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Effect of Gibberellic Acid and Cycocel on Growth, Yield and Protein Content of Pea

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Abstract: A study on the effect of pre-soaking treatments of Gibberellic acid (GA_3) and Cycocel [(2-Chloroethyl) trimethylammonium chloride] singly on growth, yield and protein content of pea (cv. Aparna and Azad-P-1) was carried out in a randomized block deign with three replications. Fertilizers used at recommended doses and pH was adjusted at 6.0. Concentrations of PGRs used 10, 100, 250, 500 and 1000 μg mL^{-1} with a control set. Length of shoots was measured at an interval of 3 days from 7 DAS. Number of branches was recorded at an interval of 7 days from 15 DAS. Chlorophyll estimation was done on 30 DAS. Yield attributing characters were recorded at proper time. Protein was estimated from harvested seeds. GA_3 irrespective of concentrations was most effective in promoting shoot growth while cycocel at all concentrations tried reduced shoot growth. Number of branches per plant was increased with both the hormones. In both the varieties chlorophyll contents were decreased by higher concentrations of GA_3 while cycocel increased it. Both the hormones significantly affected the yield characteristics. GA_3 at 250 μg mL^{-1} produced maximum number of pods per plant, seed yield, seed index and protein content in seeds in both the varieties. Cycocel at 100 and 250 μg^{-1} mL recorded maximum number of pods per plant and seed yield in cv. Azad-P-1 and cv. Aparna, respectively. Protein content in seeds was recorded highest at 500 μg mL^{-1} of cycocel. The present study clearly shows that judicious application of GA_3 and cycocel can increase yield and protein content in seeds of pea.

Key words: Gibberellic Acid (GA_3), Cycocel [(2-chloroethyl) trimethylammonium chloride], pea (*Pisum sativum* L.), yield, protein content

INTRODUCTION

Pulses provide protein to the poor people at a very cheaper rate than animal protein and hence pulses are popularly called 'poor man's meat' in developing countries. Pea (*Pisum sativum* L.) is an important pulse crop and widely grown over the world for its seeds, green pods, green foliage and young tender shoots. Though several well-tested and proven technologies to enhance the productivity of the crops are available, nutritional security continues to be a cause of concern for many developing countries. It is a paramount need to increase the productivity to feed hungry millions.

One of the most recent developments in the field of agriculture in boosting up crop production is the use of growth regulators. The applications of GAs on growth of various plants have been reported by Sarma and Deka (1977), Xu *et al.* (1997) and Matsukura *et al.* (1998). Several studies on different crops have shown that the exogenous application of GA_3, an important GAs can enhance the productivity of crops affecting the vital physiological processes (Khan *et al.*, 2002; Bora and Sarma, 2003; Rahman *et al.*, 2004).

Cycocel is a synthetic growth retarding chemicals extensively used for dwarfing of plants or plant parts (Clark and Fedac, 1977; Sarma and Mishra, 1979; Bora and Sarma, 2004). Increasing Cycocel concentration increased yield of gobhi sarson (Grewal *et al.*, 1993), cotton (Prasad and Prasad, 1994), soybean (Bora and Sarma, 2004) and protein content in wheat (Afria *et al.*, 1998).

Therefore, an experiment was conducted to study the effect of GA_3 and cycocel on growth, yield and protein content of pea in two varieties namely cv. Aparna and cv. Azad-P-1.

MATERIALS AND METHODS

The experiment was conducted in the Department of Botany of Gauhati University during 2001-2003 on a randomised block design with three replications. A suitable plot was selected and ploughed till desired tilth was obtained. Farmyard manure was mixed properly at the rate of 10 tons ha^{-1}. NPK was used at recommended doses (urea at the rate of 45 kg ha^{-1}, super phosphate at the rate of 245 kg ha^{-1} applied during ploughing) and pH was adjusted at 6 with lime. Then the plot was divided

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into three blocks each containing 11 beds of 1.0 sqm size. Certified seeds of pea (*Pisum sativum* L. cv. Aparna and cv. Azad-P-1) were collected from State Seed Corporation, Assam. Seeds were sown in mid of October and harvested in last of January. The seeds were first surface sterilised with 0.01% $HgCl_2$ solution and then washed with sterile distilled water (DW) and air-dried. GA_3 and cycocel were applied at the concentrations of 10,100, 250, 500 and 1000 μg mL^{-1}. The seeds were soaked in specific concentrations of plant growth regulators separately for 12 h. Then seeds were air-dried for one hour and sown in the experimental beds. Thirty seeds were sown per bed, in lines 30 cm apart from line to line at a distance of 10 cm between seeds. Length of shoots was recorded at an interval of 3 days for 5 times and number of branches was counted at an interval of 7 days for 3 times. Chlorophyll contents were estimated at 30 Days After Sowing (DAS) (Arnon, 1949), Yield (Bora and Sarma, 2003) and yield contributing characters like number of flowers (Rahman *et al*., 2004), number of pods per plant (Bora and Sarma, 2003; Rahman *et al*., 2004), seed yield (t ha^{-1}) (Bora and Sarma, 2004) were also recorded. Random samples were taken from thrashed seeds for determining 100 seed weight (seed index) (Bora and Sarma, 2003). Percent of protein was determined from harvested seeds (Lowry *et al*., 1951). Data collected were analysed statistically (Panse *et al*., 1985).

RESULTS AND DISCUSSION

The plant growth regulators significantly affected the growth characteristics. Data recorded on growth parameters of pea (cv. Aparna and Azad-P-1) revealed that GA_3 enhanced the shoot growth and cycocel reduced the shoot growth at all the concentrations tried. The response to the PGRs varied according to the varieties. In cv. Aparna, the maximum shoot length (73.49 cm) was recorded at 250 μg mL^{-1} of GA_3 while in cv. Azad-P-1, maximum shoot length (68.37 cm) was recorded at 500 μg mL^{-1} (Table 1). The process plant growth consists of two steps, cell divisions and subsequent cell elongation. GA_3 has been reported to increases cell wall extensibility leading to elongation growth (Matsukura *et al*., 1998; Rahman *et al*., 2004). The increase in plant height due to GA_3 application might be due to its effect on elongation of internodes (Krishnamoorthy, 1981). Huttly and Phillips (1995) suggested that GA_3 causes increase in cell number and size to produce a significant effect on growth. The application of gibberellins promote shoot elongation was also reported by Xu *et al*. (1997) and Yang *et al*. (1996). Fridborg *et al*. (2001) reported that exogenous application of GA_3 can suppress the activity of 'short internode' and 'GA insensitive' genes and hence leads to elongation growth of shoots. The present study is in the conformity

Table 1: Mean length of shoots (cm) of pea (cv. Aparna and Azad-P-1) seedlings treated with GA_3

	cv. Aparna						cv. Azad-P-1					
	Length of shoots (cm) Time in days after sowing						Length of shoots (cm) Time in days after sowing					
GA_3 conc. (μg mL^{-1})	7	10	13	16	19	Mean for GA_3	7	10	13	16	19	Mean for GA_3
0	3.17	4.95	10.33	13.53	18.07	10.01	5.13	7.50	9.80	12.80	17.60	10.57
10	4.37	10.27	12.37	18.13	21.77	13.38	7.51	10.50	13.83	17.50	23.20	14.51
100	10.89	17.00	21.33	28.20	34.43	22.37	12.50	21.03	23.50	28.13	37.10	24.45
250	12.23	21.50	44.10	50.33	73.49	40.33	15.00	26.93	32.73	47.20	61.53	36.68
500	18.00	22.93	43.00	48.47	64.37	39.35	19.50	31.87	37.13	54.00	68.37	42.17
1000	16.40	17.83	33.50	43.20	54.30	33.05	21.47	34.17	35.07	45.50	54.40	38.12
Mean for time	10.84	15.75	27.44	33.64	44.40		13.52	22.00	25.34	34.19	43.70	

CD for GA_3 (n =15), p(0.05) = 0.43, p(0.01) = 0.56, CD for GA_3, p(0.05) = 1.05, p(0.01) = 1.38, CD for time (n = 18), p(0.05) = 0.39, p(0.01) = 0.51, CD for time, p(0.05) = 0.96, p(0.01) = 1.26

Table 2: Mean length of shoots (cm) of pea (cv. Aparna and Azad-P-1) seedlings treated with cycocel

	cv. Aparna						cv. Azad-P-1					
	Length of shoots (cm) Time in days after sowing						Length of shoots (cm) Time in days after sowing					
Cycocel conc. (μg mL^{-1})	7	10	13	16	19	Mean for conc.	7	10	13	16	19	Mean for conc.
0	3.77	6.13	10.43	12.97	18.73	10.41	5.1	7.5	9.80	12.80	17.60	10.57
10	3.40	5.50	10.07	12.17	17.60	9.75	4.5	7.2	9.70	12.50	17.50	10.28
100	3.13	4.87	9.47	11.73	14.43	8.73	3.8	6.2	7.20	11.10	14.90	8.64
250	2.70	4.40	8.57	11.03	13.37	8.01	3.4	5.2	6.30	10.00	12.90	7.56
500	2.43	3.77	7.27	9.37	11.37	6.84	3.2	4.3	5.40	8.70	11.00	6.52
1000	2.03	3.43	6.43	8.27	10.43	6.12	2.8	3.8	5.00	8.30	9.90	5.96
Mean for time	2.91	4.68	8.71	10.92	14.32		3.8	5.7	7.23	10.56	13.96	

CD for cycocel (n = 15), p(0.05) = 0.20, p(0.01) = 0.26, CD for cycocel, p(0.05) = 0.41, p(0.01) = 0.54, CD for time (n = 18), p(0.05) = 0.18, p(0.01) = 0.24, CD for time, p(0.05) = 0.29, p(0.01) = 0.38

Table 3: Effect of GA_3 on number of branches of pea (cv.Aparna Azad-P-1) seedlings

GA_3 conc. ($\mu g\ mL^{-1}$)	cv. Aparna Number of branches per plant Time in days after sowing 15	21	28	Mean for GA_3	cv. Azad-P-1 Number of branches per plant Time in days after sowing 15	21	28	Mean for GA_3
0	1.13	1.43	1.67	1.41	1.25	1.92	2.33	1.83
10	1.27	1.73	1.93	1.64	1.33	2	2.33	1.89
100	1.47	2.23	2.37	2.02	1.58	2.08	2.57	2.08
250	1.7	3.07	3.3	2.69	1.58	2.08	2.83	2.17
500	1.2	1.33	1.63	1.39	1.42	1.83	2.33	1.86
1000	1.07	1.2	1.33	1.2	1.08	1.17	1.75	1.33
Mean for Time	1.31	1.83	2.04		1.38	1.85	2.36	

CD for GA_3 (n = 9), p(0.05) = 0.13, p(0.01) = 0.17, CD for GA_3, p(0.05) = 0.17, p(0.01) = 0.22, CD for time (n = 18), p(0.05) = 0.09, p(0.01) = 0.12, CD for time, p(0.05) = 0.12, p(0.01) = 0.16

Table 4: Effect of cycocel on number of branches of pea (cv. Aparna and Azad-P-1) seedlings

Cycocel conc. ($\mu g\ mL^{-1}$)	cv. Aparna Number of branches per plant Time in days after sowing 15	21	28	Mean for cycocel	cv. Azad-P-1 Number of branches per plant Time in days after sowing 15	21	28	Mean for Cycocle
0	1.13	1.43	1.67	1.41	1.25	1.92	2.33	1.83
10	1.3	1.67	1.9	1.62	1.25	1.92	2.42	1.86
100	1.57	2.33	2.57	2.16	1.58	2.42	2.92	2.31
250	1.97	3.3	3.47	2.91	2.08	2.58	3.33	2.67
500	1.93	3.23	3.43	2.87	2.42	2.92	3.58	2.97
1000	1.8	3.07	3.43	2.77	2.17	2.83	3.42	2.81
Mean for Time	1.62	2.51	2.74		1.79	2.43	2.82	

CD for cycocel (n = 9), p(0.05) = 0.11, p(0.01) = 0.14, CD for cycocel p(0.05) = 0.16, p(0.01) = 0.21, CD for time (n = 18), p(0.05) = 0.08, p(0.01) = 0.10, CD for time p(0.05) = 0.11, p(0.01) = 0.15

with these workers including Khan *et al*. (2002) and Rahman *et al*. (2004) who reported similar results.

On the other hand cycocel, irrespective of concentrations inhibited the elongation growth of shoots in both the varieties and intensity of inhibition gradually increased with the rise of concentrations. Maximum inhibition in shoot growth was recorded at 1000 $\mu g\ mL^{-1}$ (Table 2). The retardation in plant height caused by cycocel might be due to shortening of internodes by decreasing cell division and cell numbers. Child (1984) reported marked reductions in growth rate immediately following application of cycocel on oilseed rape. It antagonizes the biosynthesis of GAs and hence elongation growth is inhibited (Setia and Setia, 1990). The results are in conformity with some early reports (Bora and Sarma, 2004; Prasad and Prasad, 1994)

In both the varieties, GA_3 upto 250 $\mu g\ mL^{-1}$ was highly stimulatory on number of branches per plant and declined at higher concentrations (Table 3). On the other hand cycocel irrespective of concentrations tried was superior to the control in enhancing the number of branches per plant in both the varieties. In cv. Aparna mean number of branches per plant increased upto the concentration 250 $\mu g\ mL^{-1}$ of cycocel and then decreased at higher concentrations (Table 4). But in cv. Azad-P-1 cycocel upto the concentration 500 $\mu g\ mL^{-1}$ increased the number of branches per plant (Table 4).

Chlorophyll-b content was higher over the chlorophyll-a in all the concentrations tried. In cv. Aparna chlorophyll content decreased gradually with the rise of GA_3 concentration except in cv. Azad-P-1 where 10 $\mu g\ mL^{-1}$ of GA_3 was slightly stimulatory (Table 5). Similar results were observed in cotton (Bhatt and Ramanujam, 1970) and pea (Bora and Sarma, 2003). It was suggested that the increase in cell volume caused by GA_3 was not correlated with an increase in synthesis of chlorophyll content and thus a dilution of the chlorophyll content of the leaves was obtained. On the other hand cycocel increased the chlorophyll content in both the varieties upto 500 and 1000 $\mu g\ mL^{-1}$ was slightly inhibitory in the cv. Aparna (Table 6). The effect of cycocel in increasing chlorophyll contents may be due to the reduction in cell size resulting in denser cytoplasm (Appleby *et al*., 1966). The results are in conformity with early reports in soybean (Bora and Sarma, 2004).

At maturity, plant growth regulators significantly affected yield characteristics. GA_3 upto 500 $\mu g\ mL^{-1}$ was highly stimulatory in increasing the number of flowers and pods per plant for both the varieties. But highest number of flowers and pods were recorded at GA_3 250 $\mu g\ mL^{-1}$ and GA_3 at 1000 $\mu g\ mL^{-1}$ was slightly inhibitory (Table 7). On the other hand, both the varieties showed a varied response to cycocel. Cycocel, irrespective of concentrations was superior over the

Table 5: Chlorophyll content in pea leaves developed from GA_3 treated seeds

GA_3 conc. ($\mu g\ mL^{-1}$)	Chlorophyll content ($mg\ g^{-1}\pm SE$) cv. Aparna Chl-a	Chl-b	Total (a+b)	cv. Azad-P-1 Chl-a	Chl-b	Total (a+b)
0	1.115±0.027	1.335±0.109	2.450±0.133	1.118±0.029	1.341±0.053	2.459±0.082
10	1.102±0.023	1.237±0.057	2.339±0.075	1.131±0.017	1.351±0.059	2.490±0.072
100	1.075±0.029	1.196±0.053	2.271±0.082	1.086±0.029	1.309±0.053	2.394±0.082
250	1.010±0.034	1.191±0.049	2.201±0.062	1.054±0.025	1.233±0.054	2.287±0.079
500	1.006±0.029	1.163±0.059	2.169±0.074	1.038±0.029	1.195±0.053	2.233±0.081
1000	0.925±0.033	0.952±0.111	1.877±0.141	0.992±0.020	1.175±0.052	2.167±0.067

Table 6: Clorophyll content in pea leaves developed from cycocel treated seeds

Cycocel conc. ($\mu g\ mL^{-1}$)	Chlorophyll content ($mg\ g^{-1}\pm SE$) cv. Aparna Chl-a	Chl-b	Total (a+b)	cv. Azad-P-1 Chl-a	Chl-b	Total (a+b)
0	1.106±0.030	1.448±0.075	2.553±0.094	1.118±0.029	1.341±0.053	2.459±0.081
10	1.149±0.022	1.490±0.085	2.639±0.090	1.120±0.035	1.347±0.046	2.467±0.076
100	1.159±0.031	1.508±0.086	2.667±0.099	1.176±0.029	1.363±0.053	2.539±0.082
250	1.173±0.031	1.598±0.050	2.771±0.075	1.180±0.029	1.537±0.053	2.717±0.081
500	1.213±0.038	1.642±0.039	2.854±0.076	1.203±0.033	1.616±0.051	2.819±0.084
1000	1.076±0.069	1.532±0.040	2.607±0.106	1.120±0.046	1.428±0.038	2.548±0.076

Table 7: Effect of GA_3 on number of flowers, number of pods per plant, seed yield, seed index and protein content in seeds of pea

GA_3 conc. ($\mu g\ mL^{-1}$)	No. flowers $plant^{-1}$ cv. Aparna	cv. Azad-P-1	No. of pods $plant^{-1}$ cv. Aparna	cv. Azad-P-1	Seed yield ($q\ ha^{-1}$) cv. Aparna	cv. Azad-P-1	Seed index (g) cv. Aparna	cv. Azad-P-1	Protein (%) cv. Aparna	cv. Azad-P-1
0	17.25	17.33	17.20	17.10	11.77	11.16	21.64	22.04	21.84	21.61
10	17.38	17.50	17.33	17.23	12.32	11.20	21.76	22.10	22.44	22.20
100	19.41	19.52	19.37	19.30	13.29	13.00	22.51	23.22	27.75	27.67
250	21.83	21.93	21.73	21.79	13.79	14.05	23.05	23.35	28.90	28.90
500	17.96	17.19	17.92	17.83	11.97	11.17	22.29	22.31	24.76	24.76
1000	16.70	16.77	16.65	16.58	11.78	10.40	20.79	21.93	22.27	23.88
Sig. Level	**	**	**	**	**	**	**	**	**	**
CD										
p = 0.05	0.602	0.619	0.648	0.607	0.524	0.408	0.687	0.474	0.128	0.121
p = 0.01	0.855	0.88	0.921	0.862	0.745	0.58	0.974	0.674	0.183	0.172

Table 8: Effect of cycocel on number of flower, pods per plant, seed yield, seed index and protein content in seeds of pea

Cycocel conc. ($\mu g\ mL^{-1}$)	No. flowers $plant^{-1}$ cv. Aparna	cv. Azad-P-1	No. of pods $plant^{-1}$ cv. Aparna	cv. Azad-P-1	Seed yield ($q\ ha^{-1}$) cv. Aparna	cv. Azad-P-1	Seed index (g) cv. Aparna	cv. Azad-P-1	Protein (%) cv. Aparna	cv. Azad-P-1
0	17.25	17.20	17.20	17.10	11.76	11.16	21.85	22.04	21.93	21.61
10	17.33	17.34	17.27	17.17	12.31	11.50	22.06	22.07	22.28	22.00
100	19.32	19.30	19.26	19.23	13.19	13.53	23.01	22.99	23.73	22.28
250	22.07	18.60	22.02	18.43	14.15	11.72	23.41	23.3	25.83	23.73
500	20.94	18.49	20.88	18.37	12.56	11.5	23.14	23.37	26.01	25.83
1000	17.23	18.25	17.11	18.03	11.47	10.9	22.68	21.52	24.55	24.55
Sig. level	**	**	**	**	**	**	**	**	**	**
CD										
p = 0.05	0.448	0.476	0.357	0.488	0.681	0.651	0.475	0.545	0.121	0.077
p = 0.01	0.638	0.664	0.507	0.694	0.968	0.926	0.676	0.775	0.172	0.110

control in enhancing the number of flowers and pods per plant in cv. Azad-P-1 while in cv. Aparna 1000 $\mu g\ mL^{-1}$ was inhibitory (Table 8). Santes and Garcia (1995) reported that GA_3 controls the pod development in pea. Goto and Pharis (1999) reported that Gas not only act to normalise plant height but also stimulates development of floral organs. Cycocel increased number and length of siliqua in Indian mustard was also reported.

Seed index gradually increased with the treatment of GA_3 upto 250 $\mu g\ mL^{-1}$ and then declined in both the varieties. At 250 $\mu g\ mL^{-1}$ of GA_3 the seed index was recorded as 23.05 and 23.35g in cv. Aparna and cv. Azad-P-1, respectively (Table 7). On the other hand, 250 and 500 $\mu g\ mL^{-1}$ of cycocel were emerged as the best concentration in cv. Aparna and cv. Azad-P-1, respectively recording seed index 23.41 and 23.37 g accordingly. One value at 250 and one from 500 $\mu g\ mL^{-1}$

which are considered here (Table 8). The results are in conformity with some earlier reports by Bora *et al*. (2003) and Prasad and Prasad (1994).

Present study clearly indicated that PGRs have the potentiality to increase the yield of pea in both the varieties. The highest yield was recorded as 13.79 and 14.05 q ha^{-1} at 250 μg mL^{-1} of GA_3 as against 11.77 and 11.16 q ha^{-1} at the control in cv. Aparna and cv. Azad-P-1, respectively (Table 7). At this concentration number of branches, pods per plant and seed index were also highest. Hence, yield increased as a manifestation of increased number of branches and pods per plant along with seed index. Different concentration of cycocel differed significantly in their inherent characters to produce yield per hactre. Cycocel increased the yield upto 250 μg mL^{-1} (14.15 q ha^{-1}) and 100 μg mL^{-1} (13.53 q ha^{-1}) in cv. Aparna and cv. Azad-P-1, respectively (Table 8). The increased in seed yield might be due to increase in number of branches and pods per plant.

Among the plant growth processes, seed germination and early seedling growth are considered critical for raising a successful crop as these indirectly determine the yield of the resultant crop (Gelmond, 1978) That GA_3 enhanced the yield by better utilisation of photosynthates and metabolic machinery was also reported (Khan *et al*., 2002). Growth regulators increase the actual productivity when the plant growth is stimulated or the photosynthates are diverted to the harvested products (Setia and Setia, 1990). Also the seed production is the culmination of a number of developmental phases requiring specific nutrients to maintain the metabolic status of the flowering and seed development stages (Bhatt and Mishra, 2001). The increase in shoot length due to GA_3 treatment led to bear more leaves and thus better chance to trap more sunlight and produce more dry matter (Khan *et al*., 2002). The increase in yield in GA_3 treated plants in the present investigation corroborates such findings.

It has now been established that control of excessive vegetative growth might be beneficial for synchronized flower initiation and development of pods. Cycocel reduced the vegetative growth pertaining to the better environment for seed formation (Pando and Srivastava, 1985). Cycocyl has been used to check the abscission of flower and modify the crop canopy for improving the yield in gram (Bangal *et al*., 1982), pigeon pea (Vikhi *et al*., 1983) and soybean (Sing *et al*., 1987).

Growth regulators also caused an increase on protein content in the seeds. Irrespective of concentrations tried, the protein content increased with the application of GA_3 and cycocel. GA_3 at 250 μg mL^{-1} and cycocel at 500 μg mL^{-1} emerged as best concentrations in enhancing the protein content in seeds of pea in both varieties (Table 7 and 8).

It is established that plant hormones acted solely or in part by controlling transcription of genes (Baulcombe, 1987; MacMillan and Phinney, 1987) and thus levels of mRNA (Wu *et al*., 1993), which would, in turn regulate rates of synthesis of specific hormone induced proteins. Huizen *et al*. (1996) reported change in the polypeptide in pea fruit between molecular weight 20 and 60 kD with gibberellin treatment. On the other hand GA_3 possibly either releases the activity or inactivates some inhibitory gene, which leads to accumulation of more proteins in the seed. On the other hand cycocel reduces the elongation growth of shoots and as a result less photosynthates are utilized for vegetative growth. Grewal *et al.* (1993) reported that cycocel improves the translocation of photosynthates. More protein content stored in the seeds might be due to improvement of translocation of photosynthates to the seeds. Afria *et al*. (1998) reported that cycocel resulted in a significant increase in protein content in wheat.

In conclusion, experimental results mentioned here revealed that both GA_3 and cycocel brought about an improvement in morphological and yield attributes of pea. Highest yield and protein content under the influence of growth regulators might be due to activation of various internal mechanisms related with plant growth and metabolism. The findings of the present investigation are also in agreement with the earlier reports in soybean, *Brassica napus* (Grewal *et al*., 1993), cotton (Prasad and Prasad, 1994), mustard (Khan *et al*., 2002).

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