

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effects of Different Nitrogen Levels on the Leaf Chlorophyll Content Nutrient Concentration and Nutrient Uptake Pattern of Blackgram

¹M.U. Kulsum¹, ²M.A. Baque and ³M.A. Karim

¹Breeding Division, Bangladesh Rice Research Institute, Gazipur, Bangladesh

²Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

³Department of Agronomy, Bangabandhu, Sheikh Mujibur Rahman Agricultural University, Salna Gazipur, Bangladesh

Abstract: This study was conducted to evaluate the performance of blackgram (*Vigna mungo* L.) under various levels of nitrogen at the Agronomy Research Site of Bangabandhu Sheikh Mujibur Rahman Agricultural University during March to June 2002. Two varieties of blackgram- BARI mash 3 and BINA mash 1 and six levels of nitrogen viz. 0, 20, 40, 60, 80 and 100 kg N ha⁻¹ were the treatment variables. The experiment was laid out in a RCB Design with three replications. A best-fit positive linear relationship existed between leaf chlorophyll and leaf nitrogen content with different nitrogen levels. Unexpectedly the N, P and K accumulation in the two varieties was not affected significantly. However, there was an increasing tendency of total uptake of these elements in both the varieties. N, P and K uptake increased up to 60 kg N ha⁻¹ then decreased with the increasing nitrogen levels. Among the varieties BARI mash 3 showed better performance than BINA mash 1 for most of the parameters.

Key words: Chlorophyll, nutrient concentration, NPK uptake, nitrogen level, blackgram

INTRODUCTION

A large number of pulses are grown in Bangladesh. The main are lathyrus, lentil, chickpea, blackgram and mungbean. Among the pulses blackgram (*Vigna mungo* L.) is one of the main edible pulse crop of Bangladesh. It ranks fourth among the pulses with an area of about 70,000 hectare (BBS, 2000). Nitrogen is an essential element and important determinant of growth and development of crop plant (Tanaka *et al.*, 1984). The important plant parameters proposed for estimating nitrogen stress are leaf nitrogen, dry weight, leaf elongation, leaf area and carbondioxide rate (Green, 1976). Soils of Bangladesh are mostly deficient in nitrogen. As soil N deficiency is common in tropics and subtropics (Dakora and Keya, 1997), N supply and N management will continue to be important factors in crop production in this region. Another factor of concern is the significant environmental decline already associated with the injudicious use of fertilizer nitrogen (Vitousek *et al.*, 1997). It has been observed that photosynthesis rate decreases after flowering. The leaf nitrogen and chlorophyll content also showed a similar decline (Rao and Ghildiyal, 1985). It was suggested that decrease in photosynthesis rate after flowering was due to the mobilization and translocation of

nitrogen from leaves to seeds, owing to higher nitrogen requirement of pulse crop for seed development (Ghildiyal and Sirohi, 1986). This was further substantiated by the observation that in deflowered plants where pods were not allowed to developed, retained higher rate of photosynthesis, chlorophyll and leaf nitrogen content (Mitra and Ghildiyal, 1988). Mitra *et al.* (1988) observed that urea application retarded leaf senescence as judged by the retention of chlorophyll and leaf nitrogen. It should be possible to increase N₂ fixation by legumes through genetic improvement and management practices (Hardarson and Atkins, 2003). Since the process of nodulation and nitrogen fixation is inhibited at higher levels of fertilizer nitrogen in the soil (Lawn and Brun, 1974) and there is a high demand of inorganic nitrogen of the crop is at post flowering period, it is necessary to have clear understanding on nitrogen use efficiency of legume crop. Foliar application of N is reported to have beneficial effect on mungbean (Hamid, 1991). Despite some sporadic reports studies are insufficient to understand the mode of changes of blackgram due to external application of nitrogen. This study was therefore undertaken to find out the role of N on the leaf chlorophyll content, nutrient content and nutrient uptake pattern of blackgram under subtropical conditions.

MATERIALS AND METHODS

A field experiment was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during Kharif-1 season (from March to June, 2002). The soil of the experimental field was silty clay under salna series with PH 6.5. The experimental soil contains 0.159% total N, 0.619% organic carbon, 0.312 meq exchangeable P/100 g soil, 0.38 meq exchangeable K/100 g soil and CEC 22.54 meq/100 g soil. The experimental site is situated in a subtropical environment having frequently rainfall during March to June. The experiment was laid out in a factorial RCBD with 3 replications.

Treatment combinations: Two varieties of blackgram with six level of nitrogen fertilizer constituted the following treatment combinations.

B1 N0: BARI mash-3 with 0 kg ha⁻¹ level of nitrogen fertilizer; B1 N20: BARI mash-3 with 20 kg ha⁻¹ level of nitrogen fertilizer; B1 N40: BARI mash-3 with 40 kg ha⁻¹ level of nitrogen fertilizer; B1 N60: BARI mash-3 with 60 kg ha⁻¹ level of nitrogen fertilizer; B1 N80: BARI mash-3 with 80 kg ha⁻¹ level of nitrogen fertilizer; B1 N100: BARI mash-3 with 100 kg ha⁻¹ level of nitrogen fertilizer; B2 N0: BINA mash-1 with 0 kg ha⁻¹ level of nitrogen fertilizer; B2 N20: BINA mash-1 with 20 kg ha⁻¹ level of nitrogen fertilizer; B2 N40: BINA mash-1 with 40 kg ha⁻¹ level of nitrogen fertilizer; B2 N60: BINA mash-1 with 60 kg ha⁻¹ level of nitrogen fertilizer; B2 N80: BINA mash-1 with 80 kg ha⁻¹ level of nitrogen fertilizer; B2 N100: BINA mash-1 with 100 kg ha⁻¹ level of nitrogen fertilizer.

The well-prepared soil was fertilized at the rate of 50 and 36 kg P and K per hac. Nitrogen are six levels of 0, 20, 40, 60, 80, 100 kg ha⁻¹. Half dose of N and full dose of P and K were applied as basal at the time of seed sowing. Seeds were sown on 9 March, 2002 with 10×30 cm² spacing; light irrigation was given to establish the seedlings properly. Excess seedlings were removed on March 20 to retain one seedling per hill. The crop was top-dressed with rest half of N on April 6, 2002.

Laboratory analysis of plant samples

Leaf chlorophyll content: Leaf chlorophyll content of two blackgram varieties were measured at pre-flowering and pod filling stage. For this reason the top most leaf was collected and leaf chlorophyll was determined in the lab. by utilizing acetone method.

Oven dried plant materials (leaves+stem+petiole+grain) at final harvest were ground with a Willey grinding

machine (cap/small 1029-8, Yoshida Seisakusho Co. Ltd.) for the estimation of different mineral ions.

Total N analysis: Estimation of total nitrogen was done by colorimetric method following Lindner (1944). Plant sample was digested in Kjeldahl digestion flask with salicylic sulfuric acid and digestion catalyst. After digestion, color of the solution was developed with four different reagents (reagent B I mL, 7-10 drop of A, 5 mL of solution C and 5 mL of solution D). Then absorbance of the solution was measured at 625 nm wavelength with Double Beam Spectrophotometer (Model 200-20 Hitachi).

Total P analysis: Total P was determined by nitric-perchloric acid digestion method as described by Yamakawa (1992). The absorbance was measured at 440 nm with Double Beam Spectrophotometer (Model 200-20 Hitachi).

K analysis: K was determined following Hitachi Ltd. (1986). During K analysis dry plant sample was digested with nitric-perchloric acid solution. After digestion the sample was diluted with distilled water. Then the absorbance of that respective ion was measured with atomic absorption spectrophotometer (Model 41170-30 Hitachi).

Statistical analysis: The data recorded on different plant characters were statistically analyzed with the help of MSTAT program. The differences between the treatment means were compared by Least Significant Difference (LSD) test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Chlorophyll and leaf nitrogen content: Chlorophyll and leaf Nitrogen content of two blackgram varieties were measured at pre-flowering and pod filling stage (Fig. 1 and 2). There is a positive best fit linear functional relationship existed between leaf chlorophyll and leaf nitrogen content with different N levels. The functional relationship indicated that with the increasing N levels leaf chlorophyll and nitrogen content increased linearly. Irrespective of varieties and N levels both leaf chlorophyll and nitrogen content showed higher values at pod filling stage than pre-flowering and mature stage (Fig. 1 and 2). This finding is consistent with the findings of Mitra *et al.* (1988). They concluded that urea as an exogenous source of nitrogen was used to test the model that increased nitrogen supply during flowering and pod filling stage would retard leaf senescence and improve photosynthate and nitrogen availability for seed biomass.

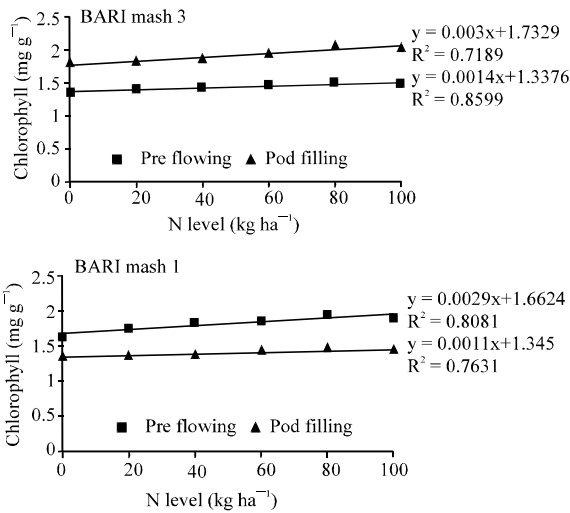


Fig. 1: Functional relationship between different N levels and chlorophyll content of two blackgram varieties at pre-flowering and podfilling stages

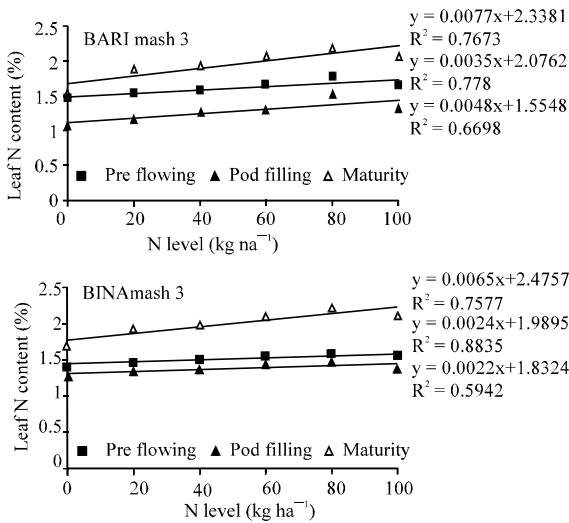


Fig. 2: Functional relationship between different N levels and leaf N content at pre-flowering, podfilling and mature stages of two blackgram varieties

Nutrient content and uptake

Nutrient concentration (N, P and K) in plants: Nitrogen is one of the important key factors in regulating the growth and yield of crops. N concentration in plant components often been used as an index of crops N requirement. Plants N content was determined at maturity stage. The levels of applied N fertilizer influenced the tissue N content. N content increased with the increases in N levels (Table 1). N content in plant parts increased in N levels upto 60 kg ha⁻¹ and it helped to sustain high concentration of N. An increase in N levels increased N

content significantly at all the growth stages, highest N content was observed at active tillering stage with higher N level (120 kg ha⁻¹) and lower at harvest with N₀ Nitrogen (Quayum *et al.*, 1994). Plants grown without nitrogen had tissue N concentration of 1.43% at harvest whereas plants treated with 60 kg ha⁻¹ had 1.57%. N content in plant tissue irrespective of varieties was highest at the same level of N treatment. Similar trend in pea was obtained by Verma *et al.* (1998). Irrespective of N levels BARI mash 3 always contain higher tissue N concentration than BINA mash 1 (Table 1). The increase in N content in blackgram at this level may be due to the accelerated metabolic activity of the fertilized plants resulting in increased absorbing power of root system.

N application also influenced P and K content in the plant tissue, but differences was not as conspicuous as was observed in case of N content (Table 1). P and K content in all plant organs increased upto 60 kg ha⁻¹ and then further increases in nitrogen caused reduction in P and K content. From the table it is clear that total N, P and K content (%) in both the blackgram varieties increased upto 60 kg N ha⁻¹ then decreased. But these nutrients are always higher in BARI mash 3 than BINA mash 1 at all levels of N. After 60 kg ha⁻¹, further increase in nitrogen caused reduction of P content also observed in pea (Verma and Bhandari, 1998).

Nutrient concentration (N, P and K) in grains: N concentration in the grains increased progressively with the increasing N levels in both the blackgram varieties (Table 2). Irrespective of varieties and N levels, seed N content increased with the increases of N levels upto 60 kg ha⁻¹. Further increased in N levels, N content in seed decreased gradually. Among the varieties BARI mash 3 always contained higher percent of N than BINA mash 1. Quayum *et al.* (1994) also showed that N uptake in grain increased with increase in N levels upto 120 kg ha⁻¹. Nitrogen uptake in rice straw also followed a similar trend as that in grain. Singh *et al.* (1992) reported, response of N of field pea upto 30 kg ha⁻¹ whereas Negi (1992) upto 20 kg ha⁻¹ only.

P and K content in seed also increased with the increase of N levels (Table 2) but the effect was not so conspicuous like as N content. Maximum P and K content in seed was observed in plant treated with 60 kg N ha⁻¹. Further increase of N levels decreased P and K content in seed. From this table it is clear that N levels influence seed N, P and K content upto 60 kg ha⁻¹. But the effect was more pronounced on N content than P and K content in seed. Among the varieties, BARI mash 3 always contained higher percent of N, P and K compared to BINA mash 1. N, P and K content in seed always lower in 0 kg N ha⁻¹ treated plants.

Table 1: Nitrogen fertilizer effect on N, P and K contents in plant of two black gram varieties at harvest

Variety	Fertilizer N (kg ha ⁻¹)	Nutrient concentration (%) in total plant at harvest stage		
		N	P	K
BARI mash 3	0	1.43	0.14	1.01
	20	1.47	0.15	1.01
	40	1.48	0.16	1.02
	60	1.57	0.18	1.24
	80	1.52	0.17	1.2
	100	1.49	0.16	1.16
BINA mash 1	0	1.35	0.12	0.93
	20	1.37	0.14	0.95
	40	1.38	0.15	0.97
	60	1.57	0.16	1.10
	80	1.47	0.15	1.01
	100	1.38	0.14	0.98
LSD (0.05)		NS	NS	NS
CV (%)		8.94	15.85	12.78

Table 2: Nitrogen fertilizer effect on N, P and K contents in seed of two blackgram varieties at harvest

Variety	Fertilizer N (kg ha ⁻¹)	Nutrient concentration (%) in total plant at harvest stage		
		N	P	K
BARI mash 3	0	2.19	0.18	0.84
	20	2.24	0.18	0.85
	40	2.25	0.19	0.86
	60	2.35	0.22	0.92
	80	2.29	0.19	0.89
	100	2.25	0.19	0.87
BINA mash 1	0	2.00	0.13	0.78
	20	2.08	0.14	0.80
	40	2.14	0.16	0.82
	60	2.33	0.18	0.90
	80	2.22	0.17	0.87
	100	2.20	0.15	0.86
LSD (0.05)		NS	NS	NS
CV (%)		8.03	6.15	2.12

Nutrient uptake (N, P and K): Uptake of nutrient elements (N, P and K) by the plant was estimated as the dry matter multiplied by their respective concentration (%). The total uptake of N, P and K by the blackgram varieties as influenced by applied N fertilizer is presented in Fig. 3. As the total dry matter increased over time, N, P and K uptake also increased. Response of N, P and K uptake to the applied N fertilizer paralleled the response of plant nutrient concentration (%). Total uptake of N, P and K varied due to nitrogen treatment variation. Plants treated with 60 kg N ha⁻¹ had the highest N, P and K uptake irrespective of both the varieties. Upto 60 kg N ha⁻¹ all the nutrient uptake increased, but further increase of N fertilizer decreased the N, P and K uptake gradually. Among the varieties BARI mash 3 always showed better uptake of these nutrients compared to BINA mash 1. This might be due to greater dry matter production by this variety. Nutrient uptake at 0 kg N ha⁻¹ was lowest in both the varieties. Similar results also was obtained by Bahl *et al.* (1996) in pea and Ferdous (2001) in edible

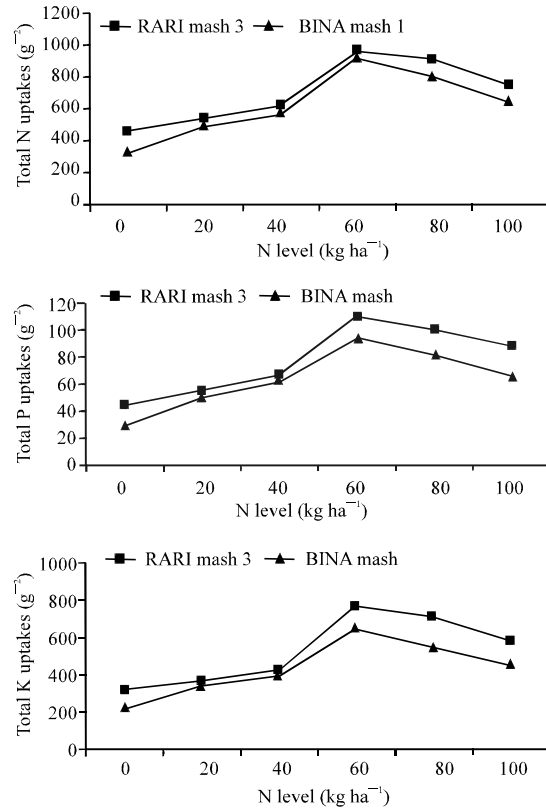


Fig. 3: Effects of different N levels on the N, P and K uptake in total plant of two blackgram varieties at harvest stage

podded pea. Irrespective of Nitrogen levels and varieties, N uptake was always higher than P and K uptake (Fig. 3).

REFERENCES

BBS, 2000. Statistical Year Book of Bangladesh. Statistics Divin. Ministry of Planning, Govt. of the People's Republic of Bangladesh.
 Bahl, G.S., N.S. Pasricha and V. Beri, 1996. Effect of nitrogen and phosphorus on nitrogenase activity; grain yield and nutrient uptake by field pea (*Pisum sativum* L.). Ind. J. Agron., 32: 24-31.
 Dakora, F.D. and S.O. Keya, 1997. Contribution of legume nitrogen fixation to sustainable agriculture in sub-saharan Africa. Soil Biol. Biochem., 29: 809-817.
 Ferdous, A.K.M., 2001. Effects of nitrogen and phosphorus fertilizers on nutrient uptake and productivity of edible podded pea. MS Thesis, BSMRAU. Salna, Gazipur, pp: 29-30.
 Ghildiyal, M.C. and G.S. Sirohi, 1986. Nitrogen utilization during growth and development in mungbean. Indian J. Exp. Biol., 24: 124-126.

- Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Res. A. Wiley-Interscience Publication 2nd Edn., Jhon Wiley and Sons. New York, pp: 680.
- Green, W., 1976. Nitrogen stress in plants. *Adv. Agron.*, 28: 1-35.
- Hardarson, G. and C. Atkins, 2003. Optimising biological N₂ fixation by legumes in farming systems. *Plant Soil*, 252: 41-54.
- Hamid, A., 1991. Foliar fertilization of nitrogen in mungbean, influence of rate and frequency of application. *Ann. Bangladesh Agric.*, 1: 33-39.
- Hitachi, Ltd., 1986. Instruction Manual for model 170-30 atomic absorption flame spectrophotometer. Tokyo. Japan.
- Lawn, R.J. and W.A. Brun, 1974. Symbiotic nitrogen fixation in soybeans. III. Effect of supplemental nitrogen and intervarietal grafting. *Crop. Sci.*, 14: 22-25.
- Lindner, R.C., 1944. Rapid analytical methods for some of the more common inorganic constituents of plant tissues. *Plant Physiol.*, 19: 76-89.
- Mitra, R., S.E. Pawar and C.R. Bhatia, 1988. Nitrogen: The major limiting factor for mungbean yield. *Proc. Second Intl. Mungbean Symposium, AVRDC, Shanhua, Tainan, Taiwan*, pp: 244-251.
- Mitra, S. and M.C. Ghildiyal, 1988. Photosynthesis and assimilate partitioning in mungbean in response to source sink alteration. *J. Agron. Crop. Sci.*, 160: 303-308.
- Negi, S.C., 1992. Effect of nitrogen and phosphorus in temperate hill-grown vegetable pea (*Pisum sativum*). *Ind. J. Agron.*, 37: 772-774.
- Quayum, M.A., A.F.M. Maniruzzaman and M.S. Islam, 1994. Effect of preceding crops on growth, nitrogen concentration and uptake in rice (*Oryza sativa*). *Ind. J. Agric. Sci.*, 64: 824-828.
- Rao, T.R.K. and M.C. Ghildiyal, 1985. Analysis of photosynthetic source and sink relationship in mungbean (*Vigna radiata* [L.] Wilczek). *Ind. J. Plant Physiol.*, 28: 135-144.
- Sing, T., K.N. Singh and K.L. Vaid, 1992. Response of 'Rachna' field pea (*Pisum sativum*) to nitrogen and phosphorus fertilization under rainfed condition in Kashmir Valley. *Indian J. Agron.*, 37: 619-620.
- Tanaka, A., J. Yamaguchi, S. Miura and H. Tamaru, 1984. Comparison of fertilizer nitrogen efficiency among field crop. *Soil Sci. Plant Nutr.*, 30: 199-208.
- Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger and D.G. Tilman, 1997. Human alteration of the global N cycle: Sources and consequences. *Ecol. Applied*, 7: 737-750.
- Verma, M.L., A.R. Bhandari and J.N. Raina, 1998. Effect of nitrogen and phosphorus application on the yield and macro nutrient concentrations of pea (*Pisum sativum* L.). *Intl. J. Trop. Agric.*, 15: 195-198.
- Verma, M.L. and A.R. Bhandari, 1998. Periodic changes in macronutrient content of pea as affected by nitrogen and phosphorus fertilizer application. *Hortic. J.*, 11: 55-65.
- Yamakawa, T., 1992. Laboratory Methods for Soil Science and Plant Nutrition. Part-2. Methods of Plant analysis. JICA-IPSA Project, pp: 6-14.