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Effects of Priming Treatments on Germination Indices of Soybean Cultivars Under Osmotic Stress

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Abstract

This study aimed to investigate the effects of priming treatments on germination indices of soybean cultivars under osmotic stress in a completely randomized design with a factorial arrangement of treatments in a botany laboratory located in Kordestan Province, Sanandaj, Iran. Experimental treatments included three treatments of priming (control and gibberellin at a concentration of 0.04%), cultivar (Clark and Hobbit), and osmotic stress (control and -1.5MPa) in three replications. The standard germination test was performed according to the guidelines presented by the International Seed Testing Association (ISTA) and some germination indices, including germination percentage, germination rate, germination uniformity, and mean germination time up to 10% germination (D10), were calculated at the end of the experiment using German program. In addition, some growth indices and enzymatic activities were also evaluated in this study. Results of the analysis of variance indicated that the triple interaction of A×B×C had significant effects on all germination indices and the lowest coefficient of variation (equal to 7.55) was obtained for germination rate while its highest value (equal to 17.22) was calculated for germination uniformity index. Concerning the effects of priming treatment, it was observed that the highest values for indices of germination percentage and germination rate (with averages of 48% and 0.12, respectively) were obtained under the application of gibberellin. In other words, gibberellin treatment led to increases equal to 15.58 and 90.44% for germination percentage and germination rate indices compared to the control treatment, respectively. In addition, increasing osmotic stress levels had a significant inverse relationship with germination indices so that the application of -1.5 MPa osmotic stress significantly Decreased the germination percentage and germination rate of both Clark and Hobbit cultivars. In general, it was concluded that the application of gibberellin mitigated adverse effects of osmotic stress of -1.5 MPa and resulted in an increasing percentage and rate of germination under stressful conditions.

Keywords: Germination; Gibberellin; Osmotic stress; Soybean.

1. Introduction

Soybean (*Glycine max* L.), a self-pollinated diploid legume, is one of the most important oilseeds that makes up nearly 60% of the oilseed production in the world and its grains contain 40% protein, 20% oil, and high doses of B vitamins. The effects of climatic, environmental, and managerial factors on soybean, including moisture, soil temperature, air temperature, day length, light intensity, soil pores, and soil conditions (soil fertility), are manifested through growth and developmental changes [1, 2]. In general, soybeans are short-day (SD) and thermophilic plants that germinate at 8 to 10 ° C; however, the optimum temperature for its germination is around 30 ° C. In addition, it was reported that this plant is compatible with arid and semi-arid climate conditions, and its seeds mostly grow at about 300 m elevation and daylength of 12 to 16 hours [3-6]. Since soybean cultivars have relatively sensitive to day length, a 15-minute difference during the day may prevent flower growth and development in a unique cultivar [7]. In addition to the above features, as a principle, early maturity groups of soybean cultivars need longer days for flowering, plant growth, and further development compared to late maturity groups [8].

Seed germination and emergence in seeds of many crops are the most sensitive stages in plants. In this regard, seed priming or priming technology, especially on soybean seeds, is one of the new methods of regulating seed germination in modern agriculture, in which seeds are first soaked in a solution for a definite time and dried before emerging from the radicle. Then, seed re-germination begins in the field at an appropriate time (as soon as conditions allow) using irrigation. Subsequently, the metabolic and physiological functions of the seeds continue, and plants begin to grow faster than expected conditions [9].

On the other hand, since Iran is located in arid and semi-arid regions, water scarcity is one of the most important agricultural problems, and drought stress is a serious threat to global food security and plant production. According to the above definition of seed priming, the primary purpose of priming is to facilitate germination and promote the physiological and metabolic functions of seeds under particular environmental conditions, which leads to improved germination percentage and germination rate indices under challenging environments [10]. Also, priming minimizes the induced dormancy periods for various seeds by temperature and increases the temperature range required for seed germination. Overall, the term priming refers to the soaking of seeds in osmotic solutions with more negative water potential and ventilated, in which environmental conditions (e.g., temperature, light, and others) have significant effects on oxygen availability, the passage of time, seed drying, and control the seed-borne microbial contaminations during the dehydration process [11-13]. In the meantime, gibberellic acid is a plant hormone and stimulant affecting the seed germination and dormancy in various plant species, which is employed as a priming solution for seeds. For more than two decades, seed priming has been applied as a standard method in many plants to alleviate the depressive effects of drought. However, studies on the optimal reactions of primed and non-primed seeds are still under investigation, and seed priming continues until its final evolution as a helpful technology [14]. Accordingly, additional and further information becomes gradually available about primed seeds

2. Materials and Methods

This study aimed to investigate the effects of priming treatment on germination indices of soybean cultivars under osmotic stress. Therefore, an experiment was performed as a three-way factorial arrangement based on the completely randomized design in the fall season, 2020, in a botanical laboratory. Experimental treatments included priming as factor A (a1: control and a2: gibberellin at a concentration of 0.04%), osmotic stress as factor B (b1: control and b2: -1.5MPa), and cultivar as factor C (c1: Clark and c2: Hobbit) in three replications. The standard germination tests were performed according to the ISTA Rules with slight modifications. Seed priming experiments (e.g., maximum germination, germination rate, and germination uniformity) were conducted as Petri experiments [15]. Accordingly, three replicates of 25 seeds (5 series of 25 healthy seeds) were first counted to determine rate, percentage, and uniformity of germination. Next, seeds were disinfected with the carboxin-thiram fungicide to maintain and improve seed quality before planting in the culture medium. The seeds were then placed in Petri dishes (between two Whatman filters) and transferred to a growth chamber at 20±1 ° C. The number of germinated seeds scored three times per day for the time necessary to achieve the final number of germinated seeds. Hence, seeds were considered as germinated when their radicle length was more than or equal to 2 mm, and counting operations continued until the number of germinated seeds for each sample remained constant for three consecutive days. It should be noted that the Germin program, which calculates the indices of D10 (time up to 10% of maximum germination), D50 (time up to 50% of maximum germination), and D90 (time up to 90% of maximum germination), was employed for calculating traits of final germination percentage and germination rate . This program calculates the above parameters for each replicate/seed treatment by interpolation from the progress of germination (%) versus the time curve. The germination rate, also called R50, was calculated in hours by the following equation [16].

Equation (1) $$R50 = \frac{1}{D50}$$

Germination uniformity (GU) refers to the time required for 90% maximum germination subtracted by the time required for 10% maximum germination and is calculated by the following equation (Equation 2). In general, a lower value of this number indicates more uniform germination (simultaneous germination) of seeds.

Equation (2) $$GU = D90 - D10$$

3. Statistical Analysis

The obtained data were first normalized for different studied traits and then analyzed using SPSS 24 and MSTAT-C 21 statistical software. Also, the LSD test was used to compare means at $p<0.05$, and charts were drawn using Excel software.

4. Results and Discussion

Drought stress is one of the most important factors limiting growth, development, and crop production in soybean and is considered a major challenging factor in many countries facing water scarcity [17, 18]. Depending on the type of the plant and stress intensity, different methods have been used by numerous researchers and farmers to overcome water stress [19, 20]. Results of analysis of variance (Table 1) showed that factor A (priming), factor B (osmotic stress), and interactions of A × B, A × C, and A × B × C had significant effects on all germination indices. In addition, factor C (cultivar) and the interaction of B × C also caused significant differences in the obtained values of germination percentage, germination rate, and time up to 10% of maximum germination; however, both factor C and the interaction of B × C had no significant effects on germination uniformity. According to the data, the lowest coefficient of variation was (equal to 7.55) obtained for germination rate, while the highest value (with an average of 17.22) was observed for germination uniformity. In general, high values of coefficient of variation for GU and D10 indicate high effects of the environment on the above traits.

Table-1. Analysis of variance (mean squares) of the studied treatments on seed germination indices

Sources of variations	df	Germination percentage (G_{max})	Germination rate (R50)	Germination uniformity (GU)	time up to 10% of maximum germination (D10)
Rep	2	50.5 *	0.0000004 ns	111.2 ns	34.14 ns
Priming (Factor A)	1	82.08 **	0.00003 **	805.52 **	420.22 **
Osmotic stress (Factor B)	1	3842.44 **	0.0032 **	1132.8 **	1128.4 **
A×B interaction	1	1546.01 **	0.00005 **	1952.22 **	381.3 **
Cultivar (Factor C)	1	1208 **	0.0001 **	8.85 ns	2018.5 **
A×C interaction	1	752.7 **	0.00003 **	988.5 **	382.3 **
B×C interaction	1	985.2 **	0.00003 **	138.3 ns	2152.1 **
A× B×C interaction	1	914.4 **	0.000011 **	1528.22 **	346.54 **
E (Error)	51	19.521	0.0000003	68.32	25.22
Coefficient of variation (%)		8.88	7.55	17.22	16.34

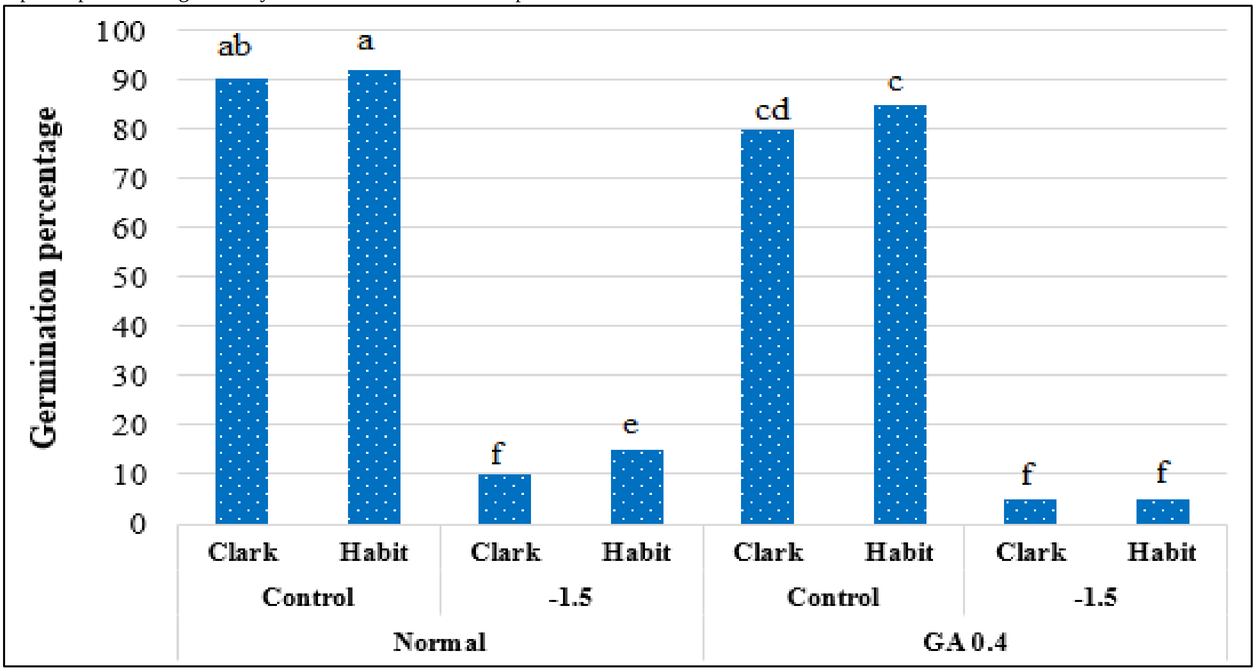
Ns: non-significant; * and **: significant at p<0.05 and p<0.01, respectively

In confirmation of the adverse effects of stressful conditions on germination indices, Yagmur and Kaydan [21] revealed that osmotic stress reduced germination traits of triticale seeds. The negative impacts of osmotic stress on the initial growth of seeds of different plants were also investigated and confirmed by other researchers [22-24]. Xu, *et al.* [24] reported that drought stress and gibberellin priming significantly affected germination percentage, seedling length, dry weight of the treated seedlings, and seedling length and weight indices at p <0.01. In addition, these traits were significantly affected by the interaction of gibberellin × drought stress (p <0.01). Results presented by Mir-Mahmoodi, *et al.* [25] also confirmed the significant effects of priming treatments on all germination indices of soybean seeds. In another study, Mohammadi and Fataei [26] also reported that GP, R50, D10, D50, and D90 were significantly affected by temperature, water potential, and their interaction at p <0.01.

5. Germination Percentage

Seed germination depends on numerous environmental factors. In the meantime, water (and its availability) is one of the most influential factors in initiating seed germination and the physical and physiological processes of seed germination. Findings of some studies also indicated an increase in growth inhibitors, such as abscisic acid, and a decrease in growth-promoting hormones, e.g., auxins, gibberellins, and cytokinins, in plants under water scarcity conditions [27, 28]. In the present study, the highest germination percentage (with an average of 90%) was recorded for the absence of gibberellin × absence of osmotic stress × Hobbit cultivar interaction, which placed in class A along with the absence of gibberellin × absence of osmotic stress × Clark cultivar interaction. In contrast, the germination percentage of seeds under treatments containing osmotic stress of -1.5 MPa was less than 10%. Results also showed that the germination percentage of seeds of Clark and Hobbit cultivars primed with gibberellin under -1.5 MPa conditions significantly increased compared to normal conditions. In other words, applying gibberellin reduced the adverse effects of osmotic stress (-1.5 MPa) and improved germination percentage (Fig. 1).

Fig-1. Effects of tripartite interaction of priming×osmotic stress×cultivar (A×B×C) on germination percentage Means sharing the same superscript are not significantly different from each other at p<0.05 of the LSD test

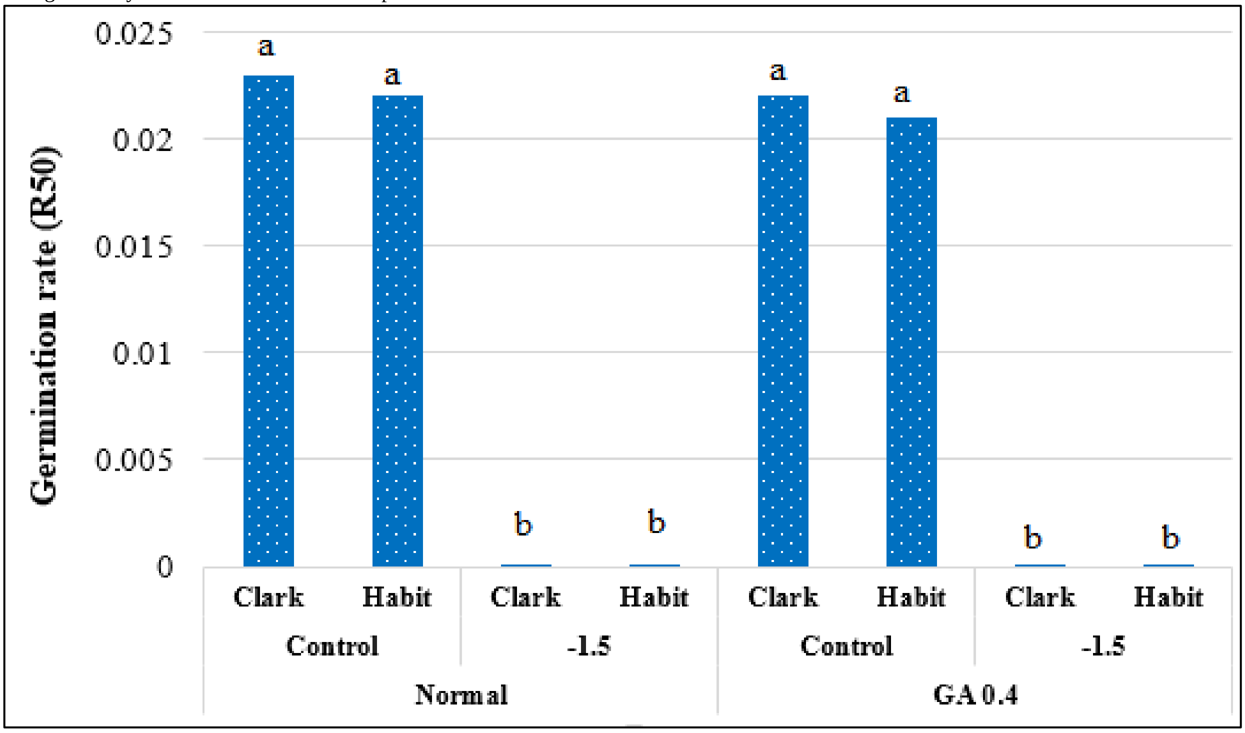


Ghassemi-Golezani, *et al.* [29], reported an increase in the germination percentage of lentil seeds under priming with distilled water. They also stated that different responses of various genotypes to drought stress might depend on several factors, e.g., seed size, seed cover, and other characteristics. Decreasing water entrance into the seed under increasing drought stress can reduce hydraulic conductivity and thus decline the rate of physiological and metabolic processes involved in seed germination [30]. Huang, *et al.* [31], reported that although increasing stress levels resulted in a significant reduction in germination percentage, gibberellin treatment significantly increased seed germination percentage.

6. Germination Rate

Regulation and completion of metabolic stages involved in seed germination are the main reasons for increasing the germination rate of plant seeds under different priming treatments. In general, since non-primed seeds spend time performing phases I and II (rapid water imbibition by seed and reactivation of metabolism, respectively) longer, primed seeds are one step ahead of non-primed seeds in terms of germination and emergence stages [32]. Therefore, the cultivation of primed seeds, especially in unfavorable environmental conditions, leads to increased germination rate and germination percentage [22]. Here, the highest germination rate was assigned to interactions of the absence of gibberellin×the absence of osmotic stress×Clark cultivar, the absence of gibberellin×the absence of osmotic stress×Hobbit cultivar, gibberellin treatment×the absence of osmotic stress×Clark cultivar, and gibberellin treatment×the absence of osmotic stress×Hobbit cultivar. Hence, all of the mentioned interactions were marked with the letter A and placed in the same group. According to the data, the application of gibberellin moderated adverse effects of drought stress-induced osmotic stress and led to increased germination rate in primed seeds of both Hobbit and Clark cultivars (Fig. 2). Decreased germination rate under stressful environmental conditions can also be directly correlated with reduced water absorption by seeds. As a whole, Hosseini and Rezvani [33] stated that seeds without priming solutions have low germination-related metabolic activities due to slow water uptake. Accordingly, the time required for radicle emergence and seed germination rate indices can be increased and decreased, respectively.

Fig-2. Effects of tripartite interaction of priming×osmotic stress×cultivar (A×B×C) on germination rate Means sharing the same superscript are not significantly different from each other at p<0.05 of the LSD test



7. Germination Uniformity and Time Up to 10% of Maximum Germination (D10)

Germination uniformity was calculated based on the time required for 90% germination subtracted by the time required for 10% germination, namely D90-D10, in which scores with a lower value indicate a shorter time interval between 10% and 90% of seed germination and more favorable germination uniformity [34]. Some researchers also stated that the lower absolute value for the obtained values indicated more uniformity for the germinated seeds [35-44]. Accordingly, since these seeds absorb water more rapidly under the same conditions, their germination rate and D10 occur higher and lower values, respectively. Based on the evidence, other researchers [45-50] stated that higher osmotic potential (less negative $\psi\pi$) and less water absorption under stressful environmental conditions could lead to reducing germination rate, decreasing germination uniformity, and increasing time up to 10% of maximum germination; accordingly, seeds reached to final germination percentage in the longer growing periods. In this regard, our findings demonstrated that the lowest GU value (namely the highest germination uniformity) was attributed to the interaction (combined treatment) of gibberellin × osmotic stress × Clark cultivar (Fig. 3).

Fig-3. Effects of tripartite interaction of priming×osmotic stress×cultivar (A×B×C) on germination uniformity Means sharing the same uperscript are not significantly different from each other at p<0.05 of the LSD test

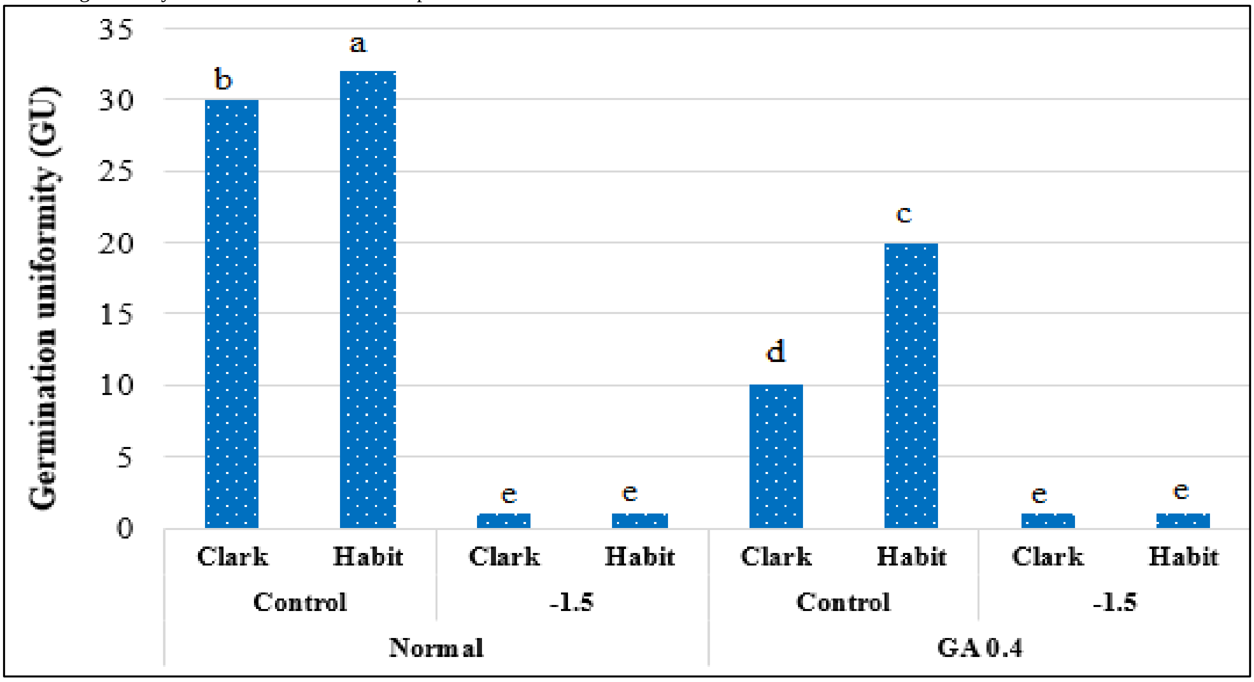
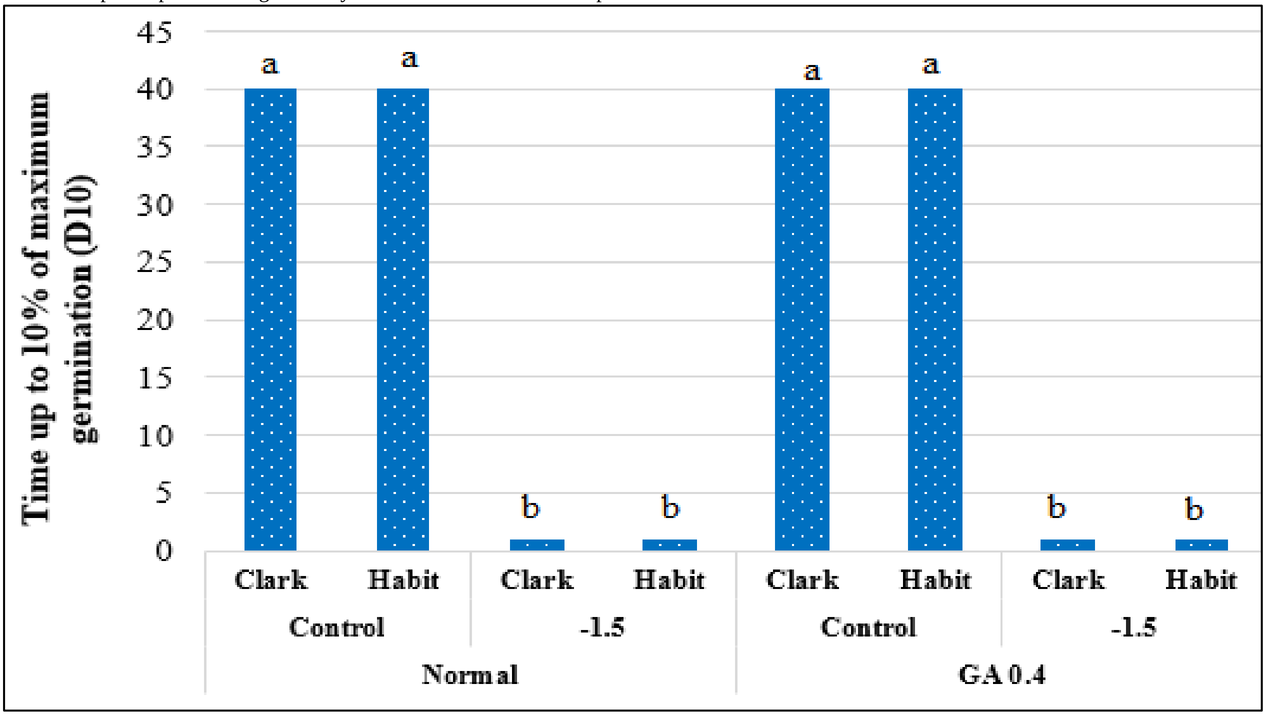


Fig-4. Effects of tripartite interaction of priming×osmotic stress×cultivar (A×B×C) on time up to 10% of maximum germination Means sharing the same superscript are not significantly different from each other at p<0.05 of the LSD test



8. Conclusion

In general, our findings showed that the highest germination percentage and germination rate (with average values of 48% and 0.12, respectively) were obtained for seeds primed with gibberellin and had increases of 18.58 and 90.44% for the above traits compared to the control treatment. On the other hand, osmotic stress had a significant inverse relationship with the percentage and rate of germination indices in both studied cultivars. In other words, the application of -1.5 MPa osmotic stress caused notable reductions in germination percentage and germination rate indices compared to the normal conditions (absence of osmotic stress).

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