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Post-waterlogging Rescue Nitrogen Improves Waterlogging Tolerance in Mungbean (*Vigna radiata*)

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Abstract

The study was conducted at the Stress Research Site of the Department of Agronomy of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh during the period from April to July 2017 to evaluate the effect of rescue nitrogen fertilizer for improving the performance of waterlogging tolerance in mungbean genotype VC-6173A. Both waterlogged and non-waterlogged mungbean plants were received varying doses of rescue nitrogen from urea fertilizer. The rescue nitrogen (N) treatments were: N_0-no rescue N; N_{30} - 20 and 10 kg ha^{-1} rescue N applied at 0-day and 15-day after removal of waterlogging (ARW); N_{40} - 30 and 10 kg ha^{-1} rescue N applied at 0-day and 15-day ARW and N_{50} - 40 and 10 kg ha^{-1} rescue N applied at 0-day and 15-day ARW. The study was laid out in a randomized complete block design with four replications. Different levels of rescue N improved plant height, leaf chlorophyll index, phonological period, plant growth, pod formation and seed yield of mungbean. N-treated plants showed higher relative SPAD chlorophyll values and the increment was higher for higher N-dose. Waterlogging reduced 40% root dry matter and 34% both shoot and total dry matter relative to non-waterlogged plants. Rescue N significantly increased both the root and shoot dry matter. The waterlogged plants without rescue N showed 29% reduction in the number of pods per plant and the reduction showed 13% for rescue N_{40} in waterlogged plants. The seed yield reduction was 25% in waterlogged plants without rescue N but with rescue N, seed yield remarkably increased particularly in N_{40}-treated plants. Therefore, the study suggests that rescue N fertilizer application may be a viable practice in improving waterlogging tolerance and increasing yield of mungbean.

Keywords: Mungbean, waterlogging, nitrogen fertilization, root and shoot growth, seed yield.

1. Introduction

Mungbean (*Vigna radiata* L.Wilczek) is one of the popular pulse crops of Bangladesh. It is rich in digestible protein (approximately 25-28%) and extensively grown in tropical and subtropical Asia because of its wider range of adaptability (Kumar *et al*., 2013). In Bangladesh, more than one lac hectare of land is covered by mungbean cultivation (BBS, 2017). The farmers of the country prefer mungbean cultivation mainly because of the rapid growth and early maturity, and its ability to fit well in rice-based cropping systems. Mungbean yield in Bangladesh is generally low due to many reasons. Different abiotic stresses are the major ones. Among the abiotic stresses, waterlogging stress is most common and restricts mungbean production, particularly when the waterlogging is encountered at the early stage of crop growth (Singh and Singh, 2011).

Waterlogging reduces oxygen concentrations around the roots of the waterlogged plants and limits nodulation and nitrogen fixation. The uptake of major nutrients (N, P and K) is also hampered (Elzenga and van Veen, 2010). Waterlogging results in a severe reduction in nutrient concentrations and leaching of nitrogen beyond the root zone and the plants suffer from nitrogen deficiency (Kisaakye *et al*., 2017; Steffens *et al*., 2005). Such deficiency is associated with the limited root activities and yellowing of leaves (Habibzadeh *et al*., 2013). The denitrification of nitrate ions and rapid volatilization in waterlogged soil may also cause nitrogen deficiency (Rasaei *et al*., 2012). Therefore, waterlogged soils need to maintain its fertility cautiously for sustainable crop production (Bhaduri *et al*., 2017).

The supply of nutrients and its availability in the soil plays a significant role in waterlogging tolerance of plants (Romheld and Kirkby, 2010). The major adaptive mechanism of plants to waterlogging is the production of adventitious roots during the post-flooding period (Islam *et al*., 2010). The development of such adventitious roots may be enhanced and accelerated through N application (Kaur *et al*. 2017; Ren *et al*., 2017). As a result, oxygen supply may increase and plants can recover quickly from flooding injury. Exogenously applied nitrogen fertilizer following waterlogging was found to improve the growth and development of corn and soybean (Kaur *et al*., 2018; Kaur *et al*., 2017), maize and wheat (Zheng *et al*., 2017) and cotton (Ashraf *et al*., 2011). Application of nitrogen to pastures just before they become waterlogged was found effective (McFarlane and Glencross, 1994). It was reported that the applied nitrate may enter anaerobically in damaged roots of waterlogged plants by passive means and translocated to the shoot (Trought and Drew, 1980). The use of N fertilizer can improve the crop root and shoot growth under soil-waterlogged conditions (Wu *et al*., 2013a, 2014) and the enhanced crop N uptake from urea top-dressed is likely to benefit grain quality, especially protein (Harris *et al*. 2016).

Nitrogen fertilization and some other management options were advocated by various scientists to overcome waterlogging stress in wheat (Hossain and Uddin, 2011), barley and oats (Setter and Waters, 2003) and maize (Zaidi *et al*., 2007) but such studies on mungbean are limited. Nitrogen applied waterlogged plants may retain more leaf area that can eventually accelerate the gas exchange processes. As a result, the waterlogged plants may produce a greater number of pods and finally increase the yield. Therefore, the study was carried out to assess the effectiveness of rescue nitrogen in improving waterlogging tolerance and yield of mungbean.

2. Materials and Methods

2.1. Experimental site and soil

The study was conducted at the Stress Research Site of the Department of Agronomy of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from April to July 2017. The topsoil was manmade having silt loam in texture with shallow red brown terrace subsoil designated as Salna soil series under Madhupur Tract (AEZ 28). The chemical properties are characterized by slightly acidic in reaction with very low and low contents of nitrogen and phosphorus, respectively. The rainfall was intermittent during the experimentation and a substantial rainfall occurred when waterlogging treatments were imposed. However, there was very low rainfall at the time of rescue N application but a heavy rainfall occurred just before a second application of rescue N. After 65 DAE, it started heavy rains and continued until termination of the experimentation. The maximum temperature ranged between 30.1 and 33.1^{0}C and did not vary significantly during the crop growing period, but minimum temperature progressively increased from 23.4 to 27.1^{0}C (Figure 1).

2.2. Treatment, layout and experimental design

A waterlogging tolerant mungbean genotype VC-6173A was used in the study. Two types of soil conditions were created viz. no-waterlogging (control) and waterlogging and both were applied with urea @ 40 kg ha^{-1}, triple superphosphate @ 85 kg ha^{-1}, muriate of potash @ 35 kg ha^{-1} and cowdung @ 10 t/ha (blanket dose) during final land preparation. Four doses of rescue nitrogen were applied in both waterlogged and non-waterlogged plants after imposing waterlogging treatments. The rescue nitrogen (N) treatments were: N_0-no rescue N; N_{30} - rescue N at 30 kg ha^{-1} of which 20 kg ha^{-1} applied at 0-day ARW (30 DAE) and 10 kg ha^{-1} at 15-day ARW (45 DAE); N_{40} - rescue N at 40 kg ha^{-1} of which 30 kg ha^{-1} applied at 0-day ARW (30 DAE) and 10 kg ha^{-1} at 15-day ARW (45 DAE) and N_{50} - rescue N at 50 kg ha^{-1} of which 40 kg ha^{-1} applied at 0-day ARW (30 DAE) and 10 kg ha^{-1} at 15-day ARW (45 DAE). The size of the experimental unit plot was 1.2×1.0 m and the total number of plots was 32. The spacing between the waterlogged and control plots was 1.5 m to avoid side entry of seepage water to the control plots. Polythene sheets were used in waterlogged treatments to avoid side leakage of water. The treatments were arranged in a randomized complete block design with four replications.

2.3. Land preparation and management practices

The experimental plots were prepared by plowing and cross plowing. Seeds of uniform size and shape were sown on 3 April 2017 after treated with Provex 200 at 3g/kg seed. Line to line and plant to plant distance were maintained 30 and 10 cm, respectively. Seedlings were emerged within 3-4 days after sowing. After thinning, one health seedling was kept for maintaining the plant to plant distance. The experimental plots were kept totally weed free from 15 DAE to 30 DAE. Insecticide Karate at 2 ml/L was applied two times maintaining intervals of 10 days at the vegetative stage, and once at the pod-filling stage for controlling thrips and pod borers. A single heavy irrigation was done just after seeding and thereafter, irrigation water was applied only when it was required.

2.4. Imposition of waterlogging stress

Waterlogging treatment was imposed to 25-day old seedlings and flooding depth was maintained 2-3 cm for three days. After removal of waterlogging, soils remained at saturation conditions for two days and altogether five days (25-30 DAE) were considered as waterlogging period. At the same time, the optimal soil moisture was maintained in plants retained as control. Rescue N fertilizer doses were applied once at the removal of waterlogging (30 DAE) and another at 15 days after removal of waterlogging (45 DAE) (Figure 1).

2.5. Data collection

Data on plant height, SPAD (Soil-Plant Analysis Development) chlorophyll index, dry matter production, and yield components were recorded for both waterlogged and non-waterlogged control plants applied with or without rescue N treatments. For this, five plants from each treatment were selected for recording the data.

2.5.1 Plant height

Periodic plant height for both waterlogged and non-waterlogged plants was measured starting from 6 days after waterlogging (DAW) with three days interval up to 18 DAW. Individual plant height was measured from the base at the ground level to the top of the main shoot.

2.5.2 SPAD chlorophyll value

SPAD chlorophyll value/index was recorded three days interval starting from 6 DAW up to 18 DAW or both waterlogged and non-waterlogged plants. A portable chlorophyll meter (Minolta SPAD 502) was used for recording SPAD chlorophyll value of leaves. SPAD value was measured at proper sunlight just prior to harvesting the plants at field conditions.

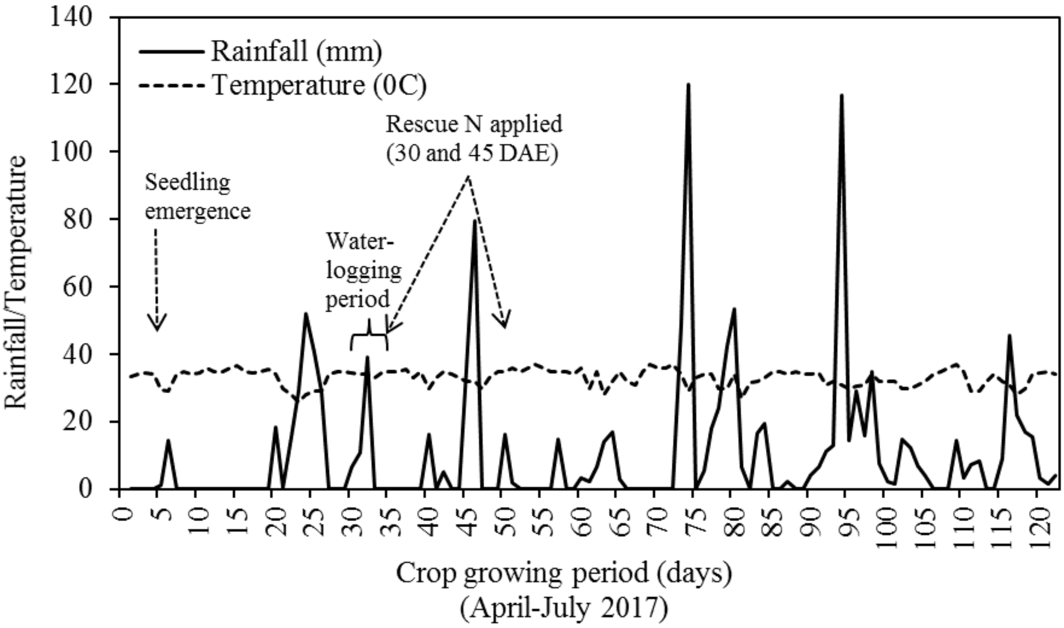

Figure 1. Trends of rainfall and maximum temperature during crop growing period

2.5.3 Dry matter production

Five plants from both waterlogged and non-waterlogged plots were harvested at 3-weeks after removal of waterlogging to determine the dry weight (DW) of different plant parts. After harvesting, plants were segmented into different components (root, stem, petiole and leaf). The segmented parts were then dried in an oven at 80ºC for 72 hours until a constant weight achieved and then the dry weight was recorded. Total DW was estimated by summing up the DW of root, stem, petiole and leaf and that of shoot DW by excluding root DW from the total DW.

2.5.4 Phenological characters

Phenological data i.e. days to first flowering, days to 50% flowering and days to maturity were recorded from the middle row of each experimental plot. Days to first flowering were counted when at last one flower was opened in a plot. Days to 50% flowering was counted when 50% of plants of each treatment plot have at least one opened flower. Days to maturity was considered when about 80% of pods showed physiological maturity.

2.5.5 Pod and seed yield

Plants were harvested at variable dates depending on maturity duration and treatments, and the yield parameters such as the number of pod/plant and seed yield were recorded. For this, both waterlogged and non-waterlogged plants were uprooted carefully by hand and then bundled and tagged. Thereafter, pods were threshed carefully in the laboratory and kept them under the open sunlight for drying. When the pod walls became brittle, the seed and straw were separated, cleaned and then dried under sunlight for desirable moisture level. Pods from selected five plants were harvested when 95% pods were matured and weighted after sundry to maintain moisture level 12%.

2.6. Statistical analysis

The data of all treatments were subjected analyzed statistically by using software Statistix

10 (Analytical software, 2018). All the recorded data were analyzed using analysis of variance (ANOVA) and means were compared by using Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

3. Results and Discussion

3.1. Plant height

The plant height of mungbean increased with the increasing age of plants in non-waterlogged control plants. A similar trend was also observed in waterlogged plants treated with or without rescue N but plant height significantly decreased in waterlogged plants applied with or without rescue N compared to non-waterlogged plants (Figure 2). Higher the N dose lower was the plant height. This indicates that N fertilizer depressed the plant height of waterlogged plants. However, when height growth was compared as a relative value between control plants and waterlogged plants with or without rescue N application, the scenarios are different (Figure 2). The relative values of different doses of rescue N applied plants significantly increased at 9 days after rescue N application (DRN).

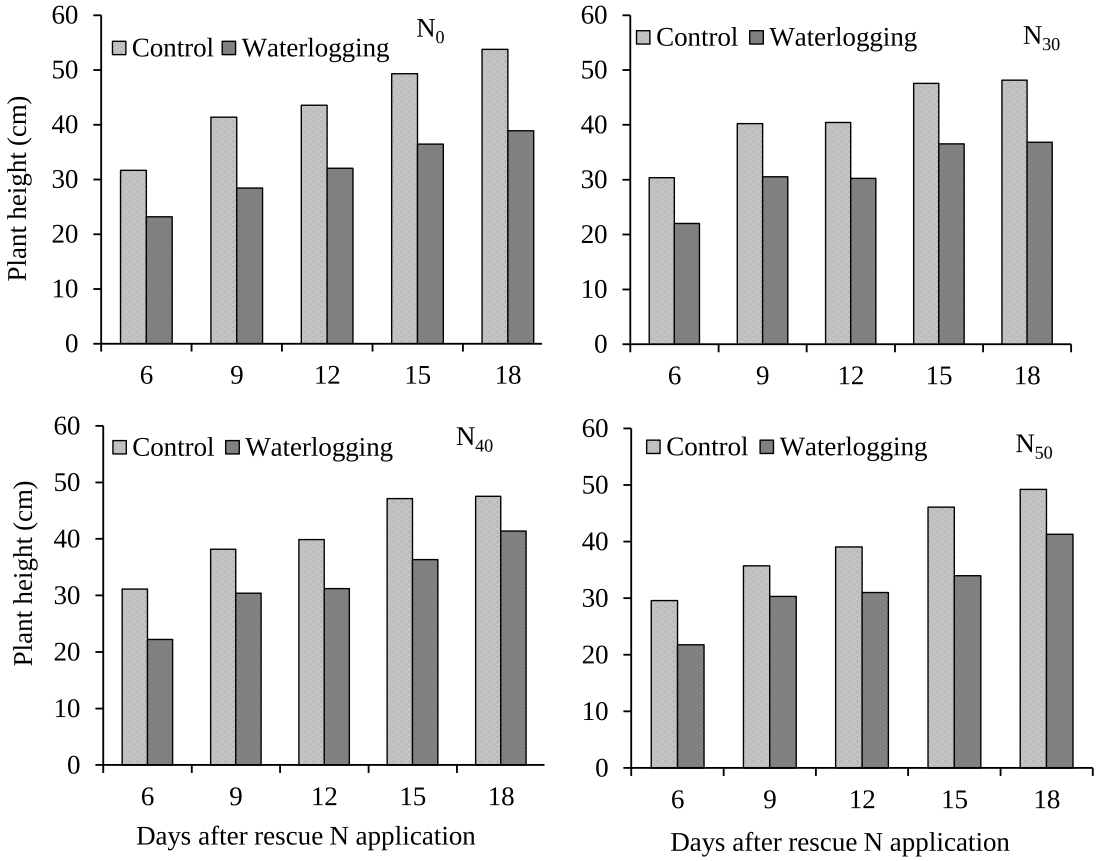

Figure 2. The response of plant height in mungbean applied with varying rate of rescue nitrogen under non-waterlogged and waterlogged conditions.

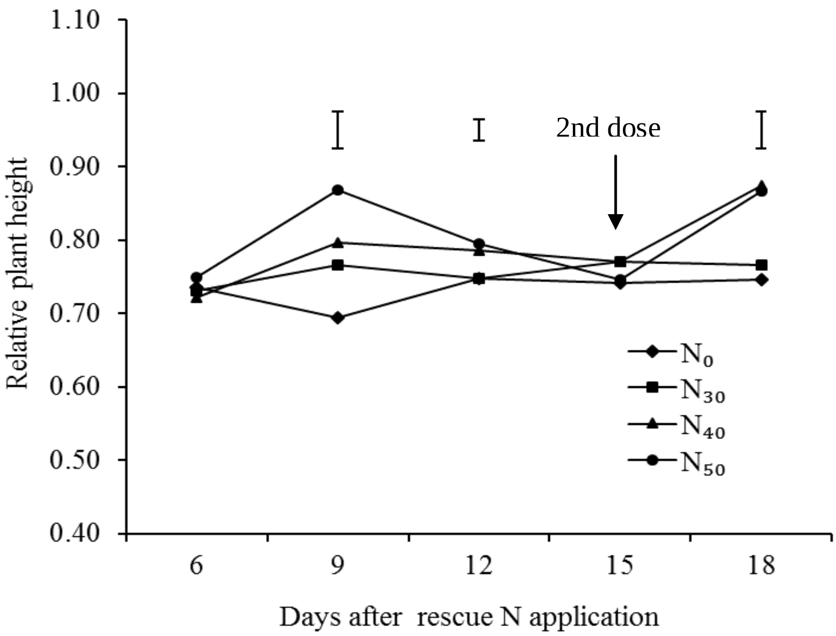

Figure 3. Relative plant height of mungbean applied with varying rates of rescue nitrogen under non-waterlogged and waterlogged conditions. Vertical bars indicate the mean difference values according to LSD at P < 0.05.

Thereafter, the values decreased but remained significantly higher compared to N_0 and N_{30} treated plants at 12 DRN. The relative plant height again started to increase after second dose rescue N application particularly in N_{40} and N_{50} treatments. A significantly higher relative plant height in both these treatments indicated that plant height increment is associated with the amount of rescue N used. Tariq *et al*. (2001) observed that the application of P and K along with N increased plant height and number of branches plant^{-1}.

3.2. SPAD chlorophyll value

The SPAD chlorophyll values were not consistent throughout the time of observation in both non-waterlogged and waterlogged plants with or without rescue nitrogen (Figure 4). Irrespective of treatments, the highest chlorophyll contents were observed at 9 DRN. In most cases, waterlogged plants applied with or without rescue N showed lower SPAD values up to 9 DRN, thereafter the values increased significantly. Interestingly, the rate of increase was as high as the values became greater than non-waterlogged plants. The lower SPAD values in waterlogged plants were commonly observed in many studies. It was noticed that the major cause of chlorophyll destruction is the formation of superoxide radicals under waterlogging situations (Malik *et al.*, 2002; De Souza *et al.* 2011).

For a clear understanding, the relative SPAD values were computed that showed increasing trends with the advance of plant age irrespective of treatments (Figure 4). Rescue N-treated plants showed higher relative SPAD value compared to the plants applied without rescue N, but varied remarkably between levels of rescue N. There were significant increases in relative SPAD values up to 12 DRN and thereafter increment was not remarkable. After the second dose of N application, the relative SPAD values again increased which indicates a positive response of N to waterlogged plants. The most remarkable observation is that high dose of rescue N (N_{50}) consistently maintained higher relative SPAD values. When medium dose (N_{40}) applied, waterlogged plants responded slowly after the first dose but a greater response was observed after second dose of rescue N. However, relative SPAD value dramatically increased for low rescue nitrogen (N_{30}) level, thereafter values decreased indicating rescue N might have been utilized by the plants within two weeks of its application and further addition of N did not improve SPAD value. On the other hand, the relative increment of SPAD value continued in plants applied with N_{40} and N_{50} levels of rescue N. Application of additional N fertilizer in waterlogged plants from various sources resulted in higher SPAD values compared to non-waterlogged plants in maize (Kaur *et al*., 2018). Nitrogen fertilizer applied as liquid form maintained chlorophyll levels either equal to or greater than that of the foliar and broadcasting (Reed and Gordon, 2005).

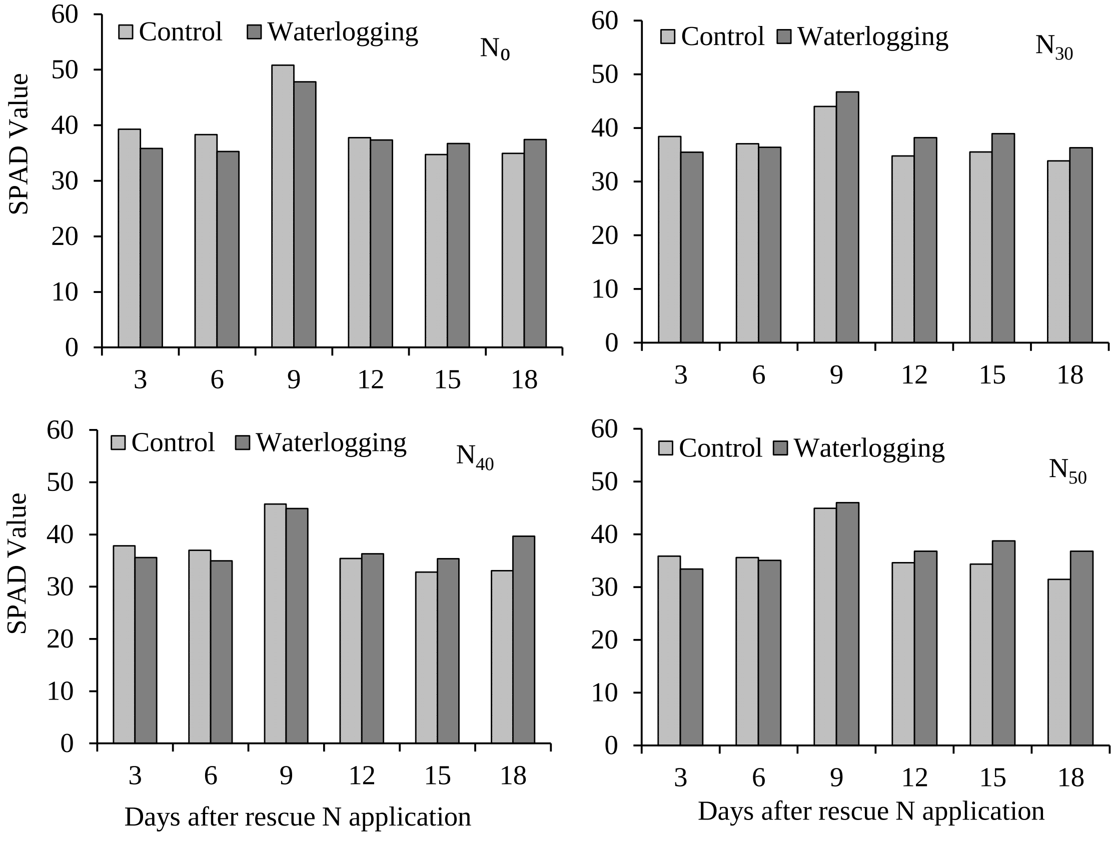

Figure 4. The response of SPAD chlorophyll value of leaves in mungbean applied with varying rate of rescue nitrogen under non-waterlogged and waterlogged conditions.

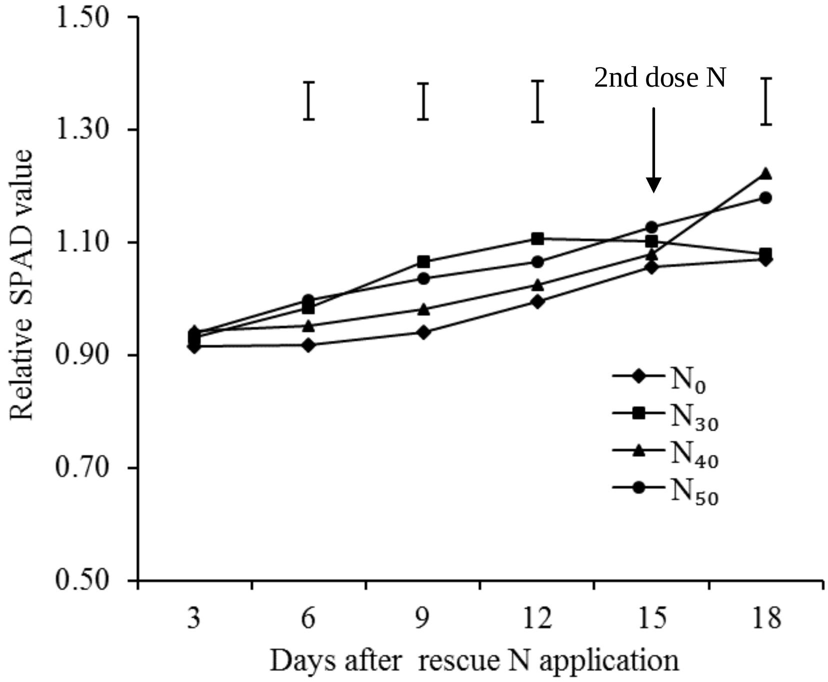

Figure 5. Relative SPAD chlorophyll values of leaves in mungbean applied with varying rates of rescue nitrogen under non-waterlogged and waterlogged conditions. Vertical bars indicate the mean difference values according to LSD at $P < 0.05$.

3.3. Root and shoot development

Effect of post-waterlogging rescue N fertilizer on the dry matter production root, shoot and total plant in mungbean are illustrated in Table 1. Relative root dry matter significantly decreased due to waterlogging showing 40% reduction relative to non-waterlogging when rescue N was not applied. Such reduction ranged from 27-29% when different doses of rescue N applied in waterlogged plants. However, rescue N application had a little tendency to increase the root dry matter with increasing N doses. Guo *et al*. (2010a) reported that N fertilization just after waterlogging enhanced root development that contributed to waterlogging tolerance in cotton. In this study, we applied rescue N two times i.e. just after removal of waterlogging (ARW) and 15 days ARW. The research results in Western Australia showed that N recovery was minimal when applied just ARW but recovery increased greatly when applied three-weeks ARW (Paterson and Palta, 2007).

The shoot dry matter also decreased largely due to waterlogging without rescue nitrogen and showed a 36% reduction based on relative values when compared with non-waterlogged control plants. However, the application of rescue nitrogen in both non-waterlogged and waterlogged plants decreased shoot dry matter. The higher relative values indicate that reduction of shoot dry weight decreased with the increase in N dose, where N_{30}, N_{40} and N_{50} doses showed 23, 18 and 12% reduction in shoot dry weight, respectively. In canola, waterlogging stress was also found to depress shoot and root growth and such adverse effects of waterlogging were alleviated by foliar application of nitrogen compounds (Habibzadeh *et al*., 2013). However, they emphasized on the appropriate amount and timely application of N fertilizer during the recovery phase in cotton. An appropriate amount may increase waterlogging resistance by altering the antioxidant enzyme activities of the root, reducing lipid peroxidation and boosting root vigor (Guo *et al*., 2010b).

Similarly, the relative total dry matter decreased by 34% in waterlogged plants without rescue nitrogen. The relative values indicate that reduction of total plant dry weight decreased with the increase of rescue N doses as observed in shoot dry matter. This indicates that additional N fertilizer enhanced dry matter accumulation in waterlogged plants. The growth improvement due to N fertilizer application in cotton seedlings affected by waterlogging was also observed by Zhou and Oosterhuis (2012). Sigua *et al*. (2012) noticed that some forage species have the ability to recover from waterlogging injury by increasing dry matter production. They also found that this increase was linearly related to the increasing amount of N fertilizer. Other findings suggested that there were beneficial effects of all tested nitrogen compounds on the growth and biochemical attributes under waterlogged conditions (Jain *et al*., 2016).

3.4. Duration of flowering and maturity

In general, flower initiation delayed in waterlogged plants compared to non-waterlogged control plants. The relative value of days to first flowering indicates that application of rescue nitrogen had a very little effect on days to first flowering (Table 2). Similar results were also observed for days to 50% flowering, although there was a tendency to increase days to 50% flowering with the increase of rescue nitrogen doses. However, maturity duration delayed for medium N dose (N_2). Amin *et al*. (2015) reported that soil waterlogging delayed flowering but the application of N and K fertilizers after the termination of waterlogging enhanced early flowering by 4 to 6 days compared to that of control.

Table 1. Effect of N fertilizer on the dry matter production of root, shoot and total plant in waterlogged mungbean

Treatment	Root DW (g/plant)		Relative root DW	Shoot DW (g/plant)		Relative shoot DW	Total DW (g/plant)		Relative total plant DW
	C	W		C	W		C	W	
N_0	0.82 ±0.07	0.49 ±0.03	0.60b	11.51 ±0.47	7.38 ±0.19	0.64c	12.34 ±0.53	7.87 ±0.16	0.64c
N_{30}	0.65 ±0.04	0.46 ±0.02	0.71a	8.45 ±0.12	6.51 ±0.26	0.77b	9.10 ±0.13	6.97 ±0.24	0.77b
N_{40}	0.68 ±0.08	0.50 ±0.0.02	0.73a	7.73 ±0.37	6.52 ±33	0.84a	8.42 ±30	7.01 ±0.31	0.83a
N_{50}	0.73 ±0.03	0.54 ±0.03	0.73a	8.48 ±0.28	7.46 ±0.16	0.88a	9.21 ±0.30	8.00 ±0.14	0.87a
LSD			0.042			0.044			0.064
CV			5.78			3.51			3.40

Note: N_0-no rescue N; N_{30}, N_{40} and N_{50} - rescue N at 30, 40 and 50 kg ha^{-1}, respectively; C-control and W-waterlogging; Means followed by the same letter (s) are not significantly different at P<0.05 by LSD; ± Standard deviation.

Table 2. Effect of rescue N fertilizer on the phenology of waterlogged mungbean plants

Treat-ment	Days to first flowering (DFF-1)		Relative value of DFF-1	Days to 50% flowering (DFF-2)		Relative value of DFF-2	Days to maturity (DM)		Relative value of DM
	C	W		C	W		C	W	
N_0	32.5 ±0.58	34.3 ±0.50	1.05	40.5 ±0.58	41.0 ±0.82	1.01	52.5 ±0.58	54.8 ±0.50	1.05ab
N_{30}	32.0 ±1.16	34.0 ±1.16	1.06	40.3 ±0.50	40.3 ±0.50	1.00	53.0 ±1.15	54.0 ±0.00	1.02b
N_{40}	33.0 ±0.82	34.0 ±1.16	1.03	39.5 ±0.58	40.5 ±1.29	1.03	52.0 ±0.00	55.3 ±0.96	1.07a
N_{50}	34.0 ±0.82	35.0 ±0.00	1.02	39.5 ±0.58	41.0 ±1.16	1.04	53.8 ±0.96	55.5 ±0.58	1.04b
LSD			NS			NS			0.03
CV			2.09			2.78			1.75

Note: N_0-no rescue N; N_{30}, N_{40} and N_{50} - rescue N at 30, 40 and 50 kg ha^{-1}, respectively; C-control and W-waterlogging; Means followed by the same letter (s) are not significantly different at P<0.05 by LSD; ± Standard deviation.

3.5. Pod formation and seed yield

Waterlogged plants without rescue nitrogen showed a 29% reduction in the number of pods per plant when compared with non-waterlogged control plants (Table 3). When waterlogged plants were applied with different doses of rescue nitrogen, the number of pods increased and reductions varied from 13-22%. However, N_{40} dose performed better in pod formation under waterlogging conditions. Similarly, the grain yield decreased by 25% due to waterlogging when plants were not applied with additional nitrogen. Grain yield was comparatively better in N_{40} followed by N_{50} treatments and yield reduction was recorded 13 and 18%, respectively. Akhtaruzzaman (1998) reported that N fertilized mungbean produced an increased number of pods per plant and seed yield in mungbean subjected to waterlogging. Ashraf *et al*. (2011) advocated post-waterlogging fertilizer application to improve growth and yield of upland cotton affected by hypoxia. Foliar-applied N can also be effectively used in reducing the detrimental effects of waterlogging at the post-anthesis stage in winter wheat yield (Wu *et al*., 2014). The cause of yield increase under waterlogging conditions for rescue N is explained by the fact that post-waterlogging N application increases photosynthetic capacity by increasing leaf area index (LAI), decreasing photo-damage to PSII and leaf chlorophyll content (Florez-Velasco *et al*., 2015; Wu *et al*., 2013b). However, the method of N application is most important and Reed and Gordon (2005) suggested using the liquid form of N fertilizer, which was found to produce greater pod fresh weight than that of normal and foliar fertility treatments.

Table 3. Pod formation and seed yield of waterlogged mungbean plant as affected by rescue N fertilizer

Treatment	Pods/plants (no.)		Relative pods/plants	Grain yield (t/ha)		Relative grain yield
	Control	Waterlogging		Control	Waterlogging	
N_0	18.78 ±0.72	13.25 ±0.29	0.71c ±0.02	1.57 ±0.02	1.17 ±0.02	0.75c ±0.02
N_{30}	17.79 ±0.47	13.48 ±0.61	0.76bc ±0.02	1.53 ±0.07	1.18 ±0.06	0.77c ±0.02
N_{40}	14.30 ±0.39	12.51 ±0.24	0.88a ±0.04	1.32 ±0.05	1.14 ±0.01	0.87a ±0.04
N_{50}	15.60 ±0.35	12.48 ±0.80	0.80b ±0.05	1.36 ±0.01	1.12 ±0.04	0.82b ±0.05
LSD			0.040			0.060
CV			3.08			4.74

Note: N_0-no rescue N; N_{30}, N_{40} and N_{50} - rescue N at 30, 40 and 50 kg ha^{-1}, respectively; Means followed by the same letter (s) are not significantly different at P<0.05 by LSD; ± Standard deviation.

4. Conclusions

The application of rescue N fertilizer was found to improve the growth and yield of mungbean subjected to waterlogging and this may be a valuable practice for the mungbean growing areas suffering from waterlogging. However, the recovery of plants from waterlogging injury after application of rescue N remarkably varied with the amount of fertilizer. Further research is needed to quantify the appropriate amount of rescue N fertilizer and time of its application to increase waterlogging tolerance in mungbean. Furthermore, the study is suggested to evaluate the potentiality of different methods of using N fertilizer during the recovery phase of waterlogged mungbean plants.

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