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Is Drought Stress Tolerance Affected by Biotypes and Seed Size in the Emerging Oilseed Crop Camelina?

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Abstract: One of the main advantages of camelina (*Camelina sativa* (L.) Crantz) is its wide environmental adaptability and extreme drought tolerance. The availability of both winter and spring camelina biotypes, characterized by different seed sizes, raises the question about possible differences in their response to drought stress at the emergence stage. To address this, a germination test was set up in controlled conditions, comparing six winter and six spring genotypes with differing seed sizes (ranging from 1.83 to 0.88 g/1000-seeds) under increasing levels of osmotic stress (0, −0.4, −0.8, −1.2, −1.4, −1.6 MPa) using polyethylene glycol (PEG). Camelina withstands mild level of osmotic stress (−0.4 MPa) without significant decrease in germination. Even at −1.2 MPa after 10 d, it still had 75% germination. Significant differences in germination were observed between biotypes, where spring biotypes performed better than winter ones. Shoot and radicle lengths were significantly diminished by imposed osmotic stress, but shoot growth seemed more impacted. In general, spring biotypes had longer shoots and radicles than winter ones. Seed size played a role in the response of camelina to drought, but it depended on biotype and stress level imposed. In particular large seeded spring types had the highest germination percentage and resulted less impaired by osmotic stress, otherwise among the tested winter types the small seeded ones were the best performing. The presented data could be useful for breeding purposes for selecting the appropriate camelina type for sowing in drought-prone regions.

Keywords: osmotic stress; polyethylene glycol (PEG); shoot length; radicle length; seed weight; mucilage

1. Introduction

Abiotic stresses, such as drought, salinity and extreme temperatures are becoming, more and more frequently, serious threats to agriculture as effects of climate change [1,2]. Abiotic stresses often account for significant production losses, even halving the potential yields of the majority of staple crops [3–6]. One of the most important abiotic factors limiting seed germination and early seedling growth is water stress [7–9]. Seed germination and seedling establishment are the most critical phases, determining successful crop production [8] and, at the same time, the most sensitive stage in the plant growth cycle [10,11]. In fact, crop establishment depends on the interaction between seedbed conditions,

like the occurrence of drought [12], and seed quality [13,14]. Seed germination can occur only if specific combinations of environmental conditions are present in the field, such as sufficient temperature, moisture availability, and light [15]. Since germination can only begin in the presence of sufficient water, this process and the initial seedling emergence are considered crucial life stages in case of drought stress. The process of seed germination is driven by the different levels of osmotic potential between the dry seeds and the water in the germination surroundings. Osmotic stress in experimental conditions is often induced by the application of a solution of polyethylene glycol 6000 (PEG 6000), causing a state of physiological drought, preventing the plant from absorbing water.

The emerging oilseed crop, camelina (*Camelina sativa* (L.) Crantz), is gaining interest worldwide in relation to its broad environmental suitability [16–18] matched with satisfactory seed yield [19], which encourages a large portfolio of end-uses, either for food, feed or non-food applications. Camelina is a native species of Eurasia [20] and is diffused worldwide. Despite being not yet extensively cultivated, camelina has been identified as a much more resilient species [21,22] than the “sister crop”, oilseed rape (*Brassica napus* L. var. *oleifera*), in relation to less input need, lower pest and disease pressure, and better resistance to abiotic stresses (i.e., temperature, drought, salinity, etc.). The availability of both spring and winter biotypes, characterized by distinctive morphological traits both at the seed and plant level [19,23], further enlarges the camelina cultivation area, making this oilseed crop one that is possibly growing almost everywhere in the world, as reported in the literature. One of the main advantages of camelina compared with oilseed rape is the extreme drought tolerance, also at germination phase [10]. The presence of mucilage in camelina seeds, which account for about 7–10% of the seed mass [24], might confer this oilseed crop a great ability to overcome drought stress at early seedling stage. Unfortunately, when camelina seeds are processed for feed uses, the mucilage sticks to the cake lowering its value, causing slow fat assimilation in the livestock [25]. For a low input species, characterized by small seed-size, like camelina, the establishment of a satisfactory stand is one of the key issues for the achievement of adequate seed yields [26]; since herbicides are not yet selective toward camelina, only space and resource competition against weeds can be a possible solution [27]. Camelina breeding is focusing on, among other traits, selecting genotypes characterized by larger seed size [16] as a way to achieve increased seed yield, as well to improve seed processing phases. Usually in annual plant species, large-seeded varieties are characterized by higher plant vigor at all growth stages since emergence [28,29]. Seed germination is also related to seed characteristics, such as seed size (weight), which varies noticeably among and within species [30]. Principally, large-seeded species compared with small-seeded ones have seedlings more tolerant to many stresses occurring at germination and establishment phases [31]. Camelina seed has a surface area of 0.062 cm², which is ×2 times smaller than that of *B. napus*, but ×17 times larger than *Arabidopsis thaliana* [32]. As well as other organs of camelina, mature seeds have a wax layer with distinctive composition [33]. According to [33], fatty alcohols in camelina seed waxes are C28–C32 in length, much longer than the predominant fatty alcohols found in the waxes of other analyzed organs. The same authors proposed that these surface waxes may confer, in part, the relative tolerance of camelina to various abiotic and biotic stresses. Camelina seeds have a large prominent columella and thin radial cell walls [34]. Upon the imbibition, camelina seeds release mucilage, which is synthesized in the seed coat [34]. Mucilage is in close contact with the seed external surface, which allows a relatively high rate of water uptake [35] and may explain camelina drought resistance at early growth stages [36].

Both spring and winter camelina genotypes with larger seed size are now available, but nothing is reported yet about their different ability to overcome drought stress at emergence and seedling stages, in comparison with normal-sized camelina genotypes. Otherwise, some studies have been conducted to compare the tolerance of different spring camelina genotypes to drought-induced osmotic stress [37]. Furthermore, in Mediterranean environments [21,38] spring camelina genotypes adapt well also to fall sowing, usually achieving higher seed yield than real winter camelina lines [39].

The aim of the present study was to investigate the effects of drought stress induced by polyethylene glycol on seed germination and early seedling growth of camelina, at the same time examining whether camelina drought tolerance can be influenced by seed size and/or biotype.

2. Materials and Methods

2.1. Genetic Material and Its Characterization

Twelve commercial camelina varieties were compared in the present study, six winter and six spring (Table 1). The set of camelina varieties has been chosen as representative of different genetic sources, and consequently breeding programs, as well as including all the commercial winter varieties available at the moment of the study. Before the start of the germination trial, the mean thousand seed weight (TSW) of each camelina variety was assessed in 8 replicates of 100 seeds each. Using a similar approach as suggested by [29], camelina varieties were then grouped with respect to their weight as: A (large seeded = $TSW > 1.27$ g), B (normal seeded = 0.98 g $< TSW < 1.27$ g), and C (small seeded = $TSW < 0.98$ g). Seed weight classes were statistically defined as: A (the upper quartile), C (the lower quartile) and B the central two quartiles. Before the start of the germination trial, the mucilage content of camelina seeds was also determined [40], on two replicates of 1 g of seeds for each genotype. The water extractable mucilage content is reported in Table 1.

Table 1. List of tested camelina varieties in the germination study with respective seed providers, biotype, thousand seed weight (TSW, g), TSW class, and mean mucilage content (%). Biotype: S = spring, W = winter. TSW class: (A) $TSW > 1.27$ g; (B) 0.98 g $< TSW < 1.27$ g; (C) $TSW < 0.98$ g.

Variety Name	Biotype	Provider	TSW (g)	TSW Class	Mucilage (%)
Midas	S	Smart Earth Camelina (Saskatoon, SK, Canada)	1.26	B	10.63
Cypress			1.60	A	10.80
EICA		Landesbetrieb Landwirtschaft Hessen (Alsfeld, Germany)	1.83	A	10.41
NS Zlatka			IFVCNS (Novi Sad, Serbia)	1.01	B
Omega		Poznan University (Poznan, Poland)	0.96	C	12.06
Calena		Saatbau Linz (Leonding, Austria)	1.13	B	7.64
Joelle	W	R. Gesch, USDA-ARS (Morris, MN, USA)	0.88	C	10.86
WG1			1.11	B	13.78
WG4		C. Rife, High Plains Crop Development (Torrington, WY, USA)	1.16	B	15.01
Bison			1.31	A	9.49
Luna		Poznan University (Poznan, Poland)	0.99	B	11.46
Merlin		J. Vollmann, BOKU University (Vienna, Austria)	0.93	C	10.86

2.2. Seed Germination Test

The study was conducted under controlled conditions at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Germination of camelina seeds was tested at 20 °C and under an 8/16 h light/dark cycle, in a completely randomized design. Testing was performed in three replicates of 100 seeds each. Germination was surveyed in Petri dishes (100 × 15 mm) on double layer filter paper, at six levels of osmotic stress (Control = 0.0, −0.4, −0.8, −1.2, −1.4 and −1.6 MPa). The osmotic potential of the solution was developed using polyethylene glycol (PEG, molecular weight 6000, Merck KgaA, Darmstadt, Germany), using a previously reported formula [41]. To prevent changes in osmotic potential during the trial, the filter paper was changed every other day. Seeds were considered germinated when radicle was at least 2 mm long. Germination was recorded on d 4 and d 10 after stress imposition. Radicle and shoot lengths were manually recorded by means of a ruler on d 10.

2.3. Statistical Analysis

Prior to analysis of variance (ANOVA), the Bartlett's test were used to verify homoscedasticity of data variance, and germination percentage data were transformed into the square root. A three-way

ANOVA was used to test the effect of: the osmotic stress levels, the biotypes and TSW classes and their interactions. When the ANOVA test produced significant results, the LSD's test was used to separate means in different groups ($p \leq 0.05$).

3. Results

3.1. Germination Results

The results of the ANOVA carried out considering as main factors: osmotic stress level (OS), biotype and seed weight class (SWC), and their interactions are reported in Table 2. Camelina germination surveyed at 4 and 10 d after stress imposition was significantly influenced by the three factors considered ($p \leq 0.05$). In particular, after either 4 or 10 d since stress imposition, the mean germination of camelina exceeded 90% under both control and the mildest stress (-0.4 MPa), without reporting significant differences (Table 3).

Table 2. ANOVA table with F values and statistical significance of the germination percentage at 4 d (Germ d 4) and 10 d (Germ d 10) after stress imposition, and of radicle and shoot length. OS = osmotic stress (0, -0.4 , -0.8 , -1.2 , -1.4 and -1.6 MPa); biotype = winter vs. spring; SWC = seed weight classes, defined as (A) $TSW > 1.27$ g; (B) 0.98 g $< TSW < 1.27$ g; (C) $TSW < 0.98$ g.

Factors	Germ d 4	Germ d 10	Shoot Length	Radicle Length
OS	3335.20 ***	576.61 ***	1153.85 ***	549.26 ***
Biotype	67.19 ***	47.20 ***	7.18 **	36.73 ***
SWC	11.42 ***	8.10 **	0.89 <i>ns</i>	0.04 <i>ns</i>
OS \times Biotype	20.21 ***	6.71 ***	5.10 ***	3.65 **
OS \times SWC	3.91 ***	1.39 <i>ns</i>	1.03 <i>ns</i>	0.58 <i>ns</i>
Biotype \times SWC	20.21 ***	6.71 ***	5.03 **	1.86 <i>ns</i>
OS \times Biotype \times SWC	11.47 ***	2.88 **	2.60 **	3.16 ***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively (LSD's test); *ns* = not significant.

Table 3. Camelina germination percentage (%), surveyed at 4 and 10 days after stress imposition, as affected by the main factors: osmotic stress level (0 vs. -0.4 vs. -0.8 vs. -1.2 vs. -1.4 vs. -1.6 MPa), biotype (winter vs. spring), and seed weight classes ((A) $TSW > 1.27$ g vs. (B) 0.98 g $< TSW < 1.27$ g vs. (C) $TSW < 0.98$ g). Mean values \pm SE. Different letters: statistically different means within the same factor (i.e., osmotic stress, biotype, seed weight class) and germination day (i.e., d 4, d 10) for $p \leq 0.05$ (LSD's test).

Main Factors	Germination d 4	Germination d 10
	%	
Osmotic stress (MPa)		
Control (0.0)	94.42 \pm 0.71 a	95.19 \pm 0.62 a
-0.4	93.22 \pm 0.93 a	94.42 \pm 0.78 a
-0.8	77.28 \pm 3.03 b	89.22 \pm 1.33 b
-1.2	1.69 \pm 0.54 c	75.72 \pm 2.44 c
-1.4	0.00 \pm 0.00 c	28.31 \pm 3.59 d
-1.6	0.00 \pm 0.00 c	0.00 \pm 0.00 e
Biotype		
Spring	46.94 \pm 4.47 a	67.47 \pm 3.60 a
Winter	41.34 \pm 4.20 b	60.15 \pm 3.76 b
Seed weight class		
A	44.85 \pm 6.15 b	64.46 \pm 5.26 b
B	43.00 \pm 4.27 b	61.80 \pm 3.67 b
C	46.87 \pm 6.39 a	67.18 \pm 5.32 a

Nevertheless, at higher stress levels (> -0.8 MPa), camelina germination was significantly impaired by osmotic stress. Under -0.8 MPa stress, germination was about 77% at 4 d, and increased to 89% at 10 d after stress imposition, showing that osmotic stress was not only reducing germination, but also slowing the whole process. This result was even more evident under higher stress (-1.2 MPa), when camelina showed only 1.7% germination after 4 d, which was significantly increased to 75% after 10 d since stress imposition (Table 3). At osmotic stress levels of -1.4 MPa camelina seeds were not able to germinate after 4 d, but 28% germination percentage was observed at 10 d, while at -1.6 MPa camelina never germinated (Table 3).

Winter and spring biotypes reported significantly different ($p \leq 0.05$) germination percentages, with the latter steadily having about 10% higher germination than the winter ones at both survey dates (Table 3). Seed weight significantly affected camelina germination (Table 3), with lighter seeds (class C = TSW < 0.98 g) steadily showing the significantly highest value, i.e., ~47% at 4 d and ~67% at 10 d.

Significant differences ($p \leq 0.05$) in camelina germination emerged also for the interactions “stress level \times biotype”, “biotype \times seed weight class”, and “stress level \times biotype \times seed weight class” (Figures 1 and 2, Table 4). Concerning the interaction “stress level \times biotype” (Figure 1A), significant differences between spring and winter types emerged after 4 d, with the latter showing always lower germination percentages than spring ones ($p \leq 0.05$). In particular, at -0.8 MPa, germination of winter types was ~25% lower than those of the spring ones (67 vs. 80%, winter vs. spring types, respectively, $p \leq 0.05$). Those differences were even more pronounced at 10 d after stress imposition and at higher stress levels (≥ -1.2 MPa, Figure 1B). Spring types confirmed their superiority in germination particularly under higher stress, in fact they reported +18% at -1.2 MPa and +46% at -1.4 MPa compared with winter types. At -1.6 MPa none of the tested camelina genotypes were able to germinate.

Interestingly, a significant interaction between “biotype \times seed weight class” was also detected (Figure 2 and Table 4). After 4 d from stress imposition (Figure 2A), winter camelina genotypes characterized by smaller seed size (class C) and spring ones with large and medium seed size (classes A and B) showed similar germination (~47%), which was significantly higher than that reported by the large seeded winter genotypes (class A, 35%, $p \leq 0.05$). This finding was also confirmed after 10 d (Figure 2B), when the large seeded spring genotypes (class A) presented the significantly highest germination percentage (71%, but not different from spring types in the class C). Meanwhile, large seeded winter genotypes (class A) reported the lowest value (51%, $p \leq 0.05$). At 10 d (Figure 2B), the winter genotypes showed significantly lower germination, in detail class C $>$ class B $>$ class A ($p \leq 0.05$). While in the spring camelina genotypes significant differences emerged only between class A and B, while C was intermediate between the other two.

At stress levels ≥ -1.2 MPa, camelina germination was highly impaired and no significant differences emerged among types and seed classes, irrespective of the stress level. After 10 d, the germination of the tested camelina genotypes was satisfactory (~90%) under control conditions, and -0.4 and -0.8 MPa for all the combinations of biotypes and seed weight classes, apart from large seeded winter types (class A) that confirmed their lower germination ($p \leq 0.05$, Table 4). Interestingly, at -1.2 MPa, spring types, irrespectively of seed size, and small seeded winter types (classes C) still presented a germination percentage of ~87%, while winter types in classes A and B had only 60% of germination ($p \leq 0.05$). At -1.4 MPa, differences were even more pronounced: spring types, in classes A and C, reported mean germination of ~46%, which was about double than that of spring types in class B and winter types in classes B and C (~24%), whilst large seeded winter types (class A) reported only 4% germination.

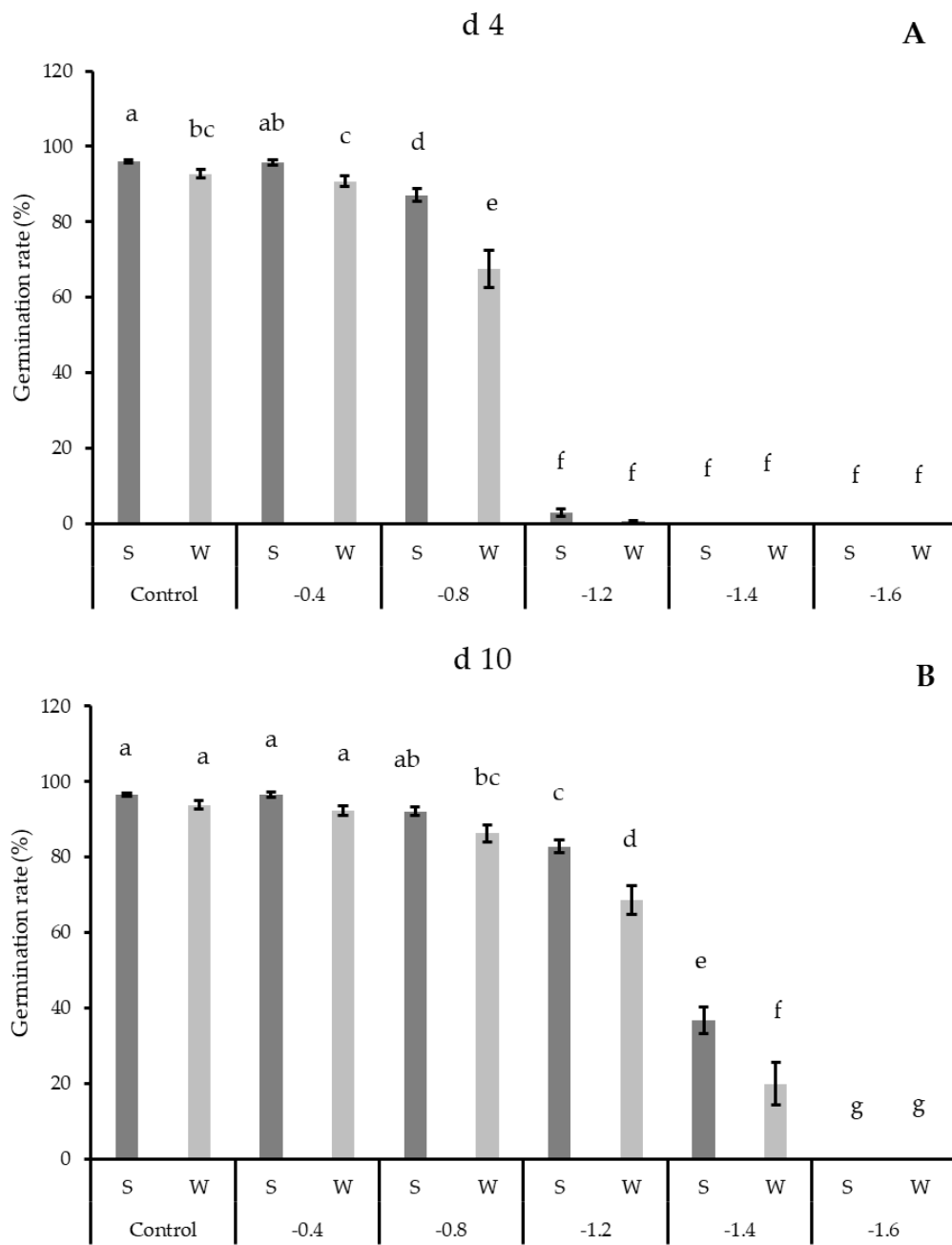


Figure 1. Camelina germination percentage (%), surveyed at 4 (A) and 10 days (B) after stress imposition, as affected by the interaction of osmotic stress level (Control = 0, -0.4, -0.8, -1.2, -1.4, -1.6 MPa) × biotype (winter, spring). Mean values ± SE. Different letters: statistically different means for $p \leq 0.05$ (LSD's test).

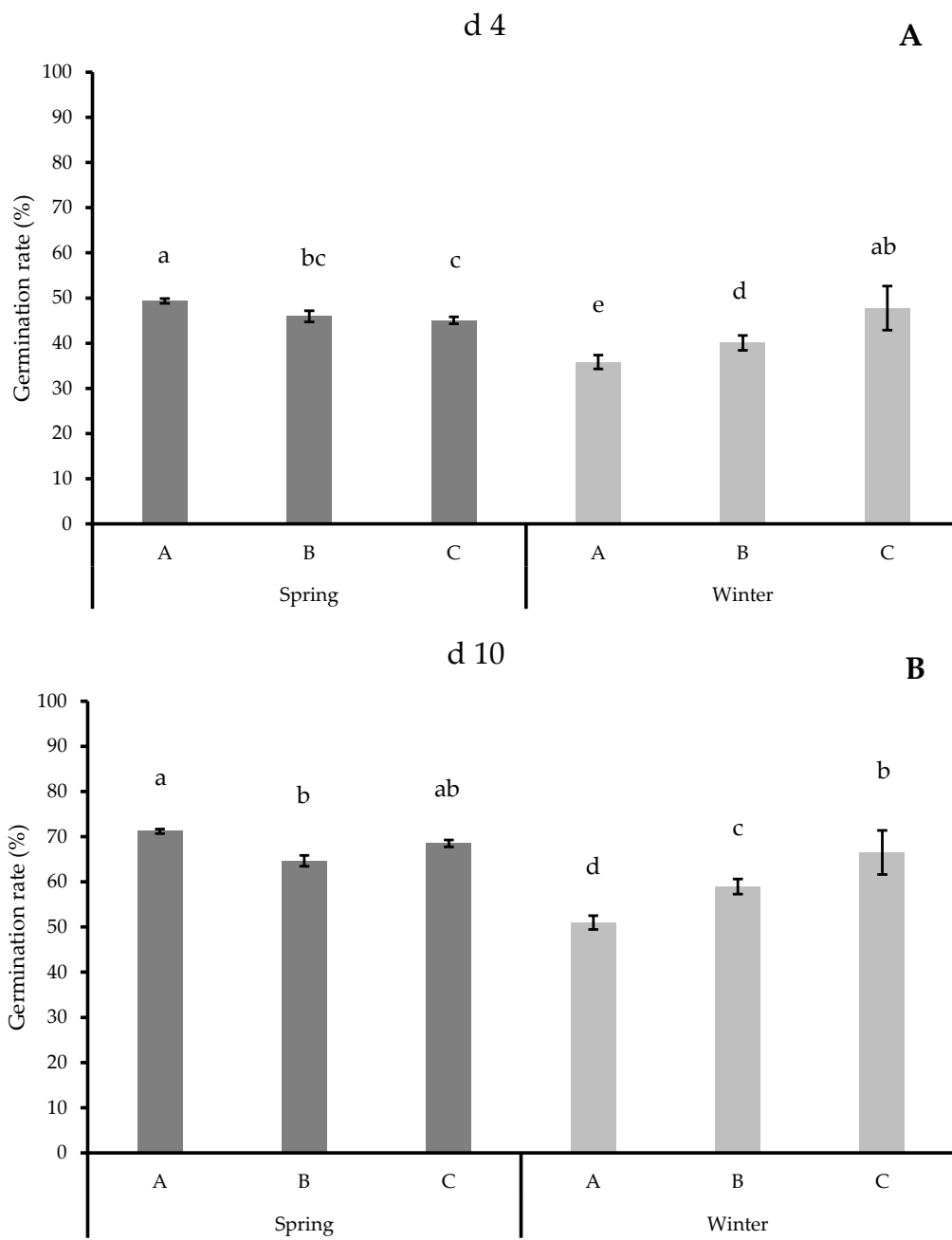


Figure 2. Camelina germination percentage (%), surveyed at 4 (A) and 10 days (B) after stress imposition, in response to the interaction biotype (winter vs. spring) × seed weight class ((A) TSW > 1.27 g vs. (B) 0.98 g < TSW < 1.27 g vs. (C) TSW < 0.98 g). Mean values ± SE. Different letters: statistically different means for $p \leq 0.05$ (LSD's test).

Table 4. Camelina germination percentage (%), surveyed at 4 and 10 days after stress imposition, as affected by the interaction osmotic stress level (0 vs. −0.4 vs. −0.8 vs. −1.2 vs. −1.4 vs. −1.6 MPa) × biotype (winter vs. spring) × seed weight class ((A) TSW > 1.27 g vs. (B) 0.98 g < TSW < 1.27 g vs. (C) TSW < 0.98 g). Mean values ± SE. Different letters: statistically different means within the same germination date for $p \leq 0.05$ (LSD's test).

Osmotic Stress	Biotype	Seed Weight Class	Germination d 4 %	Germination d 10 %
Control (0.0)	Spring	A	98.00 ± 0.52 a	98.17 ± 0.54 a
		B	95.22 ± 0.57 ab	96.11 ± 0.65 a
		C	94.67 ± 1.45 abc	94.67 ± 1.45 ab
	Winter	A	84.00 ± 1.00 def	86.33 ± 0.88 abc
		B	92.22 ± 0.78 bc	93.33 ± 0.71 ab
		C	98.00 ± 0.78 a	98.33 ± 0.33 a
−0.4 MPa	Spring	A	98.33 ± 0.33 a	98.50 ± 0.34 a
		B	94.22 ± 1.04 abc	95.44 ± 0.73 ab
		C	94.64 ± 2.03 abc	96.00 ± 2.00 ab
	Winter	A	81.00 ± 3.21 ef	83.33 ± 2.91 bcd
		B	89.56 ± 0.80 cd	91.78 ± 0.64 ab
		C	97.50 ± 0.85 a	97.50 ± 0.85 a
−0.8 MPa	Spring	A	93.33 ± 1.02 abc	95.67 ± 0.61 ab
		B	85.56 ± 2.10 de	90.78 ± 1.79 ab
		C	79.00 ± 2.52 f	89.33 ± 1.76 abc
	Winter	A	50.00 ± 2.00 h	71.33 ± 1.45 de
		B	58.67 ± 5.91 g	84.22 ± 1.31 bc
		C	89.50 ± 2.67 cd	96.83 ± 0.91 a
−1.2 MPa	Spring	A	6.50 ± 2.13 i	90.83 ± 2.12 ab
		B	0.67 ± 0.44 j	77.56 ± 1.60 cd
		C	2.00 ± 1.53 ij	82.67 ± 1.33 bcd
	Winter	A	0.00 ± 0.00 j	60.67 ± 0.88 ef
		B	0.00 ± 0.00 j	60.11 ± 5.40 ef
		C	1.67 ± 0.80 ij	85.33 ± 1.41 bc
−1.4 MPa	Spring	A	0.00 ± 0.00 j	44.00 ± 7.30 g
		B	0.00 ± 0.00 j	28.00 ± 3.70 h
		C	0.00 ± 0.00 j	48.33 ± 0.33 fg
	Winter	A	0.00 ± 0.00 j	4.33 ± 1.33 i
		B	0.00 ± 0.00 j	24.22 ± 10.49 h
		C	0.00 ± 0.00 j	21.17 ± 5.34 h
−1.6 MPa	Spring	A	0.00 ± 0.00 j	0.00 ± 0.00 i
		B	0.00 ± 0.00 j	0.00 ± 0.00 i
		C	0.00 ± 0.00 j	0.00 ± 0.00 i
	Winter	A	0.00 ± 0.00 j	0.00 ± 0.00 i
		B	0.00 ± 0.00 j	0.00 ± 0.00 i
		C	0.00 ± 0.00 j	0.00 ± 0.00 i

3.2. Seedling Morphology Results

Osmotic stress level (OS) and biotype (main effects, Table 2) significantly influenced ($p \leq 0.05$) the radicle and shoot lengths of camelina seedlings, surveyed at 10 d after stress imposition. Shoot length was significantly reduced by any level of osmotic stress compared with the control (Figure 3). In detail, camelina shoot was reduced by 33% under −0.4 MPa and by 67% under −0.8 MPa compared with the control seedlings. At stress levels ≥ -1.2 MPa, the tested camelina genotypes produced only negligible shoots (Figure 3). Otherwise, camelina was able to produce radicles up to −1.4 MPa, but a linear decrease in radicle length was evident in response to osmotic stress compared with

control (Figure 3). Significant differences between biotypes were observed for seedling morphology, in particular spring types were characterized by both longer shoots and radicles compared with the winter ones ($p \leq 0.05$, Figure 3).

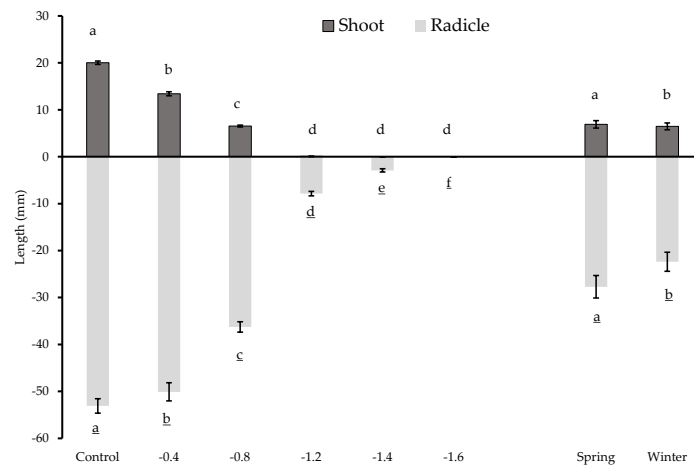


Figure 3. Shoot and radicle lengths of camelina (mm) in response to the main effects: osmotic stress (Control = 0, -0.4, -0.8, -1.2, -1.4 and -1.6 MPa) and biotype (winter and spring). Mean values \pm SE. Different standard letters: statistically different means within the same main effect for $p \leq 0.05$ (LSD's test) for shoot length. Different underlined letters: statistically different means within the same main effect for $p \leq 0.05$ (LSD's test) for radicle length. Radicle length is reported graphically as a negative value, but it corresponds to the mean of the surveyed values.

Shoot and radicle lengths were also significantly affected by the interaction “osmotic stress \times biotype” (Table 2, Figure 4). Concerning shoot length (Figure 4), significant differences between biotypes were detected only at -0.4 MPa, with spring types having longer shoots (+18%, $p \leq 0.05$) compared with winter ones. The radicle length of spring types was significantly longer than that of winter ones in the control conditions and under the two milder stresses (i.e., -0.4 MPa and -0.8 MPa); otherwise, when applying an osmotic stress ≥ -1.2 MPa differences were not significant (Figure 4).

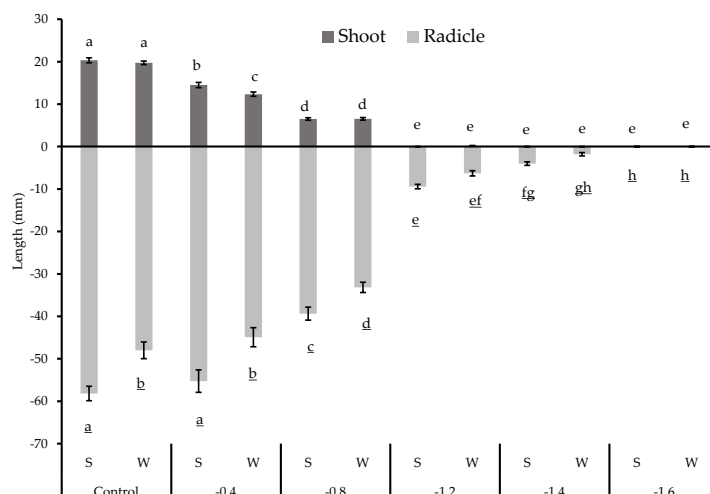


Figure 4. Shoot and radicle lengths of camelina (mm) in response to the interaction between osmotic stress (Control = 0, -0.4, -0.8, -1.2, -1.4 and -1.6 MPa) \times biotype (winter and spring). Mean values \pm SE. Different standard letters: statistically different means within the same main effect for $p \leq 0.05$ (LSD's test) for shoot length. Different underlined letters: statistically different means within the same main effect for $p \leq 0.05$ (LSD's test) for radicle length. Radicle length is reported graphically as a negative value, but it corresponds to the mean of the surveyed values.

Camelina shoot length was also significantly influenced by the interaction between biotype and seed weight class (Table 2, Figure 5). In particular, significant differences emerged between large seeded genotypes (class A) with the spring types reaching the highest value and the winter ones the lowest (7.27 vs. 5.78 mm, spring vs. the winter types, respectively $p \leq 0.05$).

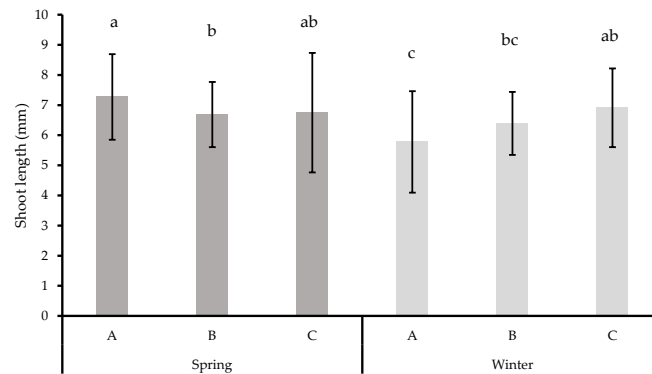


Figure 5. Shoot lengths of camelina (mm) in response to the interaction biotype (winter vs. spring) \times seed weight class ((A) TSW > 1.27 g vs. (B) 0.98 g < TSW < 1.27 g vs. (C) TSW < 0.98 g). Mean values \pm SE. Different letters: statistically different means for $p \leq 0.05$ (LSD's test).

As for germination, seedling morphology was also significantly influenced by the interaction “stress level \times biotype \times seed weight class” (Tables 1 and 5). Regarding shoot length (Table 5); the tested camelina genotypes had the same value under the control treatment, while when applying osmotic stress significant differences emerged. At -0.4 MPa, spring types, belonging to seed weight classes A and B, recorded similar values than winter types belonging to class C (mean shoot length ~ 14.6 mm). This shoot length was significantly higher than that of winter types in class A and B, which reported a mean shoot length of 8.8 and 12.3 mm, respectively. At stress levels ≥ -0.8 MPa, differences for shoot morphology in response to biotype and seed weight classes resulted negligible with lengths highly reduced by stress imposition (Table 5). Concerning radicle length, spring and winter types did not report significant differences between control and -0.4 MPa stress level, but winter types in seed classes A and B had significantly shorter radicles than the others ($p \leq 0.05$, Table 5). At stress levels ≥ -0.8 MPa, the radicle growth of camelina was significantly reduced without a clear difference in the response behavior among biotypes and seed weight classes (Table 5). As a general remark, shoot growth seemed more impacted by the imposition of osmotic stress than radicle growth; the latter was still surveyed up to -1.4 MPa, while shoot was almost completely inhibited already at -0.8 MPa.

Table 5. Shoot and radicle lengths of camelina as affected by the interaction osmotic stress level (0 vs. −0.4 vs. −0.8 vs. −1.2 vs. −1.4 vs. −1.6 MPa) × biotype (winter vs. spring) × seed weight class ((A) TSW > 1.27 g vs. (B) 0.98 g < TSW < 1.27 g vs. (C) TSW < 0.98 g). Mean values ± SE. Different letters: statistically different means within the same plant part for $p \leq 0.05$ (LSD's test).

Osmotic Stress	Biotype	Seed Weight Class	Shoot Length	Radicle Length
				mm
Control (0.0)	Spring	A	20.80 ± 1.27 a	56.05 ± 2.96 ab
		B	19.74 ± 0.64 a	58.66 ± 2.80 a
		C	21.17 ± 2.03 a	61.07 ± 1.16 a
	Winter	A	19.07 ± 1.19 a	55.40 ± 2.24 abc
		B	20.01 ± 0.62 a	49.04 ± 2.99 cd
		C	19.65 ± 0.74 a	42.73 ± 2.48 ef
−0.4 MPa	Spring	A	15.33 ± 1.19 b	51.82 ± 3.33 bcd
		B	14.26 ± 0.94 b	55.69 ± 4.70 ab
		C	13.60 ± 0.38 bc	60.87 ± 3.30 a
	Winter	A	8.83 ± 0.43 d	54.17 ± 5.09 abc
		B	12.29 ± 0.27 c	46.38 ± 3.21 de
		C	14.17 ± 0.76 b	38.10 ± 1.92 f
−0.8 MPa	Spring	A	7.48 ± 0.64 de	38.93 ± 3.11 f
		B	6.10 ± 0.23 e	40.61 ± 2.26 f
		C	5.73 ± 0.33 e	36.47 ± 2.03 fgh
	Winter	A	6.77 ± 0.03 de	27.80 ± 1.51 h
		B	6.06 ± 0.44 e	32.12 ± 1.58 gh
		C	7.17 ± 0.52 de	37.42 ± 1.24 fg
−1.2 MPa	Spring	A	0.00 ± 0.00 f	9.98 ± 1.31 i
		B	0.00 ± 0.00 f	9.69 ± 0.52 i
		C	0.00 ± 0.00 f	7.47 ± 0.72 ijk
	Winter	A	0.00 ± 0.00 f	4.93 ± 0.42 ijk
		B	0.00 ± 0.00 f	5.86 ± 0.82 ijk
		C	0.47 ± 0.23 f	7.65 ± 1.23 ij
−1.4 MPa	Spring	A	0.00 ± 0.00 f	3.43 ± 0.71 jk
		B	0.00 ± 0.00 f	3.72 ± 0.42 jk
		C	0.00 ± 0.00 f	6.00 ± 1.46 ijk
	Winter	A	0.00 ± 0.00 f	1.07 ± 0.22 jk
		B	0.00 ± 0.00 f	1.53 ± 0.62 jk
		C	0.00 ± 0.00 f	2.60 ± 0.66 jk
−1.6 MPa	Spring	A	0.00 ± 0.00 f	0.00 ± 0.00 k
		B	0.00 ± 0.00 f	0.00 ± 0.00 k
		C	0.00 ± 0.00 f	0.00 ± 0.00 k
	Winter	A	0.00 ± 0.00 f	0.00 ± 0.00 k
		B	0.00 ± 0.00 f	0.00 ± 0.00 k
		C	0.00 ± 0.00 f	0.00 ± 0.00 k

4. Discussion

One of the most important abiotic factors limiting seed germination and early seedling growth is drought. Furthermore, climate change often causes uneven precipitation patterns with periods of prolonged drought which have recently become more frequent [42]. In relation to the better resistance to abiotic stresses, camelina has been identified as a much more resilient species than “sister crop” oilseed rape [21,22], as well *Brassica carinata* and *B. juncea* [43]. As far as authors know this is the first study comparing the behavior of spring and winter camelina biotypes in response to osmotic stress at the germination phase, also considering their seed weight. In particular, seed weight is one the main

traits under study in camelina, since an improvement of the seed size is often associated to better stand establishment, higher seed yield and improved post-harvest processing [16]. Breeding effort in camelina is mainly focused on spring types [19,22], while the interest toward winter types is more recent and still geographically confined to northern USA plains, where winter camelina has been identified as a suitable cover crop [44]. Indeed, spring types are also suitable for autumn and/or winter sowing in environments characterized by mild winter temperature, i.e., Mediterranean climate, where they can produce oil with improved quality for multi-purpose applications, as recently reported by [38].

Our results showed that camelina had no reduction in germination under mild osmotic stress (-0.4 MPa) compared with the control at both dates of survey. Indeed after 10 d at -1.2 MPa, camelina germination was still above 75% (Table 3 and Figure 1). Channaoui et al. [26] reported that under similar osmotic conditions (i.e., -1.1 MPa) the germination of six oilseed rape genotypes ranged from 0.67 to 28%. Moreover, sunflower seeds did not germinate under a PEG induced osmotic stress of -1.2 MPa [45]. In a study comparing fifteen rice cultivars under osmotic stress, [46] demonstrated that the lower limit for germination ranged between -0.51 and -1.19 MPa, depending on genotype. In soybean, the threshold for germination is -1 MPa [47]. Thus, the present study confirmed the outstanding drought tolerance of camelina, as indicated by [19,43,48].

Interestingly significant differences in the response to osmotic stress were observed between spring and winter biotypes (Table 3 and Figure 2). At both surveying dates, spring biotypes had significantly higher germination percentages than winter ones, while under control conditions the same germination percentage was recorded at 10 d after stress imposition (Figures 1 and 2). This result confirmed that the germination of winter types was significantly impaired by the imposition of the osmotic stress, and it was not due to different germination vigor of the tested seed lots. However, after 4 d since stress imposition, spring genotypes were already performing slightly higher germination than winter ones. The differences in germination between the two biotypes increased with the intensification of osmotic stress in favor of spring ones. This would permit speculation that spring biotypes could perform better in dry soil conditions at establishment phase. One possible explanation of the improved germination of spring types might be the mucilage content. Indeed, the presence of mucilage in the tested camelina seeds ranged between 7.64% in Calena (spring variety) and 15.01% in WG4 (winter variety), and on average the mucilage content in winter types was higher than in the spring ones (11.9 vs. 10.3%, winter vs. spring, respectively). Thus, the improved germination of spring types cannot be explained by the total mucilage content. Further study on this aspect is needed to investigate also on the position where mucilage is localized in the seed as well as on its extrusion capacity, as reported for *Arabidopsis thaliana* by [49]. Additionally, in the desert shrub *Henophyton deserti*, also belonging to the *Brassicaceae* family, the presence of mucilage was linked to the germination prevention under unfavorable conditions [50], acting as a physical barrier for oxygen and water diffusion, and this might apply also to the present results for winter camelina biotypes.

Larger seeds are usually associated to improved germination and higher nutrient reserves, enabling seedlings to better establish under adverse conditions, which could not be tolerated by small-seeded seedlings [51]. This finding is confirmed in many plant species [52–55], including genetically modified (GM) camelina [56], and it only agrees with the present results for spring types, where large-seeded genotypes showed significantly higher germination percentage and longer shoots and roots (Figures 2 and 3). Otherwise, in winter types, small seeded genotypes had the highest germination percentage (Figure 2). This information can be useful in selecting the appropriate seed weight depending on the biotype used. The total mucilage content (Table 2) was found not to be correlated to seed weight, thus the present results confirmed that the improvement of seed size was not associated to an increase in mucilage content but it was able to confer camelina higher germination capacity under osmotic stress, at least in the spring genotypes. Thus, from a breeding perspective, this aspect might be further exploited selecting camelina lines with increased seed size and adequate mucilage content without risking a reduction in seed vigor and resilience toward drought stress.

Observing “stress level \times biotype \times seed weight class” interaction, differences in germination are much more pronounced in winter biotypes, on each stress level, and at 4 d count compared to 10 d (Table 4), presumably in relation to a slower germination in those genotypes.

Regarding seedling morphology, camelina shoot and radicle lengths were linearly diminished by increasing the level of the imposed osmotic stress (Figure 3). In particular, shoot growth seemed more impacted than radicle growth, since it was highly inhibited at -0.8 MPa and non-existent at -1.2 MPa, while radicles were still surveyed at -1.4 MPa. Regarding biotype, spring ones formed significantly longer shoots and radicles, as compared with winter ones, confirming their superiority also for this trait (Figure 4). Otherwise, seed weight showed negligible effects on seedling morphology, and mainly at shoot level (Figure 5). Interestingly, the longest shoots were surveyed in the spring types with larger seeds and the shortest in large seeded winter types, confirming the interaction effect between biotype and seed size also for shoot morphology. The present results showed that spring biotypes formed longer shoots and roots under drought conditions, with consistent results throughout seed weight classes, as compared with winter biotypes (Table 5). Forming longer radicles in a situation of early drought after germination, when water might be available at deeper soil layers, allows plants to perform better under stressed conditions.

The negative effect of osmotic stress on seedling morphology is reported in various crops by many authors. Kaya et al. [45] reported that the sunflower was not able to produce shoot under osmotic stress -0.6 MPa, while radicle formation stopped at -0.9 MPa. In a study by [26] comparing different oilseed rape genotypes, four out of six stopped producing shoots at -1.0 MPa, while radicle protrusion was impeded at -1.1 MPa. Osmotic stress induced a significant effect also on shoot and root formation of naked oat (*Avena nuda* L.), where [54] found that all tested cultivars stopped forming shoots at -0.75 MPa. In pea (*Pisum sativum* L.), osmotic stress levels ranging from -0.2 to -0.6 MPa completely prevented shoot formation, while radicle growth stopped at -0.4 to -0.8 MPa [57]. Thus, the present results confirmed the outstanding capacity of camelina to produce shoot and radicle also under significant osmotic stress compared with other crops, thus demonstrating the feasibility of growing this species under marginal soils prone to drought.

5. Conclusions

Camelina confirmed its good tolerance to drought at the germination stage. It withstands mild level of osmotic stress (-0.4 MPa) without any significant decrease in germination percentage compared with the control. Even at -1.2 MPa, it still had 75% germination percentage, which is much higher than the germination of other staple crops, as found in the literature. In this preliminary trial, camelina germination was affected by biotypes, with spring ones always having higher germination than winter ones. This might be explained by both the more intense breeding carried out in spring types, but possibly also by the epigenetic effect related to the different conditions in which the two types usually germinate.

Moreover, morphological changes were caused by osmotic stress. Shoot growth seemed more impacted than that of the radicle. Spring biotype had longer shoots and radicles than winter ones confirming their superiority also for this trait. Interestingly, seed size played a role in the response of camelina to drought, but it depended on the biotype and stress level imposed.

The present results could be useful for selecting the most appropriate camelina genotype for sowing in drought-prone regions, in particular those where both spring and winter biotypes could be sown in autumn/winter. Although the screening of traits during germination and early seedling growth for drought tolerance can provide useful information, further investigation is needed under real field conditions, to verify if these findings might be linked with increased crop productivity.

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draft preparation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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