



EFFECT OF SEED PRE-TREATMENT WITH PLANT GROWTH COMPOUND REGULATORS ON SEEDLING GROWTH UNDER DROUGHT STRESS

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ABSTRACT. The experiment aimed to evaluate the effect of different compound regulators on the germination rate, seedling morphology of two mustard (*Brassica juncea* L.) cultivars ('Felicia' and 'Prima') under simulated drought stress with PEG-6000. The eight commercial growth compound regulators (ALBIT, VERMISTIMD, ANTISTRESS, AGRINOS, REGOPLAN, BIOFOGE, STIMULATE, and FAST START) were pretreated seeds at recommended doses. The application of growth regulators promoted the growth of seedlings under drought stress but had no obvious effect on the germination rate of the two varieties. The root fresh weight, total root length, leaf area, stem length, and stem volume in 'Felicia' significantly increased with ANTISTRESS treatment by 24.28, 3.30, 24.70, 19.40, and 30.90%. In addition, the number of lateral roots reached the maximum with AGRINOS and REGOPLAN treatment compared with plants without regulators under drought conditions, which were 135.55 and 121.20%, respectively. For 'Prima', the application of FAST START had a remarkable effect on root fresh weight, total root length, lateral root number and primary root length, root surface area, leaf area, and stem volume by 17.62, 18.12, 211.20, 53.75, 28.57, 15.90, and 32.30%, respectively.

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Introduction

As the climate changes, drought is the most important natural factor, which influences plant growth and production. Drought stress caused changes in plant morphology, physiology, and gene expression (Hamidi, Safarnejad, 2010; Khan *et al.*, 2019). Available literature suggested that polyethylene glycol (PEG) can be used to simulate drought conditions and study the effects of drought stress on plants (Bressan *et al.*, 1981; Berg, Zeng, 2006). PEG is an inert long-chain polymer with high molecular weight, which has little effect on cells. Moreover, PEG osmotic stress method has the advantages of being simple, easy to control, good repeatability, and short test cycle.

Plant growth regulator (PGR) shows prominent effects on plant metabolism, resistance, growth, and productivity (Rademacher, 2015; Cao *et al.*, 2017). In current agricultural practice, the commercial growth regulators mainly include (1) Organic components, such as amino acids, humic acid, seaweed extract, organic carbon, acetic acid, sugar alkyd, chitin, chitosan, *etc.* (2) Biological components, such as nitrogen-fixation bacteria, plant growth promoting rhizobacteria, and remediation of contaminated soil microorganisms. (3) Inorganic components, such as iron, boron, calcium, magnesium, silicon, titanium, and other nutrients, and phosphate. (4) Other components, such as plant endogenous hormones. The sugarcane has



shown root improvement (from 60 to 118% in length, and 33 to 233% in surface area) by inoculation with plant growth-promoting bacteria (PGPB) combined with humic substances (Aguiar *et al.*, 2016). A study of cotton showed that the applied plant growth regulators (PGRs) had significant positive effects on the cottonseed yield, plant height, average number of open bolls, and so on (Osman *et al.*, 2010).

Brown mustard is an important cash crop, which has a long history of cultivation as an important oil crop all over the world. Meanwhile, It can also be used as a medicine, which has been proved to have a key role in cancer prevention and bactericidal, and it has attracted more and more attention (Delaquis, Mazza, 1995; Trachootham *et al.*, 2006). Most of the previous studies focused on the effects of a single endogenous hormone or nutrient on plants under drought stress (Gill, Tuteja, 2010; Yavas, Unay, 2016; Arnao, Hernández-Ruiz, 2019). However, there are few studies on the effects of compound growth regulators on the morphology of mustard. The objective of the study was to evaluate the effectiveness of PGRs on the root and shoot morphology of mustard during the seedling stage under simulated drought conditions, which would provide a theoretical basis for the practice of compound growth regulators in mustard and simplify cultivation and management.

Materials and Methods

Plant materials and treatments

Mustard cultivars 'Felicia' and 'Prima' were used in the experiment and were provided by the Department of Agronomy and Agricultural Technology of Sumy National Agrarian University, Sumy, Ukraine. The following the commercial growth regulators were used: ALBIT, VERMISTIMD, ANTISTRESS, AGRINOS, REGOPLAN, BIOFOGE, STIMULATE, and FAST START. PGRs were applied for the pre-treatment of seeds at the recommended dose (Table 1). The same size, healthy seeds were selected and coated with eight kinds of PGRs to cultivate in germination bags. Each treatment contained six germinate bags which were considered six replicates.

Each bag was added with 110 ml distilled water or 10% PEG-6000 (Sigma Chemicals Co., USA) solutions to simulate drought stress. All experiments were conducted in the growth chamber (day/night temperature at 28/20 °C) with the provision of 14 h light (350 $\mu\text{mol} (\text{m}^2 \cdot \text{s})^{-1}$) as well as 10 h dark. The germination rate was counted after 2 days of culture, and the growth parameters of root and shoot of 15 seedlings were calculated after 6 days of treatment. The fresh weight of five plants was weighed for one repetition and divided into three replicates.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by SPSS 22 (IBM, Armonk, NY, USA) with Duncan's multiple range tests ($P < 0.05$). All the collected data were shown

as the mean values \pm SD (standard deviations). The difference between control and PGR treated groups was denoted by the lowercase letters.

Table 1. Pre-treatment of mustard seeds with different plant growth regulators

| Treatment | Producer PGRs, country | PEG 6000, % | Growth regulator concentration, ml t^{-1} |
|------------------|---|-------------|--|
| CK1 | | 0 | 0 |
| CK2 | | 10 | 0 |
| T1 (ALBIT) | LLC "Research and Production Firm, Albit", Russia | 10 | 30 |
| T2 (VERMISTIM D) | PE "Bioconversion", Ukraine | 10 | 6–8 |
| T3 (ANTISTRESS) | BP "Humintech GmbH", Germany | 10 | 0.68 |
| T4 (AGRINOS) | Agrinos LLC, USA | 10 | 0.15 |
| T5 (REGOPLAN) | Agrobiotech LLC, Ukraine | 10 | 0.25 |
| T6 (BIOFOGE) | Stoller LLC, USA | 10 | 1.5–2.5 |
| T7 (STIMULATE) | Stoller LLC, USA | 10 | 0.5–1.5 |
| T8 (FAST START) | Stoller LLC, USA | 10 | 2.0–2.5 |

CK1 – distilled water; CK2 – 10% PEG-6000; T1 – 10% PEG-6000 + ALBIT; T2 – 10% PEG-6000 + VERMISTIMD; T3 – 10% PEG-6000 + ANTISTRESS; T4 – 10% PEG-6000 + AGRINOS; T5 – 10% PEG-6000 + REGOPLAN; T6 – 10% PEG-6000 + BIOFOGE; T7 – 10% PEG-6000 + STIMULATE; T8 – 10% PEG-6000 + FAST START.

Results

The effects of PGRs on germination rate under drought stress. As shown in Figure 1, the germination rate of the two cultivars changed under different treatments. In 'Felicia', the germination rate under T1 reached the minimum value (81%) compared to the CK1 (89%), CK2 (87%), and other treatments. The germination rate reached the maximum with T7 and T8, both by 90%, and was higher than normal growing conditions (89%) (Fig. 1A). For 'Prima', the germination rate of T1 (89%), T2 (88%), and T3 (87%) was slightly higher than that of CK1 (83%) and CK2 (85%) (Fig. 1B). Besides, there was a difference between the two cultivars in terms of germination rate. The germination rate of 'Felicia' was higher (89%) than that of 'Prima' (83%) under normal conditions. Although the sensitivity of 'Prima' and 'Felicia' to PGRs was different, the difference was not significant.

The effects of PGRs on fresh weight of mustard under drought stress. The results indicated that drought stress reduced the root fresh weight of 'Felicia' and 'Prima' by 22.22 and 17.93% compared with the CK1 (Fig. 2). The root fresh weight in 'Felicia' increased after the application of T3 and T5 by 24.28 and 17.85%. However, the application of T1 and T2 significantly reduced the root fresh weight of 'Felicia' by 36.43 and 20%, and the root fresh weight of T4 was not different compared with CK2. For the root fresh weight of 'Prima', the application of T5 and T8 was 23.96 and 17.62% higher than CK2. In addition, there was no significant difference between all treatments regarding the shoot fresh weight of 'Felicia' and 'Prima'. Compared with the CK1, the effect of drought stress on root fresh weight

was greater than shoot, indicating that root was very sensitive to drought stress.

The effect of PGRs on root growth of mustard under simulated drought stress. An extensive root system is advantageous to support plant growth during the early crop growth stage and absorb more water from the rhizosphere. Mustard is a straight root system, and its total root length consists of lateral roots and primary root (Fig. 3).

The root system architecture (RSA) was determined by multiple environmental factors. In 'Felicia' and 'Prima', drought stress (CK2) reduced TRL (total root length) by 12 and 15% compared to normal conditions (CK1) (Table 2), although there was no significant difference. For other root parameters, the effects of drought on the two cultivars showed opposite results. Drought significantly reduced lateral root number and primary root length in 'Prima' but not in 'Felicia'. Drought significantly reduced average root diameter

and total root volume in 'Felicia', but these indexes were not affected in 'Prima'. The responses of the two cultivars to PGRs were different under drought conditions. In 'Felicia', the application of T3 and T4 significantly increased the total root length by 3.3 and 8.2%, while other treatments were lower than CK2. In addition, the number of lateral roots reached the maximum under T4 and T5 treatment compared with that of CK2, which were 135.55 and 121.20%, respectively. For 'Prima', the PGRs increased the root length and the surface area under drought stress, except for T4 and T7. For lateral root number and primary root length, all regulators showed positive effects, and T8 treatment had the most prominent effect. Notably, the application of T8 had a remarkable effect on the root growth by increasing the root length (18.12%), surface area (28.57%), average diameter (6.06%), root volume (37.76%), lateral root number (211.20%) and primary root length (53.75%).

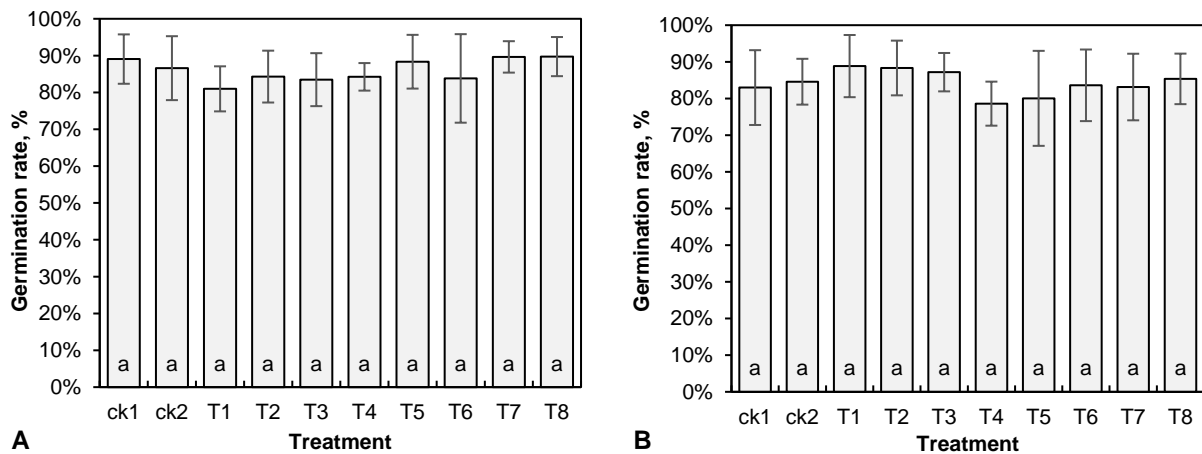


Figure 1. Seed germination rate of mustard under different treatments. A – 'Felicia', B – 'Prima'. CK1 – distilled water; CK2 – 10% PEG-6000; T1 – 10% PEG-6000 + ALBIT; T2 – 10% PEG-6000 + VERMISTIMD; T3 – 10% PEG-6000 + ANTISTRESS; T4 – 10% PEG-6000 + AGRINOS; T5 – 10% PEG-6000 + REGOPLAN; T6 – 10% PEG-6000 + BIOFOGE; T7 – 10% PEG-6000 + STIMULATE; T8 – 10% PEG-6000 + FAST START (similar lowercase letters denote non-significantly different according to Duncan's multiple range test, P >0.05)

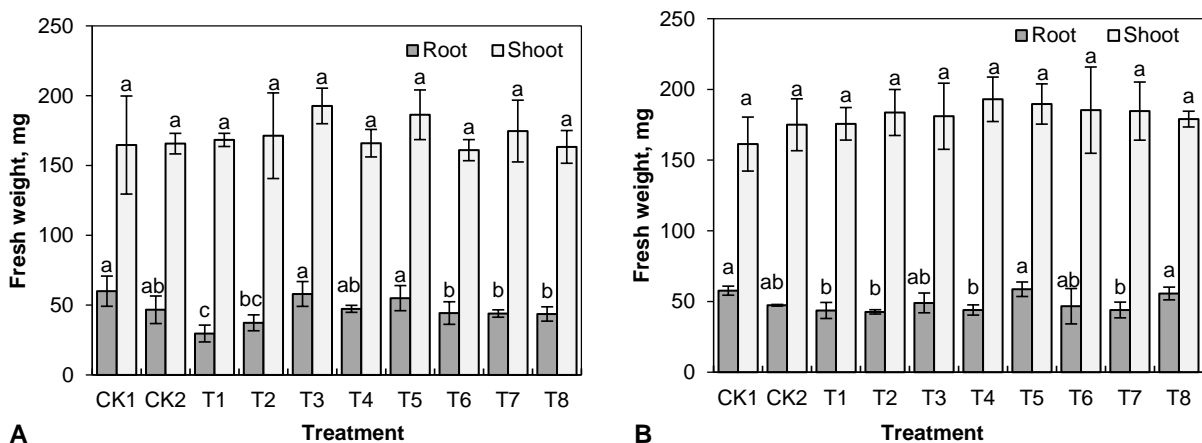


Figure 2. Fresh weight of mustard under different treatments. A – 'Felicia', B – 'Prima'. CK1 – distilled water; CK2 – 10% PEG-6000; T1 – 10% PEG-6000 + ALBIT; T2 – 10% PEG-6000 + VERMISTIMD; T3 – 10% PEG-6000 + ANTISTRESS; T4 – 10% PEG-6000 + AGRINOS; T5 – 10% PEG-6000 + REGOPLAN; T6 – 10% PEG-6000 + BIOFOGE; T7 – 10% PEG-6000 + STIMULATE; T8 – 10% PEG-6000 + FAST START (similar lowercase letters denote non-significantly different according to Duncan's multiple range test, P >0.05)

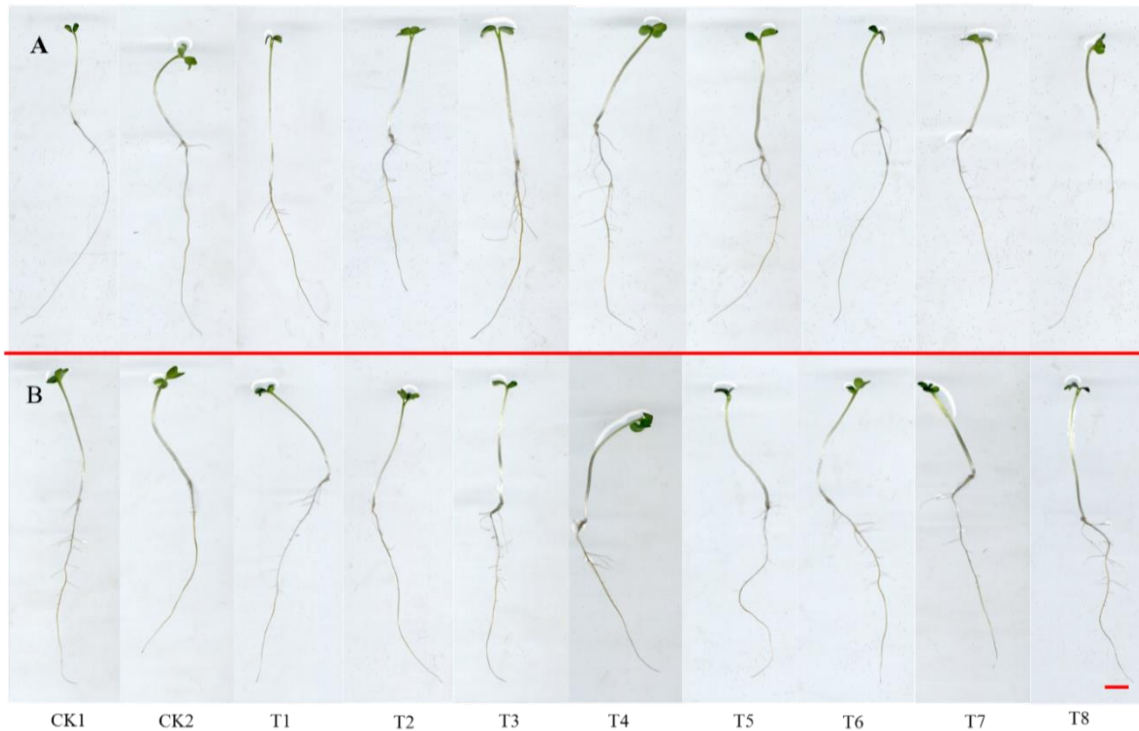


Figure 3. The appearance of the root system under the use of growth regulators. A – 'Felicia', B – 'Prima'

Table 2. Root growth parameters in different experimental setups

| Cultivars | Treatments | Total root length, cm | Total root surface area, cm ² | Average root diameter, mm | Total root volume, cm ³ | Number of first-order lateral roots | Length of primary root, cm |
|-----------|--------------------------|--------------------------|--|---------------------------|------------------------------------|-------------------------------------|----------------------------|
| 'Felicia' | CK1 | 9.18±1.37 ^{ab} | 1.07±0.07 ^a | 0.38±0.04 ^a | 10.00±1.00 ^a | 3.33±0.58 ^e | 8.81±0.17 ^a |
| | CK2 | 9.01±2.82 ^{abc} | 0.96±0.25 ^{ab} | 0.34±0.03 ^{bc} | 8.20±2.04 ^b | 4.67±0.58 ^{de} | 8.42±0.50 ^{ab} |
| | T1 | 7.19±1.35 ^c | 0.78±0.14 ^c | 0.35±0.03 ^{bc} | 6.67±1.45 ^c | 5.33±0.58 ^{cd} | 6.48±0.20 ^c |
| | T2 | 7.87±2.34 ^{abc} | 0.84±0.23 ^{bc} | 0.34±0.03 ^{bc} | 7.20±2.27 ^{bc} | 6.33±1.15 ^{bcd} | 7.34±0.36 ^{cd} |
| | T3 | 9.31±2.51 ^{ab} | 0.96±0.22 ^{ab} | 0.33±0.04 ^{bc} | 8.00±2.00 ^{bc} | 7.75±0.96 ^b | 7.12±0.54 ^{cde} |
| | T4 | 9.75±2.81 ^a | 0.98±0.18 ^{ab} | 0.33±0.04 ^c | 7.87±1.13 ^{bc} | 11.00±1.73 ^a | 6.94±0.21 ^{cde} |
| | T5 | 8.09±2.30 ^{abc} | 0.90±0.20 ^{bc} | 0.36±0.04 ^{ab} | 7.93±1.71 ^{bc} | 10.33±1.15 ^a | 5.68±0.38 ^f |
| | T6 | 7.99±2.79 ^{abc} | 0.87±0.24 ^{bc} | 0.35±0.03 ^{abc} | 7.60±1.88 ^{bc} | 5.33±0.58 ^{cd} | 7.72±0.36 ^{bc} |
| | T7 | 7.49±1.61 ^{bc} | 0.88±0.16 ^{bc} | 0.38±0.04 ^a | 8.27±1.83 ^b | 5.67±0.58 ^{cd} | 7.04±0.65 ^{cde} |
| T8 | 8.41±2.06 ^{abc} | 0.88±0.16 ^{bc} | 0.34±0.03 ^{bc} | 7.47±0.99 ^{bc} | 6.75±0.96 ^{bc} | 6.61±0.64 ^{de} | |
| 'Prima' | CK1 | 10.48±2.26 ^a | 1.04±0.23 ^{ab} | 0.32±0.03 ^{ab} | 8.33±2.44 ^b | 6.00±1.00 ^f | 9.47±1.29 ^a |
| | CK2 | 8.94±1.89 ^{ab} | 0.91±0.16 ^{bc} | 0.33±0.04 ^{ab} | 7.60±1.80 ^b | 3.75±0.96 ^b | 5.73±0.23 ^c |
| | T1 | 9.13±1.94 ^{ab} | 0.97±0.15 ^{bc} | 0.34±0.05 ^{ab} | 8.33±1.80 ^b | 8.75±0.96 ^{cd} | 7.96±0.27 ^b |
| | T2 | 9.10±1.57 ^{ab} | 0.91±0.15 ^{bc} | 0.32±0.04 ^{ab} | 7.33±1.72 ^b | 7.50±0.55 ^{de} | 6.19±0.39 ^c |
| | T3 | 9.91±3.12 ^{ab} | 0.96±0.24 ^{bc} | 0.32±0.04 ^b | 7.60±1.59 ^b | 10.33±0.58 ^{ab} | 6.36±0.47 ^c |
| | T4 | 8.47±2.65 ^b | 0.83±0.23 ^c | 0.32±0.05 ^b | 6.73±2.22 ^b | 10.67±0.58 ^{ab} | 5.58±0.50 ^c |
| | T5 | 9.11±1.74 ^{ab} | 0.95±0.15 ^{bc} | 0.34±0.05 ^{ab} | 8.07±2.09 ^b | 7.80±1.10 ^{de} | 7.53±0.08 ^b |
| | T6 | 9.18±3.05 ^{ab} | 0.94±0.24 ^{bc} | 0.33±0.04 ^{ab} | 7.80±1.74 ^b | 7.00±1.00 ^{ef} | 7.46±0.27 ^b |
| | T7 | 8.39±1.95 ^b | 0.85±0.15 ^c | 0.33±0.04 ^{ab} | 6.93±1.33 ^b | 9.33±0.58 ^{bc} | 6.37±0.47 ^c |
| T8 | 10.56±1.92 ^a | 1.17±0.19 ^a | 0.35±0.03 ^a | 10.47±2.17 ^a | 11.67±1.53 ^a | 8.81±0.51 ^a | |

Means ± SD, followed by different lowercase letters are significantly different according to Duncan's multiple range test, P < 0.05, n = 3

The effects of PGRs on the shoot growth of mustard under drought stress. For 'Felicia', the PGRs promoted the growth of the shoot under the drought condition, except for the T6 treatment group (Table 3). Leaf area, stem length, and stem volume after the application of T3 increased significantly compared with CK2 by 24.7, 19.4, and 30.9%, respectively. For the shoot growth of 'Prima', the application of T8 significantly increased the leaf area and stem volume by 15.9 and 32.3%, while there was no significant difference between other regulators and CK2.

Discussion

Drought stress is one of the most common abiotic stresses in agricultural production, and with climate change, drought stress becomes more frequent and severe in the world. The application of plant growth regulators is considered an effective strategy to improve plant stress resistance in agricultural production (Ma *et al.*, 2006; Sharma *et al.*, 2016). This study used PEG 6000 to simulate drought stress in mustard seedlings, and different types of PGRs were applied to evaluate the changes in germination rate and growth indicators of root and shoot.

Table 3. Shoot growth parameters in different experimental setups

| Cultivars | Treatments | Leaf area, cm ² | Stem length, cm | Stem diameter, mm | Stem volume, mm ³ |
|-----------|------------|----------------------------|----------------------------|--------------------------|------------------------------|
| 'Felicia' | CK1 | 0.94 ± 0.18 ^{bc} | 3.62 ± 0.67 ^{bc} | 0.82 ± 0.05 ^a | 19.40 ± 4.32 ^{ab} |
| | CK2 | 0.89 ± 0.15 ^c | 3.56 ± 0.67 ^c | 0.80 ± 0.07 ^a | 17.73 ± 3.13 ^b |
| | T1 | 1.03 ± 0.15 ^{abc} | 4.02 ± 0.47 ^{abc} | 0.81 ± 0.08 ^a | 21.07 ± 4.62 ^{ab} |
| | T2 | 1.03 ± 0.20 ^{abc} | 4.21 ± 0.92 ^{ab} | 0.79 ± 0.07 ^a | 20.33 ± 4.47 ^{ab} |
| | T3 | 1.11 ± 0.17 ^a | 4.25 ± 0.68 ^a | 0.83 ± 0.06 ^a | 23.20 ± 4.02 ^a |
| | T4 | 0.97 ± 0.14 ^{abc} | 3.96 ± 0.58 ^{abc} | 0.78 ± 0.04 ^a | 19.07 ± 2.94 ^b |
| | T5 | 1.06 ± 0.18 ^{ab} | 4.22 ± 0.81 ^{ab} | 0.80 ± 0.07 ^a | 21.07 ± 3.86 ^{ab} |
| | T6 | 0.88 ± 0.13 ^c | 3.54 ± 0.53 ^c | 0.80 ± 0.08 ^a | 17.67 ± 3.54 ^b |
| | T7 | 1.05 ± 0.24 ^{ab} | 4.14 ± 0.98 ^{abc} | 0.81 ± 0.06 ^a | 21.27 ± 5.20 ^{ab} |
| | T8 | 0.98 ± 0.27 ^{abc} | 3.78 ± 0.80 ^{abc} | 0.82 ± 0.09 ^a | 20.40 ± 8.45 ^{ab} |
| 'Prima' | CK1 | 1.00 ± 0.13 ^b | 4.03 ± 0.48 ^{ab} | 0.79 ± 0.05 ^a | 19.93 ± 3.28 ^b |
| | CK2 | 1.07 ± 0.14 ^{ab} | 4.23 ± 0.47 ^{ab} | 0.81 ± 0.08 ^a | 22.07 ± 4.67 ^{ab} |
| | T1 | 1.05 ± 0.19 ^{ab} | 4.06 ± 0.57 ^{ab} | 0.82 ± 0.08 ^a | 21.93 ± 5.92 ^{ab} |
| | T2 | 1.04 ± 0.19 ^{ab} | 4.16 ± 0.74 ^{ab} | 0.80 ± 0.08 ^a | 20.93 ± 4.70 ^b |
| | T3 | 1.03 ± 0.14 ^b | 4.15 ± 0.61 ^{ab} | 0.79 ± 0.06 ^a | 20.33 ± 3.35 ^b |
| | T4 | 1.01 ± 0.33 ^b | 3.91 ± 1.15 ^b | 0.80 ± 0.12 ^a | 21.00 ± 7.37 ^b |
| | T5 | 1.15 ± 0.16 ^{ab} | 4.64 ± 0.80 ^a | 0.79 ± 0.05 ^a | 22.93 ± 3.10 ^{ab} |
| | T6 | 1.13 ± 0.13 ^{ab} | 4.55 ± 0.61 ^a | 0.79 ± 0.07 ^a | 22.27 ± 3.45 ^{ab} |
| | T7 | 1.14 ± 0.32 ^{ab} | 4.53 ± 1.00 ^a | 0.80 ± 0.10 ^a | 23.20 ± 9.55 ^{ab} |
| | T8 | 1.24 ± 0.49 ^a | 4.51 ± 0.64 ^{ab} | 0.86 ± 0.23 ^a | 29.20 ± 24.13 ^a |

Means ± SD, followed by different lowercase letters are significantly different according to Duncan's multiple range test, P < 0.05

Seed germination is the first stage for plants to endure environmental stress. Growth regulators are used in the pre-sowing seed treatment and play an important role in regulating germination and vigour (Basra *et al.*, 1989). Previous reports suggested that seed germination and seedling vigour depend on the priming method and the concentration used (Kumari *et al.*, 2017). In this study, it has been determined that compound regulator has little effect on the germination rate of 'Felicia' and 'Prima'. This is different from previous reports that growth regulators promote germination rate of wheat (ZeQiong *et al.*, 2019) and rapeseed (Khan *et al.*, 2019). We hypothesized that it may be due to differences in PGRs. On the other hand, mustard is considered a well-adapted crop, and its germination may be related to the genotype and the ability to transform nutrients in the endosperm. To some extent, germination rate is not a good indicator to screen the effects of the regulator on mustard under drought conditions.

Roots are the first organ to sense and respond to environmental factors. In response to stress, root system changes include not only the elongation of primary roots but also the occurrence and elongation of lateral roots (Ötvös *et al.*, 2021). In the present results, although drought did not significantly reduce the total root length of the two cultivars, it did significantly reduce the lateral root number and lateral root number of 'Prima' (Table 2). Furthermore, cultivar 'Felicia' presented no significant response to 10% PEG stress regarding lateral root formation and primary root elongation, but its root diameter and total root volume were significantly reduced by the mimicked drought stress, indicating root thickening was retarded. The results suggest that 'Prima' is more sensitive to drought than 'Felicia'. The PGRs significantly promoted the root growth of cultivated 'Prima' under drought conditions.

Not like for 'Prima', T1–T8 treatments did not improve those root parameters for 'Felicia'. These results suggested that PGRs had a positive role against drought on drought sensitive cultivar; on the contrary, for drought non-sensitive cultivar, the PGRs exhibited relatively poor effects against drought. These results indicated the response of mustard to PGRs under simulated drought in the climate chamber, and the evaluation of regulators in field experiments under natural conditions needs to be further verified.

Conclusions

Drought reduced root fresh weight in both cultivars but had no effect on shoot fresh weight and germination rate. There were differences in the inhibition degree of root growth between 'Felicia' and 'Prima' under drought stress. Drought significantly reduced average root diameter and total root volume in 'Felicia', as well as the lateral root number and primary root length of 'Prima'. According to the morphological parameters of roots, 'Prima' was more sensitive to drought than 'Felicia'. The PGRs mitigated the effects of drought on seedlings to some extent, but there were differences between the two cultivars. For drought-sensitive 'Prima', PGRs had a positive role against drought; on the contrary, for drought non-sensitive 'Felicia' the PGRs exhibited relatively poor effects against drought.

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Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Author contributions

PJ – performed the data analysis and discussion of the results, drafted the manuscript;
 AM – studied the conception and interpretation of data, and is the corresponding author;
 ZZ – the author of the idea, critical revision, and approval of the final manuscript;
 SB, VK – collected data from the field, made literature search and acquisition of data.
 All authors read and approved the final manuscript.

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