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Comparison of Morphological and Physiological Traits between *Pinus brutia*, *Pinus halepensis*, and Their Vigorous F1 Hybrids

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Abstract: Hybrids between *Pinus brutia* Ten. and *Pinus halepensis* Mill. have mainly been investigated with respect to their growth rate. Less attention has been paid to factors that contribute to their resistance to drought, although the hybrids have been shown to thrive over *P. brutia* in semi-arid regions. To understand the superior hybrid phenotype, we investigated morphological traits, growth parameters, and physiological response to drought stress in *P. brutia*, *P. halepensis*, and their vigorous hybrids. Rooted cuttings were propagated from mature trees and were examined in a high-throughput gravimetric system to evaluate their response to drought. Higher root-to-shoot ratio, needle length, and leaf mass per area (LMA) were observed in the hybrids compared to the two parental species. *P. halepensis* demonstrated higher transpiration rates (E) and stomatal conductance (g_{sc}) than *P. brutia*, and an earlier reduction in E and g_{sc} in response to drought stress. Similar to *P. brutia*, the hybrids showed low E and g_{sc}, and an early stomatal closure in response to drought similar to *P. halepensis*. Our study suggests that the hybrids exhibit a unique combination of traits that may contribute to forest resilience in semi-arid regions undergoing climate change.

Keywords: *Pinus halepensis*; *Pinus brutia*; hybrids; drought; morphological traits; physiological traits

1. Introduction

The Mediterranean region is facing increased aridity and desertification according to the forecasts of global climate change [1,2]. Increased forest tree mortality is predicted to occur because of reduced precipitation and shorter winter rainy seasons [3,4]. Accordingly, drought-resistant trees are essential for sustainable afforestation and reforestation, especially in semi-arid regions. Traits related to drought adaptation are often expressed in semi-arid regions. Different tree species exhibit different growth rates and vigor [5]. It is expected that trees that grow more rapidly under water stress have morphological and physiological traits to support their rapid growth, and thus may be more resistant to drought.

Morphological traits, such as needle length, leaf mass per area (LMA), and root-to-shoot ratio, are known to be related to water availability and drought coping mechanisms [6–10]. Trees that grow under high water availability have longer needles [6,7], a larger needle cross section area [7], a larger crown, and a higher foliage density [6]. Changes in LMA occur in response to drought stress [8,9]. LMA is a proxy for plant response to drought, and it is widely used in plant ecology and forestry studies [11]. Several studies found that species with a high LMA often occur in areas with low rainfall, high temperature, and high radiation [12–14]. Plants with a high LMA have a higher nitrogen content per surface area, and as a result they potentially have a higher photosynthetic capacity [15]. Allocation of biomass between roots and shoots is a key plant-adaptive strategy, differing between species, physiological stages, and ecological environments [10]. Extensive deep

root growth, which enables increased water supply under water-limiting conditions, may be crucial for surviving the drought season [16–19].

The rate of plant growth is influenced by morphological and physiological traits. A decline in growth is a well-known response to drought [20,21]. A high relative growth rate (RGR) under water-limited conditions is a known adaptive trait in Mediterranean species that usually results in an extensive root system [22–24]. However, a low RGR may also imply resistance to drought [25–27], and may result from allocating more resources to below-ground growth than to above-ground growth [28] or as a result of conservative water use strategy [29–31].

Among the physiological changes that occur in response to a changing environment, stomatal conductance (g_{sc}) and transpiration rate (E) were shown to be indicators of drought response [32–35]. These parameters allow the estimation of the gas exchange between the plant and the atmosphere. Gas exchange parameters and weight change are used to calculate the water use efficiency (WUE), which is a proxy for the amount of water used for growth. Fluctuations in stomatal conductance occur following the opening or closure of the stomata. Open stomata allow high photosynthesis and transpiration. A high transpiration rate results from a high atmospheric demand and a high water availability. The plant starts to sense drought stress when the root water influx is lower than the leaf water outflux [36,37]. At this stage, a balance between water loss and the need for photosynthesis is achieved by regulating the stomatal aperture. Plant species vary in strategies to regulate the internal water balance at the expense of carbohydrate assimilation [30,38–42]. However, all plants reach a physiological stress point (θ) at which the plant starts closing stomata to minimize water loss. The physiological stress point is expressed by the soil water content value, which limits transpiration [37,38,43]. Water-saver species prevent damage by stomatal closure before any change in leaf water potential takes place, whereas water-spender species exhibit a decrease in leaf water potential, which is compensated by increased water uptake from the soil to compensate for the water loss [29].

Adaptation to drought through genetic change is a slow process that may be inadequate for coping with the speed and magnitude of climate change [44]. However, interspecific hybrids have been postulated to adapt to a wide range of environments due to their greater heterozygosity and their potential ability to buffer different kinds of environmental stressors [45]. Therefore, hybrids have the potential of playing a role in adaptation to the climate change [46,47].

Pinus brutia Ten. and *Pinus halepensis* Mill. are the two dominant Mediterranean conifers that are extensively used for afforestation in water limited environments [48]. The two species were planted throughout Israel over the second half of the previous century [49,50]. It was observed that *P. brutia* × *P. halepensis* hybrids occur in some *P. brutia* plantations [49–52]. In a previous study, we reported that the hybrid vigor was greater than that of *P. brutia*, and that this phenomenon was more prominent in the semi-arid region than in the sub-humid region of Israel [52]. Therefore, we hypothesized that these hybrids possess distinguishing traits allowing them to cope with drought better than their parental species. To test this hypothesis, we examined various morphological and physiological traits of rooted cuttings of *P. brutia*, *P. halepensis*, and their hybrids grown under controlled environments to identify factors related to drought resistance.

2. Materials and Methods

2.1. Plant Material and Vegetative Propagation

Verified 6- to 8-year-old mother trees were selected for vegetative propagation. Shoots were harvested in January 2019 and were used for vegetative propagation by rooted cuttings as described by Riov et al. [53]. Briefly, apical cuttings 15- to 20-cm-long were collected from the upper part of the trees and stored at 4 °C under humid conditions. After four weeks, the base of the cuttings was dipped in a rooting solution containing 5 ppm of 2-(2,4dichlorophenoxy)propionic acid-glycine methyl ester conjugate, 400 ppm of indole-3-butyric acid (IBA), and 0.025% Amistar fungicide (Syngenta) for 4 h. The cuttings were

then placed in a rooting bed that contained vermiculite No. 3: styrofoam (1:1, *v/v*) medium heated to 25 °C, with intermittent mist applied every 12 min for 10 sec. Rooted cuttings were obtained after 6–8 weeks and transferred into separate small pots. We propagated 6 *P. brutia* trees, 6 *P. halepensis* trees, and 4 F1 vigorous hybrid trees, and from each mother tree we had 5–8 cuttings for replications. Trees were tested to verify their genotypes using markers that were developed in our laboratory [52]. All cuttings were grown in 3.9 L pots filled with a growing medium of peat: perlite (80:20, *v/v*) containing 1.5 g/L Osmocote slow-release fertilizer (Even Ari Green, Beit-Elazari, Israel). The cuttings were grown for 18 months under optimal conditions before being subjected to the drought experiment. Height and stem basal diameter were measured once a month.

2.2. Morphological Traits and Anatomy

Samples for plant, root, and needle dry weight (DW) were oven dried for 48 h at 65 °C before weighing.

Leaf mass per area (LMA) was determined by collecting 10 fresh needles from each plant that were scanned for surface area and then oven dried for 48 h and weighed [54].

Stem volume was calculated using the formula V = 0.428753 $d^{2.054628}$ $h^{0.843735}$, in which *d* = diameter basal area and *h* = height [55].

Relative growth rate (RGR) was calculated from the difference in stem volume (*V*) between March 2020 (t_1) and September 2020 (t_2), according to the following equation: $RGR = \frac{lnV_2 - lnV_1}{t_2 - t_1}$.

Xylem cross-sectional area was measured in 2-cm-long stem segments sampled from each rooted cutting. The segments were then fixed and sectioned transversely using a sliding microtome SM2010R (Leica Biosystems, Deer Park, IL, USA). Cross sections of 50 μm thickness were stained with 0.1% toluidine blue O for 30 sec and then washed with distilled water.

Needle dimensions were evaluated by anatomical analysis. Fresh needles were first fixed in a solution containing 3.7% formaldehyde, 5% glacial acetic acid, 50% ethanol, and water (FAA) overnight, and then dehydrated by increasing ethanol concentrations (70%, 80%, 90%, and 100%, 30 min each), followed by increasing Histoclear solutions (25%, 50%, 75%, 100%, 60 min each) (Kaltek, K-clear) before embedding in paraffin. Cross sections were obtained by using a Microtome RM2245 (Leica Biosystems, Deer Park, IL, USA), and stained with safranin and fast green [56].

The sectioned material (xylem and needles) was examined using a NIKON Eclipse Ni microscope, and digital images were taken using a Nikon DS-Ri2 camera (Tokyo, Japan). All image measurements, needle length, leaf surface area, needle cross-sectional area, and tracheid lumen area were made using IMAGEJ (http://rsb.info.nih.gov/ij accessed on 15 November 2020).

2.3. Drought Experiment

The plants (18-month-old rooted cuttings) were acclimatized in the experimental greenhouse for one month prior to the drought experiment and measured for height and stem basal diameter. To determine the fresh weight (FW) of the plants entering the experiment, we calculated the correlation between the stem basal area and the total plant FW. The FW was measured in plants representing the two parental species and their hybrids (25 plants in total). The resulting equation (R^2 = 0.6, y = 399.63x + 21.62) was then used to calculate the initial FW of each plant in the drought experiment.

The drought experiment included 36 plants: six different *P. halepensis* genotypes, six different *P. brutia* genotypes, and 24 plants representing four different hybrids (six rooted cuttings of each hybrid). The drought experiment was carried out for 45 days, starting at the beginning of September 2020 in a greenhouse of the iCORE Center, Faculty of Agriculture, Rehovot, Israel [37,43,57,58]. During the first 7 days, the plants were watered every day at 4:00 am up to field capacity, which corresponded to 60% soil water content (SWC). The drought treatment was initiated on the eight day and continued for the following

three weeks, during which the water supply was gradually reduced. There was a significant difference in the plant weights at the beginning of the experiment (381–775 gm), with some plants having twice the weight of the others. *P. brutia* plants had the highest weight, and the hybrids had the lowest weight. To enable a standard drought treatment (i.e., a similar drying rate for all plants), drought scenarios were automatically controlled via the Plantarray's system's feedback-irrigation controller (Plantarray, PA 3.0, PlantDitech Ltd., Yavne, Israel) [59]. The plants were irrigated individually each day with a water content equal to the daily water loss through transpiration minus 100 mL. After three weeks of the drought treatment, when the SWC reached 5%, irrigation was resumed to full field capacity, and the plants were allowed to recover for the following 17 days, i.e., up to the end of the experiment.

2.4. Physiological Measurements

Using the functional telemetric platform, comprised of weighing lysimeters and soil and atmospheric sensors (Plantarray, PA 3.0, PlantDitech Ltd., Yavne, Israel), we continuously monitored the plant water balance by controlled tracking and measurement of the transpiration as described in Dalal et al. 2020 [57]. The plants were grown in the experimental greenhouse under semi-controlled conditions (natural daylight and a ventilating system) from the beginning of September 2020 up to mid-October 2020. The growing medium surface was covered with thin white plastic to minimize water loss from the medium. The weight of each pot was monitored every 30 s, and the average weight was calculated for each 3 min period and logged to a continuous data recorder. The data were processed using the SPAC analytics software embedded in the Plantarray system to calculate the whole-plant transpiration rate (E), the whole-canopy stomatal conductance (g_{sc}), water-use efficiency (WUE), and the physiological critical stress point (θ) for each plant, which is defined as the SWC threshold at which the plant starts to assess stress and begins to close its stomata [37,57,59–61]. Temperature, ambient radiance, and vapor pressure deficit (VPD) in the greenhouse were continuously recorded. All data analyses of E and g_{sc} were based on data collected at midday between 12:00 and 14:00, when the VPD was constant, and the solar radiation was at maximum. At the end of the experiment, the height, stem basal diameter, and the DW of the plant roots, stem, and needles were measured.

Predawn leaf water potential (Ψ_{PD}) during the experiment was measured by a Scholander-type pressure chamber (PMS, Corvallis, OR, USA) in order to relate the SWC to the Ψ_{PD}. Each data point plotted was from an average of three plants of the same source. Ψ_{PD} measurements began immediately after sample collection and was completed within 60 min. Samples were taken twice a week during drought stress from all plants.

2.5. Statistical Analysis

The data of the morphological and the physiological traits of *P. halepensis*, *P. brutia*, and hybrids were analyzed using one-way ANOVA. The normal distribution of errors was calculated using the Shapiro–Wilk W statistic test. For the homogeneity of variance, Levene's and Bartlett's tests were applied [62]. The assumptions of ANOVA were met. The Tukey–Kramer HSD test was used for post-hoc comparisons. To analyze the slopes and intercepts between Ψ_{PD} and SWC, the Ψ_{PD} measurements were converted to absolute values, then plotted logarithmically and analyzed using one-way ANOVA.

3. Results

3.1. Hybrids Exhibit Different Morphological and Growth Parameters from P. halepensis and P. brutia

RGR and DW of plants, branches, and needles of *P. brutia* and *P. halepensis* were significantly higher than those of the hybrids (Table 1). However, root-to-shoot ratio of the hybrids was higher than that of *P. brutia* and *P. halepensis*, but this difference was only significant for the hybrids versus *P. halepensis*. Hybrid needles were longer by 35% and 69% than those of *P. brutia* and *P. halepensis*, respectively. In addition, leaf surface area,

needle cross section area, calculated needle volume, and LMA were significantly higher in the hybrids than in *P. halepensis*. *P. brutia* demonstrated intermediate, but not significantly different, values of needle surface area and needle cross section area compared to those of the hybrids and *P. halepensis*. No significant differences were found between the two species and the hybrids in terms of tracheid lumen area and coefficient of variation (CV), and the percentage of mesophyll area in the needle cross sections. WUE, calculated from the gravimetric system data (collected prior to the imposed drought stress), was significantly lower in the hybrids compared to those in *P. brutia* and *P. halepensis*.

Table 1. One-way ANOVA of the effect of *P. halepensis*, *P. brutia*, and their hybrids on morphological and growth parameters and on WUE. Data are the means of six plants of each of *P. halepensis* and *P. brutia*, and four hybrids $\pm$ standard error. Different superscript letters indicate significant differences between the various genotypes ($p < 0.05$).

	R2	*p* Value	*P. halepensis*	*P. brutia*	Hybrid
RGR (cm^3/day)	0.37	0.0186	0.014 [a] $\pm$ 0.001	0.014 [a] $\pm$ 0.001	0.011 [b] $\pm$ 0.001
Total plant DW (gr)	0.58	0.0033	203 [a] $\pm$ 19	236 [a] $\pm$ 19	107 [b] $\pm$ 24
Total branch DW (gr)	0.57	0.0038	73 [a] $\pm$ 8.2	74 [a] $\pm$ 8.2	24.6 [b] $\pm$ 10
Root/Shoot ratio	0.38	0.0464	0.28 [b] $\pm$ 0.034	0.3 [ab] $\pm$ 0.03	0.4 [a] $\pm$ 0.034
Total needles DW (gr)	0.50	0.0112	87 [ab] $\pm$ 10	110 [a] $\pm$ 10	52 [b] $\pm$ 12
Needle length (cm)	0.92	0.0002	16 [c] $\pm$ 0.97	20 [b] $\pm$ 0.97	27 [a] $\pm$ 0.84
Needle average area (cm^2)	0.58	0.0462	2 [b] $\pm$ 0.43	3.22 [ab] $\pm$ 0.43	3.76 [a] $\pm$ 0.37
Needle cross section (mm^2)	0.63	0.0300	0.38 [b] $\pm$ 0.11	0.57 [ab] $\pm$ 0.11	0.86 [a] $\pm$ 0.09
Average needle volume (mm^3)	0.84	0.0016	55 [b] $\pm$ 24	89 [b] $\pm$ 24	233 [a] $\pm$ 21
Needle LMA (gr/m^2)	0.84	0.0014	145 [c] $\pm$ 11	183 [b] $\pm$ 11	235 [a] $\pm$ 9.6
Tracheid lumen area (μm^2)	0.32	0.2548	180 [a] $\pm$ 20	220 [a] $\pm$ 20	177 [a] $\pm$ 17
Tracheid lumen area CV	0.12	0.6200	26.3 [a] $\pm$ 3	28.5 [a] $\pm$ 3	30.4 [a] $\pm$ 2.6
% Mesophyll area in needle cross section	0.05	0.8247	0.72 [a] $\pm$ 0.02	0.73 [a] $\pm$ 0.02	0.71 [a] $\pm$ 0.01
WUE	0.42	0.0371	0.030 [a] $\pm$ 0.003	0.032 [a] $\pm$ 0.001	0.019 [b] $\pm$ 0.002

RGR, relative growth rate; DW, dry weight; LMA, leaf mass per area. CV, coefficient of variation; WUE, water use efficiency.

3.2. Physiological Parameters in Response to Drought

During the drought experiment, the maximum daily VPD fluctuated between 1.76 and 3.85 kPa, and the maximum daily radiation fluctuated between 895 and 1150 μmol/m^2/s (Figure 1a). At the beginning of the experiment, when SWC was about 60% (watering to field capacity) (Figure 1b), *P. halepensis* and *P. brutia* had a significantly higher daily transpiration rate than that of the hybrids (337, 367, and 190 g of water, respectively, Figure 1c). Under drought stress, *P. halepensis* lost water more rapidly (25.5 g/day) than *P. brutia* (19.4 g/day), whereas the hybrids lost the least water per day (9.6 g/day). At the beginning of the experiment, *P. halepensis* demonstrated significantly higher E (Figure 1d) and g$_{sc}$ (Figure 1e) than those of *P. brutia* and the hybrids. However, during the drought stress, the decline in both parameters started earlier and was steeper in *P. halepensis* (slopes were −0.0001 and −0.006 for E and g$_{sc}$, respectively) than in *P. brutia* (slopes were −0.00005 and −0.0032 for E and g$_{sc}$, respectively) and the hybrids (slopes were −0.00004 and −0.0034 for E and g$_{sc}$, respectively). *P. brutia* and the hybrids showed similar behavior in these two parameters during the experiment. *P. halepensis* reached the minimum values of E and g$_{sc}$ on the 20th day of the experiment (after 14 days of reduced watering), whereas *P. brutia* and the hybrids reached the minimum values of E and g$_{sc}$ six days later, on the 26th day of the experiment. After 16 days of reduced irrigation, the SWC reached approximately 12%. All plants reached minimum E and g$_{sc}$ values (0.0001 and 0.004 g$_{water}$/g$_{plant}$/min, respectively). Irrigation was resumed on day 29 for 17 days (Figure 1b). At the end of the recovery phase (day 45), the SWC was 55%, which is 91% of the initial value, while E was 41% of the initial values for *P. halepensis* and 54% for the hybrids and *P. brutia*.

The g_{sc} values were 20%, 29%, and 37% of the initial ones for *P. halepensis*, hybrids, and *P. brutia*, respectively.
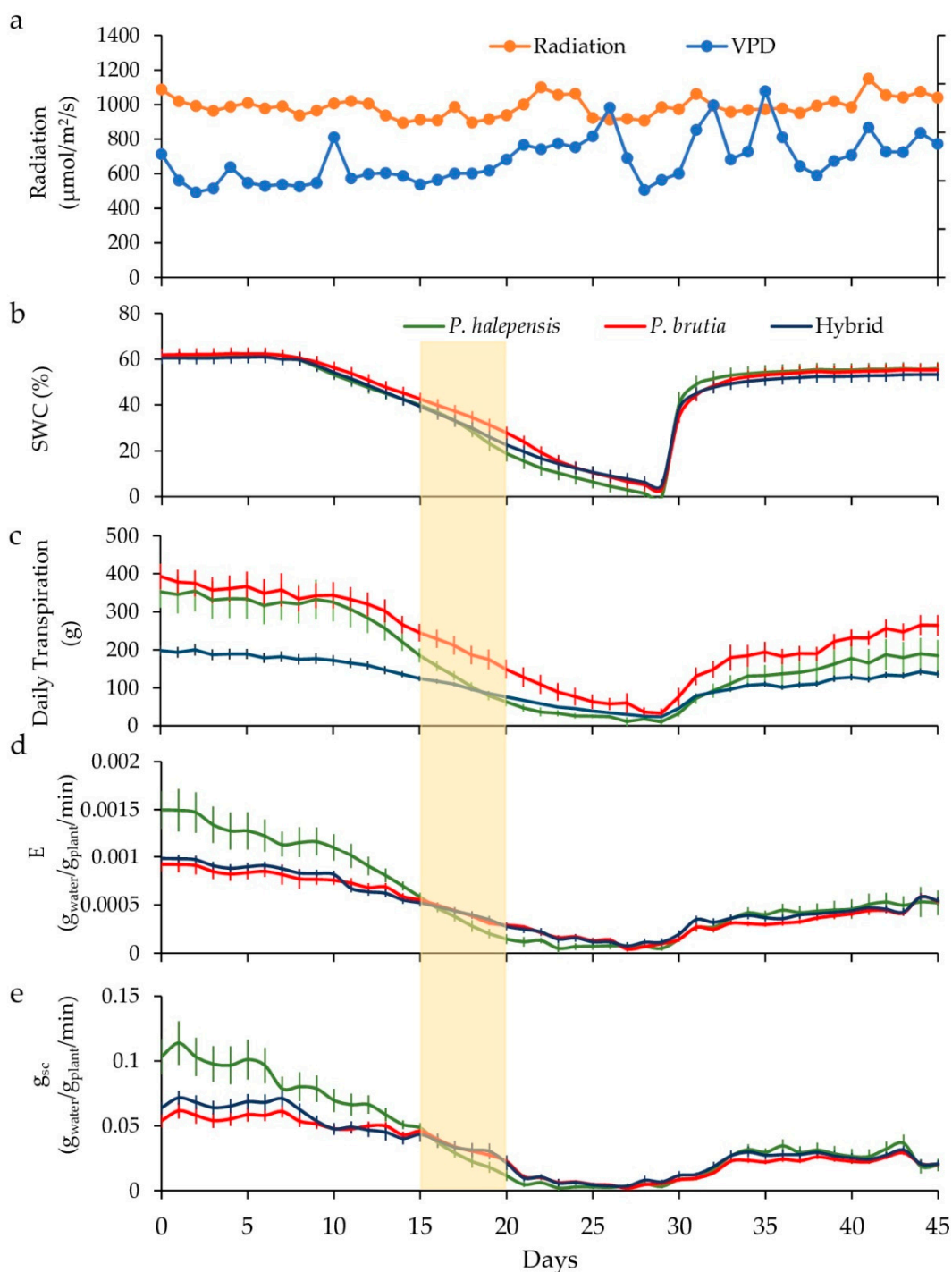

Figure 1. Environmental conditions during the drought experiment and the responses of *P. halepensis* (green), *P. brutia* (red), and their hybrids (blue) to drought stress during the 45 days of the experiment. (**a**) Maximum daily solar radiation and VPD, (**b**) soil water content (SWC), (**c**) daily transpiration, (**d**) midday whole-plant transpiration rate (E), (**e**) midday whole canopy stomatal conductance (g_{sc}). The yellow column marks the start of the reduction in the leaf water potential (Ψ_{PD}) (Figure 2).
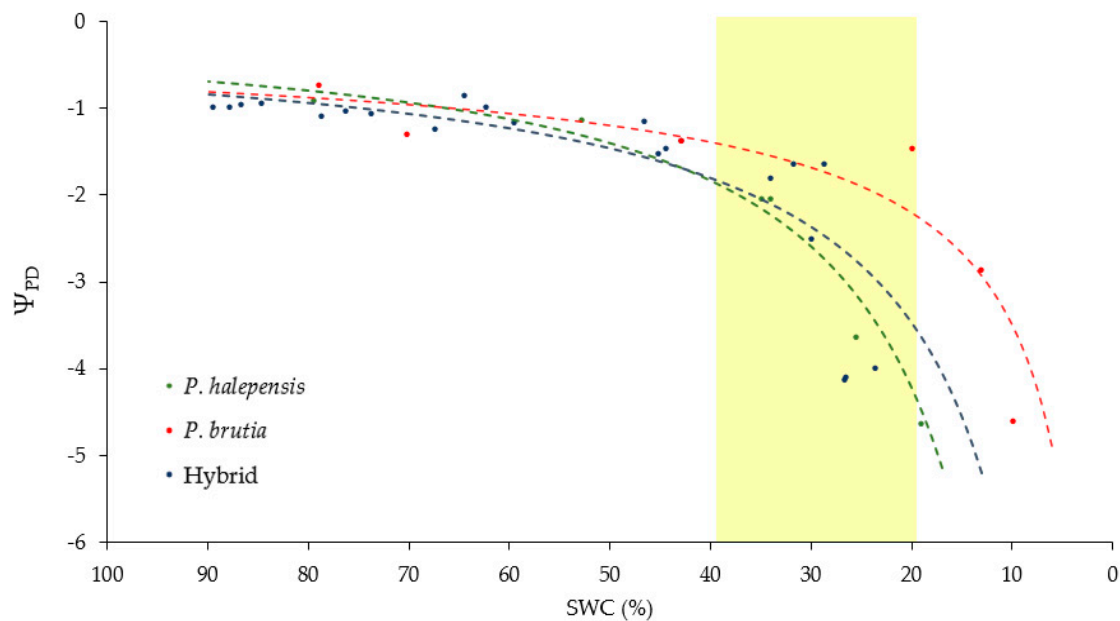
Figure 2. The relationship between soil water content (SWC) and plant predawn leaf water potential (Ψ_{PD}). The yellow region marks the range in which the plants started to reduce their leaf water potential. Each dot on the graph is an average of measurement of three different branches.

The relationship between SWC and Ψ_{PD}, revealed a decline in the Ψ_{PD} when the SWC was below 38% for *P. halepensis* and the hybrids, whereas in *P. brutia* the Ψ_{PD} declined at 20% SWC (see regression lines in Figure 2). These results were in agreement with the SWC measurements in the lysimetric system (Figure 1), in which E and g_{sc} declined when the SWC decreased below 40%. The same trend was observed for the critical stress point (θ) (Figure 3). In the hybrids, the θ was not significantly different from that of *P. halepensis* (44% and 37% SWC, respectively), whereas *P. brutia* had a significantly lower θ (30% SWC,). To examine whether the differences in Ψ_{PD} under conditions of low SWC were significant between *P. brutia* on one hand and *P. halepensis* and the hybrids on the other hand, we log transformed the two parameters and analyzed the slopes and intercepts between Ψ_{PD} and SWC. The data indicate that *P. halepensis* and the hybrids showed no significant differences in their slope values, whereas the *P. brutia* slope value was significantly lower (Table 1). The differences between the intercepts were also significant, with *P. brutia* being the highest, followed by the hybrids, and then by *P. halepensis* (Table 2).

Table 2. One-way ANOVA of the effect of *P. halepensis*, *P. brutia*, and their hybrids on the log-transformed values of Ψ_{PD} vs. SWC and their slopes and intercepts $\pm$ standard error. Different superscript letters indicate significant differences between the genotypes ($p < 0.05$).

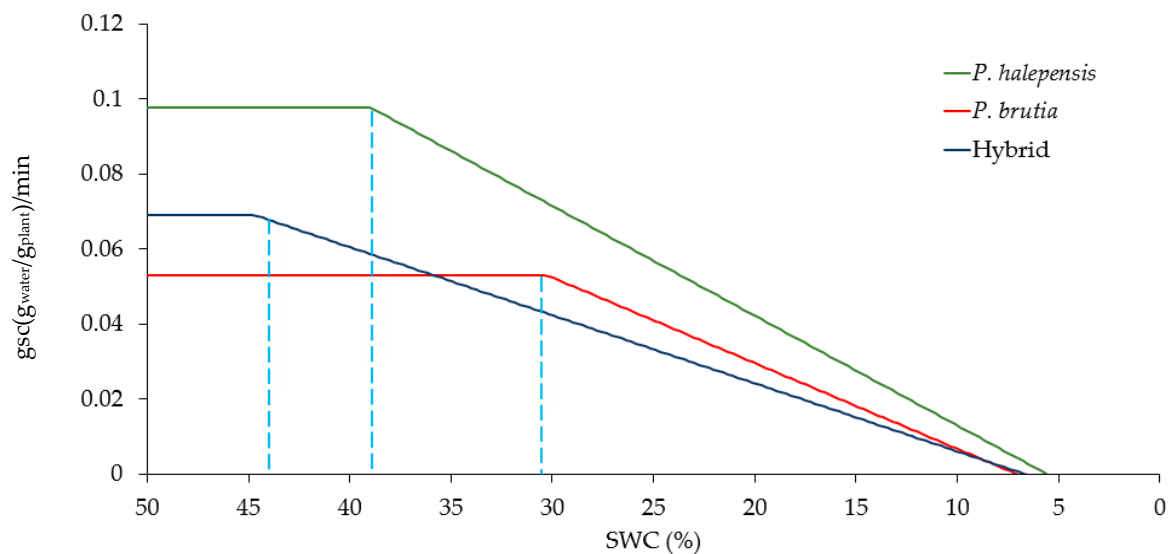|	*P. halepensis*	*P. brutia*	**Hybrid**
R^2	0.963	0.825	0.77
p value	0.0005	0.0123	<0.0001
Slope	1.2 [a] $\pm$ 0.12	0.66 [b] $\pm$ 0.15	0.94 [a] $\pm$ 0.12
Intercept	-5.05 [b] $\pm$ 0.43	-2.77 [a] $\pm$ 0.53	-4.07 [c] $\pm$ 0.45

Figure 3. Midday maximum conductance of the whole canopy (g_{sc}) as a function of soil water content (SWC) in *P. halepensis* (green), *P. brutia* (red), and their hybrids (blue). Light blue dashed lines indicate physiological critical stress points (θ).

4. Discussion

The current study focused on the comparison of morphological and physiological traits in rooted cuttings of *P. halepensis*, *P. brutia*, and their hybrids. The hybrids had a higher root-to-shoot ratio, a lower relative growth rate, longer and wider needles, and a higher LMA than both parental species. Under drought stress, the hybrids exhibited lower E and g_{sc}, similar to *P. brutia*, and an early stomatal closure, similar to *P. halepensis*. Together, these results support our hypothesis that *P. brutia* × *P. halepensis* hybrids possess a unique combination of traits as compared to their parental species. Furthermore, their traits are advantageous in dry environments, particularly as compared to *P. brutia*.

4.1. Morphological Traits and Growth Parameters

Hybrids of *P. brutia* × *P. halepensis* are known for their relatively high vigor, particularly compared to that of *P. brutia*, as expressed in tree size, foliage density, cone length, needle length, and needle width [50,63,64]. In agreement with previous studies, the current study demonstrated that several morphological traits are distinct in the hybrids compared to *P. halepensis* and *P. brutia*. These traits include needle length, needle surface area, needle cross section area, needle volume, and LMA (Table 1). LMA is a highly plastic trait. Poorter et al. (2009) [11] found that LMA values differ significantly among terrestrial plant species, and generally range from 30 to 330 g/m². The LMA values found in the current study were the highest in the hybrids, followed by *P. brutia* and *P. halepensis* (235, 183, and 145 g/m², respectively). The data in the current study are in agreement with the findings of Climent et al. (2009) [65], who showed a low range of LMA values in *P. brutia* and *P. halepensis*. Species with a high LMA invest more resources in developing a higher resilient leaf tissue with a greater longevity rather than in a large leaf surface area for light capture [11]. Plant species with a high LMA have a higher capability for coping with adverse growing conditions, and often occur in unproductive dry habitats [11,14]. A high LMA is also correlated with high solar radiation [9]. As suggested in the above-mentioned reports, it is reasonable to assume that the relatively high LMA found in the hybrids in the current study might be an advantage to thrive in marginal lands characterized by low rainfall and high solar radiation.

Similar to their mother trees in the field, we expected that the hybrid rooted cuttings will grow more rapidly compared to *P. halepensis* and *P. brutia*. However, the overall size of the hybrid rooted cuttings was smaller than that of their parental species rooted cuttings

(Table 1). Under a full (normal) irrigation regime, *P. halepensis* and *P. brutia* showed similar WUE, while their hybrids had a significantly lower WUE. The low WUE was in agreement with the relatively low RGR of the hybrids. In a previous study, Riov et al. (2020) [53] obtained rooted cuttings of *P. halepensis* trees that differ in their size in a forest stand located in a semi-arid region in Israel. Similar to the hybrid rooted cuttings, the cuttings from vigorous trees exhibited a slow growth under optimum environmental conditions, compared to that of rooted cuttings from relatively small trees. An explanation of this phenomenon could be that the higher LMA observed in the hybrids (Table 1) implies the allocation of more biomass to the leaves at the expense of stem growth [66]. In addition, the high root-to-shoot ratio indicates that the hybrids rooted cuttings allocate more biomass to their roots, probably at the expense of shoot growth at this stage. Both of these phenomena may reflect the well-known tradeoff between stress resistances versus competitiveness of plant species, as proposed by various previous studies [67–69]. We assume that this explanation might be relevant for the hybrids in the current study.

High root-to-shoot ratio is an advantage in environments with seasonal droughts, such as occurring in Mediterranean ecosystems [19,70]. Deep and extensive root systems help to maintain the water balance in trees [24,71]. High root-to-shoot ratio also supports the demand for nutrients, thus promoting the growth of plants in habitats with poor resources [16,17]. There are several studies that demonstrated the advantage of high root-to-shoot ratio especially in young seedlings of Mediterranean shrubs [70] and trees [19,72]. In the current study, we used rooted cuttings and not seedlings. Although Frampton et al. (2000) [73] showed a similar growth pattern between loblolly pine (*Pinus taeda* L.) rooted cuttings from juvenile plants to that of full-sib seedlings, we did not compare seedling growth in this study. To draw conclusions about the contribution of the root-to-shoot ratio to hybrid seedling establishment in the field, it would be necessary to germinate seeds and then genotype the naturally occurring hybrids or directly pollinated *P. brutia* cones by *P. halepensis*.

Although the hybrid needle DW was relatively low, the hybrids showed a higher LMA (Table 1). The needles had the same proportion of mesophyll in the needle cross section in the hybrids and the parental species. The hybrid plants were significantly smaller than *P. halepensis* and *P. brutia*, their shoot weight and their total needle dry weight were also significantly lower. When calculating the needle DW percentage of the total shoot DW, the hybrids had the highest percentage compared to *P. brutia* and *P. halepensis* (68%, 60%, and 54%, respectively). Again, it is evident that the hybrids allocate more resources to their needles on the expense of growth. This allocation of biomass both to the roots and to the needles may result in a plant more resilient to drought; however, further research is needed to support this assumption.

4.2. Physiological Response to Drought Stress and Recovery

To the best of our knowledge, the current study is the first to report a comparison of physiological characteristics in response to drought stress between *P. halepensis* and *P. brutia*. Before the initiation of drought stress, the daily transpiration of *P. brutia* plants was higher than that of *P. halepensis* due to their higher biomass. However, when normalizing the transpiration to the plant weight, *P. halepensis* demonstrated higher E and g_{sc} than those of *P. brutia*. The higher E and g_{sc} could be a result of higher stomatal density or stomatal opening; however, these parameters were not examined in the current study. It was shown that a relatively high E under soil field capacity conditions (E_{max}) characterizes spender versus water-saver plants [38]. In addition, E and g_{sc} are known to be positively correlated with photosynthetic capacity [74], suggesting a higher assimilation and thus a faster growth in *P. halepensis* when water is available. Upon drought stress, *P. halepensis* reduced stomata conductance earlier than *P. brutia*, suggesting that *P. halepensis* is more sensitive to reduction in SWC than *P. brutia* (Figure 3). Indeed, *P. halepensis* was shown to have a similar behavior in previous studies [34,75], which led to an increased survival rate during drought at the cost of lower biomass production [41]. In regions with a short rainy season, high E and g_{sc}

and early stomatal closure are advantageous. Trees that maintain high growth rates for longer periods during drought due to a late stomatal closure are exposed to a greater risk of drought-induced mortality if the drought persists for a long period [37,39,43]. *P. brutia* is naturally distributed in areas characterized by a stable and wetter climate conditions compared to those in the semi-arid region of Israel [76]. This can explain the risk to which *P. brutia* might be exposed in semi-arid regions by keeping its stomata open for longer periods and reaching a lower θ point, as it is less adapted to very long dry periods.

Ψ_{PD} is commonly used as a proxy for pre-dawn soil water potential (Ψ_{soil}) [42,77]. The current study did not detect differences in Ψ_{PD} between *P. halepensis* and *P. brutia* at high SWC. However, at low SWC (<40%), the differences in Ψ_{PD} were notable (Figure 2). The rate of Ψ_{PD} reduction at a low SWC in *P. brutia* was significantly lower than that of *P. halepensis* (Table 2). In agreement with these results, Gu et al. (2015) [77] reported that when trees were well hydrated, all species had similar Ψ_{PD}, but under dry conditions, different tree species exhibited different Ψ_{PD} [77]. *P. bruita* seemed to be less responsive than *P. halepensis* to the reduction in SWC and maintained a higher Ψ_{PD} at low SWC (Figure 2). These data are in agreement with the differences between *P. halepensis* and *P. brutia* in the reduction in E and g_{sc} at low Ψ_{PD}. The present results are similar to those reported by Grunwald and Schiller (1988) [78], who showed that the midday water potential (Ψ_{MD}) of *P. halepensis* was more negative than that of *P. brutia* growing in the same stand located in the Mediterranean region.

Compared to the two parental species, their hybrids showed an intermediate behavior. Their E and g_{sc} at high SWC were similar to those of *P. brutia*, while their θ and Ψ_{PD} regulation was similar to those of *P. halepensis*. In addition to the low water loss, the hybrids demonstrated a high sensitivity to the reduction of SWC, i.e., an early stomatal closure upon drought. These characteristics may indicate a unique water use strategy which is different from both of their parental species.

Although the SWC was almost fully restored at the end of the recovery stage, the g_{sc} and E did not return to their initial values in all the plants examined (Figure 1d,e). According to the g_{sc} and E measurements, the stomata were at their minimum conductance for 5 to 7 days before re-watering. Severe drought stress can lead to loss of stem hydraulic conductivity due to cavitation and embolism in the xylem [79]. Real-time native embolism measurements, using a new optic method, demonstrated the occurrence of embolism in *P. halepensis* growing in a semi-arid forest when the SWC decreased to 12% in the summer [80]. In pines, recovery from embolism requires the development of a new sapwood [71,81]. Brodribb et al. (2010) showed that lethal water potential was correlated with a tension producing a 50% loss of stem hydraulic conductivity (Ψ_{50}) in *Callitris* spp. [82]. A study of seven populations of *Pinus hartwegii* and another 513 genotypes of *Pinus pinaster* showed a low genetic variation of Ψ_{50} between climatically contrasting populations and a very limited phenotypic plasticity [83,84]. These results suggest that Ψ_{50} is a relatively stable trait in pines. When studying the relationship between Ψ_{PD} and SWC, *P. halepensis* was shown to close its stomata at a water potential between −3 to −4 MPa, close to the water potential at which it experiences 50% loss of conductivity due to embolism [85]. Thus, it is likely that the plants in the current study experienced severe embolism. Brodribb and Cochard (2009) [81] found strong evidence in four conifer species that hydraulic limitation explains the reduction in gas-exchange rate during recovery from drought stress. As previous studies have suggested [81,82], we expect that full recovery of g_{sc} and E will be achieved only following development of a new sapwood.

5. Conclusions

To the best of our knowledge, the current study is the first report of a direct comparison of morphological traits, growth parameters, and physiological response to drought stress between *P. halepensis*, *P. brutia*, and their hybrids. It was found that *P. brutia* × *P. halepensis* hybrids that were more vigorous than their neighboring *P. brutia* trees in several stands (i.e., vigorous hybrids) [52] are characterized by a unique combination of traits that can

explain their vigor, particularly in semi-arid regions. It is well known that drought resistance, as well as vigorous growth, result from a combination of several traits rather than from a single dominant trait. Subsequently, we suggest that *P. brutia* × *P. halepensis* hybrids may contribute to the genetic diversity and resilience of east-Mediterranean semi-arid forests undergoing climate change. We believe that this topic should be further studied for a better understanding of this phenomenon and the development of genetic improvement programs.

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