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Biochemical and physiological characterization for nitrogen use efficiency in aromatic rice genotypes

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ABSTRACT

In a set of 78 aromatic rice genotypes, cluster analysis was performed for yield and its related traits in field under two nitrogen (N) conditions viz., application of N fertilizer (N100) and without application of N fertilizer (N0) during wet season, 2011 and dry season, 2012. Basmati370 and Ranbir basmati were selected as high nitrogen use efficiency (NUE) genotypes and Kolajoha-3 and Ratnasundari as low NUE genotypes for characterization in terms of biochemical, physiological and agronomical aspects of NUE. A total of 32 biochemical, physiological and agronomical characters were measured in the selected four genotypes, growing in field under two N levels i.e., N0 and N100 during wet season 2012. Five efficiency parameters were also studied to determine their NUE. GS activity increased under low N and the increase was more in two high NUE genotypes (41.3%) than that of two low NUE genotypes (5.43%). NR activity increased with application of N fertilizer and low NUE genotypes expressed higher NR activity (8.8% and 2.02% more in N0 and N100 respectively). Chlorophyll content recorded maximum ($3.6\,\mathrm{mg\,g^{-1}}$) in low NUE genotypes under N100 condition, where as the chlorophyll content was minimum ($0.43\,\mathrm{mg\,g^{-1)}}$ in high NUE genotypes under N0 condition. Electron transport rate (ETR), quantum yield (Φ_{PSII}) and F_v/F_m were not affected by N levels but there were significant variations in non-photochemical quenching (q_N) (15% more in N0) and photochemical quenching (q_P) (25% more in N0). Grain yield, total dry matter and N uptake by grain and straw were higher in high NUE genotypes. Higher GS activity, maintenance of sufficient chlorophyll fluorescence and chlorophyll content in case of high NUE genotypes support their higher grain yield and total dry matter content under low N conditions by efficient N uptake, and utilization of nitrogen.

1. Introduction

Nitrogen (N) is a very important nutrient for plant growth and development. India stands third and second in N fertilizer consumption and production respectively (FAI, 2008). Nitrogen is one of the most expensive though but an essential nutrient to plant as the commercial N fertilizers represent the major cost in plant production (Singh, 2005). Though, application of N fertilizers increases crop yields, increased use of N fertilizers effects global N cycle, depletion of ozone layer and also causes nitrate leaching problems in soil (Hakeem et al., 2012). Moreover, crop plants are able to utilize only 30–40% of applied nitrogen for food production and the remaining N is left over into environment leading to hazardous environmental pollution by N contamination (Raun and Johnson, 1999). Genetic variations in N uptake and/or grain yield per unit of N applied has also been studied in different crops such as wheat, rice, maize, sorghum, and barley (Ortizmonasterio et al., 1997; Muchow, 1998; Le Gouis et al., 2000; Presterl et al., 2003; Anbessa et al., 2009; Namai et al., 2009). Nitrogen use efficiency is relatively low in rice as major part of N applied to rice is released as gaseous N, effecting environment and reducing economic efficiency of applied N (Hakeem et al., 2012). Nitrogen use efficient (NUE) genotypes/species can be defined as the plants which can

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Abbreviations: GS, glutamine synthetase; NR, nitrate reductase; NUE, nitrogen use efficiency; P_N, rate of photosynthesis; g_s, stomatal conductance; C_i, intercellular CO_2 concentration; *E, transpiration* rate; TE (P_N/T), transpiration efficiency; WUEi (P_N/g_s), intrinsic water use efficiency; P_N/C_i ratio, carboxylation efficiency; F_v/F_m, maximum photochemical efficiency of PSII; ETR, electron transport rate; Φ_{PSII}, in vivo quantum yield of PSII photochemistry; q_P, coefficient of photochemical quenching; q_N, coefficient of non photochemical quenching; chl, chlorophyll; car, carotenoids; FM, fresh mass.

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absorb and accumulate higher N content and grow well and yield better under low N conditions (Mi et al., 2007). Genetic variation in nitrogen use efficiency in rice was earlier reported (Broadbent et al., 1987; DeDatta and Broadbent, 1988; Tirol-Padre et al., 1996; Inthapanya et al., 2000; Zhang et al., 2008). It is very important to identify or develop high NUE genotypes in rice for its production under low cost crop management practices and also to protect environment (Lea and Azevedo, 2006; Hirel et al., 2007).

A comprehensive knowledge on physiological, biochemical and molecular aspects of NUE particularly in low N environment is crucial for developing NUE varieties. Plants absorb nitrogen in the form of nitrate (NO_3^-) and ammonia (NH_3) from the soil through root transporter systems and it is assimilated by a series of nitrate assimilatory enzymes, *viz*., nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthetase (GOGAT). Glutamine synthetase (GS) is a key enzyme in ammonia assimilation, converts ammonium to glutamine. Ammonium is also produced via few internal metabolic reactions such as photorespiration, nitrate/nitrite reduction, storage molecules, and amino acid conversion (Ireland and Lea, 1999; Vijayalakshmi et al., 2013). GS exists in two forms viz., cytosolic GS (GS1) and chloroplastic GS (GS2). GS1 is critical for normal growth and grain filling where as GS2 is used to recycle assimilated ammonia, derived from photorespiration and also involved in photosynthesis. In C_3 plants such as tobacco, photo respiratory ammonium was produced from oxidative decarboxylation of glycine. The amount of ammonia released during photorespiration is up to 10-fold greater than the amount of primary nitrogen taken up by the plant (Keys et al., 1978; Maheswari et al., 1993). The earlier studies revealed that the cytosolic GS played a key role in nitrogen remobilization for grain filling in wheat, rice and maize (Tabuchi et al., 2007; Martin et al., 2006). Nitrate reductase (NR), present in cytosol, is one of the foremost metabolic enzymes in plants involved in the reduction of nitrate to nitrite (Chandna and Hakeem, 2013). This enzyme shows great variability in its activity at different levels of N (Hakeem et al., 2012) and there are also genotypic differences in NR activity in rice (Chandna et al., 2010).

Total chlorophyll content increased with increasing nitrogen levels, resulting in higher photosynthesis rates leading to more sugar formation (Pramanik and Bera, 2013; Dikshit and Paliwal, 1989). Achieving higher yield with reduced nitrogen fertilization, without considerable effects on the normal physiological processes of functional leaves, has become an important challenge in rice (Long et al., 2007). The relation between slow or controlled release of N fertilizers, supporting more N absorption and related physiological mechanisms have been well studied (Liu et al., 2002; Li et al., 2004; Luo et al., 2007; Long et al., 2013). But there are only a few studies on the effect of nitrogen fertilizer on rice leaf absorption, transmission and distribution of light energy, dissipation of excess excitation energy and related mechanisms. Chlorophyll fluorescence is a quick tool to study the plant photosynthetic capacity using rapid light curve with less impact on leaf (Ralph et al., 1998; Kühl et al., 2005; Gitelson et al., 1999). Electron transport rate (ETR) of the flag leaf was affected mostly by the photosynthetically active radiation and by nitrogen levels during the initial heading stage to some extent. Chlorophyll fluorescence studies suggested a nitrogen rate of 135–180 kg/h for super hybrid rice for improving the photosynthetic electron transport rate, the effective quantum yield and the PS reaction centre openness (Long et al., 2013). The light-saturated photosynthetic rate is correlated with nitrogen content of leaf and Rubisco content. Higher photosynthetic rate at higher N levels was attributed to higher chloroplast CO_2 content, chloroplast size, Rubisco activity and carboxylation capacity (Yong et al., 2009).

Agronomic practices and environmental factors related to N use, grain yield and N accumulation can be measured by determining NUE in cereal based agro ecosystems (Hugins and Pan, 2003). Nitrogen use efficiency in rice can be divided into different efficiencies namely agronomic efficiency (AE), physiological efficiency (PE), agro physiological efficiency (APE), apparent recovery efficiency (ARE) and utilization efficiency (UE). Agronomic efficiency is defined as the response of crop/genotype to applied fertilizer or profitable production obtained per unit of nitrogen applied (Aynehband et al., 2012; Fageria et al., 2010). Physiological efficiency is defined as biological yield per unit nutrient uptake or represents grain yield or plant biomass relative to nitrogen uptake (De Datta, 1986; Peng et al., 2002). Apparent recovery efficiency, as the financial production of grain yield obtained per unit of nutrient uptake (Fageria et al., 2010) and UE as the capacity of the plant to assimilate N and remobilize the N taken up from the soil, producing into amino acids resulting in final grain yield (Moll et al., 1982; Good et al., 2004; Moose and Below, 2009). Genotypic variation in the above NUE indices exists in rice. Classifying these genotypes by using cluster analysis to disperse genotypes into qualitative groups based on response similarities was shown earlier (Yau, 1991; Rincon et al., 1996; Sezener et al., 2006). This method is based on Euclidean distances among group means and produces a dendrogram showing successive combination of individuals. Cluster analysis in the present study was undertaken for classifying 78 aromatic rice genotypes as high NUE and low NUE genotypes based on yield and its related traits performance in field under two N conditions viz., with recommended application of N fertilizer; @ 100 kg of urea (N100) and without application of N fertilizer (N0) during wet season 2011 and dry season 2012. The present work was also planned to investigate biochemical viz., GS and NR activities, photosynthetic viz., photosynthetic pigments content, leaf area and thickness, chlorophyll fluorescence, gaseous exchange parameters and NUE related parameters viz., % N content and N uptake in grain and straw and NUE indices in two genotypes with high NUE and low NUE under two N conditions, N0 and N100 during wet season 2012.

2. Materials and methods

2.1. Experimental conditions and genotypes screening

A total 78 genotypes, belong to aromatic group were evaluated for their agronomic performance under low nitrogen level (N0) and recommended nitrogen level (N100) with application of 100 kg urea/ha in field conditions during two consecutive seasons of wet (kharif) 2011 and dry (rabi) 2012 at Directorate of Rice Research (DRR), Hyderabad, India. For screening the genotypes under field conditions with two different N levels, a nitrogen deficient plot (N0) of dimensions 19.0 m length and 24.5 m width was developed and maintaining at DRR since wet season, 2010 along with a plot supplied with recommended dose of nitrogen (N100). Soil samples were collected from these plots before start of the experiment in wet season 2011and dry season 2012 to determine the initial soil properties. Soil samples were collected from 4 different areas in plot and the mixed composite was used to determine N content by using semi micro Kjeldahl method (Kjeldahl, 1883). The details of soil properties of both N0 and N100 plots were given in Table 1. The soil pH and EC are normal and organic carbon content is medium.

Hierarchical cluster analysis was carried out using Euclidean distance metric and UPGMA method (un-weighted paired group method and arithmetic averages) (web: http://darwin.cirad.fr/darwin) and data of five traits viz., panicle weight, filled grain weight, total grain weight (filled grain and unfilled grain), dry straw weight, total dry matter (dry panicle and stover) from each season and each environment. Cluster analysis was performed using the data in the following nine different pooled data sets of season and