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Genetic Variation and Trait Associations of Grain Yield and Other Quantitative Traits for Identification of Promising Wheat (*Triticum spp.*) Accessions under Nitrogen Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAD conceived, designed and supervised the study. Author MI carried out the field work, collected and analyzed the data and also reviewed the first draft. Author KD performed some of the statistical analysis and wrote the first draft. Authors PUK, RB, TNS and YJK supervised the work and critically reviewed the first draft of the manuscript. Author CPG managed the literature searches and critically reviewed the first draft. Author TAF assisted in field data collection. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Identification of superior genotypes combining high nitrogen use efficiency (NUE) and high grain yield under low nitrogen supply is imperative to the development of nitrogen use efficient wheat varieties for use by farmers in low input wheat production systems to combat the problem of nitrogen stress. The objectives of the study were to identify high yielding wheat genotypes under nitrogen stress and assess multiple morpho-physiological and yield related traits for their contribution and association to the adaptation of the accessions to nitrogen stress.
Methodology: This study assessed the response of 100 wheat accessions grown in an augmented randomized complete block design in 3 blocks under two contrasting nitrogen regimes: (1) Nitrogen stress (0 kg/ha N) and (2) recommended nitrogen supply (120 kg/ha N), in Dharwad, India during *rabi* 2014/2015.
Results: Significant genotypic responses were observed for most of the traits assessed in both treatments. Grain yield showed significant positive correlation with normalized difference vegetative index (NDVI), tillers per meter row, harvest index, biomass and NUE in both treatments. Several accessions showed adaptation to nitrogen stress by reducing their NDVI and total biomass production while increasing harvest index. Cluster analysis with Mahalanobis distance partitioned the accessions into four groups displaying high and significant inter-cluster distance, signifying that hybridization between nitrogen stress-adapted parents from the groups will produce the maximum genetic recombination for nitrogen stress tolerance in subsequent generations.
Conclusion: Accessions DDK 1025, HW 2004, HD 4709, HI 8592 and K 8020 with superior yields under nitrogen stress condition can be utilized as sources of tolerant genes in breeding programmes to improve yield performance under low nitrogen supply.

Keywords: Triticum spp.; nitrogen use efficiency; genetic variation; low soil nitrogen; cluster analysis.

1. INTRODUCTION

Wheat is a self-pollinating cereal crop belonging to the family Poaceae and a main staple food consumed by billions of people in the world [1]. It is the most important cereal crop and plays a vital role in food and nutritional security in India [2]. Substantial amounts of nitrogen (N) fertilizer is required and applied yearly to produce sustainable crop yields, owing to the importance of this nutrient. Many farmers are often resource-constrained and therefore unable to apply sufficient amounts of the recommended dose citing the high cost of this input, leading to low yields. Several studies have however established that cereals, including wheat, are not able to effectively utilize the applied N and that only an estimated 40–60% of N supplied is absorbed by crops [3,4]. This low absorption of N can influence nitrogen use efficiency (NUE) and lead to high cost of production, loss of N from the soil by leaching and pollution of surface and ground water [5] raising serious environmental concerns. The development and deployment of nitrogen use efficient wheat varieties is therefore imperative to reducing excessive input of N fertilizers along with maintaining an acceptable yield thereby increasing the profit margin for farmers and ultimately lead to a safer environment. Tolerance to nitrogen stress, once genetically encoded in the seed of a variety, can be used readily by many small holder resource constrained farmers for combating nitrogen stress effects. An in-depth knowledge of available genetic variation in nitrogen response, using field and controlled environment methods to evaluate the responsiveness of genotypes to applied N is necessary to develop new varieties. Previous attempts have been made to evaluate wheat genotypes under contrasting nitrogen supply to identify nitrogen use efficient ones using various morpho-physioloical and yield related traits [6-8]. Potential donor genotypes combining high yield under nitrogen stress conditions and high NUE could be identified from such evaluations and utilized for hybridization to provide maximum genetic recombination for NUE and tolerance to low N in subsequent generations. The objectives of the study were to identify high yielding wheat genotypes under

Table 1. List of accessions used for the study

S/N	Accession name	S/N	Accession name	S/N	Accession name	S/N	Accession name	S/N	Accession name
1	HI 8731	21	DWR-2006	41	HP 1633	61	HW 1098	81	UASDW30098
2	HD 2009	22	K 8020	42	DDK 50044-1	62	HD 4709	82	AMRUT
3	NP 715	23	VL 892	43	DBW 71	63	HD 2781	83	PBW 226
4	PBW 443	24	HPW 147	44	HD 2851	64	MACS 5022	84	GW 1280
5	K 9533	25	NIDW 295	45	DBP 01-09	65	HD 2687	85	NP 771
6	UASDW30096	26	HD 2733	46	UAS 415	66	NW 2036	86	GW 190
7	DPW 621-50	27	HD 2967	47	PBW 590	67	UASDW30076	87	UASDW30034
8	HP 1761	28	WHD 896	48	UASDW30078	68	SHARBATI SO	88	HI 8653
9	DL 153-2	29	HYB 65	49	PBW 550	69	UASDW30064	89	CHOTI LERMA
10	GW 322	30	C 306	50	GW 273	70	HI 7747	90	MACS 5008
11	NP 710	31	RAJ 4248	51	DL 784-3	71	NI 5749	91	HI 385
12	K 7903	32	HI 8498	52	HI 1531	72	NP 770	92	NIAW 301
13	UASDW30027	33	HD 2888	53	HI 8713	73	HW 2004	93	HD 2932
14	HUW 234	34	KALYANSONA	54	HUW 12	74	HINDI 62	94	UAS 234
15	HP 1209	35	HI 8725	55	Hindi Local	75	WH 147	95	DDK 1025
16	MP 4010	36	MACS 2496	56	HW 1085	76	PBW 175	96	HI 8592
17	MACS 6145	37	UASDW30045	57	NP 825	77	A 9-30-1	97	DDK1029(ck1)*
18	HD 2402	38	UASDW30016	58	NP 4	78	UP 2338	98	PBW 343(ck2)*
19	HI 1500	39	HP 1744	59	HUW 318	79	MOTIA	99	PDW 291(ck3)*
20	DBW 17	40	HI 8703	60	HP 1731	80	HS 375	100	UAS-304(ck4)*

**=checks*

nitrogen stress and assess multiple morpho-physiological and yield related traits for their contribution and association to the adaptation of the accessions to nitrogen stress.

2. MATERIALS AND METHODS

The present study was carried out at the experimental field of the Main Agricultural Research Station, University of Agricultural Science, Dharwad in the transitional tract of Karnataka state, India during *rabi* 2014/2015. The experimental site is located between 15º-31º N latitude and 76"07' E longitude and has an altitude of 750 m above sea level.

The 100 *Triticum spp.* accessions (Table 1) used for the study were obtained from the Competitive Research Project of Consultative Group of International Agricultural Research at the Directorate of wheat research, Karnal, India. All the lines were selected based on their broad adaptation to different agro-climatic zones as well as diverse growing environments. The accessions were evaluated in an augmented randomized complete block design in three blocks under two nitrogen regimes. No fertilizer was applied in the first experiment -T0 (0 kg/ha N), simulating nitrogen stress; while 120 kg/ha of N was applied to the second experiment (T1) 5 cm away from the seed row. Each genotype in each block was grown in a plot of 2 rows of 2.5 meter length with a spacing of 23 cm between rows. Weeding and other cultural practices were carried out equally for the two experiments.

The composite soil sample from each experimental site was collected from 0 to 30 cm depth before the start of the field experiment and analyzed for physical and chemical characteristics (Table 2) following the standard procedure by Kjeldhal [9].

Observations were recorded on morpho-physiological characters of five randomly taken plants at different stages of crop growth. Leaf chlorophyll content (SPAD) was measured using a SPAD-502 Plus Chlorophyll Meter (Minolta Camera Co., Ltd., Japan) on fully expanded leaves from the top of the canopy at 60 days after sowing. Normalized differential vegetative index (NDVI) was assessed at 60 days after sowing using Green-Seeker hand held optical sensor unit, model 505 (NTech Industries, Inc). Number of days to 50% flowering (DF) and days to maturity (DM) thus when more than 75% of the spikes in a plot turned golden yellow were recorded. Yield and yield components such as numbers of tillers bearing ear heads (TM) were counted at harvest per meter length of each row. Total dry weight of grains harvested from all 2 rows was taken as grain yield (YLD) per plot and expressed as kilograms per hectare (kg/ha). Total biomass (BM) at harvest was assessed by cutting plants from 1 m^2 area from ground level in each experimental unit, weighed, recorded and expressed in kg/ha.

Analysis of variance for augmented design was conducted separately for each nitrogen regime using the Augmented Complete Block Design with R (ACBD-R) software Version 3.0 [10] for

Table 2. Soil characteristics at the experimental site before sowing and after harvest of wheat crop, 2014/2015

Soil samples before sowing				
Parameters	**Treatments**			
	With nitrogen		**Without nitrogen**	
	T1R1	T1R2	T2R1	T2R2
pH (1:2:5)	7.60	8.16	7.68	7.89
Electrical conductivity (dS/sm) (1:2:5)	0.16	0.15	0.13	0.18
Average nitrogen (kg/ha)	246.40	280.20	246.40	280.00
Average phosphorus (kg/ha)	15.70	12.66	15.49	10.36
Average potassium (kg/ha)	322.50	313.75	356.05	342.05
Soil samples after harvesting				
	With nitrogen		**Without nitrogen**	
	T1R1	T1R2	T2R1	T2R2
pH (1:2:5)	8.00	7.70	7.90	7.80
Electrical conductivity (dS/sm) (1:2:5)	0.25	0.25	0.29	0.22
Average nitrogen (kg/ha)	326.00	313.60	219.00	213.60
Average phosphorus (kg/ha)	19.30	15.54	21.01	12.82
Average potassium (kg/ha)	311.28	317.24	326.16	304.72

the different characters to assess the genetic variability among the accessions. The homogeneity of error variances was tested by Bartlett test [11] before combined analysis was performed and LSD at $P \leq 0.05$ was used for mean separation. Clustering of genotypes was executed using the average linkage method, with the 12 traits evaluated under nitrogen-stress. Characters with Eigenvectors greater than or equal to 1 were considered in the cluster construction and the ideal number of clusters determined by the agreement between cubic clustering criterion, pseudo F and pseudo-t ^{2} statistics between groups. Genetic distances between the cluster centroids were estimated as standardized D^2, as indicated by Mahalanobis [12] implemented in the CANDISC procedure in SAS v. 9.4 software [13]. Spearman correlation coefficients among traits were determined with the mean trait values of the accessions using SAS v. 9.4 software [13]. Some derived variables were estimated from the primary data in addition to the direct measurements as follows:

Harvest index (HI) was calculated from the ratio of grain yield to biomass in each experimental unit and expressed as percentage.

Nitrogen use efficiency (NUE) was estimated as:

$$\frac{\text{Grain yield}}{\text{Total nitrogen supply}} * 100 \tag{1}$$

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Results from optimum Nitrogen supply (T1) and Nitrogen stress (T0) treatments were compared to assess the effect of nitrogen stress on the traits studied. There was highly significant difference (P<0.01) between the mean squares of the treatments for all the plant traits measured except tillers per meter (TM) which showed significant difference (P<0.05) (Table 3). Highly significant difference (P<0.01) were observed among the accessions for days to 50% flowering (DF), days to maturity (DM), grain yield (YLD), biomass (BM) and Nitrogen use efficiency (NUE) while harvest index (HI) showed significant difference (P<0.05). The difference amongst the accessions was however not significant (P<0.05) for leaf chlorophyll content (SPAD), normalized differential vegetative index (NDVI) and TM (Table 3). Significant genotype by treatment interactions were recorded for SPAD, DF, YLD and BM while NDVI, DM, TM, HI and NUE presented no significant difference. The above results indicate that the genotypes were not fixed and irresponsive in their expression of the traits but rather adaptable. The genotypic variation observed for grain yield and NUE under both nitrogen stress and non-stress treatments for the accessions can be explored and exploited in the development of nitrogen use efficient varieties. Genetic variation of wheat genotypes under contrasting soil nitrogen levels as observed in this study has also been reported in earlier investigations by Mahjourimajd et al. [14] and Sathisha and Desai [2].

3.2 Morpho-physioloical Traits

The impact of nitrogen stress on trait expression of the accessions varied. Some traits were more sensitive to nitrogen stress effects than others as can be seen below. With respect to photosynthesis, SPAD was ranged from 31.60 (A 9-30-1) to 54.90 (HI 8498) (Table 4) with an average of 45.83 in T0 (Table 3) and from 39.40 (HPW 147) to 59.90 (PBW 550) (Table 5) with an average of 49.36 in T1 (Table 3). A mean reduction of 7.15% was recorded when T1 was compared to T0 (Table 3). Significant reduction in chlorophyll content as a result of nitrogen stress observed in this study was also previously reported by Sathisha and Desai [2]. Filella et al. [15] and Serrano et al. [16] also reported decrease in leaf chlorophyll content as a result of low soil nitrogen and high chlorophyll content with increasing nitrogen fertilization. The low chlorophyll content observed in the T0 compared to TI is because of the low soil nitrogen content in the T0 as nitrogen is the key plant mineral nutrient required for chlorophyll production [13]. NDVI has proved useful in the assessment of early biomass of different wheat genotypes [17]. NDVI for vegetative coverage was ranged from 0.22 (HP 1731) to 0.52 (HP 1761) with a mean of 0.35 in T0, and from 0.31 (HW 2004) to 0.65 (DPW 621-50) with an average of 0.50 in T1. NDVI was affected by the nitrogen stress with a percentage reduction of 30.0 from the non-stress treatment. Cabrera-Bosquet et al. [18] found strong correlations between NDVI measurements and dry aboveground biomass and N content in wheat genotypes subjected to different water and nitrogen conditions. As observed in this study, Serrano et al. [16] also found a decrease in NDVI of wheat genotypes under nitrogen stress compared to the treatments with adequate nitrogen supply.

The accessions can therefore be said to have reduced aboveground dry matter production as an adaptive feature to the nitrogen stress imposed.

For crop phenology, DF ranged from 47.5 (NP 710) to 69.0 days (HD 4709) with a mean of 55.3 days in T0 whereas it varied from 50.5 (HI 8725) to 76.5 days (HD 4709) with an average of 58.7 in the T1. The nitrogen stress imposed resulted in a 5.64% reduction in DF. DM varied from 89.5 (HI 8725, HD 2687) to 120.0 (NP 770) with a mean of 103.72 days in T0 and from 91.50 (DL 784-3, HI 8725) to 120.00 (HW 2004) with an average of 105.97 days in T1. A mean reduction of 2.12% was recorded in T0 compared to T1 for DM. The accessions were generally early flowering and early maturing under the T0 than the T1 as also reported by Sathisha and Desai [2].

3.3 Yield Components

Yield component parameters are usually good indicators of overall nitrogen stress response. TM was ranged from 18.00 (HD 2851) to 62.00 (PBW 443) (Table 4) with a mean of 40.25 in T0 (Table 3) and from 20.00 (Hindi Local) to 72.50 (HI 8653) (Table 5) with a mean of 42.49 in T1 (Table 3). A 5.27% reduction in TM was observed in comparing T0 to T1. The increase in number of fertile tillers with increasing nitrogen supply can be ascribed to the decrease in mortality of tillers and the development of more tillers from the main stem [19,7]. As observed in this study, Kaur et al. [7] reported significant reductions in number of tillers of wheat under low nitrogen supply. BM in T0 ranged from 1981.25 kg/ha (HD 2851) to 9885.00 kg/ha (K 8020) with a mean of 5307.54 kg/ha whiles the range was from 3975.00 kg/ha (Hindi Local) to 13281.25 kg/ha (HD2402) with an average of 8723.56 kg/ha in T1. BM was highly affected by nitrogen stress, with 39.16% mean reduction from the non-stress treatment. This is in agreement with the findings of Kaur et al. [7] who reported significant reductions in biomass of wheat genotypes under low nitrogen supply. HI varied from 9.67 (MOTIA) to 30.79 (HD 2932) with a mean of 21.19% in T0, and from 10.80 (HI 385) to 27.93 (HUW 12) with a mean of 18.98 % in T1. Nitrogen stress caused 11.64% increase in HI compared to non-stressed treatment. The significantly high harvest index recorded in the T0 than the T1 indicates that the accessions increased photosynthate partitioning and or remobilization of assimilates from stems and leaves reserves to the developing grain under the nitrogen stress condition. DDK 1025 recorded the highest NUE in both treatments. NUE ranged from 7.13% (HI 7747) to 46.32% with an average of 26.25% in T0, while it varied from 13.17% (AMRUT) to 49.11% with a mean of 32.22% in T1. NUE was highly affected by the nitrogen stress imposed, with 18.53% mean reduction from the non-stress treatment. Significant reduction in NUE under the nitrogen stress treatment compared to optimum nitrogen supply in this study corroborates the observation by Ayadi et al. [20] who reported increasing NUE with increasing nitrogen fertilization levels. In another study, Todeschini et al. [6] reported a decline in NUE as the nitrogen supply level increased. This could be as a result of inconsistent increase between grain yield and nitrogen supply. Plants are therefore incapable of assimilating enough nitrogen under these conditions leading to a rise in nitrogen loss [21]. The NUE increase under maximum nitrogen supply in the present study suggests that the 120 kgN/ha is unlikely to be sufficient for the accessions evaluated to reach their optimum genetic potential for grain yield.

3.4 Grain Yield

Exposure to nitrogen stress caused a mean yield penalty of 31.54% in T0 relative to the T1 (Table 3). DDK 1025 (95) was the highest yielding accession in both T0 (2437.5 kg/ha) (Table 4) and T1 (3075.0 kg/ha) (Table 5), while HI 7747 (375.0 kg/ha) and AMRUT (825.0 kg/ha) were the lowest yielding in T0 and T1 respectively. Significant reduction in grain yield under low nitrogen supply recorded in this study has also been reported previously by Kaur et al. [7] and Mahjourimajd et al. [8]. On the basis of grain yield performance under nitrogen stress and optimal nitrogen conditions, the 100 wheat accessions can be classified into four different categories. In the first category are accessions with significantly superior ($P<0.05$) yield performance relative to the highest yielding check UAS-304 (ck4) in both treatments, namely 95 (DDK 1025), 62 (HD 4709) and 31 (RAJ 4248), 38 (UASDW 30016) (Fig 1). Three of these accessions DDK 1025, HD 4709 and UASDW 30016 had the highest mean productivities of 2756.25, 2553.13, and 2468.75 kg/ha respectively versus 2210.42 kg/ha of UAS-304, with yield advantages of 24.70, 15.50 and 11.69% (results not presented).

In the second yield category were genotypes with the lowest degree of adaptation and yield in the T0 and T1. This is the largest group with 74 accessions, notable among them were 55 (Hindi Local), 71 (NI 5749), 72 (NP 770) and 82

(AMRUT). Three of the four checks ck3 (PDW 291), ck2 (PBW 343) and ck1 (DDK 1029) were also included in this category.

The third category contained accessions that showed high yield (higher than the highest yielding check) in T0 but low yields (lower than the highest yielding check) in T1, namely; 73 (HW 2004), 33 (HD 2888), 32 (HI 8498), 96 (HI 8592), 59 (HUW 318), 22 (K 8020), 5 (K 9533), 64 (MACS 5022), 75 (WH 147) and 91 (HI 385). There was however no significant difference ($p<0.05$) between these accession in group 3 and the check (UAS-304) for grain yield in the nitrogen stressed treatment. Yield advantages of category 3 accessions over the check ranged from 0.9% (HI 385) to 24.8% (HW 2004). Category four included accessions that yielded well (higher than the check) in T1 but showed correspondingly lower yields in T0, namely 42 (DDK 50044-1), 9 (DL 153-2), 51 (DL 784-3), 2 (HD 2009), 63 (HD 2781), 88 (HI 8653), 41 (HP 1633), 57 (NP 825), 49 (PBW 550), 6 (UASDW 30096), and 28 (WHD 896). Only one accession (HD 2009) in group four had significantly higher yield ($p<0.05$) in T1 (3062.5 kg/ha) compared to the check (UAS-304) (2587.5 kg/ha), with 18.36% (475.0 kg/ha) yield advantage over UAS-304.

3.5 Clustering of Genotypes

The average linkage grouping method using the T0 variables identified by PCA produced four clusters of the 100 accessions (Fig 2). Cluster I was the largest, containing 45 accessions (45.0%) followed by cluster II, which contained 30 genotypes (30.0%). Clusters III and IV were small groups containing 7 (7.0%) and 18 accession (18.0%), respectively. Accessions with high degrees of yield adaptation under T0 were grouped in cluster IV and those with low adaptation under T0 were grouped in cluster II. The highest inter-cluster distance appeared between clusters II and III (D^2 = 124.27) followed by clusters I and III (D^2 = 68.01) and clusters II and IV (D^2 = 54.55) (Table 6). The lowest inter-cluster distance was found between clusters I and II (12.04) followed by that between clusters III and IV (17.63) and cluster I and IV (D^2=18.28). Selection of distant parental lines is a vital prerequisite for successful hybridization in crop improvement programs. The crossing of accessions from the clusters with maximum genetic distance in this study are expected yield maximum genetic recombination and diversity in successive generations.

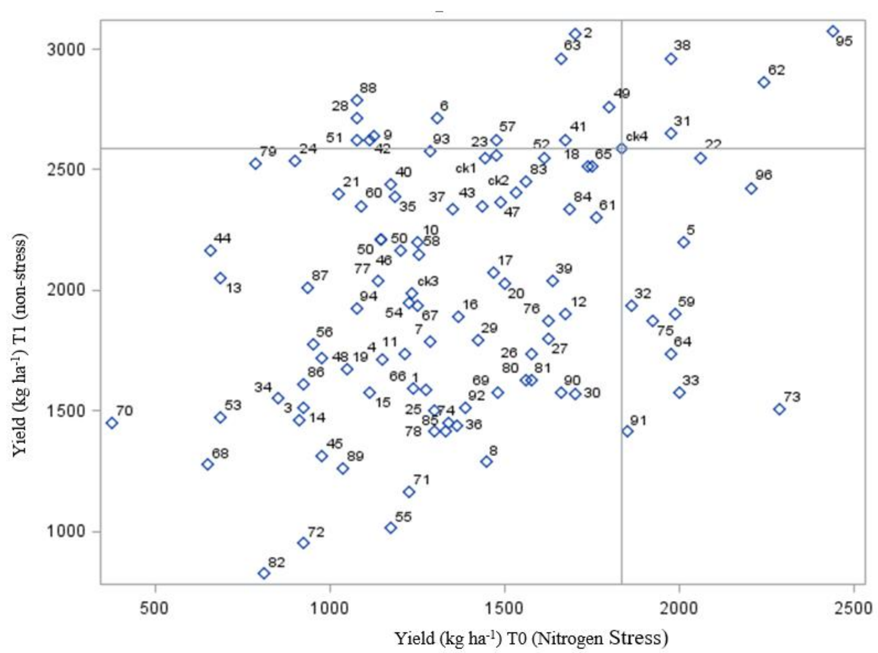

Fig. 1. Scattergram showing the identification and categorization of 100 wheat accessions on the basis of their grain yield in a comparison of nitrogen stress (T0) and non-stress (T1) growing conditions. Horizontal and vertical lines indicate mean values in T0 and T1 for the highest yielding check, ck4 (UAS-304). The numbers on the graph corresponds to the serial numbers of the accessions in tables 1, 4 and 5.

Table 3. Significance of treatment, accession, and accession-by-treatment interaction for traits evaluated in 100 wheat accessions under nitrogen stress (T0) and optimal nitrogen supply (T1) at Dharwad, 2014/2015

Traits	TRT(T) (df=1)	GENO (G) (df=99)	G x T (df=99)	CV (%)	Means		Maximum		Minimum		Percent reduced (%)
					T0	T1	T0	T1	T0	T1	
SPAD	**	ns	*	26.98	45.83	49.36	54.90	59.90	31.60	39.40	7.15
NDVI	**	ns	ns	27.79	0.35	0.50	0.52	0.65	0.22	0.31	30.00
DF	**	**	*	5.71	55.34	58.65	69.00	76.5	47.50	50.50	5.64
DM	**	**	ns	2.30	103.72	105.97	120.00	120.00	89.50	91.50	2.12
TM	*	ns	ns	22.81	40.25	42.49	62.00	72.50	18.00	20.00	5.27
YLD	**	**	*	33.85	1381.28	2017.56	2437.50	3075.00	375.00	825.00	31.54
BM	**	**	*	40.32	5307.54	8723.56	9885.00	13281.25	1981.25	3975.00	39.16
HI	**	*	ns	19.22	21.19	18.98	30.79	27.93	9.67	10.80	-11.64
NUE	**	**	ns	25.82	26.25	32.22	46.32	49.11	7.13	13.17	18.53

*TRT, treatment; GENO, accessions; SPAD, leaf chlorophyll content at 60 days after planting; NDVI, normalized differential vegetative index at 60 days after planting; DF, days to 50% flowering; DM, days to maturity; TM, tillers per meter; YLD, grain yield (kg/ha); BM, biomass (kg/ha); HI, harvest index; NUE, nitrogen use efficiency; ** Significant at P ≤ 0.01.; Significant at P ≤ 0.05.*

Table 4. Mean performance of some selected (highest and lowest plus checks) wheat accessions under the nitrogen stress treatment (T0), 2014/2015

S/N	Accession	SPAD	NDVI	DF	DM	PLHT	TM	GS	HI	SL	YLD	BM	NUE
4	PBW 443	46.7	0.4	51.0	91.5	59.0	62.0	54.0	22.0	7.9	1150.0	4087.5	21.9
7	DPW621-50	47.3	0.4	51.5	99.0	83.0	38.0	36.8	15.2	5.7	1287.5	7206.3	24.5
8	HP 1761	42.3	0.5	49.6	110.3	65.8	43.3	37.7	21.4	4.7	1446.9	5326.6	27.5
11	NP 710	41.9	0.3	47.5	106.5	53.5	39.0	41.0	18.0	6.6	1212.5	5531.3	23.0
13	UASDW 30027	45.3	0.3	58.0	113.5	65.0	28.5	34.3	17.5	10.4	687.5	3250.0	13.1
15	HP 1209	43.6	0.3	54.0	111.5	68.5	45.0	41.5	12.6	8.0	1112.5	7737.5	21.1
16	MP 4010	48.6	0.3	55.0	101.5	49.0	35.0	40.5	25.0	4.4	1368.8	4106.3	26.0
18	HD 2402	51.4	0.4	59.0	106.0	70.5	44.0	48.0	23.2	7.1	1737.5	5762.5	33.0
22	K 8020	54.3	0.3	55.0	111.5	51.5	53.5	42.0	17.4	5.4	2062.5	9825.0	39.2
24	HPW 147	45.8	0.3	57.5	108.0	57.5	46.0	35.0	20.1	7.3	900.0	3581.3	17.1
28	WHD 896	44.8	0.3	56.5	103.0	45.0	30.0	42.0	23.3	4.7	1075.0	3537.5	20.4
32	HI 8498	54.9	0.3	51.0	100.5	61.0	44.5	45.8	23.9	8.6	1862.5	5931.3	35.4
35	HI 8725	49.7	0.3	52.0	89.5	59.5	46.5	39.5	20.5	7.0	1187.5	4606.3	22.6
38	UASDW 30016	44.4	0.4	57.0	110.0	59.0	29.0	31.5	17.6	9.3	1975.0	9231.3	37.5

S/N	Accession	SPAD	NDVI	DF	DM	PLHT	TM	GS	HI	SL	YLD	BM	NUE
41	HP 1633	50.2	0.4	55.0	110.0	59.0	50.5	32.5	24.3	4.1	1675.0	5225.0	31.8
44	HD 2851	45.4	0.3	52.0	94.5	50.0	18.0	35.0	24.9	9.2	656.3	1981.3	12.5
49	PBW 550	49.6	0.3	51.5	93.5	71.0	49.5	47.5	24.0	9.2	1800.0	5706.3	34.2
51	DL 784-3	49.6	0.3	50.5	90.0	58.5	37.5	35.8	25.5	7.5	1075.0	3143.8	20.4
54	HUW 12	47.2	0.3	53.5	98.0	61.5	39.0	39.0	24.8	5.1	1225.0	3706.3	23.3
55	Hindi Local	44.1	0.3	54.0	102.0	63.0	35.0	33.8	22.8	4.2	1175.0	3981.3	22.3
62	HD 4709	50.8	0.4	69.0	110.0	60.5	45.0	35.3	23.3	7.5	2243.8	7400.0	42.6
65	HD 2687	34.3	0.3	54.0	89.5	52.5	39.0	40.3	22.5	6.1	1750.0	6043.8	33.3
67	UASDW 30076	47.3	0.3	59.5	97.0	62.0	47.0	33.5	18.7	7.0	1250.0	5437.5	23.8
70	HI 7747	43.5	0.2	56.5	107.0	50.5	32.5	33.0	15.8	4.2	375.0	1993.8	7.1
72	NP 770	41.5	0.4	55.0	120.0	67.5	40.0	41.5	18.7	5.8	925.0	4018.8	17.6
73	HW 2004	44.8	0.5	55.0	116.0	63.0	57.5	34.8	28.6	7.8	2287.5	5706.3	43.5
77	A 9-30-1	31.6	0.4	55.0	112.5	64.0	40.5	39.3	20.9	4.5	1137.5	4312.5	21.6
79	MOTIA	41.5	0.3	55.5	99.5	56.5	34.0	37.0	9.7	7.2	787.5	7356.3	15.0
80	HS 375	50.6	0.3	53.0	104.0	65.5	40.5	47.5	18.3	6.3	1562.5	6981.3	29.7
82	AMRUT	47.0	0.4	57.0	101.5	59.5	44.0	20.8	14.9	4.4	812.5	4631.3	15.4
85	NP 771	41.1	0.4	52.0	107.0	83.0	33.0	36.3	25.3	7.8	1331.3	3937.5	25.3
88	HI 8653	53.1	0.3	58.5	111.0	60.5	35.0	46.3	15.8	5.0	1075.0	5750.0	20.4
91	HI 385	47.3	0.4	60.0	112.0	54.5	57.5	28.0	27.8	7.1	1850.0	4800.0	35.2
93	HD 2932	45.6	0.3	51.0	96.5	65.5	37.5	34.0	30.8	7.2	1287.5	2893.8	24.5
95	DDK 1025	51.1	0.4	56.5	103.0	63.5	39.0	46.0	26.3	7.5	2437.5	6831.3	46.3
97	DDK1029(ck1)	45.0	0.4	68.9	110.5	68.8	37.3	40.9	20.6	8.3	1442.7	5547.9	27.4
98	PBW343(ck2)	49.3	0.4	57.3	104.8	60.4	43.5	44.0	22.7	7.5	1534.4	5193.8	29.2
99	PDW291(ck3)	47.3	0.3	58.5	106.3	58.4	36.2	36.6	21.7	7.5	1233.3	4560.4	23.4
100	UAS-304(ck4)	43.0	0.5	67.7	107.3	62.2	45.0	40.7	28.6	6.3	1833.3	4575.0	34.8
LSD(0.05)		0.1	0.1	5.8	4.2	13.8	28.0	3.5	8.6	3.4	713.9	2166.2	13.6
CV (%)		6.4	11.0	3.0	1.2	6.5	20.1	2.6	11.7	14.4	14.9	11.8	14.9

SPAD, leaf chlorophyll content at 60 days after planting; NDVI, normalized differential vegetative index at 60 days after planting; DF, days to 50% flowering; DM, days to maturity; TM, tillers per meter; YLD, grain yield (kg/ha); BM, biomass (kg/ha); HI, harvest index; NUE, nitrogen use efficiency

Table 5. Mean performance of some selected (highest and lowest plus checks) wheat accessions under the optimal nitrogen supply treatment, 2014/2015

S/N	Accessions	SPAD	NDVI	DF	DM	PLHT	TM	GS	HI	SL	YLD	BM	NUE
4	PBW 443	51.9	0.6	53.0	93.5	56.5	40.0	53.8	17.9	7.6	1712.5	7843.8	27.3
7	DPW621-50	46.4	0.6	51.5	101.0	77.5	59.5	38.5	22.7	6.2	1787.5	6093.8	28.5
8	HP 1761	51.2	0.4	54.0	117.0	61.5	32.5	41.0	21.4	7.0	1287.5	4731.3	20.6
11	NP 710	53.6	0.5	55.0	106.5	61.5	25.0	43.5	17.8	8.4	1737.5	8025.0	27.7
13	UASDW 30027	47.3	0.6	63.0	118.0	78.0	37.5	35.0	17.2	8.1	2050.0	9843.8	32.7
15	HP 1209	53.2	0.6	58.5	117.0	82.0	42.0	48.3	15.0	9.6	1575.0	8950.0	25.2
16	MP 4010	48.1	0.5	59.5	105.0	48.0	27.5	44.8	18.9	9.2	1887.5	8100.0	30.1
18	HD 2402	49.2	0.6	67.0	116.0	76.5	59.0	49.8	15.9	9.4	2512.5	13281.3	40.1
22	K 8020	46.8	0.4	58.5	113.5	55.0	39.5	48.5	19.8	8.9	2550.0	10343.8	40.7
24	HPW 147	40.7	0.5	56.0	108.0	66.5	41.0	40.0	23.8	7.2	2537.5	8125.0	40.5
28	WHD 896	50.1	0.4	55.5	114.5	58.0	38.5	39.5	20.9	7.5	2712.5	10250.0	43.3
32	HI 8498	44.4	0.5	60.5	103.5	68.0	35.0	46.5	16.1	8.2	1937.5	10093.8	30.9
35	HI 8725	51.0	0.6	50.5	91.5	68.5	37.5	41.0	16.9	9.3	2387.5	11731.3	38.1
38	UASDW 30016	54.0	0.5	65.0	113.5	54.5	65.0	31.8	18.3	10.6	2962.5	13250.0	47.3
41	HP 1633	47.5	0.5	57.5	114.0	61.5	42.5	40.5	19.6	5.3	2625.0	10793.8	41.9
44	HD 2851	50.5	0.5	51.0	99.0	66.5	37.0	40.8	21.3	9.3	2162.5	7981.3	34.5
49	PBW 550	50.1	0.5	52.0	99.0	66.0	45.5	47.8	20.3	9.7	2762.5	10812.5	44.1
51	DL 784-3	47.9	0.5	56.5	91.5	66.0	44.0	38.3	22.8	8.2	2625.0	8900.0	41.9
54	HUW 12	51.0	0.5	61.5	101.5	56.5	40.0	40.3	27.9	8.4	1950.0	5031.3	31.1
55	Hindi Local	45.7	0.5	62.0	102.5	79.5	20.0	39.3	20.3	8.5	1012.5	3975.0	16.2
62	HD 4709	55.1	0.6	76.5	108.5	58.5	45.0	34.5	19.5	9.0	2862.5	11843.8	45.7
65	HD 2687	51.8	0.4	62.0	93.0	59.5	30.0	41.5	22.0	6.0	2512.5	8906.3	40.1
67	UASDW 30076	49.7	0.4	65.0	96.0	59.5	28.5	42.0	20.5	7.1	1937.5	7531.3	30.9
70	HI 7747	49.5	0.5	57.5	109.5	52.0	42.0	32.3	15.3	4.3	1450.0	8025.0	23.2
72	NP 770	56.2	0.5	67.0	117.5	62.0	50.0	41.8	14.1	5.9	950.0	5781.3	15.2
73	HW 2004	55.7	0.3	65.5	120.0	62.0	38.0	35.3	15.6	7.8	1506.3	8125.0	24.1
77	A 9-30-1	56.5	0.5	57.5	113.0	57.5	30.0	40.8	17.5	4.5	2037.5	9575.0	32.5
79	MOTIA	47.8	0.6	55.5	100.0	70.0	40.0	39.5	19.0	7.2	2525.0	10750.0	40.3
80	HS 375	48.0	0.5	63.0	104.0	69.0	46.5	47.8	16.6	6.3	1625.0	8175.0	26.0
82	AMRUT	45.3	0.5	58.5	102.0	69.5	37.5	27.0	15.7	4.5	825.0	4431.3	13.2
85	NP 771	42.9	0.5	53.5	109.0	77.0	42.5	37.3	13.0	7.7	1412.5	9468.8	22.6

S/N	Accessions	SPAD	NDVI	DF	DM	PLHT	TM	GS	HI	SL	YLD	BM	NUE
88	HI 8653	47.7	0.6	64.0	116.0	69.0	72.5	49.5	19.9	5.1	2787.5	11200.0	44.5
91	HI 385	51.5	0.6	68.5	113.5	53.0	39.0	32.0	10.8	7.3	1412.5	11662.5	22.6
93	HD 2932	59.9	0.5	55.5	99.0	61.0	45.0	36.0	22.0	7.2	2575.0	9125.0	41.1
95	DDK 1025	44.7	0.5	55.5	105.5	64.0	36.5	48.3	21.8	7.6	3075.0	11031.3	49.1
97	DDK1029(ck1)	47.2	0.6	69.7	113.2	71.4	53.3	41.9	17.1	8.8	2545.8	12358.3	40.7
98	PBW 343(ck2)	53.6	0.5	62.7	107.0	64.2	39.0	49.5	21.4	8.2	2404.2	8764.6	38.4
99	PDW 291(ck3)	54.8	0.5	59.8	106.5	60.7	50.2	39.1	18.8	7.9	1987.5	8679.2	31.7
100	UAS-304(ck4)	52.5	0.6	69.0	106.0	72.8	49.3	45.7	24.8	7.8	2587.5	8104.2	41.3
LSD(0.05)		12.6	0.3	5.2	8.1	25.3	23.5	12.8	13.3	3.3	1281.1	3181.0	20.5
CV (%)		8.0	17.3	2.6	2.2	12.7	18.9	9.0	20.3	12.4	18.3	10.5	21.8

SPAD, leaf chlorophyll content at 60 days after planting; NDVI, normalized differential vegetative index at 60 days after planting; DF, days to 50% flowering; DM, days to maturity; TM, tillers per meter; YLD, grain yield (kg/ha); BM, biomass (kg/ha); HI, harvest index; NUE, nitrogen use efficiency

3.6 Correlation among Traits

As presented in Table 7, the correlation between YLD and NUE was highly significant and positive (r= 0.95) in both T0 and T1, suggesting that these two traits can be improved simultaneously. Highly significant negative correlation was observed between BM and HI in T0 (r= -0.51) and T1 (r= -0.37). NDVI was highly significantly correlated with PLHT and TM in T0. The correlation between NDVI and DF, and DM was significant and positive in T0. YLD and NUE individually had highly significant positive correlations with NDVI (r= 0.49), TM (r= 0.39), HI (r= 0.38), and BM (r= 0.57) in T0. YLD and NUE were individually highly significant and positively correlated with NDVI (r= 0.25), GS (r= 0.22), TM (r= 0.29), HI (r= 0.47) and BM (r= 0.60) in T1. Highly significant negative correlations were observed between PLHT and SPAD, and between DM and HI in T1.

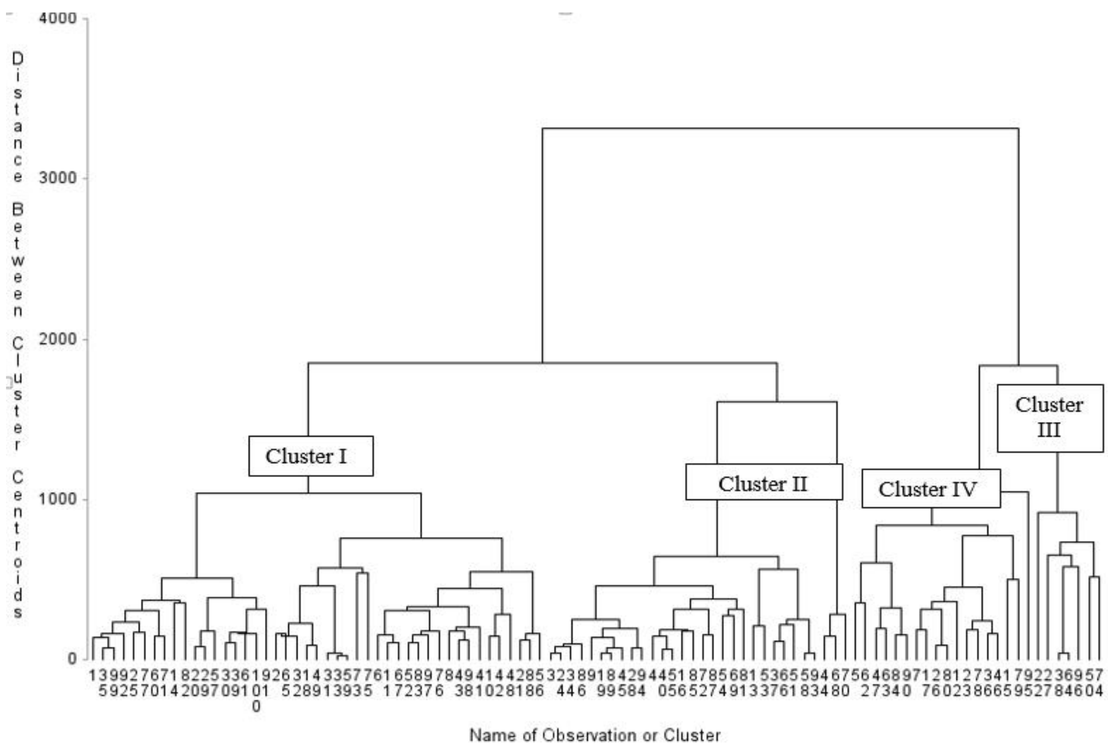

Fig. 2. Average linkage-based dendrogram displaying hierarchical grouping patterns of 100 wheat accessions in 4 clusters on the basis of 12 quantitative traits under Nitrogen-stress condition. The numbers in the figure corresponds to the serial numbers of the accessions in tables 1 and 4

Table 6. Mahalanobis squared distance to cluster of wheat accessions

From cluster	Squared distance to cluster			
	I	II	III	IV
I		12.04307**	68.01229**	18.27672**
II			124.2699**	54.55583**
III				17.62821**
IV				

*** Significant at 1% probability level*

Table 7. Spearman Correlation Coefficients between grain yield and other traits of 100 wheat accessions evaluated under nitrogen stress (upper diagonal) and optimal nitrogen supply (lower diagonal) conditions at Dharwad, 2014/2015

TRAITS	SPAD	NDVI	DF	DM	PLHT	TM	GS	HI	SL	YLD	BM	NUE
SPAD		0.13	0.17	0.03	0.01	0.17	0.22*	0.10	0.06	0.15	0.09	0.15
NDVI	0.01		0.23*	0.22*	0.49**	0.42**	-0.08	0.15	-0.09	0.49**	0.27**	0.49**
DF	0.07	0.20*		0.41**	0.06	0.19*	-0.12	0.11	0.02	0.08	-0.04	0.08
DM	0.06	0.18	0.35**		0.18	0.01	0.01	-0.02	-0.03	0.01	-0.02	0.01
PLHT	-0.25**	0.20*	-0.02	0.02		0.16	0.01	0.01	0.11	0.23*	0.20*	0.23*
TM	-0.06	0.17	-0.01	0.10	0.14		-0.04	0.14	-0.01	0.39**	0.21*	0.39**
GS	0.14	-0.03	0.02	-0.05	0.12	-0.04		0.02	0.05	0.06	0.08	0.06
HI	0.20*	-0.10	-0.07	-0.26**	-0.16	0.04	0.23*		0.10	0.38**	-0.51**	0.38**
SL	0.03	0.12	0.04	0.01	0.12	-0.09	0.16	0.06		0.05	0.01	0.05
YLD	0.12	0.25**	0.12	-0.1	0.05	0.29**	0.22**	0.47**	0.18		0.57**	0.95**
BM	-0.07	0.34**	0.14	0.15	0.17	0.24*	0.01	-0.37**	0.18	0.60**		0.57**
NUE	0.12	0.25**	0.12	-0.10	0.05	0.29**	0.22*	0.47**	0.18	0.95**	0.60**	

*SPAD, leaf chlorophyll content at 60 days after planting; NDVI, normalized differential vegetative index at 60 days after planting; DF, days to 50% flowering; DM, days to maturity; PLHT, plant height (cm); TM, tillers per meter; GS, grains per spike; YLD, grain yield (kg/ha); BM, biomass (kg/ha); HI, harvest index; SL, spike length (cm); NUE, nitrogen use efficiency ** Significant at P ≤ 0.01; *Significant at P ≤ 0.05*

4. CONCLUSION

The genotypic variation observed among the accessions for grain yield, nitrogen use efficiency and other traits under both nitrogen stress and non-stress conditions can be readily explored in the development of nitrogen use efficient wheat varieties appropriate for low input wheat production systems. Several accessions showed adaptation to nitrogen stress by reducing their biomass production while increasing harvest index thereby minimizing the impact of nitrogen stress. Accessions DDK 1025, HW 2004, HD 4709, HI 8592, and K 8020 with superior yields under the nitrogen stress condition can be utilized as sources of tolerant genes for this stress. Hybridization of accessions selected from clusters II and III will produce maximum genetic recombination and variation for tolerance to nitrogen stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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