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The effect of plant hormone gibberellic acid on germination indices *Secale montanum in vitro* and pot experiments under drought conditions

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ABSTRACT

One of the effects of drought is the reduction of germination, seedling establishment and seedling growth. Pretreatment improves some aspects of seed germination and seedling establishment. To investigation the effect of pretreatment with Gibberellic acid on the seed germination of Secale Montanum in stages of growth in vitro and pot under drought stress conditions experiment was conducted in laboratory and greenhouse Tehran University. Experiments design in factorial randomized complete block with three replications. Treatments examination included two levels of priming at 10 and 15 °C (first factor), Gibberellic acid treatments on three levels: 50, 100 and 150 ppm for 12 h (second factor) and osmotic potential (PEG 6000) in three 10 -, 12 - and 14 - times (factor III), respectively. The results showed that pretreatment with Gibberellic acid of seed in drought stress conditions in vitro increased the germination percentage, germination rate, root length and shoot length and also in medium pot, seedling establishment percentage, seedling establishment rate, root length and shoot length as influenced by different hormonal treatments, with Gibberellic acid 50 ppm at 10 °C and Gibberellic acid 100 ppm at 10 °C and - 10 times surface in the experimental vitro and pot have had the most positive effect on germination and growth factors, respectively. The seed priming treatment at 10 °C compared with 15 °C displayed a better outcome.

Key words: *secale montanum*, Gibberellic acid, water stress, seed germination and seedling establishment

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INTRODUCTION

Cerael is the most important source of food in human life that it is including 65-70% carbohydrate, proteins and too much B vitamins and trace mineral. most of Iranian food are supplied by grain and dairy products. Mountain rye (*secale montanum*) is the plant of family of grasses (poaceace). It is the most stable to cold and has long clusters and flat leaves. It grows in calearous dry land and seeds of this plant are used for making flour and bread. Seed germination is a complex physiological process that it is response to environmental signals such as water potential, light, nitrate and other factor .Unfavorable environmental conditions for seed generation and seedling emergence in dry and semi dry land is main causes of emergence and seed poor establishment. One of the limiting factors is drought condition that causes stress at the plant. Drought stress induced by high evaporation, high osmotic potential in saline soils and soil freezing. Resulting is broken thermodynamic equilibrium condition and reduce available of water. Seed germination and seedling establishment phase primarily was very sensitive to drought stress that is reduced the germination and establishment of seedling grown [1, 2, 3]. Increase performance of seed thought the used of different method promoting can achieve the final goal the same increasing seed and forage proceeds is very effective .Among the most common and efficient methods in this regard is seed treatment before planting which is called priming [4]. Studies have shown that soaking seeds with appropriate concentrations of growth hormone, has a positive effect on germination, growth and yield of different plant species and stress conditions are normal [5,6]. Gibberellic acid is a plant growth hormone that the most important role in the control of seed germination is facilitated. In previous study investigated of the effect of gibberellic acid levels (ppm 50, 100, 200, 400) on the germination of 11 species of aromatic plants and the result showed that there were significant differences between species and gibberellic acid, ppm 400 greatest effect on seeds will germinate [7]. Also investigated of the effects of hormonal priming on germination and seedling growth of lettuce, and then reported that the concentration of GA3 a statistically significant difference [8]. Mountain rye is the nutrients grasses that use for human nutrition and forage production gastronome and normally, growth in the early stages of germination and seedling stresses such as drought and low temperatures encountered Thus, we pre-treated with the hormone gibberellic acid seed germination seed crop in drought conditions improve is possible to increase the strength of the initial seed finally created the increasing in the percentage and rate of seed germination and seedling establishment. The aim of this study evaluation the gibberellic acid treatment in improving germination under drought stress in vitro and pot culture.

MATERIALS AND METHODS

1 – The Mountain rye plant seeds purchased from Isfahan Pakan Seed Company in crop year at 1391 and the experiment design to factorial in a randomized block with three replications in greenhouse Tehran University was conducted.

2- Laboratory studies:
First experimental materials (petri dishes, filter paper, forceps, etc.) sterilized for 3 hours in an autoclave and also plant seeds placed sterilized in 3% sodium hypochlorite solution for one minute for sterilization and then washed in sterilized distilled water and then the seeds treated hormonal with gibberellic acid levels at 3, 50, 100 and 150 ppm (parts per million) at 10 and 15 ° C for 12 hours. Then plant seeds were washed with distilled water and 50 seeds in each petri dish cultures were grown on two layers of filter paper and then planted seeds in three replicates at 20 °C (optimal temperature). Finally, apply a solution of poly ethylene glycol stress concentration in three 10 -, 12 - and 14 - were used in each petri dish, as this solution was added 5 ml and 7 days at 20 °C for evaluated.

3- Pot evaluation
In this step, the seeds of the plant crop, such as laboratory tests, prepadre and placed of culture petri dishes in pots (diameter 10 cm) containing sand was used for the stress of polyethylene glycol technology was used at the beginning of culture, 25 ml of solution was added to each pot and finally, after 7 days of germination parameters were measured.

4 - Parameters measured included: germination percentage, germination rate, root length and shoot length (medium petri dish), the percentage rate of seedling establishment and seedling establishment (in medium pot).

The during germination and the proper temperature germination was based on the static information. A mountain rye seed at 20 °C for 7 days in germinator was in darkness. Germination was assessed on a daily basis and are the seeds with radical length of 2 mm are considered as seed germination (AOSA, 1990), and finally the aforementioned characteristics were evaluated.

5- Data analysis:
The seed germination percentage was calculated using equation (1) [9]:

100 × (number of seeds / number of seeds germinat until the last day) = germination percentage (Equation 1)

To calculate the germination rate germination (Equation 2) was used to Timson index [10]:

Germination Rate (GR) = Σ (G / t) **(Equation 2)**

In this equation, GR is germination rate and G is estimated number seed germination per each day and t is days counted. To calculate the rate of seedling establishment and seedling establishment were used of these relations (between 1 and 2), so the relationship, was used seedling established instead of number of seeds germinated.

Probity Analysis Statistical analyzes were performed with SAS software compare data using the least significant difference (LSD) was performed with MSTATC software and graphs were plotted by Excel software.

RESULTS AND DISCUSION

The results of the evaluation of germrmination of *S. Montanum* in drought condition in vitro showed that don't have significant levels the interaction of priming temperature * surface priming * surface drought. Also the interaction of surface priming * drought stress on germination percentage and germination rate was significant at 1% level and the interaction of priming temperature * surface stress on the rate and percentage of germination and the interaction of surface primin *temperatur showed temperatur effect on length root. The overall temperature of the priming, solution of gibberellic acid and different levels of drought stress on the index germination evaluated has significant effect on the at the 1% level (Table 1-1).

Table (1-1) analysis of variance to assess traits associated with plant shoots *S. Montanum* affected by gibberellic acid stress conditions in laboratory culture

		Mean squares (MS)			
Sources changes	degrees of freedom	Germination percentage	Germination rate	Root length	During the shoot
Block	2	0.01 ns	0.03 ns	1.04**	14.51
Prime temperature	1	13.07**	8.24**	4.64**	177.85
Prime surfaces	2	6.44**	7.34**	21.27**	1014.74**
Drought stress	2	42.65**	37.36**	20.63**	403.29**
temperature* Surface priming	2	0.56 ns	0.79*	0.28 ns	5.85 ns
Temperature *Drought stress	2	0.22 ns	0.08 ns	0.62**	22.74*
Surface priming *Drought stress	4	0.02 ns	0.11 ns	1.24**	31.40**
temperature *Surface priming* stress	4	0.08ns	0.112 ns	0.13 ns	4.74 ns
Error	34	0.25	0.15	0.106	4.63
Coefficient changes	-	12.24	10.17	5.68	5.09

*Ns, *, **: Non-significant, significant at the 5 and 1%, respectively*

Analysis based on the comparison of characteristics of the interaction of gibberellic acid on germination * drought stress levels were difference significantly so that with increasing levels of gibberellic acid and whit increasing osmotic potential decreased germination percentage significantly (Figure 1-1).

The results from the analysis of the average comparison of germination percentage of seeds can be expressed the interaction of temperature priming * drought stress levels on germination percentage have significant effects and with increasing temperature priming and increasing the osmotic potential of -10 to -14 times, decreased germination percentage significantly (Figure 1-2).

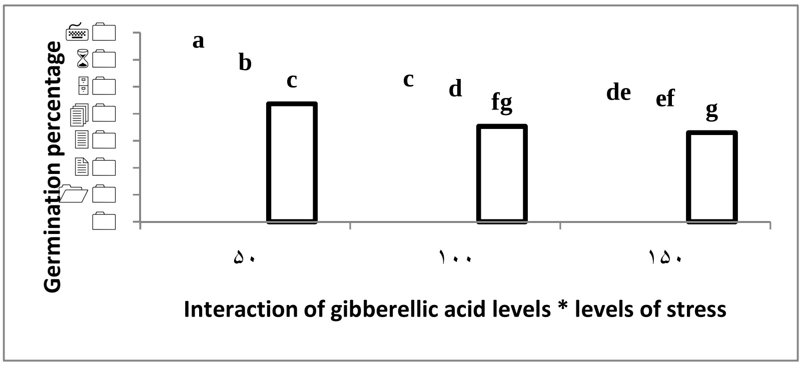


Figure (1-1): Average comparison, interactive effects of gibberellic acid * surface of drought stress on seed germination *S. Montanum* under drought stress in vitro

According to the results, the germination rate under drought stress in vitro is affected by interactions of temperature priming * drought stress levels, also the interaction of drought stress levels * gibberellic acid level. The effect of seed priming treatments on germination rate at 10 °C was than at 15 °C and also increasing levels of gibberellic ppm 50 to ppm 150 and also by increasing as well as the stress increases drought stress the germination rate diminished (Figure 1-3 and 1-4). Results based on comparison analysis revealed that the interaction of priming temperature and different levels gibberellic acid and concentrations of a different solution has a significant effect on root length and maximum length of root was in priming treatments 10 °C gibberellic acid ppm 50 with the potential and the osmotic concentration of -10 times (1-5 and 1-6). Based on the results, the priming effect of gibberellic acid on shoot length as well as the positive effect of this treatment on root length was similar to seed priming treatment at 10 °C with a solution of ppm 50 and osmotic concentration - 10 time had the greatest impact (Figure 1-7 and 1-8 and 1-9).

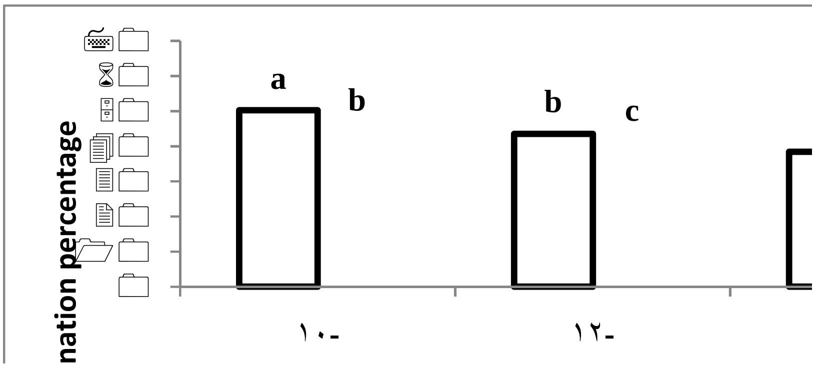


Figure (1-2): Average comparison, interactive effects of temperature priming * drought stress on germination seeds of *S. Montanum* under drought stress in vitro

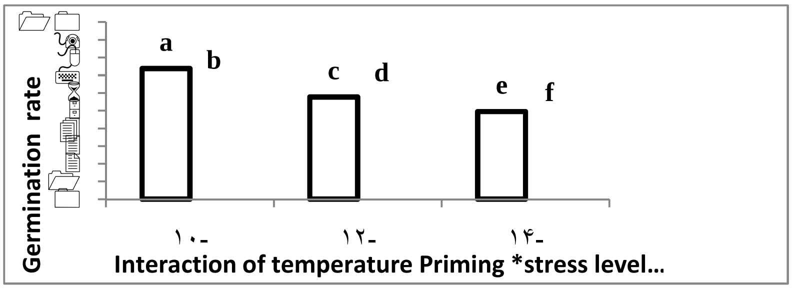


Figure 1-3: Average comparison, interactive effects of temperature priming * surface drought stress on germination rate of the seeds of *S. Montanum* under drought stress in vitro

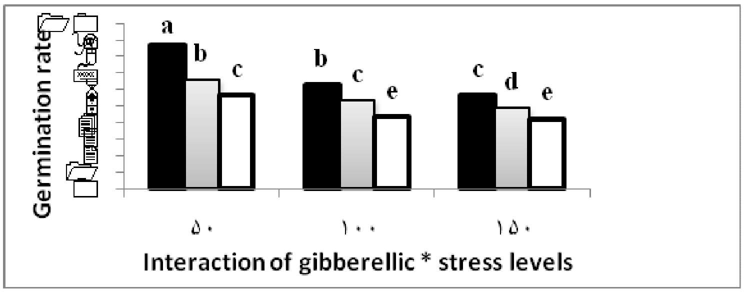


Figure (1-4): Average comparison, interactive effects of gibberellic acid * surface of drought stress on germination rate of seeds *S. Montanum* under drought stress in vitro

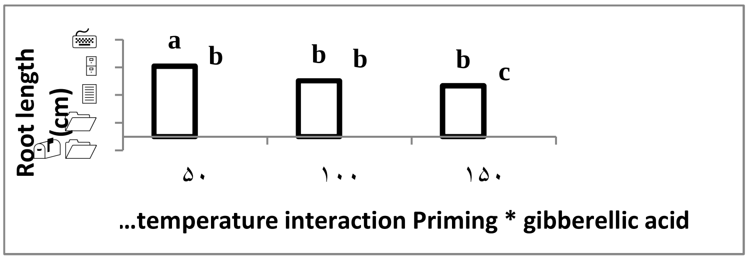


Figure (1-5): Average comparison, interactive effects of priming temperature * gibberellic acid on root Length *S. Montanum* under drought stress in vitro

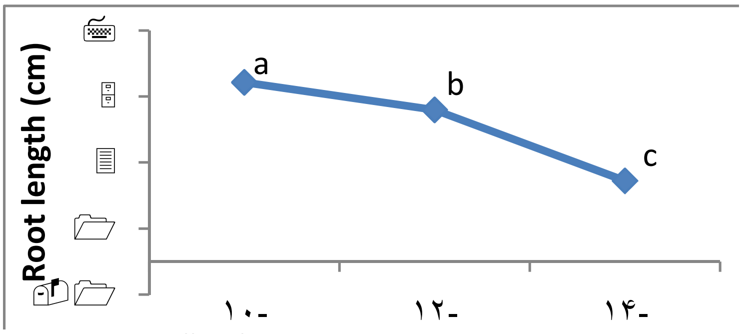


Figure (1-6): Average comparison, the effect of drought stress on root Length *S. Montanum* under drought stress in vitro

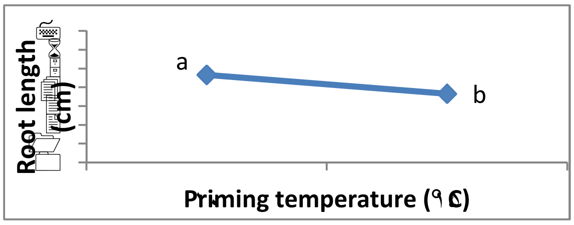


Figure (1-7): Average comparison the average temperature of the priming effect (° C) on both seed length S. Montanum under drought stress in vitro

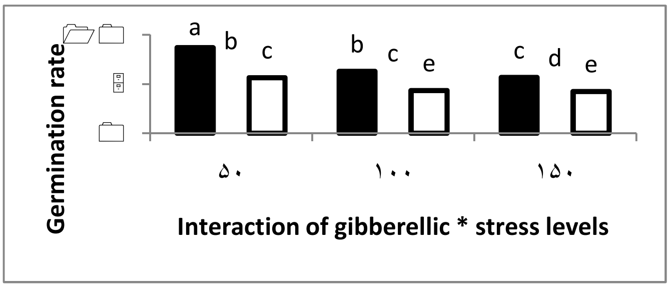


Figure (1-8): Average comparison, effect of gibberellic acid levels (ppm) in shoot length *S. Montanum* under drought stress in vitro

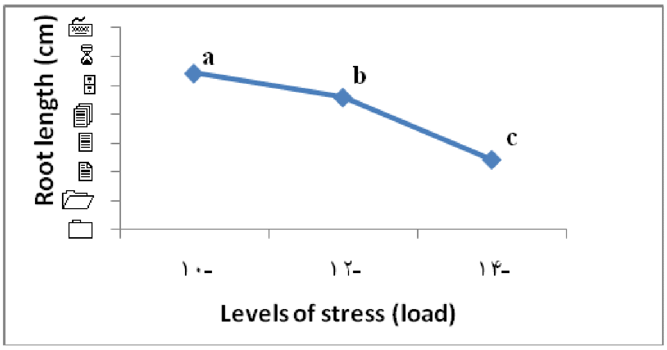


Figure (1-9): Average comparison, effect of levels of stress (load) on the shoot length *S. Montanum* under drought stress in vitro

According to analysis of variance analysis in pot culture under conditions of water stress, the interaction priming level * drought stress on root length and shoot length (5% level), the interaction priming temperature * surface gibberellic acid on seedling establishment rate (5% level) and temperature effects prime temperature, prime levels and different levels of drought stress on the components of the germination (percentage, rate, root length and shoot length) was significant at 1% level (Table 1-2).

The results of the comparison of characteristics of seed germination of *S. Montanum* the medium vases, and priming under drought stress affects different temperatures, with different levels of gibberellic acid and also reported that the percentage of seedlings decreased with increasing osmotic solution concentration and generally the highest establishment of gibberellic acid ppm 50 treatments in the 10 °C temperature and stress - 10 times was observed (Figure 1-10, 1-11 and 1-12). Average Comparison showed that the interaction of priming temperature * gibberellic acid levels on the rate of seedling establishment in drought conditions have different significantly and priming treatments at 10 °C and by gibberellic acid 100 and 150 (ppm) was more effective in increasing the rate of seedling establishment has been in drought conditions (Figure 1-13) and also reported that the increase in osmotic potential rate of seedling establishment was significantly reduced (Figure 1-14).

Table (1-2) analysis of variance to assess characteristic associated with plant germination *S. Montanum* affected by gibberellic acid in drought stress conditions in pot culture

		Mean squares (MS)			
Sources changes	degrees of freedom	Seedling establishment (%)	Rate of seedling establishment	Root length	During the shoot
Block	2	0.60*	0.80**	0.10^{ns}	14.29*
Prime temperature	1	5.22**	3.94**	3.86**	20.29**
Prime surfaces	2	9.87**	9.39**	2.49**	163.18
Drought stress	2	29.10**	22.27**	24.16**	757.40**
temperature* Surface priming	2	0.007^{ns}	0.06^{ns}	0.32*	7.18^{ns}
Temperature * Drought stress	2	0.52^{ns}	0.29^{ns}	0.08^{ns}	1.40^{ns}
Surface priming *Drought stress	4	0.63*	0.45*	0.19^{ns}	5.62^{ns}
temperature* Surface priming* stress	4	0.13^{ns}	0.07^{ns}	0.05^{ns}	0.29^{ns}
Error	34	0.16	0.14	0.07	4.02
Coefficient changes	-	8.68	9.32	5.80	4.78

*Ns, *, **: Non-significant, significant at the 5 and 1%, respectively*

The based on the assessment of the root in potting medium under constant tension throughout the plant root *S. Montanum* influenced by the interaction of gibberellic acid levels * stress levels and gibberellic acid ppm 100 treatment on stress -10 times was highest and gibberellic acid treatment ppm 50 on stress - 14 times was lowest root length and seed priming in temperature at 10 °C was positive effect than 15 °C (1-15 and 1-16). The length change, the study compared an analysis similar to reported changes so that the maximum shoot length in seed priming treatment at 10 °C and a solution of gibberellic acid 100 and 150 (ppm) and potential at -10 times were observed (Figure 1-17 and 1-18).

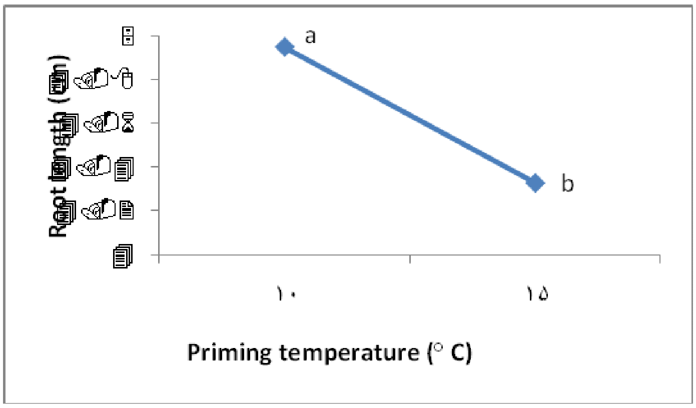


Figure (1-10): Average comparison, the effect of priming temperature on seed germination S. *Montanum* under drought stress conditions in pot

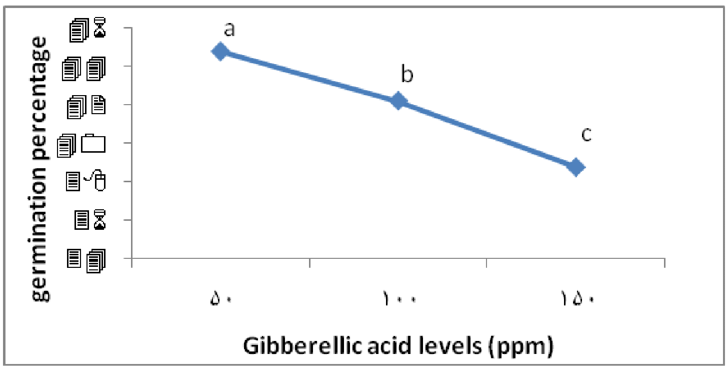


Figure (1-11): Average comparison, the effect of gibberellic acid on the germination of seeds of *S. Montanum* under drought stress conditions in pot

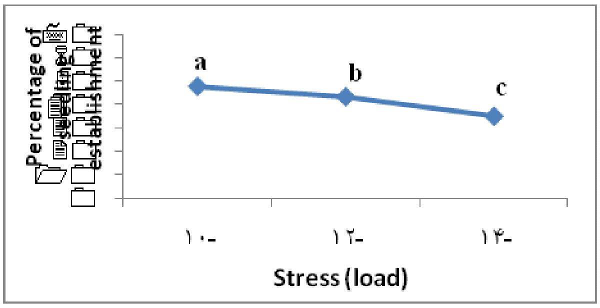


Figure (1-12): Average comparison, the effect of drought stress on seed germination *S. Montanum* under drought stress conditions in pot

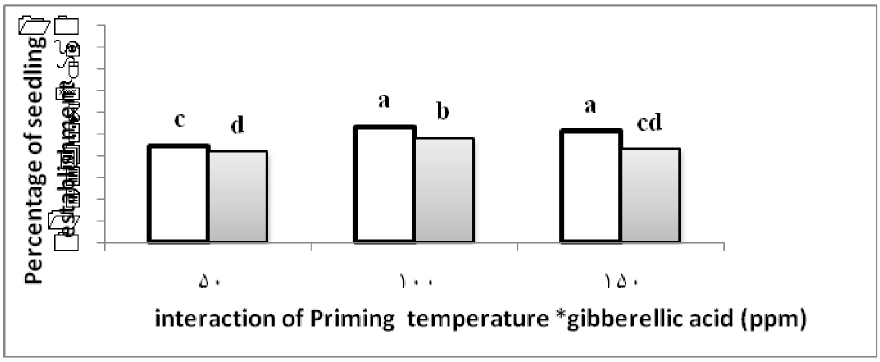


Figure (1-13): Average comparison, the interaction of priming temperature * gibberellic acid surface on the rate of seedling establishment of *S. Montanum* under drought stress conditions in pot

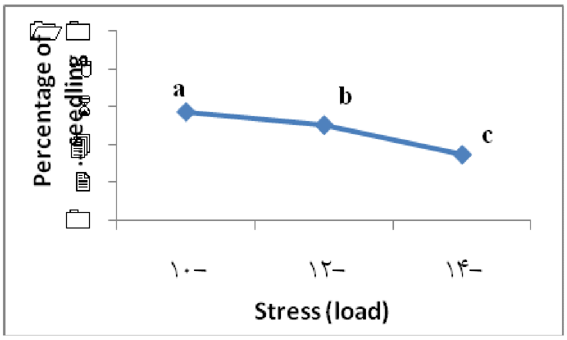


Figure (1-14): Average comparison, the effect of surface stress on the rate of seedling establishment of *S. Montanum* under drought stress conditions in pot

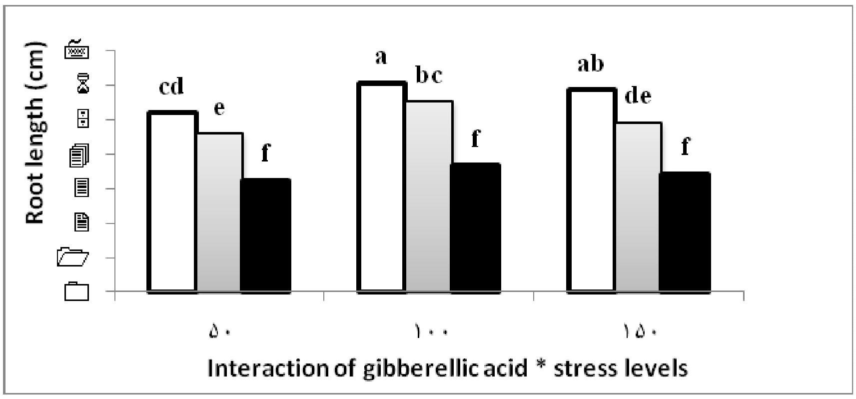


Figure (1-15): Average comparison, the interaction of gibberellic acid levels * drought stress levels on root length of seeds of *S. Montanum* under drought stress conditions in pot

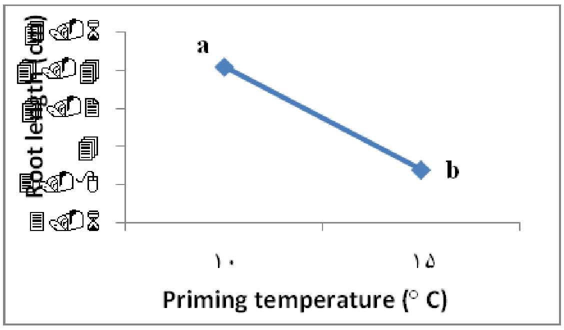


Figure (1-16): Average comparison, the effect of temperature priming on the length of the root or seed *S. Montanum* under drought stress conditions in pot

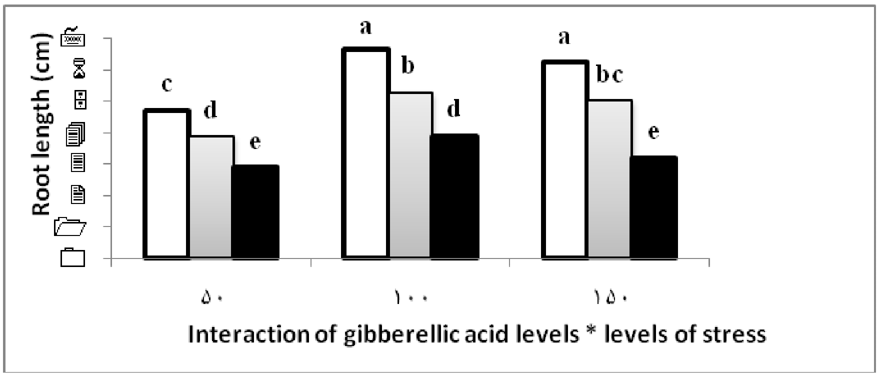


Figure (1-17): Average comparison, the effect of gibberellic acid levels * levels of drought stress on the shoot seeds of *S. Montanum* under drought stress conditions in pot

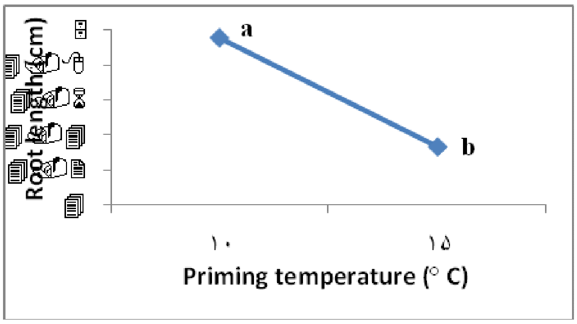


Figure (1-18): Average comparison, the effect temperature priming on shoot length *S. Montanum* under drought stress conditions in pot

In drought conditions may be impaired water absorption by seeds, which in the case of metabolic function in seed germination will occur slowly, the time of root growth and seed germination rate of this decrease, To cope with stress disorders and improve seed germination, pretreated seed plant hormone gibberellic acid in particular will have a positive impact. In this study, the effect of the hormone gibberellic acid on seed germination traits, including the percentage and rate of germination, root and shoot gibberellic acid 50 and 100 (ppm), respectively, in vitro laboratory and greenhouse conditions with the greatest impact on improving seed germination of stress.

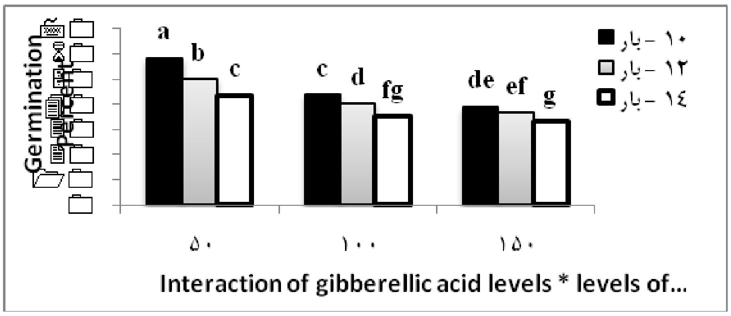
Gibberellic acid hormone secreted by the embryo seed encoding the genes enzymes involved in seed germination, especially the alpha-amylase enzyme is active and this is done by increasing the mRNA encoding the enzyme that way in germination and early seedling establishment plays a role.

Based on published reports, many researchers showed that the effect of gibberellic acid on improvement germination on the stress, that results are in agreement by this study [11, 12, and 13]. Many reports suggest the improved germination behavior and related indicators such as mean time to germination, seed vigor, root length, shoot length, the rate of seed germination and initial establishment of the priming seed [14,15]. Also in this case study can be regarding the positive role of gibberellic acid on lettuce seed germination and promote [16]. On sorghum and sunflower seeds to increase germination under drought, salinity and frost point be.

It seems that the technique of priming to transcribe early transcription of DNA and increased RNA and protein synthesis to the seeds that grow embryos also increases, the parts damaged seed repair would discharge its metabolites reduce the rate and uniformity of seed germination and seedling emergence can improve [16].

CONCLUSION

Results from this study showed that pretreated of *S. Montanum* seeds by hormone gibberellic acid improves germination under drought stress so that the priming seed treatment with gibberellic acid ppm 50 at 10 °C in petri dish culture, germination percentage, germination rate, root length and shoot length increased significantly in the stress three levels. The results indicate that the expression of characteristic in the pot priming treatments had a significant effect on germination componentsso that the seed treatment with gibberellic ppm 100 at 10 °C is shown the maximum positive impact in increasing seedling establishment, seedling establishment rate, root length and shoot length.



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