





## Article

# Assessing Drought Tolerance of Newly Developed Tissue-Cultured Canola Genotypes under Varying Irrigation Regimes

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**Abstract:** Drought is a major abiotic stress that greatly affects canola growth, production, and quality. Moreover, water scarcity is projected to be more severe and frequent as a result of climate change, in particular in arid environments. Thereupon, developing drought-tolerant and high-yielding canola genotypes has become more critical to sustaining its production and ensuring global food security with the continuing population growth. In the present study, ten canola genotypes comprising six developed tissue-cultured canola genotypes, two exotic genotypes, and two commercial cultivars were evaluated under four irrigation regimes. The applied irrigation regimes were well-watered (100% crop evapotranspiration, ET<sub>c</sub>), mild drought (80% ET<sub>c</sub>), moderate drought (60% ET<sub>c</sub>), and severe drought (40% ET<sub>c</sub>) conditions. Drought-stress treatments (80, 60, and 40% ET<sub>c</sub>) gradually reduced the chlorophyll content, relative water content, flowering time, days to maturity, plant height, number of pods, number of branches, seed yield, and oil percentage, and increased proline, phenolic, anthocyanin, and glycine betaine contents. The evaluated genotypes exhibited varied responses to drought-stress conditions. The developed tissue-cultured genotypes T2, T3, and T1, as well as exotic genotype Torpe, possessed the highest performance in all evaluated parameters and surpassed the other tested genotypes under water-deficit conditions. Overall, our findings elicited the superiority of certain newly developed tissue-cultured genotypes and exotic ones compared with commercial cultivars, which could be exploited in canola breeding under water-deficit conditions.

**Keywords:** arid environment; agronomic traits; cluster analysis; drought tolerance indices; Mediterranean region; physiological parameters; principal component analysis

## 1. Introduction

Canola (*Brassica napus* L.) is a valuable crop grown mainly for edible oil [1]. Its oil is a healthy ingredient providing polyunsaturated fatty acids, and its by-product is rich in its

protein content [2]. Canola can be used as an alternative break crop in semi-arid regions where the cereal-based crop is the predominant cropping system [3]. This is due to its high water-use efficiency and relative tolerance to drought stress [4]. Nevertheless, the global demand for vegetable oil is increasing and drought represents a threat to oilseed production in several parts of the world, in particular under the current climate change [5]. Canola, as with other temperate crops, is vulnerable to drought stress [6]. Its plant growth is regulated by various factors, of which water plays a crucial role. The first common undesirable effect of drought on canola may be a reduction in yield-related traits and a reduction in oil and seed quality [7]. The impacts of drought stress depend on its severity, timing, and duration. Canola is more sensitive to water deficiency during the reproductive stage; even a short drought stress could penalize the seed yield. However, during the vegetative growth stage, drought exhibits less damage [8]. Consequently, drought reduces the seed yield when it occurs in the reproductive stage more than at the beginning of the plant cycle [9]. Thus, the genotypes that have the ability to maintain a good cell water status at the final plant cycle are significantly important for drought-prone regions [10,11].

Water-deficit stress alters agronomic and physiochemical traits at any period of plant growth. Early drought restricts germination, emergence, and early canola seedling growth, which leads to crop failure [12–16]. Water-deficit stress at anthesis reduces the plant height, number of branches, thousand seed weight, number of pods per plant, number of seeds per pod, and seed size, which ultimately leads to low seed and oil yields [17–20]. At the physiological level, water-deficit stress increases chlorophyll degradation, decreases photosynthesis activity, unbalances mineral nutrient contents, reduces cell turgidity, and causes oxidative damage due to the increasing production of reactive oxygen species [21,22]. The negative impacts of water-deficit stress on different physiological processes inactivate metabolic enzymes, impair nucleic acids, and damage membrane lipids, which ultimately result in the death of plant cells [23–25].

Canola is a promising oil crop that delivers an opportunity to diminish the great gap between consumption and oil production in low-income countries. It is commonly cultivated in marginal areas that suffer from predominant environmental stresses at both vegetative and reproductive growth stages [26–28]. Accordingly, developing drought-tolerant canola genotypes that can cope with prolonged water-deficit stress is the best solution to reduce the great shortage of edible oil in low-income countries. In this regard, the present study aimed to: (i) assess the response of the physiochemical and agronomic traits of newly developed tissue-cultured canola genotypes to various drought severities; and (ii) identify tolerant and high-yielding genotypes with high seed and oil yields compared with commercial cultivars.

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Site

Ten canola genotypes (*Brassica napus* L.) were used in this study (Table 1). The evaluated genotypes comprised three tissue-cultured genotypes regenerated from the exotic genotype Siberian (S1, S2, and S3), three genotypes regenerated from the exotic genotype Torpe (T1, T2, and T3), two exotic genotypes (Siberian and Torpe), and two drought-tolerant commercial cultivars (Serw-4 and Pactol). The tissue-cultured canola genotypes were developed under osmotic stress (180 mM NaCl) by Abdrabou et al. [29]. A field experiment was conducted during two growing seasons of 2018–2019 and 2019–2020 at Nubaria Agricultural Research Center (ARC), El-Behira governorate, Egypt (30°55' N and 29°56' E). According to the optimal period of canola cultivation in the region, the seed sowing was performed on the first week of November in both seasons. This region is dry, with low precipitation; the annual rainfall is below 30 mm (Table S1). Before sowing, soil samples were taken from 0–30 cm depth to determine the physical and chemical properties according to Horwitz and Latimer [30]. The analysis indicated that the soil of the experimental site could be classified as sandy clay (i.e., 54.0% sand, 20.6% silt, and 24.8% clay) (Table S2).

**Table 1.** Origin of the used canola genotypes.

Accession	Origin
Serw-4	Local variety obtained from Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt
Pactol	Adopted French cultivar in Egypt
Siberian	Accessions from the gene bank of the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
S1	
S2	Regenerated genotypes developed from Siberian genotype
S3	
Torpe	Accessions from the gene bank of the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
T <sub>1</sub>	
T <sub>2</sub>	Regenerated genotypes developed from Torpe genotype
T <sub>3</sub>	

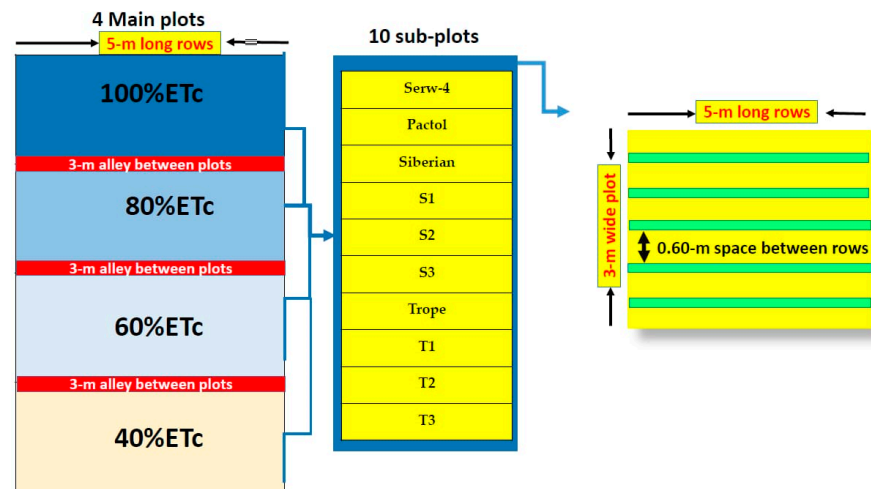
### 2.2. Experimental Design and Crop Management

The experiment was performed in a randomized complete block design with a split-plot arrangement with three replicates. The irrigation regimes were allocated in the main plots and canola genotypes were assigned to the sub-plots (Figure 1). The ten canola genotypes were assessed under four water irrigation regimes based on an evapotranspiration (ET<sub>c</sub>) replacement, which was based on the crop coefficient approach of Allen et al. [31]. The applied irrigation regimes were well-watered (100% ET<sub>c</sub>), mild drought (80% ET<sub>c</sub>), moderate drought (60% ET<sub>c</sub>), and severe drought (40% ET<sub>c</sub>). The drip irrigation system was applied using drip laterals with a 0.6 m space between them and an emitter spacing of 0.30 m. The cumulative amounts of the applied aforementioned regimes in the first season in the same order were 461.3, 368.8, 276.6, and 184.4 mm ha<sup>-1</sup>; in the second season, these were 481.0, 385.3, 289.0, and 192.6 mm ha<sup>-1</sup>. Each plot included five 5 m long rows with a 0.60 m space between the rows and a 0.15 m space between the plants. These distances provided an experimental plot size of 15 m<sup>2</sup> and a total area of the whole experiment of 2322 m<sup>2</sup>. Each hill was sown with several seeds and thinned to two seedlings after two weeks to achieve complete germination to provide 400 plants/plot. In both growing seasons, the canola plants were sowed during the optimum period, which was the second week of November. Phosphorus (P) and potassium (K) were added before sowing at the rate of 25 kg P ha<sup>-1</sup> as superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and 80 kg K ha<sup>-1</sup> as potassium sulfate (48% K<sub>2</sub>O). Nitrogen fertilizer was applied by fertigation at 70 kg N ha<sup>-1</sup> as ammonium sulfate (20.6% N) and fractioned into 4 equal doses at 10 day intervals after sowing.

### 2.3. Measured Traits

Canola leaves were collected from 10 random plants of each plot (second leaf from above of the main stems) 90 days after sowing for both seasons to assess the physiological parameters. The relative water content (RWC%) was determined according to Schonfeld et al. [32], the total chlorophyll as outlined by Moran [33], the proline content following Bates et al. [34], the total phenolic content as described by Jindal and Singh [35], the anthocyanin content in accordance with Mirecki and Teramura [36], and the glycine betaine content as presented by Grieve and Grattan [37]. The days to flowering (DTF, days) and days to maturity (DTM, days) were recorded. At physiological maturity (16th and 10th of April in the first and second seasons, respectively) the following agronomic traits were recorded from ten random plants per each plot: plant height (cm), number of branches per plant, number of pods per plant, and number of seeds per pod. The seed yield (t ha<sup>-1</sup>)

was estimated based on the harvested plot and converted to  $\text{ton ha}^{-1}$ . Furthermore, the oil percentage was quantified after extraction by the Soxhlet extraction method.



**Figure 1.** Layout of the experiment, presenting four irrigation regimes in main plots and assessed genotypes in sub-plots.

#### 2.4. Drought Tolerance Indices

Nine indices related to drought tolerance were calculated, as presented in Table 2.

**Table 2.** The drought tolerance indices applied to discriminate the assessed canola genotypes under well-watered and drought conditions.

Index	Formula
Drought tolerance index [38]	$(Y_p - Y_s)$
Harmonic mean [39]	$(Y_p \times Y_s) / (Y_p + Y_s)$
Mean productivity [38]	$(Y_s + Y_p) / 2$
Stress susceptibility index [39]	$1 - (Y_s / Y_p) / SI$ , $SI = 1 - (\bar{Y}_s / \bar{Y}_p)$
Geometric mean productivity [40]	$\sqrt{(Y_p \times Y_s)}$
Stress tolerance index [40]	$(Y_p \times Y_s) / (\bar{Y}_p)^2$
Modified stress tolerance index [41]	$(YI)^2 \times [(Y_p \times Y_s) / (\bar{Y}_p)^2]$
Yield stability index [42]	$Y_s / Y_p$
Yield index [43]	$Y_s / \bar{Y}_s$

$Y_s$ : seed yield under severe drought stress;  $Y_p$ : seed yield under well-watered conditions;  $\bar{Y}_s$ : average seed yield of all genotypes under severe drought stress;  $\bar{Y}_p$ : average seed yield of all genotypes under non-water stress.

#### 2.5. Statistical Analysis

The data were subjected to a normality distribution test and variance homogeneity using Shapiro–Wilk and Bartlett’s tests, respectively. A combined analysis of variance (ANOVA) was applied for the split–split plot design in three replicates across two growing seasons. The differences among the irrigation regimes, canola genotypes, and their interaction were discriminated according to the Tukey HSD test ( $p \leq 0.05$ ). All analyses were applied using R statistical software version 3.6.2.

### 3. Results

#### 3.1. Physiological Parameters

The ANOVA analysis revealed that the season interaction with the genotype and irrigation regime was not significant for most traits (Table S1). Accordingly, the measured traits were combined over the two seasons. The drought-stress regimes displayed a substantial impact on all evaluated physiological parameters compared with the well-watered conditions (Table 3). Severe drought decreased the total chlorophyll content and RWC by 50.9

and 27.6%, respectively, and increased the contents of proline, anthocyanin, glycine betaine, and total phenolic by 102.2, 726.4, 374.8, and 291.9%, respectively, compared with the well-watered conditions. The canola genotypes significantly differed in all physiological and agronomic parameters, and presented a significant interaction with the irrigation regimes, except RWC (Table 3). A significant interaction effect between the irrigation regimes and assessed canola genotypes was observed for the contents of chlorophyll, proline, anthocyanin, glycine betaine, and total phenolic content. Overall, under the well-watered treatment, less variation was observed among the genotypes for most traits compared with severe drought (Figure 2). Under drought conditions, T1, T2, and T3 had the highest values of most evaluated physiological parameters (Figure 2). Otherwise, the genotypes Siberian, S1, and S2 possessed the lowest values for the physiological parameters. The growing seasons and their interactions with the irrigation regimes and genotypes had non-significant effects on most of the studied traits (Table S3).

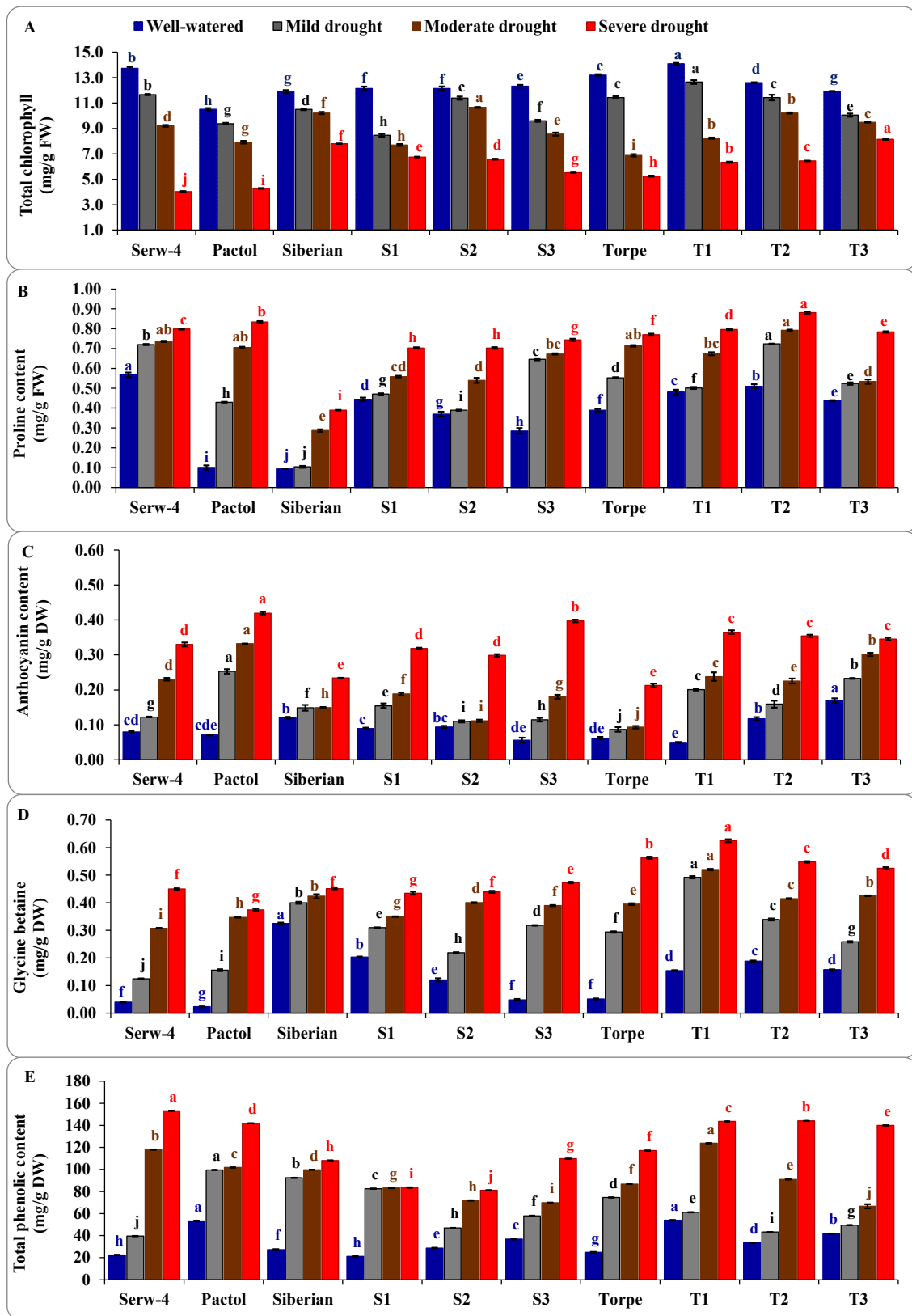
### 3.2. Agronomic Traits

Drought stress, particularly the severe conditions, significantly decreased all agronomic traits (Table 4). The number of pods per plant and seed yield per ha were the traits most decreased by severe drought stress, roughly by 53.3% and 52.9%, respectively, compared with the well-watered conditions. The oil percentage was less affected by severe drought stress; it decreased by only 5.2% compared with the well-watered conditions. The canola genotypes behaved differently for all agronomic traits and they ranked differently across the assessed irrigation regimes (Table 4). A significant interaction effect between the irrigation regimes and assessed canola genotypes was observed for the days to flowering and oil percentage (Table 4 and Figure 3). It is worth mentioning that T1, T2, and T3 produced higher seed yields than the other genotypes under drought-stress conditions. In addition, T2 and T3 had the highest oil percentage under drought stress. Accordingly, the Torpe tissue-cultured genotypes displayed the highest values for all agronomic traits (Table 4).

**Table 3.** Impact of irrigation regimes on physiological parameters of canola genotypes averaged over two growing seasons.

Studied Factor	Total Chlorophyll (mg/g DW)	Relative Water Content (%)	Proline Content (mg/g DW)	Anthocyanin Content (mg/g DW)	Glycine Betaine (mg/g DW)	Total Phenolic Content (mg/g DW)
Irrigation (I)						
Well-watered	12.46 <sup>a</sup>	75.74 <sup>a</sup>	0.368 <sup>d</sup>	0.091 <sup>d</sup>	0.131 <sup>d</sup>	34.38 <sup>d</sup>
Mild drought	10.66 <sup>b</sup>	69.51 <sup>b</sup>	0.506 <sup>c</sup>	0.158 <sup>c</sup>	0.291 <sup>c</sup>	64.77 <sup>c</sup>
Moderate drought	8.91 <sup>c</sup>	62.71 <sup>c</sup>	0.621 <sup>b</sup>	0.205 <sup>b</sup>	0.398 <sup>b</sup>	91.25 <sup>b</sup>
Severe drought	6.12 <sup>d</sup>	54.85 <sup>d</sup>	0.744 <sup>a</sup>	0.752 <sup>a</sup>	0.622 <sup>a</sup>	134.74 <sup>a</sup>
Genotype (G)						
Serw-4	9.66 <sup>e</sup>	70.12 <sup>a</sup>	0.706 <sup>a</sup>	0.263 <sup>e</sup>	0.231 <sup>e</sup>	103.34 <sup>a</sup>
Pactol	8.02 <sup>i</sup>	65.30 <sup>c</sup>	0.517 <sup>c</sup>	0.354 <sup>b</sup>	0.225 <sup>e</sup>	99.16 <sup>b</sup>
Siberian	9.90 <sup>d</sup>	61.66 <sup>e</sup>	0.219 <sup>d</sup>	0.337 <sup>c</sup>	0.472 <sup>b</sup>	101.15 <sup>c</sup>
S1	8.76 <sup>h</sup>	68.75 <sup>ab</sup>	0.597 <sup>b</sup>	0.313 <sup>d</sup>	0.347 <sup>cd</sup>	67.62 <sup>h</sup>
S2	10.19 <sup>b</sup>	64.31 <sup>cd</sup>	0.501 <sup>c</sup>	0.253 <sup>e</sup>	0.524 <sup>a</sup>	57.16 <sup>i</sup>
S3	8.99 <sup>g</sup>	69.14 <sup>ab</sup>	0.587 <sup>b</sup>	0.262 <sup>e</sup>	0.307 <sup>d</sup>	68.60 <sup>g</sup>
Torpe	9.20 <sup>f</sup>	62.79 <sup>de</sup>	0.607 <sup>b</sup>	0.264 <sup>e</sup>	0.477 <sup>b</sup>	75.85 <sup>e</sup>
T1	10.33 <sup>a</sup>	63.13 <sup>d</sup>	0.590 <sup>b</sup>	0.360 <sup>b</sup>	0.354 <sup>c</sup>	96.62 <sup>d</sup>
T2	10.17 <sup>bc</sup>	67.83 <sup>b</sup>	0.706 <sup>a</sup>	0.196 <sup>f</sup>	0.349 <sup>cd</sup>	68.93 <sup>g</sup>
T3	10.10 <sup>c</sup>	64.03 <sup>cd</sup>	0.569 <sup>b</sup>	0.413 <sup>a</sup>	0.319 <sup>cd</sup>	74.48 <sup>f</sup>
ANOVA	df					
Irrigation (I)	3	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype (G)	9	<0.001	0.041	<0.001	<0.001	<0.001
I × G	27	<0.001	0.705	<0.001	<0.001	<0.001

Means followed by different letters under the same factor were significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

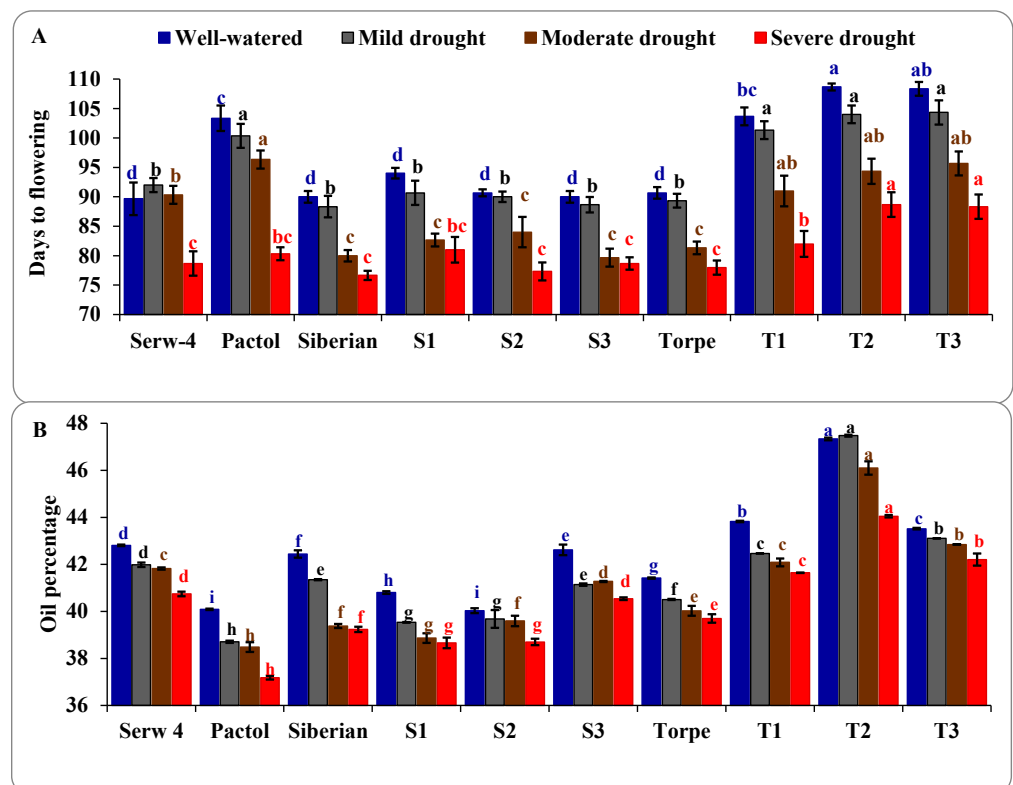


**Figure 2.** Response physiological parameters of ten canola genotypes to four irrigation regimes averaged over two growing seasons. The bars on the top of the columns represent the SE, and different letters on the column of each irrigation regime (with the same color) show the significant difference using Tukey’s HSD test ( $p \leq 0.05$ ).

**Table 4.** Impact of irrigation regimes on agronomic traits of canola genotypes averaged over two growing seasons.

Studied Factor	Days to Flowering	Days to Maturity	Number of Branches/Plant	Number of Pods/Plant	Plant Height (cm)	Seed Yield (t/ha)	Oil Content (%)
Irrigation (I)							
Well-watered	96.90 <sup>a</sup>	173.47 <sup>a</sup>	7.45 <sup>a</sup>	290.8 <sup>a</sup>	150.67 <sup>a</sup>	2.25 <sup>a</sup>	42.49 <sup>a</sup>
Mild drought	94.90 <sup>a</sup>	171.33 <sup>a</sup>	6.70 <sup>b</sup>	243.0 <sup>b</sup>	144.33 <sup>ab</sup>	2.03 <sup>b</sup>	41.59 <sup>b</sup>
Moderate drought	87.53 <sup>b</sup>	164.27 <sup>b</sup>	6.02 <sup>c</sup>	185.5 <sup>c</sup>	139.00 <sup>b</sup>	1.54 <sup>c</sup>	41.05 <sup>c</sup>
Severe drought	80.97 <sup>c</sup>	157.63 <sup>c</sup>	4.82 <sup>d</sup>	135.9 <sup>d</sup>	131.17 <sup>c</sup>	1.06 <sup>d</sup>	40.26 <sup>d</sup>
Genotype (G)							
Serw-4	87.67 <sup>b</sup>	167.42 <sup>abc</sup>	6.12 <sup>ab</sup>	211.1 <sup>bcde</sup>	136.12 <sup>bc</sup>	1.77 <sup>bc</sup>	41.84 <sup>d</sup>
Pactol	95.08 <sup>a</sup>	167.58 <sup>abc</sup>	6.00 <sup>ab</sup>	223.0 <sup>abcd</sup>	139.62 <sup>abc</sup>	1.79 <sup>b</sup>	38.61 <sup>i</sup>
Siberian	83.75 <sup>b</sup>	165.50 <sup>bc</sup>	6.04 <sup>ab</sup>	206.3 <sup>def</sup>	145.79 <sup>ab</sup>	1.63 <sup>d</sup>	40.60 <sup>f</sup>
S1	87.08 <sup>b</sup>	164.75 <sup>bc</sup>	5.71 <sup>b</sup>	208.7 <sup>cdef</sup>	141.33 <sup>abc</sup>	1.50 <sup>e</sup>	39.47 <sup>h</sup>
S2	85.50 <sup>b</sup>	162.58 <sup>c</sup>	6.08 <sup>ab</sup>	190.6 <sup>f</sup>	141.92 <sup>abc</sup>	1.44 <sup>e</sup>	39.50 <sup>h</sup>
S3	84.25 <sup>b</sup>	167.83 <sup>ab</sup>	6.04 <sup>ab</sup>	201.6 <sup>ef</sup>	137.71 <sup>abc</sup>	1.68 <sup>cd</sup>	41.39 <sup>e</sup>
Torpe	84.83 <sup>b</sup>	164.75 <sup>bc</sup>	6.04 <sup>ab</sup>	209.2 <sup>bcdef</sup>	129.58 <sup>c</sup>	1.78 <sup>bc</sup>	40.41 <sup>g</sup>
T1	94.50 <sup>a</sup>	167.92 <sup>ab</sup>	6.58 <sup>ab</sup>	228.4 <sup>ab</sup>	143.25 <sup>abc</sup>	1.82 <sup>ab</sup>	42.50 <sup>c</sup>
T2	98.92 <sup>a</sup>	171.17 <sup>a</sup>	7.08 <sup>a</sup>	232.1 <sup>a</sup>	150.42 <sup>a</sup>	1.93 <sup>a</sup>	46.24 <sup>a</sup>
T3	99.17 <sup>a</sup>	167.25 <sup>abc</sup>	6.75 <sup>ab</sup>	227.1 <sup>abc</sup>	147.17 <sup>ab</sup>	1.87 <sup>ab</sup>	42.92 <sup>b</sup>
ANOVA	df						
Irrigation (I)	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype (G)	9	<0.001	<0.001	0.006	<0.001	<0.001	<0.001
I × G	27	0.041	0.450	0.990	0.122	0.125	<0.001

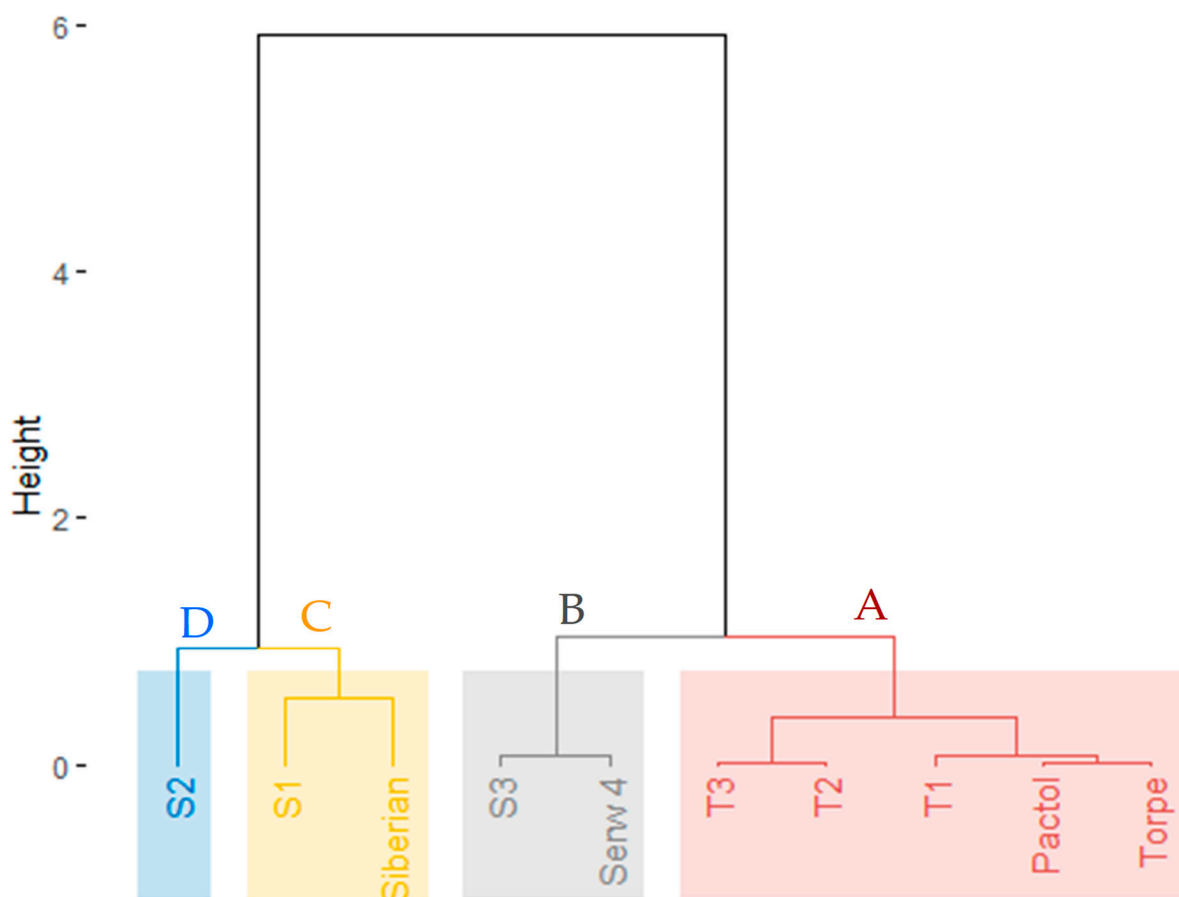
Means followed by different letters under the same factor were significantly different according to Tukey’s HSD test ( $p \leq 0.05$ ).



**Figure 3.** Response days to flowering and oil percentage of ten canola genotypes to four irrigation regimes averaged over two growing seasons. The bars on the top of the columns represent the SE, and different letters on the column of each irrigation regime (with the same color) show the significant difference using Tukey’s HSD test ( $p \leq 0.05$ ).

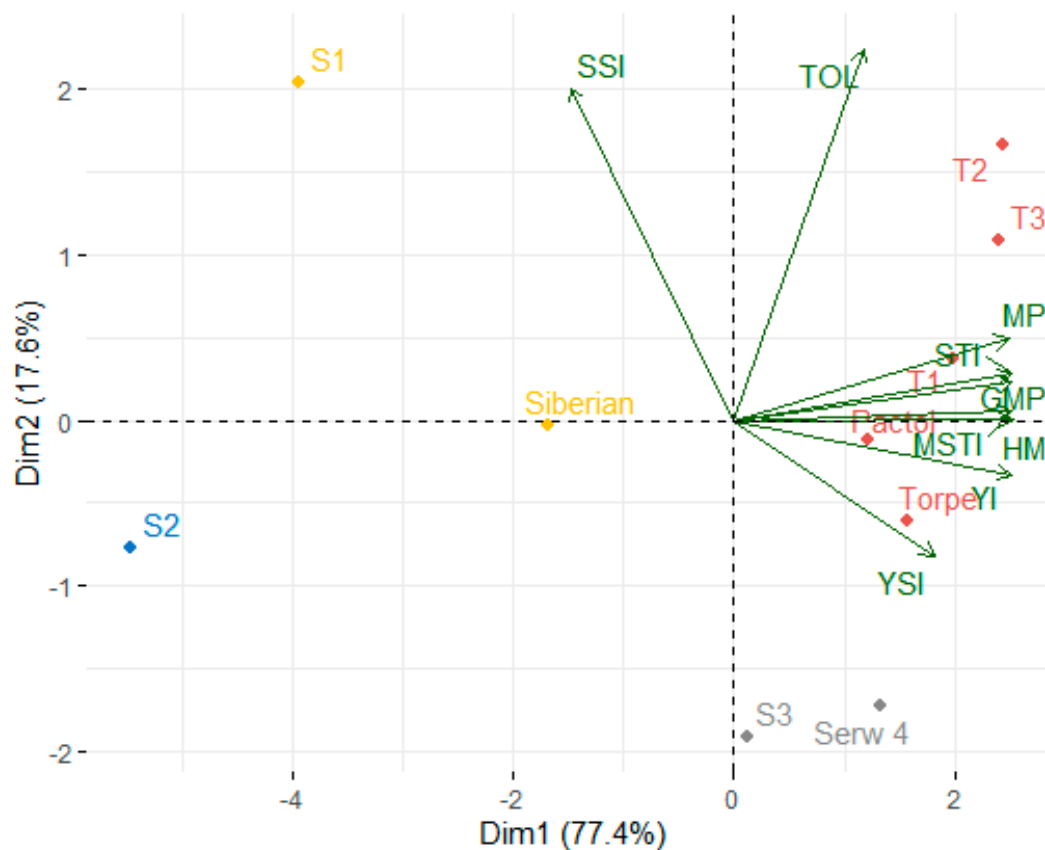
### 3.3. Genotypic Classification

Nine drought indices were calculated based on the seed yield under well-watered and severe drought conditions. A hierarchical cluster was employed to classify the assessed canola genotypes based on these nine tolerance indices (Figure 4). According to the cluster analysis, the genotypes were classified into four groups. Group A consisted of five genotypes (T1, T2, T3, Pactol, and Torpe), Group B included two genotypes (Serw-4 and S3), Group C comprised two genotypes (S1 and Siberian), and Group D had one genotype (S2). Group A displayed favorable tolerance indices and was considered to have drought-tolerant genotypes. In contrast, Group C and D represented the worst values of tolerance indices and, accordingly, were considered to have drought-sensitive genotypes. The tissue-cultured genotypes from Torpe showed a better drought tolerance compared with those developed from Siberian. A principal component analysis was employed to explore the relationship among the studied tolerance indices and assessed genotypes (Figure 5). The results displayed that most drought indices were grouped on PC1 (77.4%), except the SSI and TOL indices, which were located on PC2 (17.6%). Moreover, PC1 divided the assessed genotypes into two groups. The genotypes T1, T2, T3, Pactol, Torpe, Serw-4, and S3 were located on the positive side and were associated with all tolerance indices, except SSI and TOL. On the other hand, S1, S2, and Siberian were situated on the negative side and were associated with SSI and TOL.



**Figure 4.** Dendrogram of the phenotypic distances among ten canola genotypes based on nine drought tolerance indices.





**Figure 5.** Principal component analysis for canola genotypes based on drought indices. The genotypes in the same color represent the same group of genotypes.

### 3.4. Relationships among Assessed Treatments and Traits

A principal component analysis was employed to explore the association among the assessed treatments and studied traits (Figure 6). The first two PCs explained 78.36% of the variability. PC1 displayed 69.79% of the variation and was associated with the irrigation regimes from well-watered to severe drought conditions (Figure 6). PC1 divided the irrigation regimes into two groups; the severe and moderate drought conditions were located on the negative side, but those of the mild drought and well-watered conditions were situated on the positive side. PC2 exhibited 8.57% of the variation and seemed to correspond with the assessed canola genotypes. The canola genotypes were more dissimilar, with plots under drought conditions (severe, mild, and moderate) compared with well-watered conditions. All agronomic traits, in addition to the chlorophyll content and relative water content, were positively correlated with well-watered and mild drought conditions on the positive side of PC1. However, the proline, anthocyanin, glycine betaine, and total phenolic contents were positively associated with severe and moderate drought stress, but negatively associated with well-watered and mild drought conditions as well as agronomic traits, chlorophyll content, and relative water content.

Furthermore, a correlation network was performed for the further exploration of the association among the evaluated traits (Figure 7). The agronomic traits were positively intercorrelated; the seed yield presented strong associations with most agronomic traits, but a weak relationship with the plant height (PH) and oil percentage (Figure 7). Conversely, most physiological parameters were negatively intercorrelated with agronomic traits. With an exception, positive correlations were observed for anthocyanin content (AC) with glycine betaine (GB) and total phenolic content (TPC) as well as total chlorophyll (Chl) content and relative water content (RWC). Moreover, the total chlorophyll content showed positive correlations with agronomic traits, especially with the seed yield (SY), number of pods

per plant (NoP), and number of branches per plant (NoB). Similarly, the relative water content was positively correlated with the seed yield, number of pods per plant, and days to maturity (DTM).

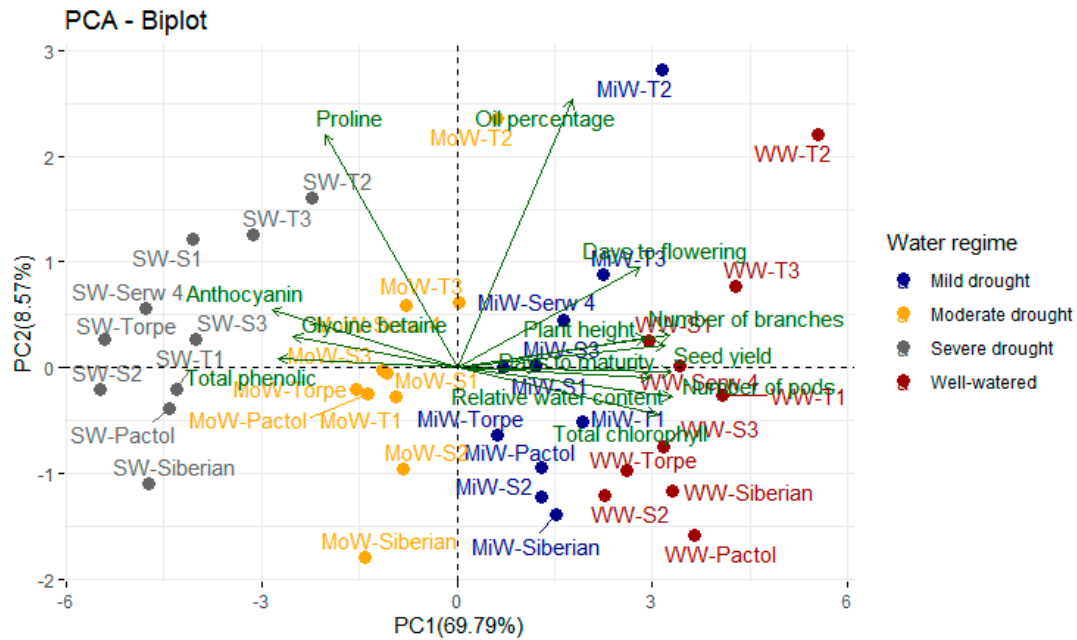


Figure 6. PC biplot for the studied traits of canola under four irrigation regimes (well-watered (WW), mild drought (MiW), moderate drought (MoW), and severe drought (SW)) over two growing seasons.

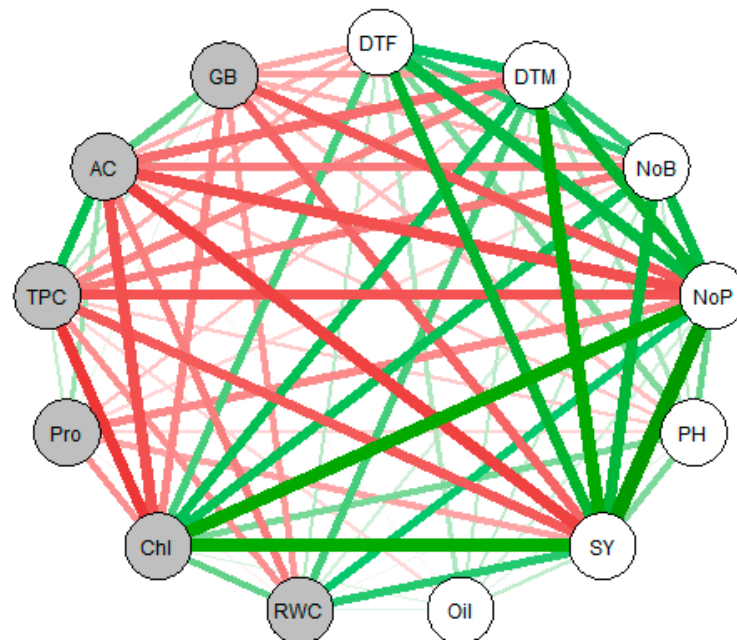


Figure 7. Correlation network of physiological (grey) and agronomic traits (white). The red lines represent negative correlations and the green lines represent positive correlations. Line width indicates the strength of the relationship. Only significant ( $p < 0.05$ ) correlations are shown. Chl: total chlorophyll; RWC: relative water content; Pro: proline content; AC: anthocyanin content; GB: glycine betaine; TPC: total phenolic content; DTF: days to flowering; DM: days to maturity; NoB: number of branches per plant; NoP: number of pods per plant; PH: plant height; SY: seed yield; Oil: oil percentage.

#### 4. Discussion

Drought is one of the major constraints of canola production in Mediterranean semi-arid environments and is expected to be more severe under the current climate change [44–46]. In the present study, water-deficit conditions adversely affected all recorded traits compared with well-watered conditions. Drought-impaired physiological traits such as the total chlorophyll and relative water content were reflected in the seed yield and its components. The adverse impact of drought stress on agronomic performance can be partly explained by reducing the nutrient uptake, limiting its movement from the roots to the shoot and impairing membrane permeability [47,48]. In this respect, Jamshidi et al. [18], Rad and Zandi [20], Eyni Nargeseh et al. [49], and Fard et al. [50] elucidated that drought stress considerably reduces the plant height, number of branches, number of pods, and canola seed yield. Otherwise, a water deficit increases the proline, anthocyanin, glycine betaine, and total phenolic contents. Under drought stress, organic solutes called osmoprotectants are increased in the plants to cope with the induced stress [51,52]. Likewise, Ahmad et al. [53], Khan et al. [54], and Khan et al. [55] disclosed an increase in osmoprotectant contents under drought stress. The accumulation of osmoprotectants may serve as a readily accessible source of nitrogen, carbon, and energy during recovery after drought stress [56]. These osmoprotectants (such as glycine betaine and proline) are exogenously applied to improve the growth, survival, and tolerance of plants under various environmental stresses [57].

Under the current climate change and continuing population growth, it has become more crucial to develop new drought-tolerant canola genotypes with high oil yields to ensure global food security. Sakhanokho and Kelley [58] and Orbović et al. [59] disclosed that *in vitro* plant tissue culture has become one of the most effective approaches in recent decades to induce selective conditions. Several drought-tolerant genotypes have been developed based on tissue cultures supplemented with selective agents for different species, as proved by Purushotham et al. [60], Gangopadhyay et al. [61], Ochatt et al. [62], and Errabii et al. [63]. In the present study, six developed tissue-cultured canola genotypes alongside two exotic genotypes and two commercial cultivars were assessed under four irrigation regimes. The evaluated canola genotypes displayed an appreciable variability for all measured traits. This finding indicated an adequate genetic variability among the used genotypes and the possibility of breeding new canola cultivars. The detected considerable genotypic variations were in consonance with the results of Kandil et al. [64]. The evaluated set of canola genotypes was clustered into four different groups according to drought tolerance indices. The variable response of canola genotypes to drought stress was also demonstrated by Zali et al. [65]. The tissue-cultured genotypes T2 and T3 regenerated from Torpe were the most drought-tolerant genotypes. These two genotypes were more vigorous and productive in the field, as expressed by the high values for the plant height, number of branches per plant, and number of pods per plant. These two genotypes were more productive, with the highest seed yields under drought-stress conditions even compared with the commercial cultivars Serw-4 and Pactol. The evaluated commercial cultivars were previously demonstrated to be more tolerant in the study of Kandil et al. [64]. Furthermore, T2 and T3 reached flowering and maturity later than the other developed genotypes and commercial cultivars. The late flowering and maturity seemed to be favorable for high seed and oil yields, as elucidated by Riffkin et al. [66]. This indicated that these genotypes maintained their photosynthesis activity and growth longer than the other genotypes, which could lead to a better seed set and filling, and ultimately to a high seed yield under drought conditions. Moreover, T2 and T3 accumulated more proline compared with the other genotypes. The accumulation of proline contributes to osmotic modifications under water scarcity due to acclimatization to recompense for plant survival and, accordingly, assists in tolerating drought stress [67–69]. Therefore, the proline accumulation was detected to be higher in the most tolerant genotypes [70]. The favorable performance exhibited by the tissue-cultured genotypes T2 and T3 was different from the mother parent Torpe. This indicated that the improvement in these

genotypes was most likely caused by a somaclonal variation that had occurred through the tissue-culture process under osmotic stress. Consequently, the traits that were altered during the tissue-culture process under salt stress offered more tolerance to the tissue-cultured genotypes. Plants develop diverse activated defense mechanisms and stress-responsive signaling pathways to adapt to drought and salinity stresses [71]. In this context, Golldack et al. [72] and Ma et al. [73] deduced that plant tolerance to drought and salinity stress was similar in multicomponent signaling pathways, plant metabolism, biochemical responses, and energy supply to restore cellular homeostasis and promote survival. Likewise, Agarwal et al. [74] elucidated that the regulatory processes of plant tolerance to drought and salinity involve the control of cellular osmotic adjustments and water flux via the biosynthesis of osmoprotectants.

Understanding the relationship between the studied traits is a vital aspect that can provide valuable information [75–77]. The formation of pods, including the seed set, is highly impacted by drought stress [8]. The obtained results showed that the number of pods and branches per plant were highly associated with the seed yield. Additionally, the chlorophyll content was positively associated with the seed yield, indicating that an increased chlorophyll content under drought conditions would improve the seed yield. In this respect, Khodabin et al. [78] deduced that tolerant canola cultivars exhibited a higher chlorophyll content under water-stress conditions. Germchi et al. [79] manifested that a higher number of branches could be attributed to improving the seed yield. Moreover, Diepenbrock [80] depicted that the number of siliqua per plant was highly related to the seed yield. Accordingly, improving the traits that exhibit high associations with the seed yield would improve canola productivity under drought stress.

## 5. Conclusions

A water shortage significantly reduced the total chlorophyll, relative water content, days to flowering, days to maturity, number of branches per plant, number of pods per plant, and plant height, which ultimately led to a poor performance in the seed yield and oil percentage. The newly developed tissue-cultured genotypes possessed genetic diversity, which was reflected in their different responses to the studied irrigation regimes. The genotypes T2, T3, T1, and Torpe were less affected by drought stress and exhibited a better performance in all evaluated parameters. These genotypes surpassed the other tested genotypes and commercial cultivars under different drought levels. Conclusively, these genotypes displayed a superiority in the physiological and agronomic parameters compared with the commercial cultivars, which could be exploited in improving canola production in water-limited environments.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13030836/s1>, Table S1. Climatic data for the experimental site in 2019 and 2020 cropping seasons. Table S2. Soil properties of the experimental site for 2018–2019 and 2019–2020 growing seasons Table S3. Analyses of variance of agronomic and physio-chemical traits as affected by canola genotypes, irrigation regimes and their interactions.

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