



## Influence of seedling age and nitrogen application on photosynthesis and yield of rice (*Oryza sativa*) grown under waterlogged condition

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**Abstract** Rice seedlings of three different age groups, *i.e.*, 30, 45 and 60 days old were grown with three different N levels, *i.e.*, 0, 20 and 40 kg ha<sup>-1</sup> for two consecutive years under waterlogged condition. The highest yield was obtained with 40 kg N ha<sup>-1</sup> in crop grown with 60 day old seedlings in both the years. Higher dosage of N application as mud ball showed higher stomatal conductance, net photosynthesis and transpiration. The response of N was better in crop grown with older seedlings. Grain yield showed significant positive relationship with net photosynthesis rate ( $r = 0.82^{**}$ ) and carboxylation efficiency ( $r = 0.81^{**}$ ) than with stomatal conductance ( $r = 0.66$ ), and could be critical determinant for better yield in crop grown with older seedlings at higher N level under waterlogged condition.

**Keywords** Nitrogen · Photosynthesis · Rice · Waterlogging

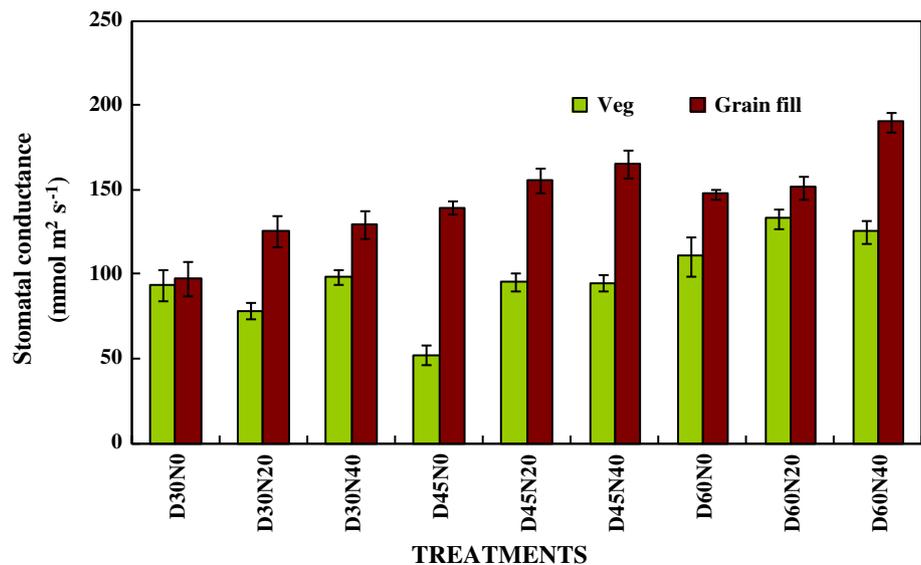
Maintaining yield under flood prone low lying condition is challenging due to unfavorable water regime. Yield is uncertain due to unpredictable water regime and lack of proper management practices causing reduction in yield. Apart from use of suitable cultivars, adoption of appropriate management practices can also enhance yield under such condition. Under low lying waterlogged condition use of mature rice seedlings has been found to be beneficial for rice cultivation, as aged seedlings provided better crop stand and yield (Sharma and Panda 1989; Ghosh 2006).

The higher carbohydrate reserve pool in older seedlings provides better crop stand compared to younger seedlings (Chaturvedi et al. 1995; Mallick et al. 1995). Under excess water condition slower rate of depletion of carbohydrates in older rice seedlings facilitated better survival and improved crop stand than crop grown with younger seedlings (Das et al. 2009). Moreover, under rising water level situation tall older seedlings have physical advantage of height to avoid submergence for longer duration than short younger seedlings. We have reported earlier that older seedlings showed better panicle growth, especially at higher nitrogen level (Roy Chowdhury et al. 2011). The panicle length, panicle weight, spikelet number per panicle and number of filled grains per panicle showed significant improvement in crop raised from older seedlings than from younger seedlings of 30 days. In this paper we report the changes in stomatal characteristics and photosynthesis behavior of rice seedlings of different age groups under different N level under waterlogged condition.

Experiments were conducted during monsoon season from June to December at Experimental Research Farm, Directorate of Water Management, Mendhasal, Bhubaneswar in the years 2007 and 2008 under rainfed condition. Seedlings of a local, tall, long duration and photosensitive rice cultivar 'Pauli' were grown in nursery for 30, 45 and 60 days. These seedlings of different age groups were used for transplanting on 20th and 16th August in 2007 and 2008, respectively. The experiment was conducted under split-plot design with age of seedling as main plot and level of nitrogen (N), in the form of urea, as sub plot with three replications. The nitrogen was given at 0, 20 and 40 kg ha<sup>-1</sup> by mud ball method of application. The desired amount of urea was mixed with clay and handful size urea mixed clay balls were prepared and were evenly placed in entire plot at the time of each application. Phosphorus

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**Fig. 1** Changes in stomatal conductance at vegetative and grain filling stages in rice crop grown from 30, 45 and 60 days old seedlings at 0, 20 and 40 kg N ha<sup>-1</sup>. Each value is mean of three replications

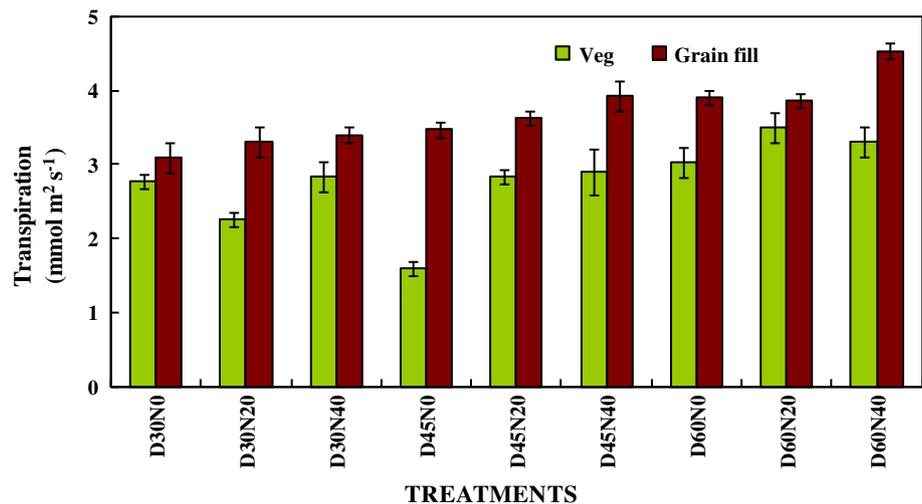


(P) and potassium (K) fertilizers were applied at 40 kg ha<sup>-1</sup>. The experimental plot size was 10 × 5 m with a depth of 1.5 m so as to maintain waterlogged condition. The spacing was maintained at 20 × 2.0 cm and. The entire dosage of P and K was applied as basal at the time of sowing. Nitrogen was applied in three split doses, at 7 days after planting, maximum tillering stage (58 days after transplanting) and panicle initiation stage (93 days after transplanting) at the rate of 50, 25 and 25 % of applied dose, respectively. The crop was harvested in the last week of December and yield of both grain and straw was recorded. The depth of water in experimental field was measured with a measuring scale. The stomatal conductance, transpiration and other parameters in 2007 were monitored at 60 days after planting (DAP) (tillering stage/vegetative) and 100 DAP (reproductive stages/grain filling stage) with a steady state porometer (PMS 2, PP System Ltd, USA). In the year 2008 in addition to stomatal conductance ( $g_s$ ) and transpiration, net photosynthesis rate was also measured at grain filling stage with a portable photosynthesis system (CIRAS2, PP System Ltd, USA). The carboxylation efficiency ( $g_m$ ) was calculated as ratio of net photosynthesis and substomatal CO<sub>2</sub> concentration following (Sidique et al. 1999).

In 2007 stomatal conductance ( $g_s$ ) at vegetative stage showed increase with increase in dosage of N in crops raised from seedlings of all age groups. However, crop raised from 60 days old seedlings in general maintained higher stomatal conductance than crop raised either from 30 to 45 day old seedlings. The  $g_s$  during grain filling stage was higher than at vegetative stage under all the N levels and seedling types. The increase was greater in crop raised from 60 day old seedlings at 40 kg N level compared to other treatments. Even though stomatal conductance did not show specific

trend during vegetative stage but at reproductive stage stomatal conductance was significantly higher at higher N level in crop raised from older seedlings (45 and 60 day old seedlings) (Fig. 1). The transpiration rate also showed similar trend in plants under higher N level both at vegetative and reproductive stages. In crop raised from 60 day old seedlings the highest transpiration rate at vegetative stage was 3.03 mmol without N which increased to 3.31 mmol m<sup>-2</sup> s<sup>-1</sup> at 40 kg N level. At grain filling stage the transpiration rate in crop raised from 60 day old seedlings with zero N was 3.90 mmol, which increased to 4.51 mmol m<sup>-2</sup> s<sup>-1</sup> at 40 kg N level. However the response of applied N was more at reproductive stage than at vegetative stage (Fig. 2). This difference in trend at two stages might be due to regulatory influence of sink strength (growing panicle) on stomatal conductance. The stomatal conductance and transpiration showed significant positive correlation both at vegetative ( $r = 0.98^{**}$ ,  $R^2 0.96$ ) and at reproductive stages ( $r = 0.94^{**}$ ,  $R^2 0.88$ ), suggesting that transpiration at both the stages was under stomatal control even under highly humid rice growing environment. High humidity (low vapor pressure deficit) has been reported to improve leaf water status (Luo and Strain 1992) and favored better plant growth facilitating leaf expansion (Nagarajah and Schulze 1983, Wong 1993). In swamp taro, higher RH linearly improved stomatal conductance ( $R^2 0.69^{**}$ ) (Roy Chowdhury et al. 2004) indicating that even under highly humid waterlogged environment higher RH favored stomatal opening. However the response of plant growth to humidity level is not universal and is dependent on type of plants. In some plants low VPD is beneficial for growth (Luo and Strain 1992), whereas in plants like alfalfa exposure to low VPD reduced leaf area under ambient CO<sub>2</sub> condition (De Luis et al. 2002). Stomatal conductance and yield were reported to have positive

**Fig. 2** Changes in transpiration at vegetative and grain filling stages in rice crop grown from 30, 45 and 60 days old seedlings at 0, 20 and 40 kg N ha<sup>-1</sup>. Each value is mean of three replications



**Table 1** The net photosynthesis ( $P_n$ ), stomatal conductance, transpiration, substomatal CO<sub>2</sub> concentration ( $C_i$ ), carboxylation efficiency ( $P_n/C_i$ ) and yield of rice crop grown from 30, 45 and 60 days old seedlings at 0, 20 and 40 kg N ha<sup>-1</sup>. Each value is mean of three replications

Treatments	Net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	Stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ )	Transpiration ( $\text{mmol H}_2\text{O}$ $\text{m}^{-2} \text{ s}^{-1}$ )	Sub-stomatal CO <sub>2</sub> concentration (ppm)	Carboxylation efficiency ( $P_n/C_i$ )	Yield (t ha <sup>-1</sup> )	
						2007-08	2008-09
D30N0	9.53	322.67	6.01	315.67	0.030	1.08	1.01
D30N20	18.47	394.33	6.72	279.33	0.066	1.51	1.20
D30N40	19.60	380.33	6.34	268.67	0.073	1.99	1.31
D45N0	10.53	299.33	6.02	310.33	0.034	1.15	1.15
D45N20	17.63	368.00	6.30	275.33	0.064	1.28	1.47
D45N60	20.33	292.67	6.15	249.00	0.082	1.39	1.83
D60N0	12.03	289.67	6.33	307.00	0.039	1.38	1.37
D60N20	20.30	424.33	6.97	274.67	0.074	1.81	1.80
D60N40	24.63	644.67	7.66	274.00	0.090	2.24	2.14
lsd <sub>05</sub> I						0.21	0.24
lsd <sub>05</sub> F						0.16	0.23
lsd <sub>05</sub> IxF						ns	0.40

correlations in several crops, like in wheat (Fischer et al. 1998), sweet potato (Roy Chowdhury and Varma 1998), swamp taro (Roy Chowdhury et al. 2004). In this study, photosynthesis rate and stomatal conductance showed positive correlation ( $r = 0.72^*$ ) at reproductive stage. Under well watered conditions stomatal conductance is a major determinant of photosynthetic rate in rice (Kusumi et al. 2012). The net photosynthesis was higher in crop raised from older seedlings and increased further at higher N application (Fig. 2). Single leaf photosynthesis and  $g_s$  were found to have close correlation ( $r = 0.80$  or more) across large number of rice cultivars, suggesting that the varietal difference in single leaf photosynthesis was mainly influenced by  $g_s$  (Kuroda and Kumura 1990). In our study grain yield was highly correlated with net photosynthesis rate ( $r = 0.82^{**}$ ) as well as carboxylation efficiency ( $g_m$ ,  $r = 0.81^*$ ) than with

stomatal conductance ( $r = 0.66$  ns). The relationship between stomatal conductance and yield has not always been straight forward (Richards 2000), emphasizing that other non-stomatal factors might also be critical for determining photosynthesis rate and yield. About 30 % of the apparent effect of  $g_s$  on single leaf photosynthesis was attributable to the effect of the mesophyll photosynthetic activity, which changed in parallel with  $g_s$  (Kuroda and Kumura 1990). The carboxylation efficiency ( $g_m$ ) plays a major role in determining photosynthesis rate in rice (Centritto et al. 2009) and helps in maintaining higher photosynthesis rate under stressed condition. Both the ratio of total surface area of mesophyll cells to leaf area and intercellular air spaces have been shown to increase with increasing N application (Chonan 1970). The carbon isotope discrimination study have also shown increased carboxylation efficiency with

increasing leaf N content in rice (von Caemmerer and Evans 1991). In our study also the  $g_m$  consistently increased with increased dosage of applied N irrespective of age of seedlings (Table 1). The  $g_m$  was highest in crop raised from 60 day old seedling at 40 kg N ha<sup>-1</sup> followed by 45 day old seedling and least in crop raised from 30 days old seedlings with 40 N kg ha<sup>-1</sup>. The stomatal conductance and transpiration at grain filling stage in 2008-09 also showed significant correlation ( $r = 0.94^*$ ). The stand density and yield have been reported to improve in rice through N application under deep water situation (Ghosh 2007). The higher sink strength represented by longer panicle, higher spikelet number and better grain filling (Roy Chowdhury et al. 2011) might have promoted the photosynthesis rate in crop raised from older seedlings due to greater demand from sink, particularly at higher N level. The higher  $g_m$  in older seedlings grown crop under higher N level and close association of both  $P_n$  and  $g_m$  with grain yield suggested those as two critical determinants for higher yield in aged rice seedlings at higher N level under waterlogged environment. However the interaction effect of age  $\times$  N did not show significant difference between treatments.

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