

Germination and Seedling Growth of a Set of Rapeseed (*Brassica napus*) Varieties under Drought Stress Conditions

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Abstract— Drought stress is one of the major abiotic factors affecting seed germination and plant growth especially in arid and semi-arid regions. In this study, we investigated the effects of drought stress on seed germination and seedling growth of five varieties of rapeseed. Seven drought stress levels of zero (control), -3, -5, -7, -9, -11 and -13 bars were performed using polyethylene glycol-6000 (PEG-6000). A completely randomized design with three replications was used for this experiment. Germination percentage (GP), germination rate (GR), mean germination time (MGT), root length (RL) and shoot length (SL) were measured to evaluate the varieties response to PEG-induced drought stress. Drought stress, variety and the interaction drought × variety had a significant effect on all studied parameters. GP and GR decreased with the increase in stress level, while MGT increased. There were no seeds germinated for all varieties at -11 bars and -13 bars. Shoot length decreased with increasing drought stress but different varieties show different performance under stress environment. Root length decreased with increasing level of severe drought stress. However, the presence of moderate drought stress could even improve the root growth of the investigated varieties. The varieties 'INRA-CZH2' and 'INRA-CZH3' exhibited the highest germination percentage and the best early seedling growth. Thus, they could be recommended for environments with early cropping cycle drought.

Keywords— Drought stress, Germination, Rapeseed, Seedling growth.

I. INTRODUCTION

Rapeseed (*Brassica napus* L.), belonging to the family of *Brassicaceae*, is one of the most important sources of vegetable oils and protein-rich meals worldwide. Rapeseed is the world's third most important source of vegetable oil after palm and soybean [1]. Also, rapeseed

oil is one of the most interesting edible oils in the world. Its nutritive value is excellent due to the abundant unsaturated fatty acids. Rapeseed meal (remains after oil extraction) is used for livestock feed industry. Its amino acid content is ideal and it has a high content of fiber, several minerals and vitamins [2, 3].

Drought was always present in Morocco's history, but during this last decade, it has become more frequent, with a net reduction in precipitation and an increasing temperature trend. Climatic data during the period 1961-2004 showed an increase in drought frequency, severity and spatial distribution [4]. In Morocco with an arid and semi-arid climate, rapeseed is more often planted in late autumn and harvested in early summer. Accordingly, this stress is also considered as an essential limiting factor for rapeseed growth and production due to poorly distributed rainfalls over the crop growing season. Although drought can occur at any time during the growing season, two main periods of drought are more likely, the early one that coincides with seed germination and seedling emergence and the terminal drought that is more frequent and affects grains set and growth [5].

Drought can be most simply defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system [6, 7]. In the field, drought is a severe limitation of plant growth, development and productivity, particularly in arid and semi-arid regions [8], where the rainfall varies from year to year. However, depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for drought stress. Seed germination is first critical and at the same time the most sensitive stage in the life cycle of plants [9] and the seeds exposed to unfavorable environmental conditions like drought stress may have to compromise the seedlings establishment [10]. Soil water supply is an important environmental factor controlling seed germination [11]. If the osmotic

potential is reduced, seed germination will be delayed or prevented, depending on the extent of its reduction [12]. One technique for studying the effect of drought stress on germination is to simulate stress conditions using artificial solutions to provide variable osmotic potentials [13, 14]. Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator [15, 16, 17, 18] as an inert osmoticum in germination tests [19] and a non-penetrating solute [20]. This results in osmotic stress that inhibits seed germination through the prevention of water uptake.

The present research was carried out to study the effect of water stress (PEG) on rapeseed germination and early seedling growth, evaluating various levels of PEG and different rapeseed varieties.

II. MATERIALS AND METHODS

The plant material used in this study consisted of five varieties of rapeseed (*Brassica napus* L.) from the collection of the National Institute for Agricultural Research (INRA) of Morocco (Regional Center of Meknes). These are 'INRA-CZH2', 'INRA-CZH3', 'INRA-CZSyn1', 'Moufida' and 'Narjisse'.

The experiment was conducted in a double factorial completely randomized design, with three replications. The first factor was the variety, with five levels, and the second was the stress solution of PEG, with seven levels. Drought stress was induced by polyethylene glycol (PEG-6000) solutions. Application of six levels of drought stress, with osmotic potentials of -3, -5, -7, -9, -11 and -13 bars, was prepared as described by [21]. Distilled water was used as control. For each treatment, 100 seeds were sterilized in a solution of 5% sodium hypochlorite for 1 min and then carefully rinsed with distilled water to remove any traces of sterilizing agent, and were allowed to germinate in a Petri dish double lined with filter paper moistened with 15 ml of the appropriate level of PEG-6000 at 25 ± 1 °C for 8 days. Germination parameters were counted after 2, 4, 6 and 8 days following seeds placement in Petri dishes for germination. Seeds were considered germinated when the radicle was at least 3 mm long. For germination percentage, the number of seeds germinated on day 8 was considered. The germination rate index was determined by $GR = \sum (Ni/Di)$ as described by Carlton et al. 1968 [22], where Ni is the number of seeds germinated between two counts and Di represents the day of counting. Mean Germination Time (MGT) expressed in d, is the inverse of GR ($MGT = 1/GR$). Root and shoot length were measured on the eighth day after germination (end of experiment). Shoot length was measured from the cotyledons to the collar, and the root length was measured from the collar to the root tip.

Statistical analysis was conducted with the software package SPSS for Windows (Version 22). Data were subjected to an analysis of variance (ANOVA) to determine statistically significant differences among varieties, drought levels and their interaction levels. Duncan's new multiple range test (DMRT) was applied to compare treatment means.

III. RESULTS

3.1 Drought stress effects on germination

Results of analysis of variance indicated that drought stress affected significantly rapeseed seed germination percentage (GP), germination rate index (GR) and mean germination time (MGT). There was also a significant effect of variety and its interaction with drought on these parameters (Table 1). GP and GR decreased, while MGT increased with the increase in drought level (Table 2). The highest GP (97.40 %) was observed in absence of drought stress (control), and the most significant decline was recorded at -9 bars (64.20%). Below this osmotic potential, i.e. at -11 and -13 bars, no germination was recorded. The germination percentage at the -5 and -7 bars was 90.33 and 85.00 %, respectively.

In absence of stress (control treatment), significant differences among varieties were observed for GP. 'INRA-CZH2', 'INRA-CZH3' and 'Moufida' were the most interesting, having a GP of 100, 99 and 98.70%, respectively, while 'INRA-CZSyn1' and 'Narjisse', had 94% and 95%, respectively (Fig. 1). Under moderate stress (-3 bars), 'INRA-CZH2' had the highest GP (100%), whilst 'INRA-CZSyn1' had the lowest one (87%). Under severe-intermediate stress (-9 bars), again, 'INRA-CZH2' maintained the highest GP (93%), while 'Narjisse' showed the most drastic reduction in GP (40%) (Fig. 1). 'INRA-CZH3' exhibited quite high GP (80%), whilst 'Moufida' and 'INRA-CZSyn1' had too much lower GP, with an average of 58% and 50%, respectively (Fig. 1). This indicated that, in terms of germination, 'INRA-CZH2' and 'INRA-CZH3' were the most tolerant to such stress, and 'Narjisse' was the most sensitive. The other varieties were intermediate.

Mean germination time (MGT) of all experimented varieties was significantly delayed by increasing the drought level (Fig. 2). 'INRA-CZH2' and 'INRA-CZH3' were the least affected as they showed the lowest MGT values for all drought levels (Fig. 2). By increasing drought stress, germination rate (GR) decreased significantly in all varieties (Fig. 3). Again, 'INRA-CZH2' and 'INRA-CZH3' confirmed their highest tolerance to various drought levels, maintaining the highest GR values, compared to the other varieties. Overall, they had a GR of about 30 and 28.6%, respectively, which was significantly higher than 23.3,

18.2 and 17.8%, observed in 'Moufida', 'INRA-CZSyn1' and 'Narjisse', respectively.

3.2 Drought stress effects on seedling growth

There were significant differences among varieties and between drought stress levels for shoot length (SL) and root length (RL) (Table 1). Also, the effect of drought stress \times variety interaction on both parameters was significant, indicating that varieties reacted differently to the drought levels.

Shoot length decreased with increase in drought level. The highest average shoot length, 4.77 cm, was observed in absence of drought (control). At the osmotic potential of -3 bars, corresponding to the moderate drought stress, the average shoot length was 2.42 cm. The first drastic reduction was recorded at -7 bars (0.67 cm) and the lowest shoot length ever observed was 0.28 cm, recorded at -9 bars.

Overall, the varieties 'Narjisse' and 'INRA-CZSyn1' showed the highest average shoot length (1.62 cm) while the variety 'INRA-CZH2' showed the lowest one (1.04 cm). The other varieties were intermediate (Fig. 4). In absence of stress, 'Narjisse' and 'INRA-CZSyn1' had the highest average shoot length (5.70 cm). At moderate stress (-3 bars), all the varieties were comparable, with an average of about 2.40 cm. 'Narjisse' maintained the highest SL (2.55 cm) at -5 bars followed by 'INRA-CZH3', 'INRA-CZSyn1', 'Moufida' and 'INRA-CZH2', having an average SL of 1.99, 2.03, 1.80 and 1.46 cm, respectively. However, under intermediate-severe stress (-9 bars), 'INRA-CZSyn1' and 'Narjisse' had the lowest average SL (0.21 cm) while 'INRA-CZH3' exhibited the highest one (0.41 cm). 'INRA-CZH2' and 'Moufida' were comparable, with a mean value of 0.27 cm (Fig. 4). On the other hand, and for all drought stress levels combined, the variety 'INRA-CZH3' developed the longest root, with an overall average of 4.23 cm, whilst

the variety 'Narjisse' had the lowest average root length, i.e. 3.58 cm. Unlike shoot length, root length (RL) increased with moderate and moderate-intermediate drought stress (-3 and -5 bars, respectively). The highest average RL, 7.70 cm, was observed for moderate-intermediate stress (-5 bars). The varieties 'INRA-CZH2' and 'Narjisse' exhibited the highest mean RL, i.e. 8.35 cm. At the osmotic potential of -3 bars, corresponding to the moderate drought stress, the average RL was 6.70 cm. 'INRA-CZH3' was the most interesting variety, with RL of 7.95 cm. In absence of drought (control), average RL was 5.10 cm, and 'INRA-CZSyn1' developed the longest root (about 6 cm). This indicates that moderate up to intermediate drought stress did not affect the root growth, but could even improve it. Under intermediate drought stress (-7 bars), RL was unchanged for the varieties 'INRA-CZH3' and 'Moufida', decreased for 'Narjisse' and increased for 'INRA-CZH2'. Finally under severe-intermediate stress (-9 bars), a decrease of this parameter was observed for all varieties (Fig. 5). However, the variety 'INRA-CZH2' was the least affected, maintaining a RL of about 3.50 cm, compared to 3.80 cm recorded in absence of drought stress (control).

IV. DISCUSSION

Seed germination and early seedling growth are critical stages for plant establishment [23, 24], and plants are more sensitive to drought stress during these stages. Drought stress greatly affects seed germination, but the response intensity and harmful effects of such stress depend on the species. In the present study, all the seed germination parameters measured were affected by drought stress. Particularly, GP and GR decreased with the increase in stress levels. This is in agreement with findings of previous studies in *Brassica napus* [18] and in *Brassica juncea* [15]. MGT increased with the increase in stress levels.

Table 1. Analysis of variance (mean squares) for seed germination and seedling growth related traits of five rapeseed varieties evaluated under different drought stress levels.

| Source of variation | Degree of freedom | Germination percentage | Germination rate | Mean germination time | Root length | Shoot length |
|---------------------|-------------------|------------------------|------------------|-----------------------|-------------|--------------|
| Variety (V) | 4 | 1003.32*** | 681.11*** | 0.0018*** | 3.45* | 20.10*** |
| Drought (D) | 6 | 28360.17*** | 5142.93*** | 0.0103*** | 383.33*** | 748.11*** |
| V \times D | 24 | 200.20*** | 62.16*** | 0.0005*** | 3.35*** | 9.26*** |

*, **, *** Significant at 0.05; 0.01 and 0.001 probability levels, respectively.

Table.2: Overall averages of seed germination and seedling growth related traits for each PEG applied osmotic potential.

| | Germination percentage | Germination rate | Mean germination time | Root length (cm) | Shoot Length (cm) |
|-----|------------------------|------------------|-----------------------|------------------|-------------------|
| 0 | 97.40 a | 42.74 a | 0.026 d | 5.09 c | 4.77 a |
| -3 | 94.67 b | 42.51 a | 0.024 d | 6.72 b | 2.42 b |
| -5 | 90.33 c | 36.05 b | 0.030 c | 7.70 a | 2.00 c |
| -7 | 85.00 d | 27.28 c | 0.040 b | 4.71 c | 0.67 d |
| -9 | 64.20 e | 16.49 d | 0.077 a | 2.66 d | 0.28 e |
| -11 | 0.00 f | 0.00 e | 0.00 e | 0.00 e | 0.00 f |
| -13 | 0.00 f | 0.00 e | 0.00 e | 0.00 e | 0.00 f |

Mean values, in each column, followed by the same letter are not significantly different.

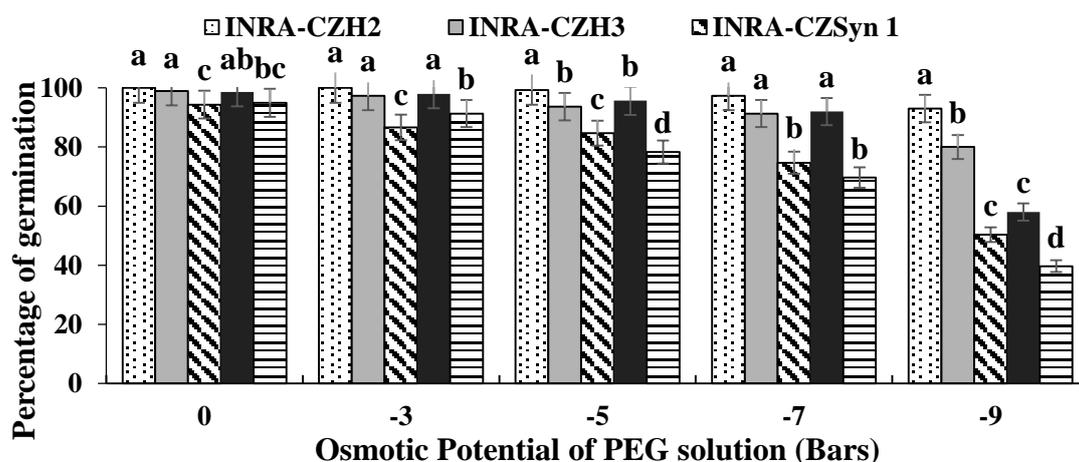


Fig. 1: Effect of PEG induced drought stress on germination percentage of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

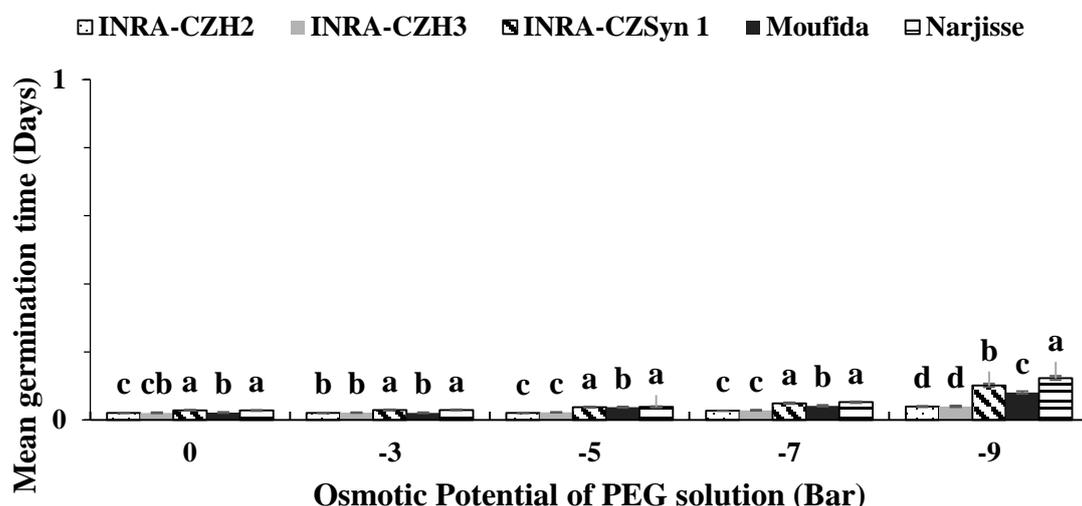


Fig. 2: Effect of PEG induced drought stress on mean germination time (MGT) of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

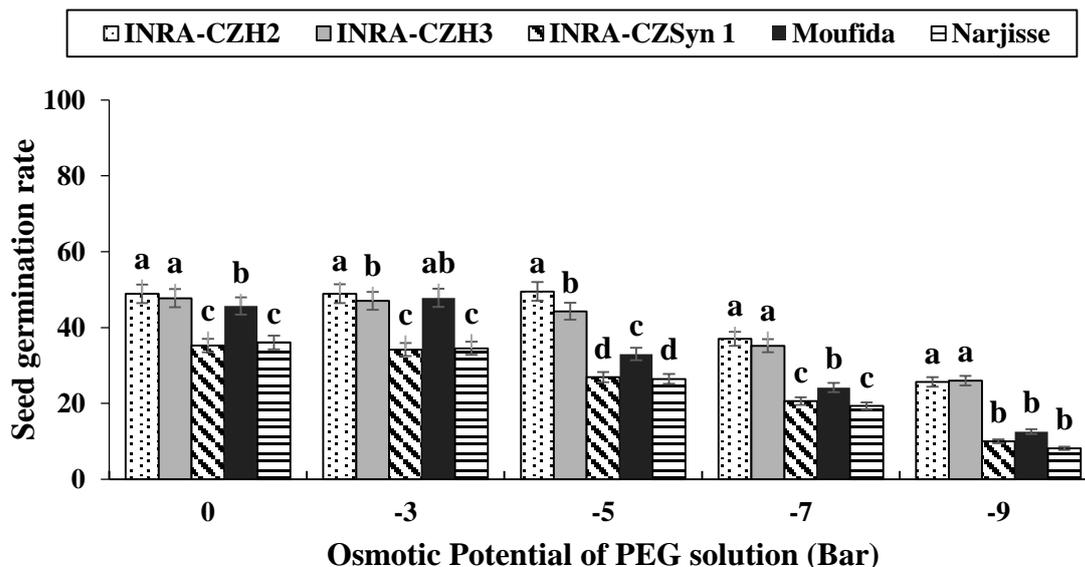


Fig. 3. Effect of PEG induced drought stress on germination rate of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

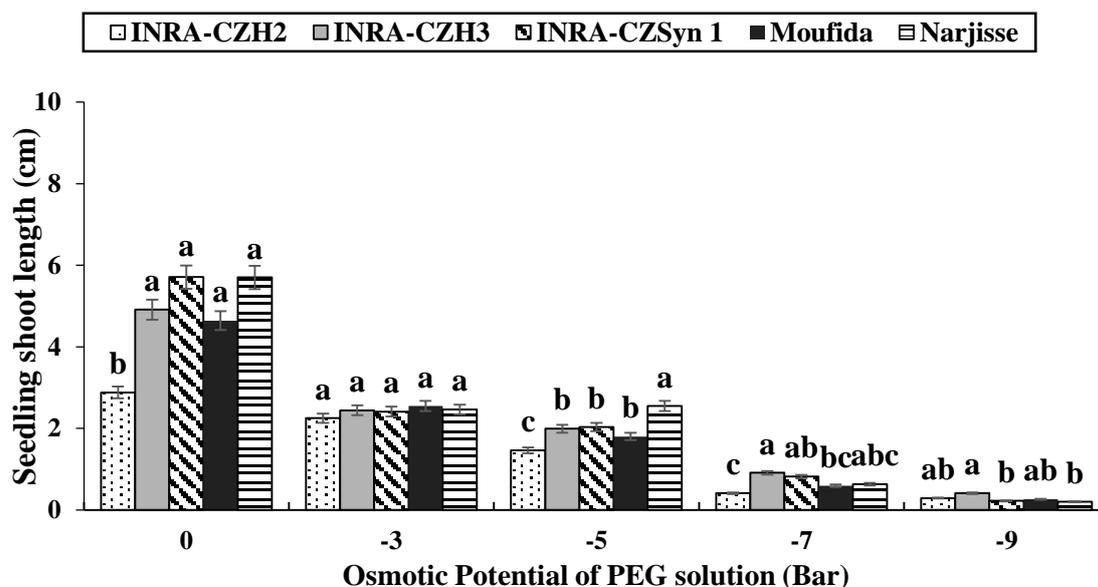


Fig. 4. Effect of PEG induced drought stress on seedling shoot length of five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

The same result had been found in safflower [25] and in sesame [26]. The germination of 'INRA-CZH2' and 'INRA-CZH3' was less affected than that of 'INRA-CZSyn1', 'Moufida' and 'Narjisse', indicating that, during germination, 'INRA-CZH2' and 'INRA-CZH3' were more tolerant to drought stress than the other varieties. To germinate, all varieties of rapeseed could tolerate until -9 bars, and from -11 bars, no germination was recorded. Toosi et al. [15] reported that -10 and -12 bars completely inhibited seed germination in *Brassica juncea* Var. Ensabi. Kaya et al [27] found that none of the sunflower seeds could germinate at -12 bars, and in a

recent study on sesame, it was also shown that seeds ceased to germinate from -12 bars [26]. However, Zraibi et al. [25] reported that safflower seeds ceased to germinate already from -2.5 bars. These findings indicate that rapeseed is less tolerant than sunflower and sesame and more tolerant than safflower to drought stress during germination stage.

For all varieties, drought stress affected the germination and early seedling growth of rapeseed. This may be due to alteration of enzymes and hormones found in the seed [28] or to the metabolic disorders induced by stress and generation of Reactive Oxygen Species [29].

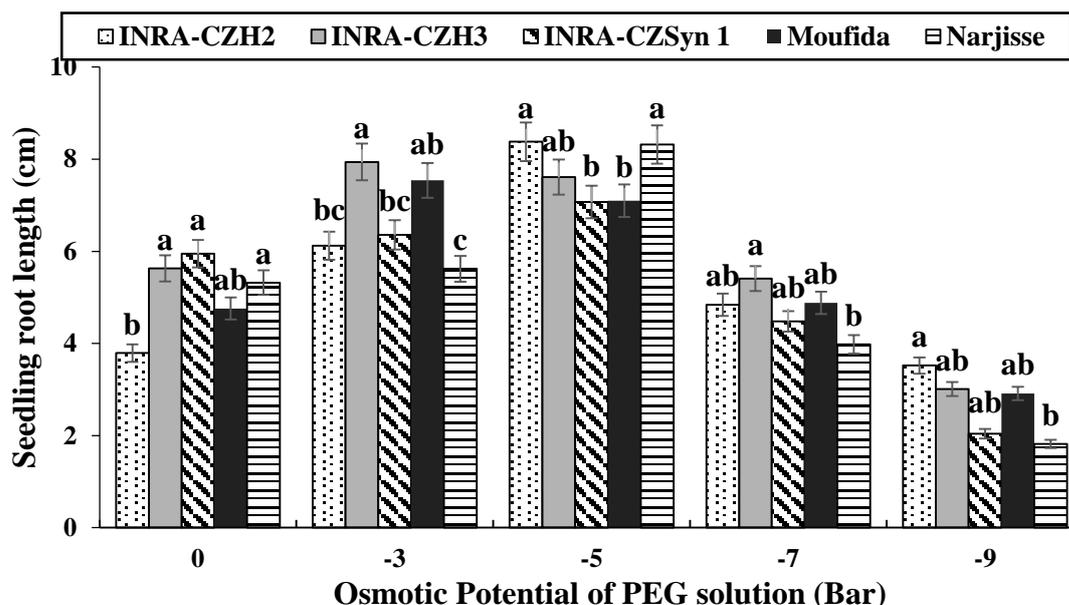


Fig. 5. Effect of PEG induced drought stress on seedling root length of five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

It could also be a deficit of hydration of the seeds due to high osmotic potential causing inhibition of the mechanisms leading to the output of the radicle out of the coat and therefore a seed germination delay [30].

In our study, shoot length generally decreased with increased drought levels. There are many reports on various crops that are in accordance with this finding [15, 25, 26]. However, we found that the varieties investigated showed different performances under this stress. In absence of stress, 'Narjisse' and 'INRA-CZSyn1' had the highest average shoot length, whilst under elevated drought stress, 'INRA-CZH3' was the most tolerant, having exhibited the highest shoot growth. Similar results were previously shown in other crops, including *Brassica juncea* Var. Ensabi [15], safflower [25] and sesame [26]. Also, root length decreased with the increased drought levels. However, the presence of moderate drought stress could even improve the root growth of the varieties investigated. These results agree with those reported in *Brassica juncea* Var. Ensabi [15], in cowpea [31], in pearl millet [32] and in triticale [33]. Our investigation revealed that, in absence of any stress, the varieties 'Narjisse', 'INRA-CZSyn1' and 'INRA-CZH3' developed the longest root. However, for all drought stress levels combined, the variety 'INRA-CZH3' was the most tolerant, exhibiting the longest average root,

The variation in rapeseed varieties performance determined by some seedling growth parameters such as shoot length and root length indicated that seedling growth is a reliable and efficient stage for the study of rapeseed genotypes reaction to moisture stress. The varieties having genetic potential to maintain higher

seedling growth under moisture stress conditions are drought tolerant in this particular stage, and their tolerance should be confirmed at adult plant stages.

V. CONCLUSION

Based on the results of this study, the varieties 'INRA-CZH2' and 'INRA-CZH3' germinated better than the other varieties under drought conditions. The observed variation among varieties is a reliable indicator of genotypic differential for drought tolerance in rapeseed. This suggests that the choice of the rapeseed variety to be planted in a given environment should depend upon the presence and the degree of the stress observed in such environment. In a non-stressed environment, the varieties, 'INRA-CZSyn1' and 'Narjisse', even with an intermediate germination percentage, should be planted due to their best seedling growth (root and shoot length). In drought stressed environments, the varieties 'INRA-CZH2' and 'INRA-CZH3', exhibiting the highest germination percentage, should be recommended.

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