International Journal of
Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 2 (3): 238-245, 2007
ISSN 1816-4897

Physiological Effect of Some Bioregulators on Vegetative Growth, Yield and Chemical Constituents of Pearl Millet (*Pennisetum typhoides* (Burm) Stapf. and Hubb)

Nishi Mathur and Anil Vyas
Department of Botany, Microbial Biotechnology and Biofertilizer Laboratory,
J.N.V. University, Jodhpur, India-342003

Abstract: A field study was conducted to estimate the effect of salicylic acid (1, 2 and 3 mM), sitosterol as well as putrescine concentrations (0.05, 0.10 and 0.15 mM) on vegetative growth characters, yield and its components and grain biochemical constituents of pearl millet (*Pennisetum typhoides* (Burm) Stapf. and Hubb) at elongation and milky stages. The application of bioregulators led to significant increases of vegetative growth characters such as plant height, leaf area, leaf area index, dry weight/plant, specific leaf weight, crop growth rate and net assimilation rate except plant height, which significantly reduced with increasing salicylic acid concentration Increasing vegetative character values were obtained at salicylic acid (3 mM), sitosterol or putrescine (0.15 mM) at elongation and milky stages of pearl millet plantsyield and its components, i.e., ear length, ear diameter, grain yield/plant, grain yield/feddan, crop index and 100-grain weight of pearl millet were significantly increased at certain level of applied bioregulators application Salicylic acid (3 mM), sitosterol (0.15 mM) and putrescine (0.15 mM) gave the highest values of the previous yield components. Bioregulators varied of their effect on grain biochemical constituents of pearl millet plant. Sitosterol (0.15 mM) was more effective on crude protein and total carotenoids while putrescine (0.15 mM) was also more effective on total sugars and oil content of grains. Grain yield/feddan showed highly significant and positive correlation with the majority of growth parameters under sitosterol and putrescine application but not with salicylic acid treatments.

Key words: Bioregulators, pearl millet, growth, grain yield

Introduction

Salicylic acid 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 and Prithiviraj, 2003; Shakirova *et al*., 2003). The effect of salicylic acid on the physiological processes is variable, promoting some processes and inhibiting others depending on its concentration, plant species, developmental stages and environmental conditions (El-Mergawi and Abdel Wahid, 2004). Low salicylic acid doses enhanced growth of wheat and maize (Shakirova *et al*., 2003; Shehata *et al*., 2001).

The diamine putrescine occurred widely in the higher plants. It was suggested to be involved in a variety of growth and developmental processes such as cell division (Bueno and Matilla, 1992), fruit set and growth (Biasi *et al*., 1991) and senescence (Kao, 1994). The interaction of polyamines with the macromolecules was responsible for physiological effects on plant growth and development (Smith, 1985). Polyamines were effective on inhibiting RNase and protease activities (Kaur-Sawhney and Galston, 1982). Also, Serafmi-Fracassini (1991) found that polyamins are activators of protein process that was related to genes encoding (Imai *et al*., 2004).

Corresponding Author: Anil Vyas, Department of Botany, Microbial Biotechnology and Biofertilizer Laboratory, J.N.V. University, Jodhpur, India-342003 Tel: +91-09414128040

Sitosterol is the major constituent of steroids in plant that plays a critical role in membrane function (Bloch, 1983). Sterols have a main role in plant development Brassinosteroids have been found to evoke both cell elongation and cell division resulting in elongation, swelling, curvature and splitting of the internode (Mandava, 1998). Physiological functions proposed for Brassinosteroids included cell elongation, cell division, leaf bending, vascular differentiation, proton pump-mediated membrane polarization, sink/source regulation responses (Sasse, 1999). In addition, brassinosteroids caused changes in enzymatic activities, membrane potential, DNA, RNA, protein synthesis, photosynthetic activity and changes in the balance of endogenous phytohorornones (Steven and Jenneth, 1998). Particular interest in sterols was elicited by enhanced growth characters and yield of maize and soybean plant (Abdel-Wahed *et al*., 2000, 2005). Recently, these studies provided strong evidence that sterols could be essential for normal plant growth and development (Ozedemir *et al*., 2004).

The aim of present research was to study the effect of the three bioregulators (Salicylic acid, sitosterol and putrescine) on growth, yield and some biochemical constituents of pearl millet plants.

Materials and Methods

Grains of pearl millet cultivar (CZI 9623) sown on May 2004 and 2005, respectively at the Gangana village, Jodhpur, Rajasthan. The plot area was 10.5 m^2 (3.5×3 m) in rows 60 cm apart and 20 cm apart between hill along the row. The experiments were arranged as a complete randomized block design with six replicates. The plants were sprayed at elongation stage (45 days after sowing) with the following bioregulators: Salicylic acid ($C_7H_6O_3$) (1, 2 and 3 mM), sitosterol (stigmasta-5-en-β-ol: (24 R)-24 ethylcholest-5-en-3o1) and putrescine (NH_4 $(CH_2)_4$ NH_2) (0.05, 0.10 and 0.15 mM). Control treatment sprayed with distilled water at the same period. Pre-sowing at rate of 100 kg/fed. Calcium superphosphate (15.5% P 205) was applied to the soil. Nitrogen fertilizer as ammonium nitrate (33.5% N) was added at a rate of 120 kg N/feddan in two equal doses the 1st half at 21 and the 2nd at 36 days after sowing.

Growth Characteristics

The following growth characters were measured at elongation stage (60 days after sowing) and milky stage (90 days after sowing). The vegetative growth characters were plant height, number of leaves/plant, stem diameter, dry weight/plant, Leaf area/cm^2 according to Bremner and Taha (1966), leaf area index (Watson, 1952), specific leaf weight (Pearce *et al*., 1969), crop growth rate (Abdel-Gawad *et al*., 1980) and net assimilation rate (Watson, 1958).

Yield and its Components Characteristics

Yield and its components such as ear length, ear diameter, number of row/ear, number of grains/row, grain weight/plant, grain yield/fed., crop index, 100-grain weight and shelling percentage were determined at maturity stage.

Chemical Analysis of Grains

Grain samples were dried at 70°C for constant weight and ground to determine the chemical constituents such as crude protein (Chapman and Pratt, 1978), total sugars (Dubios *et al*., 1956), oil percentage (AOAC, 1988) and total carotenoids (AOAC, 1956).

Statistical Analysis

The data were statistically analyzed in randomized complete block design according to Snedecor and Cochran (1990) and then combined analysis as well as simple correlation coefficients between growth characters and yield attributes were done according to Gomez and Gomez (1984). The treatments means were compared using LSD test at 5% of significant.

Results and Discussion

Growth Characters

Data presented in Table 1-2 show that vegetative growth characters of pearl millet such as plant height, leaf area/plant, stem diameter, leaf area index, dry weight/plant, specific leaf weight, net assimilation rate and crop growth rate significantly increased at elongation or milky stages as a result to foliar application of salicylic acid, sitosterol and putrescine bioregulators. However, number of leaves/plant was insignificantly affected by bioregulators application. Plant height significantly decreased with increasing salicylic concentration up to 3 mM. This mean that salicylic acid might be cause an inhibitory effect plant elongation, while, sitosterol and putrescine bioregulators were more effective on all vegetative growth characters. The maximum values of these characters were obtained by the concentration (3 mM) of all bioregulators at elongation and milky stages. It appeared from the results that sitosterol was more effective substance on pearl millet growth than the others. This effect

Table 1: Effect of some bioregulators on vegetative growth of pearl millet cv. CZI 9623 at elongation stage (60 days after sowing) (Average of two seasons, 2004-2005)

Growth regulators treatments (mM)	Plant height (cm)	No. of leaves/plant	Stem diameter (cm)	Dry weight (g/plant)	Leaf area (cm^2)	Leaf area index (LAI)	Specific leaf weight (mg cm^{-2})
Control	200.15	13.60	1.52	148.50	492.34	2.91	6.29
Salicylic acid							
1 mM	194.60	13.68	1.79	177.46	513.42	3.39	6.84
2 mM	191.87	13.90	1.94	190.28	611.43	3.75	7.45
3 mM	189.10	14.17	2.20	197.54	625.78	3.82	8.31
Sitosterol							
0.05 mM	209.44	13.77	1.72	172.94	575.96	3.57	7.71
0.10 mM	212.49	14.08	1.77	184.30	609.15	3.97	7.84
0.15 mM	228.41	14.16	1.95	195.97	682.86	4.17	8.38
Putrescine							
0.05 mM	215.52	13.78	1.75	165.38	566.83	3.50	7.75
0.10 mM	211.95	14.17	1.83	190.36	587.78	3.98	8.18
0.15 mM	226.39	14.07	1.95	199.64	637.87	4.26	8.56
LSD at 5%	12.80	NS	0.34	12.76	2.64	0.63	0.96

Table 2: Effect of some bioregulators on vegetative growth of pearl millet cv. CZI 9623 at milky stage (90 days after sowing) (Average of two seasons, 2004-2005)

Growth regulators treatments (mM)	Plant height (cm)	No. of leaves/ plant	Stem diameter (cm)	Dry weight (g/plant)	Leaf area (cm^2)	Leaf area index (LAI)	Specific leaf weight (mg/cm^2)	Net assimilati on rate (mg/cm^2/day) (60-90 days)	Crop growth rate mg/ (cm^2/day) (60-90 days)
Control	264.52	13.98	1.80	211.01	581.54	3.67	8.23	3.06	4.80
Salicylic acid									
1 mM	260.83	14.76	2.12	253.56	610.29	4.48	8.00	3.59	5.74
2 mM	257.39	14.13	2.31	271.31	630.88	4.66	8.84	3.23	6.04
3 mM	250.57	14.38	2.45	276.21	649.7	4.89	9.82	3.81	6.35
Sitosterol									
0.05 mM	267.32	14.27	2.05	252.21	683.18	4.29	9.63	3.97	5.16
0.10 mM	271.78	14.59	2.20	254.83	701.54	5.00	9.99	4.09	6.19
0.15 mM	276.86	14.88	2.31	270.10	745.76	5.26	10.87	4.37	6.44
Putrescine									
0.05 mM	263.09	14.6	1.87	227.47	679.86	4.57	9.74	3.17	5.72
0.10 mM	278.12	14.46	2.15	268.09	698.52	5.16	10.59	4.39	6.12
0.15 mM	283.38	14.96	2.24	278.90	736.67	5.33	11.03	4.34	6.39
LSD at 5%	6.45	NS	0.22	17.65	76.43	0.97	1.31	0.57	1.03

Table 3: Effect of some bioregulators on yield and its component of pearl millet cv. CZI 9623 (Average of two seasons, 2004-2005)

Growth regulators treatments (mM)	Ear length	Ear diameter (cm)	No. of (cm)	No. of grains/row rows/ear	100-grain weight (g)	Grain yield/ plant (g)	Grain yield (ton/fed.)	Crop index
Control	18.81	2.03	13.97	56.64	13.78	177.01	2.92	0.43
Salicylic acid								
1 mM	20.52	2.40	14.75	57.58	16.18	203.58	3.12	0.45
2 mM	21.31	2.51	15.12	57.90	18.20	212.86	3.22	0.46
3 mM	19.43	2.78	15.68	58.31	18.46	230.37	3.72	0.46
Sitosterol								
0.05 mM	19.63	2.16	14.94	57.80	16.52	215.83	3.76	0.44
0.10 mM	20.63	2.42	14.97	58.42	17.48	221.79	3.91	0.46
0.15 mM	22.05	2.52	15.20	59.14	19.06	235.45	4.36	0.50
Putrescine								
0.05 mM	19.33	2.24	14.88	57.76	15.91	206.36	3.67	0.48
0.10 mM	20.56	2.39	15.03	58.27	17.33	218.15	4.07	0.46
0.15 mM	21.40	2.48	15.55	59.52	18.90	226.10	4.20	0.50
LSD at 5%	1.18	0.38	NS	NS	2.59	28.52	0.74	0.10

could be attributed to bioregulators effect on physiological processes in plant such as, ion uptake, cell elongation, cell division, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity. Present results are in agreement with Shehata *et al*. (2001) revealed that low salicylic acid doses led to significant increases in growth characters of maize. Similar results were obtained by Khan *et al*. (2003) on corn and soybean and Shakirova *et al*. (2003) of wheat. Also, El-Mergawi and Abd El-Wahed (2004) found that the types of plants varied in their response to salicylic acid application as a result to genotype variation. Whereas, steroids have a role on cell elongation, cell division, leaf bending and enzymatic activities of plant (Sasse, 1999; Ozdemir *et al*., 2004). Consequently, the results of putrescine effect on vegetative growth of pearl millet are similar with those obtained by Abd El-Wahed (2000) on maize, Abd El-Wahed and Gamal El-Din (2005) on chamomile.

Yield and its Components

Data in Table 3 show that yield and its components such as ear length, ear diameter, 100-grain weight, grain yield/plant, grain yield/fed and crop index were significantly enhanced with foliar application of bioregulators (salicylic acid, sitosterol and putrescine) compared with control. However, number of rows/ear and number of grains/row had no significant increase by all bioregulators applications. Increasing yield and its components was related to bioregulators concentration up to 3 mM except ear length character. It appeared from the results that salicylic acid was more effective than sitosterol and putrescine application on ear diameter enhancement. Ear length, 100 grains weight, grain yield/plant and grain yield/fed, were also more affected by sitosterol than the other bioregulators. However, sitosterol and putrescine treatments did not show significant differences in their effect on crop index. The favourable effects of bioregulators were obtained at concentration of salicylic acid of 3 mM, sitosterol of 0.15 mM and putrescine of 0.05 mM. It might be due to bioregulators stimulation of physiological processes that were reflected on improving vegetative growth that followed by active translocation of the photosynthesis products from source to sink in pearl millet plant. The previous studies confirmed the present obtained positive responses of salicylic acid foliar application on soybean (Gutierrez-Coronado *et al*., 1998). In addition, brassinosteroids increased peanut yield (Vardhini and Seeta, 1998) as well as maize and chamomile yield after foliar or injection application of both stigmasterol or spermidine (Abdel-Wahed, 2000; Abdel-Wahad and Gamal El-Din, 2004). Whereas, increasing or decreasing putrescine content controlled pistillate flower and pistillate differentiation (Lin *et al*., 2001).

Table 4: Effect of some bioregulators on some biochemical constitutes of pearl millet cv. CZI 9623 (Average of two seasons, 2004-2005)

Growth regulators treatments (mM)	Crude protein (%)	Total carbohydrate (%)	Oil (%)	Total carotenoids (%)
Control	8.50	74.52	5.52	60.23
Salicylic acid				
1 mM	9.15	81.65	5.84	72.57
2 mM	9.21	82.30	6.67	75.83
3 mM	10.04	80.08	6.58	80.40
Sitosterol				
0.05 mM	9.41	81.84	6.05	73.77
0.10 mM	10.75	80.75	7.61	81.59
0.15 mM	11.57	77.01	6.58	88.07
Putrescine				
0.05 mM	9.11	84.53	6.07	76.10
0.10 mM	10.75	79.92	6.88	80.90
0.15 mM	11.46	75.90	7.78	89.00
LSD at 5%	1.65	4.90	1.01	6.73

Table 5: A simple correlation between grain yield (ton/fed.) and some growth characters under putrescine application

Growth regulators treatments (mM)	Grain yield (ton/fed.)	Ear length (cm)	Ear diameter (cm)	No. of rows/ ear	No. of grains/ ear	100 grain weight (g)	Grain yield/ plant (g)	Shelling (%)	Crop index
Grain yield (ton/fed.)	1	0.737**	0.793**	0.714**	0.558	0.530	0.932**	0.911**	0.630*
Ear length (cm)	0.737**	1	0.518	0.546	0.296	0.643*	0.728**	0.563	0.570
Ear diameter (cm)	0.793**	0.518	1	0.698*	0.396	0.465	0.802**	0.829**	0.547
No. of rows/ear	0.714**	0.546	0.698*	1	0.149	0.408	0.735**	0.783**	0.223
No. of grains/ear	0.558	0.296	0.396	0.149	1	0.325	0.502	0.526	0.510
100-grain weight (g)	0.530	0.643*	0.465	0.408	0.325	1	0.429	0.493	0.220
Grain yield/plant (g)	0.932**	0.728**	0.820**	0.735**	0.502	0.429	1	0.892**	0.686*
Shelling%	0.911*	0.563	0.829**	0.783**	0.526	0.493	0.892**	1	0.588*
Crop index	0.630*	0.570	0.547	0.223	0.510	0.220	0.686*	0.588*	1

* Significant, ** Highly significant

Chemical Constituents

Data in Table 4 indicated that foliar application of salicylic acid, sitosterol and putrescine bioregulators led to significant effect on some biochemical constituents of pearl millet seed. Crude protein, oil and total carotenoids content of pearl millet grain tended to increase while the total sugars content were decreased with enhancement bioregulators concentration. The maximum values of biochemical content in pearl millet seed were obtained by sitosterol treatment (0.15 mM) on crude protein and total carotenoids by putrescine (0.05 or 0.15 mM) on total sugars and oil. It appeared from the results that both sitosterol and putrescine are more effective on seed biochemical contents than salicylic acid. This effect might be due to these substances on enzymatic activity and translocation of the metabolites to pearl millet seed. These results are agreement with those obtained by Vardhini and Seeta (1998) they found that the exogenous application of brassinosteroids enhanced levels of DNA, RNA, soluble proteins, various carbohydrate and fat content of peanut seed. Also, Abd El-Wahed *et al*. (2000) and Abd El-Wahed (2001) found that stigmasterol or spermidine application increased the previous mentioned contents of maize seeds. This might be due to their conjugation with sugars and their effect on α-amylase. So, putrescine or salicylic application promoted nitrate reductase activity that was positively correlated with total organic nitrogen of maize (Shankar *et al*., 2001). In this current study increasing carotenoids content may due to convert these substances to pyruvic acid that led to increase biosynthesis of two general classes of carotenoid pigments carotene and xanthophylls (Martin-Tanguy, 2001; Raskin, 1992).

Correlation Study

Data in Table 5-7 show that grain yield (ton/fed.) recorded highly significant and positive correlation with each of plant height, stem diameter, ear length, grain yield/plant as well as, shelling

Table 6: A simple correlation between grain yield (ton/fed.) and some growth characters under sitosterol application.

Growth regulators treatments (mM)	Grain yield (ton/fed.)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of grains/ear	100 grain weight (g)	Grain yield/ plant (g)	Shelling (%)	Crop index
Grain yield (ton/fed.)	1	0.794**	0.651*	0.298	0.727**	0.455	0.923**	0.917**	0.588*
Ear length (cm)	0.794**	1	0.731**	0.409	0.568	0.625*	0.729**	0.636*	0.570
Ear diameter (cm)	0.651*	0.731**	1	0.449	0.273	0.587*	0.598*	0.564	0.762**
No. of rows/ear	0.298	0.409	0.449	1	0.451	0.451	0.334	0.187	0.276
No. of grains/ear	0.727**	0.568	0.273	0.451	1	0.078	0.621*	0.528	0.434
100-grain weight (g)	0.455	0.625*	0.587*	0.451	0.078	1	0.309	0.411	0.401
Grain yield/plant (g)	0.923**	0.729*	0.598*	0.334	0.621*	0.309	1	0.887**	0.571
Shelling%	0.917**	0.636*	0.564	0.187	0.528	0.411	0.887**	1	0.634*
Crop index	0.588*	0.570	0.762**	0.276	0.434	0.401	0.571	0.634*	1

* Significant, ** Highly significant

Table 7: A simple correlation between grain yield (ton/fed.) and some growth characters under salicylic application

Growth regulators treatments (mM)	Grain yield (ton/fed.)	Ear length (cm)	Ear diameter (cm)	No. of rows/ear	No. of grains/ear	100 grain weight (g)	Grain yield/ plant (g)	Shelling (%)	Crop index
Grain yield (ton/fed.)	1	-0.427	0.271	-0.073	-0.055	-0.151	0.104	0.003	0.206
Ear length (cm)	-0.427	1	0.486	0.387	0.622*	0.464	0.536	0.516	-0.067
Ear diameter (cm)	0.271	0.486	1	0.646*	0.584*	0.463	0.814**	0.727	0.334
No. of rows/ear	-0.073	0.387	0.646*	1	0.041	0.671*	0.525	0.697	0.006
No. of grains/ear	-0.055	0.622*	0.584*	0.041	1	0.078	0.621	0.528	0.434
100-grain weight (g)	-0.151	0.464	0.463	0.671*	0.078	1	0.402	0.607*	0.056
Grain yield/plant (g)	0.104	0.536	0.814**	0.525	0.621*	0.402	1	0.859**	0.531
Shelling%	0.003	0.516	0.727**	0.697*	0.528	0.607*	0.859**	1	0.578*
Crop index	0.206	-0.067	0.334	0.006	0.434	0.056	0.531	0.578*	1

percentage. Significant and positive correlation of grain yield was obtained with crop index and insignificant and positive correlation with 100-grains weight under sitosterol and putrescine application. On the other hand, grain yield (ton/fed.) showed insignificant and positive correlation with plant height, stem and ear diameter, grain yield/plant as well as, crop index. In addition, insignificant and negative correlation of grain yield with ear length, number of rows/ear, number of grains/ear and 100-grains weight under salicylic acid application are shown in Table 4. It appears that the improvement of growth reflected on increasing grain yield/fed. of pearl millet that showed high significant correlation between grain yield, stem diameter, ear diameter, number of rows fear and grain yield/plant under treating pearl millet with sitosterol and putrescine.

From the above mentioned results, it could be concluded that foliar application of sitosterol at 0.15 mM followed by putrescine at 0.15 mM, then salicylic acid at 3 mM to pearl millet CZI 9623 pioneer plant at elongation stages improved growth, yield and its components as well as, some biochemical constituents of pearl millet plants.

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