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Investigating the impact of salt and drought stress on germination and early seedling growth stages of Rapeseeds (*Brassica napus* L.)

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Abstract

The most sensitive stage to abiotic stress is considered seed germination. These stressors comprise high soil salinity, drought, and irrigation water, which can negatively affect seeds' germination and plant growth, especially in semi-arid and arid regions. Rapeseed is an oilseed crop adapted to the Mediterranean area; however, it is reportedly sensitive to salt and water stress during seed germination. In the present study, we tested under controlled conditions. Two randomized complete block factorial experiments were conducted under simulated drought and salinity stress conditions using polyethylene glycol PEG 6000 and NaCl, respectively. The experiment used different concentrations of saline solution, including 0 (distilled water), 50, 100, and 150 mmol.l-1 of NaCl. The following osmotic potentials were applied to simulate water deficit in PEG 6000: 0 (distilled water), 5%, 10%, 15%, and 20% (w/v). This study investigated Germination Percentage, Mean Daily Germination, Seedling Vigor Index, and root and shoot length.

There were notable variations among the treatments ($p<0.01$) for all the traits. The impact of water and salt stress was responsible for a decrease in all the identified germination and seedling parameters. Increasing NaCl levels significantly impacted the germination process, particularly under salinity levels of 150, 200, and 250 mM NaCl and 20% PEG. A higher concentration of NaCl (250 mM) and PEG (20%) resulted in a significant decrease in rapeseed yield. Higher PEG and NaCl concentrations decrease shoot and root length. Osmotic stress affects seedling elongation more than germination percentage. This indicates that seedlings prioritize elongation over germination in response to osmotic stress.

Keywords: Rapeseed, Drought stress, Salt stress, Germination, Seedling growth.

1. INTRODUCTION

Environmental stresses impacting agriculture, including drought, salt, high temper PEG concentrations, and stress. These conditions roughly cut agricultural production in half, and salinity and drought-related water stress are major issues worldwide. Still, salt and drought similarly impact plants and equally affect Khayatnezhad et al. (2010).

Because both generate osmotic stress and most stressed plants have comparable metabolic responses, salinity and drought stress are physiologically connected (Kumar et al., 2011).

Severe drought conditions can significantly hinder plant growth, development, and production, as the annual rainfall levels vary greatly, especially in arid and semi-arid regions. Drought is one of the most significant limitations on agricultural output, with a significant impact on plant growth, distribution, gene expression, quality, and yield (Yang et al., 2004; Shi et al., 2009).

Stress from drought and salt affects plants at all phases of their growth cycle, with seed germination being the most sensitive phase to environmental stress during plants' development and growth (Endo et al., 2014; Luan et al., 2014). Seed germination, which marks the start of a plant's life cycle, is essential for the plant's future vital activities. Many plants require ideal circumstances for germination, most of which are not salinized (Cassaniti et al., 2012, 2013). Salt and drought stress are two significant abiotic stressors that restrict seedling evolution (Almansouri et al.,2001). These stressors reduce germination rates and slow seedling growth (Farooq et al., 2009, 2015). Seed germination happens differently for every plant, depending on the ambient factors and the seed's morphological, genetic, and physiological properties. Critical phases for crop establishment in dry and semi-arid areas are seed germination and seedling emergence. Indeed, crop stand density and ultimate production are established during these crucial phases (Hadas, 1976).Water stress has been shown to either halt germination or significantly slow it down (Turk et al., 2004).

Another significant barrier to seed germination is salinity. Due to either osmotic stress or an ion-toxic effect, the soil's salinity impacts both sides of germination. The primary adverse consequence of salt concentration in soils is the creation of osmotic potentials, hindering the absorption of a sufficient amount of water to sprout, given that the germination process depends on water. Furthermore, significant Na^+ and Cl^- ions quantities are absorbed by seeds and seedlings in saline environments, which harm plant cells (Khajeh-hosseini et al., 2003; Zhou et al., 2022).

The rapeseed *Brassica napus* L. belongs to the Brassicaceae family and is one of the world's primary sources of protein-rich foods and vegetable oils. Protein is abundant in its by-product, and its oil is a nutritious source of polyunsaturated fatty acids. (Onacik-Gür and Bikowska, 2020). The livestock feed industry uses rapeseed meal (remains after oil extraction). It contains a high fiber content, a perfect amount of amino acids, and several minerals and vitamins. **(**Onacik-Gür and bikowska, 2020; Hamzei and Soltani, 2012**)**. Even though rapeseed cultivation is not well-known in Algeria, it should be noted that the Algerian consumer consumes rapeseed oil daily on their plate (Belaid, 2014).

Rapeseed can be utilized as an alternative break crop in semi-arid areas where cereal-based crops predominate. (Hamzei and Soltani, 2012). Its excellent water-use efficiency and relative resistance to drought stress are the causes of this (Wu et al., 2018).

In the Mediterranean, Algeria included, rapeseed exhibits good adaptability as a promising oilseed crop. It has tremendous potential to improve vegetable oil production in these nations. In this country's dry and semi-arid regions, rapeseed, planted in late fall and harvested in early summer, is frequently subjected to various drought stress phases, ranging from germination to seed filling, which impacts plant growth and development.

Salinity significantly affects canola seed germination and plant growth. Salinity inhibits root growth, germination, and imbibition. Nevertheless, NaCl still affects these vital activities similarly, whether through an osmotic action or specific ion toxicity (Katembe et al., 1998). According to earlier research, the sensitivity of Brassica species to water stress during germination and the early phases of seedling development are genetically variable. (Mohammadi and Amiri, 2010; Toosi et al., 2014; Channaoui et al., 2017).

Therefore, it is worth instigating how seeds grow in salty and dry conditions to understand plant resistance and distribution in arid and semi-arid ecosystems.

This paper presents two experiments on the germination of *Brassica napus* L. seeds in Petri dishes. The experiments aim to investigate the effect of water deficit and salinity on seedling growth. The aim is to identify (salt toxicity or PEG-induced water stress) variables that limit seed germination and confirm how these abiotic stressors may restrict crop growth in the early phases of the growing season.

2. Materials and methods

The experiment was conducted in the Plant Biology laboratory of the Department of Agronomy at Mohamed El Bachir El Ibrahimi University in Bordj Bou Arréridj, Algeria, in 2022.

2.1. Plant materials and growing conditions

The study utilized two *Brassica napus* L. varieties, namely, "InVigor" and "ES Hydromel."

To test the ability of Rapeseed (*Brassica napus* L.) seeds to withstand salt or water stress during germination, experiments were conducted using distilled water, Polyethylene Glycol solutions (PEG-6000), and NaCl solutions. Each experiment involved soaking 20 seeds in NaCl solutions of varying concentrations (50, 100, 150, 200, and 250 mM NaCl) or in solutions of different water potentials (PEG 6000) ranging from 5% to 20% (w/v).

After disinfecting the seeds of *Brassica napus* L. (rapeseed) for ten minutes with 2% sodium hypochlorite, the seeds were adequately washed and soaked in distilled water. Using two sheets of filter paper wet with either 10 ml distilled water, an aqueous chloride salt, or a PEG solution, seeds germinated at 25 °C in the dark for eight days. Rehman et *al.* (1996) said the papers were swapped every two days to avoid salt or PEG buildup. A seed was deemed to have germinated at one millimeter of radicle emerging from the seed. The control was distilled water (0 MPa).

2.2. Measurement of germination parameters

The following formula is used to estimate the Germination percentage;

Germination % age (GP) = (Total seeds germinated /Total seeds planted) x 100.

We calculated the Mean Daily Germination (MDG) based on (Rubio-Casal et al., 2003): MDG = Final germination percentage/number of days to final germination.

Seedling Vigor Index (SVI) was calculated using this equation (Dezfuli et al., 2008; Memon et al., 2013): S V I = Seedling length (cm) x germination % age.

At the end of the experiment, the length of the roots and shoots was measured on the eighth day following germination. Root length was measured from the base (collar) to the tip of the root, while shoot length was measured from the base to the cotyledons.

2.3. Statistical analysis

Three factorial components were set in a randomized design for the experiment, with three replications and twenty seeds for each replicate. Variety was the first factor, with two levels ("InVigor" and "ES Hydromel"). NaCl or PEG was the second factor, with two levels. The third factor was the osmotic potential levels (5%, 10%, 15%, and 20% of PEG) and salinity solutions (50, 100, 150, 200, and 250 mM NaCl). An analysis of variance (ANOVA) was conducted on the germination data. Moreover, LSD test ($P < 0.05$) were used to compare mean differences.

3. Results

Both genetic and environmental variables influence seed germination and early seedling growth, and various species have developed distinct defense mechanisms against unfavorable environments. As a result, even in comparable environments, the seeds of multiple species may germinate differently.

3.1. Drought and salt stress effects on germination

The study investigated the effects of reduced water potential in PEG or NaCl solution on the germination characteristics and early embryo growth of Rapeseed (*Brassica napus* L.) under laboratory conditions. The ANOVA results indicated that drought and salt stress significantly affected rapeseed seed Germination percentage (GP) and Mean Daily Germination (MDG). There was also a significant effect of variety and its interaction with drought and salt stress on GP (Table 1, 2). However, there was no significant effect of varieties on the MDG.

Table 1. Analysis of variance (mean squares) for seed germination and seedling-related traits of two rapeseed genotypes evaluated under salt stress conditions.

Source of variation	**DDL**	**GP**	**MDG**	**SVI**	**SL**	**RL**
Variety(V)	1	44,44**	16,78^{ns}	1640.2 ^{ns}	0,49**	1,69*
Salt stress (S)	5	2755,55***	929,95***	9224218.8***	108,72***	382,67***
Vx S	5	113,88**	54,51^{ns}	18887.3 ^{ns}	0,83667*	0,43^{ns}

DDL: Degree of freedom, GP: germination percentage, MDG: Mean Daily Germination, SVI: Seedling vigor index, SL: Shoot length, RL: Root length. *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. Analysis of variance (mean squares) for seed germination and seedling-related traits of two rapeseed genotypes evaluated under drought stress conditions.

Source of variation	**DDL**	**GP**	**MDG**	**SVI**	**SL**	**RL**
Variety(V)	1	13,33^{ns}	0,468^{ns}	4060.0 ^{ns}	0,048^{ns}	0,225^{ns}
Drought (D)	4	1378,33***	324,20***	7829726.0***	120,24***	296,243***
VxD	4	145,00*	7,083**	16729.8 ^{ns}	0,382^{ns}	2,404*

DDL: Degree of freedom, GP: germination percentage, MDG: Mean Daily Germination, SVI: Seedling vigor index, SL: Shoot length, RL: Root length. *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively.

The final GP of the control (absence of stress) reached about 100% for two genotypes. Conversely, the GP decreased under stress conditions with increasing salt or PEG in solution (Figure1).Under saline conditions, for the concentrations used (50mM; 100mM), the GP remains relatively high with values around (95%, 96.6%) and (98.3%, 93.3%) for the InVigor and ES Hydromel varieties, with minimum values of (76.6%, 68.3%) for a stress of (250mM) respectively. The varieties exhibit sensitivity to salt.

In the PEG solution, the PG at 5% and 10% PEG solution was (100%, 95%) and (95%, 96.67%) for the InVigor and ES Hydromel varieties, respectively. The most significant decline was recorded at 15% and 20% (73.33% and 70%) for the InVigor and Hydromel varieties. In NaCl and PEG solutions, In Vigor had higher GP than ES Hydromel at most osmotic potentials.

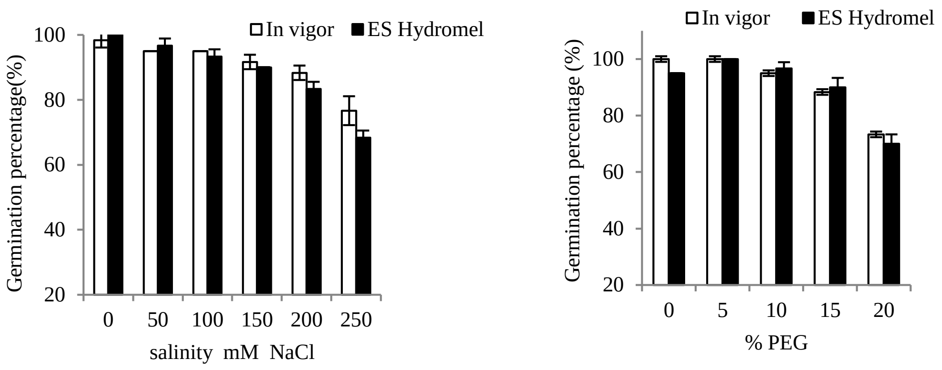

Figure1. Germination percentages of two varieties of rapeseed (*Brassica napus* L.) at different concentrations of NaCl and different osmotic potentials of PEG. Values are mean ± SE.

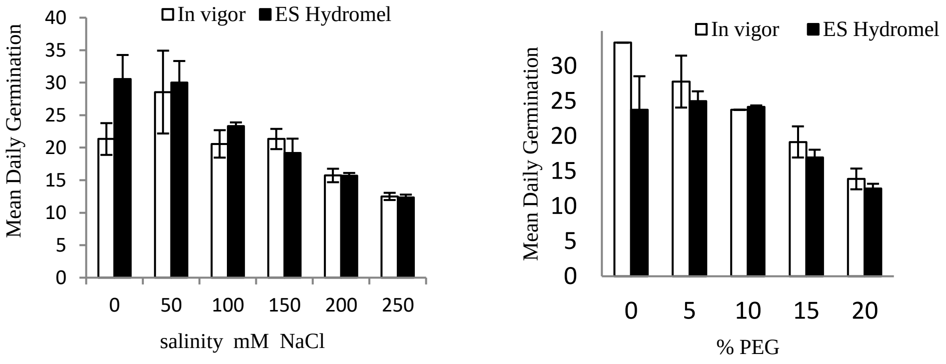

Figure2.Germination percentages of two varieties of rapeseed (*Brassica napus* L.) at different concentrations of NaCl and different osmotic potentials of PEG. Values are mean ± SE.

The MDG of *Brassica napus* L. seedlings grown in different concentrations of NaCl and PEG are presented in Figure 2. The MDG of seedlings significantly decreased due to the increase in salinity and drought levels compared to the control group (Figure2).Under salt treatment, no significant difference was observed between 50, 100, or 150Mm NaCl and the control. The MDG was noticeably decreased by 15%

and 20% PEG but not by 5% and 10% PEG. A higher concentration of NaCl (250mM) and PEG (20%) caused a significant reduction in the MDG of rapeseed. Moreover, the ES Hydromel varieties were the most sensitive to salt stress, and the InVigor varieties were the most sensitive to drought stress.

3.2. Effect of PEG and NaCl in vigor index (SVI) and seedling growth (root and shoot length)

The analysis of variance revealed a significant influence of drought and salt stress on the Vigor Index, shoot length, and root length (Tables 1 and 2). Additionally, varieties under salt stress significantly affected shoot length and root length (Tables 1 and 2). Although the genotype and stress were analyzed together, the results showed no significant impact on the studied parameters.

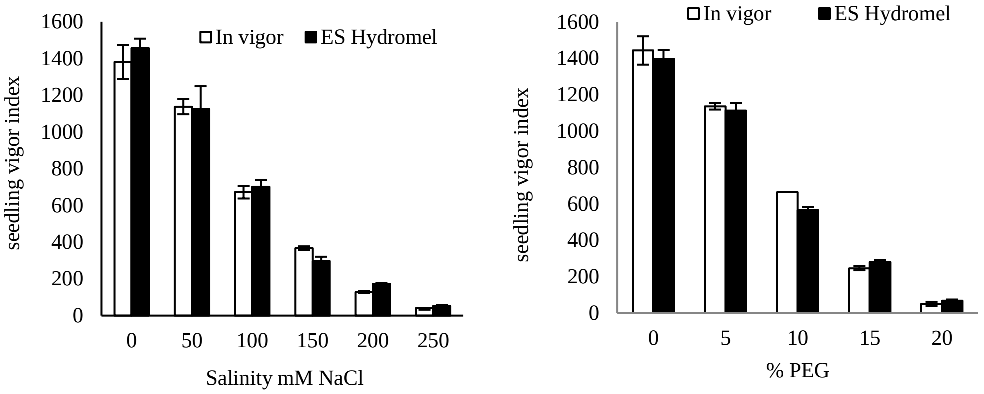

Figure 3. Seedling Vigor index of two varieties of rapeseed (*Brassica napus* L.) at different concentrations of NaCl and different osmotic potentials of PEG. Values are mean ± SE.

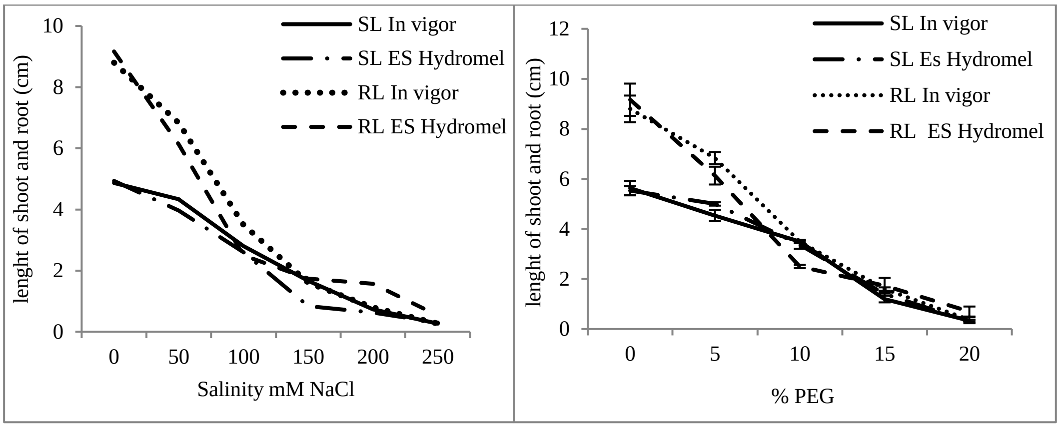

Figure 4. Shoot length (SL) and Root length (RL) of two varieties of rapeseed (*Brassica napus* L.) at different concentrations of NaCl and different osmotic potentials of PEG.Values are mean ± SE.

The data presented in Figure 3 revealed that the average seedling vigor index of different PEG concentrations ranged between 1443,33 to 51,76, and the index of different concentrations of NaCl varied between 1456,67 to 40,33. The same figure depicts the differences between NaCl and PEG treatments for the vigor index of Rapeseed (Figure 3). SVI is considerably decreased with increasing NaCl and PEG concentrations. Based on the results, the control is associated with the highest Vigor Index, compared to low values noted at 250 mM NaCl and 20% PEG (Figure 3).

Tables 1 and 2 show notable variations in shoot length (SL) and root length (RL) among different varieties and between different drought and salt stress levels. Figure 4 depicts the longest radicles in seedlings grown under non-stress conditions. In contrast, the shortest radicles were observed in those exposed to the highest PEG or NaCl. Increasing the PEG and NaCl concentrations resulted in a decrease in the shoot and root length. The root length of ES Hydromel varieties was lower than that of InVigor varieties when the seeds were treated with 5% PEG + 50 mM NaCl or 10% PEG + 100 mM NaCl. Conversely, no difference was observed in the shoot length of early seedlings for both species.

There was an inverse relationship between the levels of PEG and NaCl and germination characteristics. Germination was influenced by increased NaCl levels, especially under salinity of 150, 200, and 250mM NaCl, and 20% PEG. All determined germination and seedling parameters decreased in response to water and salt stress.

4. Discussion

The germination process is crucial for the survival of many species, including crops like rapeseed. Nevertheless, drought and salinity can negatively affect these species' germination, seedling growth, and yield. Salinity and drought are the most critical soil abiotic limiting factors for germination and seedling growth (Atak et al., 2006). The research presented here indicated differences in the two species studied's GP, MDG, SVI, and root and shoot lengths. Water deficit and salt stress cause harmful biochemical and physiological changes in germinating seeds.

In this study, drought and salt stress affected all the measured seed germination parameters. Increased stress levels mainly decreased GP, MDG, SVI, and root and shoot lengths. This agrees with previous studies findings in *Brassica napus* (Channaoui et al., 2017, 2019) and *Brassica juncea* (Toosi et al., 2014). The same result was found in safflower (Kaya et al., 2003) and in pea (*Pisum sativum* L.) (Sai Kachout et al., 2021)**.** Based on the measured seed germination parameters, it was found that InVigor varieties were less affected by stress compared to ES Hydromel varieties. This suggests that InVigor varieties are more drought and salinity-stress tolerant than ES Hydromel varieties. Drought and salinity stress can have a severe impact on seed germination by hindering water absorption and causing toxicity due to sodium and chloride ions. These factors may delay or completely stop germination and seedling growth (Ashraf and Foolad 2005). In our experiment studies analyzing the germination of specific Rapeseed species, it was reported that germination in *Brassica napus* was decreased by PEG and NaCl. The decrease in osmotic potential led to a significant reduction in GP and MDG of *Brassica napus* L. seedlings. Salt and drought stress have no noticeable effect on GP at low concentrations of NaCl and PEG. However, athigher stress levels, the germination percentagein the two rapeseed varieties significantly decreased compared to the control group. Our results aligned with Bialecka and Keoczynski (2010) and Mohammadizad et al., 2013.

The seed germination delay and the decrease in the daily average germination across all varieties associated with the increase in saline and drought levels are translated by the time needed for the seed to establish mechanisms to adjust its internal osmotic pressure to the environment (Bliss et al., 1986). Salinity and drought can decrease crop growth, development, and yield by delaying germination and reducing its velocity, enhancing germination events and dispersal (Heshmat et al., 2011).

Previous research has indicated that various seed priming treatments can mitigate the negative effects of salinity and water stress on germination (Janmohammadi et al., 2008). Macar et al. (2009) discovered that PEG did not have a toxic effect on sunflower seeds, as all the seeds germinated after removing polyethylene glycol. The researchers concluded that polyethylene glycol had an impact on germination and seedling growth by hindering water entry into plant organs. Additionally, compared to PEG, Na+ and Cl- could pass through plant cells and accumulate in the vacuole or cytoplasm. Moreover, according to Òeèajeva and Ievinsh (2007), the reduction in the germination percentage of Chenopodium glaucum seeds is due to decreased seed water absorption during the stages of imbibitions and turgescence. It seems that at 100mmole NaCl and 10% PEG concentration or higher, the water potential inside the germinating seed became higher than that outside, i.e., in the NaCl substrate.

As a result of extreme salinity levels, the seed’s coat cannot absorb water, causing germination failure. It is also assumed this salinity rate can also be toxic to the seed embryo, significantly delaying the germination. This observation align with Bahrami et al. (2012) finding, as they reported for some sesame cultivars, a sharp decline in germination, from 50-56% to 0-5%, when the salinity level went from 14.65 to 18.45 dS m-1. A drastic reduction was also observed in rapeseed germination, from 80% at -0.9 MPa to 0% at -1.2 MPa (Pace and Benincasa, 2010).

The study revealed that both salinity and drought had adverse effects on the germination percentage, root length, seedling length, and seedling vigor index. Furthermore, the various canola varieties that were exposed to increasing doses of NaCl and PEG also showed different response. These results agree with other researchers (Channaoui et al., 2017, 2019; Osman and İlbaş, 2023; Kayaçetin, 2021, 2022). According to various studies, including the present work, the decrease in root and shoot lengths can be considered as an important indicator of salt stress. Jamil et al. (2005) also suggested that the length of roots and shoots can provide crucial information about a plant's response to salt stress. Furthermore, Kumar *et* al. (2011), reported that drought stress reduced the seedling vigor index in pigeon peas.

Our experiment showed that osmotic stress led to a reduction in seedling vigor index. While both PEG and salinity decreased seedling length and germination percentage values, osmotic stress was observed to affect seedling elongation more than germination percentage. This could be an adaptive response to osmotic stress. Similar to physiological mechanistic responses during plant growth, drought and salinity stress are able to delay or inhibit the seed germination and seedling establishment (Farooq et al., 2009; Sai Kachout et al., 2021; Osman and İlbaş, 2023).

CONCLUSION

It is essential to learn about genetic variation in plant tolerance to salinity and drought to ensure efficient use of agricultural soils facing these issues from environmental and economic perspectives. The study's findings reveal significant differences among the tested cultivars in their response to drought tolerance. Gradually increasing the NaCl and PEG treatments resulted in a reduction of all the analyzed parameters in the chosen cultivars. The most significant decrease was observed at higher salt and PEG treatments (15% and 20% PEG + 150, 200, and 250 mM NaCl), demonstrating that the tested varieties are sensitive to stressful conditions. Studying the response of various cultivars in limiting environments such as salinity and drought can help enhance plant tolerance to these conditions. This research is of great importance for improving breeding and selection efficiency. Additionally, future studies should focus on the physiological, molecular, and metabolic changes induced by priming agents under salt and water stress in rapeseed plants.

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