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The effect of seed priming with salicylic acid on growth and grain yield of pinto bean under nitrogen levels

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Abstract

A field experiment was carried out to study the effects of seed priming by salicylic acid on growth and grain yield of pinto bean (*Phaseolus vulgaris* L. cv. Khomain) were investigated under different nitrogen application conditions. This experiment was carried out in factorial experiment in completely randomized block design with three replications in 2013 growing season at research station of Zanjan city (university of Zanjan) and Khoramdareh city of Iran. The experimental treatments included nitrogen fertilizer with four levels (included 0, 20, 40 and 80 kg ha^{-1}) and seed priming with salicylic acid with five levels (included 0 (control), 750, 1500 and 2250 µM and also hydro-priming). The result showed that nitrogen (N) and salicylic acid (SA) had significant effects on morphology traits and grain yield. Mean comparison showed that chlorophyll index, dry weight of stem and leaves, stem length, leaves area and grain yield increased in SA compared to control treatment. Means comparison showed that the treatment of 2250 µM SA had the highest morphology traits and yield which was higher than that other treatments. As well as morphology characteristics such as dry weight of leaves were significantly increased with the increasing nitrogen levels and also improvement on grain yield. Furthermore, application of 20, 40 and 80 kg ha^{-1} N increased grain yield by 10%, 3.5% and 5.4% respectively, compared to the control plants. However, hydro priming could not affect this grain yield and not signification by control.

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Introduction

Pinto bean (*Phaseolus vulgaris* L.) is the most important legume crop and a major source of protein. Cultivation of pinto bean leads to the increase of soil nitrogen compounds (Hungria and Vargas, 2000). Different techniques could be used to enhance crop performance, particularly under unfavorable environmental conditions. One of these simple techniques is seed priming (McDonald, 2000; Finch-Savange, 2000; Halmer, 2004). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops (Parera and Cantliffe, 1994; Habibi and Abdoli, 2013). Pre-sowing seed treatments, which during those seeds hydration are done outside of field and under control condition, can accelerate seed germination, improve the seedling establishment and increase water and nutrients absorption by roots (Anuradha and Rao, 2007).

Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development and regeneration of physiological processes in plants (Sakhabutinova *et al.*, 2003; Krantev *et al.*, 2008). Improvement or modification of plant growth and development can occur by the direct application of SA to seeds (Arfan *et al.*, 2007; Azooz and Youssef, 2010; Mansouri and Aboutalebian, 2013). Ion uptake and transport (Wang *et al.*, 2006), photosynthetic rate, membrane permeability (Afzal *et al.*, 2006) and transpiration (Khan *et al.*, 2003) could also be affected by SA application. Khodary (2004) found that SA treatment increased the chlorophyll and carotenoid contents in maize plants. Sakhabutdinova *et al.* (2003) established that treatment of wheat plants with 0.05 M SA increased the level of cell division within the apical meristem of seedlings roots which caused an increase in plant growth. Application of SA as a priming agent improved salt tolerance in *Vicia faba* due to enhanced catalase, ascorbate peroxidase, peroxidase and glutathione reductase activities (Azooz, 2009). El Tayeb and Ahmed (2010) showed that dry weight of wheat seedlings treated with SA was increased compared with untreated seedlings and higher contents of sugar, protein and minerals were recorded in SA treated plants.

Many factors affect grain yield of plant crop such as fertilization. Among the main nutrients required for plans, nitrogen (N) had significant effect on growth and development also direct relationship of N with plant growth and yield of crops has been proven (Andrade *et al.*, 1993; Fageria and Baligar, 2005; Rahmati, 2012; Sorkhi and Fateh, 2014). Bangarwa *et al.* (1998) have been reported that, leaf area index, photosynthesis and finally dry matter accumulation were increased due to high level of N. Studies done by Farzanjoo *et al.* (2002) indicate that there is a high correlation between the rate of dry matter production in sorghum and the application of different levels of N fertilizer. Researchers have reported that the main effect of N on increasing grain yield is through the number of grains. Moreover, in many cases grain weight is affected by nitrogen and has increased (Ghasemi, 2002; Rezaei and Ramazani, 2010). Moosavi *et al.* (2014) reported that the nitrogen utilization significantly increased flower yield, water use efficiency (WUE) and plant height of marigold (*Calendula officinalis* L.), but there was not any significant difference between 120 and 180 kg N ha^{-1} treatments. Although some researchers have separately studied various aspects of priming with SA and N fertilizers (Gholizadeh, 2011; Pak Mehr *et al.*, 2011; Habibi and Abdoli, 2013). This study was therefore conducted to evaluate the effect of seed priming by salicylic acid on growth and grain yield of pinto bean (*Phaseolus vulgaris* L.) were investigated under different levels of nitrogen.

Materials and methods

Study side and plant material

Two field experiments were carried out at the research station of the Zanjan University, Zanjan city (36°, 41′/N; 41°, 27′/E), 1620 meter elevated from sea level and Khoramdareh city (36°, 25′ to 36°, 10′/N; 49°, 25′ to 48°, 55′/E), 1575 meter elevated from sea level of Iran during the spring and summer of 2013 on pinto bean (*Phaseolus vulgaris* L.) cultivar “Khomain”.

Treatments

The experimental treatments included nitrogen fertilizer with four levels (included 0, 20, 40 and 80 kg ha^{-1}) and priming with salicylic acid with five levels (included 0 (control), 750, 1500 and 2250 µM and also hydro-priming). The seeds were soaked in 750, 1500 and 2250 µM SA and water (hydro-priming) at 25°C for 24 hours. The nitrogenous fertilizer as urea ($CO(NH_2)_2$) was applied in the two to three leaving stage.

Cultivation

The land was plowed and disked before planting. Each plot included 3 rows 50 cm apart, 5 meters long, 2 and 1 meters distances were taken between test plots and replicates, respectively. Seeds were sown at a density of 35 seeds m^{-2} on the 17th of June 2013. The soil samples were collected at random from the top 0-30 cm soil depth before sowing and some of the physico-chemical properties of this soil are presented in Table 1. Humidity and moderate temperatures during the crop season is presented in Table 2.

Sampling for stem and leaf traits measurements

In the measuring stem and leaf traits such as: stem dry weight, leaves dry weight, stem length and leaves area, 10 plants on each plot was harvested at flowering stage and were dried at 75 °C for 48 h and their dry weights were measured.

Chlorophyll index (SPAD) measurements

Leaf chlorophyll index was obtained by portable chlorophyll meter (SPAD-502, Minolta, Japan) from five individual leaves per plots on the flowering stage.

Grain yield measurements

Grain yield for each treatment were measured by harvesting 2 m^2 of the central part of each plot at crop maturity.

Statistical analysis

All data were statistically analyzed based on a factorial in completely randomized block design with three replications for field experiment, using MSTAT-C and SAS software (version 8.0). The means of the treatments were compared using the Duncan's range test at $P<0.05$ (SAS Institute Inc. 2004). The figures were drawn using Excel.

Results and discussion

Stem and leaves dry weight

The results of analysis of variance showed that location, nitrogen and seed priming by salicylic acid significantly affected stem dry weight and leaves dry weight (Table 3). Interaction between location and salicylic acid was significant for dry weight of stem in the flowering stage (Table 3). In the present study the averages of stem dry weight and leaves dry weight of different treatments in Zanjan location were 13.5 g and 25.0 g respectively, while under Khoramdareh location these values significantly increased to 14.9 g and 34.0 g, respectively (Table 4).

Table 1. Physico-chemical properties of the soil used in the experiment.

Physical and chemical property (Saturation extract)	Zanjan	Khoramdareh
K (mg kg^{-1})	280	433
Available P for plant (mg kg^{-1})	9	13
N (%)	0.092	0.087
pH	8.2	7.7
Bulk density (g cm^{-3})	1.4	-
Texture	Clay loam	Clay loam

Result revealed that the highest values of stem dry weight were recorded in control and 80 kg ha^{-1} N treatments (14.4 and 14.2 g, respectively), also the lowest stem dry weight was seen in N of 40 kg ha^{-1} (13.9 g) (Figure 2 A). But, highest and lowest values of leaves dry weight were recorded in 40 and 80 kg ha^{-1} N treatments with 29.8 and 29.3 g, respectively (Figure 2 B).

Table 2. Minimum, maximum and mean of air temperature and precipitation in the Zanjan and Khoramdareh regions of Iran during 2013.

Location	Month	Temperature (°C)			Precipitation (mm)
		Min	Max	Mean	
Zanjan	Apr.	3.1	18.2	10.6	14
	May.	5.7	19.8	12.7	48
	Jun.	10.3	27.6	19.0	6
	Jul.	13.1	31.6	22.3	0
	Aug.	14.7	31.8	23.2	2
	Sep.	11.9	31.0	21.5	3
Khoramdareh	Apr.	5.5	19.1	12.3	16
	May.	7.3	20.6	13.9	55
	Jun.	13.0	27.3	20.1	12
	Jul.	15.3	31.3	23.3	0
	Aug.	15.4	30.7	23.0	2
	Sep.	14.1	31.2	22.7	4

Source: Meteorological Office, Iran.

Table 3. Analysis of variance of the effects of location, nitrogen levels, seed priming by salicylic acid and their interactions on morphology traits and grain yield in pinto bean.

Sources of variations	df	Mean of squares		
		Stem dry weight	Leaves dry weight	Stem length
Location (L)	1	58.7 **	2401.7 **	1980.5 **
R (L)	4	0.461	0.584	14.9
Nitrogen (N)	3	0.953 **	1.291 *	44.9 ns
Salicylic acid (SA)	4	107.6 **	105.6 **	17717.4 **
N×SA	12	0.374 ns	0.636 ns	25.6 ns
L×N	3	0.378 ns	0.119 ns	2.73 ns
L×SA	4	0.746 *	0.100 ns	6.61 ns
L×N×SA	12	0.231 ns	0.149 ns	1.91 ns
Error	76	0.231	0.396	22.0
CV (%)		3.40	2.13	3.95

Seed priming with SA increased the dry weight of stem and leaves in the flowering stage (Figure 1 A, B). Means comparison showed that the treatment of 2250 µM salicylic acid (SA) had the highest dry weight of stem (17.2 g) which was higher than that under the treatments of 1500 and 750 µM SA by 15.4 and 13.6 g, respectively (Figure 1 A). The lowest stem dry weight was obtained at control (no priming with SA and water); however it had not any significant difference with hydro priming (Figure 1 A). Also, the result showed that dry weight of leaves in plant grown under 750, 1500 and 2250 µM of SA showed 6.7%, 10.8% and 17.5% increase when compared with control, respectively (Figure 1 B). These results endorse the findings of Basra *et al.* (2003) and Rashid *et al.* (2002) who reported that primed treatment significantly increased total biomass and dry weight when compared with control. SA in plants generating a significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport and also induces specific changes in leaf anatomy and chloroplast structure (Khan *et al.*, 2003; Arfan *et al.*, 2007; Azooz and Youssef, 2010). It is supposed that the protective and growth promoting effect of SA are due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth. Hosseinzadeh *et al.* (2013) reported that foliar application of 700 and 1400 µM SA solution led to 56% and 127% increase in fresh weight, respectively.

Figure 4 shows the stem dry weight in location and different SA treatment. The dry weight of stem was always higher in 2250 µM SA treatment in Zanjan and Khoramdareh conditions. But, were no significant differences in the stem dry weight among control (no using SA) and hydro priming (Figure 3).

Correlation of stem dry weight with shoot length and leaves area was significantly positive at 1% level of probability (Table 5).

Continued table 3.

Sources of variations	df	Mean of squares		
		Leaves area	Chlorophyll index (SPAD)	Grain yield
Location (L)	1	14107172 ns	116.8 **	2028000 *
R (L)	4	2075344	3.51	188445
Nitrogen (N)	3	6498476 **	1.636 ns	408642 **
Salicylic acid (SA)	4	123736358 **	694.7 **	10653185 **
N×SA	12	2657632 *	1.587 ns	60000 ns
L×N	3	5979121 **	0.200 ns	8049 ns
L×SA	4	2056171 ns	0.038 ns	2814 ns
L×N×SA	12	2541028 ns	0.152 ns	10518 ns
Error	76	1220575	2.087	98464
CV (%)		10.6	7.01	10.7

ns, * and **: Non significant, significant at 5 % and 1 % levels of probability, respectively.

Table 4. Effect of location on stem dry weight, leaves dry weight, stem length, SPAD and grain yield in pinto bean.

Location	Stem length (cm)	Stem dry weight (g)	Leaves dry weight (g)	Chlorophyll index (SPAD)	Grain yield (kg/ha)
Zanjan	114.8 b	13.5 b	25.0 b	19.6 b	2807 b
Khoramdareh	122.9 a	14.9 a	34.0 a	21.6 a	3067 a

Means followed by the same letter within columns are not significantly different (P < 0.05) according to Duncan's test.

Stem length and leaves area

The results of analysis of variance showed that location and salicylic acid significantly affected stem length of pinto bean were significant at 1% level (Table 3). As well as the results of analysis of variance showed that nitrogen and salicylic acid significantly affected leaves area (Table 3). The averages of stem length of different treatments in Zanjan location were 114.8 cm, while under Khoramdareh location these values significantly increased to 122.9 cm (Table 4).

Table 5. Correlation coefficients among grain yield and morphology traits in pinto bean under two locations.

Traits	Location	SPAD	LA	SL	SDW	LDW	GY
Chlorophyll index (SPAD)	Zanjan	1					
	Khoramdareh	1					
Leaves area (LA)	Zanjan	0.94**	1				
	Khoramdareh	0.74**	1				
Stem length (SL)	Zanjan	0.92**	0.93**	1			
	Khoramdareh	0.93**	0.75**	1			
Stem dry weight (SDW)	Zanjan	0.93**	0.95**	0.95**	1		
	Khoramdareh	0.89**	0.69**	0.93**	1		
Leaves dry weight (LDW)	Zanjan	0.89**	0.90**	0.93**	0.95**	1	
	Khoramdareh	0.87**	0.84**	0.92**	0.88**	1	
Grain yield (GY)	Zanjan	0.82**	0.80**	0.89**	0.84**	0.83**	1
	Khoramdareh	0.81**	0.65**	0.90**	0.84**	0.83**	1

* and **: Significant at 5% and 1% levels of probability, respectively.

Nitrogen levels significantly affected the area of leaves of pinto bean. The highest leaves area was observed in control (Non application of nitrogen), 20 and 40 kg ha^{-1}, while N at 80 kg ha^{-1} decreased significantly leaves area (Figure 2 C). In addition to, using of 20, 40 and 80 kg ha^{-1} N decreased leaves area by 2.9%, 1.6% and 9.8% respectively, compared to the control plants (Figure 2 C). But, Abadi *et al.* (2014) reported that the highest leaf area index was achieved by applying 240 kg N ha^{-1} of sorghum.

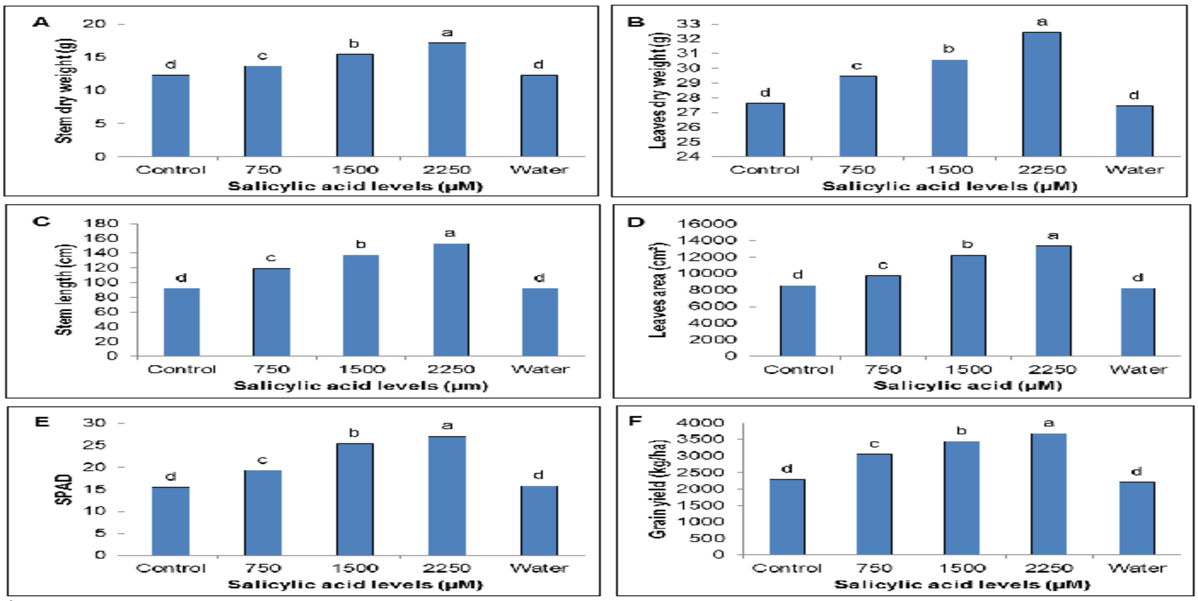

Fig. 1. Effect of seed priming by salicylic acid on stem dry weight (A), leaves dry weight (B), stem length (C), leaves area (D) and chlorophyll index or SPAD (E) in the flowering stage and grain yield (F) of pinto bean. Within each salicylic acid level, means with different letters are significantly different ($P < 0.05$).

Stem length and area of leaves in the flowering stag were significantly increased with the increasing SA levels (Figures 1 C, D). Seed priming with 2250 µM SA the better compared to other salicylic acid levels and hydro-priming on morphology traits and agronomy characteristics of pinto bean (Figure 1). Primed seeds after sowing had faster germination, rapid establishment, and uniformity growth. Such a plant expands root system at short time compared to the control and with up taking more water and nutrient materials produce photosynthetic organs that rapidly reach to autotrophic stage (Duman, 2006).

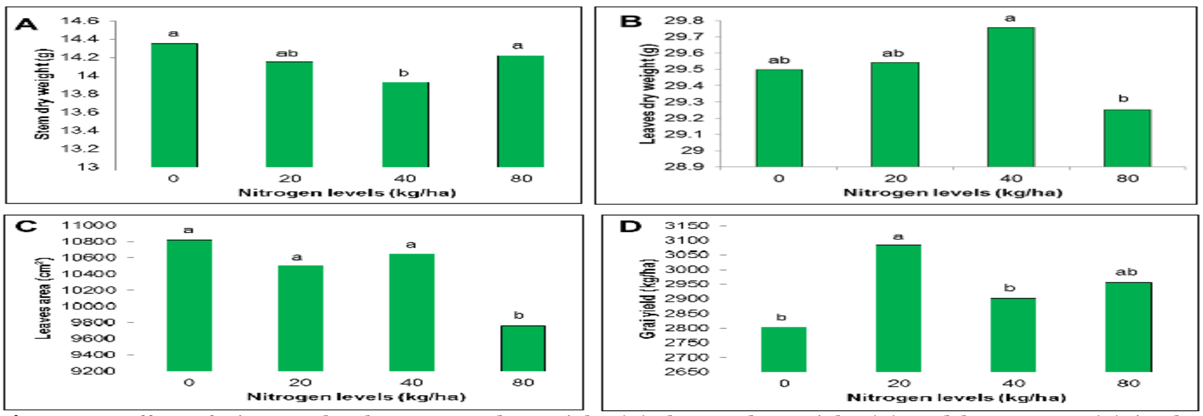

Figure 2. Effect of nitrogen levels on stem dry weight (A), leaves dry weight (B) and leaves area (C) in the flowering stage and grain yield (D) of pinto bean. Within each nitrogen level, means with different letters are significantly different ($P < 0.05$).

The result showed that the interaction between location and nitrogen was significant for leaves area in the flowering stage (Table 3 and Figure 4). Also, Interaction between nitrogen and salicylic acid was significant only for leaves area in the flowering stage (Table 3 and Figure 5). Seed priming with 2250 µM SA increased area of leaves compared to other treatments in different nitrogen conditions were 13213, 13304, 13346 and 13529 cm^2 leaves area achieved in control (no application nitrogen), 20, 40 and 80 N kg/ha, respectively (Figure 5). Gholizadeh (2011) reported that the general research treatment that using salicylic acid in dosage of 700 and 1400 µM in care along with using nitrogen fertilizer (75 kg ha^{-1}) had the best efficiency.

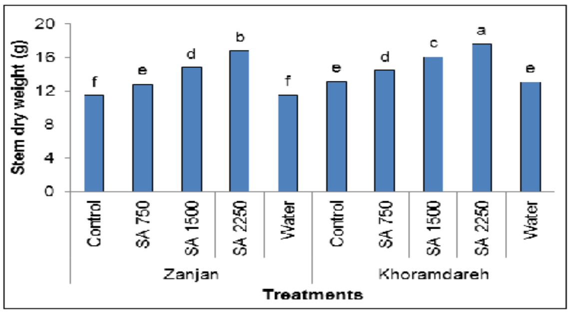

Fig. 3. Mean comparison of interactions between location and salicylic acid levels on stem dry weight in the flowering stage. Means with different letters are significantly different ($P < 0.05$).

Chlorophyll index

According to the analysis of variance (ANOVA) results, location and salicylic acid significantly affected chlorophyll index (Table 3). The averages of chlorophyll index of different treatments in Zanjan location were 19.6 unit by SPAD, while under Khoramdareh location these values significantly increased to 21.6 unit by SPAD (Table 4).

The lowest chlorophyll index was observed in control (no priming) and hydro priming, while SA at 2250 µM increased significantly chlorophyll index. So that, chlorophyll content in plant grown under 750, 1500 and 2250 µM of SA showed 24.7%, 64.0% and 74.7% increase when compared with control, respectively (Figure 1 E). However, Moharekar *et al.* (2003) reported that salicylic acid increases the synthesis of carotenoids and xanthophylls and also enhanced the rate of deep oxidation with a concomitant decrease in chlorophyll pigments in wheat and moong. It seems that SA treatment induces biosynthesis the some specific carotenoids in plants.

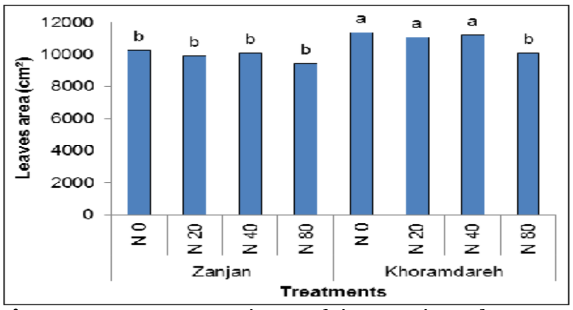

Fig. 4. Mean comparison of interactions between location and nitrogen levels on leaves area in the flowering stage. Means with different letters are significantly different ($P < 0.05$).

Correlation of chlorophyll index with shoot length and dry weight of stem and leaves was significantly positive at 1% level of probability (Table 5).

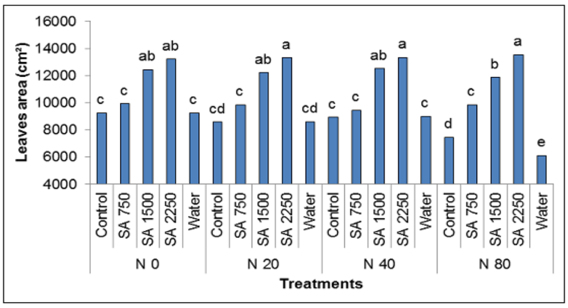

Fig. 5. Mean comparison of interactions between nitrogen levels and salicylic acid levels on leaves area in the flowering stage. Means with different letters are significantly different ($P < 0.05$).

Grain yield

The results of analysis of variance showed that location, nitrogen and seed priming by salicylic acid significantly affected grain yield (Table 3). The averages of grain yield of different treatments in Zanjan location were 2807 kg ha^{-1}, while under Khoramdareh location these values significantly increased to 3067 kg ha^{-1} (Table 4).

Increased nitrogen level also increased the grain yield of pinto bean, although the increased were high for 20 kg ha^{-1} N than for 80 kg ha^{-1} (Figure 2 D). Furthermore, application of 20, 40 and 80 kg ha^{-1} N increased grain yield by 10%, 3.5% and 5.4%

respectively, compared to the control plants (Figure 2 D). Our results are in line with findings of Rabieyan *et al.* (2011) who reported that both vegetative and grain yield of chickpea increased by application of nitrogen fertilizer and bio-fertilizer inoculation. Jalali *et al.* (2010) with perform tow experiment in two years about effect of nitrogen fertilizer and organic matters on yield component of maize was recorded that, the highest grain yield, number of grains per ear, 100 grain weight from 250 kg ha^{-1} urea fertilizer.

In the present study the seed priming with SA increased the chlorophyll index, leaves area, dry weight of leaves, stem length and dry weight of stem in the flowering stage and improvement grain yield (Figure 1). Seed priming with 2250 µM SA increased grain yield compared to other treatments were 2289, 3054, 3444 and 3683 kg ha^{-1} yield achieved in 0, 750, 1500 and 2250 µM SA, respectively (Figure 1 F). However, hydro priming could not affect this grain yield and not signification by control. Pak Mehr *et al.* (2011) reported that seed priming with salicylic acid increased pods length, number of pods, number of seeds, 100 seed weight, biomass, yield and harvest index of cowpea (*Vigna unguiculata* L.) in both irrigated and water deficit conditions. Harris *et al.* (1999) and Mansouri and Aboutalebian (2013) reported that seed priming improves crop establishment in many crop which results in faster development, earlier flowering and maturity and higher yield.

Correlation of grain yield with all morphology and physiology traits was significantly positive at 1% level of probability in the two locations (Table 5).

Conclusion

According to the results obtained, seed priming with salicylic acid increased the chlorophyll index, leaf area, dry weight of leaf, stem length and dry weight of stem in the flowering stage and improvement grain yield. Seed priming with 2250 µM SA the better compared to other salicylic acid levels and hydro-priming on agronomy characteristics of pinto bean. Also, increased nitrogen level also increased the grain yield of pinto bean, although the increased were high for 20 kg ha^{-1} N than for 80 kg ha^{-1}.

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