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Effect of Boron Molybdenum and Nickel on Photosynthetic Pigments and Photosynthetic Rate of Blackgram (*Vigna mungo* L. Hepper)

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ABSTRACT

A field experiment was conducted on the effect of B, Mo and Ni as basal on photosynthetic pigments and photosynthesis rate of blackgram (*Vigna mungo* L. Hepper) at college farm, Agricultural college, Bapatla during *Rabi* 2017-18 in randomized block design with eight treatments of micronutrient application viz., control (T_1), B (T_2), Mo (T_3), Ni (T_4), B+Mo (T_5), B+Ni (T_6), Mo+Ni (T_7) and B+Mo+Ni (T_8) replicated thrice. The results revealed that soil application of B, Mo and Ni individually as basal resulted in increase of all photosynthetic pigments at vegetative stage. At later stages, Mo application had no effect, while B and Ni had the increasing effect on chlorophyll 'b'(7.2 & 8.8%) and carotenoids (5.6 %). The impact of Ni application on total chlorophyll was on par with that of combination treatments at pod development stage. All combination treatments increased the content of photosynthetic pigments. B, Mo and Ni application in combination (T_5 to T_8) enhanced the chlorophyll 'a' by 13.7, 7.2, 9.4 & 16.5 %, chlorophyll 'b' by 26.4, 19.2, 24.0 & 40.8 %, carotenoids by 22.2, 16.7, 19.4 & 27.8 % and total chlorophyll content by 16.9, 13.6, 15.6 & 18.9 % respectively. Photosynthetic rate increased continuously and this increase was declined at pod development stage. The increase in photosynthetic rate in T_2 and T_5 to T_8 at vegetative stage was 1.3, 1.5,1.5, 1.4 and 1.6 folds, respectively. At flowering stage, it was 1.2, 1.5, 1.4 1.3 and 1.5 folds, respectively. At pod development stage, B and Mo application showed 1.2 folds increase, B+Ni and Mo+Ni application showed 1.4 folds increase, while B+Mo and B+Mo+Ni application increased the photosynthesis rate by 1.6 folds.

Keywords: *Blackgram, Boron, Chlorophyll, Molybdenum, Nickel, Photosynthetic rate*

Blackgram (*Vigna mungo* L. Hepper) is the third important pulse crop grown throughout India, known as urdbean, urd and urad occupying an area of 31 lakh ha producing 14 lakh tonnes with a productivity of 451.61 kg ha^{-1} (Indiastat, 2016-17). In Andhra Pradesh, it is grown in an area of 5.03 lakh ha with a production and productivity of 3.29 lakh tonnes and 659 kg ha^{-1} respectively (Indiastat, 2016-17) and in coastal regions mainly cultivated under rice fallows. The most important constraint limiting crop yield in developing nations worldwide especially among resource poor farmers is maintaining soil fertility status. Pulses production is largely confined to marginal and submarginal lands of low fertility status and inadequate soil moisture. Without restoration of soil fertility in marginal and submarginal lands, farmer will gain little benefit from the use of improved varieties. The overall strategy for improving soil fertility, escalating crop yields and sustaining them at high level must include the management of soil nutrients including both macro and micro.

Micronutrients plays role in balanced crop nutrition by orchestrating a range of physiological functions. A lack of any one of the micronutrients in soil can limit growth even when all other nutrients are present in adequate amounts. Boron (B) influences the absorption and equilibrium of nitrogen, phosphorous and potassium, play a key role in sugar translocation, nodulation, nitrogen fixation, protein synthesis, sucrose synthesis, cell wall composition, membrane stability, K^+ transport, viability of pollen, pollen germination, pollen tube growth, pollination and seed set. Molybdenum (Mo) is important for good foliage growth, nitrogen fixation, nitrogen assimilation as a constituent of nitrogenase, nitrate reductase and inhibitor of acid phosphatase. Mo deficiency is one of the important nutritional problems limiting crop production in pulse growing areas and about 11% of Indian soils are deficient in available Mo (Singh, 2011). In greengram, application of graded levels of Mo (0.025 to 0.1 mg kg^{-1} soil) positively influenced the chlorophyll content, the highest was observed at 0.075 mg Mo kg^{-1} soil (Velmurugan and Mahendran, 2015). Greater levels of chlorophyll was obtained in plants supplied with borax @ 3.75 kg ha^{-1} and ammonium molybdate @ 0.75 kg ha^{-1} (Singh *et al*., 2014). B @ 2 mg kg^{-1} soil or Mo @ 1.2 mg kg^{-1} soil or both significantly increased the chlorophyll content and photosynthetic activity of leaves (Duyingqiong *et al.,* 2002) in ground nut. Nickel (Ni) is essential for plant growth as a constituent of several metallo-enzymes. Legumes are one of the crops found most sensitive to Ni deficiency. It has been reported that application of Ni at low concentrations increased the chlorophyll a, chlorophyll b, carotenoids and total chlorophyll in blackgram (Das *et al*., 2015; Singh *et al.,* 2013; Dubey and pandey, 2011), greengram (Jagetiya *et al.,* 2013), chickpea (Naz *et al.,* 2018; Khan *et al.,* 2014) and maize (Hussain *et al.,* 2013) increase in photosynthesis rate with Ni application at low concentrations was reported in blackgram (Das *et al*., 2015; Dubey and Pandey, 2011) and maize (Hussain *et al.,* 2013). Chlorophyll concentration and net photosynthesis rate were reduced in rice grown in nutrient solution when Mo or Ni were omitted Since Ni is needed in small quantities, more research needs to be done. Keeping all these in view, the present study was undertaken to assess the impact of application of B, Mo and Ni as basal on photosynthesis rate in blackgram.

MATERIAL AND METHODS

A field experiment was conducted on effect of B, Mo and Ni as basal on photosynthetic pigments and photosynthesis rate of blackgram (*Vigna mungo* L. Hepper) at college farm, Agricultural college, Bapatla during *Rabi* 2017-18. The experiment was laid out in randomized block design with eight treatments of micronutrient application in three replications. Crop variety PU-31 was used as test crop. The experiment was comprised of following treatments. T_1- no micronutrient application (control), T_2- Borax @ 2.5 kg ha^{-1}, T_3- Ammonium molybdate @ 1.5 kg ha^{-1}, T_4- Nickel chloride @1 kg ha^{-1}, T_5- Borax @ 2.5 kg and Ammonium molybdate @ 1.5 kg ha^{-1}, T_6- Borax @ 2.5 kg and Nickel chloride @1 kg ha^{-1}, T_7- Ammonium molybdate @ 1.5 kg and Nickel chloride @1 kg ha^{-1}, T_8- Borax @ 2.5 kg, Ammonium molybdate @ 1.5 kg and Nickel chloride @1 kg ha^{-1}.

The properties of the experimental soil were pH 7.21, EC 0.63 dS m^{-1}, organic carbon 5.2 %, available nitrogen (N) 206.3 kg ha^{-1}, available phosphorus (P) 86.0 kg P_2O_5 ha^{-1}, available potassium (K) 284.6 kg K_2O ha^{-1} and texture sandy clay loam. Crop was sown on well prepared land in lines using seed rate of 12-15 kg ha^{-1}. The row to row and plant to plant distance was 30 cm and 10 cm, respectively. At sowing time fertilizer was applied to the crop @ 50 kg phosphorus (P_2O_5) and 20 Kg nitrogen (N) ha^{-1} in the form of urea and single super phosphate, respectively. All micronutrient treatments were imposed as basal. Experimental plots were protected from pests and diseases by spraying chlorpyriphos @ 2.5 ml L^{-1} at the initial stage of crop growth. The other standard package of cultural practices including irrigation and weed control were followed throughout crop growth period and were kept normal for all the treatments. The matured third leaf from the top was sampled plot wise in replications to estimate the photosynthetic pigments. Chlorophyll was extracted by non macerating technique using dimethyl sulphoxide as described by Hiscox and Stam (1979). The optical density of the extract was measured using spectrophotometer (Systronics Model 105) and the content of chlorophyll a, chlorophyll b, carotenoids and total chorophyll was calculated with Arnon's formulae. Photosynthesis rate in the target leaf was measured between 10.00 AM to 12.00 noon using a portable infrared gas analyser (TPS-2 PP system). The data were analysed statistically by following. (OP STAT).

RESULTS AND DISCUSSION

Photosynthetic pigments

Chlorophyll 'a'

Application of micronutrients B, Mo and Ni significantly influenced the content of photosynthetic pigments in leaves of blackgram. Chlorophyll 'a' content in leaf at vegetative stage (20 DAE) varied from 0.65 to 1.08 mg g^{-1} due to micronutrient application (Table 1). Maximum chlorophyll 'a' (1.62) content was observed in B+Mo+Ni application and minimum(1.39) in control, which differed significantly with all other treatments. The next higher chlorophyll 'a' superior to control was noted in plants treated with Boron+Molybdenum (T_5), Nickel (T_4), Boron+Nickel (T_6) and Molybdenum+Nickel (T_7). Besides this, chlorophyll 'a' content observed in Boron (T_2) and Molybdenum (T_3) treated plants was found on par to each other and third in exhibiting the impact.

At flowering stage chlorophyll a content increased, varied from 1.39 to 1.62 mg g^{-1}. The lowest value was observed in control plants, which was on par with B, Mo and Ni individual application (Table 1). In plants grown with the three micronutrients (T_8), chlorophyll 'a' content was significantly higher than other treatments except Boron+Molybdenum (T_5) application, which showed parity. Boron+Nickel (T_6) and Molybdenum+Nickel (T_7) treated plants had the chlorophyll 'a' on par with each other and found superior to control and inferior to T_8 and T_5. At pod development stage, chlorophyll 'a' content observed in treatments T_4 to T_8 was on par with each other. The lowest value was observed in control, which was on par with Molybdenum (T_3) application. The impact of B application in increasing the content of chlorophyll 'a' was superior to T_1 and T_3 and inferior ad T_4 (Ni) and other combination treatments T_5 to T_8. This clearly indicates that B, Mo and Ni application in blackgram increases the chlorophyll a content in leaf. The impact of individual application of these three micronutrients in enhancing chlorophyll 'a' content in leaf was remarkable only at vegetative stage and later negligible. But when applied in combinations, remarkable increase was observed at all stages. The combination treatments T_5 to T_8 enhanced the chlorophyll 'a' by 13.7, 7.2, 9.4

and 16.5 per cent respectively. Similar enhancing effect on photosynthetic pigments was also reported by Velmurugan and Mahendran (2015) in greengram.

Chlorophyll 'b'

Chlorophyll 'b' content in leaf at vegetative stage (20 DAE) varied from 1.16 to 1.54 mg g^{-1}. The lowest value was observed in control, which differed significantly with all other treatments (Table 1). The highest value of Chl 'b' was observed in B+Mo+Ni application followed by B+Mo application, which showed parity with the treatments B+Ni and Mo+Ni. Among the treatments T_2 to T_8, less impact was observed in Boron (T_2) and Molybdenum (T_3) application. Compared to T_2 and T_3, T_4 (Nickel) showed greater impact in enhancing chlorophyll 'b' content, but found inferior to combination treatments. Chlorophyll 'b' content at flowering stage (40 DAE) increased, varied from 1.25 to 1.76 mg g^{-1}. The lowest value was observed in control plants which showed parity with B and Mo treated plants. Among T_4 to T_8, the significantly highest and lowest values were observed in combination of micronutrients B+Mo+Ni (T_8) and Ni (T_4) alone, respectively. Application of Boron+Molybdenum (T_5), Boron+Nickel (T_6) and Molybdenum+Nickel (T_7) recorded the chlorophyll 'b' content on par with each other, superior to T_4 and inferior to T_8. At pod development stage (60 DAE), chlorophyll 'b' content decreased compared to that at flowering stage, varied from 1.13 to 1.43 mg g^{-1}. The highest value was recorded in B+Mo+Ni application, which showed parity with Mo+Ni and B+Mo application. Besides this the next higher value noted in B+Ni (T_6), Ni (T_4) and B (T_2) application was on par with each other. The lowest value was observed in control followed by Mo application. This suggests that B, Mo and Ni application could improve the photosynthetic ability of the crop by enhancing the chlorophyll 'b' in leaf, but such effect was not found in individual application of B and Mo. Application of three nutrients in combination caused remarkable increase (40.8 %); B+Ni, Mo+Ni and B+Mo application resulted in 19.2, 24.0 and 26.4 percent increase compared to control. The impact of B and Mo alone was noted at early stages, but later it was not up to considerable extent compared to control. Similar effect with application of Ni was reported by Das *et al.*(2015), Singh *et al.*(2013) and Dubey and Pandey (2011) in blackgram.

Carotenoids

Carotenoid content in leaf was significantly influenced by micronutrient application (Table 2). At vegetative stage (20 DAE), it varied from 0.51 to 0.64 mg g^{-1}. The lowest value was observed in control, which showed parity with B (T_2), Mo (T_3) and Ni (T_4) application. The significantly highest value was observed in T_8(B+Mo+Ni) followed by T_5(B+Mo). Application of B+Ni and Mo+Ni recorded the carotenoid content on par with each other, superior to control and inferior to T_8 and T_5. Besides this, carotenoid content observed in B (T_2) and Ni (T_4) application was also at par with Mo +Ni application.

At flowering stage, carotenoid content varied from 0.72 to 0.92 mg g^{-1} and found that it was higher than the vegetative stage. The lowest value was observed in control which was on par with Mo application. In plants grown with three micronutrients, carotenoid content was significantly higher than other treatments. The next higher value was observed in T_5 (B+Mo) and T_7 (Mo +Ni), which were found on par. The variation in carotenoid content observed between T_6 (B+Ni) & T_7 (Mo +Ni) and T_2 & T_4 was not significant, but all were found superior to control. At pod development stage (60 DAE), micronutrient application in combinations (T_5 to T_8) exhibited better performance than individual application of B and Mo. The carotenoid content observed in T_5 to T_8 was on par with one another. Plants treated with Ni application alone also had significantly higher carotenoid content than control plants but inferior to T_5 to T_8 and on par with Mo application.

It is evident from the results that B, Mo and Ni increases the content of carotenoids. During crop growth, the higher level of carotenoid content was observed at 40 DAE. At this stage, increase in carotenoid content was more in application of micronutrients in combination than in individual treatments and control. The treatments B+Mo, B+Ni, Mo+Ni and B+Mo+Ni increased the carotenoids level in leaf by 22.2, 16.7, 19.4 and 27.8 percent respectively, while B and Ni application alone increased it by 5.6 percent. Mo application has no enhancing effect on carotenoid content. B, Mo and Ni helps in increasing carotenoid contents as per reports of Singh *et al.* (2013) and the above results sounds same with the findings of Das *et al.*(2015).

Total chlorophyll

Significant difference was observed among the treatments pertaining to total chlorophyll content in leaf (Table 2). At 20 DAE, it varied from 2.2 to 3.44 mg g^{-1}, the lowest value was observed in control, which differed significantly with all other treatments. Among T_2 to T_8, the highest value was observed in T_8 and the lowest in T_2 and T_3. B+Mo(T_5), B+Ni(T_6) and Mo+Ni (T_7) recorded the total chlorophyll content on par with each other and next to T_8. Besides this chlorophyll content noted in T_4 was superior to T_2 & T_3 and inferior to other treatments.

Table 1. Effect of micronutrients (B, Mo & Ni) on Chlorophyll 'a' and Chlorophyll 'b' (mg g^{-1}) in Blackgram

Treatments	Chlorophyll 'a' (mg g^{-1})			Chlorophyll 'b' (mg g^{-1})		
	20 DAE	40 DAE	60 DAE	20 DAE	40 DAE	60 DAE
T_1: Control	0.65	1.39	1.27	1.15	1.25	1.13
T_2: Borax @ 2.5 Kg ha^{-1}	0.85	1.44	1.37	1.26	1.34	1.23
T_3: Ammonium molybdate @ 1.5Kg ha^{-1}	0.86	1.41	1.33	1.25	1.31	1.21
T_4: Ni Cl_2. $6H_2O$ @1.0 Kg ha^{-1}	0.96	1.45	1.42	1.35	1.36	1.26
T_5: Borax @ 2.5&Ammonium molybdate @ 1.5 Kg ha^{-1}	1	1.58	1.47	1.48	1.58	1.4
T_6: Borax @ 2.5 & Ni Cl_2. $6H_2O$ @1.0 Kg ha^{-1}	0.96	1.49	1.46	1.42	1.49	1.32
T_7: Ammonium molybdate @ 1.5 & Ni Cl_2. $6H_2O$ @1.0 Kg ha^{-1}	0.95	1.52	1.46	1.45	1.55	1.34
T_8: Borax @ 2.5 & Ammonium molybdate @1.5 & Ni Cl_2.$6H_2O$ @1.0 Kg ha^{-1}	1.08	1.62	1.47	1.54	1.76	1.43
SE (m)±	0.02	0.03	0.03	0.02	0.03	0.03
CD (0.05)	0.06	0.09	0.09	0.07	0.1	0.09
CV (%)	3.87	3.26	3.9	3.1	3.4	3.9

Table 2. Effect of micronutrients (B, Mo & Ni) on carotenoid contents and Total chlorophyll (mg g^{-1}) in Blackgram

Treatments	Carotenoids (mg g^{-1})			Total Chlorophyll contents (mg g^{-1})			Photosynthetic Rate (m mol CO_2 m^{-2} s^{-1})		
	20 DAE	40 DAE	60 DAE	20 DAE	40 DAE	60 DAE	20 DAE	40 DAE	60 DAE
T1: Control	0.51	0.72	0.69	2.2	3.6	2.5	9.5	16.8	13.8
T2: Borax @ 2.5 Kg ha^{-1}	0.53	0.76	0.7	2.75	3.71	2.58	12.5	20.5	17.05
T3: Ammonium molybdate @ 1.5Kg ha^{-1}	0.52	0.74	0.71	2.65	3.65	2.55	11.8	19.5	16
T4: Ni Cl_2. $6H_2O$ @1.0 Kg ha^{-1}	0.53	0.76	0.76	2.88	3.82	2.66	10.5	17.8	14.5
T5: Borax @ 2.5&Ammonium molybdate @ 1.5 Kg ha^{-1}	0.59	0.88	0.84	3.21	4.21	2.7	14.2	24.67	21.5
T6: Borax @ 2.5 & Ni Cl_2. $6H_2O$ @ 1.0 Kg ha^{-1}	0.56	0.84	0.83	3.18	4.09	2.68	13.8	23.5	19.5
T7: Ammonium molybdate @ 1.5 & Ni Cl_2. $6H_2O$ @ 1.0 Kg ha^{-1}	0.55	0.86	0.83	3.29	4.16	2.69	13	22	19
T8: Borax @ 2.5 & Ammonium molybdate @ 1.5 & Ni Cl_2.$6H_2O$ @1.0 Kg ha^{-1}.	0.64	0.92	0.84	3.44	4.28	2.78	15.3	26	22.5
SE(m)±	0.01	0.01	0.02	0.04	0.08	0.05	0.93	0.93	0.53
CD(0.05)	0.03	0.03	0.06	0.12	0.24	0.15	2.81	2.83	1.62
CV (%)	3.5	2.61	3.73	2.24	3.63	3.24	12.75	7.57	5.15

Total chlorophyll content at flowering stage increased, varied from 3.68 to 4.28 mg g^{-1}. The lowest value was observed in control, which showed parity with T_2, T_3 and T_4. Micronutrients application in combinations (T_5 to T_8) resulted higher total chlorophyll content and exhibited better performance, at later stage (60 DAE), total chlorophyll decreased compared to that at 40 DAE, varied from 2.5 to 2.78 mg g^{-1}. The lowest value was observed in control, found on par with T_2 and T_3. The highest value was observed in T_8, which showed parity with T_4, T_5, T_6 and T_7.

It is evident from the results that B, Mo and Ni application alone showed remarkable increase in total chlorophyll only at initial stages. Whereas application of these in combinations showed better performance in increasing the total chlorophyll. At 40 DAE, where the total chlorophyll reached to maximum, application of B+Mo, B+Ni, Mo+Ni and B+Mo+Ni resulted in enhancement of total chlorophyll by 16.9, 13.6, 15.6 and 18.9 per cent respectively.

B, Mo and Ni application individually as basal resulted in increase of all photosynthetic pigments at vegetative stage. At later stages, Mo application had no effect, while B and Ni had the increasing effect on chlorophyll b and carotenoids. The impact of Ni application on total chlorophyll was on par with that of combination treatments at pod development stage. All combination treatments increased the content of photosynthetic pigments. Singh *et al.*(2014) reported that application of B and Mo as borax @ 3.75 kg and ammonium molybdate @ 0.75 kg ha^{-1} respectively resulted in greater levels of chlorophyll in chickpea. Similar enhancing effect on photosynthetic pigments was also reported by Velmurugan and Mahendran (2015) in greengram with Mo, Duyingqiong *et al.*(2002) in groundnut with B, Mo and B+Mo. Similar effect with application of Ni was reported by Das *et al.*(2015), Singh *et al.*(2013) and Dubey and Pandey (2011) in blackgram; Naz *et al.* (2018) and Khan and Prakash (2014) in chickpea; Jagetiya *et al.* (2013) in greengram and Hussain *et al.* (2013) in maize. Omission of either Mo or Ni or both resulted in reduction chlorophyll concentration in rice grown in hydrophonics (Milton *et al.*, 2009).

Photosynthesis rate (m mol Co_2 m^{-2} s^{-1})

B, Mo and Ni application significantly affected the photosynthesis rate of blackgram leaves (Table 2). At vegetative stage (20 DAE), among all the treatments, T_8 (B+Mo+Ni) recorded higher photosynthetic rate (15.3 m mol Co_2 m^{-2} s^{-1}), which was on par with other combination treatments (T_7, T_6, T_5) and individual application of boron (T_2). The lower photosynthesis rate was recorded by control (9.5 m mol Co_2 m^{-2} s^{-1}) and the impact of Mo and Ni individually application was on par with control.

At flowering stage, B+Mo+Ni application recorded maximum Photosynthetic rate (26.0 m mol Co_2 m^{-2} s^{-1}), which showed parity with B+Mo and B+Ni application Minimum Photosynthetic rate was recorded by control (16.8 m mol Co_2 m^{-2} s^{-1}) and it showed parity with Mo (T_3) and Ni (T_4) application. The impact of boron application on photosynthesis was greater than control, on par with Mo+Ni application and lower than other combinations.

At pod development stage (60 DAE), B+Mo+Ni treated plants showed higher Photosynthetic rate followed by B+Mo treated plants. The next higher Photosynthetic rate was observed in B+Ni and Mo+Ni treated plants, which were at a par, superior to control and individual treatments. The lower Photosynthetic rate was observed in control (13.8 m mol Co_2 m^{-2} s^{-1}) followed by Ni treated plants. B and Mo application recorded the Photosynthetic rate at a par, superior to control and inferior to combination treatments.

Photosynthetic rate increased continuously and this increase was declined at pod development stage(Table 2). The increase in photosynthetic rate in T_2 and T_5 to T_8 at vegetative stage was 1.3, 1.5,1.5, 1.4 and 1.6 folds, respectively. At flowering stage, it was 1.2, 1.5, 1.4 1.3 and 1.5 folds, respectively. At pod development stage, B and Mo application showed 1.2 folds increase, B+Ni and Mo+Ni application showed 1.4 folds increase, while B+Mo and B+Mo+Ni application increased the photosynthesis rate by 1.6 folds. The increase in Photosynthetic rate can be attributed to the increase of chlorophyll content and also might be due to increase in leaf area, leaf water status, stomatal conductance, internal Co_2 concentration and enzyme activity with supplementation of B, Mo and Ni. Das *et al.*(2015) reported that application of Ni up to 200 µM increased the chlorophyll a, chlorophyll b, carotenoids by four times and there by increased the photosynthetic responses. Similarly, the increase in photosynthetic activity was reported by Duyingqiong *et al.* (2002) in groundnut with B and Mo application.

CONCLUSION

From the present study it can be informed that , individual application of B, Mo and Ni had less increase in photosynthetic pigments when compared to same micronutrients applied as combination. PS rate increased continuously and this increase was declined at pod development stage. The increase in photosynthetic rate in B, B+Mo, B+Ni, Mo+Ni and B+Mo+Ni at vegetative stage was 1.3, 1.5, 1.5, 1.4 and 1.6 folds, respectively.

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