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EFFECTS OF GROWTH REGULATORS ON PROXIMATE COMPOSITION, ASCORBIC ACID AND AMINO ACID CONTENT OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.)

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Abstract

Crude protein content of fresh leaf, pod and dry seed; and ascorbic acid, crude fiber and ash contents of fresh pods of French bean were significantly increased with GA_3 (50 to 110 ppm) and NAA (50 to 90 ppm) application, whereas carbohydrate and crude fat contents decreased. GA_3 and NAA increased lysine, glutamic acid, aspartic acid, arginine and tyrosine contents, while GA_3 decreased leucine in dry seeds. Amino acids like isoleucine, leucine, lysine, methionine, threonine, valine, histidine, aspartic acid, glutamic acid, arginine, glycine, serine, alanine and tyrosine were increased by 50 ppm NAA treated seeds, which was followed by 70 ppm GA_3 except isoleucine and leucine which were decreased.

Introduction

French bean is a major source of dietary protein in the countries of South America and many others of the third world. It is easily available and cheaper compared to the animal based protein. Its premature succulent pods and raw grains are consumed as vegetables which contain amples amount of biochemical properties along with ascorbic acid content of crop plants (Rashid 1999). Growth regulators at the gene level; profoundly stimulate the transcriptional and translational mechanisms of protein synthesis (Key 1969, Fattah 1971). Gibberellic acid (GA_3), if applied in low concentration increases the nutritive qualities, including ascorbic acid content of the edible portions of the cultivated species (Ouzounidou *et al.* 2010, Singh *et al.* 2011). Similarly, NAA is a synthetic plant growth regulator in the auxin family, which enhances these parameters in a number of crops (Sridhar *et al.* 2009). GA_3 increases amino acids in seed and thus improves protein quality in cashew (Hariharan and Unnikrishnan 2005) and in Bengal gram (Desai and Khanvilkar 1977). In the same light, NAA increases amino acids in dry seed of legume crops (Dhingra *et al.* 1995, Karim *et al*. 2006, Ullah *et al*. 2011).

However, information regarding the effect of PGRs especially of GA_3 and NAA on ascorbic acid, proximate composition and amino acid contents of French bean are scanty. Therefore, the present investigation was carried out to study the effects of GA_3 and NAA on leaf, pod and seed crude protein; pod crude fiber, ash, ascorbic acid and amino acids in dry seeds of French bean.

Materials and Methods

A field experiment was conducted at the research field (23°44′23.3″ N latitude and 90°23′03.8″ E longitude) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka during the Rabi season of November 2010 to February 2011 in a RCBD with three replications. The unit plot size was 2.0 m × 1.5 m. There were nine treatments, *viz.* T_0: control

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(distilled water spray), T_1 : 50 ppm GA_3, T_2 : 70 ppm GA_3 , T_3 : 90 ppm GA_3, T_4 : 110 ppm GA_3, T_5 : 50 NAA, T_6 : 70 ppm NAA, T_7 : 90 ppm NAA and T_8 : 110 ppm NAA. The foliar treatments were sprayed at 18 days after sowing (DAS). Cow-dung was applied at the rate of 2.0 Kg/plot, and N, P, K, S and B were applied at 58.8, 22.8, 15.0, 16.7 and 1.8 g/plot in the form of urea, triple super phosphate, muriate of potash, gypsum and borax respectively, according to Fertilizer Recommendation Guide (2005). After adding cow-dung, the soil was left for two weeks to condition it. Along with cow dung, P, B and S containing fertilizers were added entirely as basal. One third of N and K were applied at final land preparation and, rest two third were top dressed at 20 and 40 DAS, respectively. Seeds were sown on November 15, 2010 using a spacing of 30 cm × 15 cm at a depth of 3.00 cm. Furrow irrigation was given at an interval of 7 days. Weeding was done at an interval of 10 days.

Quantitative determination of crude protein of fresh leaf samples and ascorbic acid, total carbohydrate, crude protein, ash, crude fat and crude fiber contents of fresh pods samples at 58 DAS (pod setting stage) and crude protein of dry seed samples at harvest of French bean were measured following methods of AOAC (2005).

The amino acid composition in dry seeds sample (at harvest) were determined for some selected amino acids like isoleucine, leucine, lysine, methionine, threonine, valine, histidine, aspartic acid, glutamic acid, arginine, glycine, serine, alanine and tyrosine by an Amino acid analyzer (Schimadzu, Japan), according to Alfasane *et al*. (2008).

All the data except those of amino acid were subjected to ANOVA and the treatment means were compared by LSD test at 5% level of significance (Gomez and Gomez 1984).

Results and Discussion

GA_3 with 50 ppm concentrations (T_1) increased the fresh leaf crude protein contents (4.04%) while, NAA at 50 ppm (T_5) showed the highest value (4.15%) at 58 DAS (Fig. 1). The findings are in agreement with Jeyakumar *et al*. (2008) who recorded higher soluble protein in black gram leaf sprayed with 40 ppm NAA at pre-flowering stage compared to the non sprayed plants. Similarly, T_1 showed the highest crude protein in fresh pods (4.80%) which were significantly higher than the other treatments. T_3 and T_4 showed similar values as was observed in the control. Likewise T_5 showed significantly the highest crude protein in fresh pods (5.05%) which was also significantly higher than the other treatments. T_0 and T_8 had identical values which were even lower than T_6 (4.62%). T_3 and T_4 showed the similar values (4.53 and 4.51%) which were identical as was observed with the control (3.50%).These findings agree well with the results of Fattah and Jahan (1976) who working on ripened tomato reported that 50 ppm GA_3 significantly increased protein content (26.19%) over the control. Olaiya *et al*. (2010) worked on tomato (genotype JM 94/47) and found that the crude protein was increased by the application of all the bioregulators tested (IAA, IBA and NAA at 60, 100 and 140 mg/l, respectively) compared to the control.

NAA with 50 ppm increased dry seed crude protein in this study (Fig. 2). The findings are in close accord with the results of Ullah (2006), who found highest crude protein (27.48%) over that obtained with 50 and 70 ppm NAA treatments in cowpea seed. Begum (2010) recorded the maximum protein content in mustard seeds spraying 70 ppm NAA as foliar application. It was found in another report that, GA_3 with a wide range of concentration increased seed protein percentages in chickpea cultivars (Kaya *et al*. 2010). This might be attributed to the higher N content in leaf which mobilized to the seeds through pods of the French bean of this study. Growth regulators increase DNA and subsequently this led the increase in RNA synthesis, which might

have resulted in a considerable increase in protein synthesis and seed protein (Nakamura and Takahashi 1971, Key 1969).

Total carbohydrate content of fresh pod (% of fresh wt.) of French bean reduced significantly due to GA_3 and NAA treatments at 58 DAS growth stage. It ranged from 5.718 to 8.028% (Table 1). The maximum decrease of total carbohydrate content of fresh pod was recorded for T_5, which was similar to T_1 (5.726%), showed a decrease of 28.77 and 28.68%, respectively in comparison with the control. However, the maximum value was observed for control (T_0). Similarly, Olaiya *et al*. (2010) reported that the total carbohydrate content in the plants treated with the bioregulators were statistically lower for treated tomato compared to the control.

From the results of experiment it was found that, GA_3 and NAA significantly increased fresh pod crude fiber of French bean. The maximum values (1.843%) were recorded from T_1, followed by T_5 (1.764%). These were increased by 18.14 and 13.08%, respectively over the control. The minimum was found for T_8 (1.490%) (Table 1). GA_3 (50 to 110 ppm) significantly increased pod fiber compared to the control. The evidence of increasing pod crude fiber due to GA_3 application in French bean is scanty, but it was found to be increased in fruits of lady's finger (Fathima and Balasubramanian 2006) which supports the results as observed in the present study.

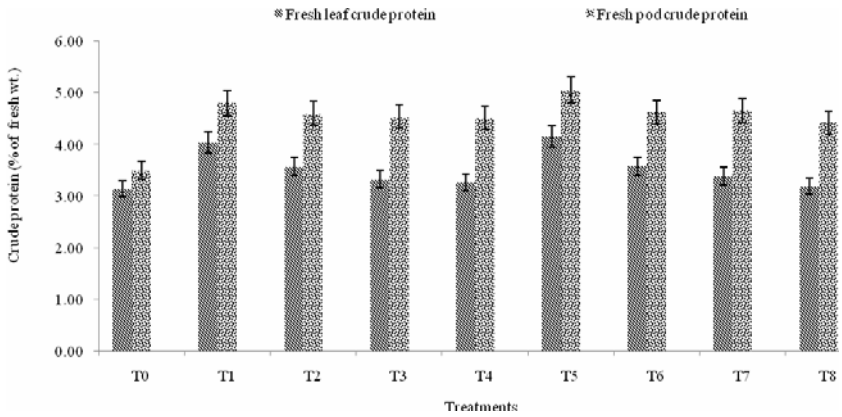

Fig. 1. Effect of GA_3 and NAA on fresh leaf and pod crude protein contents (% of fresh wt.) of French bean (Mean ± standard error); T_o = Control, T_1 = 50 ppm GA_3 , T_2 = 70 ppm GA_3, T_3 = 90 ppm GA_3, T_4 = 110 ppm GA_3, T_5 = 50 ppm NAA, T_6 = 70 ppm NAA, T_7 = 90 ppm NAA, T_8 =110 ppm NAA.

The data in Table 1 indicated that, GA_3 and NAA had significant influence on fresh pod ash content of French bean. It ranged from 0.615 to 0.851% at 58 DAS. The maximum was observed for 50 ppm NAA, followed by 50 ppm GA_3, which were 38.37 and 35.12% higher over those for the control . The findings of this parameter was supported by the work of Olaiya *et al.* (2010), who reported that the ash content was higher compared to the control due to the application of IBA, IAA and NAA (60 to 140 mg/l) in tomato.

GA_3 and NAA treatments significantly decreased crude fat of fresh pod showing the lowest for T_5 compared to T_0. Low values for fat content in treated tomatoes were also reported by Olaiya *et al.* (2010).

GA_3 and NAA with different concentrations slightly increased pod moisture, which ranged from 87.62 to 88.83%. It was maximum in 50 ppm NAA treated plants, followed by 50 ppm GA_3. On the contrary the minimum was recorded for the control. Previously it was reported that PGRs increased leaf moisture, plant's fresh and dry weight of rapeseed (Begum 2010).

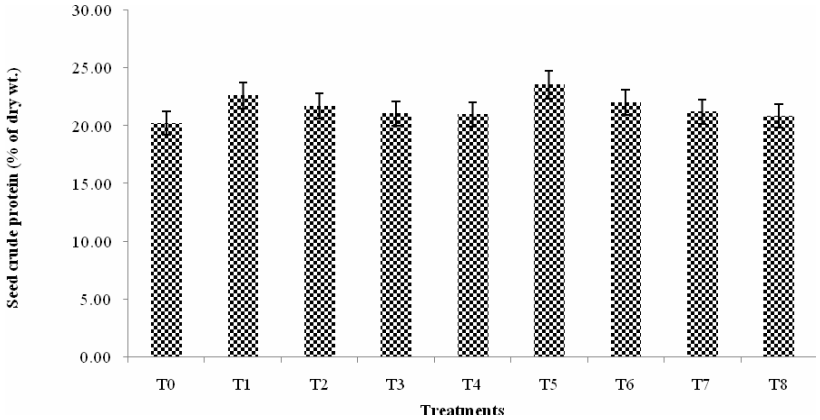

Fig. 2. Effect of GA_3 and NAA on dry seed crude protein content (% of dry wt.) of French bean (Mean ± standard error).

Ascorbic acid contents of fresh pod (% of fresh wt.) ranged from 16.009 to 20.407 mg/100g (Table 1). The highest value of it (20.407 mg/100 g) was recorded for T_1 (50 ppm GA_3) and was followed by T_5, T_2, T_6, T_3, T_7 and T_4 showing 8.67 to 27.47% increased over the control indicating evidence of biosynthesis of ascorbic acid due to these growth regulators. These findings closely agree with those of Ouzounidou *et al.* (2010) who worked in green pepper reporting that 100 µM GA_3 treated plants increased 13% ascorbic acid content over the non treated ones. In another work it was observed that the application of 25 and 50 ppm NAA as foliar spray showed the highest ascorbic acid in paprika chilli (Kannan *et al*. 2008). Olaiya (2011) also observed that 100 mg/l NAA treated genotype NHLy 13 of tomato showed maximum value (19.88 mg/100 g) of ascorbic acid content.

Table 1. Effect of GA_3 and NAA on total carbohydrate, crude fiber, ash, crude fat and ascorbic acid contents of fresh pod (% of fresh wt.) of French bean.

Treatments	Total carbohydrate (%)	Crude fiber (%)	Ash (%)	Crude fat (%)	Moisture (%)	Ascorbic acid (mg/100 g)
T0	8.028	1.560	0.615	0.178	87.620	16.009
T1	5.726	1.843	0.831	0.158	88.790	20.407
T2	6.208	1.753	0.765	0.168	88.520	19.964
T3	6.717	1.673	0.733	0.169	88.233	18.853
T4	6.901	1.614	0.722	0.169	88.080	16.736
T5	5.718	1.764	0.851	0.156	88.830	19.971
T6	6.198	1.730	0.767	0.167	88.550	19.793
T7	6.887	1.657	0.750	0.169	88.133	17.397
T8	7.191	1.490	0.677	0.171	88.050	16.408
LSD (0.05)	0.036	0.012	0.004	0.002	0.053	0.257

Out of fourteen amino acids, GA_3 and NAA with 50 to 110 ppm increased lysine, aspartic acid, glutamic acid, arginine and tyrosine in dry seeds of French bean. On the other hand, leucine contents were decreased by GA_3 application.

Isoleucine content in dry seeds varied from 0.74 to 0.92% (% of dry weight) the highest for T_5 (50 ppm NAA) which was 17.95% higher over the control. However, the lowest was for T_8 (110 ppm NAA). Leucine content ranged between 0.29 and 0.56%. Application of GA_3 treatments decreased the leucine of dry seeds (15.22 to 36.96%). Among the treatments, it was maximum for T_5, followed by T_6 and T_7, while the minimum was for T_1. T_5 increased it 21.74 % over that of the control (Table 2).

Lysine content ranged from 2.23 to 2.78% showing the highest for T_6 (70 ppm NAA) and was followed by T_7, T_5, T_2 and T_1, respectively. It was increased 24.66% by T_6, compared to the control. Application of both the growth regulators increased its content in treated seeds of French bean. Methionine content in dry seeds ranged from 0.36 to 0.47%. It was highest for T_1 (50 ppm GA_3), followed by T_5, whereas the lowest for T_8 (110 ppm NAA) showing 23.68% higher over the control.

Threonine content of dry seeds ranged between 0.38 and 0.66% showing the highest for T_2 followed by T_3, T_4, T_1 and T_5, respectively (Table 2). T_2 increased it 24.53% over the control. However, the lowest was found for T_8. Valine content ranged from 0.10 to 0.21% the highest was recorded for T_5 (50 ppm NAA), followed by T_1, while the lowest was for T_8. T_5 increased valine by 75% compared to the control . Histidine content in dry seeds ranged from 0.65 to 0.88% which was maximum for T_2, followed by T_1, while the minimum for T_8. T_2 increased it 12.82%, while T_8 decreased it 16.67% compared to the control. The amount of aspartic acid varied from 1.26 to 1.62%. The highest was observed for T_5, followed by T_2. Glutamic acid content ranged from 0.70 to 1.38%. Both of GA_3 and NAA increased its content in seeds showing highest for T_2, followed by T_1 , T_3 and T_4, respectively. T_2 (70 ppm GA_3) increased it by 97.14% over the control . Arginine content in dry seeds varied from 0.96 to 1.15%. Like glutamic acid, both the growth regulators enhanced arginine content which was maximum for T_2 showing an increase of 19.79% over the control.

The results on glycine content of dry seeds of French bean indicated that foliar application of GA_3 and NAA increased its content with the increased concentration up to the level below that of T_8. T_4 showed the highest glycine content of French bean seeds which was 30.56% over that of the control. Like with arginine the serine content in dry seeds ranged was maximum for T_2 , while minimum for T_4. T_2 showed 26.90% more serine over the control. Alanine content in dry seeds ranged from 0.59 to 0.89%. T_5 (50 ppm NAA) in the growth regulator treated French bean plants, the highest with T_5 (0.89%) and was followed by T_2 . The lowest (0.59%) was for T_6 which showed 32.84% decreased alanine than the control. Tyrosine content ranged from 0.40 to 0.56%, the highest was found in T_4 (40% higher over that of the control) while the lowest in T_8 which was identical to that of the control.

It may be summarized that the amino acids profile of the dry seeds harvested from the treated French bean crop showed that 50 ppm NAA (T_5) increased 14 tested amino acids in the present study. These results are in agreement with those of Ullah *et al*. (2011) who worked in cowpea seeds spraying NAA at two concentration (40 and 50 ppm) reporting increased glutamic acid, aspartic acid, lysine, arginine and serine contents compared to the control. In another work, Desai and Khanvilkar (1977) reported for Bengal gram that arginine, tryptophan, histidine, threonine and tyrosine contents were increased due to the use of 100 ppm NAA. Similarly, Dhingra *et al.* (1995) and Karim *et al*. (2006) found increased amino acids in chickpea seeds following the application of 20 ppm NAA.

In this study 50 ppm GA_3 (T_1) increased all the tested amino acids except decreasing leucine. These findings also agree well with those as reported by Desai and Khanvilkar (1977) who worked in Bengal gram showing increased contents on arginine, cystine, histidine, isoleucine, leucine,

lysine, methionine, threonine, tryptophan, tyrosine and valine following sparaying 50 ppm GA. It can be suggested that the negative effect of GA_3 on leucine content in pulse seeds may also vary depending on the species as. Moreover, Hariharan and Unnikrishnan (2005) spraying 40 and 50 ppm GA_3 on foliage of cashew kernel reported varying amount of different amino acids along with total protein although not with uniform pattern.

Table 2. Effect of GA_3 and NAA on dry seed amino acids content (% of dry wt.) of French bean.

Tr.*	Ile	Leu	Lys	Met	Thr	Val	His	Asp	Glu	Arg	Gly	Ser	Ala	Tyr
T_0	0.78	0.46	2.23	0.38	0.53	0.12	0.78	1.26	0.70	0.96	0.72	1.45	0.67	0.40
T_1	0.88	0.29	2.47	0.47	0.63	0.17	0.86	1.46	1.32	1.10	0.82	1.58	0.78	0.42
T_2	0.76	0.39	2.61	0.43	0.66	0.15	0.88	1.52	1.38	1.15	0.92	1.84	0.88	0.47
T_3	0.81	0.35	2.46	0.40	0.65	0.15	0.80	1.51	1.30	1.00	0.93	1.40	0.83	0.50
T_4	0.81	0.34	2.44	0.39	0.65	0.16	0.79	1.50	1.29	1.00	0.94	1.36	0.82	0.56
T_5	0.92	0.56	2.74	0.44	0.59	0.21	0.84	1.62	0.88	1.14	0.84	1.64	0.89	0.50
T_6	0.86	0.54	2.78	0.42	0.42	0.14	0.80	1.49	0.86	0.98	0.81	1.66	0.59	0.46
T_7	0.80	0.52	2.76	0.40	0.40	0.13	0.79	1.47	0.85	1.08	0.79	1.61	0.60	0.44
T_8	0.74	0.44	2.41	0.36	0.38	0.10	0.65	1.36	0.82	1.09	0.68	1.53	0.60	0.40

*Tr. = Treatments, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Thr = Threonine, Val = Valine, His = Histidine, Asp = Aspartic acid, Glu = Glutamic acid, Arg = Arginine, Gly = Glycine, Ser = Serine, Ala = Alanine, Tyr = Tyrosine.

Scientists opined that activities of nitrate reductase, transaminase, glutamate dehydrogenase, adenosine triphosphate, phosphorylase and amylase in different crops were influenced by appropriate growth regulators along with potassium naphthenate, NAA and GA_3 which could facilitate greater accumulation of amino acids contributing to protein biosynthesis in plants (Fattah 1971, Cruz *et al.* 1970). Reviewing the above described results of this study, it might be concluded that applying 50 ppm NAA produced the best nutritious fresh pods and dry seeds of French bean.

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