Effect of Bioregulators on Growth, Yield and Chemical Constituents of Soybean (*Glycine max*)

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Received: December 23, 2010 Accepted: January 7, 2011 Published: December 1, 2011
doi:10.5539/jas.v3n4p151 URL: http://dx.doi.org/10.5539/jas.v3n4p151

The authors are thankful to Indian Council of Agricultural Research (ICAR) for funding the All India Coordinated Research Project on Soybean, Dr. S. N. Puri, hon'ble Vice-chancllor and Incharge Director of Research for technical support to carry out this field experiment.

Abstract

A three-year study was conducted during 2006-2008 in India (Manipur) to study the response of soybean variety JS 335 to salicylic acid @ 50 ppm, Ethrel @ 200 ppm, Cycocel @ 500 ppm and control (water spray) applied as foliar spray at different stages viz. flower-initiation (40 DAS), pod-initiation (60 DAS) and flower-initiation + pod-initiation. The study revealed that application of Ethrel @ 200 ppm at both flower-initiation (40 DAS) + pod-initiation (60 DAS) gave higher vegetative growth, yield, net returns and B:C ratio as compare to salicylic acid @ 50 ppm, Cycocel @ 500 ppm and control. However, maximum chlorophyll content and carotenoids were obtained from cycocel @ 500 ppm treated plants.

Keywords: Bioregulators, Cycocel, Ethrel, Salicylic Acid, Soybean

1. Introduction

Plant growth regulators are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and can enhance the effective partitioning of accumulates from source and sink in the field crops (Solaimalai*et al.,* 2001).

Salicylic acid ($C_7H_6O_3$) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plant, such as stomatal closure, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan *et al*., 2003 and Shakirove*et al*., 2003). Foliar application of salicylic acid exerted a significant effect on plant growth metabolism when applied at physiological concentration and thus acted as one of the plant growth regulating substances (Kalarani*et al*., 2002). Salicylic acid increased the number of flowers, pods/plant and seed yield of soybean (Gutierrez-Coronado *et al*., 1998); enhanced wheat growth (Shakirova*et al*., 2003) and maize growth (Shehetaet *al*., 2001; Abdel-Wahed*et al*., 2006; El-Mergawi and Abdel-Wahed, 2007).

Ethylene released from ethrel (2-Chloroethylphosphonic acid) could possibly be utilized for promoting pod growth as Abbas (1991) has shown that early pod development is related to higher ethylene levels, thus decreasing flower and pod shedding and thereby reducing abscission and improving better pod set. Ethrel induced increase in cell division, resulting in increased fruit size and yield have been reported in tomato fruits (Atta – Aly*et al*., 1999). In cucurbits ethrel application at 250 ppm promoted pistillat flower formation (Robinson *et al*. 1970).

Cycocel (2-Chloroethyl, trimethyl ammonium chloride) has been used to check the abscission of flower and modify the crop canopy for improving the yield in gram (Bangal*et al*., 1982), pigeonpea (Vikhi*et al*., 1983) and soybean (Singh *et al*., 1987). 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.

Agricultural application of vegetal bio-regulators is becoming a useful practice that has improved the yield of beans, corn and soybean (Vieira and Castro, 2004). The aim of present work was to study the effect of the three bioregulators (Salicylic acid, Ethrel and Cycocel) and their stages of application on growth, yield and biochemical constituents of soybean.

2. Materials and methods

Fieldexperiments wereconducted for 3 consecutive years at the Agricultural Research Station of Central Agricultural University, Imphal during 2006-2008 under All India Coordinated Research Project on Soybean. The soil was clay loam in texture with pH of 5.4. The treatments consisted of three bioregulators viz. Salicylic acid @ 50 ppm, Ethrel @ 200 ppm and Cycocel @ 500 ppm with three different stages of application (flower-initiation, pod-initiation and flower-initiation + pod-initiation). Control treatment was sprayed with distilled water at the same stages. The experiment was laid out in factorial randomized block design with three replications. Recommended dose of NPK for soybean 20 kg N, 60 kg P_2O_5 and 40 kg K_2O/ha was applied at the time of sowing through urea, single super phosphate and muriate of potash. The seeds were treated with Bavistin @ 2.5 g/kg seed before sowing. The plot area was 21.6 m^2 (6 x 3.6 m) and seeds of soybean cultivar JS 335 was sown on 8^{th} June, 10^{th} June and 8^{th} June 2006, 2007 and 2008 respectively in rows 45 cm apart and 10 cm between plants. The crops were harvested on 12^{th}, 18^{th} and 15^{th} October 2006, 2007 and 2008 respectively.

2.1 Growth characteristics

Plant height and number of braches/plant were recorded at the time of maturity whereas dry weight/plant and leaf area index were recorded at 75 days after sowing.

2.2 Yield and its components characteristics

Yield and its components such as number of pods/plant, number of seeds/plant, seed index, grain yield, straw yield and harvest index were determined at maturity stage.

2.3 Chemical analysis

Grain samples were dried at 70^oC for constant weight and ground to determine the chemical constituents such as protein and oil percentage. The oil content of soybean seeds was estimated by adopting Soxhlet Ether Extraction method (Sadasivam and Manickam, 1996). Protein content was determined by the Kjeldahl method (total nitrogen), as recommended by the Association of Official Analytical Chemists (AOAC, 1975), with modifications. Nitrogen contents were multiplied by dry matter-based factor 6.25 to determined total protein content (Gupta &Varshaney, 1994).

2.4 Estimation of photosynthetic pigments

The blade of the third leaf from tip (terminal leaflet) was taken at 75 days after sowing to determine photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) by the spectrophotometic method recommended by Metzner*et al.* (1965). Taking into consideration the dilution made, it was possible to determine the concentration of the pigment fraction (chlorophyll a, chlorophyll b and carotenoids) as ug/ml using the following equations:

(1) Chlorophyll a = 10.3 E 663 – 0.918 E 644 = ug /ml,

(2) Chlorophyll b = 19.7 E 644 – 3.870 E 663 = ug /ml,

(3) Carotenoids = 4.2 E 452.5 – 0.0264 Chl. A + 0.426 Chl. B = ug/ml

Where, E is equal optical density at the given wave length

The average data of 3 years were subjected to statistical analysis in factorial randomized block designed as per method of Gomez and Gomez (1984).

3. Results and discussion

3.1 Growth parameters

Data presented in Table 1 showed that foliar application of Salicylic acid @ 50 ppm and Ethrel @ 200 ppm significantly increased the plant height over Cycocel @ 500 ppm and control (Table 1). Cycocel is an anti-gibberellin dwarfing agent, and foliar spray of this may induce deficiency of gibberellins in the plant and reduce the growth by blocking and conversion of geranyl pyrophosphate to coponyl pyrophosphate which is the first step of gibberellins synthesis (Moore, 1980). Significantly higher number of branches, dry weight and leaf area index was observed with bioregulators over the control. Ravinchandran and Ramaswami (1991), also indicated that the application of mepiquat chloride, cycocel and TIBA significantly increase the amount of dry matter production in soybean.

3.2 Photosynthetic pigments

The data recorded in Table 2 showed that Photosynthetic pigments content were increased significantly with the application of bioregulators at flower-initiation + pod-initiation stages than the other stages. The increased chlorophyll content of Cycocel treated plants could be referred to hormonal effects as it has been noted earlier that ancymidol stimulate chlorophyll biosynthesis through acceleration of chloroplasts differentiation and stimulating photosynthetic enzymes (Chung Jaedong*et al*., 1999). The present results are in agreement with those obtained by many investigators (Bekheta 2004 and Bekheta*et al*., 2006). The effect of cycocel in increasing chlorophyll contents may be due to the reduction in cell size resulting in dense cytoplasm (Appleby *et al*., 1966). The results are in conformity with early reports in soybean (Bora and Sarma, 2004).

3.3 Yield and its components

Data recorded in Table 3 and 4 showed that spraying of bioregulators caused a marked effect on number of pods per plant, seed yield, straw yield and harvest index in comparison to the untreated plants. Application of Ethrel @ 200 ppm gave significantly higher yield (1.75 t/ha) over the other treatments when applied at both flower-initiation and pod-initiation stages. Ethrel has been reported to improve productivity of pulse crops like cowpea, pigeonpea, mungbean and soybean by increasing the number of pods, seed weight and seed yield (Chandra 1985, Bora and Bahra 1989). Beneficial effect of Ethrel on increased flowering, fruit size and fruit yield have also been reported in tomato (Atta-aly*et al*., 1999). Ethylene released from Ethrel could possibly be utilized for promoting pod growth as Abbas (1991) has shown that early development is related to higher ethylene levels, thus decreasing flowering and pod shedding and thereby reducing abscission and improving better pod set. Harvest index was increased due to spraying the plants with bioregulators as compared to control. The increase in the yield recorded in this investigation could be a reflection of the effect of bioregulators on growth and development, it might be due to (a) marked increase in the number of branches per plant (Table 1) which gave a chance to the plant to carry more flowers, pods and hence more seeds (b) marked increase in the photosynthetic pigments content (Table 2), which could lead to increase in photosynthesis, resulting in greater transfer of assimilates to the seeds and causing increase in their weight (Table 3).

3.4 Oil and Protein content

Growth regulators also caused an increase on oil and protein content in the seeds (Table 5). Grewal*etal*., (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.

3.5 Economics

The highest net returns (Rs.69525) and B:C ratio (4.49) were obtained from Ethrel @ 200 ppm when applied at flower-initiation + pod-initiation stages (Table 5).

4. Conclusion

From the above findings it can be concluded that application of Ethrel @ 200 ppm as foliar spray at flower-initiation (40 days after sowing) + pod-initiation (65 days after sowing) stages gave higher vegetative growth, yield, net returns and B:C ratio as compare to Salicylic acid @ 50 ppm , Cycocel @ 500 ppm and control.

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Table 1. Effect of bioregulators on vegetative growth parameters of soybean (average for three years)

Characters	Bioregulators	FI	PI	FI + PI	Mean
Plant height (cm)	Salicylic acid @ 50 ppm	51.60	51.09	50.69	51.13
	Ethrel @ 200 ppm	50.60	50.71	50.93	50.75
	Cycocel @ 500 ppm	32.56	35.67	33.09	33.77
	Control	37.96	34.71	35.58	36.08
	Mean	43.18	43.05	42.57	
	CD (P=0.05) A	3.30			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			
Braches/plant	Salicylic acid @ 50 ppm	3.8	4.1	3.7	3.9
	Ethrel @ 200 ppm	4.3	4.0	4.4	4.2
	Cycocel @ 500 ppm	3.9	3.8	3.7	3.8
	Control	2.0	2.0	2.2	2.1
	Mean	3.5	3.5	3.5	
	CD (P=0.05) A	0.34			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			
Dry weight/plant (g) at 75 DAS	Salicylic acid @ 50 ppm	18.27	18.75	18.54	18.52
	Ethrel @ 200 ppm	20.98	21.83	22.59	21.80
	Cycocel @ 500 ppm	17.07	16.11	16.46	16.55
	Control	16.21	15.30	13.82	15.11
	Mean	18.13	18.00	17.85	
	CD (P=0.05) A	0.90			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	1.56			
Leaf area index at 75 DAS	Salicylic acid @ 50 ppm	3.27	3.17	3.40	3.28
	Ethrel @ 200 ppm	3.30	3.24	3.44	3.33
	Cycocel @ 500 ppm	3.42	3.34	3.60	3.45
	Control	2.62	2.50	2.64	2.59
	Mean	3.15	3.06	3.27	
	CD (P=0.05) A	0.24			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			

A = Bioregulators; B = Stages of application (FI = Flower initiation; PI = Pod initiation ; FI + PI = Both flower initiation and pod initiation)

Table 2. Effect of bioregulators on photosynthetic pigments of soybean (average for three years)

Characters	Bioregulators	FI	PI	FI + PI	Mean
Chlorophyll ‘a’ (ug/ml)	Salicylic acid @ 50 ppm	5.46	4.83	5.93	5.41
	Ethrel @ 200 ppm	6.32	6.28	6.85	6.48
	Cycocel @ 500 ppm	7.83	7.26	7.96	7.68
	Control	3.27	3.44	3.23	3.31
	Mean	5.72	5.45	5.99	
	CD (P=0.05) A	0.32			
	CD (P=0.05) B	0.28			
	CD (P=0.05) A x B	NS			
Chlorophyll ‘b’(ug/ml)	Salicylic acid @ 50 ppm	1.83	1.62	1.93	1.79
	Ethrel @ 200 ppm	2.15	2.35	2.43	2.31
	Cycocel @ 500 ppm	2.69	2.46	2.94	2.70
	Control	1.85	1.84	1.83	1.84
	Mean	2.13	2.07	2.28	
	CD (P=0.05) A	0.08			
	CD (P=0.05) B	0.07			
	CD (P=0.05) A x B	0.15			
Chlorophyll ‘a + b’(ug/ml)	Salicylic acid @ 50 ppm	7.29	6.45	7.86	7.20
	Ethrel @ 200 ppm	8.46	8.63	9.28	8.79
	Cycocel @ 500 ppm	10.53	9.71	10.90	10.38
	Control	5.12	5.28	5.06	5.15
	Mean	7.85	7.52	8.28	
	CD (P=0.05) A	0.36			
	CD (P=0.05) B	0.31			
	CD (P=0.05) A x B	0.62			
Carotenoids(ug/ml)	Salicylic acid @ 50 ppm	2.29	2.02	2.21	2.17
	Ethrel @ 200 ppm	2.55	2.94	3.07	2.85
	Cycocel @ 500 ppm	3.18	3.03	3.54	3.25
	Control	2.04	1.99	2.11	2.05
	Mean	2.52	2.50	2.73	
	CD (P=0.05) A	0.09			
	CD (P=0.05) B	0.08			
	CD (P=0.05) A x B	0.16			

A = Bioregulators; B = Stages of application (FI = Flower initiation; PI = Pod initiation ; FI + PI = Both flower initiation and pod initiation)

Table 3. Effect of bioregulators on yield components of soybean (average for three years)

Characters	Bioregulators	FI	PI	FI + PI	Mean
Pods/plant	Salicylic acid @ 50 ppm	47	52	56	52
	Ethrel @ 200 ppm	54	55	63	57
	Cycocel @ 500 ppm	40	38	28	35
	Control	24	24	25	24
	Mean	41	42	43	
	CD (P=0.05) A	3.29			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	5.69			
100 seed weight (g)	Salicylic acid @ 50 ppm	11.63	11.57	11.98	11.73
	Ethrel @ 200 ppm	12.32	12.33	12.67	12.44
	Cycocel @ 500 ppm	11.27	11.19	11.39	11.28
	Control	10.32	10.48	10.48	10.43
	Mean	11.39	11.39	11.63	
	CD (P=0.05) A	0.16			
	CD (P=0.05) B	0.14			
	CD (P=0.05) A x B	NS			
Seeds/pod	Salicylic acid @ 50 ppm	2.46	2.42	2.47	2.45
	Ethrel @ 200 ppm	2.47	2.44	2.65	2.52
	Cycocel @ 500 ppm	2.55	2.49	2.54	2.53
	Control	2.20	2.09	2.11	2.13
	Mean	2.42	2.36	2.44	
	CD (P=0.05) A	0.13			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			

A = Bioregulators; B = Stages of application (FI = Flower initiation; PI = Pod initiation ; FI + PI = Both flower initiation and pod initiation)

Table 4. Effect of bioregulators on yield of soybean (average for three years)

Characters	Bioregulators	FI	PI	FI + PI	Mean
Seed yield (t/ha)	Salicylic acid @ 50 ppm	1.45	1.41	1.68	1.51
	Ethrel @ 200 ppm	1.69	1.68	1.88	1.75
	Cycocel @ 500 ppm	1.22	1.19	1.27	1.23
	Control	1.08	1.05	1.09	1.07
	Mean	1.36	1.33	1.48	
	CD (P=0.05) A	0.11			
	CD (P=0.05) B	0.10			
	CD (P=0.05) A x B	NS			
Straw yield (t/ha)	Salicylic acid @ 50 ppm	2.38	2.32	2.57	2.42
	Ethrel @ 200 ppm	2.23	2.21	2.59	2.34
	Cycocel @ 500 ppm	1.53	1.60	1.61	1.58
	Control	1.92	2.12	2.18	2.07
	Mean	2.02	2.06	2.24	
	CD (P=0.05) A	181			
	CD (P=0.05) B	157			
	CD (P=0.05) A x B	NS			
Harvest index (%)	Salicylic acid @ 50 ppm	38.01	37.80	39.60	38.47
	Ethrel @ 200 ppm	42.94	43.16	42.07	42.72
	Cycocel @ 500 ppm	44.51	41.69	42.52	42.91
	Control	35.90	33.16	36.89	35.31
	Mean	40.34	38.95	40.27	
	CD (P=0.05) A	0.03			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			

A = Bioregulators; B = Stages of application (FI = Flower initiation; PI = Pod initiation ; FI + PI = Both flower initiation and pod initiation)

Table 5. Effect of bioregulators on oil and protein content of soybean seed (average for three years)

Characters	Bioregulators	FI	PI	FI + PI	Mean
Oil content (%)	Salicylic acid @ 50 ppm	17.66	17.59	17.64	17.63
	Ethrel @ 200 ppm	17.51	17.60	17.50	17.54
	Cycocel @ 500 ppm	17.53	17.53	17.52	17.53
	Control	16.52	16.58	16.65	16.58
	Mean	17.31	17.33	17.33	
	CD (P=0.05) A	0.18			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			
Protein content (%)	Salicylic acid @ 50 ppm	34.17	34.90	34.73	34.60
	Ethrel @ 200 ppm	34.92	36.08	36.11	35.70
	Cycocel @ 500 ppm	34.81	34.66	36.05	35.17
	Control	33.12	34.13	33.28	33.51
	Mean	34.26	34.94	35.04	
	CD (P=0.05) A	1.09			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			
Net return (Rs/ha)	Salicylic acid @ 50 ppm	51532	49860	59068	53485
	Ethrel @ 200 ppm	61973	61465	69525	64321
	Cycocel @ 500 ppm	40663	39003	42599	49755
	Control	34900	33836	43254	37330
	Mean	47267	46041	53611	
	CD (P=0.05) A	4703			
	CD (P=0.05) B	4073			
	CD (P=0.05) A x B	NS			
B:C ratio	Salicylic acid @ 50 ppm	3.77	3.65	3.54	3.65
	Ethrel @ 200 ppm	4.38	4.34	4.59	4.44
	Cycocel @ 500 ppm	2.81	2.70	2.44	2.65
	Control	2.59	2.51	3.20	2.77
	Mean	3.39	3.30	3.44	
	CD (P=0.05) A	0.33			
	CD (P=0.05) B	NS			
	CD (P=0.05) A x B	NS			

A = Bioregulators; B = Stages of application (FI = Flower initiation; PI = Pod initiation ; FI + PI = Both flower initiation and pod initiation); DAS = Days after sowing