



Article

Tolerance to Drought and Water Stress Resistance Mechanism of Castor Bean

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Received: 10 September 2020; Accepted: 14 October 2020; Published: 16 October 2020



Abstract: Castor bean (*Ricinus communis* L.) is a multipurpose crop; its oil has numerous applications worldwide and the last decade demonstrated a growing international demand. The aim of this work was to investigate the level of castor bean tolerance to drought and its possession of a water stress resistance mechanism by applying three different water regimes in a glasshouse pot experiment conducted for two years. The treatments applied were 70% (T₇₀-control), 55% (T₅₅) and 40% (T₄₀) of the available soil moisture. The results showed that the growth parameters height, trunk diameter, and fresh and dry weights of leaves and stems were not affected by the moderate water scarcity (T_{55}) , while they were significantly decreased by T₄₀. Significant decrease in leaf number was observed in both T₅₅ (17%) and T₄₀ (27%) plants, with a delay of 4 weeks in the lower treated plants. Leaf area was decreased by 54% and 20% in T₅₅ and T₄₀ respectively, indicating that its reduction was mainly due to a reduction of leaf size than of leaf number. The leaf water potential was increased negatively with increasing stress, showing a water loss and decrease of turgidity in cells. Stomatal resistance was significantly higher at the higher water scarcity and this response indicates a water stress resistance mechanism. This result was also confirmed by the regression analysis performed between stomatal resistance and leaf water potential. In conclusion, castor bean showed a tolerance ability under water stress conditions and its early physiological reaction allows its acclimatization to drought conditions.

Keywords: *Ricinus communis* L.; water scarcity; leaf water potential; stomatal resistance; leaf area; growth parameters

1. Introduction

Water scarcity is one of the most concerning environmental issues of the world. Apart from agriculture, which is the main water-consuming sector by using 70% of the world's water resources, there are several other factors that exert considerable pressure on the available water reserves, such as climate change, industrialization, rapid demographic growth and urbanization, evolution of economic development, pollution, etc. [1–7]. However, since agriculture is the main factor which affects water scarcity, it is essential to find ways to reduce the water consumption of this sector. One solution is to identify drought-tolerant crops and castor bean is an important candidate.

Castor bean (*Ricinus communis* L.) is a non-edible multipurpose oilseed species of the Euphorbiaceae family. It is a C3 fast-growing monotypic crop with chromosome number of 2n = 20 [8]. It originates from Eastern Africa and most probably from Ethiopia, as in this country the higher number of wild and semi-cultivated types worldwide are found [9,10]. Nowadays, it is cultivated all over the tropical and semi-tropical regions for its highly valued oil [11–13]. Castor bean plants can vary greatly in their

morphological characteristics, i.e., they may be shrubs or trees that can grow above 10 m high, they may have different color of foliage and stems, seed size and color, and oil content [10,14,15]. Even though it is a perennial species, in the Mediterranean region it is usually cultivated as an annual crop reaching a height of 60–120 cm [12,16]. It can be cultivated in marginal lands unsuitable for food production and has low input requirements and production cost [13,17,18]. These attributes make it a valuable crop that could contribute to the low indirect land-use change and increase farmers' incomes through access to new markets.

Castor bean is a quite important crop as its oil is rich (80–85%) in the only commercially available natural hydroxylated fatty acid, ricinoleic acid, which can form the unsaturated aliphatic polyester, poly(ricinoleic acid) with many industrial applications and a growing international demand [10,11,15,19–23]. The production share of castor oil seed by region is in Europe 0.1%, in Africa 4.9%, in the Americas 5.9% and in Asia 89.1% [24]. The most important producers of castor seeds are India with 1.14 million tons, China with 175.8 thousand tons, Brazil with 72.4 thousand tons and Mozambique with 46.0 thousand tons [24]. Europe is the main user of castor oil, consuming 40% of world production. Castor oil is of great importance for the chemical, pharmaceutical and polymer industries. It is used for the production of high-quality lubricants, polyurethane, paints, coatings, textile dyes, surfactants, resins, plastics, soaps, medications, cosmetics, etc. as well as for biodiesel production [10,16,20,25,26].

It is obvious that the possibilities to spread the cultivation and increase the production of this crop in Europe are very challenging. It is well documented that castor bean can grow quite well under the pedo-climatic conditions of the Mediterranean region [12,27–29]. The purpose of this research work was to study castor bean tolerance to water scarcity that is a critical cultivation constraint for the Mediterranean region.

2. Materials and Methods

2.1. Soil

The soil used was surface, obtained from an agricultural site of the Kopais plain, Boeotia. It was air-dried under room temperature and ground to pass through a 2-mm sieve. Peat was added to the soil in a 1:4 ratio and the mixture was well homogenized. Samples from the mixture were analyzed for their texture by the wet sieving and pipette method [30], pH by the use of standard glass/calomel electrodes in 1:1 w/v soil—water ratio, organic matter content by the Walkley–Black method [31], electrical conductivity by using a conductivity meter on 1:2 soil/water suspension, and for equivalent CaCO₃ by the Rowell method [32].

The texture of the used soil was Sandy Loam (SL), having 58.3% sand, 27.6% silt and 14.1% clay respectively. The organic matter content was measured to be 4.1%. The pH was 7.48, even though it was expected to be lower due to the addition of peat. However, this increased value could be explained by the high concentration of equivalent CaCO₃ (21.7%) that reacts antagonistically with the acids of organic matter. The electrical conductivity was determined to be 2.6 dS/m, indicating that the soil was slightly saline and most crops could grow on it.

2.2. Experimental Setup

The experiment was conducted for two successive years (2017 and 2018) in a glasshouse at the Agricultural University of Athens (37°59′ N, 23°42′ E and altitude 33 m). Each year, castor seeds were sown in small plastic pots (two seeds per pot) and were irrigated every two days. Sixty-four days later, 45 uniform plantlets were selected, having a height of 15.0 ± 1.5 cm and bearing 6 ± 1 leaves each. The plantlets were transplanted into plastic pots filled with 11.5 kg of the already prepared soil (one plantlet per pot). All plants received uniform irrigation in order to ensure their good establishment before the beginning of the treatments. Fifteen days later, the pots were divided into three groups and

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each group received a different irrigation treatment (Table 1). The experimental design used was the completely randomized with five replications.

Group of 15 Pots	Treatment	Pot Weight (kg)	Available Soil Moisture (%)
A	Well-watered/Control—T ₇₀	13.50	70
В	Moderately stressed—T ₅₅	13.10	55
С	Highly stressed—T ₄₀	12.70	40

Table 1. Irrigation treatments applied to the pots.

2.3. Treatments

Three irrigation treatments were applied in this experiment. In order to calculate the irrigation dose per treatment, the Available Soil Moisture (ASM) was determined, i.e., the difference in soil moisture between the Water Holding Capacity (WHC) and the permanent wilting point (PWP). The ASM was measured to be 2.9 L of water per pot. The weight of each pot, when the soil was at 100% of the ASM, was 14.4 kg (i.e., 11.5 kg of soil + 2.9 kg of water). The treatments applied were 70%, 55% and 40% ASM (Table 1). The irrigation dose per treatment was calculated every 2 days by weighing the pots so as to keep their weight constant.

2.4. Measurements

A total number of five plants per group of pots were randomly chosen to run the growth measurements, namely the plant height, the number of leaves, the trunk diameter (at 10 cm from the soil surface) and the leaf area. The growth measurements were repeated at weekly intervals. At the end of both experiments (26 weeks after sowing the seeds), all plants were harvested and the fresh and dry (oven-dried at 75 °C for 48 h) weights of the above ground biomass per plant were measured.

Another five plants within each group of pots were used for the determination of the plant water status by measuring the midday leaf water potential (Ψ_{leaf}) and the leaf stomatal resistance (Rs), at intervals of seven days. On each measurement day, the leaf water potential was measured in three mature and fully expanded leaves of one plant per treatment. The leaves were excised at the petiole junction with the stem and were immediately enclosed in sealed plastic bags. Thereinafter, each bag was enclosed in another aluminum vapor deposition film bag. All bags were placed in an incubator maintained at 25 °C until measurement, which was done by using a Scholander-style pressure chamber. All pots used for this measurement remained in place and they were irrigated so us to avoid alterations in the experimental arrangement.

The remaining five plants per group were used to measure the leaf water potential at different hours during the day, namely at 6:30, 9:00, 11:30, 14:00 and 16:30.

Leaf Rs was measured in plants on the third leaf from the top of the stem, using a porometer (AP4, Delta-T Devices).

The leaf area (LA) of castor bean plants was measured by a non-destructive method as described by Chaudhary et al. [33].

2.5. Statistical Analysis

The experiment was repeated twice during the years 2017 and 2018 with qualitatively similar results. The data presented in this work is the average result from both years. One-way analysis of variance (ANOVA) and Duncan test were conducted in order to investigate the effect of water stress on plant growth. In order to identify the relation between Rs and leaf water potential for each stress level, a linear regression model with indicator variables was applied and a linear model was estimated for each stress level. Furthermore, significance tests for differences among the slopes of the models were performed. The ANOVA and linear regression assumptions (error normality and homoscedasticity)

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were tested. Data processing and statistical analysis were conducted using STATGRAPHICS Plus (Statgraphics Technologies Inc., The Plains, VA, USA).

3. Results and Discussion

Water scarcity has a direct impact on the growth, productivity and quality of crops, causing a variety of pressures on agricultural production, and on increasing food demands [34,35]. The first reaction of plants is the decrease of cell size and growth rate. The reduction of cell volume is the most sensitive function under water scarcity and causes a reduction in plant growth rate, in the elongation of plant parts and leaves, and in stomatal opening [36–39].

The results showed that the applied levels of water stress began to affect the plant height six weeks after the beginning of the first application (Figure 1A). Plant height was positively affected by the increase of the available soil moisture. Six weeks after treatment application the height of the T_{40} plants was statistically significantly smaller than that of the T_{70} plants, while the T_{55} plants gave intermediate values, which however did not differ significantly from the T_{40} treated plants nor from the T_{70} plants (p < 0.05). Thereafter, the control plants were higher than those of T_{55} , but the differences were not statistically significant, indicating a relative tolerance of castor bean to reduced water availability. However, all plants of both groups differed significantly from the highly stressed plants. Therefore, only 40% ASM significantly reduced the height of the plants. Similar results are reported by [28,29] in field experiments and at irrigation levels of (i) 0, 50, 75 and 100% and (ii) 33, 66 and 100% of evapotranspiration, respectively. In both cases, the increase in ASM caused a statistically significant linear increase in the height of the plants.

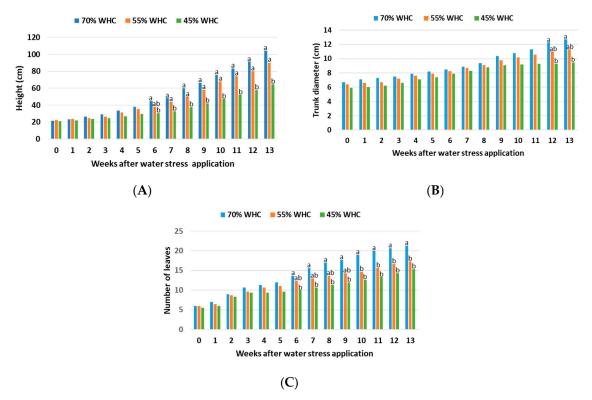


Figure 1. Effect of water stress on plant growth parameters: (**A**) height, (**B**) diameter, (**C**) number of leaves (ANOVA, Duncan test, p < 0.05, n = 5).

Plant diameter was not significantly affected by the treatments, apart from the last two weeks of the experiments, when the diameter of the highly stressed plants differed statistically only from the control plants (Figure 1B).

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The total number of leaves was affected by the treatments during both experimental years (Figure 1C). Significant decrease in leaf number was observed in both T_{55} and T_{40} plants, respectively 6 and 10 weeks after treatment. It is well known that prolonged water stress in many plant species leads to aging and cutting of the older (lower) leaves [40]. This mechanism is considered to be the first line of defense against water stress, as it reduces respiratory losses. In castor plants, a shedding of the older leaves was observed in all treatments, with a significant upward trend as the lack of ASM increased.

The fresh and dry weights of both leaves and stems were statistically significantly reduced only at the highest stress level (Figure 2). The biomass production of the plants remained unaffected not only at the 70% of the available soil moisture, but also at the 55%, indicating a relative resistance of the plants at these levels of water scarcity. These observations are also confirmed by the results concerning the Rs.

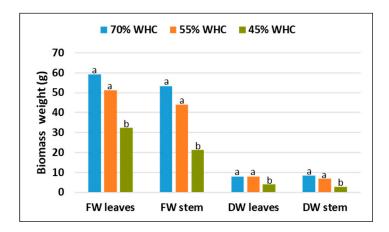


Figure 2. Effect of water stress on the fresh (FW) and dry (DW) weights of castor plants (ANOVA, Duncan test, p < 0.05, n = 5).

Plants adapted to grow in dry regions are armed with constitutive morphological and anatomical modifications that help to conserve water [41]. One of the morphological adaptations of plants to different environmental conditions is the shape and the size of their leaves. The leaf is the main organ which responds to environmental conditions more clearly in respect to other organs, such as the stem and roots [42,43]. Thus, leaf area is a critical trait in plant growth; in general, plants tend to decrease their LA when coping with water stress in order to minimize water losses through transpiration [44]. In castor bean plants of this work, the LA was significantly affected by water scarcity (Figure 3). The leaves of T_{40} plants had a 54% (mean value) lower LA than the control plants, while the LA of the T_{55} plants was reduced by 20%. The corresponding reduction in the number of leaves was 27% and 17%, respectively, indicating that LA reduction was mainly due to a reduction of leaf size than of leaf number. This result is in accordance with [45] who reported that castor seedlings grown in containers of different volume had less change in the leaf number (1.4-fold) than in the leaf size (2.8-fold). Indeed, reduction of leaf size is usually the main cause for LA changes and not the decrease of leaf number [45–47].

These results confirm that water availability plays a primary role in plant development [43,48].

The leaf water potential values throughout the experiments showed a clear gradation of the plant water status between the three levels of stress (Table 2). In all measurements, the water potential of the control plants (T_{70}) had the least negative values, i.e., closer to zero (turgid point) indicating that the turgor pressure increased and was close to the osmotic pressure of the cell.

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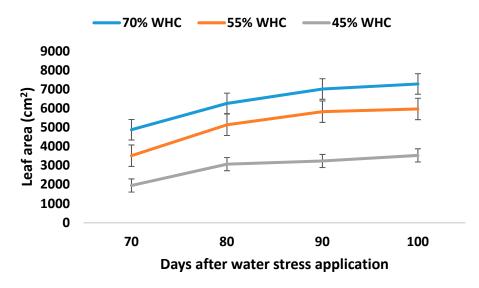


Figure 3. Castor leaf area as affected by the water deficit.

Table 2. L	.eaf water	potential c	of castor p	lants (in	MPa, mean v	values, $n = 5$).
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Stress Levels	Days after Water Stress Application					
Stress Levels	23	33	43	53	63	73
Control (70% ASM)	-1.23	-1.08	-0.93	-0.83	-1.39	-1.10
Low (55% ASM)	-1.34	-1.27	-1.35	-1.14	-1.49	-1.23
High (40% ASM)	-1.47	-1.64	-1.58	-1.33	-1.65	-1.41
Difference between T_{70} and T_{40}	-0.24	-0.56	-0.65	-0.50	-0.26	-0.31

The values of the water potential increased negatively with increasing stress, showing a water loss and decrease of turgidity in cells. More specific, in plants of T_{40} , the lowest values were determined, while in the T_{55} , intermediate values of water potential were observed. Similar results are reported by Sausen and Rosa [49]; in their experiment pre-dawn values of leaf water potential were recorded and varied between -0.7 and -0.9 MPa in well-watered plants ($\cong 70\%$ of field capacity, i.e., 0.30 g g⁻¹ water/soil ratio) and showed a progressive reduction to -2.1 MPa in water–stressed plants (0.18 g g⁻¹ water/soil ratio).

It is worth noting that 43 days after treatment application, the maximum difference in water potential values between the low and high stressed plants was observed (Table 2), while at the same period, the first statistically significant differences in the plant growth measurements were determined (Figure 1).

The changes in water potential values over a day (i.e., 60 days after the beginning of treatments) showed that T_{70} and T_{55} treated plants at 14:00 (highest temperature) exhibited the worst water status (highest negative water potential values) (Table 3). In T_{40} plants, the water status reached its lowest level earlier (11:30), demonstrating the inability of plants to tolerate water deficit during the day.

Table 3. Leaf water potential (MPa) of castor plants during the day (in MPa, mean values, n = 5).

Stress Levels			Time		
Stress Levels	6:30	9:00	11:30	14:00	16:30
Control (70% ASM)	-0.69	-0.47	-0.85	-0.98	-0.91
Low (55% ASM)	-0.83	-0.61	-1.19	-1.25	-1.13
High (40% ASM)	-1.15	-1.23	-1.46	-1.37	-1.35

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It is well known that leaf stomatal characteristics are greatly influenced by environmental conditions [50–54]. Water stress causes closure of leaf stomata in order to control the water losses of plants by reducing stomatal aperture and thereby the transpiration rate. Under adequate water availability, the stomatal pores are open since turgor increases, the thin wall of the stomata guard cells bulges away from the aperture, while the front wall (facing the pore) becomes straight or concave. The reverse mechanism takes place under water scarcity. Stomatal opening and closing can be assessed through the evolution of Rs which defines the plant–atmosphere interactions, strongly influencing the rate of gas exchange, and hence, photosynthesis and transpiration [55,56].

The results of Rs measurements showed statistically significant differences (p < 0.05) between the treatments (Figure 4). The increase in Rs was significantly higher at the higher water scarcity (T_{40}) and this response indicates a water stress resistance mechanism [18,57]. Three weeks after the water stress application, the Rs of the highly treated plants (T_{40}) started to show significant differences from the other plants, indicating that the physiological response had already begun. This phenomenon is of great agronomic interest, as this reaction also means a clear decrease in the photosynthetic mechanism and hence lower growth and productivity [58,59]. The Rs of moderately treated plants (T_{55}) did not differ statistically significantly from the control ones, indicating a tolerance ability of castor bean plants to relatively low soil moisture conditions. Therefore, the stomata remain open under relatively reduced ASM and the effects of such levels of water stress on the final growth and yields of castor bean may not be significant. However, these results need further verification under field conditions.

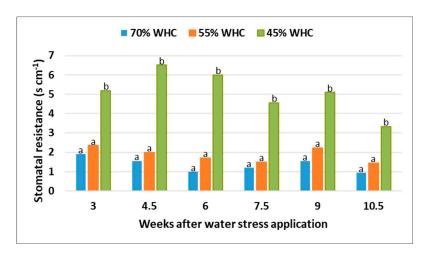


Figure 4. Effect of water stress on the leaf stomatal resistance of castor plants. Different letters show statistically significant differences (ANOVA, Duncan test, p < 0.05, n = 5).

In order to evaluate the castor bean response to water scarcity and to estimate the degree of stomatal control, a regression analysis was performed between Rs and leaf water potential [43,60,61]. More specifically, a linear regression model with indicator variables was applied having $R^2 = 74.20\%$ (adjusted $R^2 = 70.89\%$) and a linear model was estimated for each stress level. The equation of the fitted model is:

$$y = 0.72 - 0.44x - 10.70I_{40} - 2.69I_{55} - 9.46xI_{40} - 2.32xI_{55}$$

where y is the dependent/response variable (stomatal resistance, Rs), x is the predictor variable (leaf water potential, Ψ) and I_i , i = 40, 55 are indicators variables where $I_i = 1$ if the data belongs to stress level T_i and $I_i = 0$ otherwise.

This equation describes three linear models that are shown in Table 4 and Figure 5. The results show that the slopes of these models are statistically different (p < 0.0001).

Stress Level	Linear Model	Pearson's Correlation Coefficient	<i>p</i> -Value
T ₇₀ Control (70% ASM)	y = 0.72 - 0.44x	0.00	0.9824
T ₅₅ Low (55% ASM)	y = -1.97 - 2.76x	-0.59	0.0207
T ₄₀ High (40% ASM)	y = -9.98 - 9.91x	-0.70	0.0034

Table 4. Regression analysis between stomatal resistance and leaf water potential under different stress levels

 $y = \text{stomatal resistance (Rs)}, x = \text{leaf water potential }(\Psi).$

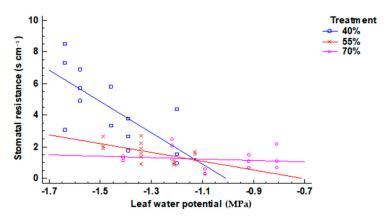


Figure 5. Linear models for regression between stomatal resistance and leaf water potential under different stress levels.

For T_{40} and T_{55} treatments the results show statistically significant negative linear correlation between Ψ and Rs. On the contrary, for the T_{70} treatment, no statistically significant linear or any other type of correlation was found. As is shown in Figure 5, the line representing treatment T_{70} is almost parallel to the horizontal axis (estimated slope of -0.44) indicating that for a range of Ψ between -1.7 and -0.7 MPa, the variation in Rs is quite small, probably due to the fact that the well-watered control plants absorbed water from the soil fast enough to balance their transpiration and stomatal closure. On the contrary, the line of T_{40} showed the most negative slope (estimated to be -9.91) indicating that under severe water scarcity, a small variation of Ψ induces a rapid stomatal closure in castor bean plants. This rapid stomatal response supports the result stated before that castor plants show a water stress resistance mechanism. The correlation between Ψ and Rs in T_{55} is not so tight and the slope of the line representing this treatment is much less negative (estimated to be -2.76).

4. Conclusions

Castor bean plants showed a tolerance ability under water stress conditions. The results of both experimental years showed that plant growth decreased under severe lack of water availability (T_{40}) , while under moderate water scarcity (T_{55}) it remained unaffected. The lower leaf water potential and the stomatal closure under severe water stress reflect a physiological reaction that allows the acclimatization of plants to drought conditions. This work will be continued for further investigation and validation under field conditions.

Author Contributions: Conceptualization, E.G.P.; methodology, E.G.P.; formal analysis, G.K.P. and E.G.P.; investigation, E.G.P. and E.A.; data curation, E.G.P.; writing—original draft preparation, E.G.P.; writing—review and editing, E.A., G.K.P. and G.E.-A.; funding acquisition, E.G.P. All authors have read and agreed to the published version of the manuscript.

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Funding: This research has received funding from the European Union's Horizon 2020 projects MAGIC and PANACEA, under the grant agreements No. 727698 and No. 773501 respectively.

Conflicts of Interest: The authors declare no conflict of interest.

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