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Physiology of Pgr's and Nitrogen on Crop Growth Rate, Net Assimilation Rate, Nitrate Reductase Activity and Indole Acetic Acid Oxidase Activity of Black Gram (*Vigna Mungo* L.)

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Abstract The field experiment were undertaken to study the effect of nitrogen in combination with foliar spray of bioregulators and micronutrients on growth and productivity of CO5 black gram. Photosynthetic pigments and foliage soluble protein content were estimated at different phenological phases of black gram. Seed yield were assessed at the time of harvest. Significant increase in the CGR (Crop Growth Rate) and NAR (Net Assimilation Rate) due to basal application of nitrogen 25 kg per hectare with foliar spray of urea 2% and 0.1 ppm brassinolide. The NA-ase and IAA-oxidase enzymes were also greatly altered by the basal application of nitrogen 25 kg per hectare with foliar spray of urea 2% and 0.1 ppm brassinolide treatment.

Keywords Black gram; PGR (Plant Growth Regulator); CGR (Crop Growth Rate); NAR (Net Assimilation Rate); NR-ase; IAA-oxidase; Nitrogen and yield

Introduction

Pulses are the most important crops in India because of its low cost and high quality protein. They play a major role in providing a balanced protein component in the diet of the people. Pulses contain a higher level of quality protein, nearly three times as much as cereals; therefore they are the cheapest and rich source of protein and essential amino acids and thus share a major protein of the vegetarian diet. Besides, the crops enrich the soil fertility and health in terms of addition of nitrogen and organic matter. Among pulses, black gram (*Vigna mungo* L. *Hepper*), occupies a unique place for its use as vegetable, and it is grown both as pure and mixed crop along with maize, cotton, sorghum and other millets. It is also known as urd bean, and it is an important pulse crop grown all over the world. It is a major component of the daily Indian diet and serves as a rich protein source (23.9%) besides; it also contains 60.4% carbohydrates. As per the World Health Organization every man needs 80 g of pulses per day and as per the Indian Council of Medical Research, every man needs minimum consumption of 47 g of protein per day to meet requirement of the body. But at present, the per capita availability of pulses is only 30~35 g per day. Therefore, there is a need for three fold increase in pulse production as that of current production. Black gram is indeterminate in its flowering and fruiting habits and there is a competition for available assimilates between vegetative and reproductive sinks. There is limitation of source (leaves) particularly at flowering and fruiting stage. Hence, there is a need to improve LAI, CGR, NAR and LAD. Being a C_3 plant, CGR and RGR are relatively less than cereals and the major yield components are pods per plant, seeds per plant and test weight of seeds. Apart from this genetic

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makeup, the major physiological constraints limiting its production are flower drop and fruit drop (Ojeaga and Ojehomon, 1972). This performance of the crops can be overcome by foliar application of growth regulating chemicals at the crucial stages of the crop, which is one of the latest trends in agriculture. The growth regulating chemicals bioregulators can improve the LAI, SLW and SLA and play a significant role in improving the productive potential of the crop. With this above background, the present investigation was carried out.

1 Result

1.1 Crop growth rate (CGR) ($g \cdot m^{-2} \cdot day^{-1}$)

Crop Growth Rate recorded between vegetative to flowering stage (30~45 DAS) flowering to pod filling stage (45~60 DAS) and pod filling to harvest stage (60~75 DAS) is presented in (Table 1). The crop growth rate ranged from 0.54 to 0.78, 1.22 to 1.84 and 1.02 to 1.36 $g \cdot m^{-2} \cdot day^{-1}$ at 30~45, 45~60 and 60~75 DAS, respectively. The treatment T_7 (N 25 kg/ha + Urea 2% + BR 0.1 ppm) recorded the maximum CGR (0.78, 1.84 and 1.36 $g \cdot m^{-2} \cdot day^{-1}$) at all the stages of growth. There was no significant variation among the treatments at pod filling to harvest stage.

1.2 Net assimilation rate (NAR) ($mg \cdot cm^{-2} \cdot day^{-1}$)

The trend of NAR decreased from 30~45 DAS of crop growth to 60~75 DAS (Table 2). However, the declining trend was reduced by the application of nutrients and growth regulators. The NAR at various stages of crop growth viz., 30~45 DAS, 45~60 DAS and 60~75 DAS stages ranged from 0.54 $mg \cdot cm^{-2} \cdot day^{-1}$ to 0.66 $mg \cdot cm^{-2} \cdot day^{-1}$, 0.50 $mg \cdot cm^{-2} \cdot day^{-1}$ to 0.65 $mg \cdot cm^{-2} \cdot day^{-1}$ and 0.50 $mg \cdot cm^{-2} \cdot day^{-1}$ to 0.63 $mg \cdot cm^{-2} \cdot day^{-1}$ respectively. The treatment T_7 (N 25 kg/ha + Urea 2% + BR 0.1 ppm) recorded the highest NAR values at all the stages of crop growth, followed by T_3 and T_4 treatments.

Table 1 Effect of nitrogen nutrition and growth regulators on crop growth rate ($g \cdot m^{-2} \cdot day^{-1}$) in black gram at different growth stages

Treatment	30~45 DAS	45~60 DAS	60~75 DAS
T_1	0.54	1.22	1.02
T_2	0.56	1.28	1.07
T_3	0.71	1.62	1.20
T_4	0.60	1.53	1.21
T_5	0.61	1.44	1.03
T_6	0.55	1.51	1.09
T_7	0.78	1.84	1.36
T_8	0.59	1.42	1.01
T_9	0.60	1.58	1.04
Mean	0.61	1.49	1.11
SEd	0.02	0.06	0.04
CD (P=0.05)	0.05	0.13	0.98

Note: T_1: Control; T_2: N 25 kg/ha + Urea 2% + NAA 40 ppm; T_3: N 50 kg/ha + CCC 200 ppm; T_4: N 25 kg/ha + Urea 2% + CCC 200 ppm; T_5: N 25 kg/ha + Urea 2% + Humic acid 0.1%; T_6: N 25 k/ha + Urea 2% + Salicylic acid 100 ppm; T_7: N 25 kg/ha + Urea 2% + Brassinosteriod 0.1 ppm; T_8: N 25 kg/ha + Urea 2% + ZnSO4 0.5% + FeSO4 0.5% + Borax 0.2%; T_9: N 25 kg/ha + Water spray

1.3 Nitrate reductase activity (μg $NO_2 \cdot g^{-1} \cdot hr^{-1}$)

The trend of NRase activity estimated at four stages of growth is presented in Table 3. Higher NRase activity was observed at the pod filling stage (60 DAS) for all the treatments including control. At the same stage, significant increase (56.2 μg $NO_2 \cdot g^{-1} \cdot hr^{-1}$) was observed with N 25 kg/ha + urea 2% + 0.1 ppm BR (T_7) treated plants compared to other treatments. The enzyme activity recorded for treatment T_4 was found to be statistically comparable with the values of treatment T_7.

1.4 Indole acetic acid oxidase activity (IAAO) (μg of unoxidised auxin$\cdot g^{-1} \cdot hr^{-1}$)

The data on IAA oxidase estimated at specific stages and expressed in terms of unoxidized auxin content is presented in Table 4. The unoxidised auxin content was significantly increased by the foliar application of nutrients and growth regulators. Application of N 25 kg/ha + urea 2% + 0.1 ppm BR (T_7) proved to be superior (65.8, 98.5, 105.6 and 74.8 μg of unoxidised auxin·g^{-1}·hr^{-1}) in its activity compared to all other treatments. It is also known that all the treatments were significantly superior over the control (88.1, 121.4, 147.2 and 116.0 μg of unoxidised auxin·g^{-1}·hr^{-1}) at all the stages of growth.

Table 2 Effect of nitrogen nutrition and growth regulators on net assimilation rate (mg·m^{-2}·day^{-1}) in black gram at different growth stages

Treatment	30~45 DAS	45~60 DAS	60~75 DAS
T_1	0.54	0.50	0.48
T_2	0.60	0.58	0.57
T_3	0.65	0.64	0.61
T_4	0.64	0.60	0.59
T_5	0.59	0.57	0.55
T_6	0.57	0.54	0.52
T_7	0.66	0.65	0.63
T_8	0.62	0.59	0.56
T_9	0.56	0.52	0.50
Mean	0.61	0.58	0.56
SEd	0.026	0.025	0.024
CD (P=0.05)	0.055	0.054	0.052

Note: T_1: Control; T_2: N 25 kg/ha + Urea 2% + NAA 40 ppm; T_3: N 50 kg/ha + CCC 200 ppm; T_4: N 25 kg/ha + Urea 2% + CCC 200 ppm; T_5: N 25 kg/ha + Urea 2% + Humic acid 0.1%; T_6: N 25 k/ha + Urea 2% + Salicylic acid 100 ppm; T_7: N 25 kg/ha + Urea 2% + Brassinosteriod 0.1 ppm; T_8: N 25 kg/ha + Urea 2% + ZnSO4 0.5% + FeSO4 0.5% + Borax 0.2%; T_9: N 25 kg/ha + Water spray

Table 3 Effect of nitrogen nutrition and growth regulators on NRase activity (μg NO_2·g^{-1}·hr^{-1}) in black gram at different growth stages

Treatments	30 DAS	45 DAS	60 DAS	Harvest stage
T_1	14.4	19.4	28.1	9.8
T_2	16.2	22.8	41.7	11.9
T_3	18.3	28.6	49.8	10.6
T_4	19.9	34.6	52.8	17.3
T_5	16.7	22.9	42.8	10.7
T_6	17.6	26.4	46.5	10.8
T_7	21.2	36.3	56.2	18.3
T_8	15.8	22.4	38.4	15.4
T_9	15.1	21.0	30.4	11.6
Mean	17.21	26.09	43.58	12.91
SEd	0.72	1.10	1.82	0.54
CD (0.05)	1.54	2.34	3.87	1.15

Note: T_1: Control; T_2: N 25 kg/ha + Urea 2% + NAA 40 ppm; T_3: N 50 kg/ha + CCC 200 ppm; T_4: N 25 kg/ha + Urea 2% + CCC 200 ppm; T_5: N 25 kg/ha + Urea 2% + Humic acid 0.1%; T_6: N 25 k/ha + Urea 2% + Salicylic acid 100 ppm; T_7: N 25 kg/ha + Urea 2% + Brassinosteriod 0.1 ppm; T_8: N 25 kg/ha + Urea 2% + ZnSO4 0.5% + FeSO4 0.5% + Borax 0.2%; T_9: N 25 kg/ha + Water spray

Table 4 Effect of nitrogen nutrition and growth regulators on IAA-oxidase in black gram at different growth stages (μg unoxidised auxin·g^{-1}·hr^{-1})

Treatments	30 DAS	45 DAS	60 DAS	Harvest stage
T_1	88.1	121.4	147.2	116.0
T_2	71.3	118.7	128.6	78.4
T_3	69.0	109.1	114.9	89.3
T_4	68.7	101.5	109.4	78.6
T_5	73.8	116.9	124.3	84.2
T_6	80.7	111.0	120.4	92.5
T_7	65.8	98.5	105.6	74.8
T_8	78.4	105.2	117.6	86.4
T_9	85.6	120.7	138.5	109.5
Mean	75.54	111.22	122.67	89.75
SEd	3.33	4.83	5.42	4.05
CD (0.05)	7.07	10.24	11.50	8.58

Note: T_1: Control; T_2: N 25 kg/ha + Urea 2% + NAA 40 ppm; T_3: N 50 kg/ha + CCC 200 ppm; T_4: N 25 kg/ha + Urea 2% + CCC 200 ppm; T_5: N 25 kg/ha + Urea 2% + Humic acid 0.1%; T_6: N 25 k/ha + Urea 2% + Salicylic acid 100 ppm; T_7: N 25 kg/ha + Urea 2% + Brassinosteriod 0.1 ppm; T_8: N 25 kg/ha + Urea 2% + ZnSO4 0.5% + FeSO4 0.5% + Borax 0.2%; T_9: N 25 kg/ha + Water spray

2 Discussion

Crop production is determined by crop growth rate as a function of light interception by the leaf area of a crop (Whigham, 1983). Shibles and Weber (1966) also stated that as CGR is a linear function of intercepted irradiance, maintaining higher LAI has a positive effect for higher dry matter production due to increased CGR. As observed in the present study, CGR of black gram exhibited an increasing trend from vegetative to pod filling stage followed by a decline thereafter. Since CGR is the product of NAR and LAI, this trend indicated that in black gram CGR is closely related to LAI rather than NAR. In supporting this finding, Shibles and Weber (1966) observed a strong positive correlation between CGR and LAI in soybean. Plant growth hormones played a significant role on GRR as observed in the present study. BR 0.1 ppm + Urea 2% spray in addition to recommended dose of N (25 kg/ha) strongly influenced CGR with more than 50 per cent increase over control, particularly between flowering and pod formation stages. This finding is in close confirmity with the results of Prakash et al. (2007) in sesamum. Umadevi (1998) also quoted the significant effect of BR in improving CGR in sesamum. Cycocel (200 ppm) spray in addition to N (50 kg/ha) also showed remarkable enhancement in CGR with about 30% increase over control in black gram of the present study. This finding was corroborated with the result of Vijayakumar and Abdulkhadar (1997) in cassava. This enhancement in CGR was due to fast development of the sources as well as sink.

The net assimilation rate is a measure of net photosynthesis of leaves in crop community. In the present investigation, net assimilation rate slightly declined from vegetative to final stage of the crop. Watson (1958) suggested that the cause for the decrease in NAR with increased leaf area in crop plants was due to mutual shading of the leaves, which would decrease the leaf photosynthesis. Briggs et al. (1920), however, viewed that the decreased NAR with age was due to losses in respiration in all parts of the plants. NAR was significantly influenced by hormonal manipulation, particularly brassinolide. BR (0.1 ppm) along with Urea (2%) increased the assimilation rate by 22% during vegetative to flowering stage and 30% during flowering to pod filling stages over control. Similarly, N (50 kg/ha) + CCC (200 ppm) spray resulted in 20% and 28% improvement in NAR over control in these two stages respectively. These findings were in confirmity with the results of Kalita et al. (1994). Nemchenko et al. (1981) demonstrated the positive rate of CCC in increasing net assimilation rate of potato. Baghal and Yadhav (1992) explained the significant role of BR in enhancing the NAR in black gram besides arriving strong correlation with seed yield. The increase in NAR due to BR could be assigned to its growth regulating activity, since its activity is analogous to auxin and gibberellins.

The nitrate reductase is the rate-limiting enzyme in nitrogen assimilation and is a key point of metabolic regulation (Eilrich and Hageman, 1973) in crop plants. Thus, NRase is intimately associated with the plant growth and development (Sinha and Nicholas, 1981). While observing the pattern of NRase activity in groundnut throughout the growth period, Sung and Sun (1990) reported that NRase activity declined rapidly during post flowering stages. However in the preseh foliage. The enzyme activity was further triggered by combined effect of BR. Similar to this finding, Sairam (1994) study high NRase activity was extended up to pod filling stage, which might be due to the additional dose of Urea applied throug observed a significant increase in NRase activity due to brassinolide application in wheat. Jaisingh et al. (1993) also recorded an increased rate of enzyme activity due to brassinolide application in *Cicer aurietinum*. Besides brassinolide, CCC was also found effective in triggering this enzyme activity as revealed in the present study. Wasnik and Bagga (1992) also observed the significant effect of CCC in enhancing the enzyme activity in mungbean.

The IAA oxidase activity determines the auxin levels and thereby apical dominance. It was also reported that IAA oxidase activity was low in region of high auxin content and high in region of low auxin content (Galston and Dalberg, 1954). In the present investigation, the time trend of IAA oxidase activity revealed a decreasing trend up to pod filling stage. Hormonal treatments played their differential role in altering the auxin level. The treatment combination with brassinolide effectively suppressed the enzyme activity and maintained higher level of auxin in leaves. This finding was in close confirmity with the result of Han et al. (1988), in which foliar application of brassinosteroid increased IAA synthesis in tobacco. Helmy et al. (1997) also had similar finding with the view that brassinisteroid application at 30 mg/L had enhanced the auxin content in broad bean. Bindu Joseph (2000) also revealed the significant influence of BR on lowering the activity of IAA oxidase in groundnut up to flowering stage of the crop. Thus increase in auxin content by BR application would have contributed to increased reproductive growth. BR acted synergistically with auxin in stimulating cell elongation (Katsumi, 1985) and ethylene production (Arteca et al., 1995) suggesting that effects of BR are mediated through auxin (Takeno and pharis, 1982) or that BRs enhance tissue sensitivity to auxin (Mandava, 1988). BR induced increase in fresh weight of squash cotyledons was accompanied by an increase in auxin and decrease in ABA levels (Eun et al., 1989).

3 Materials and Methods

The present investigation was undertaken under field condition to study the effect of nutrients and plant growth regulators on growth and productivity of black gram variety CO5. The research experiment was carried out at Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore during July to October, 2007. Growth regulators like Naphthalene Acetic Acid (NAA), Salicylic Acid (SA), Cycocel (CCC), Brassinosteriod (BR), Humic Acid and the nutrients such as Nitrogen, DAP, Boric Acid, Ferrous Sulphate, Zinc Sulphate were used. The data were statistically analyzed with the Design of Randomized Block Design with three replication and the Plot size of 4 × 3 m with Spacing of 30 × 10 cm. In this research study has nine treatments and the details are T_1: Control; T_2: N 25 kg/ha + Urea 2% + NAA 40 ppm, T_3: N 50 kg/ha + CCC 200 ppm; T_4: N 25 kg/ha + Urea 2% + CCC 200 ppm; T_5: N 25 kg/ha + Urea 2% + Humic acid 0.1%; T_6: N 25 kg/ha + Urea 2% + Salicylic acid 100 ppm; T_7: N 25 kg/ha + Urea 2% + Brassinosteriod 0.1 ppm; T_8: N 25 kg/ha + Urea 2% + $ZnSO_4$ 0.5% + $FeSO_4$ 0.5% + Borax 0.2%; T_9: N 25 kg/ha + Water spray. The CGR was calculated by employing the formula of Watson (1956) and expressed as $g \cdot m^{-2} \cdot day^{-1}$. NAR (Net Assimilation Rate) was calculated by using the formula of Gregory et al. (1917) and subsequently modified by Williams (1946) and expressed as $mg \cdot cm^{-2} \cdot day^{-1}$. Employing the formula of Nicholas et al. (1976), the NR'ase enzyme activity was calculated and expressed as µg $NO_2 \cdot g^{-1} \cdot hr^{-1}$. The IAA-oxidase enzyme activity was estimated by using the formula of Parthasarathy et al. (1970) and the enzyme activity was expressed as μg of unoxidised auxin·g^{-1}·hr^{-1}.

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