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Efficacy of Putrescine and IBA on biochemical and yield contributing parameters of black gram

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Abstract
In order to examine effects of different concentrations of growth regulators (putrescine and IBA) on biochemical and yield contributing parameters on black gram a field experiment was carried out in the botany farm, college of agriculture, Nagpur during 2017-18. Foliar application of putrescine and IBA 25, 50, 75 and 100 ppm each was given at vegetative Stage (25 and 40 DAS) and the data were taken at 25, 40 and 55 DAS coinciding with vegetative stage, flowering stage and before harvest stage. Application of putrescine and IBA enhanced biochemical parameters *viz.*, chlorophyll content, NPK content in leaves and protein content in grains, yield contributing characters *viz.*, number of seeds pod^{-1},weight of 100 seeds, seed yield plant^{-1}, plot^{-1} and ha^{-1}. Analysis of data revealed that 100 ppm IBA considered as a most effective concentration in enhancing all biochemical and yield contributing parameters. But considering the B: C ratio two foliar sprays of 25 ppm IBAat 25 and 40 DAS was found more effective and economical treatment with increased yield of 30.47% having B:C ratio of 2.97 as compared to 2.54 in control.

Keywords: Black gram, putrescine, IBA, foliar application, biochemical parameters, and yield

Introduction
Black gram is chief constituent of dal (husked or unhusked), Papad, Idali, Dahiwada, and imarati. The grain of black gram is superior in nutritive value, black gram contain about 24% proteins, 61% carbohydrate, 1.8% fat, 345 Kcal energy, 3.5% minerals, 385 mg 100 g^{-1} phosphoric acid and 16.2% total diatory fibre. Black gram rich in minerals like Zinc, Calcium and also rich in Vitamins like thiamine, riboflavin and niacin.
In India, black gram traditionally grown in *kharif* season, but in south it also grown as *rabi* crop. It is widely grown in India and Central Asia. In India generally grown in area of which received annual rainfall of 800 mm. It is a hardy and drought resistant plant. Major black gram producing state in India is Maharashtra. Maharashtra is a leading producer of black gam followed by Andra Pradesh, Utter Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Rajasthan and Orrisa.
The yield of black gram may be enhanced through physiological manipulation such as foliar application of putrescine and IBA. Plant growth regulators are shown to change leaf resistance by altering stomatal aperture, the rate of photosynthesis could be manipulated through this technology. Putrescine, IBA, IAA, GA, kinetin, phenolics and aliphatic alcohols are reported to increase and stimulate the rate of photosynthesis. 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) [4], fruit set and growth (Biasi *et al*.,1991) [3] and senescence (Kao, 1994) [11].
Considering the above facts present investigation was undertaken to study the effect of putrescine and IBA on biochemical and yield and yield contributing parameters of black gram.

Materials and Methods
A field experiment was conducted at botany farm, college of agriculture, Nagpur to know the response of black to growth regulators on its biochemical and yield contributing parameters. The experiment was laid out in complete randomized block design with three replications. The experiment consisted of nine treatments *viz*., IBA and putrscine applied at 25, 50, 75 and 100 ppm each with control (water spray). The seeds were sown with a spacing of 30 x 10 cm. Growth promoters were sprayed twice at 25 and 40 DAS. First Observation (25 DAS) was made before the spray and other observations were made at 40 and 55 DAS. Five randomly selected plants were tagged and observations were taken on biochemical parameters (leaf chlorophyll content, N, P, K content in leaves and protein content) and yield attributing characters (Number of seeds pod^{-1}, weight of 100 seeds and yield plot^{-1}). Determination of nitrogen and protein was carried out by micro-kjeldhal method as given by Somichi *et al.*

(1972) [16]. Phosphorous and potassium estimated by vanadomolybdate yellow colour method, (using calorimeter and flame photometer respectively) given by Jackson, (1967) [9]. The observed data were analyzed statistically using analysis of variance at 5% level of significance (Panse and Sukhamate, 1967).

Results and Discussion

Chlorophyll content

The chlorophyll content differed significantly between control and growth regulators applied plants (table 1). The content varied with the lowest value of 1.28 mg g^{-1}in control (T_1) to highest value of 1.94 mg g^{-1} in 100 ppm IBA treatment (T_9) at 40 DAS and lowest value of 1.19 in control (T_1) to highest value of 1.49 in 100 ppm IBA treatment (T_9) at 55 DAS among the treatments studied. Foliar application of all the growth regulator treatments increased the chlorophyll content over control except application of 75 ppm putrescine (T_4), 50 ppm IBA (T_7), 50 ppm putrescine (T_3), 25 ppm IBA (T_6) and 25 ppm putrescine (T_2). These treatments were found at par with control. Among the treatments, the highest chlorophyll content was recorded in treatment T_9 (100 ppm IBA) followed by the treatments T_5 (100 ppm putrescine) and T_8 (75 ppm IBA) when compared with treatment T_1 (control) at both the stages of observations. Putrescine or IBA treatments retard senescence and chlorophyll loss by altering the stability and permeability of membranes and protecting chloroplast from senescing (Gonzalez-Aguilar *et al*., 1997) [8]. A possible explanation for the promoting effect of putrescine on photosynthetic pigment of black gram plant in the present work is that PAs might retard the chlorophyll destruction and / or increase their biosynthesis or stabilize the thylakoid membrane. Similar effects of PAs on photosynthetic pigments had been observed by Pinkey *et al.* (2016) [14]. They conducted a field experiment to access the effect of putrescine in mitigating the water stress in wheat and reported that foliar sprays of 50 and 100 ppm putrescine significantly increased the chlorophyll content.

Nitrogen content

Leaf nitrogen content differed significantly among the treatments and varied from a minimum of 3.96% at 40 DAS and 3.56% at 55 DAS in control treatment to a maximum of 5.81% at 40 DAS and 4.77% at 55 DAS in treatment T_9 (100 ppm IBA). Next to this treatment the treatments were T_5 (100 ppm putrescine), T_8 (75 ppm IBA) and T_4 (75 ppm putrescine) when compared with treatment T_1 (control). Whereas, treatments T_3 (50 ppm putrescine), T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with treatment T_1 (control).

From this data it is observed that leaf nitrogen content was increased up to 40 DAS and reduced thereafter, at 55 DAS. The decrease in nitrogen content might be due to fact that younger leaves and developing organs, such as grains act as strong sink demand and may draw heavily nitrogen from older leaves (Gardner *et al*., 1988) [7]. Results recorded by Poonkodi (2003) [15] also stated that decrease in nitrogen content at later stage might be due to translocation and utilization of nutrient for flower and pod formation.

Ahmed *et al*. (2013) [1] tried putrescine and humic acid on cotton. These growth regulators were sprayed eight times started at 45 DAP and repeated every after 15 days on cotton and reported that foliar application of 2 ppm putrescine and 1% humic acid significantly enhanced inorganic nitrogen.

Phosphorous content

Leaf phosphorus content at 40 DAS differed among the treatments and varied from a minimum of 0.833% in control to a maximum of 0.938% in treatment T_9 (100 ppm IBA). Treatment T_5 (100 ppm putrescine) also showed their significance and enhanced phosphorus content in leaves when compared with treatment control and other treatments under study. While, foliar application of 75 ppm IBA (T_8), 75 ppm putrescine (T_4), 50 ppm IBA (T_7) and 50 ppm putrescine (T_3) also increased phosphorus content significantly over control. Treatments T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with control (T_1).

Leaf phosphorus content at 55 DAS differed among the treatments and varied from a minimum of 0.812% to a maximum of 0.911% in treatment T_9 (100 ppm IBA) followed by the treatments T_5 (100 ppm putrescine) T_8 (75 ppm IBA) and T_4 (75 ppm putrescine) when compared with treatment T_1 (control) and remaining treatments under study. But treatments T_7 (50 ppm IBA), T_3 (50 ppm putrescine), T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with control in leaf phosphorus content.

Wagh (2015) [17] observed that foliar sprays of putrescine and IBA @ 100 ppm significantly enhanced phosphorus content in soybean leaves.

Potassium content

Potassium is an essential macronutrient for plants involved in many physiological processes. It is important for crop yield as well as for the quality of edible parts of crops. Although potassium is not assimilated into organic matter, potassium deficiency has a strong impact on plant metabolism. Plant responses to low potassium involve changes in the concentrations of many metabolites as well as alteration in the transcriptional levels of many genes and in the activity of many enzymes.

Data pertaining to potassium content in leaves were estimated at various stages of observations *viz*., 25, 40 and 55 DAS. Significant results were recorded at all the stages of observations *viz*., 40 and 55 DAS except 25 DAS.

At 40 DAS significantly highest leaf potassium content was observed in treatment T_9 (100 ppm IBA) followed by treatments T_5 (100 ppm putrescine) T_8 (75 ppm IBA) and T_4 (75 ppm putrescine). Similarly treatments T_7 (50 ppm IBA) and T_3 (50 ppm putrescine) were also found significantly superior over treatment T_1 (control) in potassium content. Treatments T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with treatment T_1 (control). The range of potassium content at 40 DAS in blackgram was 0.98-1.91%.

At 55 DAS significantly highest leaf potassium content was observed in treatment T_9 (100 ppm IBA) followed by treatments T_5 (100 ppm putrescine) and T_8 (75 ppm IBA). Similarly treatments T_4 (75 ppm putrescine), T_7 (50 ppm IBA) and T_3 (50 ppm putrescine) were also found significantly superior over treatment T_1 (control).Treatments T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with treatment T_1 (control). The range of potassium content at 50 DAS in blackgram was 0.82-1.62%.

It is clear from the data, that leaf potassium content was decreased gradually in later stage of crop growth i.e.55 DAS. It might be because of diversion of potassium towards developing parts i.e. pod formation in blackgram.

Deotale *et al.* (2016) [6] conducted a field experiment to investigate the foliar sprays of putrescine and IBA on soybean and noted that two foliar sprays of 100 ppm putrescine and

100 ppm IBA at two stages i.e. before flowering and 10 days after flowering were found to be most effective in enhancing potassium content in leaves.

Protein content in seed

Significant variation in protein content was observed among the treatments studied (table 2). Foliar application of growth regulator 100 ppm IBA registered the highest protein content (26.50%) followed by treatments T_5 (100 ppm putrescine), T_8 (75 ppm IBA), T_4 (75 ppm putrescine) and T_7 (50 ppm IBA) when compared with treatment T_1 (control) and rest of the treatments under study. Similarly, treatments T_3 (50 ppm putrescine) and T_6 (50 ppm IBA) were also increased seed protein significantly over treatment T_1 (control). Treatment T_2 (25 ppm putrescine) was found at par with treatment T_1 (control) in protein content.

It is observed from the results that putrescine and IBA are more effective in seed protein content. This might be due to enhancement of enzymatic activity and translocation of metabolites to the blackgram seeds.

Mathur and Vyas (2007) [12] conducted an experiment to study the effect of salicyclic acid, sisterol as well as putrescine on pearl millet (*Pennisetum thyphoides*). Results showed that application of salicyclic acid at 3 mM and sisterol or putrescine at 0.15 Mm significantly increased protein content.

Yield contributing parameters

Results presented in table 2 showed that both growth regulators (putrescine and IBA) have a promoting effect on yield contributing characters *viz.*, number of seeds pod^{-1}, 100 seed weight, Yield plant^{-1} and yield plot^{-1}of black gram. Significant variation in seed weight was noticed among the treatments. The highest 100 seed weight (4.93 g) and the lowest 100 seed weight (4.10 g) were recorded in 100 ppm IBA (T_9) and control (T_1) respectively. However, 100 seed weight was significantly maximum in treatments T_5 (100 ppm putrescine), T_8 (75 ppm IBA), T_4 (75 ppm putrescine) and T_7 (50 ppm IBA) when compared with treatment T_1 (control). But treatments T_3 (50 ppm putrescine), T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) were found at par with treatment T_1 (control).

Number of seeds pod^{-1} varied among the treatments and ranged a minimum of 4.51 to a maximum of 6.18. Among all the treatments the highest seeds pod^{-1} was obtained in treatment T_9 (100 ppm IBA) followed by treatments T_5 (100 ppm putrescine), T_8 (75 ppm IBA) and T_4 (75 ppm putrescine) when compared with treatment T_1 (control) and rest of the treatments under observations. Foliar application of 50 ppm putrescine, 25 ppm IBA and 25 ppm putrescine exhibited significantly minimum number of seeds pod^{-1} and remained at par with control treatment.

Seed yield plant^{-1}varied among the treatments. Foliar application of growth regulators significantly increased seed yield plant^{-1}over control. The highest seed yield (4.05 g plant^{-1}) was recorded in treatment T_9 (100 ppm IBA) as compared to (2.36 g plant^{-1}) treatment T_1 (control).Seed yield plot^{-1}also varied among the treatments. Foliar application of growth regulators significantly increased seed yield plot^{-1}over control. The highest seed yield (0.57 kg plot^{-1}) was recorded in treatment T_9 (100 ppm IBA) as compared to (0.33 kg plot^{-1}) treatment T_1 (control).

The highest seed yield of 13.49 q ha^{-1}was obtained by the treatment T_9 (100 ppm IBA) which registered 52.25% higher seed yield over control (8.86 q ha^{-1}).Treatments T_5 (100 ppm putrescine), T_8 (75 ppm IBA),T_4 (75 ppm putrescine), T_7 (50 ppm IBA), T_3 (50 ppm putrescine), T_6 (25 ppm IBA) and T_2 (25 ppm putrescine) in a descending manner also enhanced seed yield ha^{-1}significantly over control.

The increase in yield and yield attributes may be due to altering the hormonal balance and improved water relation in plants. PA namely putrescine is involve in stabilization of D_1 and D_2 polypeptides of photosystem second which is the source of electron for NADP+ reduction at photosystem one (Taiz and Zeiger, 1991) [18]. It is also prevented the lipid peroxidation, proteolytic attack and inhibitors of ethylene synthesis through inhibition of ACC synthase and conversion of ACC to ethylene, which is a common phenomenon occurred during senescence. The present results also in line with the report of (Kabir *et al*., 1992) [10]. They stated that PAs improved the yield and yield attributes in tomato. The increments in yield components due to putrescine treatments may be attributed to the increasing growth rate, in this respect, Davies (1995) [5] reported that polyamines play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits. It is worthy to mention that there is a close relationship between the effect of PAs and the stimulated growth, endogenous Phyto hormones, the photosynthetic output (soluble sugars, polysaccharides and total carbohydrates) and the nitrogen constituents. These results might increase the efficiency of solar energy conversion which maximize the growth ability of black gram plant and consequently increased its productivity and yield components.

Amin *et al*. (2013) [2] explained the effect of two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1}applied either alone or in combinations. Spraying of putrescine and IBA @100 mg l^{-1} significantly increased number of pods, seed yield, straw and biological yield feed^{-1} of chickpea (*Cicer arientinum*).

Table 1: Effect of putrescine and IBA on leaf chlorophyll, nitrogen and phosphorus contents

Treatments	Chlorophyll Content in leaves (mg/g)			Nitrogen Content in leaves (%)			Phosphorous Content in leaves (%)		
	25 DAS	40 DAS	55 DAS	25 DAS	40 DAS	55 DAS	25 DAS	40 DAS	55 DAS
T_1 (control)	1.22	1.28	1.19	3.48	3.96	3.56	0.632	0.833	0.812
T_2 (25 ppm putrescine)	1.28	1.35	1.21	3.70	4.12	3.74	0.653	0.847	0.826
T_3 (50 ppm putrescine)	1.24	1.46	1.33	3.49	4.59	4.15	0.678	0.869	0.842
T_4 (75 ppm putrescine)	1.32	1.58	1.38	3.82	5.19	4.63	0.629	0.892	0.876
T_5 (100 ppm putrescine)	1.29	1.78	1.47	3.57	5.52	4.87	0.664	0.921	0.897
T_6 (25 ppm IBA)	1.23	1.42	1.24	3.73	4.22	3.83	0.667	0.856	0.834
T_7 (50 ppm IBA e)	1.34	1.52	1.34	3.66	4.72	4.27	0.618	0.878	0.857
T_8 (75 ppm IBA)	1.26	1.65	1.46	3.83	5.34	4.77	0.646	0.903	0.893
T_9 (100 ppm IBA)	1.33	1.94	1.49	3.67	5.81	5.17	0.658	0.938	0.911
SE (m) ±	0.08	0.11	0.07	0.21	0.23	0.27	0.04	0.01	0.02
CD at 5%	-	0.32	0.20	-	0.68	0.80	-	0.03	0.05

Table 2: Effect putrescine and IBA on leaf potassium content, seed protein, seeds pod^{-1}, 100 seed weight, seed yield plant^{-1}, plot^{-1}, ha^{-1},per cent increase in yield and B:C ratio

Treatments	Leaf potassium content (%)		Protein content (%)	Seeds pod^{-1}	100 seed weight (g)		Seed yield plant^{-1} (g)	Seed yield plot^{-1} (kg)	Seed yield ha^{-1} (q)	Percent increase over control	B: C ratio
	25 DAS	40 DAS	55 DAS								
T_1 (control)	0.81	0.98	0.82	24.20	4.51	4.10	2.36	0.33	8.86	-	2.54
T_2 (25 ppm IBA)	0.87	1.05	0.89	24.86	4.70	4.23	3.27	0.46	10.89	22.91	2.37
T_3 (50 ppm IBA)	0.92	1.46	1.07	25.54	5.22	4.50	3.62	0.51	12.06	36.11	2.11
T_4 (75 ppm IBA)	0.84	1.69	1.32	25.98	5.61	4.66	3.79	0.53	12.63	42.50	1.85
T_5 (100 ppm IBA)	0.89	1.88	1.49	26.25	6.05	4.80	4.03	0.56	13.43	51.58	1.69
T_6 (25 ppm putrescine)	0.86	1.24	0.98	25.32	4.98	4.30	3.47	0.49	11.56	30.47	2.97
T_7 (50 ppm putrescine)	0.91	1.54	1.22	25.68	5.31	4.65	3.69	0.52	12.29	38.71	2.86
T_8 (75 ppm putrescine)	0.85	1.74	1.42	26.10	5.74	4.70	3.85	0.54	12.83	44.80	2.73
T_9 (100 ppm putrescine)	0.94	1.91	1.62	26.50	6.18	4.93	4.05	0.57	13.49	52.25	2.64
SE (m) ±	0.06	0.09	0.07	0.31	0.24	0.14	0.21	0.03	0.56	-	-
CD at 5%	-	0.26	0.20	0.92	0.71	0.41	0.62	0.08	1.67	-	-

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