the current study, we studied trees that are growing in the semi-arid Yatir forest, where climate conditions are considered suboptimal for *P. halepensis* (Schiller and Atzmon 2009). Trees at this forest demonstrate a high phenotypic variation due to the significant effect of the environment on overall growth. It is assumed that vigorously growing trees are better adapted to semi-arid conditions than poorly growing trees. By using the vegetative propagation method that was developed in the current study, we observed that the rooted cuttings exhibited contradictory growth parameters to their mother tree as observed in the forest. That is, clones from large-type trees were significantly smaller than clones from small-type trees (Fig. 4C-D). Although this difference is unexplained, we speculate that the two types differ in their intrinsic growth rate. The small-type trees from the forest demonstrate their full growth capacity under optimal conditions in the greenhouse, and thus grow fast, while under drought conditions they often experience drought stress, which leads to growth cessation and thus they remain small. The large-type trees, on the other hand, display a slow growth habit, and thus experience less stress under limited water conditions, which allows a continuous growth. Despite these clear differences, further study is needed to unravel the genotype by environment interaction (G×E) of these trees (White et al. 2007). Nevertheless, based on various observations it seems that genetic slow-growth rate is an advantage under semi-arid climate conditions. Surprisingly, the two contrasting tree types that were selected based on their size demonstrated different rooting ability, with the small-type exhibiting a higher rooting rate than the large-type. This difference might be an outcome of unbalanced hormonal status in the mother trees, that is probably affected by the abiotic stress that the harsh environment at the Yatir forest provides. It might be interesting to investigate whether the small-type trees undergo a temporary transition to juvenility while under drought stress.

Conclusion

The vegetative propagation method for rooting of cuttings from mature and old *P. halepensis* trees and its hybrids that was developed in this study is of a great importance, as this is the only way that enables to utilize superior genotypes for forest plantings or preserve unique trees from native or planted populations. Superior genotypes in mature planted pine forests are selected based on their tolerance to biotic and abiotic cues after they have reached their full growth potential. Native genetic resources, which are important for their unique genetic makeup, are currently under threat of gene flow contamination from afforestation, and of climate change effects (such as drought and fires). It is now possible to use both genetic resources for forest program plantations.

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