Abstract

Nitrogen efficiency (NE) is an important approach to develop nitrogen use efficient (NUE) genotypes in rice growing regions globally. Identification of nitrogen (N) tolerant genotype is the foremost step to develop Nitrogen Use Efficient (NUE) genotypes. In order to identify the N tolerant genotypes in popular indica rice a hydroponic experiment was conducted in glass house with various concentrations of N and 26 genotypes were screened by measuring growth dynamics, biomass accumulation and NE traits. The data revealed that growth dynamics viz., morphological (TNL, RL and SL), biomass (RFW, SFW, RDW, SDW and TDM) and NE traits showed high genotypic variation for N treatments. Of which root and shoot traits were significantly influenced the nitrogen efficiency trait of genotypes. Based on NE; genotypes were categorized as High Efficient; Medium efficient and Low efficient genotypes. Growth dynamics and NE traits were higher in DRRH3 at low N condition; while BPT5204 responds very poor under low N which confirmed that DRRH3 as most N-efficient and BPT5204 as most N-inefficient genotypes. Further experimental data was subjected to multivariate statistical analysis by means of Pearson correlation, PCA and hierarchical clustering of heatmap analysis. The statistical data is in tune with the experimental data and strongly supported that root and shoot traits are most contributing traits for NE of genotypes. The data generated in the present study is very useful for the selection of genotypes as parents for the development of NUE genotypes of rice.

Key words: Rice genotypes, N treatments, growth dynamics, Nitrogen efficiency, Multivariate statistical analysis, N-efficient and N-inefficient genotypes.

Introduction

Rice is a staple food crop of the world; sixty percent of the global population depends on it for more than 22% of their daily calories (20). Ninety percent of the global rice is produced especially from Asian countries i.e., China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Pakistan, Philippines, Korea and Japan. Among the rice growing countries India occupies largest place with 44.8 million hectares of cultivation followed by China and Indonesia. Over the past half-century a marked increase in rice production attributed due to high yielding genotypes, usage of more folds of N-chemical fertilizers and modern agricultural techniques (25).

To steady the food demands; increased use of N- fertilizer content in the world from 3.5 million metric tons in 1960 to projected 187.7 million metric tons in 2015 and 223.1 million metric tons by 2030 (37). A major portion of global N-fertilizer would be used for the production of the rice (42). Rice based cropping system is one the most
incompetent N-user; only 30% - 50% of applied N-fertilizer utilized and in some cases less than it (27)(31); unutilized N-fertilizer dissipates by leaching, de nitrification, immobilization and volatilization (40) resulted drastic effect on environment and all living life forms (6) (11) (28) (33) (39). The efficient absorption and utilization of nitrogen is the key factor for the development high yielding varieties in rice. As the nutrient uptake and utilization are co-dependent careful integration of both physiological and agronomic traits are very important to develop nitrogen efficient genotypes.

Genetic variability for efficiency in N-absorption and utilization have been documented in several crops (18). Development of a reliable selection criterion is very important for identification of genetic variability Nitrogen Use Efficient (NUE) genotypes. This will confidently help us to enhance the nitrogen uptake and utilization efficiency which eventually leads to the Nitrogen Use Efficiency in crop plants (17).

Hydroponics is a foremost controlled and elementary modeling tool for plant research, it is also denoted as ‘water culture of plants’ and has been widely used in research as well as in commercial farming since from 18th century onwards. Hydroponics is very important for plant research as it helps to identify essential elements, their optimum levels and plant uptake form of element under well controlled nutrient solutions. It is also helpful for the identification of intra and inter specific genetic variations with respect to different treatment levels and to identify the elements deficiency, toxicity symptoms under more controlled conditions (34).

To determine the limits of concentration of nitrogen in rice genotypes is an important aspect as the rate of absorption and assimilation of applied nitrogen varies in the genotypes. Thus the present study aimed to screen the rice genotypes for their nitrogen efficiency with different nitrogen concentrations as KNO₃ as source of nitrogen in a hydroponic experiment. This preliminary screening experiment would identify the Nitrogen Use Efficient genotypes which would be further helpful to the breeders for development of high nitrogen efficient genotypes.

**Materials and methods**

**Genotypes for study:** Twenty six rice (*Oryza sativa* L.) genotypes viz., KMR3R, RPHR-1096, DRRH3, B-95-1, RPHR-111-3, AJAYA-R, EPLT-104, DR714-1-2R, SC5-2-2-1, KRH2, BCW-56, RPHR-1005 and EPLT-109 were supplied by the Directorate of Rice Research (DRR), Rajendranagar, Hyderabad, RAMAPPA, JAYA, JGL-MASURI, BADRAKALI, NLR-3042, WGL-347, JGL-1798, SWARNA, MTU1010, BPT5204, ERRAMALLELU and IMPROVED SAMBA MASURI were supplied by the Agricultural research station, Utukuru, Kadapa and BI 33 was supplied by the GKVK, Bangalore.

**Experimental design:** The experiment was conducted in a completely randomized block design (CRBD) with two replicates. Four levels of nitrogen viz., nitrogen deficiency/T1 treatment (0 mM L⁻¹), low nitrogen/T2 treatment (1 mM L⁻¹), medium nitrogen/T3 treatment (4 mM L⁻¹) and high nitrogen/T4 treatment (10 mM L⁻¹) were supplied in the form of potassium nitrate (KNO₃). Surface sterilized seeds (0.05% HgCl₂) were germinated in germination boxes on filter paper for one week. After, similar sizes of seedlings were placed in nutrient solution (17) containing macronutrients of 5.6 mM L⁻¹ K₂SO₄, 3.4 mM L⁻¹ CaCl₂.2H₂O, 0.9 mM L⁻¹ MgSO₄.7H₂O, 0.9 mM L⁻¹ NaH₂Po₄ and micro nutrients of 21.5 mM L⁻¹ FeCl₃.6H₂O, 23.0 mM L⁻¹ H₃BO₃, 9 mM L⁻¹ MnCl₂.4H₂O, 0.3 mM L⁻¹ (NH₄)₆Mo₇O₄.4H₂O, 0.9 mM L⁻¹ CuSO₄.4H₂O, 3.5 mM L⁻¹ ZnSO₄.7H₂O in the culture room maintained at 26/22°C day/night temperature, 60% relative humidity and 16/8 hrs light/dark photoperiod with 2650 luxs of output light intensity. The nutrient solution was aerated with sterile air to provide sufficient O₂ and changed every week up to 30 days.

**Experimental observations**

**Growth dynamics (GD):** Growth dynamics were separated into morphological and biomass traits. **Morphological traits:** Plant samples were taken out from nutrient solution and separated in to root...
and shoot. The morphological traits were partitioned into total number of leaves (TNL), root length (RL) and shoot length (SL). The root length was measured from the root-shoot junction to tip of the longest root and shoot length was measured from the soil above ground level up to uppermost longest fully expanded leaf (1). The root and shoot lengths were measured by using metric scale and expressed in terms of centimeters (cm).

**Biomass traits**: Biomass was partitioned into root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW) and total dry matter (TDM). For fresh weights, root and shoot samples were dried with blotting sheets and recorded by using electronic weighing balance (Model AX200, SHIMADZU, JAPAN) and for dry weights plant samples were dried at 65°C for 72 hrs in hot air oven (proper care taken to avoid burning of the samples) and recorded by using electronic weighing balance (Model AX200, SHIMADZU, JAPAN). The fresh and dry weights were expressed in terms of grams (g) per plant.

**Nitrogen efficiency (NE)**: Nitrogen efficiencies of 26 rice genotypes were calculated based on their mean of shoot dry weight values, which were applied into following formula according to Chandna et al. (2010) (8) and expressed in terms of percentage (%).

\[
\text{Nitrogen efficiency (\%)} = \left( \frac{\text{Biomass accumulation at N-insufficient condition}}{\text{Biomass accumulation at N-sufficient condition}} \right) \times 100
\]

**Multivariate statistical analysis**: All data were subjected to statistically analyzed by means of experimental treatments average mean to growth dynamics and nitrogen efficiency traits for observing response with different nitrogen levels and multivariate statistical analysis by means of Pearson correlation coefficient (19) for relation of traits with respect to different nitrogen levels among genotypes, principal component analysis (PCA) (32) for identification of most contributing of traits for higher nitrogen efficient genotypes under low nitrogen levels and finally done the hierarchical heatmap and cluster analysis (32) for better screening of nitrogen efficient genotypes with graphical representation and genotypes grouping with respect to different nitrogen treatment levels.

**Results**

**N-treatment effect on growth dynamics (GD)**: Effect of various concentrations of nitrogen on growth dynamics (GD) and nitrogen efficiency (NE) of 26 rice genotypes were assessed and genotypes were categorized using multivariate statistical analysis by means of Pearson correlation coefficient, principal component analysis (PCA) and hierarchical heatmap clustering. The growth dynamics i.e., total number of leaves (TNL), root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW) and total dry matter (TDM) displayed high genotypic variations across nitrogen treatments. In the present study, the concentration of nitrogen frailly affects the growth and development of leaves. However, a significant genotypic variation observed for total number of leaves (TNL/P) with a mean data ranged from 1.95 (BPT5204) to 3.58 (DRRH3) under various nitrogen treatments and an average of greater number of leaves 4.0 (TNL/P) were observed in the genotype DRRH3 at medium N rate (4 mM L⁻¹) and fewer number leaves in genotype BPT5204 at N deficient (0 mM L⁻¹) condition. Root length sturdily affected by the N treatment. Mean root lengths of genotypes varied from 2.24 cm (BPT5204) to 9.16 cm (DRRH3), with an average value of 9.58 cm in genotype DRRH3 at medium N rate (4 mM L⁻¹) and 1.50 cm by the RPHR-111-3 at high N rate (10 mM L⁻¹). In contrast high root fresh weight (RFW) was recorded in DRRH3 (0.025 g) and lowest in B-95-1 (0.007 g). The root dry weight (RDW) was ranged from 0.002 g (B-95-1, AJAYA-R, EPLT-104 and BPT5204) to 0.025 g (DRRH3) observed at T2 and T3 levels and with a mean data the highest RDW was recorded in DRRH3 (0.019 g) and lowest in BPT5204 (0.003 g).

The shoot lengths were significantly affected by the N treatments and mean shoot lengths varied from 3.68 cm (BPT5204) to 10.05 cm (DRRH3).
The genotype JGL-MASURI recorded an average of 13.00 cm at high N level (10 mM L\(^{-1}\)) and 2.90 cm by BPT5204 at low N levels (0 mM L\(^{-1}\)). Shoot fresh weights (SFW) were ranged from 0.004 g (B-95-1) to 0.052 g (EPLT-104) observed at T1 levels and with a mean data the highest SFW was recorded in EPLT-104 (0.025 g) and lowest in B-95-1 (0.006 g). The shoot dry weight (SDW) were ranged from 0.002 g (B-95-1, EPLT-104 and BPT5204) to 0.015 g (JAYA) observed at T1, T2 and T3 levels and with a mean data the highest SDW was recorded in JAYA (0.012 g) and lowest in B-95-1 (0.003 g). The total dry mater (TDM) was ranged from 0.004 g (B-95-1 and BPT5204) to 0.032 g (DRRH3) observed at T2 level and with a mean data the highest TDM was recorded in DRRH3 (0.028 g) and lowest in B-95-1 (0.007 g) (Table 1).

Simple correlation data analysis revealed the relationship between traits among genotypes with respect to nitrogen treatments. RL significantly correlated with TNL/P (\(p < 0.01\)) and SL (\(p < 0.05\)) with a correlation coefficient (r) of 0.610, 0.424 respectively. RFW was highly significant (\(p < 0.01\)) with RL and SL with a correlation coefficient (r) of 0.564 and 0.422 respectively. SFW was highly significant (\(p < 0.01\)) with RFW with a correlation coefficient (r) of 0.576, RDW was highly significant (\(p < 0.01\)) with RL and RFW with correlation coefficients (r) of 0.553 and 0.698 respectively, SDW was positive significant with all measured traits, TDM was highly significant (\(p < 0.01\)) with RL, SL, RFW, RDW and SDW significantly (\(p < 0.05\)) correlated with TNL with a correlation coefficients (r) of 0.624, 0.580, 0.676, 0.933, 0.914 and 0.415 respectively (Table 2 a-d).

Treantments effect on nitrogen efficiency (NE): Screening of genotypes to nitrogen tolerance was determined based on the nitrogen efficiency (NE), which is derived from the shoot dry weight NE is a widely considered parameter for identification of genotypic variation in tolerance to nutrient deficiency (2). In the present study, NE data displayed high genotypic variation under different nitrogen regimes. NE values were ranged from 22.22% to 200.00% at T3 treatment (4 mM L\(^{-1}\)) and 20.00% to 166.67% T4 treatment (10 mM L\(^{-1}\)). This data clearly indicates that plants were unable to up take the excess N source. At medium nitrogen conditions NE significantly positively correlated with root dry weight (RD) (\(p < 0.01\)) and root length (RL) (\(p < 0.05\)) and negatively correlated with shoot dry weight (SDW) (\(p < 0.01\)) with correlation coefficients (r) of 0.499; 0.457 and -0.545 respectively. Similarly, NE at high nitrogen (10 mM L\(^{-1}\)) conditions had significant positive correlation with RDW (\(p < 0.05\)), RL (\(p < 0.01\)) and negative correlation with SDW (\(p < 0.05\)) with correlation coefficients (r) of 0.491; 0.526, and -0.486 respectively. It indicates both at medium and high nitrogen conditions root length and root dry weight are key contributing traits for genotypes NE. Further, as per Hakeem et al. (2012) (17), based on the NE data at both N treatments (4 mM L\(^{-1}\)and 10 mM L\(^{-1}\)) in the present study genotypes were grouped into three categories viz., high nitrogen efficient (HNE) genotypes (the genotypes contained more than 90% of NE values), moderate nitrogen efficient (MNE) genotypes (the genotypes contained between 65.00-89.99% of NE values) and low nitrogen efficient (LNE) genotypes (the genotypes contained below 65.00% of NE values). Accordingly, KMR3R, RPHR-196, DRRH3, NLR-3042, SC5-2-2-1, KRH2 and SWARNA were grouped as high nitrogen efficient (HNE) genotypes, the RPHR-111-3, B133, RAMAPPA, BCW-56, RPHR-1005, JAYA, JGL-MAHSURI, MTU-1010, BADRAKALI, EERRAMALLELU and IMPROVED SAMBA MAHSURI were grouped as moderate nitrogen efficient (MNE) genotypes and the B-95-1, AJAYA-R, EPLT-104, DR714-1-2R, WGL-347, EPLT-109, JGL-1798 and BPT5204 were grouped as low nitrogen efficient (LNE) genotypes. Overall at T3 and T4 treatments DRRH3 considered as high nitrogen efficient genotype and the BPT5204 as low nitrogen efficient genotype (Table 1).

Principal Component Analysis (PCA): Principal Component Analysis (PCA) was performed to accurately identify the most contributing traits in categorizing the genotypes in response different nitrogen treatments. At deficient nitrogen (0 mM L\(^{-1}\)) condition the PCA of first four components contributed 90.325% of...
variability and rest of the components contributed 9.676% of variability among genotypes for growth dynamic traits. Principal component PC 1 had the Eigen value 4.550 and contributed 56.880% of total variability which is obtained from the traits such as TDM (0.445), SDW (0.412), RDW (0.411), RFW (0.373), RL (0.354), SL (0.316) and TNL (0.257), the PC 2 had Eigen value 1.301 and contributed 16.261% of total variability obtained SFW (0.716) and RFW (0.317), the PC 3 had Eigen value 0.792 and contributed 9.897% of total variability obtained from SL (0.622), TNL (0.355), SFW (0.354) and RL (0.286), TDM, SDW and RDW of PC 1, SFW of PC 2, TNL of PC 3 and SL of PC 4 were had the high positive values indicated more contribution towards total variability. At deficient nitrogen (0 mM L⁻¹) condition based on PCA analysis genotype were grouped into two categories, high tolerant genotypes DRRH3, KRH2, DR714-1-28, BI 33 and JAYA with high higher ranking values, and sensitive genotypes BPT5204, B-95-1, AJAYA-R, EPLT-104, WGL-347, JGL-1798 and RAMAPPA with low PC values (Figure 1a).

At low nitrogen (1 mM L⁻¹) condition the PCA of first four components contributed for 90.457% of genotypic variability and rest of the components were for 9.543% of variability. Principal component PC 1 had the Eigen value 5.217 and contributed 65.210% of total variability obtained from TDM (0.408), SFW (0.383), RDW (0.378), RFW (0.366), SDW (0.345), RL (0.324), TNL (0.315) and SL (0.295), the PC 2 had Eigen value 0.804 and contributed 10.047% of total variability obtained from major contribution of SL (0.622), TNL (0.355), SFW (0.354) and RL (0.286), TDM, SDW and SFW of PC 2, TNL of PC 3 and SL of PC 4 were had the high positive values indicated more contribution towards total variability. Genotypes based the PCA values high ranking value genotypes such as DRRH3, KRH2 and BI33 considered as high tolerant genotypes and low PCA value genotypes BPT 5204, B-95-1, JGL-MAHSURI, DR714-1-28, WGL-347 and EPLT-104 considered as more sensitive genotypes to moderate nitrogen levels (Figure 1b).

At medium nitrogen (4 mM L⁻¹) condition the PCA of first four components contributed 88.593% of genotypic variability contributed by the first four components of PCA and 11.407% of variability by the rest of the components. PC 1 had the Eigen value 4.438 and contributed 49.307% of total variability obtained from the traits RDW (0.422), TDM (0.418), TNL (0.414), RL (0.375), RFW (0.351), SL (0.338), NE (0.238) and SFW (0.204), the PC 2 had Eigen value 2.064 and contributed 22.937% of total variability obtained from SDW (0.638) and SFW (0.538), the PC 3 had Eigen value 0.816 and contributed 9.065% of total variability obtained from SL (0.655), RL (0.255) and TNL (0.201) and the PC 4 had Eigen value 0.656 and contributed 7.284% of total variability obtained from RFW (0.640), SL (0.385) and NE (0.332). RDW, TDM and TNL of PC 1, SDW and SFW of PC 2, SL of PC 3 and RFW of PC 4 were had the high positive values indicated more contribution towards total variability. Genotypes based the PCA values high ranking value genotypes such as DRRH3, KRH2, BI33 considered as high tolerant genotypes and low PCA value genotypes BPT 5204, B-95-1, WGL-347, RPHR-1005 and JGL-1798 considered as more sensitive genotypes to moderate nitrogen levels (Figure 1c).

At high nitrogen (10 mM L⁻¹) condition the PCA of first four components contributed 88.947% of genotypic variability and rest of the components were 11.053% of variability. PC 1 had the Eigen value 4.747 and contributed 52.746% of total variability obtained from TDM (0.424), TNL (0.378), RDW (0.375), RFW (0.361), SFW (0.341), SL (0.325) and SDW (0.207), the PC 2 had Eigen value 2.004 and contributed 22.269% of total variability obtained from NE (0.589), RL (0.312) and SDW (0.292),
Fig. 1(a). Principal component analysis for various growth dynamic traits in 26 rice genotypes under nitrogen deficient condition.

Fig. 1(b). Principal component analysis for various growth dynamic traits in 26 rice genotypes under low nitrogen condition.

Fig. 1(c). Principal component analysis for various growth dynamic and nitrogen efficiency traits in 26 rice genotypes under moderate nitrogen condition.

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the PC 3 had Eigen value 0.710 and contributed 7.884% of total variability obtained from SL (0.678) and RL (0.213), and the PC 4 had Eigen value 0.544 and contributed 6.048% of total variability obtained from SFW (0.486), NE (0.348), TNL (0.338) and SL (0.233). At higher nitrogen levels TDM, NE, SL and SFW contributed more for the genetic variability. High PCA value genotypes such as DRRH3, KRH2, BI33, BCW-56, DR714-1-2R, SC5-2-2-1, JAYA, MTU1010, EPLT-104 and JGL-MAHSURI were considered as high tolerant genotypes and low PCA value genotypes B-95-1, SWARNA, AJAYA-R, IMPROVED SAMBA MAHSURI, BPT 5204, JGL-1798, RPHR-1005 and WGL-347 were considered as sensitive to high nitrogen levels (Figure 1d).

Hierarchical heatmap and cluster analysis

Hierarchical heatmap and cluster analysis was carried out in twenty six genotypes to get an accurate confirmation on categorization of genotypes based on growth dynamics and NE traits. Fine graphical representations are very useful to examine the genotypes and their contributed traits for judgment of the experimental results.

Under deficient nitrogen source (0 mM L⁻¹) the twenty six rice genotypes mainly divided into two clusters viz., cluster I and cluster II, they again sub divided into two sub-clusters individually viz., cluster 1A, cluster 1B and cluster II A, cluster II B. Fourteen genotypes grouped in cluster I A viz., KMR3R, EPLT-109, SWARNA, BADRAKALI, RPHR-1096, BCW-56, DRRH3, KRH2, JAYA, RPHR-111-3, SC5-2-2-1, NLR-3042, JGL-1798 and MTU1010, which showed maximum response to low nitrogen conditions, only one genotype was there in cluster I B viz., EPLT-104 performed well but comparatively low to class IA under low nitrogen conditions, genotypes under cluster II A viz., B-95-1, JGL MAHSURI and BPT5204 responded poorly and cluster II B genotypes viz., AJAYA-R, RAMAPPA, DR714-1-2R, WGL-347, IMPROVED SAMBA MAHSURI, BI33, RPHR-1005 and ERRAMALLELU performed moderately under nitrogen deficit conditions. The cluster analysis data confirmed that cluster I A and I B genotypes were considered as high tolerant genotypes, cluster II A genotypes considered as high sensitive genotypes and cluster II B genotypes considered as moderate genotypes at nitrogen deficient conditions. In this treatment, the contributed traits were distinguished into three groups viz., group I, group II and group III. The group I have morphological traits of SL, RL and TNL, group II have biomass traits of SDW, TDM and RDW and group III have biomass traits of RFW and SFW. NE trait wasn’t considered in this grouping for this treatment because there is no statistical value (Figure 2a).

Under low nitrogen source (1 mM L⁻¹) the twenty six rice genotypes were mainly divided into two clusters viz., cluster I and cluster II, cluster I again sub divided into two sub-clusters viz., cluster 1A, cluster 1B. There were 18 genotypes
grouped into cluster I viz., KMR3R, SC5-2-2-1, RPHR-1096, RAMAPPA, DR714-1-2R, SWARNA, IMPROVED SAMBA MAHSURI, RPHR-1005, ERRAMALLELU, BADRAKALI, EPLT-109, RPHR-111-3, NLR-3042, JGL MAHSURI, MTU1010, BI33, BCW-56 and JAYA, DRRH3, KRH2 which exhibited moderate response to nitrogen, genotypes in the cluster I B viz., B-95-1, AJAYA-R, EPLT-104, WGL-347, BPT5204 and JGL-1798 displayed poor response and cluster II remains in single group contained DRRH3 and KRH2 genotypes which performed very well and displayed maximum response under low nitrogen level. This data clearly established that cluster I A genotypes as moderate genotypes, cluster I B genotypes as sensitive genotypes and cluster II genotypes as tolerant genotypes at low nitrogen level. Under low nitrogen condition the contributed traits were distinguished into two group’s viz., group I and group II. The group I have morphological traits of SL, RL and TNL. NE wasn’t considered in this group because as there is no statistical value. Group II have biomass traits of RFW, SDW, SFW, TDM and RDW (Figure 2b).

At moderate nitrogen source (4 mM L$^{-1}$) the twenty six rice genotypes were mainly divided in to two clusters viz., cluster I and cluster II, they again sub divided into two sub-clusters individually viz., cluster 1A, cluster 1B and cluster II A, cluster II B. The cluster I A contains genotypes viz., KMR3R, RPHR-1096, RAMAPPA, JGL MAHSURI, RPHR-111-3, SC5-2-2-1, NLR-3042, MTU1010 and SWARNA which showed moderate response to nitrogen and cluster I B contains genotypes viz., B-95-1, AJAYA-R, RPHR-1005, BADRAKALI, WGL-347, BPT5204, EPLT-109, ERRAMALLELU and JGL-1798 which are very poor responders to nitrogen at this concentration. The genotypes in the cluster II A viz., DRRH3 and KRH2 II B are high responsive genotypes to medium nitrogen concentration, genotypes of cluster II B such as BI33, EPLT-104, IMPROVED SAMBA MAHSURI, DR714-1-2R, BCW-56 and JAYA performed well at this concentration but comparatively lesser than group II A. This data clearly confirmed that cluster I A genotypes considered as moderately tolerant.
and cluster I B genotypes as sensitive, cluster II A genotypes as highly tolerant and cluster II B genotypes considered as tolerant at moderate nitrogen level. In this treatment, the contributed traits were distinguished into four group's viz., group I, group II, group III and group IV. The group I have morphological traits of SL, RL and TNL, group II have biomass traits of RFW, SDW, RDW and TDM, group III has NE and group IV have SFW and SDW (Figure 2c).

At high nitrogen source (10 mM L⁻¹) twenty six rice genotypes were mainly grouped in to two clusters viz., cluster I and cluster II, they again sub divided into two sub-clusters individually viz., cluster 1A, cluster 1B and cluster II A, cluster II B. Cluster I A consisting of KMR3R, RPHR-111-3, WGL-347, AJAYA-R, EPLT-109, RAMAPPA, RPHR-1005 and BPT5204 which displayed moderate response to high nitrogen levels, cluster I B genotypes viz., RPHR-1096, NLR-3042, B-95-1, SWARNA, JGL-1798 and IMPROVED SAMBA MAHSURI., exhibited less performance, cluster II A genotypes viz., DRRH3 and KRH2 performed very well and high response to high nitrogen levels and cluster II B viz., BI33, BADRAKALI, ERRAMALLELU, EPLT-104, MTU 1010, DR714-1-2R, BCW-56, SC5-2-2-1, JAYA and JGL MAHSURI performed well but comparatively lesser than group II A. This data clearly confirmed that cluster I A genotypes considered moderate and cluster I B genotypes were considered as sensitive, cluster II A genotypes were considered as high tolerant and cluster II B genotypes were considered as tolerant at moderate nitrogen level. In this treatment, the contributed traits were distinguished into three groups viz., group I, group II and group III. The group I have RFW, TDM, RDW, RL and TNL, group II have only NE and group III have SFW, SDW and SL (Figure 2d).

Discussion

Nitrogen is an essential element for growth and other physiological functions of the plant body. The amount of nitrogen can influence the absorption of light, light use efficiency and accumulation of dry matter in various parts of the plants (5). In general, plants absorb and utilize nutrients very rapidly at their early stages of growth as the availability of nutrients are very high in the soil. Genotypes differently absorb and utilize the nutrients. Differential absorption of nutrients by the genotypes depends on several factors such as genotype nature, size and morphology of the root, nutrients requirement, availability of nutrients, growth stage of the plant, uptake and allocation efficiency of the genotype and nutrient use efficiency (26). Though it is unwarranted, several crop plants unavoidably uptake excess nitrogen fertilizer from the soil for their growth and development. However, only 50% or less of the absorbed nitrogen is used and rest of the nitrogen vanished into the environment and cause severe environmental pollution. To overcome this, identification of N-efficient genotypes which can absorb and efficiently utilize the accumulated high nitrogen or genotypes which can grow and yield under low N conditions are very important in the agronomical context. In a previous study based the nitrogen uptake kinetics and biochemical analysis several rice genotypes were screened and identified high NE genotypes. The high NE genotypes had greater amount of nitrogen contents than low NE genotypes (8) (16).
Table 1. Effects of nitrogen treatments on growth dynamics and nitrogen efficiency of the tested rice genotypes.

<table>
<thead>
<tr>
<th>Nitrogen Treatment</th>
<th>Biomass (g)</th>
<th>Leaf Length (cm)</th>
<th>Root Length (cm)</th>
<th>Basal Width (cm)</th>
<th>Root Diameter (g)</th>
<th>Shoot Fresh Weight (g)</th>
<th>Shoot Dry Weight (g)</th>
<th>Total Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5 ± 0.5</td>
<td>2.0 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>1mNΔ</td>
<td>4.0 ± 1.0</td>
<td>2.5 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.0 ± 0.2</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>1mNΔ+</td>
<td>4.5 ± 1.5</td>
<td>3.0 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>2.0 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.0 ± 0.5</td>
<td>0.0 ± 0.5</td>
</tr>
<tr>
<td>2mNΔ</td>
<td>5.0 ± 2.0</td>
<td>3.5 ± 1.0</td>
<td>3.0 ± 1.0</td>
<td>2.5 ± 1.0</td>
<td>2.0 ± 1.0</td>
<td>0.5 ± 1.0</td>
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<td>0.0 ± 1.0</td>
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<td>5.5 ± 2.5</td>
<td>4.0 ± 1.5</td>
<td>3.5 ± 1.5</td>
<td>3.0 ± 1.5</td>
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Continued
Identification of Nitrogen Efficient Indica Rice
Table 2 (a). Correlation matrix for various growth dynamic traits under nitrogen deficient condition.

<table>
<thead>
<tr>
<th></th>
<th>TNL/P</th>
<th>RL(Cm)</th>
<th>SL(Cm)</th>
<th>RFW(g)</th>
<th>SFW(g)</th>
<th>RDW(g)</th>
<th>SDW(g)</th>
<th>TDM(g)</th>
</tr>
</thead>
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<tr>
<td>TNL/P</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RL(Cm)</td>
<td>.610**</td>
<td>1</td>
<td>.564**</td>
<td>.422'</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>SL(Cm)</td>
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</tr>
<tr>
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<td>.603**</td>
<td>.675**</td>
<td>.542**</td>
<td>.184</td>
<td>.708**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SDW(g)</td>
<td>.415'</td>
<td>.624**</td>
<td>.580**</td>
<td>.676**</td>
<td>.312</td>
<td>.933**</td>
<td>.914**</td>
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<tr>
<td>TDM(g)</td>
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<td>.624**</td>
<td>.580**</td>
<td>.676**</td>
<td>.312</td>
<td>.933**</td>
<td>.914**</td>
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* and ** Significant at 5% and 1% levels.

Table 2(b). Correlation matrix for various growth dynamic traits under low nitrogen condition.

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<th>SFW(g)</th>
<th>RDW(g)</th>
<th>SDW(g)</th>
<th>TDM(g)</th>
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<tr>
<td>RL(Cm)</td>
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<td>.450'</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL(Cm)</td>
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<td>.450'</td>
<td>1</td>
<td>.518''</td>
<td>1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RFW(g)</td>
<td>.407*</td>
<td>.642''</td>
<td>.518''</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SFW(g)</td>
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<td>.603''</td>
<td>.649''</td>
<td>.632'</td>
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<td></td>
<td></td>
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<tr>
<td>RDW(g)</td>
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<td>.599''</td>
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<tr>
<td>SDW(g)</td>
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<tr>
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<td>.578''</td>
<td>.485'</td>
<td>.777''</td>
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<td>.935''</td>
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* and ** Significant at 5% and 1% levels.

Table 2(c). Correlation matrix for various growth dynamic and nitrogen efficiency traits under moderate nitrogen condition.

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<th>SFW(g)</th>
<th>RDW(g)</th>
<th>SDW(g)</th>
<th>TDM(g)</th>
<th>NE (%)</th>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL(Cm)</td>
<td>.667''</td>
<td>.568''</td>
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<td>.405''</td>
<td>.405'</td>
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<tr>
<td>SFW(g)</td>
<td>.392'</td>
<td>.175</td>
<td>.439'</td>
<td>.466'</td>
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<td></td>
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<tr>
<td>RDW(g)</td>
<td>.709''</td>
<td>.677''</td>
<td>.449'</td>
<td>.643''</td>
<td>.122</td>
<td>1</td>
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<tr>
<td>SDW(g)</td>
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<td>.166</td>
<td>.023</td>
<td>.057</td>
<td>.611''</td>
<td>.153</td>
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<tr>
<td>TDM(g)</td>
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<td>.596''</td>
<td>.449'</td>
<td>.652''</td>
<td>.365</td>
<td>.917''</td>
<td>.253</td>
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<td>NE (%)</td>
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<td>.457'</td>
<td>.275</td>
<td>.350</td>
<td>-212</td>
<td>.409''</td>
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Krishna Kumar Guduru et al
In the present investigation, we assessed twenty six rice genotypes for their growth dynamics and nitrogen efficiency with respect to different nitrogen treatment levels viz., nitrogen deficiency (0 mM L⁻¹)/T1, low nitrogen (1 mM L⁻¹)/T2, medium nitrogen (4 mM L⁻¹)/T3/ and high nitrogen (10 mM L⁻¹)/T4 treatments.

Our findings indicated that there was a high genotypic variation among genotypes for growth dynamics traits viz., morphological (TNL, RL and SL) and biomass (RFW, SFW, RDW, SDW and TDM) different nitrogen levels. High growth performance was observed in DRRH3 and low in BPT5204. As reported in previous studies the concentration of nitrogen sternly affects the total number of leaves, in DRRH3 total number of leaves increased with the concentration of nitrogen, however at higher concentration the number of leaves declined slowly. It is attributed that nitrogen concentration modulates the hormone gibberellins indirectly through cytokinins and enhance the number of leaves per plant (10) (23) (38). Maximum total dry matter accumulation recorded in the DRRH3 and minimum dry matter accumulation was observed in B-95-1 and high genotypic variation was observed among the genotypes. However, as reported earlier (14) not much variation was observed within the genotype among four treatments. Root trait is an important one which is influenced by both genetically and surrounding environments. In the present study root length significantly influenced nitrogen concentration. Most of the genotypes recorded greater root lengths at nitrogen deficient conditions and low nitrogen conditions. In contrast, most of genotypes displayed low growth rates at high nitrogen concentrations. This is probably at low nitrogen concentrations roots penetrate into deep soil layers for nutrients absorption.

Root is an essential plant organ which absorbs water and nutrients from soil and plays a significant role in growth and development of plants. The concentration of N significantly affects the root growth and its functional ability. Rice genotypes significantly exhibited variations in shoot-root ratio; root length and root dry weight for N treatments. Rice genotypes such as DRRH3 recorded higher biomass in their roots at low N levels as well at high N levels. High nitrogen genotypes recorded higher biomass than low nitrogen efficient genotypes of rice (17). The reason for the accumulation high biomass in the high efficient genotypes could be the effective utilization of accumulated N in protein synthesis (17). In the present study, nitrogen concentration increases the biomass of the roots. As reported earlier nitrogen treatment level significantly improved the root growth in terms of root dry weight of upland rice genotypes (13). The increase of root dry weight

### Table 2(d). Correlation matrix for various growth dynamic and nitrogen efficiency traits under high nitrogen condition.

<table>
<thead>
<tr>
<th>TNL/RLCM</th>
<th>RL(Cm)</th>
<th>SL(Cm)</th>
<th>RFW(g)</th>
<th>SFW(g)</th>
<th>RDW(g)</th>
<th>SDW(g)</th>
<th>TDM(g)</th>
<th>NE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNL/RLCM</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RL(Cm)</td>
<td>.467**</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SL(Cm)</td>
<td>.565**</td>
<td>.452*</td>
<td>1</td>
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<td></td>
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<tr>
<td>RFW(g)</td>
<td>.648**</td>
<td>.520**</td>
<td>.275</td>
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<td></td>
<td></td>
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<tr>
<td>SFW(g)</td>
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<td>.464*</td>
<td>.560**</td>
<td>.577**</td>
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<td>.457**</td>
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<tr>
<td>SDW(g)</td>
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<td>-.046</td>
<td>.497**</td>
<td>.335</td>
<td>.599**</td>
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<tr>
<td>TDM(g)</td>
<td>.683**</td>
<td>.626**</td>
<td>.645**</td>
<td>.694**</td>
<td>.588**</td>
<td>.853**</td>
<td>.539**</td>
<td>1</td>
</tr>
<tr>
<td>NE (%)</td>
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<td>.526**</td>
<td>.069</td>
<td>.332</td>
<td>-.056</td>
<td>.491*</td>
<td>-.486*</td>
<td>.160</td>
</tr>
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</table>
significantly enhanced yield in upland rice genotypes by improving water and nutrient efficiency (13). The plant growth is regulated by supply of carbon from shoot to root via phloem transport as well as nitrogen from root to shoot via xylem transport (35). The fluxes are possibly depends on the concentration gradients of carbon, nitrogen, mineral nutrients in the shoot, roots and various environmental factors (41). According to Cooper and Clarkson (1989)(9) the increase of carbon transportation and root-shoot dry weight under N limited condition is the important aspect to improve the nitrogen efficiency in the genotypes.

Based on NE data in the present study of 26 genotypes were categorized into high nitrogen efficient (HNE) genotypes viz., KMR3R, RPHR-196, DRRH3, NLR-3042, SC5-2-2-1, KRH2 and SWARNA, moderate nitrogen efficient (MNE) genotypes viz., RPHR-111-3, BI33, RAMAPPA, BCW-56, RPHR-1005, JAYA, JGL-MAHSURI, MTU-1010, BADRAKALI, ERRAMALLELU and IMPROVED SAMBA MAHSURI and low nitrogen efficient (LNE) genotypes viz., B-95-1, AJAYA-R, EPLT-104, DR714-1-2R, WGL-347, EPLT-109, JGL-1798 and BPT5204 at both N treatment (4 mM L⁻¹ and 10 mM L⁻¹) levels. The high NE genotypes probably efficiently utilize the accumulated nitrogen in protein synthesis resulted in high biomass accumulation. Our data suggested that at low soil nitrogen conditions high nitrogen efficient genotypes (HNE) showed higher nitrogen efficiency than medium (MNE) and low (LNE) nitrogen efficient genotypes. Therefore HNE genotypes were highly desirable at low soil nitrogen levels (4).

Further, statistical significance was done by multivariate statistical analysis which gave a clear, confident and accurate analysis on association of multiple complex traits of genotypes at different N treatments. Multivariate analysis is a widely used technique for identification, separation of genotypes and germplasm based on morphological, biochemical or molecular markers (22). Multivariate analysis includes Pearson correlation, principal component analysis, hierarchical heatmap and clustering analysis (24).

In the present Pearson correlation analysis indicated that rice root and shoot traits are major contributed traits for tolerant to different nitrogen regimes. Root and shoot organs were directly involved in acquisition and assimilation of nitrogen in different parts of the plants. Directly and indirectly contributed traits would more useful for selection process and definitely helpful to the breeders to develop better genotypes (29).

PCA is one of the multivariate statistical technique for simplify complex data sets (7) (12). PCA reduces the “n” variables to “r” new variables in a given “m” observations (30). The PCA identified most contributed traits and their variability with response to nitrogen treatment levels among genotypes. In the present study high variability was observed in the range of 88-90% among the measured traits such as growth dynamics and nitrogen efficiency traits at different nitrogen treatment levels. Based on this variability it was concluded that DRRH3 as nitrogen tolerant and BPT5204 as nitrogen sensitive genotypes among 26 genotypes at various nitrogen regimes. PCA data also revealed that most contributed traits for genotypic variation were root, shoot dynamics, leaves number and dry matter. The PCA data is in agreement with correlation studies. Earlier findings also reported that the high level variability among genotypes and measured traits resulted in high level of positive effect, which is further useful for improvement of genotypes during breeding programme (15) (3).

Hierarchical clustering of heatmap is a graphical representation and visual method that can be used to observe the complex associations of multiple traits and variations in the genotypes irrespective of their treatment levels. The heatmap and hierarchical clustering was measured with hierarchy based on the distance or similarity way (21). In the present study genotypes and traits measured traits were clustered into different groups, from which it was concluded that the DRRH3 is a nitrogen tolerant genotype where as BPT5204 is a sensitive genotype. Measured traits such as growth dynamics and nitrogen efficiency traits are more responsible traits for the genotypic
variation and these traits were grouped into three
groups such as group I, group II, and group III at
each treatment levels.

Conclusion
In conclusion of our data, based on growth
dynamics, biomass as well as nitrogen efficiency
traits confirmed DRRH3 as high nitrogen efficient
genotype and BPT5204 as low nitrogen efficient
 genotype among the genotypes studied at various
nitrogen levels. These results were further strongly
supported by multivariate statistical analysis. The
root and shoot parameters were significantly
positively correlated with nitrogen efficiency trait
at different N levels. These root and shoot
parameters were significantly influenced by
genotypes as well as N treatments. Hence, while
developing NUE rice genotypes breeders should
focus on these traits using adequate N rate.

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Research (CSIR), New Delhi, India.

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40.7.753.
loss in conjunction with translocation from
leaves as influenced by growth stage, leaf
efficiency “ State of the art. Agronomy “
Faculty Publications. Agronomy and
Horticulture Department of Nebraska –


19. IBM SPSS Statistics version 20.0.0.


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