Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 6, p. 477-486, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

The effect of application of different levels of axing and gibberellins at different growth stages on quantitative and qualitative production components of field bean

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Article published on June 19, 2014

Key words: Field bean, production components, auxin and gibberellins hormones.

Abstract

This experiment was carried out in Shahid Salemi experimental field of Islamic Azad University of Ahvaz, In Northeast of Ahvaz at latitude 31°20´ N and longitude 48°40´ E and 22.5 m above the sea level in the summer of 2010. The experiment was carried out as split plot in the form of randomized complete block design with three replications. The main treatment included three different growth stages of crop (V_0 = vegetative phase, V_1 = flowering phase, V_2 = podding and grain filling phase), and the sub treatment included 4 levels of auxin hormone (A_0 = 0, A_1 = 3, A_2= 7, A_3 = 20 ppm) and three levels of gibberellins (G_0 = 0, G_1 = 5, G_2 = 15 ppm). Grain yield, biological yield, harvest index, and protein percentage of grain were affected by experimental treatments. In evaluating the main effects, the highest rate of studies traits was obtained at growth stages in V_2 phase. Moreover, due to the application of 7ppm auxin and 15ppm gibberellins the highest rate of studied traits was obtained. In studying the interactive effect of growth stages and auxin hormone, the highest rate of grain yield was obtained during podding and grain filling stage and application of 7ppm auxin. In addition, investigating the interactive effect of growth stages and gibberellins showed that the highest rate of grain yield was obtained at V_2 phase and application of 15ppm gibberellins. Examining the interactive effect of auxin and gibberellins showed that the highest rate of grain yield was obtained through the application of 7ppm auxin and 15ppm gibberellins.

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Introduction

Legumes are the main diet of many poor people around the world because the rich amount of protein in grain of such crops in combination with grain cereals can provide a valuable bio food product. In poor and populous countries of the world like India by the consumption of 7.11 kg per capita, the contribution of legumes to peoples' diet is more than other countries. Also, in Iran legumes play an important role in people nutrition by the consumption of 8.4 kg per capita, even though its consumption is 6.1 kg lower that the average world consumption. In addition to high amount of protein and nutrition, the consumption of beans is highly useful for health. Bean with high level of production and cultivation, which is ranked in the first place among legumes, is one of the most valuable legumes in terms of palatability and protein quality. To achieve maximum crop yield external growth regulators can be effective because these hormones can develop production components of plant and its yield components such as the increase of podding and grain weight, prevention of flowers loss given that the flower loss in legumes is about 30% to 50% and application of different levels of hormones can reduce the loss (In Khuzestan this problem is exacerbated due to the heat). Determining the best period of time for application of hormone in bean fields, increasing the growth period and preventing the loss of leaves (due to hot weather in Khuzestan, aging and loss of leaves occur very quickly and the growth period is decreased which causes the decrease of dry matter accumulation and yield) are important parts of qualitative components of protein yield and the change of different levels of this component is evaluated in different treatments.

Sumer Field *et al.,* (1981) have stated that official estimation of cowpea seed production is unreliable and it is already impossible to achieve accurate information about it because most of the world's crops are grown on subsistence farming in developing countries and are not reported. Cowpea has not much entered the world trade even though it has been available for several years in European retail sales. In released statistics on world food production no information is recorded except the recent data published by FAO on legumes or Brazilian crops entitled "dry beans (Frijoles Secos)" which is mostly related to common beans. Even there is no statistics on bean production in China and the data which is reported as legumes production in the Indian subcontinent involves a large number of legumes so that the ratio of cowpeas might not be inferred (Steel, 1985).

Gibberellic acid is a hormone which plays a very important role in different processes of plants physiology including seeds germination, endosperm stimulation for preparing assimilates to be used by various organs of plants, stem growth, flowering, and enhancing flowering capability.

Negatia *et al.,* (2002) investigated the effect of levels and timing of application of gibberellic acid on growth and yield components of common bean and concluded that leaf area index greatly changed after the application of gibberellic acid at different times. Moreover, the best time to spray gibberellic acid was determined to be 14 days after germination, because the highest increase of growth factors such as leaf area index occurred during this time, so that the comparison of grain yield with concentration of 7.5 mg/l showed that the yield of the crops which had received gibberellins 14 days after flowering stage was nearly three times as much as those that had received the hormone 7 days after flowering stage. Moreover, the harvest index increased in beans which had received gibberellins 14 days after germination. As a result, application of gibberellins will cause the increase of leaf area, dry weight, number of grains and pods, and consequently total dry weight of bean and grain yield of legumes.

By studying the effect of levels and timing of application of gibberellic acid on growth and yield components of common bean via spraying gibberellic acid with concentration of 2.5, 5, 7.5 mg/l to the whole crop of common bean 7, 14, and 28 days after

planting, Negatia *et al.,* (2002) stated that gibberellins led to the increase of stem height and leaf area index and the decrease of sunlight absorption in common bean.

Studying the yield of common bean in different weather conditions, Mebora *et al.,* (1963) stated that it is possible to increase the large mass of dry matter in crops through the selection of appropriate genotypes, application of sufficient nitrogen, and effective irrigation. Moreover, application of external plant growth regulators such as gibberellic acid stimulates dry matter accumulation and consequently increases the crop.

Sabio *et al.,* (2003) have stated that the most important effect of auxin on crops is the growth of stem length. There is an overlap between gibberellic acid and auxin in Arabidopsis and the simultaneous application of gibberellins and auxin lengthens the roots; however, since auxin accumulates in shoot apical meristem and causes apical dominance, by cutting off the meristem which is the source of auxin, the root growth increases compared to the time when gibberellins alone causes the root growth and this reaction is due to the interactive effect of gibberellic acid and auxin.

Setterfield (1963) examined the effect of gibberellins with concentration of 0.1-1 mg/l along with auxin on artichoke and stared that as gibberellic acid didn't have a positive effect on mitotic division and together with high concentration of auxin it led to the increase of plant fresh weight due to the increase of cells volume and size.

Wagner *et al.,* (2004) investigated the effect of gibberellic acid and auxin on soybeans that had low height and low yield. They concluded that spraying gibberellins with the concentration of 50 mg/l onto soybean leaves at vegetative stage would lead to the increase of stem length and early internodes of stem and also the increase of stem thickness and consequently, the increase of total dry matter.

Shadad *et al.,* (1990) declared that spraying gibberellic acid onto bean leads to the increase of leaf area, rate of relative growth, and continuity of leaf area. Gibberellins also cause intercellular differentiation. In woody plants, gibberellins cause the stimulation of vascular cambium to produce late phloem. In general, grain growth results from metabolism reaction changes from catabolism to anabolism form and gibberellins will increase activities or synthesis of a particular group of enzymes which will change the metabolism of two-carbon pieces and lead to the synthesis of intermediate compounds.

Specifications of the Experiment Site

The experiment was carried out in the summer of 2010 in experimental field of Shahid Salemi belonging to Islamic Azad University of Ahvaz, in the northeast of Ahvaz at latitude 31°20´ N and longitude 48°40´ E and 22.5 m above the sea level.

Soil Profile of the Experiment Site

In order to determine physical and chemical characteristics of soil in the area, separate sample were taken from 6 spots of the experimental filed soil and a compound sample was prepared by mixing the samples and was carried to the laboratory for analyzing the soil (Table 1).

Field Experiments

Cultivation was done in Shahid Salemi field in Ahvaz and before sowing the land was completely smoothed with disc and then the resulted clods were crushed so that the planting ground become quite hollow and soft. The seeds already got disinfected by chemicals and were sowed in isles. The seed planting depth was about 2 to 3 cm. the plants were spaced about 30 to 40 cm from each other and the space between two planting lines was 90 cm. Immediately after sowing the seeds, and before the beans germination, chemical struggle got started to destroy the weeds. It should be noted that the land should be slightly wet while spraying. In general, in planting beans, weeding is required 2 or 3 times.

The experiment was carried out as split plot in the form of randomized complete block design with three replications. The main treatment included three different growth stages of crop (V_0 = vegetative phase, V_1 = flowering phase, V_2 = podding and grain filling phase), and the sub treatment included 4 levels of auxin hormone (A_0 = 0, A_1 = 3, A_2= 7, A_3 = 20ppm) and three levels of gibberellins (G_0 = 0, G_1 = 5, G_2 = 15ppm). The treatments were designed based on the amount of the hormones in plant and included little, medium and much amounts. All treatment hormones were sprayed on plant during different growth stages. In order to spray little amounts of hormone manual sprays were used during the research.

Estimating Dry Matter Accumulation

In order to study the dry matter accumulation trend every 14 days samples were taken from each plot and biological yield was calculated in final harvest by calculating the grain yield by the following formula, the harvest index was measured too.

HI (%) = Ys / Yb× 100

After the final harvest, 10 g grain from each treatment was ground and sent to the laboratory to measure the percentage of protein.

Statistical Calculations

The obtained results were analyzed by SAS software and the means were compared via LSD Test. To draw diagrams, Excel software was used.

Results and Discussion

Grain Yield

Three factors of growth stages, auxin and gibberellins significantly increased the grain yield of common bean compared to the control treatment (Table 2). Growth enhancer hormones particularly different kinds of auxin, gibberellins, and cytokinin will affect growth and yield of crops (Zahir *et al.,* 2004). The interactive effects of growth stages x auxin, growth stages x gibberellins, and also auxin x gibberellins caused significant differences on grain yield at level of 5 % (Table 2). In some cases, the effect of growth regulator hormones affected the ecological needs for the emergence of vegetative phase. For instance, Gibberellins can provide the conditions for the plant to enter vegetative phase as an alternative to photoperiodic needs of plant and even sometimes it can be an alternative to vernalization. This could be due to special vital and biochemical reactions (Tajbakhshi and Ghiasi, 2008).

Table 1. The results of physical and chemical characteristics of experimental field soil.

Soil tissue	Constituent soil (%) particles			of Organic material (%)	Saturation Percentage (SP)	PH	Ec	Soil depth (cm)
	sand	silt	clay				mmhos/cm	
Silty	24	35	38	0/574	48/6	6/67	3/75	0-30
clay loam	23	33	39	0/598	50/1	6/87	5/32	30-60

In grain filling stage, auxin concentration has a major role in regulating grain filling and growth (Brenner and Cheikh, 1995). Different growth stages of bean affected the grain yield and the highest grain yield was obtained at podding and grain filling stage (phase), and the lowest grain yield was observed at vegetative stage. The treatment with 3 ppm auxin had no significant effect on grain yield compared to the control treatment while the treatment with 7ppm auxin had a significant effect on grain yield and showed the highest yield and then in the treatment with 20 ppm auxin the grain yield decreased. Gibberellins also affected the grain yield and the highest grain yield belonged to the treatment with 15ppm gibberellins by 1965 kg/ha and also the lowest grain yield belonged to the control treatment by 1611 kg/ha. The mean comparison results of the interactive effects of growth stages and auxin showed that the highest grain yield belonged to the treatment with 7ppm auxin at podding and grain filling stage (phase)

by 2455 kg/ha and the lowest grain yield belonged to the control treatment at vegetative stage by 1507.5 kg/ha. There are some reports that indicate seed priming with optimal concentrations of plant growth hormones has effectively increased germination, emergence, growth, and yield of different species of crops (Pakmehr, 2009; Lee *et al.,* 1998).

Table 2. The ANOVA of quantitative and qualitative production components of field bean.

Mean square				
Protein percentage	Harvest index (%)	Biological yield	Grain yield	Variation source
0/72 ns	0/83 ns	2/47 ns	1/03 ns	Replication
10/28*	289/45**	259/23 **	32/51**	Growth stages (V)
17/93 *	137/41*	120/32 **	11/51*	Auxin hormone (A)
19/32 *	197/41*	98/51 *	27/41*	Gibberellins hormone (G)
11/21*	82/5**	111/51**	99/51 *	Growth stages × Auxin
19/53 *	102/11*	43/21*	17/21*	Gibberellins × Auxin
27/11**	92/33 *	72/51**	19/71*	Growth stages × G
5/17	9/11	17/21	11/51	Variations coefficient (%)

**: significant at 1% level *: significant at 5% level Ns: non-significant difference.

Application of appropriate concentration of gibberellins highly affects the yield and yield components of lots of crops such as soybean (Hedden and Philips, 2000). The highest grain yield belonged to growth stages treatments. Mukherjee and Prabhakar (1980) reported that the increase of gibberellic acid concentration up to 10 ppm led to the increase of rice yield per pot, but at concentration of 100ppm gibberellic acid the grain yield decreased.

The mean comparison results of the interactive effects of growth stages and gibberellins showed that the highest grain yield belonged to the treatment with 15ppm gibberellins at podding and grain filling stage by 2212.5 kg/ha and the lowest grain yield belonged to the control treatment at vegetative stage by 1530.5 kg/ha. The mean comparison results of the interactive effects of auxin and gibberellins showed that the highest grain yield belonged to the treatment with 7 ppm auxin and 15 ppm gibberellins. High rates of gibberellins, auxin, abscisic acid and cytokinin at grain development stage have been reported by plenty of researchers. The level of hormones changes during the grain development stage. The results of the experiment showed that hormone treatments used in this experiment improved the growth and yield of crop and finally increased the grain weight in the heap and prevented the weight loss under stress conditions particularly in sensitive cultivar of Hoveizeh (Mukherjee and Prabhakar, 1980). Madah *et al.,* (2006) concluded that salicylic acid spray rather than its irrigation had a better effect on yield and yield components of pea and among different concentrations of salicylic acid the concentration of 0.7mM had often the best effect and the lowest yield and yield components belonged to the treatment with concentration of 0.1 mM and through irrigation. Yang *et al.,* (2003) reported that the low concentration of auxin and abscisic acid in rice grains is directly associated with producing small grains and ultimately the reduction of grain yield.

Biological Yield

Growth stages, auxin and gibberellins and also their interactive effect on biological yield of field bean were significant (Table 2). The highest biological yield belonged to the treatment with 7 ppm auxin by 5250 kg/ha and then it decreased at concentration of 20 ppm. The lowest biological yield belonged to the

treatment without application of gibberellins (table 3). Among plant hormones, auxins and ethylene play an important role in development of root system and ultimately the yield of plants (Torres Rubio *et al.,* 2000). Excessive amount of auxin can have an inhibitory effect on growth indices particularly on root (Etesami and Alikhani, 2011) and ultimately crops yield.

Table 3. Mean comparison of the main effects of quantitative and qualitative production components of field bean.

Mean square				
Protein percentage	Harvest index (%)	Biological yield	Grain yield	Variation source
				Growth stages (V)
17/81 ^{b}	33/14 ^{b}	4375 ^{c}	1450 ^{c}	V_0
18/71 ^{b}	46/96 ^{a}	4525 ^{b}	2125 ^{b}	V_1
24/51 ^{a}	47/49 ^{a}	5180 ^{a}	2460 ^{a}	V_2
				Auxin (A)
16/2 ^{c}	37/16 ^{c}	4211 ^{d}	1565 ^{d}	A_0
17/5 ^{bc}	37/74 ^{c}	4425 ^{c}	1670 ^{c}	A_1
19/21 ^{a}	46/67 ^{a}	5250 ^{a}	2450 ^{a}	A_2
18/1 ^{b}	43/41 ^{b}	5010 ^{ab}	2175 ^{b}	A_3
				Gibberellins (G)
18/5 ^{c}	40/12 ^{b}	4015 ^{b}	1611 ^{c}	G_0
20/5 ^{b}	41/09 ^{b}	4125 ^{b}	1695 ^{b}	G_1
22/7 ^{a}	43/41 ^{a}	4526 ^{a}	1965 ^{a}	G_2

Similar letters in each column mean non-significant difference at 5% level via LSD Test.

The mean comparison results showed that different growth stages of been affected the biological yield. The lowest biological yield was observed at vegetative stage and the highest biological yield was observed at podding and grain filling stage by 5180 kg/ ha. As auxin concentration increased, biological yield increased too. The highest biological yield belonged to the treatment with 7 ppm auxin by 5250 kg/ha. The increase of gibberellins concentration led to the increase of biological yield so that the highest biological yield belonged to the treatment with 15 ppm gibberellins. The mean comparison results of the interactive effects of different growth stages and auxin showed that the highest biological yield belonged to the treatment with 7 ppm auxin at podding and grain filling stage by 5215 kg/ha. The mean comparison results of the interactive effects of different growth stages and gibberellins showed that different concentrations of gibberellins at vegetative and flowering stages were not significantly different in terms of biological yield and the highest biological yield belonged to the treatment with 7 ppm gibberellins at podding and grain filling stage by 4853 kg/ha. The results showed that biological yield in control treatment and 3 ppm gibberellins treatment was not significantly different. The highest biological yield belonged to the treatment with 7ppm auxin and 15 ppm gibberellins by 5250 kg/ha.

Harvest Index

Harvest index is one of the most important biological indices which indicates assimilates mobilization from vegetative organs of plant into grains. It seems that this index will change differently under environmental stress and particularly its occurrence time. Harvest index is controlled by genetic and

environment but the role of genetic in controlling this trait is relatively more (Zecevic and Knezevic, 2005). The harvest index indicates the percentage of organic matter mobilization from the source to the reservoir; as a result, plants with high harvest index are able to transfer more carbohydrates from green organs to grains and consequently have high yield (Pakmehr *et al.,* 2011). The effect of growth stages and the interactive effects of auxin and growth stages on harvest index were significant at 1% level and the effect of different concentrations of auxin, gibberellins and the interactive effect of auxin x gibberellins and growth stages x gibberellins on harvest index of bean were significant at 5% probability level (Table 2). The maximum rate of free auxin was related to the time before the root growth and simultaneous with the onset of swelling and water absorption (Bialk *et al.,* 1992). Auxin is released from its storage during the germination Scottish pine seeds (Lai and Liang, 2000). The highest rate of bean harvest index was obtained at the last stage of growth stages (podding and grain filing) and the lowest rate of harvest index was observed at the first stage of bean growth stages (vegetative phase) (Table 3). Fischer *et al.,* (1998) reported that the yield improvement is correlated with the increase of harvest index and particularly with the increase of grain/m^2.

Pakmehr (2009) reported that priming seeds with salicylic acid led to the increase of green index and percentage, leaf area, relative water content, photosynthesis rate, transpiration intensity, stomatal conductance, chlorophyll content, leaf proline content, cell membrane stability, and reduction of the number of remained days to flowering, co2 rate within the stomata and soluble sugar content of the leaf. Inhibition of gibberellins biosynthesis may delay the pollination so that adding external gibberellins removes this effect (Jacobson and Olszewski, 1993). Since various hormones like auxin might increase the germination ability of seeds and seedling vigor in some plants in salinity conditions (Balestri and Bertini, 2003), it could be said that some effects of salinity on germination such as the growth of rootlets and stemlet are due to its effect on hormonal activities. It is reported that some auxins such as IAA during the seed germination increase percentage of germination and growth of coleoptiles (Rekoslavskayal *et al.,* 1999). The increase of stem length in treatment with gibberellic acid might be due to the role of this substance in facilitating plant growth, so that by stimulating and accelerating cellular division, increasing the cell length and enlarging it, gibberellic acid affects the plant growth rate (Al-Khassawneh *et al.,* 2006). Gibberellins are a group of plant growth enhancers which interfere at many stages of plant growth and development including stem elongation, seeds germination, and growth of reproductive organs. Growth facilitation by gibberellic acid is due to high demands for total soluble sugars due to the increase of expression of invertase. Different hormones such as auxin and gibberellic acid affect the expression of invertase. Hexoses of 6-carbon sugars glucose and fructose, which are produced in target tissues by invertase activity, are not only the source of carbon and energy for plant growth but also provide the necessary force and energy for cell elongation by reducing the osmotic potential of the cell and increasing water absorption. The growth of tissue depends on both cell expansion and cell proliferation. This process depends on a complex network of messages such as hormones like gibberellic acid. Moreover, active growth of tissue causes phloem unloading through increasing the activity of target consumption spot which is indirectly related to the increase of invertase activity. Therefore, with regard to sucrose metabolism there should be a balance between developmental messages including hormones and real metabolic status of cells (Gonzalez *et al.*, 1999).

The mean comparison results showed that there was no significant difference between treatment with 3 ppm auxin and control treatment and the highest harvest index was observed in treatment with 7 ppm auxin. The lowest harvest index belonged to vegetative stage. There was no significant difference

between flowering, podding and grain filling stages. Different concentrations of gibberellins had a significant effect on harvest index. As gibberellins concentration increased, harvest index increased, too so that the highest rate of harvest index belonged to treatment with 15 ppm gibberellins by 43.41%. The highest rate of harvest index due to the interactive effects of growth stages and auxin was related to the treatment with 7 ppm auxin at podding and grain filling stage. The lowest rate of harvest index was observed in control treatment at vegetative stage. The mean comparison results showed that there was no significant difference between the harvest index of treatments at flowering stage ×gibberellins and podding and grain filling stage × gibberellins. The highest rate of harvest index due to the interactive effects of auxin and gibberellins belonged to treatment with 7ppm auxin ×15 ppm gibberellins by 45.16%.

Table 4. Mean comparison of interactive effects of quantitative and qualitative production components of field bean.

Mean square				
Protein percentage	Harvest index (%)	Biological yield	Grain yield	Variation source
				Growth stages ×Auxin
17 ^{c}	35/11	4293	1507/5	$V_0 A_0$
17/65 ^{bc}	35/45	4400	1560	$V_0 A_1$
18/51 ^{b}	40/51	4812/5	1950	$V_0 A_2$
17/95 ^{b}	38/62	4690/5	1812/5	$V_0 A_3$
17/45 ^{bc}	42/23	4368	1845	$V_1 A_0$
18/1 ^{b}	42/4	4475	1897/5	$V_1 A_1$
18/96 ^{b}	46/8	4887/5	2287/5	$V_1 A_2$
18/4 ^{b}	90/23	4767/5	2150	$V_1 A_3$
20/35 ^{a}	42/86	4695/5	2012/5	$V_2 A_0$
21 ^{a}	42/99	4802/5	2065	$V_2 A_1$
21/86 ^{a}	47/07	5215 ^{a}	2455 ^{a}	$V_2 A_2$
21/3 ^{a}	45/48	5095	2317/5 ^{b}	$V_2 A_3$
				Growth stages × Gibberellins
18/15 ^{d}	36/48 ^{d}	4195 ^{f}	1530/5 ^{f}	$V_0 G_0$
19/15 ^{cd}	37 ^{d}	4250 ^{f}	1572/5 ^{f}	$V_0 G_1$
20/25 ^{c}	38/36 ^{c}	4450/5 ^{d}	1707/5 ^{e}	$V_0 G_2$
18/6 ^{d}	43/74 ^{b}	4270 ^{ef}	1868 ^{d}	$V_1 G_0$
19/6 ^{c}	44/16 ^{ab}	4325 ^{e}	1910 ^{c}	$V_1 G_1$
20/7 ^{c}	45/18 ^{a}	4525/5 ^{c}	2045 ^{b}	$V_1 G_2$
21/5 ^{b}	44/27 ^{ab}	4597/5 ^{c}	2035/5 ^{b}	$V_2 G_0$
22/5 ^{b}	44/65 ^{ab}	4652/5 ^{b}	2077/5 ^{b}	$V_2 G_1$
23/6 ^{a}	45/59 ^{a}	4853 ^{a}	2212/5 ^{a}	$V_2 G_2$
				Auxin × Gibberellins
17/35	38/6 ^{g}	4113 ^{h}	1588 ^{g}	$A_0 G_0$
18/35 ^{b}	39/1 ^{f}	4168 ^{gh}	1630 ^{f}	$A_0 G_1$
19/45 ^{a}	40/4 ^{e}	4368/5 ^{f}	1765 ^{e}	$A_0 G_2$
18 ^{b}	38/87 ^{f}	4220 ^{g}	1640/5 ^{f}	$A_1 G_0$
19 ^{ab}	39/35 ^{f}	4275 ^{g}	1682/5 ^{ef}	$A_1 G_1$
20/1 ^{a}	40/6 ^{e}	4475/5 ^{e}	1817/5 ^{d}	$A_1 G_2$
18/85 ^{ab}	43/83 ^{c}	4632/5 ^{c}	2030/5 ^{b}	$A_2 G_0$
19/85 ^{a}	44/21 ^{b}	4687/5 ^{c}	2072/5 ^{b}	$A_2 G_1$
20/95 ^{a}	45/16 ^{a}	4888 ^{a}	2207/5 ^{a}	$A_2 G_2$
18/3 ^{b}	41/95 ^{d}	4512/5 ^{d}	1893 ^{c}	$A_3 G_0$
19/3 ^{a}	42/36 ^{d}	4567/5 ^{cd}	1935 ^{c}	$A_3 G_1$
20/4 ^{a}	43/41 ^{c}	4768 ^{b}	2070 ^{b}	$A_3 G_2$

Similar letters in each column mean non-significant difference at 5% level via LSD Test.

Grain Protein Percentage

The mean comparison results showed that different concentrations of auxin, gibberellins, and growth stages caused the increase of grain protein percentage in field bean (Table 3). There was significant difference between the effect of different concentrations of auxin, gibberellins, and growth stages and also the interactive effects of auxin × growth stages, and auxin × gibberellins on protein percentage of field bean at 5% level. The interactive effects of growth stages × gibberellins on harvest index were significant at 1% level (Table 2).

The rate of grain protein in rice increased as the concentration of gibberellic acid increased up to 10ppm and then it decreased (Mukherjee and Prabhakar, 1980). In this experiment, as the concentration of gibberellins increased up to 15 ppm the highest rate of grain protein in field bean was obtained. Gilroy and Jones (1994) inserted gibberellins as direct microinjection into single protoplasts of barley aleurone and controlled the expression and secretion of amylase with special glucuronidase structures or starch digestion and concluded that microinjection of a little gibberellins (250 μM) didn't stimulate amylase formation while the treatment of one protoplast with internal gibberellins stimulated amylase formation. Obviously, when gibberellins and abscisic acid appear in aleurone protoplast this way, these compounds don't become active; now, there is credible evidence that verify the presence of gibberellins in plasma membrane for receiving gibberellins in aleurone cells. All these experiments provide evidence that gibberellins do not really work like steroid hormones. A binding protein that is able to link a gibberellins receiver has not been known so far. There was a significant difference between the rate of grain protein in bean at vegetative stage and podding and grain filling stage. The highest percentage of grain protein was obtained at podding and grain filling stage by 24.51%. However, the rate of grain protein was not significantly different at vegetative stage and flowering stage and was 17.81 and 18.71% respectively. As auxin concentration increased up to 7ppm, grain protein percentage increased compared to the control treatment and reached 19.21% and then through the increase of auxin from 7 to20 ppm it reduced to 18.1%. Gibberellins could affect the rate of grain protein in field bean, so that as gibberellins increases grain protein percentage increases, too. The lowest grain protein percentage belonged to the control treatment by 16.2% and the highest grain protein percentage belonged to the treatment with 15 ppm gibberellins by 22.7%. The mean comparison results of the interactive effects of growth stages and different concentrations of auxin on grain protein percentage of field bean showed that there was not any significant difference between different concentrations of auxin at each one of vegetative, flowering, podding and grain filling stages compared to the control treatment. The mean comparison results of the interactive effects of growth stages and different concentrations of gibberellins showed that the highest percentage of grain protein belonged to the treatment of 15 ppm gibberellins at podding and grain filling stage. Moreover, the results of the interactive effect of auxin and gibberellins showed that the highest percentage of grain protein was observed in the treatment with 7 ppm auxin on grain protein percentage of field bean showed that there was not any significant difference between × 15ppm gibberellins by 20.95% (Table 4).

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