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Genetics of nitrogen use efficiency and its component traits under nitrogen-rich environment in wheat (*Triticum aestivum*)

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ABSTRACT

Minimizing the footprints of overuse of nitrogen (N) on the ecosystem is the need of an hour. There is little information on the genetic inheritance of NUE and its component traits. The present study was therefore designed to study the genetic behaviour of NUE traits under abundant N supply. Diallel analysis of 21 F_1 along with 7 parents was carried out for NUE traits, viz. shoot dry weight (SDW), root dry weight (RDW), maximum root length (MRL), N% in shoot, gram N in shoot (gN) and nitrogen use efficiency (NUE) under high N in hydroponic condition. Analysis of variance revealed significance for both general combining ability (GCA) and specific combining ability (SCA) for all NUE traits indicating the importance of both additive and non-additive gene action for these traits. Baker's ratio revealed a preponderance of additive genes over non-additive for SDW, RDW and MRLsuggesting thereby effectivity of selection for SDW, RDW, gN, and MRL in the early segregating generation of high GCA lines can improve NUE under high N. Crosses with high SCA effects for SDW, RDW, gN and N% were from the parent combination of high × low GCA effects, whereas for MRL, low × low GCA parents resulted in crosses with high SCA effect indicating the dispersal of favourable alleles in the different parents. Our study indicates the feasibility of improvement of NUE through direct selection for the component traits under N rich environment.

Keywords: GCA, Nitrogen use efficiency, Root traits, SCA, Wheat

Increased nitrogen (N) fertilization was one of the major factors for the increase in wheat (*Triticum aestivum* L.) yield during the 20th century (Yadav *et al*. 2010). The N fertilizer application has increased drastically since 1970 around the globe and is assumed to increase further by 2050 (Ranjan and Yadav 2019) particularly to harness the potential yield beyond 7 tonnes/ha (Yadav *et al*. 2017). Despite N being the most abundantly available in the atmosphere (78%), the plant is not able to use this largely because of non-availability of a mechanism in plants to break the triple bond between N atoms. Plants, therefore, carry out their function by taking up the N from the soil in the form of nitrate ion. However, a large proportion of applied N (50-70%) from the plant-soil framework is lost due to leaching, volatilization, denitrification, and runoff (Malyan *et al.* 2016).

To ensure food security for a still-growing population with minimum ecological footprints, improving nitrogen use efficiency (NUE) of the cropping system is inevitable. Globally, NUE of cereals is around 33% (Raun and Johnson 1999) with significantly higher (42%) in developed countries than developing countries (29%). In an empirical plant breeding approach knowledge of gene action governing the trait of importance (NUE traits) helps the breeder to design the breeding programme properly. Germplasm bases used by Indian breeders have hardly been evaluated for these traits and only limited information is available on the genetics of NUE (Yildirin *et al*. 2007, Gorny *et al.* 2011, Al-Naggar *et al.* 2015, Ranjan and Yadav 2020) in wheat. As most of these studies were on European winter wheat and there was an urgent need for such study on Indian breeding material to develop N responsive wheat varieties. The wheat breeders are reluctant to include these objectives in their breeding programme not only due to the complexity involved in the assessment of NUE traits but also due to a lack of knowledge about their genetics. The present study was, therefore, designed to work out the genetics of NUE and its component traits under N non-limiting/rich environment in Indian wheat germplasm.

MATERIALS AND METHODS

The materials used in this study consist of 175 spring wheat genotypes including commercially released cultivars of India and advanced breeding lines (Ranjan *et al.* 2021). All the genotypes were grown under controlled conditions of

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the hydroponic system maintained at the National Phytotron Facility at Indian Agricultural Research Institute (IARI), New Delhi, India under N non-limiting solution with three replications in *rabi* 2014–15. The relationship between NUE component traits in hydroponic and natural conditions (pipe) under non input limiting in the subset genotypes showed good agreement under two conditions (Ranjan *et al.* 2019b).

The details of hydroponic condition and procedure for screening the material is presented in the earlier publications (Ranjan *et al.* 2021). To create N non-limiting/N rich environments, the normal concentration of N containing compounds like (NH_4NO_3, KNO_3, $CaNO_3$) were doubled. Data were recorded for shoot dry weight (SDW), root dry weight (RDW), maximum root length (MRL), gram N in the shoot (gN) and nitrogen use efficiency (NUE). Based on mean gram N in the shoot (gN), extreme seven genotypes (High gN and low gN) were selected (Table 1) where gram N in the shoot (gN) = N % in shoot × Shoot dry weight (g). The N % in the shoot was estimated by the Micro-Kjeldahl method (Ranjan and Yadav 2020). The MRL (cm) was measured at harvest from the shoot base to the longest root tip. Root and shoot portion was separated after harvest and were dried in an oven at 60ºC for 4 days before measuring the dry weights (gm). NUE is calculated as (Ranjan *et al.* 2019b)

$$\text{Nitrogen use efficiency (NUE): } \frac{\text{Shoot dry weight (gm)}}{\text{N supplied in g per plant}}$$

Making diallel cross and evaluation of F_1 along with parents: To know about the genetics of NUE and its component traits under high N level, a half diallel was generated by crossing seven contrasting genotypes based on mean gN leading to 21 F_1s during off season (2015) at IARI Regional Station, Wellington, Tamil Nadu and *Rabi* (2015–16), IARI, New Delhi to generate enough F_1seeds for carrying out the experimentation. The F_1 seeds along with the parents were raised in two replication under hydroponic in controlled conditions maintained at the National Phytotron Facility at IARI, under N non-limiting/rich environment. The diallel was raised for 8 weeks in hydroponics and the data were recorded on maximum root length (MRL), root dry weight (RDW), shoot dry weight (SDW), shoot nitrogen (N) %, gram N in shoot (gN) and nitrogen use efficiency (NUE) for the mean plant.

Table 1 List of extreme genotypes used for making diallel crosses of this study

Table S1	Pedigree	
154	HD2824/VL796	
144	DL672/P66.270//DL894/3/CUMYN	High
151	HD2687/HP1896//WH542	
152	CSW44	
84	HD2967/DBW17	
128	HD 3090	Low
5	HD2953/HS365	

Statistical analysis: Analysis of variance of 175 genotypes was carried out by OpStat (Online software, CCSHAU, Hisar). Griffing's (1956) diallel Method 2 Model I (fixed effects were used to analyze ANOVA for the general combining ability (GCA) and specific combining ability (SCA).

The relative importance of GCA and SCA was assessed by estimating the components of variance and expressed in the ratio.

$$\text{Bakers Ratio: } \frac{2\ \text{MSGCA}}{2\ \text{MSGCA} + \text{MSSCA}} \quad \text{(Baker 1978).}$$

The closer the ratio to unity, the greater is the magnitude of additive gene action and vice versa. MSGCA and MSSCA are mean sum of square due to GCA and SCA.

RESULTS AND DISCUSSION

Genetic variability for traits influencing NUE under hydroponic: Analysis of variance showed significant ($P<0.001$) main effects due to genotypes for all the traits studied (Ranjan *et al*. 2021). The variability parameter, viz. mean performance, the range of different traits revealed large variability for all the traits under study. Several similar studies on genetic variation for NUE parameters in modern cereals have reported the significance of genotypes and their interaction for NUE traits (Barraclough *et al*. 2010, Bingham *et al.* 2012, Sadras and Lawson 2013, Fageria and Santos 2014, Hawkesford 2017, Guttieri *et al.* 2017, Ranjan *et al.* 2019b, Ranjan *et al.* 2021). Association analysis too revealed a positive and significant correlation of NUE with SDW, NUpE, RDW, and MRL under N non-limiting environment (Ranjan *et al.* 2019b).

Analysis of variance for combining ability for different traits: The analysis of variance for combining ability for different characters under study is presented in (Table 2). The significance of treatments (N non-limiting) revealed the existence of sufficient genetic variability among parents and its different cross combinations for all of the traits studied in the present investigation. Similarly, the significance of GCA and SCA for all the traits under study suggests the importance of both additive and non-additive gene action in their inheritance. A similar result was also reported by Le Gouis *et al.* (2002), Souza *et al.* (2008) and Silva *et al.* (2014). Baker's ratio for SDW/NUE (0.72), RDW (0.808) and MRL (0.735) suggest a preponderance of additive gene action, whereas a ratio of 0.48 and 0.42 for gram N in a shoot (gN) and N% suggest equal importance of additive and non-additive gene action in their inheritance. The preponderance of additive gene action for NUE was also reported by Gorny *et al.* (2011). In this study, the higher value of additive effects compared to non-additive effects for SDW, RDW, and MRL suggests that selection for these traits might be effective in the early segregating generations. However for gN, even with the equal contribution of additive and non-additive gene action, it has been suggested to delay early generation selection as the dominance nature of genes will result in false selection in an early generation. Similarly, the

Table 2 ANOVA for treatment and Combining ability for 6 traits in 7 × 7 diallel cross of wheat

Source of Variation	MSS					
	SDW	RDW	MRL	N%	gN	NUE
Replication	0.001*	$1.14E^{-06}$	24.4	0.138*	$1.03E^{-05}$	2.608*
Treatment	0.043***	0.0005***	49.1***	1.295***	$3.72E^{-05}$***	81.235***
GCA	0.053***	0.000848***	62.7***	0.534***	$1.92E^{-05}$***	100.490***
SCA	0.040***	0.000401***	45.2***	1.512***	$4.24E^{-05}$***	75.734***
Error	0.002	0.00011	8.0	0.024	$2.99E^{-06}$	3.578
Baker Ratio	0.72	0.808	0.735	0.41	0.48	0.72

***, significant at 0.1% level of significance; GCA, general combining ability; SCA, specific combining ability

preponderance of dominance gene action for N% in shoot which was in tune with earlier reports by Al-Naggar *et al.* (2015) suggest for population improvement programme to overcome the masking effect of non-additive gene action and to fix the additive and additive × additive variability and selection should be delayed for the later generation.

Mean performance of parents and F_1 hybrids: The per se performance of parents and crosses for NUE and its attributing traits is presented in Tables S1 and S2. The mean value shows a wide range of values for different NUE traits. SDW is an important component trait for NUE. Among the parents, line-2 (HD 2687/HP 1896//WH 542) recorded the maximum value, while line-7 (HD 2953/HS 365) recorded the least. Among the F_1 hybrids evaluated, the maximum value for SDW was noticed in cross combination, line-2 × line-4 (0.707g). Root traits like RDW and MRL are important as the plants absorb nutrients and water from the soil. Mean RDW is maximum for line 1 and least in line 4. Among different cross combinations, hybrid line 1 × line 3 exhibits maximum RDW. Gram N in the shoot (gN) in shoot indicates the ability of the plant to uptake N from the soil which depends on the number of factors like root traits, transporter protein present in the root hairs and soil microorganism present in the rhizosphere (Ranjan and Yadav 2019). Mean gN in the shoot is maximum in line 2 (HD 2687/HP 1896//WH 542) and among crosses inline 2 × line 4 (0.022 g). Similarly for NUE, which was also maximum for line 2 (HD 2687/HP 1896//WH 542) with 33.76. Among the hybrids evaluated, the maximum value was noticed in cross combination, line 2 × line 4 (30.71).

The gene action involved in the expression of different characters is decided by combining ability analysis and thus helps the breeder to decide the breeding procedure for trait improvement. The additive effects and breeding value are the results of GCA. Similarly, SCA effect is proportional to the non-additive effect.

Combining ability effects of parents and F_1 hybrids: The estimates of general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of hybrids for six characters are presented in Tables S1 and S2, respectively. For SDW, two parents have a significant positive effect and four parents have a significant negative effect. The highest significant positive GCA effect was observed in line 2 (0.074) and was followed by line 1(0.074). The significant GCA effect in the negative direction was observed in line 6 and line 7 (-0.029). Among different cross combinations, SCA effects ranged from 0.242 (3×4) to 0.153 (2×4). Eighteen out of 21 crosses recorded significance to this trait in which five were positive or in a desirable direction and 14 crosses were negative or undesirable direction. For gN in shoot, line 2 (0.002) showed the highest positive significant GCA effect and line 7 (-0.001) showed a significant negative GCA effect. Two parents exhibited significant positive effects and one parent has a significant negative effect and others have a non-significant GCA effect. Similarly, for NUE, the highest positive GCA effect was observed in line 2 (3.224) and the highest negative GCA effect was observed in line 5 (-2.914). Among parents, two parents have a significant positive effect and four parents have a significant negative effect. Whereas, SCA effects ranged between - 10.53 in cross 3×4 to 6.655 in 2×4.The 18 out of 21 crosses recorded significance to this trait in which five were positive or in desirable direction and 14 crosses were in an undesirable direction. The direction and magnitude of combining ability effects are known to be useful in selecting parent plants in crop improvement programs (Mather and Jinks 1971). The ranking of seven parents on the basis of GCA effect for six traits (Table S3) revealed that line 2 (HD 2687/HP 1896//WH 542) and line 1(DL 672/P66.270//DL 894/3/CUMYYN) have a high overall score which indicates potential inflow of desirable alleles of different genes controlling these traits from the parents into the offspring. The segregating generations from the crosses of these lines are more likely to respond to selection.

In the present study, crosses showing high SCA effects (Table S4) for SDW/ NUE (2×4), RDW (5×6), MRL (1×2), N% in shoot and gN in shoot (3×6) were derived from parents with various types of GCA effects (high × high, high × low). High SCA effect in crosses from parents with high × high occurs largely because of additive × additive gene actions which can be fixed through direct selection in the segregating generation. The high × low GCA parents cross having high SCA effects is due to lacking additive effect in low GCA parents compared with high GCA parents, and its heterozygote which is highly responsive to high N environment due to non-additive effects such as dominance and epistasis (Jinks and Jones 1958). Thus, it has been suggested that repeated intermating of the desirable segregants followed by selection may be a useful strategy for obtaining transgressive segregants in crosses from high × low GCA parents.

To minimize environmental and economic issues due to the overuse of N fertilizer, the research was carried to assess the variability for NUE traits in Indian wheat germplasm and to know the inheritance of NUE traits in segregation generation. Analysis of variance revealed the abundance of genetic variability for NUE traits largely because of an absence of selection for or against NUE traits during the development of these breeding lines. Combining ability analysis for N responsiveness analyzed under N rich environment indicates the importance of both additive and non-additive gene action in their inheritance. Additive gene effects were found more important for SDW, RDW, and MRL suggesting early generation selection under high N conditions for these traits in the segregating population generated by crossing lines with high GCA such as HD 2687/HP 1896//WH 542 and DL 672/P66.270//DL 894/3/CUMYYN. The N% and gN were largely governed by non-additive gene effects and can be improved through the exploitation of heterosis or recurrent selection in wheat.

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