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Rapeseed Morpho-Physio-Biochemical Responses to Drought Stress Induced by PEG-6000

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Abstract: Rapeseed is a valuable oil crop due to its high nutritious value and ample oil content. The current study provides a comparative analysis of 24 cultivars to better understand the performance and predict the adaptative mechanisms of drought-tolerant and drought-sensitive cultivars based on germination and morphophysiological traits during the early seedling stage using PEG-6000 simulated drought conditions. JYZ 158 and FY 520 (tolerant cultivars) and YG 2009 and NZ 1838 (sensitive cultivars) were selected to further explore the role of osmolytes and enzymatic activity in improving drought tolerance. This investigation illustrated that drought stress negatively influenced all studied cultivars; however, the degree of influence was different for each cultivar, suggesting their different potential for drought tolerance. Moreover, enzymatic and osmoregulatory mechanisms were highly efficient in tolerant cultivars compared to sensitive cultivars. Additionally, tolerant cultivars showed higher chlorophyll and lower malondialdehyde (MDA) contents versus sensitive cultivars under drought stress conditions. Higher drought tolerance coincided with higher enzymatic activity and osmolyte content. This work showed that JYZ 158 and FY 520 cultivars had higher drought tolerance, and might be a significant germplasm resource for breeding programs developing drought-tolerant rapeseed.

Keywords: rapeseed; drought; early seedling stage; osmolytes; antioxidant enzymes

Citation: Batool, M.; El-Badri, A.M.; Wang, Z.; Mohamed, I.A.A.; Yang, H.; Ai, X.; Salah, A.; Hassan, M.U.; Sami, R.; Kuai, J.; et al. Rapeseed Morpho-Physio-Biochemical Responses to Drought Stress Induced by PEG-6000. *Agronomy* **2022**, *12*, 579. https://doi.org/10.3390/agronomy12030579

Academic Editor: María del Pilar Cordovilla

Received: 14 January 2022
Accepted: 15 February 2022
Published: 26 February 2022

1. Introduction

Rapeseed (*Brassica napus* L.) is a valuable and economically important oilseed crop globally, occupying a large cultivation area in China with more than 7 million hectares [1]. It is one of the most important crops for global oil production and is a multipurpose edible crop [2]. Rapeseed meal is a valuable animal feed in the feed industry. Moreover, it has nutritional importance due to its ideal amino acid content, higher fiber content, and contents of essential vitamins and minerals [3]. It is susceptible to drought stress, which is detrimental at each developmental phase of the plant life cycle [4].

Water deficit is one of the crucial limiting factors which reduces crop growth and productivity [5]. China is hit badly by drought events, which directly affect the economy, causing losses higher than 4.78 billion euro (according to the 2018 price level). An area

of more than 200 thousand km^2 was affected between 1984 and 2018 (China Meteorological Administration, 2019). Drought stress is a critical abiotic factor that damages plants, increases oxidative stress and reduces plant height [6]. Furthermore, it negatively affects morpho-physiochemical processes and metabolic responses [7]. Drought-stressed rapeseed seedlings show a decrease in germination percentage, poor growth and vigor index with lower biomass accumulation [8], along with severe oxidative damage and impaired antioxidant defense systems [9].

Seed germination is an essential biological process in the growth cycle of plants [10]. In semi-arid areas, successful crop production is mainly dependent on optimum seed germination and early seedling growth that is closely linked with the capacity of seeds to sprout under drought stress [11]. Several physiochemical processes associated with moisture availability, stored material mobilization, hormonal activities and protein structure are affected under drought conditions, affecting seedling survival and growth [12]. It is widely documented that initial drought stress restricts seed germination leading to poor stand establishment of seedlings during development, hence impairing the crop growth [13,14].

Drought conditions have inhibitory effects on rapeseed growth, impairing photosynthetic processes, leaf water content and subsequent developmental processes [15]. Additionally, the decline in photosynthetic pigments can be associated with a lower water supply, which reduces leaf water content [16]. Water deficiency causes chloroplasts to become oval to round in shape and move toward the center of the cell, indicating that drought impairs structural integrity [17]. Malondialdehyde (MDA) is the product of peroxidation of lipids in the membrane and an indicator of various stresses [18].

The generation of reactive oxygen species (ROS) is a responsive action taken by plants [19,20]. The equilibrium between synthesis and degeneration of ROS is not maintained under drought stress; hence, ROS (free radicals) accumulate in the cells, leading to cell membrane dysfunction [21]. Drought stress-induced lipid peroxidation enhances ROS production and breaks down unsaturated fatty acids, ultimately causing structural degradation of the seed and arresting seed germination [22]. Malondialdehyde (MDA) is the product of lipid peroxidation, and proline is one of the antioxidants which maintain cell turgor via osmotic adjustment and regulate redox metabolic processes to scavenge ROS [11].

Plants have a complicated enzymatic defensive mechanism against oxidative stress to suppress ROS overproduction that is correlated with tolerance against unfavorable conditions [23]. Osmotic substances play protective roles for membrane and assist the plant in water intake for maintaining physiological functioning [24]. Moreover, antioxidant enzymes, including superoxide dismutase (SOD) and peroxidase (POD), defend the cell membrane from oxidative damage by removing excessive ROS from cells under stress conditions [22]. Notably, catalase (CAT) and ascorbate peroxidase (APX) alleviate the damaging effects of stress [25]. The accumulation of osmolytes, such as proline, soluble sugars and protein, upon drought stress is linked to stress tolerance [18,26]. Moreover, total soluble sugar (TSS) and total soluble protein (TSP) are two important osmo-protectants that can help the plant withstand unfavorable environments [19].

It is important to identify drought-tolerant germplasm before developing a drought tolerance breeding program. Therefore, the current study aimed to increase understanding of the influence of drought stress on morphophysiological attributes of rapeseed by measuring key factors such as seedling growth, photosynthetic pigments, osmolytes accumulation, lipid peroxidation and enzymatic antioxidants. Diversity in the ability of the most common rapeseed cultivars to withstand drought stress during seed germination and the early seedling stage was examined. Our results can be used for further analysis and subsequent research.

2. Material and Methods

2.1. Plant Materials and Growth Conditions

A panel comprised of 24 rapeseed cultivars with different genetic backgrounds was selected based on agronomic performance, economic importance and cultivated area to study the deleterious effects of drought stress during the early seedling stage (Table S1). The experiment was carried out in bifactorial design using three replications with four biological replications. The first factor contained 24 cultivars, and the second factor involved drought stress using polyethylene glycol 6000 (PEG-6000). Polyethylene glycol 6000 is a high molecular weight compound that is unable to pass through the cell wall; therefore, it can regulate water potential in the cells by outward water flow from plant tissues into a concentrated solution [27].

2.2. Germination Trails

A pilot study was conducted to select the concentration of PEG-6000 that should be used for inducing drought levels in the screening of cultivars. Three cultivars, randomly selected, were subjected to different levels of drought (0, 5, 10, 15, 20 and 25% PEG-6000) for seven days. The results were noted for final germination percentage (FG%) and it was found that PEG-6000 with 5% concentration was similar to 0%, where the FG% was 99.44, 99.44 and 93.88% (normal conditions) and 99.44, 98.33 and 95.00% (5% PEG-6000) in YYZ 3, XZY 518 and GZ 1, respectively, indicating that a 5% concentration is too low. A 20% concentration showed significantly reduced FG% (85.55, 88.88 and 80.55%) in YYZ 3, XZY 518 and GZ 1, respectively, and inhibited seedling growth. The severe drought effect caused stunted growth and could not use to measure required plant attributes. By comparison, 25% PEG-6000 showed highly significantly lower FG% (8.888, 20.00 and 24.44%) in YYZ 3, XZY 518 and GZ 1, respectively, suggesting severe stress without growth (Table S3). The maximum visible response was noted at 15%, and a slight difference noted at 10% PEG-6000, which were used for further study.

Mature seeds of 24 cultivars were carefully selected and hand-picked based on uniform size, surface sterilized using 70% ethanol (5 min), rinsed (5 times) with distilled water, and dried using blot paper until constant weight. Sixty uniform and healthy seeds were sown in polyethylene boxes (12 × 12 × 6 cm) with three-layered sterilized filter paper with 15 mL of a solution of 0, 10 or 15% PEG-6000 in each germination box. The experiment was carried out for seven days in a growth chamber (day/night temperature at 25/20 °C) with 12 h light (13,000 lx) and 12 h dark, at Huazhong Agricultural University, Wuhan, Hubei, China.

2.3. Assessment of Morphological Traits

Seeds with a minimum radicle extrusion of 2 mm were considered germinated and were counted daily in each box for seven days. Final germination percentage (FG%), germination rate (GR), vigor index I (VI (I)) and vigor index II (VI (II)) were measured at the seventh day of the early growth stage. A description is given in (Table S2), according to the equation provided by [28]. Seedlings were harvested on the seventh day, and 10 seedlings with a uniform appearance from each replication were used to measure root and shoot length. Shoot and root fresh weight were calculated from the same seedlings, then dry weight was measured after the samples were dried at 80 °C to constant weight.

2.4. Determination of Photosynthetic Pigments

After 7-days of treatment, chlorophyll (chl) and carotenoid contents (μg g^{-1} FW) in fresh leaves were determined. First, 0.1 g FW was mixed with 80% acetone in test tubes, kept overnight, and then centrifuged. Afterwards, absorbance was noted using an ultraviolet spectrophotometer (UV-2100, UNIC, Shanghai, China) at 646, 663 and 480 nm [10].

2.5. Determination of Relative Water Content (RWC)

After sampling, small leaves from whole plants were weighed, maintained in distilled water overnight, then dried with blotted paper and the saturated leaves weighed (turgor

weight). The weighed samples were dried for 48 h at 80 °C, and the dry weight was noted. Leaf relative water content was calculated using the equation according to [29].

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{dry weight})}{(\text{Turgor weight} - \text{dry weight})} \times 100$$

2.6. Determination of Total Soluble Sugar, Total Soluble Protein, Proline, and MDA Contents

Total soluble sugar was estimated in samples using the anthrone sulfuric acid method. Briefly, 0.1 g fresh weight of sample was mixed thoroughly with 10 mL water. Afterwards, the mixture was boiled for 30 min at 100 °C followed by centrifugation. The supernatant was collected and mixed in sulfuric acid-anthrone reagent, then boiled for 10 min at 95–100 °C in a water bath and cooling. The absorption value was read on a spectrophotometer at 620 nm following the method of [30]. The Coomassie brilliant blue (CBB) method was used to estimate total soluble protein in the fresh sample, the absorbance value at 595 nm being read on a spectrophotometer following the method of [31].

Proline content was measured using the method described by [32]. Fresh shoots (0.1 g) were mixed with 3% aqueous sulfosalicylic acid and the homogenate was centrifuged. Then supernatants were mixed with glacial acetic acid and ninhydrin reagent and shaken thoroughly, then placed in a water bath for 30 min followed by cooling. The mixture was centrifuged at 10,000 rpm for 5 min, extracted with 4 mL toluene followed by vortex mixing, and the absorption value was noted using a UV-spectrophotometer at 520 nm. Proline content was measured using a standard curve [32].

Malondialdehyde (MDA) content measures lipid peroxidation, assessed by the Heath and Packer method [33]. Fresh shoot sample (0.5 g) was homogenized with 5 mL of 0.1% (*w/v*) trichloroacetic acid (TCA) and centrifuged for 20 min. Supernatants were collected, and 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. Then, the reaction solution was subjected to heating for 30 min at 95 °C followed by cooling, centrifugation for 15 min and supernatants were collected carefully. The MDA content was calculated using a UV-spectrophotometer at 450, 532 and 600 nm.

2.7. Measurement of Antioxidant Enzyme Activities

The activities of antioxidant enzymes were assessed by homogenizing 0.1 g of crushed frozen samples with potassium phosphate buffer (PPB) (pH 7.8). The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C to collect the supernatant. SOD, CAT, POD and APX activities were determined in the supernatant using a spectrophotometer with respective wavelengths according to the manufacturer's instructions, respectively, following the methods of [10].

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assessed by inhibiting photochemical reduction by nitro blue tetrazolium (NBT). The reaction mixture contained 50 mM PPB (pH 7.8), 13 mM methionine, 75 mM NBT, 2 mM riboflavin, 0.1 mM EDTA and 0.1 mL of enzyme extract in a 3 mL volume. One unit of SOD activity was measured as the amount of enzyme required to cause 50% inhibition of NBT reduction and was measured spectrophotometrically at 560 nm.

To assay peroxidase (POD; EC 1.11.1.7) activity, 0.1 mL enzyme extract was mixed with 50 mM PPB (pH 7.0), 1% (*m/v*) guaiacol, and 0.4% (*v/v*) H_2O_2. The absorbance was measured at a 470 nm.

The assay for ascorbate peroxidase (APX; EC 1.11.1.11) was conducted using a reaction mixture (3 mL) containing 100 mM phosphate (pH 7), 0.1 mM EDTA-Na2, 0.3 mM ascorbic acid, 0.06 mM H_2O_2, and 0.1 mL enzyme extract. The change in absorption was quantified at 290 nm for 30 s after adding H_2O_2.

The method to measure catalase (CAT; EC 1.11.1.6) activity used H_2O_2 (extinction co-efficient 39.4 mM^{-1} cm^{-1}), 3 mL reaction mixture containing 50 mM PPB (pH 7.0), 2 mM EDTA-Na_2, 10 mM H_2O_2, and 0.1 mL enzyme extract, the spectrophotometric assay recorded at 240 nm.

2.8. Microstructural Analysis

Fresh leaf samples were cleaned with distilled water and cut into uniform slices, fixed with 4% glutaraldehyde and 0.2 M sodium phosphate buffer (pH 6.8) and then distilled with 0.1 M sodium phosphate buffer (pH 6.8). Phosphate buffer (0.2M, pH 6.8) was used to fix the sample. Afterwards, dehydration was done in a gradient ethanol series. Slices were examined using a transmission electron microscope after staining with lead citrate and 2% uranyl acetate [34].

2.9. Statistical Analysis

The experiment was carried out as a bifactorial design, and measurements were made with three replications. Statistical analysis for germination and growth-related traits was conducted using Statistix 8.1 software with linear models. Significant differences (LSD) were calculated to examine differences at $p < 0.05$. Differences among treatments were determined using ANOVA. Graphical presentation was carried out using GraphPad prism (V: 5.0.1) and RStudio software.

3. Results

3.1. Variation in Seed Germination Traits under Drought Stress

The impact of drought stress on various 24 cultivars of rapeseed using different concentrations of PEG-6000 (0, 10 and 15%) was studied. The mean values of FG%, GR, VI (I) and VI (II) were measured to estimate the negative effect of drought stress on seed germination. Results showed that the mean values of all measured traits were significantly reduced at the higher level of drought stress (15% PEG-6000) compared to the control (Table 1). Remarkably, few cultivars showed better performance under 10% PEG-6000-induced drought than under normal conditions. Box and whisker charts showed the variation in germination traits for all 24 rapeseed cultivars, measured under 0, 10 and 15% PEG-6000 treatments. Additionally, the box and whisker charts showed substantial variations of germination-related traits between treatments, especially at 15% PEG 6000, indicated by the lower and upper limits of box plot for each trait (Figure 1). The mean of the measured traits showed a significant reduction at the higher level of drought stress (15% PEG-6000). The mean values of FG%, GR, V(I) and V(II) were 94.67%, 33.00, 921.3 and 36.87 (normal conditions), 94.49%, 27.02, 1056 and 26.49 (10% PEG-6000), 83.07%, 19.44, 706.0 and 15.65 (15% PEG-6000), respectively (Table 1).

Table 1. Seed germination traits under different concentrations of PEG-6000-induced drought stress.

Variety	FG%			GR			VI (I)			VI (II)		
	CK	10%	15%	CK	10%	15%	CK	10%	15%	CK	10%	15%
CY 81	91.67 $^{b–e}$	90.55 ^{cde}	57.77 ^{j}	20.76 ^{m}	20.76 ^{j}	9.840 ^{l}	976.7 ^{de}	1045 ^{fgh}	592.3 ^{hi}	33.33 $^{h–k}$	27.38 $^{d–h}$	10.33 ^{k}
YYZ 3	99.33 ^{a}	98.88 ^{ab}	99.44 ^{a}	43.42 ^{cd}	32.47 ^{c}	28.28 ^{bc}	1061 ^{cd}	1120 $^{c-f}$	878.7 ^{cd}	31.66 ^{klm}	20.55 ^{j}	16.54 ^{efg}
YY 28	97.77 ^{abc}	96.11 ^{abc}	78.33 ^{h}	35.49 ^{f}	27.69 ^{efg}	14.28 ^{k}	347.3 ^{j}	520.3 ^{l}	336.7 ^{j}	31.33 ^{lm}	24.68 ^{hi}	13.67 ^{hij}
CY 36	99.33 ^{a}	98.88 ^{ab}	89.44 ^{def}	30.95 ^{hi}	28.16 ^{ef}	23.44 ^{ef}	804.3 ^{gh}	1102 $^{d-g}$	820.4 ^{de}	32.66 ^{jkl}	27.32 $^{e–h}$	18.33 ^{b}
JYZ 158	96.67 $^{a–d}$	98.33 ^{ab}	97.77 ^{ab}	51.33 ^{a}	39.24 ^{a}	30.82 ^{ab}	1243 ^{ab}	1361 ^{a}	1041 ^{a}	45.66 ^{ab}	34.67 ^{a}	22.33 ^{a}
ZY 50	98.67 ^{a}	99.44 ^{ab}	96.66 ^{abc}	28.29 ^{ij}	27.64 ^{efg}	22.53 ^{fg}	942.1 $^{d-g}$	1245 ^{bc}	813.3 ^{de}	32.66 ^{jkl}	26.66 $^{e–h}$	14.80 $^{f–i}$
QY 33	96.67 $^{a–d}$	100.0 ^{a}	85.56 ^{efg}	42.54 ^{cd}	33.48 ^{c}	20.36 ^{ghi}	1133 ^{c}	1388 ^{a}	881.7 ^{efg}	37.66 $^{d–h}$	23.68 ^{l}	14.67 $^{f-i}$
ZY 51	96.67 $^{a-d}$	96.11 $^{a–d}$	67.67 ^{i}	25.22 ^{k}	24.72 ^{hi}	13.99 ^{k}	873.7 ^{efg}	976.5 ^{hi}	509.6 ^{i}	40.33 ^{cd}	26.89 $^{e–h}$	13.67 ^{ij}
XZY 518	99.33 ^{a}	99.44 ^{ab}	91.30 $^{b-e}$	41.25 ^{d}	31.56 ^{cd}	22.36 ^{fg}	973.7 ^{def}	1087 ^{gh}	712.3 ^{fg}	37.66 $^{d–i}$	27.01 $^{f–h}$	18.46 ^{bc}
GHY 8	89.33 ^{e}	89.44 ^{def}	88.88 ^{def}	24.94 ^{kl}	24.95 ^{hi}	20.83 ^{gh}	746.3 ^{h}	788.1 ^{j}	687.5 ^{gh}	35.86 $^{i–l}$	23.60 ^{l}	13.66 ^{ij}
ZYZ 108	91.11 ^{cde}	88.33 ^{ef}	79.33 ^{hg}	33.28 ^{fg}	24.79 ^{hi}	17.63 ^{j}	595.1 ^{i}	976.6 ^{hi}	617.3 ^{h}	37.58 $^{e–i}$	29.38 ^{cde}	15.79 $^{e–h}$
NZ 1838	90.00 ^{de}	83.88 ^{f}	55.67 ^{j}	16.93 ^{n}	15.26 ^{k}	7.251 ^{m}	839.1 ^{fgh}	921.1 ^{i}	259.3 ^{j}	32.64 ^{jkl}	20.55 ^{j}	7.336 ^{l}
XZY 553	98.67 ^{ab}	99.44 ^{ab}	96.67 ^{abc}	43.86 ^{c}	32.37 ^{c}	25.47 ^{de}	987.7 ^{de}	1070 $^{e-h}$	922.3 ^{bc}	41.66 ^{bc}	32.28 ^{bc}	18.51 ^{bc}
YY 9	99.33 ^{a}	96.66 ^{abc}	78.33 ^{h}	31.69 ^{gh}	22.32 ^{il}	14.99 ^{k}	810.7 ^{gh}	710.6 ^{jk}	352.7 ^{j}	38.66 ^{def}	20.33 ^{j}	12.67 ^{j}
HYZ 62	96.67 $^{a–d}$	97.77 ^{ab}	88.33 ^{def}	41.83 ^{d}	29.98 ^{de}	19.90 ^{hij}	973.7 ^{de}	985.7 ^{ghi}	607.3 ^{hi}	48.43 ^{a}	32.28 ^{ab}	17.33 ^{cde}
QY 3	95.67 $^{a–e}$	94.44 $^{a–d}$	82.67 ^{fgh}	38.62 ^{e}	28.58 ^{ef}	14.10 ^{k}	1127 ^{c}	1298 ^{ab}	818.2 ^{de}	38.66 ^{def}	26.67 $^{e–h}$	16.67 ^{cde}
QY 7	90.00 ^{de}	90.55 ^{cde}	90.00 ^{cde}	33.35 ^{fg}	25.61 ^{gh}	22.65 ^{fg}	822.7 ^{gh}	976.0 ^{hi}	883.3 ^{bcd}	28.66 ^{m}	20.66 ^{j}	16.33 ^{cde}
ZS 11	96.11 $^{a–e}$	97.77 ^{b}	93.67 $^{a–d}$	32.70 ^{gh}	26.60 ^{fgh}	26.67 ^{cd}	956.3 $^{d–g}$	1072 ^{fgh}	1033 ^{a}	35.47 $^{g–j}$	29.33 ^{def}	16.33 ^{cde}
YG 2009	66.67 ^{f}	66.66 ^{g}	28.67 ^{k}	9.352 ^{o}	10.51 ^{l}	4.661 ^{n}	540.3 ^{i}	606.4 ^{kl}	162.4 ^{k}	21.69 ^{n}	14.36 ^{k}	3.330 ^{m}
HYZ 72	93.33 $^{a–e}$	96.66 ^{abc}	88.33 ^{def}	27.65 ^{j}	25.31 ^{gh}	17.84 ^{ij}	1046 ^{cd}	1351 ^{ab}	787.7 ^{ef}	37.69 $^{d-g}$	27.59 ^{fgh}	15.67 $^{f–i}$
QY 1	98.67 ^{ab}	96.11 $^{a–d}$	88.33 ^{fgh}	31.31 ^{gh}	27.68 ^{efg}	20.64 ^{gh}	1133 ^{c}	1176 ^{cd}	972.3 ^{a}	36.39 $^{e–i}$	26.33 ^{ghi}	17.33 ^{cde}
TYZ 283	96.67 $^{a–e}$	97.77 ^{ab}	82.67 ^{cde}	22.64 ^{lm}	20.90 ^{j}	13.92 ^{k}	1036 ^{cd}	1137 ^{cde}	587.7 ^{hi}	35.68 $^{f–j}$	27.51 $^{d–g}$	14.51 ^{ghi}
GZ 1	93.67 $^{a–e}$	93.88 $^{b–e}$	90.00 ^{cde}	38.35 ^{e}	31.55 ^{cd}	22.59 ^{fg}	1136 ^{bc}	1325 ^{ab}	974.3 ^{ab}	38.77 ^{de}	29.30 ^{cd}	17.68 ^{bcd}

Table 1. *Cont.*

Variety	FG%			GR			VI (I)			VI (II)		
	CK	10%	15%	CK	10%	15%	CK	10%	15%	CK	10%	15%
FY 520	98.88 ^{a}	100.0 ^{a}	98.30 ^{ab}	46.36 ^{b}	36.43 ^{b}	32.28 ^{a}	1281 ^{a}	1088 $^{d–g}$	896.7 ^{bcd}	47.44 ^{a}	32.67 ^{ab}	23.78 ^{a}
Mean	94.67	94.49	83.07	33.00	27.02	19.44	921.3	1056	706.01	36.87	26.49	15.65

FG%: final germination percentage; GR: germination rate; VI (I): vigor index (I) and VI (II): vigor index (II). According to Fisher's least significant difference (LSD) test, data are presented as mean values with different letters that denote statistically significant differences between means within each indicator column among cultivars.

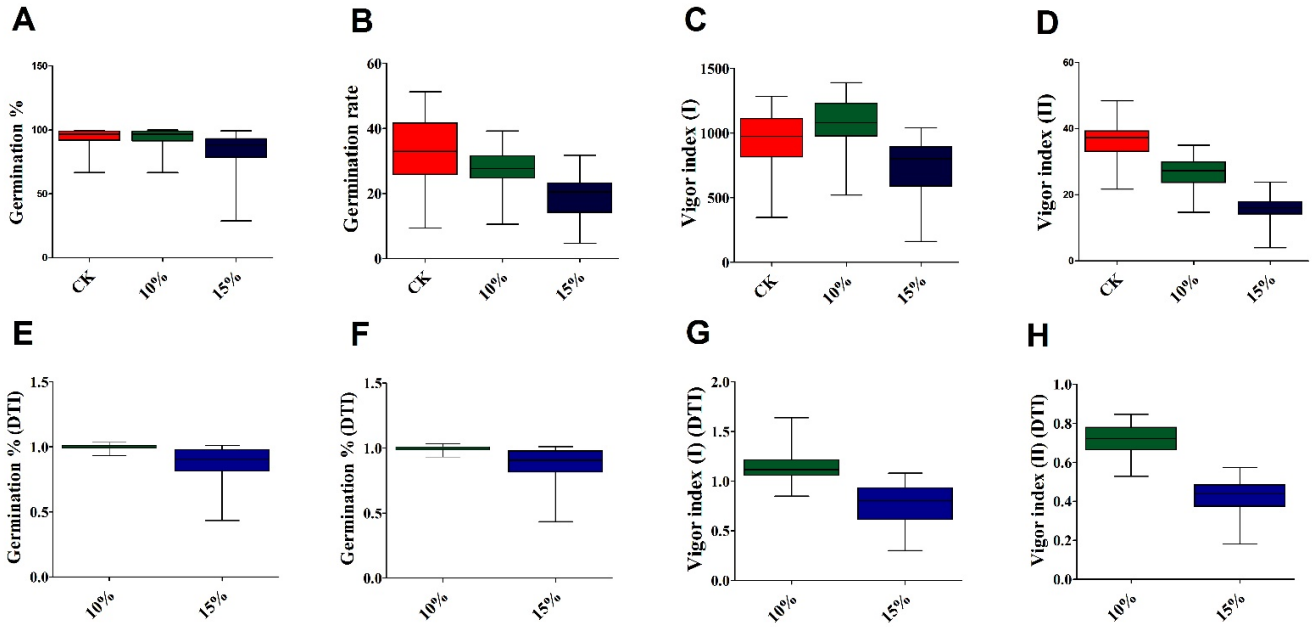

Figure 1. Box and whisker charts showing variation in germination-related traits: (**A**) final germination %, (**B**) germination rate, (**C**) vigor index (I) and (**D**) vigor index (II), under CK, 10%, and 15% PEG-6000 treatments. Reduction in DTI values of (**E**) final germination %, (**F**) germination rate, (**G**) vigor index (I), and (**H**) vigor index (II) under drought stress in rapeseed seedlings.

FG% was greatly affected by the 15% PEG-6000 concentration. YG 2009, NZ 1838 and CY 81 had the lowest values at 28.67, 55.67 and 57.77%, respectively. Some cultivars had higher values, including YYZ 3 (99.44%), JYZ 158 (97.77%) and FY 520 (98.30%) under 15% PEG-6000. The germination rate (GR) was lowest in YG 2009 and NZ 1838, at 4.661 and 7.251, respectively, while few cultivars showed better performance of GR, including JYZ 158 and FY 520 with 30.82 and 32.28 values, respectively, under the 15% PEG-6000 treatment (Table 1).

Vigor index decreased under the higher PEG-6000 concentrations (especially 15% PEG-6000), while, cultivars with comparatively higher vigor index values showed better adaptability. JYZ 158 and FY 520 had the highest values of 1041 and 896.7 (VI (I)), 22.33, and 23.78 (VI (II)) under 15% PEG-6000, respectively. On the other hand, few cultivars showed lower values of VI (I) and (II): YG 2009 (162.4 and 3.330) and NZ 1838 (259.3 and 7.336) under 15% PEG-6000, respectively (Table 1).

3.2. Variation in Seedling Growth Traits under Drought Stress

The box and whisker charts revealed substantial variations of seedling growth traits between treatments, especially at 15% PEG 6000, indicated by lower and upper limits of box plot for each trait (Figure 2). Under normal conditions, the mean values were recorded as 0.266, 0.122, 0.089, 0.0084, 2.235 and 7.470, while being 0.188, 0.090, 0.032, 0.0094, 1.672 and 9.463 under 10% PEG-6000 treatment, and 0.140, 0.045, 0.031, 0.0056, 1.337 and 6.968 under the 15% PEG-6000 treatment for shoot fresh weight (ShFW), root fresh weight (RFW),

shoot dry weight (ShDW), root dry weight (RDW), shoot length (ShL) and root length (RL), respectively (Table 2).

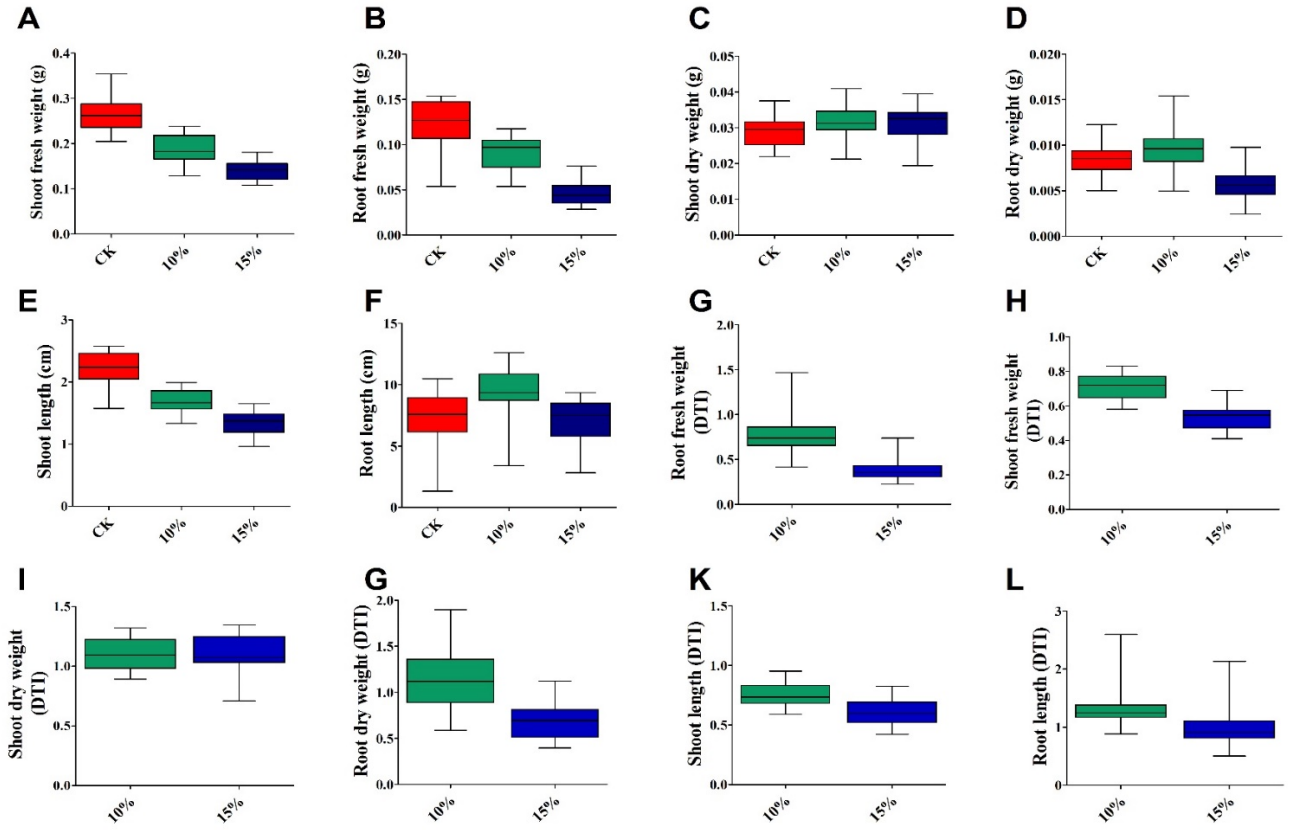

Figure 2. Box and whisker charts showing the variation of traits. (**A**) shoot fresh weight, (**B**) root fresh weight, (**C**) shoot dry weight, (**D**) root dry weight, (**E**) shoot length, (**F**) root length in rapeseed seedlings under control, 10%, and 15% PEG-6000 treatments. Boxplots illustrate reduction in DTI values of (**G**) shoot fresh weight, (**H**) root fresh weight, (**I**) shoot dry weight, (**J**) root dry weight, (**K**) shoot length and (**L**) root length under drought stress in rapeseed seedlings.

ShFW had the highest values in JYZ 158 (0.177) and FY 520 (0.181), and the lowest values in YG 2009 (0.108) and NZ 1838 (0.112) under 15% PEG-6000. For ShDW, HYZ 62 showed a highest value of 0.039, while the lowest value occurred in ZY 50, of 0.019, under 15% PEG-6000 (Table 2). For RFW, the values were highest for FY 520 (0.076) and JYZ 158 (0.054), while the lowest values were obtained for YG 2009 (0.028), TYZ 283 (0.029) and NZ 1838 (0.029) under 15% PEG-6000. The highest values of RDW were in JYZ 158 (0.0097), and the lowest values in YG 2009 (0.0024) and NZ 1838 (0.0033) under stress (15% PEG-6000) (Table 2). Under 15% PEG-6000, ShL was higher in FY 520 (1.466) and JYZ 158 (1.463) and lowest in QY 7 (1.193), while RL was higher in JYZ 158 (9.183) and FY 520 (8.143), and lower in YY 28 (2.817) (Table 2). Increased sensitivity of germination to PEG-6000 treatments for different cultivars was indicated by lower drought tolerance index (DTI) values. A large variation among genotypes was detected concerning their responses to drought stress. For DTI, the cultivar's mean values under 10% PEG-6000 were 0.711, 0.752, 1.101, 1.153, 0.751 and 1.342, while under 15% PEG-6000 the mean values were 0.532, 0.38, 1.101, 0.680, 0.600 and 0.991 for ShFW, RFW, ShDW, RDW, ShL and RL, respectively (Table 3, Figure 2). Furthermore, the drought tolerance index (DTI) values were much lower in some cultivars, including the sensitive cultivars YG 2009 and NZ 1838.

Table 2. Seedling growth-related traits under PEG-6000 induced drought stress.

Variety	ShFW			RFW			ShDW			RDW			ShL			RL		
	CK	10%	15%	CK	10%	15%	CK	10%	15%	CK	10%	15%	CK	10%	15%	CK	10%	15%
CY 81	0.235 $^{f–i}$	0.188 ^{efg}	0.135 $^{g–j}$	0.133 $^{c–f}$	0.117 ^{a}	0.055 ^{cd}	0.025 $^{e–h}$	0.030 $^{f–l}$	0.026 ^{g}	0.0084 $^{d–g}$	0.0098 $^{c–f}$	0.0087 ^{b}	1.965 ^{hij}	1.873 ^{cde}	1.620 ^{a}	8.691 ^{bcd}	9.681 $^{d–g}$	8.469 $^{a–d}$
YYZ 3	0.205 ^{i}	0.129 ^{m}	0.114 ^{kl}	0.117 $^{f–i}$	0.075 $^{h–k}$	0.050 ^{def}	0.024 ^{ghi}	0.021 ^{n}	0.025 ^{h}	0.0082 ^{fgh}	0.0087 ^{fg}	0.0046 ^{jk}	2.160 $^{f–j}$	1.473 ^{klm}	1.186 $^{b–f}$	8.450 ^{bcd}	10.18 ^{def}	7.597 $^{c–f}$
YY 28	0.270 $^{c–f}$	0.196 ^{def}	0.142 $^{d–g}$	0.054 ^{m}	0.064 $^{i–l}$	0.040 ^{ghi}	0.032 ^{b}	0.034 $^{d–j}$	0.033 ^{cd}	0.0050 ^{l}	0.0081 ^{g}	0.0057 ^{hij}	2.236 $^{d-h}$	1.993 ^{a}	1.486 ^{abc}	1.320 ^{j}	3.427 ^{l}	2.817 ^{l}
CY 36	0.225 ^{hi}	0.182 $^{f-i}$	0.155 $^{d–f}$	0.106 $^{h-k}$	0.094 ^{efg}	0.059 ^{bc}	0.027 ^{cde}	0.032 $^{ge–k}$	0.033 ^{cd}	0.0091 ^{def}	0.0096 ^{efg}	0.0075 ^{bc}	2.007 $^{g-j}$	1.729 ^{def}	1.482 ^{ab}	5.987 ^{fgh}	9.250 ^{fgh}	7.689 $^{b–e}$
JYZ 158	0.323 ^{ab}	0.235 ^{ab}	0.177 ^{a}	0.154 ^{a}	0.117 ^{a}	0.054 ^{cd}	0.037 ^{a}	0.035 $^{b–f}$	0.034 ^{cd}	0.0113 ^{ab}	0.0154 ^{a}	0.0098 ^{a}	2.505 $^{a–d}$	1.887 ^{bcd}	1.463 ^{abc}	10.46 ^{a}	11.89 ^{abc}	9.183 ^{abc}
ZY 50	0.228 ^{ghi}	0.176 $^{g-j}$	0.118 $^{h–l}$	0.106 ^{ijk}	0.098 ^{cde}	0.035 ^{ij}	0.032 ^{b}	0.031 $^{f–l}$	0.033 ^{cd}	0.0068 ^{hij}	0.0128 ^{b}	0.0046 ^{kl}	1.883 ^{ij}	1.613 $^{f-j}$	1.450 $^{a–d}$	7.563 $^{c–f}$	10.91 ^{bcd}	6.853 $^{d–h}$
QY 33	0.274 ^{cde}	0.159 ^{jkl}	0.115 ^{jkl}	0.124 $^{f-i}$	0.081 ^{fgh}	0.061 ^{abc}	0.024 ^{ghi}	0.024 ^{mn}	0.019 ^{i}	0.0081 $^{e–h}$	0.0107 ^{cd}	0.0063 ^{gh}	2.363 $^{a–d}$	1.397 ^{mn}	1.226 $^{a–f}$	9.020 ^{abc}	12.62 ^{a}	8.063 $^{b–e}$
ZY 51	0.274 ^{cde}	0.183 ^{fgh}	0.158 $^{b–e}$	0.147 ^{ab}	0.098 ^{de}	0.049 ^{def}	0.030 ^{bc}	0.036 $^{b–e}$	0.038 ^{ab}	0.0107 ^{bc}	0.0095 $^{c-g}$	0.0054 ^{ij}	2.353 $^{a–e}$	1.733 $^{d–h}$	1.536 ^{ab}	6.587 $^{e-h}$	8.481 ^{hi}	5.984 $^{f-i}$
XZY 518	0.243 $^{e–h}$	0.165 $^{i-l}$	0.147 $^{c–g}$	0.138 $^{b–e}$	0.105 $^{a-e}$	0.054 ^{cd}	0.032 ^{b}	0.030 $^{h–l}$	0.034 ^{ab}	0.009 ^{def}	0.0109 ^{cde}	0.0074 ^{cd}	1.877 ^{jk}	1.346 ^{n}	1.193 $^{b–f}$	7.621 ^{cde}	9.353 ^{fgh}	6.637 $^{e–h}$
GHY 8	0.288 ^{bc}	0.182 $^{f-i}$	0.118 $^{i–l}$	0.098 ^{kl}	0.077 ^{ghi}	0.039 ^{ghi}	0.026 ^{cde}	0.029 ^{jkl}	0.027 ^{c}	0.0099 ^{cd}	0.0058 ^{h}	0.0046 ^{lm}	2.576 ^{a}	1.886 ^{bcd}	1.087 ^{f}	5.563 ^{h}	6.857 ^{jk}	6.657 $^{e–h}$
ZYZ 108	0.342 ^{a}	0.238 ^{a}	0.158 $^{a–d}$	0.067 ^{m}	0.098 ^{de}	0.039 ^{ghi}	0.037 ^{a}	0.041 ^{a}	0.026 ^{fg}	0.0050 ^{kl}	0.0094 $^{c–g}$	0.0046 ^{lm}	2.566 ^{a}	1.686 $^{e–i}$	1.203 $^{b–f}$	3.911 ^{i}	9.371 ^{fgh}	6.517 $^{e–h}$
NZ 1838	0.262 $^{c–g}$	0.165 $^{i-l}$	0.112 ^{kl}	0.101 ^{jkl}	0.079 ^{gh}	0.029 ^{j}	0.031 ^{b}	0.030 $^{i–l}$	0.031 ^{gh}	0.0062 ^{ijk}	0.0055 ^{h}	0.0033 ^{n}	2.003 $^{g–j}$	1.566 ^{jkl}	0.966 ^{ef}	7.357 $^{d-g}$	9.317 ^{fgh}	3.717 ^{jkl}
XZY 553	0.288 ^{c}	0.222 ^{bcd}	0.147 $^{c–g}$	0.147 ^{ab}	0.102 $^{a–e}$	0.044 ^{fg}	0.033 ^{b}	0.034 $^{c–g}$	0.034 ^{c}	0.0108 ^{ab}	0.0083 ^{fg}	0.0056 ^{ghi}	2.316 $^{c–g}$	1.663 $^{e–j}$	1.516 ^{ab}	7.560 ^{def}	9.073 ^{fgh}	8.003 $^{b–e}$
YY 9	0.262 $^{c–g}$	0.158 ^{kl}	0.129 $^{g–k}$	0.129 ^{def}	0.054 ^{l}	0.035 ^{hij}	0.031 ^{b}	0.033 $^{d–i}$	0.032 ^{cde}	0.0073 ^{hij}	0.0054 ^{h}	0.0037 ^{mn}	2.166 $^{d–h}$	1.473 ^{lm}	1.049 $^{c–f}$	5.993 ^{gh}	5.877 ^{k}	3.463 ^{kl}
HYZ 62	0.354 ^{a}	0.227 ^{ab}	0.164 ^{abc}	0.147 ^{ab}	0.105 $^{a–e}$	0.035 ^{ij}	0.029 ^{bcd}	0.036 ^{bcd}	0.039 ^{a}	0.0123 ^{a}	0.0098 $^{c–f}$	0.0057 ^{ghi}	2.316 $^{b–g}$	1.573 $^{h–l}$	1.286 $^{a–e}$	7.583 $^{b–e}$	8.597 ^{ghi}	5.573 ^{hij}
QY 3	0.261 $^{c–g}$	0.175 $^{g-k}$	0.142 $^{d–g}$	0.149 ^{ab}	0.113 ^{abc}	0.064 ^{ab}	0.028 ^{def}	0.028 ^{lm}	0.030 ^{ef}	0.0091 ^{def}	0.0097 $^{c–f}$	0.0064 ^{ef}	2.226 $^{d–h}$	1.566 ^{jkl}	1.407 $^{a–e}$	9.251 ^{ab}	12.19 ^{a}	8.483 $^{a–d}$
QY 7	0.206 ^{i}	0.156 ^{l}	0.142 $^{e–h}$	0.113 $^{g–j}$	0.074 $^{h–k}$	0.044 ^{fg}	0.022 ^{i}	0.029 ^{klm}	0.027 ^{fg}	0.0080 $^{e–h}$	0.0091 $^{d–g}$	0.0051 ^{ijk}	1.576 ^{k}	1.333 ^{n}	1.193 $^{a–f}$	7.567 ^{def}	9.260 ^{fgh}	8.491 $^{a–d}$
ZS 11	0.244 $^{d–h}$	0.188 ^{efg}	0.135 $^{f–i}$	0.125 $^{d–g}$	0.115 ^{ab}	0.041 ^{gh}	0.025 ^{fgh}	0.030 $^{g–l}$	0.031 ^{de}	0.0087 $^{d–g}$	0.0097 $^{c–f}$	0.0068 ^{de}	2.353 $^{a–f}$	1.580 $^{g–k}$	1.653 ^{ab}	7.397 $^{d–g}$	9.387 $^{e-h}$	9.070 ^{abc}
YG 2009	0.238 $^{f–i}$	0.158 ^{jkl}	0.108 ^{l}	0.086 ^{l}	0.062 ^{jkl}	0.028 ^{j}	0.027 ^{def}	0.031 $^{f–l}$	0.032 ^{cde}	0.0062 ^{jk}	0.0054 ^{h}	0.0024 ^{o}	2.241 $^{d–g}$	1.581 $^{i–l}$	1.007 ^{def}	5.876 ^{gh}	7.437 ^{ij}	4.613 ^{ijk}
HYZ 72	0.285 ^{c}	0.226 ^{abc}	0.142 $^{f–i}$	0.124 $^{e–h}$	0.059 ^{kl}	0.035 ^{hi}	0.031 ^{b}	0.038 ^{ab}	0.036 ^{b}	0.0075 ^{ghi}	0.0107 ^{c}	0.0052 ^{sjk}	2.493 ^{abc}	1.961 ^{ab}	1.399 $^{a-e}$	8.460 ^{bcd}	12.097 ^{ab}	7.393 $^{d-g}$
QY 1	0.221 ^{hi}	0.168 $^{h–l}$	0.143 $^{d–g}$	0.152 ^{ab}	0.102 $^{b–e}$	0.054 ^{de}	0.025 ^{efg}	0.029 $^{i–l}$	0.033 ^{cd}	0.009 ^{def}	0.0127 ^{b}	0.0063 ^{fg}	2.156 $^{f–i}$	1.678 $^{e–j}$	1.573 ^{ab}	9.103 ^{ab}	10.65 ^{cde}	9.357 ^{a}
TYZ 283	0.248 $^{d–h}$	0.206 ^{sde}	0.147 $^{c–g}$	0.128 $^{d–g}$	0.075 ^{hij}	0.029 ^{j}	0.032 ^{b}	0.037 ^{abc}	0.037 ^{ab}	0.0081 $^{e–h}$	0.0098 $^{c–f}$	0.0038 ^{mn}	2.166 $^{e–i}$	1.837 ^{cde}	1.317 $^{a-e}$	8.481 ^{bcd}	9.993 ^{def}	5.707 ^{ghi}
GZ 1	0.277 ^{cd}	0.206 ^{cde}	0.156 $^{b–f}$	0.141 $^{a–d}$	0.113 $^{a-s}$	0.044 ^{efg}	0.023 ^{hi}	0.030 $^{g–l}$	0.031 ^{de}	0.0095 ^{cde}	0.0124 ^{b}	0.0067 ^{de}	2.576 ^{a}	1.967 ^{abc}	1.350 $^{a-d}$	9.169 ^{ab}	12.08 ^{ab}	9.323 ^{ab}
FY 520	0.332 ^{a}	0.237 ^{a}	0.181 ^{ab}	0.148 ^{abc}	0.095 ^{ef}	0.076 ^{a}	0.032 ^{b}	0.034 $^{c–h}$	0.037 ^{ab}	0.0086 $^{d–h}$	0.0082 ^{g}	0.0065 ^{ef}	2.580 ^{a}	1.746 $^{d–g}$	1.466 ^{abc}	10.32 ^{a}	9.147 ^{fgh}	8.143 $^{c-f}$
Mean	0.266	0.188	0.140	0.122	0.090	0.045	0.089	0.032	0.031	0.0084	0.0094	0.0056	2.235	1.672	1.337	7.470	9.463	6.968

ShFW, shoot fresh weight; RFW, root fresh weight; ShDW, shoot dry weight; RDW, root dry weight, ShL, shoot length; and RL, root length. Data are presented as mean values with different letters, which denote statistically significant difference between means within each indicator column among cultivars according to Fisher's least significant difference (LSD) test.

Table 3. Drought tolerance index of germination and seedling growth traits of rapeseed cultivars under drought stress.

Variety	FG%		GR		VI (II)		VI (I)		ShFW		RFW		ShDW		RDW		ShL		RL	
	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%	10%	15%
CY 81	0.98 ^{cd}	0.63 ^{k}	0.99 ^{b}	0.47 ^{kl}	0.82 ^{a}	0.32 ^{m}	1.07 ^{gh}	0.59 ^{i}	0.80 ^{ab}	0.57 ^{cde}	0.88 ^{c}	0.41 ^{de}	1.18 ^{cde}	1.06 ^{fg}	1.16 ^{d}	0.95 ^{b}	0.95 ^{a}	0.82 ^{a}	1.11 ^{i}	0.97 ^{ef}
YYZ 3	0.99 ^{bcd}	1.00 ^{a}	0.74 ^{ghi}	0.65 ^{cd}	0.62 ^{i}	0.50 ^{b}	1.09 $^{e–h}$	0.82 ^{ef}	0.63 ^{jkl}	0.56 ^{def}	0.64 ^{ij}	0.42 ^{de}	0.89 ^{k}	1.05 ^{fg}	1.06 ^{d}	0.56 ^{jk}	0.68 $^{f-i}$	0.55 ^{ij}	1.20 ^{ghi}	0.89 ^{fg}
YY 28	0.98 ^{cd}	0.80 ^{i}	0.78 ^{efg}	0.40 ^{mn}	0.78 ^{ab}	0.45 ^{efg}	1.49 ^{b}	0.96 ^{bc}	0.72 $^{d–h}$	0.52 $^{f–j}$	1.19 ^{b}	0.74 ^{a}	1.07 ^{fgh}	1.06 ^{gh}	1.61 ^{b}	1.12 ^{a}	0.89 ^{b}	0.66 ^{de}	2.59 ^{a}	2.13 ^{a}
CY 36	0.99 ^{bcd}	0.89 ^{efg}	0.90 ^{d}	0.75 ^{b}	0.82 ^{a}	0.58 ^{a}	1.36 ^{c}	1.03 ^{ab}	0.81 ^{ab}	0.68 ^{a}	0.88 ^{c}	0.55 ^{b}	1.20 ^{bcd}	1.23 $_{c}$^{de}	0.98 ^{ef}	0.82 ^{de}	0.86 ^{b}	0.74 ^{bc}	1.54 ^{c}	1.28 ^{c}
JYZ 158	1.02 ^{abc}	1.01 ^{a}	0.76 ^{fgh}	0.60 ^{efg}	0.75 ^{bcd}	0.49 ^{bc}	1.08 ^{fgh}	0.83 ^{ef}	0.73 $^{d–g}$	0.55 $^{e-h}$	0.76 ^{def}	0.35 ^{fg}	0.93 ^{jk}	0.90 ^{i}	1.37 ^{c}	0.86 ^{cd}	0.74 ^{cde}	0.58 ^{ghi}	1.13 ^{i}	0.87 ^{gh}
ZY 50	1.00 $^{a–d}$	0.98 ^{abc}	0.97 ^{bc}	0.79 ^{ab}	0.82 ^{a}	0.44 ^{efg}	1.33 ^{c}	0.85 ^{e}	0.77 ^{bcd}	0.52 $^{g-j}$	0.92 ^{c}	0.33 ^{ghi}	0.97 $^{h–k}$	1.04 ^{gh}	1.89 ^{a}	0.68 ^{jk}	0.85 ^{b}	0.76 ^{b}	1.44 ^{cd}	0.90 ^{fg}
QY 33	1.03 ^{ab}	0.88 $^{e-h}$	0.78 ^{efg}	0.48 ^{ghi}	0.62 ^{i}	0.39 ^{jk}	1.27 ^{cd}	0.72 ^{g}	0.58 ^{l}	0.42 ^{mn}	0.65 ^{hij}	0.49 ^{c}	0.99 $^{g-j}$	0.81 ^{i}	1.32 ^{c}	0.77 ^{hi}	0.59 ^{j}	0.52 ^{jk}	1.39 $_{d}$^{ef}	0.89 ^{fg}
ZY 51	0.99 ^{bcd}	0.70 ^{j}	0.98 ^{b}	0.55 ^{hij}	0.66 ^{hi}	0.34 ^{lm}	1.13 $^{e–h}$	0.58 ^{i}	0.66 ^{ij}	0.57 ^{cde}	0.66 ^{hi}	0.33 ^{gh}	1.23 ^{bcd}	1.27 ^{bcd}	0.89 ^{fgh}	0.50 ^{lm}	0.73 $^{c-f}$	0.65 ^{def}	1.28 ^{fgh}	0.90 ^{fg}
XZY 518	1.00 $^{a–d}$	0.92 ^{de}	0.76 ^{fgh}	0.54 ^{hij}	0.71 $^{c-h}$	0.48 ^{bcd}	1.12 $^{e–h}$	0.75 ^{g}	0.68 $^{g–j}$	0.60 ^{c}	0.76 $^{d-g}$	0.39 ^{ef}	0.95 ^{ijk}	1.08 ^{fg}	1.21 ^{d}	0.82 ^{ef}	0.72 $^{d-h}$	0.63 ^{efg}	1.22 ^{ghi}	0.87 ^{gh}
GHY 8	1.00 $^{a–d}$	0.99 ^{ab}	1.00 ^{b}	0.83 ^{a}	0.67 ^{ghi}	0.40 ^{hij}	1.07 ^{fgh}	0.94 ^{c}	0.63 ^{jkl}	0.41 ^{n}	0.78 ^{def}	0.39 ^{ef}	1.09 ^{fg}	1.03 ^{gh}	0.58 ^{j}	0.46 ^{n}	0.73 $^{c-g}$	0.42 ^{n}	1.23 ^{gh}	1.19 ^{c}
ZYZ 108	0.96 ^{de}	0.87 ^{fgh}	0.74 ^{ghi}	0.52 ^{ij}	0.79 ^{ab}	0.42 $^{g–j}$	1.65 ^{a}	1.04 ^{a}	0.69 $^{f–i}$	0.46 ^{kl}	1.46 ^{a}	0.59 ^{b}	1.09 ^{fg}	0.70 ^{j}	1.87 ^{a}	0.91 ^{bc}	0.66 ^{i}	0.47 ^{lmn}	2.39 ^{b}	1.66 ^{b}
NZ 1838	0.93 ^{e}	0.61 ^{k}	0.90 ^{d}	0.42 ^{lm}	0.62 ^{i}	0.24 ^{n}	1.08 ^{fgh}	0.30 ^{k}	0.63 ^{jkl}	0.43 ^{lmn}	0.78 ^{de}	0.28 ^{ij}	0.94 ^{jk}	0.99 ^{h}	0.88 ^{gh}	0.54 ^{l}	0.78 ^{c}	0.48 ^{klm}	1.26 ^{gh}	0.50 ^{k}
XZY 553	1.00 $^{a–d}$	0.98 ^{abc}	0.74 ^{ghi}	0.58 ^{fgh}	0.74 $^{b–f}$	0.42 $^{g–j}$	1.09 ^{fgh}	0.94 ^{cd}	0.77 ^{bcd}	0.51 ^{hij}	0.69 ^{ghi}	0.29 ^{hij}	1.03 ^{fgh}	1.03 ^{fg}	0.76 ^{i}	0.51 ^{lm}	0.71 $^{d-h}$	0.65 ^{def}	1.20 ^{hi}	1.05 ^{de}
YY 9	0.97 ^{de}	0.78 ^{i}	0.70 ^{i}	0.47 ^{kl}	0.52 ^{j}	0.33 ^{m}	0.87 ^{i}	0.43 ^{j}	0.60 ^{kl}	0.49 ^{jk}	0.41 ^{k}	0.27 ^{jkl}	1.07 ^{fgh}	1.01 ^{gh}	0.74 ^{i}	0.50 ^{l}	0.68 $^{f–i}$	0.48 ^{klm}	0.98 ^{j}	0.57 ^{k}
HYZ 62	1.01 $^{a–d}$	0.91 ^{def}	0.72 ^{hi}	0.47 ^{kl}	0.67 ^{ghi}	0.36 ^{kl}	1.04 ^{h}	0.63 ^{hi}	0.64 ^{jk}	0.46 ^{kl}	0.71 $^{f-i}$	0.24 ^{kl}	1.25 ^{bc}	1.34 ^{a}	0.79 ^{hi}	0.46 ^{n}	0.68 ^{ghi}	0.55 ^{hij}	1.13 ^{i}	0.73 ^{ij}
QY 3	0.98 ^{cd}	0.86 ^{gh}	0.74 ^{ghi}	0.36 ^{n}	0.69 ^{fgh}	0.44 $^{e–h}$	1.18 ^{def}	0.74 ^{g}	0.67 ^{hij}	0.54 $^{e–i}$	0.75 $^{d–g}$	0.43 ^{de}	0.97 ^{ghi}	1.05 ^{ef}	1.08 ^{de}	0.71 ^{i}	0.70 $^{e–i}$	0.63 ^{efg}	1.32 ^{efg}	0.92 ^{fg}
QY 7	1.00 $^{a–d}$	1.00 ^{a}	0.76 ^{fgh}	0.68 ^{c}	0.72 $^{c–g}$	0.58 ^{a}	1.16 ^{efg}	1.05 ^{a}	0.75 $^{b-e}$	0.68 ^{a}	0.65 ^{hig}	0.39 ^{ef}	1.32 ^{a}	1.25 ^{ab}	1.12 ^{d}	0.63 ^{k}	0.84 ^{b}	0.75 ^{b}	1.22 ^{ghi}	1.12 ^{d}
ZS 11	1.02 ^{abc}	0.97 ^{abc}	0.81 ^{ef}	0.81 ^{a}	0.83 ^{a}	0.46 ^{cde}	1.14 ^{efg}	1.07 ^{a}	0.77 ^{bcd}	0.55 ^{efg}	0.91 ^{c}	0.32 ^{ghi}	1.23 ^{ab}	1.26 ^{ab}	1.12 ^{d}	0.78 ^{fg}	0.67 ^{hi}	0.70 ^{cd}	1.26 ^{gh}	1.22 ^{c}
YG 2009	1.00 $^{a–d}$	0.43 ^{l}	1.12 ^{a}	0.49 ^{jk}	0.67 ^{ghi}	0.18 ^{o}	1.11 $^{e–h}$	0.30 ^{k}	0.66 ^{ij}	0.45 ^{lm}	0.71 $^{e-h}$	0.32 ^{ghi}	1.17 ^{cde}	1.20 $^{c-e}$	0.80 ^{hi}	0.39 ^{o}	0.70 $^{d–i}$	0.44 ^{mn}	1.26 ^{gh}	0.78 ^{hi}
HYZ 72	1.04 ^{a}	0.94 ^{cd}	0.91 ^{d}	0.64 ^{cde}	0.72 $^{c–h}$	0.41 ^{hij}	1.32 ^{c}	0.76 ^{fg}	0.78 ^{abc}	0.49 ^{jk}	0.47 ^{k}	0.28 ^{ijk}	1.23 ^{bc}	1.18 ^{de}	1.43 ^{c}	0.68 ^{jk}	0.78 ^{c}	0.56 ^{hij}	1.43 ^{cde}	0.87 ^{gh}
QY 1	0.97 ^{de}	0.89 ^{e}	0.88 ^{d}	0.65 ^{cd}	0.70 $^{c–h}$	0.47 ^{cde}	1.06 ^{gh}	0.86 ^{e}	0.76 $^{b–e}$	0.64 ^{b}	0.66 ^{hi}	0.35 ^{fg}	1.16 ^{def}	1.32 ^{bc}	1.41 ^{c}	0.69 ^{jk}	0.78 ^{c}	0.73 ^{bc}	1.17 ^{hi}	1.03 ^{de}
TYZ 283	1.01 $^{a–d}$	0.85 ^{h}	0.92 ^{cd}	0.62 ^{def}	0.75 $^{d–h}$	0.40 ^{ij}	1.12 $^{e-h}$	0.56 ^{i}	0.83 ^{a}	0.59 ^{cd}	0.59 ^{j}	0.22 ^{l}	1.18 ^{bcd}	1.17 ^{e}	1.20 ^{d}	0.47 ^{mn}	0.84 ^{b}	0.60 ^{fgh}	1.17 ^{hi}	0.67 ^{j}
GZ 1	1.00 $^{a–d}$	0.95 ^{bc}	0.82 ^{e}	0.59 ^{fgh}	0.76 ^{bc}	0.46 ^{def}	1.19 ^{de}	0.87 ^{de}	0.74 $^{c–f}$	0.56 ^{def}	0.80 ^{d}	0.31 $^{g–j}$	1.31 ^{a}	1.34 ^{a}	1.30 ^{c}	0.70 ^{ghi}	0.76 ^{cd}	0.52 ^{jk}	1.32 ^{efg}	1.01 ^{e}
FY 520	1.01 $^{a–d}$	0.99 ^{ab}	0.78 ^{efg}	0.68 ^{c}	0.70 $^{e-h}$	0.48 ^{bcd}	0.85 ^{i}	0.69 ^{gh}	0.71 $^{e–i}$	0.54 $^{e–h}$	0.64 ^{ij}	0.51 ^{cd}	1.06 ^{fgh}	1.16 ^{e}	0.94 ^{fg}	0.74 ^{fg}	0.67 ^{ghi}	0.56 ^{hij}	0.88 ^{j}	0.78 ^{ij}
Mean	0.99	0.87	0.84	0.58	0.72	0.42	1.16	0.76	0.71	0.53	0.76	0.38	1.10	1.10	1.15	0.68	0.75	0.60	1.34	0.99

FG%: final germination percentage; GR: germination rate; VI (I): vigor index (I); VI (II): vigor index (II); ShFW: shoot fresh weight; RFW: root fresh weight; ShDW: shoot dry weight; RDW: root dry weight; ShL: shoot length, and RL: root length. Data are presented as mean values with different letters, which denote statistically significant difference between means within each indicator column among cultivars according to Fisher's least significant difference (LSD) test.

3.3. Correlations of Traits under Control and PEG-6000 Stress

Pearson's correlations between the cultivars under normal and stressed conditions showed differences in response to drought stress. Correlations (r-value) of the 10 studied traits under 0, 10 and 15% PEG-6000 treatments are presented in Figure 3A–C. Stronger correlations can be seen among traits, where r-values $\geq$ 0.7 showed highly positively stronger relationship and r-values $\geq$ 0.5 showed positively strong interaction. Under the nonstressed conditions, highly positive r values $\geq$ 0.70 were recorded for ShFW (0.73), RDW (0.81) and VI (I) (0.95) with ShL, RFW and RL, respectively. RFW was highly correlated with VI (I) and RL. Additionally, positive r values $\geq$ 0.50 were scored for ShDW with ShFW, and VI (II) with RDW, RFW and VI (I). FG% showed a positive correlation with GR and VI (II), and GR with VI (II) and VI (I) (Figure 3A).

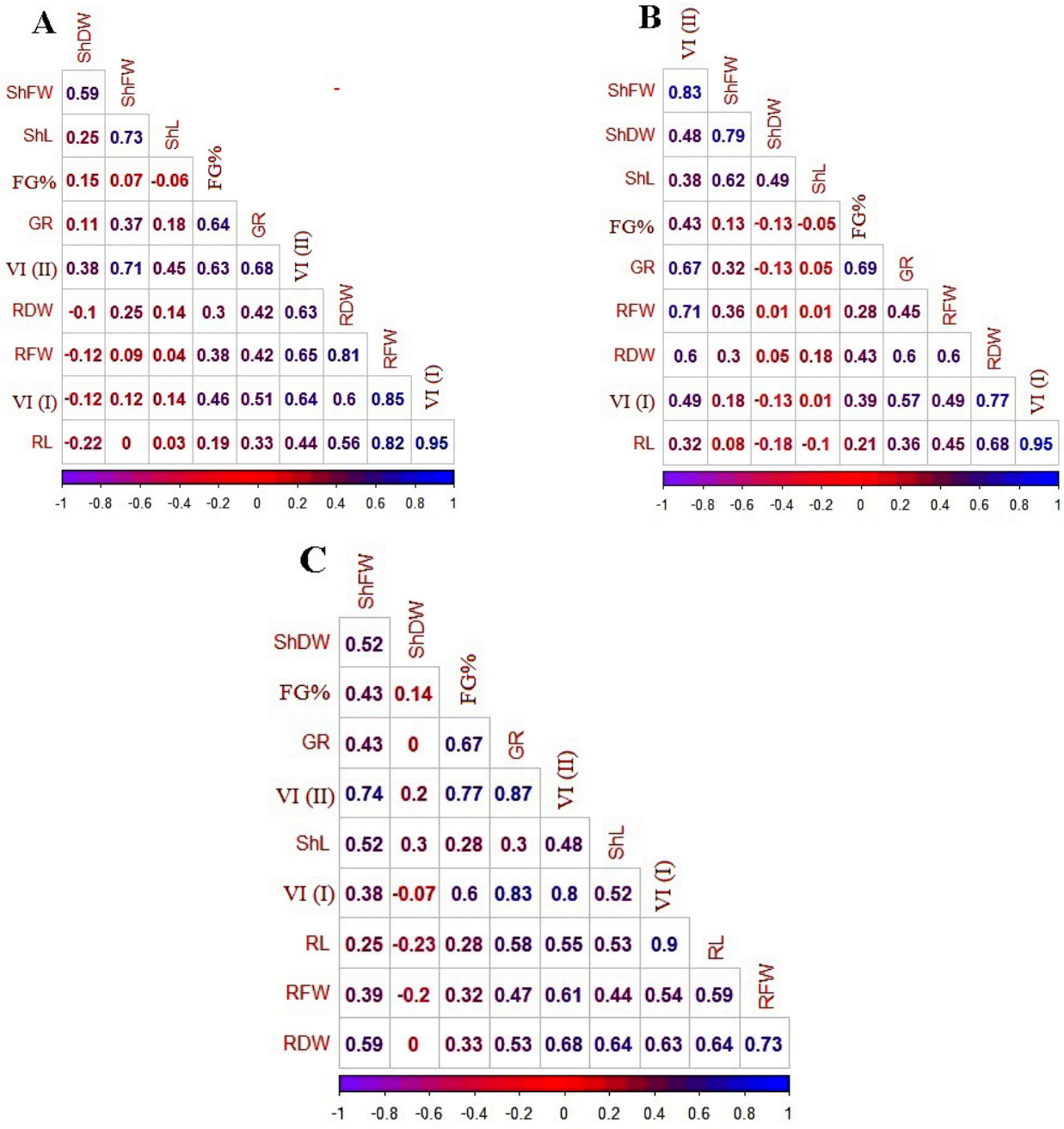

Figure 3. Correlation indicators of rapeseed cultivars based on mean values: under (**A**) control, (**B**) 10% PEG-6000 and (**C**) 15% PEG-6000.

Positive correlations were also observed for most of the same 10 traits under the 10% PEG-6000 treatment, as indicated by the red and yellow cells in the correlation triangle. The r values were a little higher for some traits compared to their corresponding values under normal condition. Highly positive r-values were $\geq$ 0.70 for VI (II) (0.83), ShFW (0.79), RDW (0.77) and VI (I) (0.95) seedlings with ShFW, ShDW, VI (I) and RL, respectively, indicating stronger correlation. Additionally, positive r values $\geq$ 0.50 were scored for FG%, RFW and RDW with GR, RDW and RL, respectively. Lower values were observed for ShL, ShDW with all traits, except with ShL (Figure 3B). Furthermore, VI (II) was highly correlated with ShFW and FG% with GR compared to control.

Under the 15% PEG-6000 treatment, highly positive r values were $\geq$ 0.70 for VI (I) (0.90) and RFW (0.73) with RL and RDW, respectively. FG% was correlated with VI (II) (0.77), while GR (r-value 0.83 and 0.87) was correlated with VI (I) and VI (II), with a stronger correlation. Meanwhile, positive r values $\geq$ 0.50 were recorded for ShFW with ShDW and ShL; RL with RFW and RDW, while lower values were obtained for ShDW with all attributes (Figure 3C).

Detailed inspection of the morphological traits showed that JYZ 158 and FY 520 cultivars had the best performance in several traits, while YG 2009 and NZ 1838 showed poor performance. According to the results, four cultivars were selected as sensitive and tolerant based on differences in drought tolerance and were further investigated with more measurements.

3.4. Variation in Growth-Related Traits of Rapeseed Seedlings

Based on germination and morphological traits analysis of 24 rapeseed cultivars, JYZ 158 and FY 520 were classed as highly tolerant cultivars, and YG 2009 and NZ 1838 classed as least tolerant cultivars. Results showed a significant reduction of the shoot and root length of rapeseed under drought stress, which was more prominent in sensitive cultivars (Figure 4). Highly tolerant cultivars (JYZ 158 and FY 520) and highly sensitive cultivars (YG 2009 and NZ) 1838 were selected to explore the role of osmolytes and antioxidant enzyme activity in improving drought tolerance.

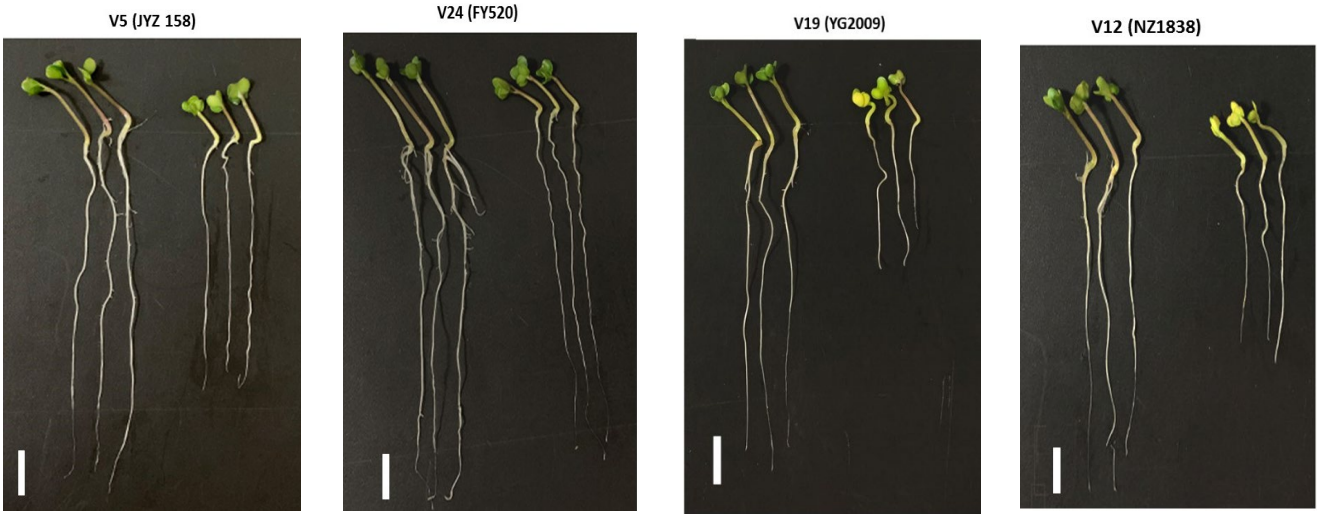

Figure 4. Effect of drought stress (15% PEG-6000) on the shoot and root growth of tolerant cultivars (JYZ 158 and FY 520) and sensitive cultivars (YG 2009, and NZ 1838). Scale bar: 1 cm.

3.5. Variations in Photosynthetic Pigments under Drought Stress

Compared to seedling growth under normal conditions, a significant decrease was noted in photosynthetic pigment levels under drought stress. Under 15% PEG-6000, Chl a content was reduced by 21.22 and 23.21% in JYZ 158 and FY 520 and decreased by 27.32 and 26.61% in YG 2009 and NZ 1838, respectively, while Chl b was decreased by 24.11% (JYZ 158), 31.30% (FY 520), 47.11% (YG 2009) and 45.40% (NZ 1838) (Figure 5A,B). Under

stress, total chlorophyll in JYZ 158 and FY 520 (tolerant cultivars) was decreased by 21.93 and 25.21%, while in YG 2009 and NZ 1838 (sensitive cultivars) it was reduced by 32.00 and 31.22%, respectively (Figure 5C). Carotenoid content was reduced by 24.90% (JYZ 158), 24.80% (FY 520), 30.16% (YG 2009) and 29.02% (NZ 1838) under 15% PEG-6000 (Figure 5D).

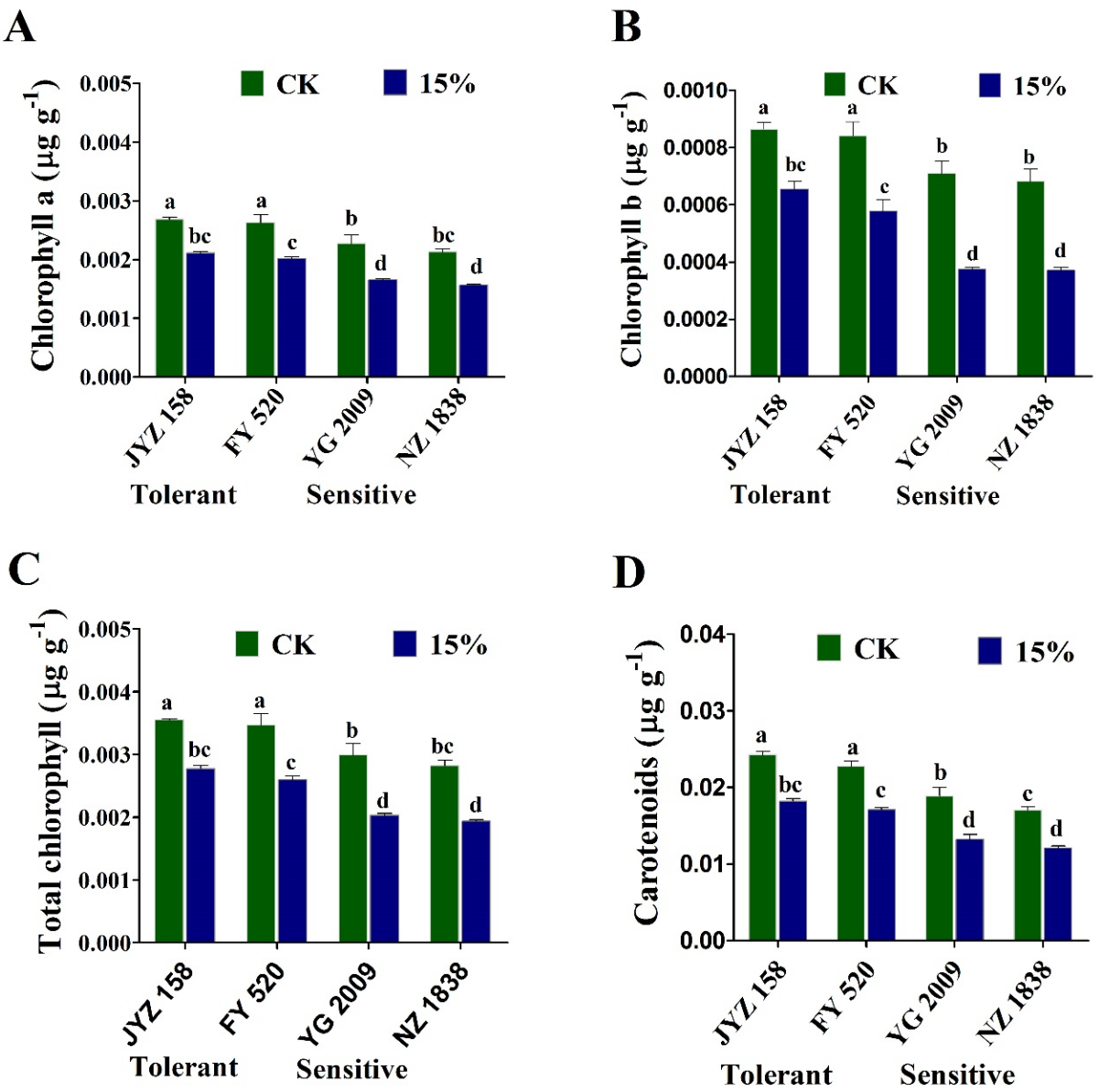

Figure 5. Effects of drought stress (PEG-6000-induced) on (**A**) chlorophyll a, (**B**) chlorophyll b, (**C**) total chlorophyll and (**D**) carotenoids in four rapeseed cultivars during the early seedling stage. Bars represent mean ± SE of three replicates. The different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range tests.

3.6. Variation of Osmo-Protectants, MDA, Proline and RWC Contents in Rapeseed Seedlings

Tolerant cultivars had higher levels of TSS and TSP than sensitive cultivars. Furthermore, TSS increased by 57.50, 45.83, 48.42 and 45.51% in JYZ 158, FY 520, YG 2009 and NZ 1838 under drought stress, respectively, versus normal conditions. TSP was increased in JYZ 158 and FY 520 (tolerant cultivars), and YG 2009 and NZ 1838 (sensitive cultivars) by 14.81, 16.22, 26.72 and 24.21%, respectively, over controls in relation to drought (Figure 6A,B).

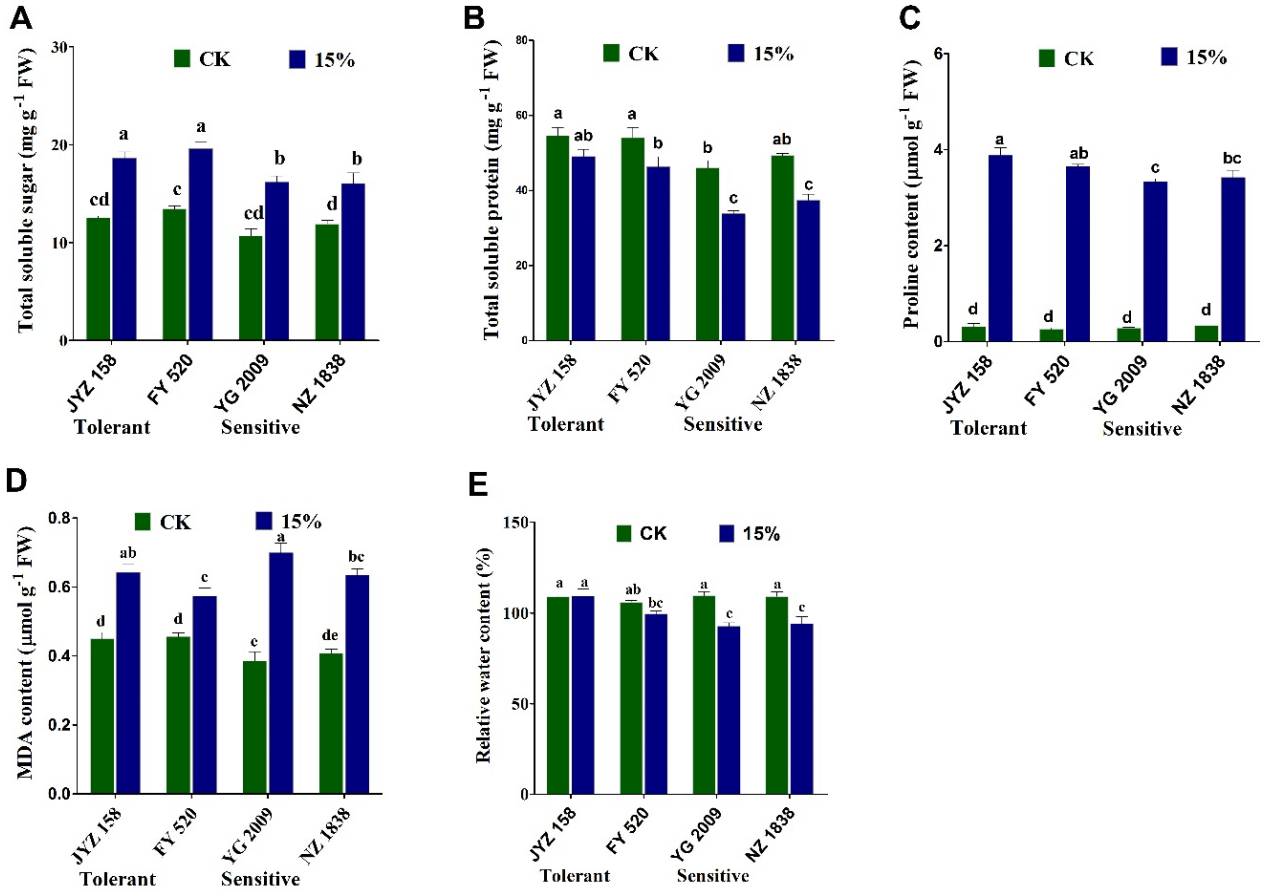

Figure 6. Effects of drought stress (PEG-6000-induced) on (**A**) total soluble sugar (TSS), (**B**) total soluble protein (TSP), (**C**) proline content, (**D**) MDA content and (**E**) water content in four rapeseed cultivars during the early seedling stage. Bars represent mean ± SE of three replicates. Different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range tests.

The contents of proline and MDA under stress conditions were recorded as increases of 1161 and 42.72% (JYZ 158), 1282 and 25.63% (FY 520), 1072 and 81.51% (YG 2009), 922.9 and 55.81% (NZ 1838), respectively, versus the normal condition (Figure 6C,D). RWC was stable in the tolerant cultivars under drought stress conditions compared to control; however, turgor was reduced significantly in sensitive cultivars due to weak tolerance. Water content was slightly enhanced by 0.42% in JYZ 158, slightly reduced by 6.101% in FY 520, and significantly reduced by 15.61 and 13.85% in YG 2009 and NZ 1838, respectively, compared to control (Figure 6E).

3.7. Activities of Enzymatic Antioxidants under PEG-6000 Induced Drought Stress

Enzymatic antioxidants (Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX)) showed marked changes under drought stress in rapeseed seedlings of tolerant and sensitive cultivars. For SOD, JYZ 158 and FY 520 showed an increase of 116.3 and 167.4%, respectively, while YG 2009 and NZ 1838 showed an increase of 85.62 and 71.31%, respectively, under drought stress compared to control. POD activity increased under drought stress in all cultivars, by 89.92 and 69.50% in tolerant cultivars (JYZ 158 and FY 520), 96.91 and 79.23% in sensitive cultivars (YG 2009 and NZ 1838) compared to control, indicating that POD activity might be a significant participant in the defense system (Figure 7A,B). CAT activity increased under drought stress by 108.8% (JYZ 158), 107.9% (FY 520), 92.92% (YG 2009), and 97.51% (NZ 1838) compared to control, whereas APX activity increased by 143.5, 151.6, 34.31 and 212.1% in JYZ 158, FY 520, YG 2009 and NZ 1838, respectively, under drought stress compared to control (Figure 7C,D).

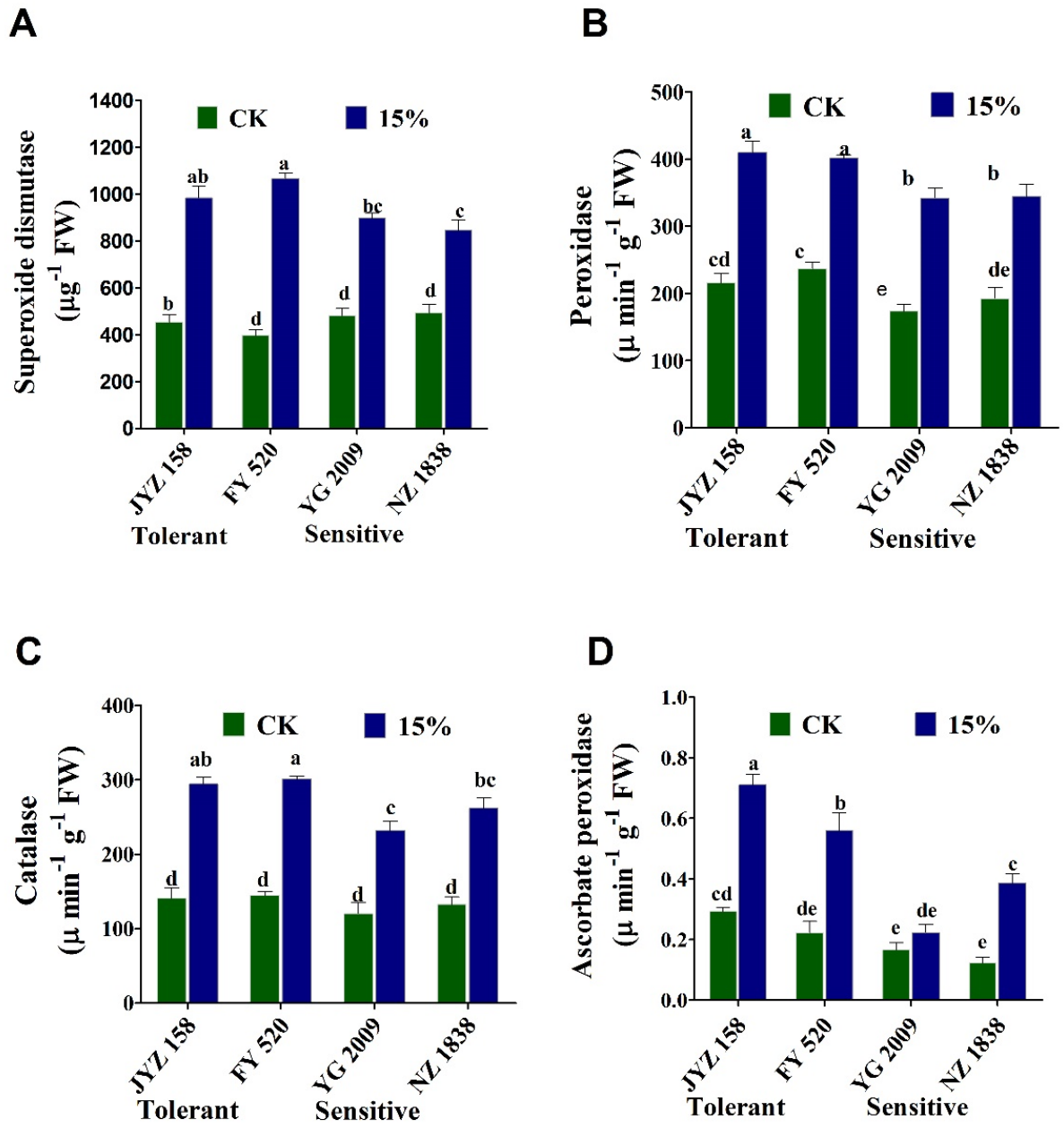

Figure 7. Effects of drought stress (PEG-6000 induced) on (**A**) superoxide dismutase (SOD), (**B**) peroxidase (POD), (**C**) catalase (CAT) and (**D**) ascorbate peroxidase (APX) in tolerant and sensitive rapeseed cultivars. Bars represent mean ± SE of three replicates. The different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range tests.

3.8. Microstructural Variation in Rapeseed Seedlings under Drought Stress

To further investigate the effects of drought stress on the chloroplast, the ultrastructure of the chloroplast in two cultivars, FY 520 (highly tolerant) and YG 2009 (highly sensitive), was observed using transmission electron microscopy (TEM). Under normal conditions, the form of chloroplast was well organized with elliptic with clear edges and was positioned near the properly developed cell wall (Figure 8A,B). Under drought stress, the shape of chloroplasts was as well-organized as in the tolerant cultivars, with well-developed lamella having normally stacked grana and thylakoids, and the cell wall had a proper configuration (Figure 8C). However, the structure of chloroplasts in leaves of sensitive cultivars was abnormal, with several vesicles instead of thylakoids, and chloroplasts moved toward the

center of the cell. Non-visible and incomplete cell boundaries were observed in sensitive cultivars under drought treatment (Figure 8D).

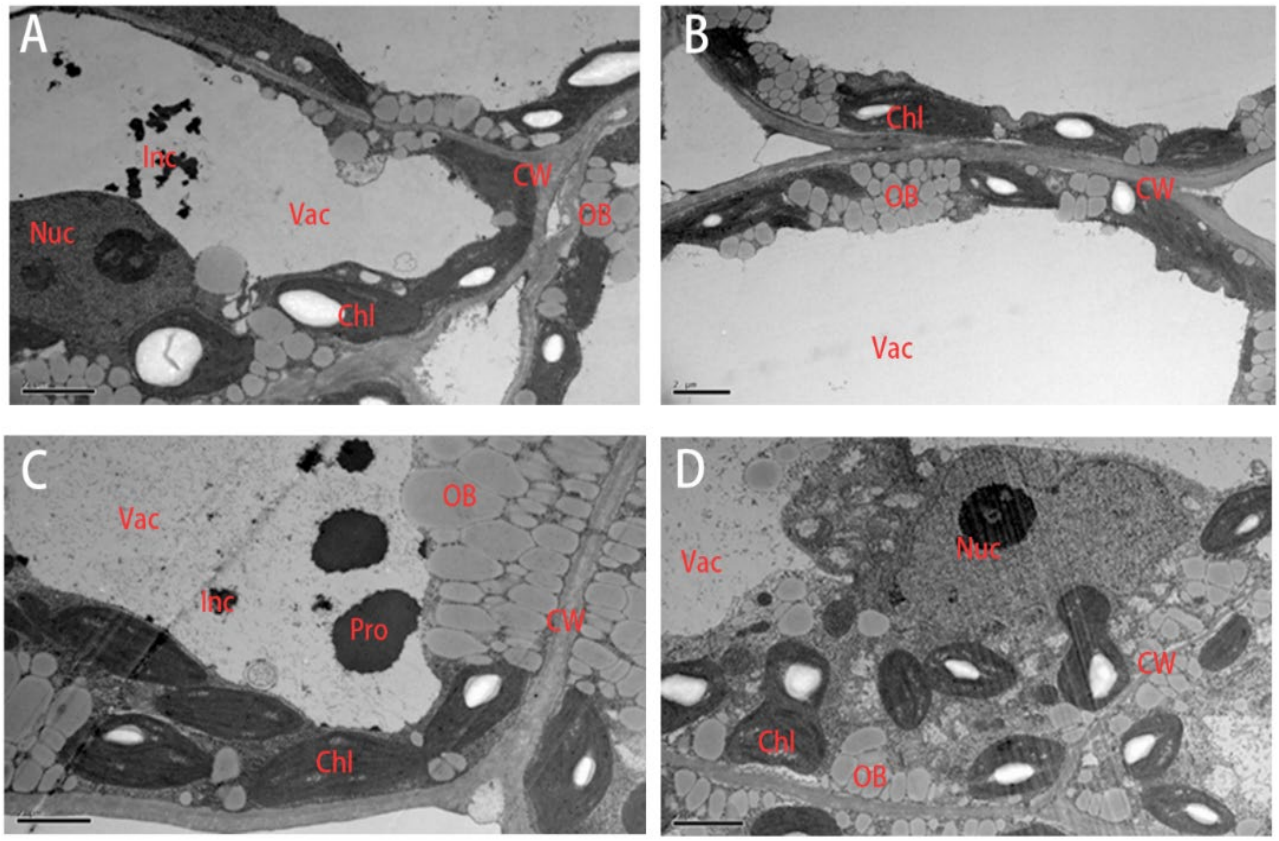

Figure 8. Effects of drought stress (PEG-6000-induced) on the internal structure of seedlings. (**A**) control of tolerant variety, (**B**) control of sensitive variety, (**C**) tolerant variety under 15% PEG-6000 treatment and (**D**) sensitive variety with 15% PEG-6000 treatment.

4. Discussion

Germination is a key step of seedling development during the plant life cycle [3]. A non-conducive environment, such as water stress, contributes towards poor seed germination and inhibits seedling development [35]. Rapid germination and successful seedling establishment are crucial for the normal growth and profitable production of *Brassica napus* L. [36,37]. The current study showed that germination percentage, germination rate and seedling growth were considerably decreased in all 24 studied cultivars under PEG-6000-simulated drought stress, but the negative effect was higher in sensitive cultivars compared to tolerant cultivars. Hence, germination percentage and germination speed was reduced under stress condition, which would lead to poor stand establishment [3,38]. Reduced germination was due to reduced water uptake, lower energy supply and impairment of enzymatic activities [39].

Drought stress reduces the water potential gradient between the internal and external environment of seeds [40] and reduces water movement through the seed coat and water absorption [41], resulting in reduction and delayed seed germination [3]. Drought stress reduced germination and seedling growth in *B. napus* and enhanced ROS production, which has damaging effects on structural components of cells and metabolic processes [11]. The slower hydrolysis of materials present in the endosperm leads to a lower transportation rate of hydrolyzed material to the developing embryonic axis, reducing germination and growth [11,42].

The current investigation showed that the shoot length of seedlings was reduced under drought stress due to a reduction in water availability [43]. Moreover, a substantial

reduction in plant height, leaf size and chlorophyll content occurred under water deficiency in rapeseed [44]. Our results show that mild drought stress increased root length, which indicates that mild water deficit might cause alterations in root structure to prevent dehydration [45]. On the other hand, severe drought stress shortened root length and reduced development in the 24 studied cultivars, indicating that a significant reduction in root length was due to reduction in cell division and expansion [46]. Drought stress causes disturbance of several physiochemical process, with a complex mechanistic action that limits plant development [47–50]. A few cultivars maintained or had greater germination and seedling growth under 10% PEG-6000, suggesting that plants possess an effective defense system that may be stimulated with higher efficacy under moderate stress, ultimately enabling the plants to grow better under drought stress conditions [51].

Photosynthetic pigments decreased in the four studied cultivars, but the reduction was less in drought-tolerant than sensitive cultivars, indicating that photoinhibition of photosystem II was higher [52]. A decline in chlorophyll content is usually observed during drought exposure, and it causes a significant reduction in carotenoids and chlorophyll biosynthesis [53]. Drought stress causes dysfunction/destruction of the thylakoid structural membrane, which leads to a drastic decrease in chlorophyll content under water stress [54]. Additionally, drought affects chlorophyll-based chiral macro-aggregates of the harvesting complex, which cause oxidative stress [55]. The level of Chl a and b were significantly higher for irrigated plants than water-stressed plants in rapeseed [56], which supports our results that seedlings under normal conditions had significantly higher chlorophyll contents than drought treated seedlings. A detrimental effect of water deficiency was degeneration of chlorophyll, which causes a decline in the energy transfer between chlorophyll and the reaction center [54] and induces the overproduction of electrons through the electron transport chain, damaging the photosynthetic apparatus [57].

The present study showed that drought stress greatly influenced synthesis of the plant cell wall. The structural integrity of cells in the leaves of sensitive cultivars was greatly affected and abnormally formed compared to the tolerant cultivar, with oval to round chloroplasts moving toward the center of the cell. Furthermore, nonvisible and incomplete boundaries of the cell were observed under drought stress. These results coincide with those of maize seedlings, where the chloroplast structure varied from oval to circular due to plasmolysis caused by drought stress [17]. Additionally, chloroplast degeneration showed variation between tolerant and sensitive cultivars under drought stress [58–60].

The plant is a sessile organism and its responds to an unfavorable environment, such as drought, by a signaling pathway resulting in adaptation [61]. Water shortage causes oxidative stress in tissues and induces electron leakage within mitochondria and chloroplasts that leads to excitation of triplet oxygen, and enhanced ROS. This disorganizes the structure of photosynthetic pigments, consequently reducing photosynthesis and biomass production [62].

Substantial damage by ROS was recorded, which favor lipid peroxidation and structural degradation in stressed plants [22]. Osmotic adjustment and compatible solutes play a key role against drought stress by stabilizing cellular structure and function and maintaining turgor [63]. Accumulated solutes of different lower molecular weights, including TSS, proline, glycine betaine (GB), organic acids (OA) and trehalose, protect cell structure, thereby maintaining functional activity [64,65]. Proline is an important metabolite that accumulates under drought stress and confers protection on the sub-cellular structure and increases the activity of anti-oxidants [64], leading to an appreciable increase in drought tolerance. TSS and proline levels increased under drought stress in the four studied cultivars, signifying the role of osmolyte in all cultivars under drought stress, and indicating that several metabolites accumulated to relieve osmotic stress [66], and enhance plant survival.

Drought tolerance is correlated with an efficient scavenging system that helps maintain low ROS, thus preventing membrane peroxidation [9]. The response mechanism in plants against drought conditions depends on antioxidative enzymatic activity and osmolytes accumulation. CAT, SOD, POD and APX are essential enzymes in the defensive mechanism

that scavenges ROS. For the destruction of H_2O_2, several antioxidative enzymes act in synchrony. SOD is involved in the conversion of O_2^- into H_2O_2 and O_2, while CAT and POD convert H_2O_2 into O_2 and H_2O, and APX is involved in the AsA–GSH cycle, that supports the H_2O_2 removal [24]. This study demonstrated that such enzymatic activities increased under drought stress in the four studied cultivars, and the enhancement was higher in tolerant than in sensitive cultivars, suggesting that tolerant cultivars have a more efficient defense system, including enhanced scavenging activity [67].

5. Conclusions

Germination and growth-related traits showed variation among all studied cultivars, showing that tolerance against drought stress varied with exposure level and cultivar. The results show that drought negatively affects seed germination and seedling growth. Rapeseed seedlings respond to stress conditions with adaptive and acclimatization strategies, ranging from seemingly simple morphological responses to complex physiochemical changes that serve as important stress tolerance markers. The tolerance capacity was different for different cultivars. JYZ 158 and FY 520 had greater drought tolerance, while YG 2009 and NZ 1838 had lower drought tolerance. The study showed that drought stress imparted negative impacts on development and induced defense mechanisms for protection against drought-induced injuries. Drought tolerance in tolerant cultivars was due to higher antioxidant activity through enzymes and osmotic adjustment by accumulating osmotic substances such as proline, total soluble sugar and protein. Our findings provide insight into the drought-responsive mechanisms that can assist the researchers in improving the tolerance of rapeseed cultivars. The outcomes of this investigation have important implications for research on rapeseed during seed germination and at the early seedling stage during drought stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12030579/s1. Table S1: List of 24 cultivars examined under PEG-6000-induced drought stress. Table S2: Germination and seedling traits description and abbreviations under control and polyethylene glycol 6000 (PEG-6000) drought treatments, and the drought tolerance indices (DTIs) used to evaluate traits response to drought treatments. Table S3: Final germination percentage of three cultivars to select the concentration of PEG-6000.

Author Contributions: Conceptualization, M.B. and A.M.E.-B.; methodology, M.B.; software, I.A.A.M. and H.Y.; formal analysis, Z.W., X.A. and A.S.; investigation, B.W. and J.K.; resources, B.W. and G.Z.; data curation, R.S.; validation, M.U.H.; writing—original draft preparation, M.B. and A.M.E.-B.; writing—review & editing, M.B. and A.M.E.-B.; supervision, B.W.; project administration, B.W.; funding acquisition, B.W. and G.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by "National Key Research and Development Program of China, 2020 YFD1000904" and "Technical Innovation Project in Hubei Province, 2020 BBB061, and 2020 BBB062".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to all lab mates for their support.

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

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