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EFFECT OF POLYAMINES APPLICATION ON GERMINATION AND PHYSIOLOGICAL CHARACTERISTICS OF BORAGE (*Borago officinalis* L.)

SUMMARY

The aim of study was determine the advantages of re-drying after seed priming by polyamines. As biologically active compounds, polyamines (PAs) have been considered as modulator of plant growth and development, they play a significant role in plant response to environmental stress. The effects of polyamines priming on seed germination, emergence and seedling growth of borage plants was investigated by a laboratory experiment in factorial layout with complete randomized design (CRD) conducted in three replications. The seeds were classified into five sub-samples one of which was kept as control (unprimed) while the rest of them were primed with polyamines. Seeds pre-treatments included: control (unprimed), water pretreatment for 4 and 8 h, spermidine at 5 and 5.5 mM for 4 and 8 h, spermine at 2.5 and 3 mM for 4 and 8 h, putrescine at 2.5 and 3 mM for 4 and 8 h. Sseed treatments with polyamine led to earlier and enhanced germination. Improved seedling length, seedling fresh and dry weight as well as vigor index were found in polyamine-treated seeds. Moreover, the majority of priming treatments enhanced seedling emergence percentage, emergence energy and coefficient of uniformity of emergence (CUE) as compared with control samples. Non-primed seeds (control samples) significantly showed the least α-amylase and β-amylase activity (0.293 and 4.923 $U.mg^{-1}$ Protein, respectively) and shortest of plant height. 4-hour seed treatment by 3mM putrescine and 8-hour treatment with 3mM spermine were recognized as the most effective treatments in most of the studied traits.

Key words: Pre-treatment, Polyamines (PAs), Germination, Vigor index, Amylase enzymes, Emergence.

INTRODUCTION

As one of the precious medicinal plants, Borage is capable of wide cultivation in the semi-arid regions. However, its seedling establishes in the field with difficulty. High plant crops yield is achievable by high seedlings establishment which enables the plant to can cope with the environment and produce high rate of crops (Kamithi et al., 2016). Fast and uniform germination

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as well as seeds emergence and vigorous seedlings are important factors in its establishment and therefore increase of the yield quality and quantity (Cantliffe, 2003). Seed germination performance has been improved by several techniques; these techniques are applicable for environmental stresses and/or aged seeds.

One of the simple and low-cost techniques to break dormancy, expand germination and stand establishment is seed priming which controls seeds hydration followed by their redrying (Afzal et al., 2009). Primed seeds usually exhibit developed germination percent (Nathawat et al., 2007); such an enhanced performance can be attributed to numerous physiological, biochemical and molecular modifications (Taylor et al., 1998; Powell et al., 2000; Afzal et al., 2008). Haigh and Barlow (1987) reported faster imbibition, enhanced extensibility of radicle cell walls and weakened endosperm as a result of priming which could shorten the lag phase of tomato before its radicle emergence. Application of plant growth regulators during priming and other pre-sowing treatments can improve their seed germination performance (Farooq et al., 2007). Seed priming can be conducted in various media including tap water (hydropriming), aerated low water potential solutions like polyethylene glycol or a salt solution (KNO_3, KCl, K_3PO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$ and NaCl) (osmopriming), solid matrix (matripriming), plant growth regulators and polyamines (hormonal priming) (Chiu et al. 2002; Basra et al. 2006; Farooq et al. 2006a, b, c, d, 2007).

As biologically active compounds, polyamines (PAs) can modulate plant growth and development; they play a crucial role in plant responses to environmental stress. Putrescine (Put – diamine), spermidine (Spd – triamine) and spermine (Spm – tetramine) are widely used PAs in higher plants and exist in free, soluble conjugated, and insoluble bound forms (Gill and Tuteja, 2010). Increasing evidences have revealed the role of PAs in regulating plants' responses to different environmental stresses such as drought or osmotic stress, salinity, heat and chilling through direct binding to membrane phospholipids, direct scavenge of free radicals, osmotic adjustment, maintaining a cation-anion balance and binding to the antioxidant enzymes which can enhance their function (Alcazar, 2010; Puyang et al., 2015). Furthermore, PAs may be involved in accumulation of seed protein storage and its maturation (Santanen and Simola 1999) which can in turn promote seed germination (Sińska and Lewandoska 1991, Zeid and Shedeed 2006). PAs are proven to activate protein synthesis in early germination stages (Takahashi and Kakehi, 2010). Seed priming by PAs solutions could improve germination and stress resistance of seedlings under abiotic stress. PAs biosynthesis dramatically elevates under stresses; its function has been recognized as a protective response as it scavenges free radicals (Bagni and Pistocchi, 1991; Kuznetsov et al. 2002). Xu et al. (2011) expressed that Put priming treatment will enhance germination percentage and chilling tolerance of tobacco seedlings. In a similar study by Farooq et al., effective improvement of germination and early seedling growth of sunflower seed priming by Spd (Farooq et al., 2007) and rice (Farooq et al., 2008) were observed. Use of putrescine on

Syngonium plants resulted in significant enhancement of leaves fresh and dry weights and leaf area (El-Quensi et al., 2010).

This study was conducted on the basis of this hypothesis that PA-treatment of the seeds that are would enhance their germination and hence, the borage seeds performance would be improved due to subsequent seedling emergence. T the best of our knowledge, the majority of the previous studies has been devoted to improvement of germination and emergence though seed priming by PAs. In this content, no study has addressed the possibility of borage seed invigoration by polyamines treatment of seeds. In this regard, the aim of this study was to examine seed PA-treatment potential for improving vigor in borage. Moreover, the present study wants to evaluate the possible benefits (if any) of seed priming by PAs for borage by studying the responses at seed germination (vigor) and early growth stages.

MATERIAL AND METHODS

The impact of polyamines priming on seed germination, emergence and seedling growth of borage plants under the saline condition was assessed through a laboratory experiment in University of Maragheh, Iran. For this purpose, a complete randomized design (CRD)-based factorial experiment with three replications was performed. Borage seeds were provided from Pakan Bazr Co., Isfahan, Iran. These seeds were divided into 5 sub-samples. One of them was considered as control (unprimed) and the other four experienced polyamines priming as follows:

1: Control (unprimed)
2: Priming with water for 4 (h_1) and 8 (h_2) hours
3: Priming with spermidine at 5 (sd_1) and 5.5 (sd_2) mM for 4 and 8 hours
4: Priming with spermine at 2.5 (sm_1) and 3 (sm_2) mM for 4 and 8 hours
5: Priming with putrescine at 2.5 (p_1) and 3 (p_2) mM for 4 and 8 hours

After priming, the seeds were washed with tap water and dried for ~ 2 h at room temperature (20-25 $^{\circ}$C). Two experiments were performed at the laboratory. The first experiment involved seeds placement in petri dishes (40 seeds per petri dish) between layers of moistened Whatman paper at 25 $^{\circ}$C in germinator. While in the second experiment, the seeds were cultured in pots (25 seeds per pot). Petri dishes and pots were drenched with water. Germination observation was daily performed as mentioned in association of official seed analysts (AOSA) method (AOSA, 1990).

The field environment was simulated by pot planting, so, the results of this step could be compared with those of petri dishes. In this regard, the pre-treated seeds (P2, P3, P4 and P5) and control samples (P1) were quantitatively and qualitatively tested. Physiological measurements were conducted on petri dish seeds at day 7 of germination; while the other batch was sampled at day 10 of germination to determine the seedling growth. This study determined various traits including final germination (FGP) and emergence (FGE) percentage, germination rate, energy of germination (GE) and emergence (EE), mean germination time (MGT), seedling fresh weight (SeFW) and dry weight (SeDW), seedling length (SeL), vigor index (VI), plant height and coefficient of uniformity of emergence (CUE). For evaluation of physiological traits, amylase enzymes activities were assessed.

The traits were measured by following methods:

Final Germination Percentage (FGP):

FGP = (the number of germinated seed up to the day)/(the total number of seeds) ×100

Mean germination time (MGT) was calculated by equation developed by Ellis and Roberts (1981):

$$\mathrm{MGT} = \frac{\sum \mathrm{Dn}}{\sum \mathrm{n}}$$

In which, n shows the number of seeds germinated on day D, and D denotes the day number from the initiation of germination.

The germination energy measurement was conducted on 4th day of planting. This parameter is defined as the percentage of germinated seeds 4 days after planting compared to the total number of studied seeds (Farooq et al., 2005).

Seeds germinated Vigor Index (VI):

VI = [seedling length (cm) × germination percentage] / 100.

The coefficient of uniformity of emergence (CUE) was calculated by formulae proposed by Bewley and Black (1994):

$$\mathrm{CUE} = \sum n \,/\, \sum[(\bar{t} - t)^2 . n]$$

Here, t represents the time in days, starting from day 0, the day of sowing and n denotes the number of seeds which completed the emergence on day t; while $\bar{t}$ is MET.

Amylase enzymes activities were evaluated by the method developed by Tarrago and Nicolas (1976) and Kishorekumar et al. (2007). In a typical experiment, the seeds (0.1 g) were ground with distilled water (8 mL) at 4 °C. Then the extract was obtained by 25-min centrifugation at 20,000 *g* at 4 °C. The supernatant was then applied to assess the activities of α-amylase and β-amylase. 3 mL of supernatant was mixed with 3 mL of $CaCl_2$ (3 mM) followed by 5-min incubation at 70 °C. The reaction mixture (consisting 0.1 mM citrate buffer, 2% soluble starch solution, 0.7 mL hot enzyme extract) underwent 6-min incubation at 30 °C followed by 5-min heating at 50 °C. Spectrophotometric measurements at 540 nm were employed to assess α-amylase activity. β-amylase activity was evaluated after α-amylase inactivation at pH value of 3.4. The reaction solution included 0.1 mM citrate buffer, 2% soluble starch, 0.7 mL EDTA treated enzyme extract; 2 mL of the mentioned mixture was incubated at 30 °C for 5 min after adding starch. Activity of β-amylase was then measured by the method similar to that of α-amylase.

Suitable analysis of variance was performed by SPSS and MSTATC software. Means of each trait were compared as mentioned in Duncan multiple range test at P value of 0.05. Excel software was also employed for plotting the figures.

RESULTS AND DISCUSSION

According to the obtained results, seed germination was significantly enhanced upon PAs application (Figure 1a). Seeds soaking in Put at 2.5 and 3 mM for 4h resulted in better and higher than germinations as compared with other treated seeds (Figure 1a). Mean germination time (MGT day) declined by prolonging the soaking duration (Figure 1b). Incorporation of PAs into priming media resulted in MGT decrease as compared with control samples. In PAs treated samples, the minimum MGT was observed in seeds primed with Spm and Spd (Figure 1b).As suggested by Figure 1c, seed priming with PAs resulted in increased energy of germination. Seeds primed in Put for 4h, 3mM Spm for 4h and Spd for 8h exhibited the highest energy of germination; while the control sample showed lowest value of the mentioned trait.

Seed germination is a complex physiological process which can be modulated by phytohormones or physiological activators like abscisic acid (Finkelstein et al., 2000), nitric oxide (Beligni and Lamattina, 2000) or polyamines (Zapata et al., 2004). Vigorous seedlings responded to seed priming by polyamines and evidently showed enhanced resistance against the adverse effects of environmental stresses (Li et al., 2014). Moreover, polyamine priming resulted in significant improvement of germination and early seedling growth in borage. Our study also revealed that polyamine pretreatment can stimulate borage germination. Earlier and more uniform germination were detected in PA-pretreated seeds. Enhanced germination percentage due to priming could be attributed to breakdown of reserve food material, elevated cell division and embryonic axis expansion (Basra et al., 2006).

The earlier and more synchronized germination could be also due to enhanced metabolic activities of the treated samples (yang et al., 2016). Previous studies have also reported the positive impacts on seed germination through Spd priming (Rebecca et al., 2010; Sedagahat and Rahemi, 2011). Recently, it has been shown that Spd soaking can significantly improve seed germination in corn, while exogenous application of cyclohexylamine (CHA; an inhibitor of Spd biosynthesis) resulted in significant inhibition of seed germination and declined seed vigour (Huang et al., 2017).

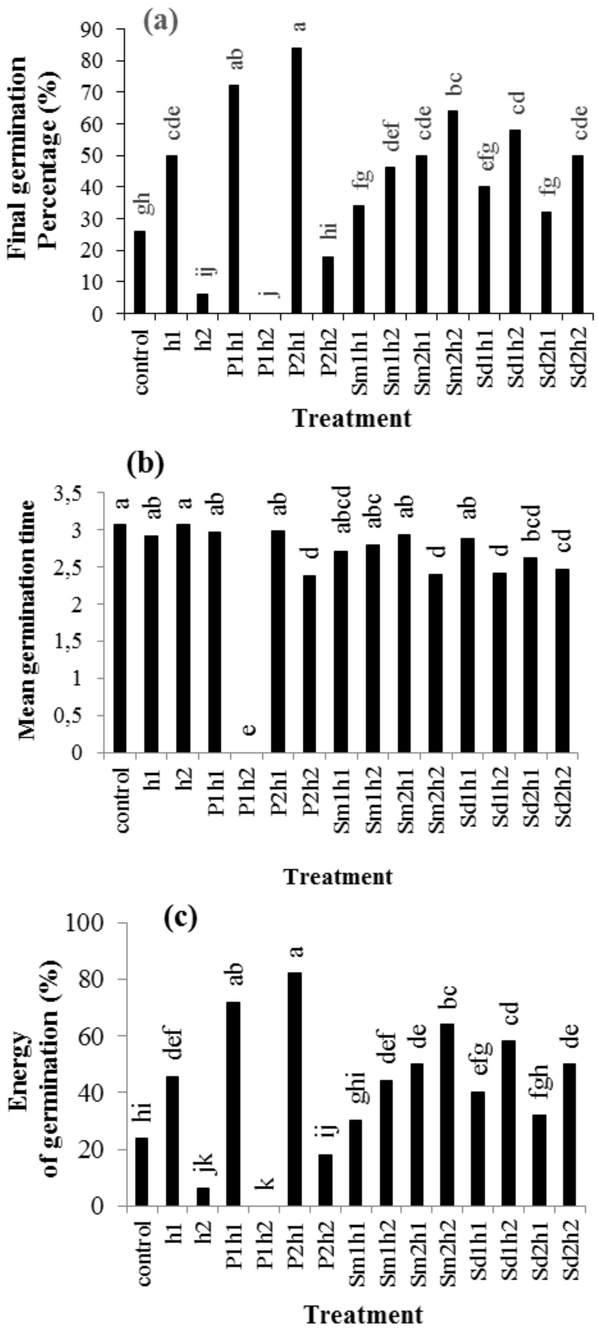

Figure 1- Effect of PA- priming strategies on (a) final germination percentage (b) mean germination time (c) and energy of germination of the seeds

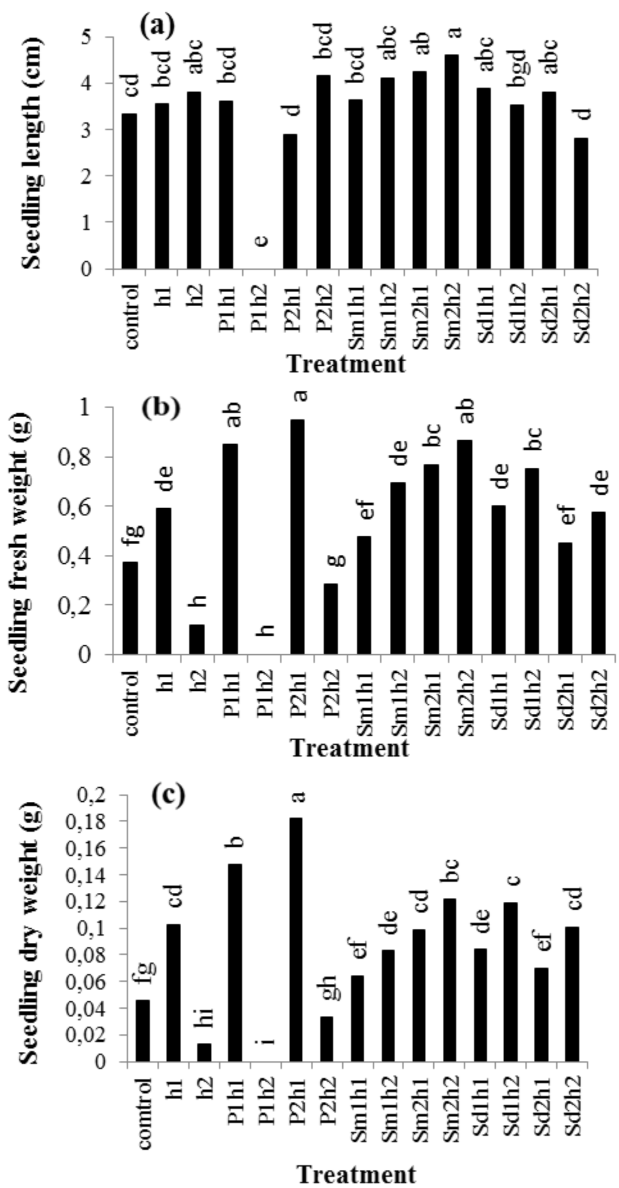

Figure 2- Effect of PAs priming strategies on (a) seedling length (b) seedling fresh (c) and dry weight of the seeds

In comparison with control, seeds pre-soaking in polyamine solutions drastically improved the seedling growth. Seedling length was also dramatically influenced by priming treatments. Priming with 3mM of Spm for 4 and 8 h resulted in the highest seedling length (Figure 2a). Furthermore, polyamine seed treatments led to significant enhancment of seedling fresh and dry weights. Among various tested treatments, the Put-treated seeds exhibited more vigorous seedlings. Substantially higher seedling fresh and dry weights were observed in sampled treated by Put (3mM) for 4 h (Figure 2b,c). While statistical minimums of seedling fresh and dry weights were measured in samples pretreated with water for 8 h and Put (3mM) for 8 h (Figure 2b, and c).

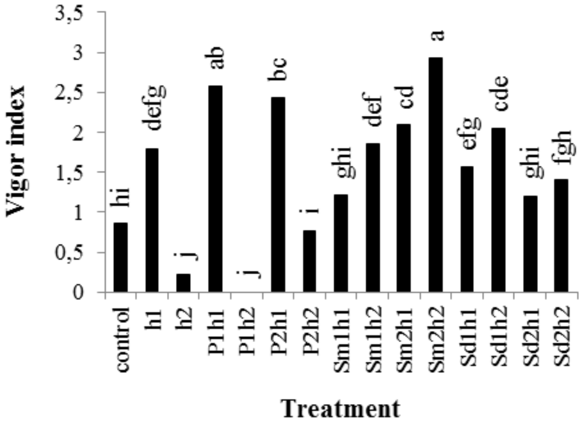

Figure 3- Effect of seed priming with polyamine on vigor index.

Similarly, vigor index measurments showed high values in polyamines-treated samples (Figure 3). Almost all of the polyamine-treated samples revealed similar results in terms of vigor index improvement with little variation among concentration levels. The maximum vigor index was recorded in samples treated with Spm (3 mM) for 8 h (Figure 3). The results also indicated that seed priming by polyamines will increase seed vigor as suggested by seedling fresh and dry weights comparison with control samples. The enhanced growth could be assigned to earlier germination and emergence (Hammad et al., 2012). Such earlier synchronized and faster emergence could be the consequence of the improved DNA, RNA and protein syntheses during priming, which will lead to augmented seedling growth (Huang and Villanueva, 1992; Farooq et al., 2011). This shows the polyamines significance for plant development justifying the performance of polyamines in this study. According to the obtained results, all the PA treatments led to the seed germination and seedling growth stimulation as compared with control; among which, Put showed more effectiveness. The findings of this study are consistent with those obtained by Farooq et al. (2008) indicating that Put at lower concentrations will result in effective improvement of germination performance as compared with other Pas. Our results are however in contrast with the findings reported by Farooq et al. (2007) and Farooq et al. (2011). Exogenous Spd enhanced seed germination in numerous plant systems, as well. For example, based on the studies conducted with white clover indicated that Spd-primed seeds not only led to enhanced germination percentages and shortened mean germination, but also improved the seed vigor shown by longer root length and higher seedling fresh and dry weights in comparison with control (Li et al., 2014).

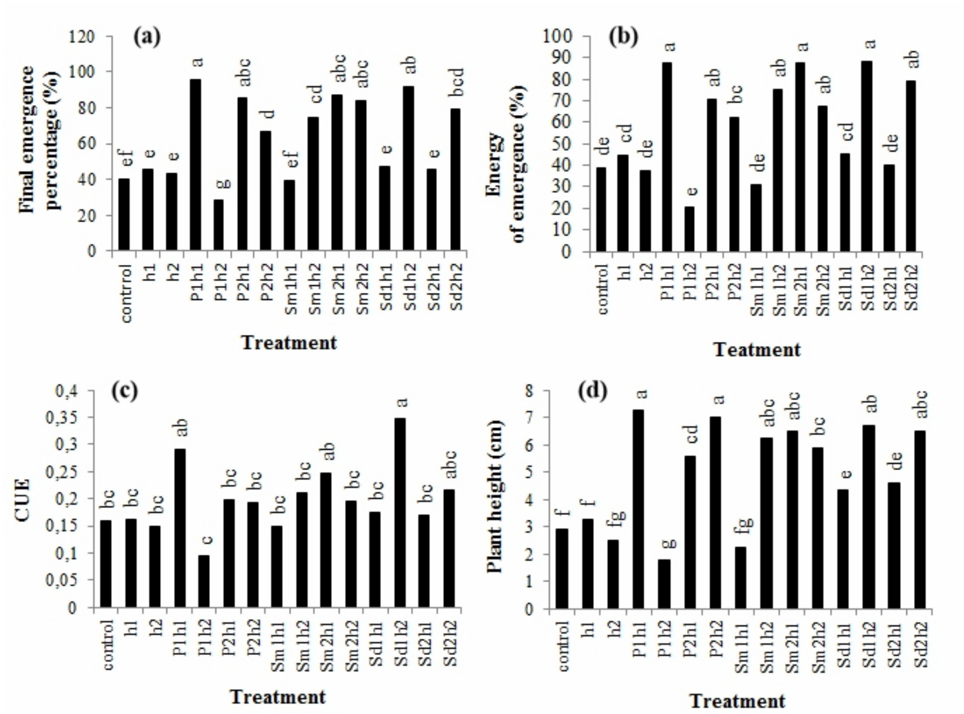

Figure 4- Effect of polyamine treatment on (a) Emergence percentage (b) Energy of emergence (c) CUE (d) Plat height of samples

Most of the priming treatments resulted in improved seedling emergence percentage, energy of emergency and coefficient of uniformity of emergence (CUE) (Figure 4a,b,c). Among the mentioned priming methods, the maximum and minimum percentage of emergence were measured in seeds treated with Put (2.5 mM) for 4 h and 8h, respectively. The maximum energy of emergence was observed in seeds primed by Spd (5mM) for 8h which showed a 2.5-fold increase relative to control (Figure 4a, and b). Pre-sowing of the seeds with polyamines led to enhanced coefficient of uniformity of emergence (CUE) where the maximum value was recorded for samples undergoint Spd (5mM) treatment for 8h (Figure 4c). Seed soaking in PAs also improved the plant height (Figure 4d). Apparently, improvement of seedling emergence and growth through polyamines priming can be assigned to stimulation of germination metabolism (Figure 1) and increased seed vigor (Figure 3). Seed germination is the most important period of seedling establishment (Hubbard et al. 2012; Shi et al. 2014). Polyamine was involved in seed germination of plants. PA content increased during the first 15 days of Ocotea catharinensis seed germination it was then decreased and stabilized between 30 and 60 day of germination (Dias et al. 2009). Exogenous polyamines showed enhancing effects on seed germination of the hot pepper (Khan et al. 2012). A signifcant relationship was observed between PAs and hormones in plant growth regulation. So, enhanced polyamines may rise the plant hormones and enzymes (Pieruzzi et al., 2011; yang et al., 2016). Moreover, Increased EE (energy of emergence), FEP (Final emergence percentage), CUE (coefficient of uniformity of emergence) and plant height seemed to be attributed to efficient mobilization and use of seed reserves and improved genetic repair, i.e., earlier and faster DNA, RNA and proteins syntheses (Srivastava 2002). These changes could be due to earlier initiation of germination suggested by lower E_{50} values (Basra et al. 2006). Boothe et al. (2010) showed that accumulation of the various seed storage compounds plays an important role because: (i) these reserves support the early growth of seedlings after germination, thus affecting the seedling vigor; and (ii) they are widely applied as food and feed. A sturdy relationship between seed metabolites and CUE (coefficient of uniformity of emergence) and MET (mean emergence time) supported this assumption that faster starch metabolism may take part in uniform and early emergence and vigorous seedling growth.

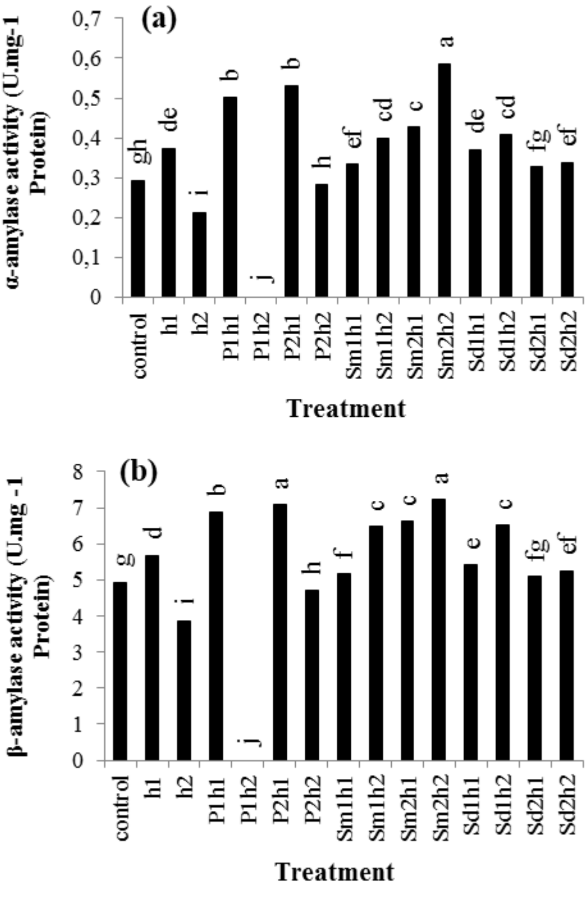

Figure 5- Effect of polyamine treatment on (a)α-amylase and(b) β-amylase activities

Maximum (0.587 U.mg $^{-1}$ Protein) and minimum (0.293 U.mg $^{-1}$ Protein) values of alpha-amylase activity were observed when borage seeds were primed with spermine (3mM) for 8 h and Put(2.5 mM) for 8 h (Figure 5a). Accordingly, polyamine seed treatments resulted in significant improvement of β-amylase activity. Maximum β-amylase activity was detected for seeds soaked in 3 mM Spm solution for 8 h (Figure 5). The polyamines-induced improvement of seed germination, seedling growth, vigor index and seed emergence was assigned to germination metabolism stimulation as a result of increased α-amylase and β-amylase activities. Priming may significantly affect the enzyme activities necessary in fast seed germination (Varier et al., 2010). Amylases are essential enzymes with prominent role in hydrolyzing the seeds starch reserve, which will provide sugar for developing embryo (Andoh and Kobata, 2002; Kamithi et al., 2016). Li et al. (2014) stated that seed priming with Spd could enhance starch metabolism possibly because of elevated α- and β-amylase activities. Farooq et al. (2011) reported a strong correlation between amylase and soluble sugars supporting this hypothesis that fast starch metabolism could be helpful in the early emergence of seeds and vigorous seedling growth observed by Spd priming. Starch metabolites such as glucose play crucial role in seed germination since they act as osmolytes in cellular turgor maintenance and energy sources. That's why PAs plays a significant role in accelerating starch metabolism due to enhanced α-amylase and β-amylase activity during seed germination. Seed germination potential is determined by cellular metabolism during germination (Bewley and Black 1994; Holdsworth et al. 2008a; Rajjou and Debeaujon 2008); thus the success of the new plant establishment. Deeper understanding of these mechanisms will result in development of molecular and biochemical markers, which can be employed as quality markers of seed sector in high-vigor seed lots markets (Catusse et al. 2011; yang et al. 2016).

CONCLUSIONS

Based on the results of this study, seed priming by polyamine techniques can significantly enhance the emergence uniformity coefficient through improving the seeds vigor; although its effectiveness significantly varied for different polyamines and their concentrations. Seed priming treatments exhibited higher beneficial impacts on stimulating amylase enzymes activities and improvement of the seedling emergence and growth. It can be concluded that seed priming by PAs could be regarded as a promising approach to improve the seed germination especially under adverse conditions. Furthermore, for practical uses, investigation of the polyamines effects on different crops under biotic and abiotic conditions sounds necessary. This interesting topic could be considered for future studies.

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