Article

Assessment of Germination Response to Salinity Stress in Castor through the Hydrotime Model

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Abstract: Germination of castor seeds from five dwarf hybrid genotypes, compared to a 'Local' genotype selected by the University of Catania from a Tunisian population well adapted to the Mediterranean environment, was studied at five different salt levels (0, −0.3, −0.6, −0.9, and −1.2 MPa) in order to assess seed germination performance under stress conditions. The results confirmed that optimum moisture (0 MPa) ensured 100% of germination; on the contrary, salt concentration negatively influenced the final germination percentage (FGP) and radicle elongation, causing severe consequences for plant establishment. At a level of −1.2 MPa, no germination occurred, while a level of −0.3 MPa slightly affected the seed germination of the dwarf genotypes, which achieved 77.3% of germination, contrary to the 'Local' genotype, in which germination was kept stable. Higher levels of salt (−0.6 and −0.9 MPa) caused a linear decrease in FGP and radicle elongation. Overall, the dwarf hybrid 'C1019' performed better at higher salt impositions, as did 'C857', confirming these genotypes were the most tolerant among the dwarf hybrids. Conversely, 'C1013' turned out to be the most susceptible genotype, followed by 'C1008'. On the other hand, the 'Local' castor genotype was the best-performing genotype at −0.3 MPa and the most tolerant genotype in terms of salt concentration and germination time, which were accurately predicted by the hydrotime model, validating it as a valid method of assessing the germination response of castor seeds to Ψ.

Keywords: dwarf hybrids; marginal land; radicle length; *Ricinus communis* L.

1. Introduction

The world is currently experiencing one of the greatest crises in modern history, characterized by significant climate change and a severe drop in agricultural production [1]. Salinity, drought, and high or low temperature are the main abiotic stresses that threat worldwide agriculture [2]. In depth, salinization affects about 3 million hectares of land in Europe and 30% of irrigated land in the world [3], representing an extreme obstacle for food security. Given the reduction in total arable land, research is pushing towards the selection of tolerant crops or the development of salt-tolerant plants by breeding [4].

This scenario broadens the possibilities of castor being globally exploited. Castor (*Ricinus communis* L.) is an oilseed crop belonging to the Euphorbiaceae family and originating from Asia or, most likely, Africa [5].

The chemical and pharmaceutical industries have positively re-evaluated this crop because of its wide range of uses, mainly as an oil producer (35–65% oil content) [6,7]. The extensive applications of castor [8], which include biofuels, biolubricants, antimicrobials or antioxidants in varnishes and paints, raw material for the production of sebacic acid,

nylon, and other resins, and also as feed for animals and as an ornamental plant [8,9], are the reasons for the growing interest in this crop.

Furthermore, castor is a rustic, low-maintenance crop because of its low nutrient requirements and great land and climate adaptations [8]. Thus, the chance of using marginal areas for its cultivation, avoiding competition with plants used for food production, and the necessity of reducing fossil fuel usage according to the 2030 Agenda [10], make castor the ideal plant to be exploited in the decades to come.

Marginal lands, which are increasing at an annual rate of 1–2%, are often characterized by high saline levels [11]. As seed germination and seedling emergence and development are the most crucial and sensitive stages during the plant life cycle [12], soil salinization is a severe obstacle to adequate plant growth [13]. Salt stress, induced by numerous causes such intense evaporation, rising sea levels, and incorrect irrigation practices, induces a reduction in water absorption (osmotic stress) and an excessive accumulation of Na^+ and Cl^- ions (toxic effects) [14]. These stresses adversely affect overall metabolism, seed germination, and later plant establishment and development [15], while also being responsible for the production of reactive oxygen species (ROS) which are responsible for cell membrane damage [16].

Therefore, assessing the seed germination response of castor to salinity stress is important in order to predict seed performance under different soil salinity conditions that the plant may experience during the first stages of its growing season. In this context, this study was conducted to evaluate the relationship between salt stress conditions and the seed germination characteristics of castor through hydrotime analysis and compare the germination responses of different genotypes on the basis of their stress tolerance.

2. Materials and Methods

2.1. Seed Material

Seeds of castor (*Ricinus communis* L.) were used for the laboratory experiment, carried out at the University of Catania (Italy) and studying two experimental factors: genotype and salt level. Six genotypes were assessed: five dwarf hybrids selected and bred by Kaiima Company, Campinas—São Paulo, Brazil, for commercial purposes, and a local genotype, used as control, mass-selected from a wild Tunisian population for its adaptation to the Mediterranean basin at the Di3A of the University of Catania (Italy).

Seeds used for the experiment were produced in an open field at the experimental farm of the University of Catania (37°24′31″ N; 15°3′33″ E, Italy) using the above genotypes. Seeds were harvested in September 2022, stored in paper bags, and kept at room temperature (10–20 °C) until use for this study in October 2022. Seeds were surface-sterilized before germination tests with 1% sodium hypochlorite solution (NaClO) (Sigma-Aldrich s.r.l., Milano, Italy) for 3 min, rinsed in distilled water, and then dried at room temperature for 24 h. Seed moisture contents ranged between 3.5 and 4%.

2.2. Salt Solutions

Five salt solutions were used for germination tests, prepared by dissolving NaCl in deionized water to generate five water potentials (ψ): 0 (control), −0.3, −0.6, −0.9, and −1.2 MPa. These solutions had electrical conductivity (*EC*) values of 0, 8.2, 13.7, 16.7, and 11.9 dS/m, respectively. Their osmotic potential was verified using an automatic cryoscopic osmometer (Gonotec Osmomat 030 model, Berlin, Germany). Electric conductivity (*EC*) was measured in a portable conductivity meter (Mod. CyberScan CON 400/410, Eutech Instruments, Pte Ltd., Singapore).

2.3. Germination Tests

Beforehand, germination was tested and was as high as 100%. Seeds were germinated at a constant temperature (*T*) of 25 °C, which is considered the optimum for castor seed germination [12,17], maintained in a thermostatically controlled incubator (±1 °C) in dark conditions. For each genotype and salt level, four replicates of 20 seeds each were placed

in Petri dishes (Ø 120 mm), containing two filter papers—one above and one below the seeds—to allow a complete seed hydration. Each paper filter was moistened with 5 mL of one of the NaCl solutions. Petri dishes were hermetically sealed with parafilm to prevent evaporation, then randomized in the incubator.

The seeds were kept in the dishes to assess germination. This was scored when radicles emerged from the seeds (at least 2 mm long). Data were collected daily until no additional germination occurred for one week.

2.4. Radicle Length Measurement

Radicle length was measured for each genotype and salt level. For this purpose, on the fourth day from the recorded start of germination (i.e., first radicle appearance), five seeds were randomly chosen from those that germinated first in each Petri dish. Only 5 seeds were considered in order to allow for the evaluation of the same number in all the treatments. Seeds were photographed to document radicle length at the different salt levels.

Photos were taken using an iPhone X smartphone. Scanned images were then analyzed using ImageJ software Java 8, an open-source Java image program used for processing digital images, developed at the National Institutes of Health (NIH) [18]. Radicle elongation was measured just by using the cursor. To this end, the program required the setting of the correct metric in the image, easily calibrated using a ruler.

2.5. Calculations and Data Analysis

Data on final germination percentage (FGP, %), previously arcsine-transformed, and those on root length were checked for normality and homogeneity of variance and statistically analyzed by a completely randomized two-way ANOVA. The data were processed using CoStat version 6.003 (CoHort Software). ANOVA was conducted considering osmotic potential and genotype as fixed factors. When 'F' ratios were significant, means were separated by Tukey's test ($p < 0.05$).

The course of cumulative germination over time was described separately for each genotype and salt level using a sigmoidal equation with three parameters (SIGMAPLOT 9.0 software, Systat Software Inc., San Jose, CA, USA):

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \tag{1}$$

where a is the maximum value of germination, x is the time after seed imbibition (hours), x_0 is the time (hours) to 50% of seed germination, b is a fitting parameter of the curve. The x values on the curve that corresponded to specific y values on the curve (g fractions of seed germination), i.e., 10, 30, and 50%, the latter when achieved, were assumed as theoretical time (t_g) to 10, 30, and 50% germination.

To estimate the base water potential (ψ_b) to attain germination of g fraction at each salt level, the reciprocal of germination time ($1/t_g$ or GR_g) was regressed vs. ψ. As the abscissa intercept on the temperature axis is assumed as an estimate of the theoretical minimum temperature for germination [19], likewise, the intercept on ψ axis (that of abscissa) may be assumed as an estimate of the theoretical minimum water potential for germination of g [$\psi_{b(g)}$] [20,21].

To estimate $\psi_{b(g)}$ of all the observed germination percentages at each t_g, the equation which describes the hydrotime model [22]:

$$\theta_H = [\psi - \psi_{b(g)}]\, t_g \tag{2}$$

where θ_H is the hydrotime (MPa h) needed for g seed fraction to germinate, ψ is the actual water potential, $\psi_{b(g)}$ is the base water potential ψ for germination of g, and t_g is the time to germination of g, was modified as follows [23]:

$$\Psi_{b(g)} = \psi - \left(\frac{\theta_H}{t_g}\right) \tag{3}$$

where θ_H is the mean hydrotime of g fractions (10, 30 and 50%) calculated as a reciprocal of the slope (b coefficient) of the regression line of GR_g ($1/t_g$ vs. ψ) [24].

The time courses of the observed cumulative germination percentages were linearized on a probability scale and regressed vs. $\Psi - (\frac{\theta_H}{t_g})$, which corresponds to $\psi_{b(g)}$, according to the following equation [23]:

$$\text{probit}\,(g) = [\psi - (\frac{\theta_H}{t_g}) - \psi_{b(50)}]/\sigma\psi_b] \tag{4}$$

which models the germination time course at different water potentials.

Different values for θ_H were used in repeated probit regressions and the value which best fit to all data was assumed as the optimal one. The reciprocal of the slope (b coefficient) of the linear regression corresponded to the standard deviation $\sigma\psi_b$ of the seed lot. The $\psi_{b(50)}$ (i.e., ψ_b for 50% germination) corresponded to the value on the regression line at which probit is equal to zero. The estimated values for θ_H, $\psi_{b(50)}$, and $\sigma\psi_b$ were assumed as the hydrotime parameters.

3. Results

3.1. Cumulative Germination Time Course

Since no germination occurred at -1.2 MPa, this salt level was not considered for any of the following analyzed traits.

The cumulative seed germination time course of the six genotypes of castor is illustrated in Figure 1. The course is well described ($R^2 > 0.99$) by the three-parameter sigmoidal function (Equation (1)), whose trend reveals a short initial phase of low germination, negligible at 0 MPa, followed by a sharp increase in germination up to the maximum in all genotypes. The 'Local' castor, which reached maximum germination (>90%) after 48–96 h, was the fastest to germinate at low levels of salt stress (in distilled water and at -0.3 MPA). Similarly, the genotypes 'C1019' and 'C1008' were fast to germinate at -0.3 MPa, reaching 50% germination after 96 h.

Overall, further increases in salt concentration slowed down and depressed germination. At -0.6 MPa, the 'C1019' genotype reached 50% germination after 168 h (7 days), and the 'C857' and 'Local' genotypes germinated to 50% in 192 h (8 days). At the maximum level of salt stress at which seeds germinated (-0.9 MPa), 'C1019' and 'C857' exhibited the highest tolerance, approaching 50% germination in approximately 200 h (less than 10 days).

3.2. Final Seed Germination Percentage (FGP) under Salt Stress

The final seed germination percentage (FGP) was full (100%) under optimal conditions (0 MPa, control) in all the genotypes (Figure 2). The FGP was slightly reduced to 81% at -0.3 MPa, but at lower ψ (-0.6 and -0.9 MPa), the germination decrease was evident (on average to 47 and 46%, respectively).

Across salt concentrations, the FGP significantly differed by genotype (G, $p < 0.001$), being the highest in the 'Local' and 'C1019' genotypes (84.3 and 85%). Good levels of germination were also achieved by 'C857' and 'C856' (75.8 and 68.3%, respectively). The lowest germination occurred in 'C1013' (44.3%) and in 'C1008' (53.3%) and was not significantly different.

Significant S × G interactions ($p < 0.001$) indicated different FGP responses to salt levels depending on genotype. The 'Local' castor was the only one to germinate to the maximum level (100%) at both 0 and -0.3 MPa, indicating a tolerance to low levels of salt stress. Under the same stress conditions, seed germination was also high in 'C856', 'C1019' (93.3% in both), and 'C857' (90%). Genetic differences in FGP became extremely evident when higher levels of salt stress were imposed (-0.6 and -0.9 MPa). This result was clearer at -0.9 MPa, under which conditions 'C1008' and 'C1013' germinated to 23.2 and 16.8%, respectively, confirming their scarce tolerance to salt stress.

3.3. Radicle Length Measurements

No stress conditions (control, 0 MPa) allowed a regular development of the radicle, which reached 1.72 cm in length on the fourth day across all genotypes (Figure 3). Interestingly, a low level of salt stress (−0.3 MPa) induced a slight but not significant decrease in radicle length (1.53 cm) with respect to the control. The length decrease became significant at −0.6 MPa (1.20 cm), and at −0.9 MPa, radicle length was reduced to 0.95 cm (i.e., 45% lower than the control at 0 MPa).

'Genotype' also significantly influenced radicle length (G, $p < 0.05$). Across salt concentrations, the longest radicles ($\geq$1.52 cm) were measured in 'C1019' and 'C856'. The smallest radicle (0.95 cm) was measured in 'C1013'.
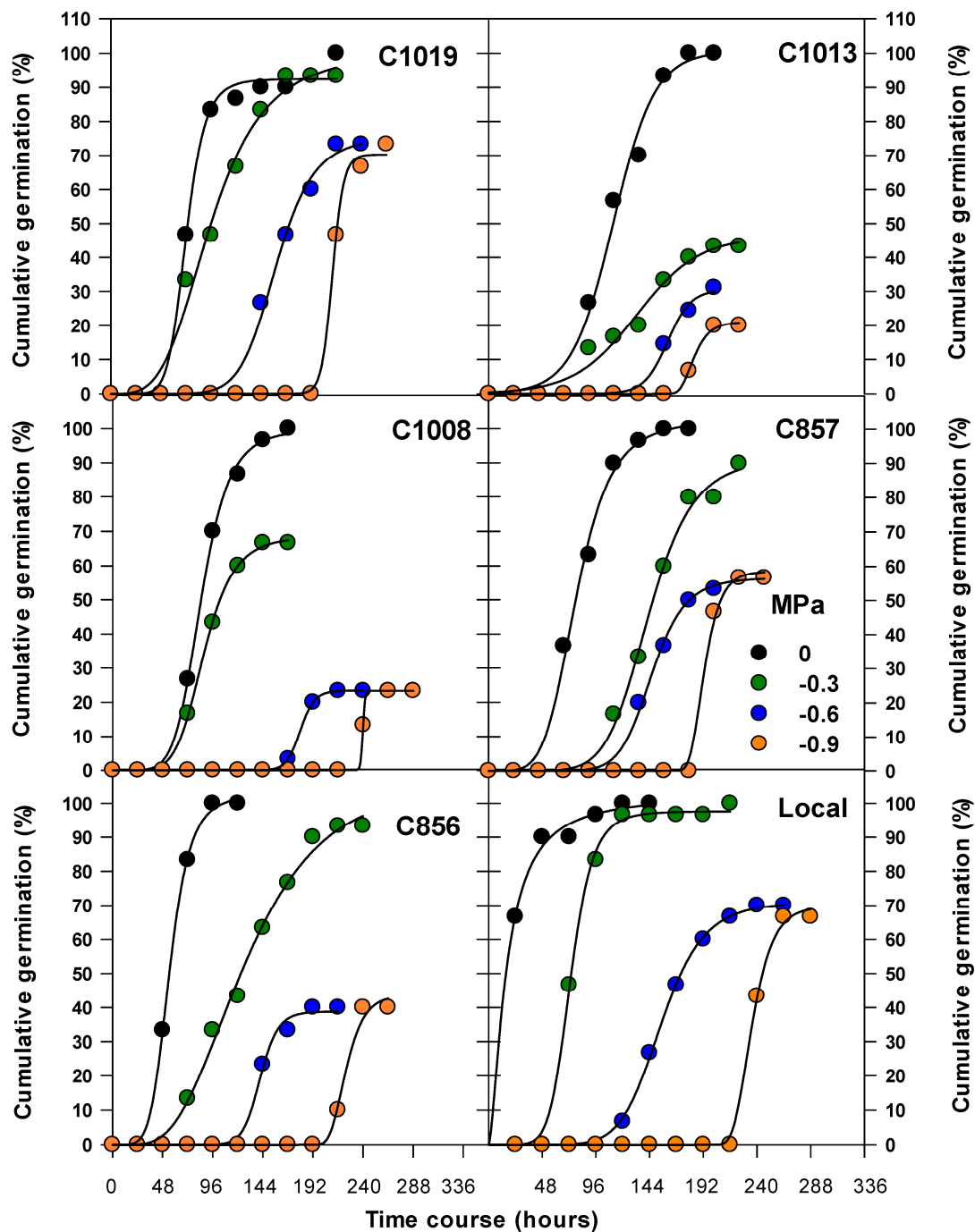
Figure 1. Cumulative germination time courses (solid curves) at different temperatures in 6 genotypes of castor. Symbols represent the observed percentages at 0, −0.3, −0.6, and −0.9 MPa vs. time.
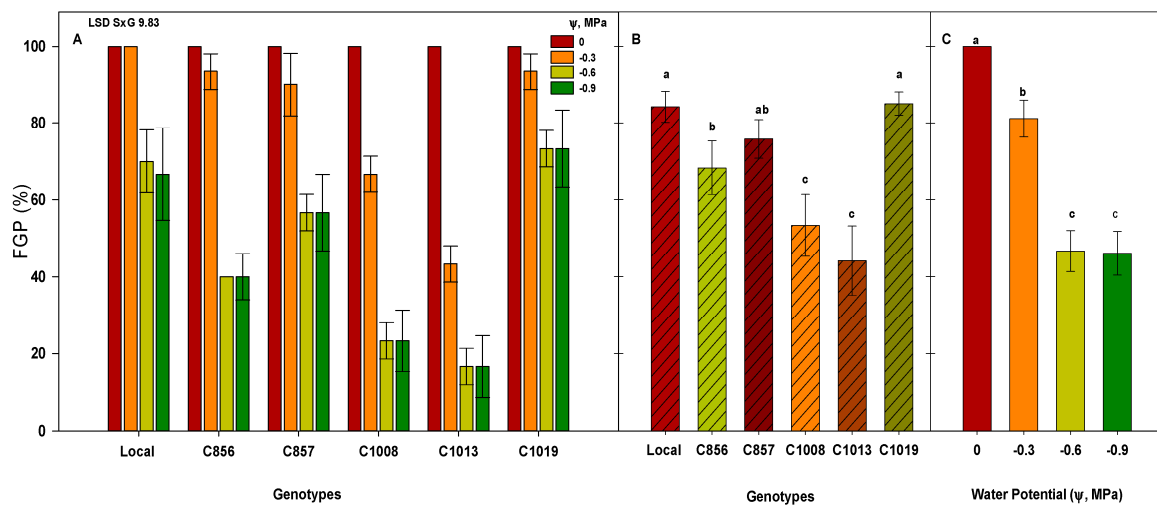
Figure 2. Final germination percentage (FGP, %) in six genotypes of castor at four different salt levels (0, −0.3, −0.6, −0.9 MPa) (*n* = 4) (**A**). Average values are reported per genotype (**B**) and water potential (**C**). Values with the same letter are not significantly different at *p* < 0.05 level. LSD value for S × G (*p* < 0.05) is reported.
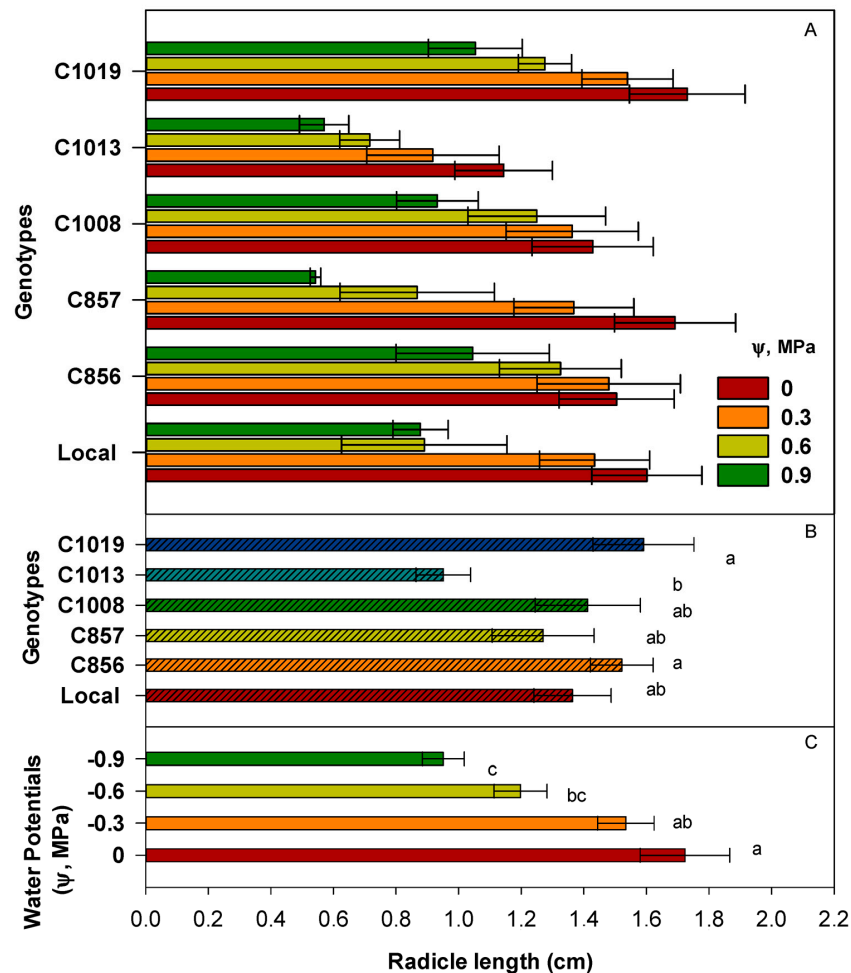
Figure 3. Radicle length (cm) in six genotypes of castor at four different salt levels (0, −0.3, −0.6, −0.9 MPa) (*n* = 4) (**A**). Average values are reported per genotype (**B**) and water potential (**C**). Values with the same letter are not significantly different at *p* < 0.05 level. LSD value for S × G (*p* < 0.05) is reported.

3.4. Hydrotime Model and Estimated Parameters

The seed germination response to salt stress of each castor genotype was assessed by hydrotime analysis (Figure 4). The analysis evidenced a wide genetic variability for $\Psi_{b(50)}$, as estimated by the model (Table 1). The lower the Ψ_b, the higher the tolerance to salinity stress. In particular, the results highlighted that 'C1019' was the most tolerant genotype, having a -1.39 MPa $\Psi_{b(50)}$, rather close to that calculated by the *x*-axis intercept of GR_{50} vs. ψ (-1.19 MPa). High salt tolerance was also found for 'C857', which had a -1.23 MPa $\psi_{b(50)}$, as estimated by the model (-1.07 MPa as calculated). 'C856' was the most susceptible to salt stress during germination, with a -0.75 MPa $\psi_{b(50)}$ (estimated). A -0.91 MPa $\psi_{b(50)}$ was estimated by the hydrotime model for the 'Local' castor (very close to the calculated -0.92 MPa), which, therefore, exhibited a moderate tolerance to salt stress.
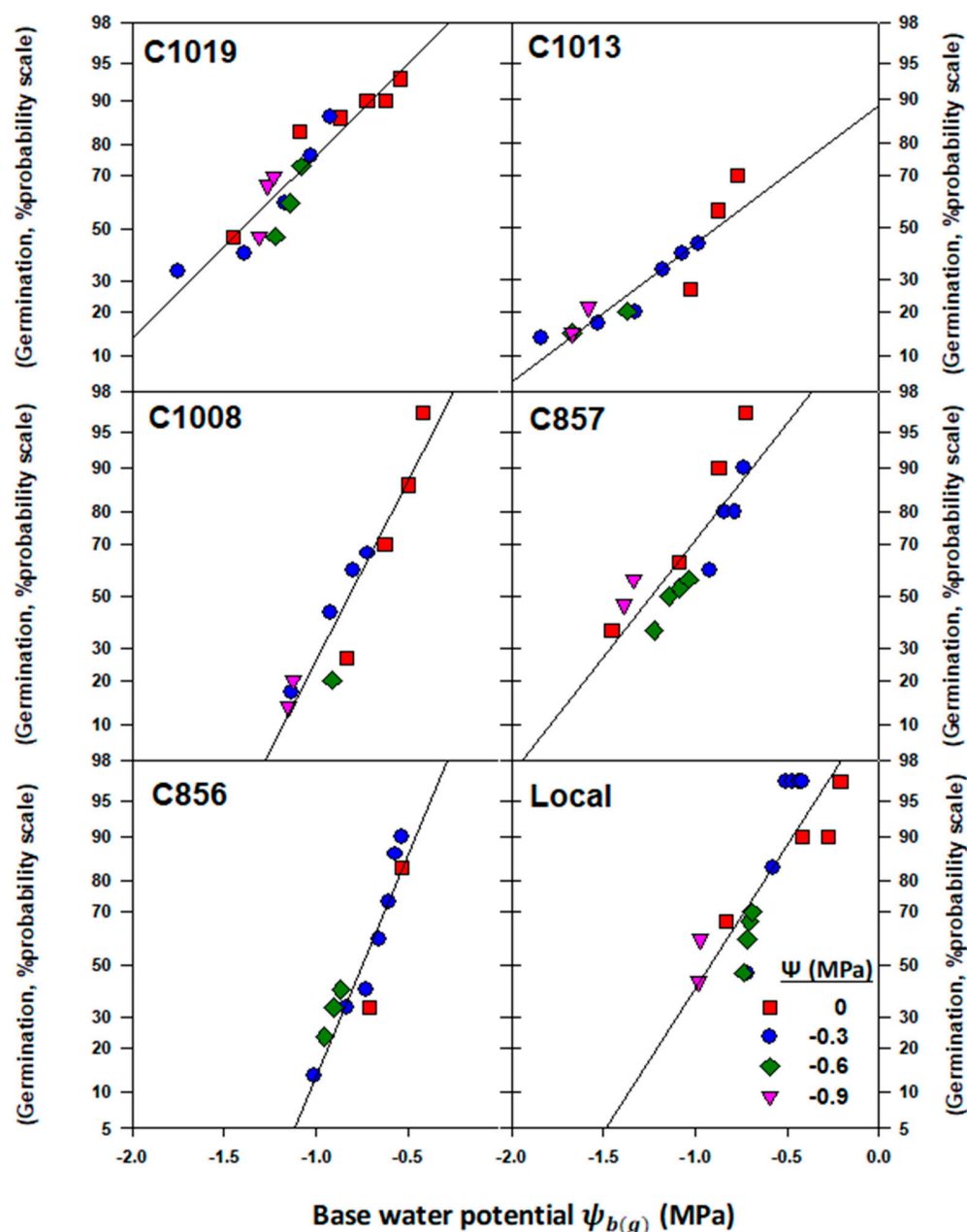
Figure 4. Probit analysis of germination of dwarf and 'Local' castor seeds at different water potentials (Ψ). Seeds were germinated at 25 °C, testing four different salt levels (0, -0.3, -0.6, -0.9 MPa). Percentages (symbols) of germination time courses from Figure 1 were plotted as a function of $\Psi - (\theta_H/t_g)$, which is equivalent to $\Psi_{b(g)}$. The parameter values of the fitted line are reported in Table 1.

Table 1. Calculated (from *x*-axis intercept of GR$_{50}$ vs. Ψ) and estimated (from hydrotime model) values of base water potential for 50% germination [Ψ$_{b(50)}$] and calculated (from the inverse of the slope *b* of *x*-axis intercept of GR$_{50}$ vs. Ψ) and estimated (from model) values of hydrotime (θ_H) in six genotypes of castor. Standard deviation ($\sigma\Psi_b$) is the estimated parameter of the hydrotime model, obtained from the reciprocal of the slope of probit germination regression against ψ_b. Coefficient of determination (R^2) is also reported.

Genotype	Ψ$_{b(50)}$ (MPa)	Ψ$_{b(50)}$ (MPa)	$\sigma\Psi_b$ (MPa)	R^2	θ_H (MPa h)	θ_H (MPa h)
	Calculated	**Estimated**			**1/b ± σ**	**From Model**
Local	−0.92	−0.91	0.35	0.71	30.6 ± 0.09	20.0
C856	−0.55	−0.75	0.22	0.91	38.1 ± 0.40	51.0
C857	−1.07	−1.23	0.42	0.74	90.5 ± 0.66	110.0
C1008	−0.53	−0.82	0.28	0.91	45.9 ± 0.18	60.0
C1013	−0.53	−0.88	0.72	0.83	87.7 ± 0.07	147.5
C1019	−1.19	−1.39	0.55	0.87	88.6 ± 0.33	104.5

The analysis highlighted that θ_H, as estimated by the model, was the highest in 'C1013' (147.5 MPa h). This genotype also exhibited a low $\psi_{b(50)}$, both estimated and calculated (−0.88 and −0.53 MPa, respectively). A similarly high θ_H (<100 MPa h) was also estimated for 'C1019' and 'C857', i.e., the genotypes that, in turn, exhibited the highest tolerance to salt stress in terms of $\psi_{b(50)}$. Quite a low θ_H was estimated (20.0 MPa h) and calculated (30.6 MPa h) for 'Local' castor.

Figure 5 shows how the ψ_b of each seed lot varies, following a Gaussian distribution. The uniformity of germination is described by $\sigma\psi_b$, calculated as the inverse of the slope of the fitted line (probit analysis). A wide $\sigma\psi_b$ was calculated for the genotypes 'C1013' (0.72) and 'C1019' (0.55), both also exhibiting the highest ψ_b (the least negative).
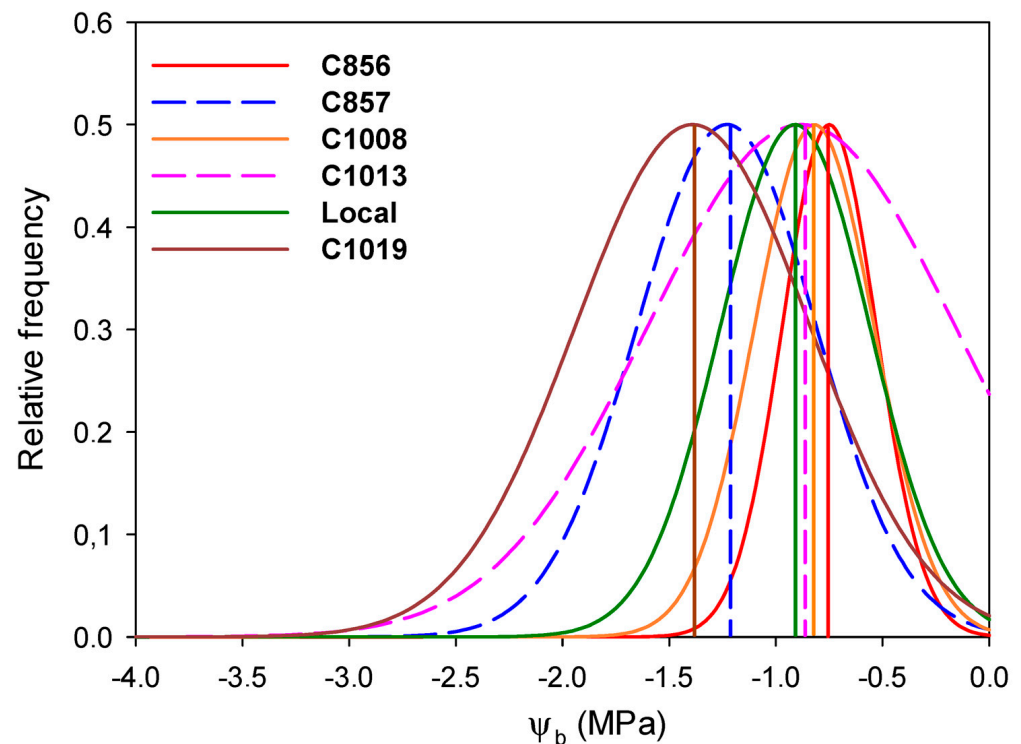
Figure 5. Normal distribution of relative frequencies of Ψ$_{b(g)}$ values for different genotypes of castor. Vertical lines indicate Ψ$_{b(50)}$ values in *x*-axis for castor seeds as estimated by hydrotime model.

4. Discussion

The impact of abiotic stresses, such as salinity, on seed germination and later on seedling growth has been widely studied [25–27], but still remains a considerable issue in agricultural crops.

Salt (NaCl) stress inhibits the water uptake of seeds due to an increase in osmosis activity, which negatively influences both the rate of germination, thus delaying seed germination, and subsequent plant establishment and growth and, ultimately, final yield [28].

Numerous studies have highlighted how germination and radicle growth are the most critical stages of crop life [26,29,30], including castor [12]. Despite some studies documenting castor as a salt-tolerant crop [31,32], most research has always focused merely on its agronomic traits, such as seed productivity and oil yield, content, and quality [33,34]. Little is known about the effects of salt stress on the very early stages of its growing season, i.e., germination and seedling emergence. In the present research, seed germination performance under salt stress conditions was assessed in different genotypes of castor.

In this study, in most genotypes, a slight stress (-0.3 MPa) resulted in a small delay in the start of seed germination, while final germination approached the same levels as under no stress conditions. Similarly, a slight delay in the start of germination but with the same final germination as that under no salt stress was recorded at -0.5 MPa water potential in seeds of several genotypes of carob [35]. However, higher levels of salt stress (up to -0.9 MPa) prolonged the initial phase of low germination and significantly depressed germination in all genotypes of castor in our study, indicating a great susceptibility of castor to salinity. At -1.2 MPa, germination was totally suppressed. Similarly, Han et al. (2022) [36], working on castor seeds germinated under different levels of alkalinity, reported a low tolerance to osmotic stress in terms of germination, which was reduced by 65% even at 150 mM $NaHCO_3$.

The mechanism of plants' water uptake from the soil when their water potential is lower than that of the soil is extensively known [37]. Salt limits plant growth due to an osmotic impact and a toxic effect. Indeed, an excess of soluble salts reduces the water potential of the soil around the roots by limiting water absorption, causing both osmotic stress and a water deficit [13].

Tolerance to osmotic stress manifests itself in a decrease in cellular expansion in the roots and leaves, decreasing stomatal conductance to save water [38]. Moreover, according to the literature, the delay and decrease in seed germination due to increasing levels of salt stress are strictly attributable to Na^+ accumulation. Secondly, the ability of Na^+ accumulated by seeds to compete with K^+ for binding sites leads to severe ionic stress and to an interference with essential cellular activities [13,39].

According to Farrooq et al., 2017 [40], who worked on grain legumes, a higher salt concentration in the soil inhibits seed germination by creating a low osmotic potential around the seed, which limits water uptake while also impacting seed metabolic processes. Similarly, Ullah et al. [41], by evaluating seed germination in barley, found a proportional decrease in final seed germination as water potential reduced and confirmed that stress correlated to Ψ influences seed germination.

Overall, across all levels of salt concentration, all the genotypes studied showed the same radicle growth rate, as measured on the fourth day after the start of germination, except 'C1013', whose radicle growth was rather low. A similarly slow radicle growth was recorded in this genotype in a previous work by Cafaro et al. [12].

Increasing salt stress levels in this study also adversely affected radicle growth, potentially preventing normal plant establishment. Similar adverse effects on radicle growth were reported by Li et al. [42], who, working with seeds of sunflower (*Helianthus annus* L.), found that soil salinization strongly affected seed germination and seedling growth and development.

The hydrotime model provides useful information on seed germination characteristics under stress conditions, defining the tolerance or sensitivity of a variety to salt stress [43]. Its parameters are hydrotime requirements (θ_H), which define the speed of germination,

and base water potential (Ψ_b), which indicates the minimum potential (Ψ) of a seed lot to reach germination. Therefore, these parameters greatly influence subsequent plant establishment (plant population) and potential productivity in unfavourable environment, such as marginal lands.

The model highlights that genotypes that have a similar ψ_b can have a different θ_H to germinate or, on the contrary, a different ψ_b but a similar θ_H. In this study, the 'Local' and 'C1013' genotypes of castor had a ψ_b of -0.91 and -0.88 MPa, respectively, but their θ_H was quite different (20 and 147.5 MPa d, respectively). Differently, the genotypes 'C857' and 'C1019' had a different ψ_b (-1.23 and -1.39 MPa, respectively) but a similar θ_H (110 and 104.5 MPa d, respectively).

$\sigma\Psi_b$ is another important parameter of the hydrotime model. It indicates the germination distribution of a seed lot depending on the ψ_b of each g fraction, i.e., the susceptibility of a given genotype to salt stress during germination. A high value of $\sigma\Psi_b$ indicates a higher chance of germinating at low (more negative) ψ values. In this regard, the genotypes 'C856' and 'C1008' had the smallest $\sigma\Psi_b$; therefore, their chance to germinate at a low ψ is restricted. On the other hand, 'C1019' and 'C1013', having a high $\sigma\Psi_b$, may better germinate under increasing salt stress conditions.

The variability in salinity tolerance during germination found allows us to select the most tolerant genotypes among those studied. Likewise, a wide genetic variability for tolerance to low temperatures during germination was found in a previous work by Cafaro et al. [12]. Indeed, the 'Local' castor was described as the most tolerant genotype to the lowering of temperatures. Similarly, in this study, the 'Local' genotype had the lowest θ_H requirements, thus being appointed as the best candidate genotype to be cultivated in soils affected by salt and for early sowings [6] as long as soil ψ_b does not drop below levels of -0.9 MPa.

On the contrary, the dwarf genotype 'C1019' was slower to germinate due to its high value of θ_H. However, in the previous work, it showed a great tolerance to high temperatures [12], which sped up the germination and water uptake processes of the seeds [20], making it the best candidate to be used in late sowings and in very low ψ_b conditions, which can easily be found in marginal lands.

5. Conclusions

Castor, like all crops, suffers with salinity stress, and the main phenological stages affected are the earliest ones, i.e., germination and subsequent seedling growth. As salinization is one of the leading obstacles to the exploitation of marginal lands, which could be enhanced by non-food cultivation, evaluating castor's tolerance capacity and its methods of assessment is fundamental.

This study highlighted that the genotypes 'C856', 'C1013', and 'C1008' are more susceptible to salt stress compared to the genotypes 'C1019' and 'C857' in terms of the different agronomic traits studied, confirming these latter two genotypes are the most tolerant among the dwarf hybrids. The 'Local' castor, selected for the Mediterranean environment, had greater tolerance to salinity stress with respect to some dwarf genotypes, as demonstrated by the hydrotime model, which accurately predicted germination and germination time, thus being a valid candidate for marginal land exploitation.

Author Contributions: Conceptualization, V.C. and C.P.; Methodology, V.C.; Software, V.C.; Validation, E.A., S.L.C. and C.P.; Formal analysis, V.C.; Investigation, V.C. and C.P.; Resources, S.L.C.; Data curation, V.C. and C.P.; Writing—original draft, V.C.; Writing—review & editing, S.L.C. and C.P.; Visualization, V.C.; Supervision, E.A. and S.L.C.; Project administration, S.L.C.; Funding acquisition, S.L.C. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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

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