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Research Article

Nitrogen Deficiency Tolerance and Responsiveness of Durum Wheat Genotypes in Ethiopia

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Development of low-nitrogen (N) tolerant and N-responsive durum wheat genotypes is required since nitrogen efficiency has emerged as a highly desirable trait from economic and environmental perspectives. Two hundred durum wheat genotypes were evaluated at three locations under optimum (ON) and low (LN) nitrogen conditions to screen genotypes for low-nitrogen tolerance and responsiveness to an optimum N supply. The results showed significant variations among the durum wheat genotypes for low-N tolerance and responsiveness. The average reduction in grain yield under the LN condition was 48.03% across genotypes. Only 17% of the genotypes tested performed well (grain yield reduction <40%) under LN conditions. Based on the absolute grain yield, biomass yield, and normalized difference vegetative index value, on average, 32, 14, 17, and 37% of the tested genotypes were classified as efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive, respectively. Considering the absolute and relative grain yield, biomass yield, normalized difference vegetative index values, and stress tolerance indices as selection criteria, 17 genotypes were chosen for subsequent breeding. Among the screening indices, geometric mean productivity, stress tolerance index, yield index, and stress susceptibility index exhibited positive and significant correlations with grain yield under both N conditions; hence, either of these traits can be used to select low-N-tolerant genotypes. The common genotypes identified as LN-tolerant and responsive to N application in this study could be used as parental donors for developing N-efficient and responsive durum wheat varieties.

1. Introduction

Durum wheat (*Triticum turgidum* var. durum Desf) is an important food crop in the world and an endemic species of Sub-Saharan Africa. It has been grown for many years by smallholder farmers in the Ethiopian Highlands to ensure production for their own consumption [1] and income generation as input to food processing industries. Prior to the introduction of improved bread wheat varieties, durum wheat was the dominant (60–70%) wheat crop produced in Ethiopia. However, due to the introduction of bread wheat from international breeding programs into the country and its widespread adaptation with satisfactory yield potential, farmers have given less attention to durum wheat cultivation, despite the crop's importance in various aspects. Currently, it accounts for 20% of total wheat production and 30% of both cultivated land and smallholder wheat-producing households across the entire area covered [2]. According to [3], bread and durum wheat were produced in Ethiopia by approximately 4.94 million households during the "meher" and "belg" (rain and dry) seasons on an estimated 2.13 million ha of land, with an annual production of 6.23 million tons and a mean national yield of 3.05 t·ha^{-1}. Regardless of the long history of durum wheat cultivation and its importance in Ethiopian agriculture, its average productivity remains far below the world average (3.5 t·ha^{-1}) [4]. This is partly due to the lack of varieties that are resistant to biotic and abiotic stresses [5, 6].

Among the abiotic stresses, nitrogen deficiency is one of the most important crop production constraints in Ethiopia, where soils are generally deficient in nutrients [7–9]. Nitrogen is an important nutrient for plant growth, development, and productivity, as well as for efficient and profitable crop production. Thus, limited nitrogen supply to the crop substantially reduces plant physiological activities, morphological growth, and hence grain as well as biomass yields [10, 11]. Currently, nitrogen efficiency in crop production has emerged as a highly desirable trait from economic and environmental perspectives [12]. Furthermore, with increased awareness of environmental protection and sustainable agricultural production, it is more important than ever to include selection for low-nitrogen-tolerant wheat cultivars for high yield and quality in the breeding process [13].

Genotypes that perform well under optimal or high nitrogen conditions may not perform well under low N conditions. As a result, selection in both low and high N environments is critical for identifying high nitrogen use-efficient and/or tolerant wheat genotypes with the potential to perform well in both N environments [14]. Such information is very important, particularly in the case of resource-poor farmers, since it enables them to target appropriate cultivars that can result in optimum yields under low N supplies. It also avoids significant yield reduction from using inefficient cultivars and economic loss and environmental degradation due to the application of high amounts of nitrogen in the case of nonresponsive cultivars.

Since such information is scanty in Ethiopia in particular and in Sub-Saharan Africa (SSA) countries in general, resource-poor farmers have still been applying the same quantity of nitrogen fertilizer regardless of the existence of substantial diversities in nitrogen efficiency among the available cultivars, as reported by several studies [10, 15–18]. Thus, in the context of continuous nutrient mining without equivalent replenishment to the soil in Ethiopia and SSA in general, where suboptimal fertilizers application is a very common practice, identification and availing of N-efficient and/or N-responsive cultivars among resource-poor farmers, as well as increasing yield, are invaluable for sustaining wheat production and productivity. The availability of information for such cultivars that can produce high yields under optimum N conditions while also performing better under low N conditions is of great significance to the small-landholding farmers in Ethiopia because it allows them to simultaneously address the needs of both low- and high-input production systems.

The most widely used concept of nitrogen efficiency in plant breeding is to exploit existing genetic variations under nitrogen stress conditions and select superior genotypes based on their yield, yield components, physiological traits, and stress screening indices [19, 20]. The conventional plant breeding technique of selecting for such traits has significantly increased wheat productivity under both optimum and low nitrogen conditions. Different studies [13, 21] showed the presence of genetic variability in nitrogen use efficiency in terms of N uptake and N utilization, which has been used to develop low-N-tolerant wheat varieties.

Despite the availability of huge durum genetic resources, little research has been conducted in Ethiopia on the variation of durum wheat genotypes for low-nitrogen tolerance and responsiveness to nitrogen application. Consequently, there is a need to screen the available durum wheat genotypes for N-efficiency as well as for their responsiveness to N application and provide information useful for the breeding program. In line with this, we hypothesized that the two hundred durum wheat genotypes covered in this study show substantial genetic diversity for N-efficiency and N-responsiveness. Therefore, the main objectives of this study were (i) to evaluate and select durum wheat genotypes for low-nitrogen tolerance and responsiveness to N application and (ii) to determine the most effective stress tolerance indices useful for the selection of low-N-tolerant durum wheat genotypes.

2. Materials and Methods

2.1. Description of the Study Areas. The experiments were carried out at Debre Zeit, Chefe Donsa, and Minjar/Memhir Hager/research sites in Ade'a, Gimbichu, and Minjar Shenkora districts, respectively, in the central highlands of Ethiopia during the main cropping season of 2020. The study sites are located at 8°44′–8°57′ N, 38°58′–39° 16′ E, and 1900–2435 meters above sea level (Table 1). The mean annual rainfall of the study areas ranged from 865–1020 mm, while the mean maximum and minimum temperatures varied from 20–28.8°C and 8–12.3°C, respectively (Table 1). The main rainy season lasts from June to September at all sites. The major soil order in the study areas was black vertisol with high wet aggregate stability and water logging capacity [22].

2.2. Soil Sampling and Analysis. Before sowing, three composite soil samples were collected from each of the three sites, and soil nitrogen analysis was performed according to the standard procedure (Table 2). The collected soil samples were air-dried, crushed using a mortar and pestle, and sieved to pass through a 2 mm mesh. The soil samples were analyzed for textural class, soil pH, total nitrogen (N), available phosphorous (av. P), organic matter (OM) contents, and cation exchange capacity (CEC) at the soil laboratory of the Debre Zeit Agricultural Research Center (DZARC). The pH of the composite soil samples was measured in 1 : 2.5 soil water suspensions. The total N, available P, and OM contents of the soil were determined by the semi-micro-Kjeldahl [23, 24] and wet digestion [25] methods, respectively. The neutral ammonium acetate (CH_3COONH_4) saturation method [26] was employed to determine the CEC of the soils. The results of the physicochemical properties of the soils are shown in Table 2. The total N contents of the studied

Table 1: Description of the study areas.

S/ No	Sites	Districts	Location				Weather	
			Latitude (N)	Longitude (E)	Alt. (masl)	RF (mm)	Max. temp. (°C)	Min. temp. (°C)
1	Debre Zeit	Ade'a	8° 44′	38° 58′	1900	984	26.8	11.4
2	Chefe Donsa	Gimbichu	8° 57′	39° 16′	2435	1020	20.0	8.0
3	Minjar/memhir Hager	Minjar Shenkora	8° 46′	39° 16′	2257	865	28.8	12.3

Table 2: Presowing soil physicochemical properties of experimental fields.

Locations	pH (1 : 2.5 H_2O)	Total nitrogen (%)	Available phosphorous (mg kg^{-1})	Organic matter (%)	Soil texture	Cation exchange capacity (meq 100 g^{-1})
Debre Zeit	6.78	0.1	15.19	1.51	Clay	51.6
Chefe Donsa	6.84	0.08	5.85	1.68	Clay	40.4
Minjar	6.79	0.12	10.09	2.07	Clay	45.8

soils ranged from 0.08–0.12%, thereby belonging to the very low to low category [27]. Consequently, the initial status of the soils was found suitable for establishing the experiments.

2.3. Treatments and Experimental Design. Two hundred durum wheat genotypes obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), the International Maize and Wheat Improvement Centre (CIMMYT), the Ethiopian Biodiversity Institute (EBI), and the durum wheat breeding program of the Debre Zeit Agricultural Research Center (DZARC) were evaluated under low and optimum nitrogen (N) conditions (Table 3 and Table S1). The experiments were conducted on a field that had previously been cropped with tef (*Eragrostis tef* (*Zucc.*) Trotter).

The N treatments consisted of two levels: unfertilized (low N), and 92 kg·N·ha^{-1} (optimum N). The experiments were laid out in an alpha-lattice design with two replications. To accommodate both the N fertilized and unfertilized plots, each block was divided into two adjacent 1.5 meters apart from sub-blocks. The entire test genotypes were sown separately in the adjacent sub-blocks with and without N. Hand sorting was used to select clean seeds of each genotype to a reasonably uniform size before sowing. Planting was carried out on July 24, 2020, July 25, 2020, and August 6, 2020, at the Debre Zeit, Minjar, and Chefe Donsa locations, respectively. The plots were 1 m × 1.2 m (1.2 m^2) in size and spaced 0.5 m apart. One of the sub-blocks in each block received 92 kg·N·ha^{-1} fertilizer in splits, with one-third of the total amount applied at the time of sowing and the remaining two-thirdstop-dressed during the tillering stage of the crop development, while the other sub-block was not fertilized. The recommended rate of phosphorus fertilizer (10 kg·P·ha^{-1}) in the form of triple supperphosphate was uniformly applied to all plots in order to reduce the confounding effect of other nutrients. Within each block, the test genotypes were assigned to plots at random. Weeding was carried out by hand, so the test fields were weed-free.

To control stem, leaf, and yellow rust infestations, the fungicide Nativo 300SC (200 g/l Tebuconazole + 100 g/l Trifloxystrobin) was used, and all other crop management techniques were applied uniformly to all plots as per the recommendations. Experimental fields were harvested when all genotypes reached harvest maturity on December 12, 2020, at Debre Zeit; on December 17, 2020, at Minjar; and on January 4, 2021, at Chefe Donsa.

2.4. Data Collection. Data on days to 50% heading (DH), days to 90% physiological maturity (DM), plant height (PH), number of fertile tillers per plant (NFT), spike length (SL), number of spikelets per spike (SPS), and number of seeds per spike (NSPS) were collected following the procedures used by [28]. The measurements of PH, NFT, SPS, SL, and NSPS were taken from ten randomly selected plant samples per plot. After plants were manually harvested, data on aboveground biomass yield (BY) and grain yield (GY) were recorded and converted to a hectare basis. The BY was measured in the field using spring balance during harvesting. The harvested biomass was air-dried and threshed, and the grain yield (GY) was determined using an analytical balance and adjusted to a standard moisture content of 12.5%. Harvest index (HI) was calculated as the ratio of grain to the total biomass yield. The normalized difference vegetative index (NDVI) was measured using a hand-held green seeker optical sensor. The relative GY, BY, and NDVI readings were calculated by dividing the GY, BY, and NDVI readings of a genotype under low N by the GY, BY, and NDVI readings of the same genotype under optimal N. The stress tolerance indices were computed as described by [29] as per the following equations:

Table 3: Sources, numbers, and identification codes of durum wheat genotypes tested under an optimum and low-nitrogen environment at Debre Zeit, Chefe Donsa and Minjar areas during the 2020 main cropping season.

Sources	Number of genotypes	Genotypes with identification codes
ICARDA	13	1, 2, 3, 4, 5, 6, 7, 12, 14, 15, 16, 17, and 19
CIMMYT	83	86–168
EBI	67	8, 9, 10, 11, 13, 18, 20, 21, 22, 23, 24, 25, 28, 29, 31, 32, 33, 34, 35, 36, 38, 39, 40, and 42–85
DZARC released varieties	25	26, 27, 30, 37, 41, and 169–188
DZARC breeding lines	12	189–200
Total	200	

$$\text{Stress susceptibility index (SSI)} = \frac{\left(Y_{ns} - Y_{st}\right)}{\left(Y_{ns} * \left(1 - \left[\mu Y_{st}/\mu Y_{ns}\right]\right)\right)}, \tag{1}$$

where Y_{ns} and Y_{st} are the yields of a given genotype under optimum and low N conditions, respectively; whereas μY_{ns} and μY_{st} are the mean yields of all the tested genotypes under optimum and low N conditions, respectively.

$$\begin{aligned}
&\text{Stress tolerance index (STI)} = \frac{\left(Y_{ns}\right)\left(Y_{st}\right)}{\left(\mu Y_{ns}\right)^2},\\
&\text{Yield stability index (YSI)} = \frac{Y_{st}}{Y_{ns}},\\
&\text{Mean productivity (MP)} = \frac{\left(Y_{ns} + Y_{st}\right)}{2},\\
&\text{Yield index (YI)} = \frac{Y_{st}}{\mu Y_{st}},\\
&\text{Tolerance index (TOL)} = Y_{ns} - Y_{st},\\
&\text{Geometric mean productivity index (GMP)} = \sqrt{Y_{ns} * Y_{st}},\\
&\text{Relative reduction due to stress (RRS)} = 1 - \left(\frac{P_{st}}{P_{ns}}\right),
\end{aligned} \tag{2}$$

where P_{ns} and P_{st} are performances of a given genotype unstressed and stressed conditions.

2.5. Screening Procedure for N-Efficiency and N-Responsiveness. The durum wheat genotypes were classified for N-efficiency and responsiveness using the procedure set by [30]. The genotype performances under optimum N were plotted against their performances under low N. This categorization enabled one to differentiate between N-efficient and N-inefficient genotypes based on above- and below-average performances under low N, respectively. Similar categorization was also made for the N-responsive and N-nonresponsive genotypes, relying on above- and below-average performances under optimum N, respectively [10, 30]. Eventually, the durum wheat genotypes were classified as efficient or inefficient, responsive or non-responsive to N fertilization based on the aforementioned criteria using sigma plot software.

2.6. Data Analyses. The *F*-max ratio for homogeneity of variance was carried out to determine the validity of the experiment and to combine the data over locations [31]. Since the error variances for all traits were homogeneous, the data were pooled and analyzed across locations. The data were subjected to a combined analysis of variance using Meta-R software [32]. The phenotypic correlation coefficients were calculated using R-software version 4.1.3 [33] to determine the relationships between tolerance indices and grain yield, as well as the other quantitative and physiological traits and grain yield under optimum and low N conditions. The factoextra R package was used to create correlation plots.

3. Results and Discussion

3.1. Yield, Yield Components, and Physiological Traits. The combined analysis of variance across the three locations revealed that the tested genotypes varied significantly in all of the measured variables for yield, yield components, and other traits under both N unstressed (Y_{ns}) and stressed (Y_{st}) conditions (Tables S6 and S7). Likewise, the genotype by environment interaction effects were also highly significant for all the measured traits in both environments except for the number of fertile tillers plant^{-1} (NFT), spike length (SL), the number of seed spike^{-1} (NSPS), and harvest index (HI) under optimum nitrogen (N) conditions. This variation could be due to genetic variability among genotypes.

Grain yield differed significantly ($P < 0.01$) between durum wheat genotypes grown under optimum and low-N environments (Table S6). This demonstrated that the genotypes responded differently to the N application. The interaction of genotypes and environments was also significant ($P < 0.01$) indicating that genotypes performed differently in various environmental conditions.

Averaged over locations, the top yielder genotypes under optimum N were 131, 172, 10, 142, 179, 101, 180, 27, 16, 132, 83, 84, and 155, with grain yields of 5.08, 4.85, 4.83, 4.75, 4.75, 4.73, 4.70, 4.65, 4.63, 4.63, 4.58, 4.52, and 4.52 $t{\cdot}ha^{-1}$, respectively. However, under low N conditions, genotypes 155, 121, 175, 27, 196, 191, 105, 14, 100, 55, 101, 157, and 140 exhibited grain yield means of 2.78, 2.75, 2.70, 2.67, 2.63, 2.63, 2.60, 2.58, 2.58, 2.58 and 2.53 $t{\cdot}ha^{-1}$, respectively. Among the top thirteen genotypes, three genotypes, 155, 101, and 27, exhibited higher grain yields under both optimum and low-N environments (Table S2). This suggests that the low performance of genotypes is not necessarily exclusive of high productivity under high N conditions. In line with the present results, [10, 13, 34, 35] found a significant variation among wheat genotypes for grain yield under high and low N conditions. Moreover, [36] reported significant differences in grain yields among maize cultivars grown in different N environments.

The average reduction in grain yield (GY) under low N compared to optimum N conditions was 48.03% across all the genotypes and three locations. The genotypes with the lowest reduction percentage (<32%) in GY under low N were 175 (22.5%), 100 (24.6%), 167 (27.9%), 14 (28.5%), 17 (29.9%), 146 (30.4%), 57 (30.4%), 168 (31.2%), 166 (31.5%), 105 (31.6%), and 55 (31.7%). In contrast, the genotypes with the highest reduction in GY were 22 (70.4%), 16 (67.9%), 74 (67.8%), 171 (66.8%), 132 (66.2%), 128 (66.2%), 79 (64.8%), 2 (63.8%), 29 (63.1%), and 179 (61.1%) (Table S2). In general, about 17% of the tested genotypes performed well under low N conditions, which was consistent with the findings of [37], who reported that when plant material performs relatively well under low N input, yield reduction does not exceed 35–40%. Genotypes with the lowest yield reduction percentages are considered tolerant to low N conditions, whereas genotypes with the highest yield reduction percentages are sensitive to low N conditions. Therefore, the tolerant genotypes could serve as potential donors in the development of N-efficient durum wheat varieties.

The biomass yield (BY) of durum wheat was also significantly ($P < 0.01$) affected by the genotypes and their interaction with the environment under both optimum and low-N environments (Table S6). The BY was relatively higher under both N conditions for genotypes 181 and 72 as compared to genotypes 101 and 5, which produced low biomass yields (Table S2). The genotypes with the highest biomass yield may have a higher tillering capacity and higher N uptake efficiency. Moreover, NDVI significantly ($P < 0.001$) varied among genotypes and locations under low N and among genotypes under optimum N (Table S6). In line with this study, [38] noted significant variation in plant height (PH), NSPS, BY, HI, and the normalized difference vegetative index (NDVI) among wheat cultivars. [10, 34] also found significant genotype by environment interactions for PH and NDVI in wheat under optimum and low N conditions. Moreover, [39] found significant variation in wheat germplasm grown for semiarid climate adaptability in growth, yield, and yield-related traits.

3.2. Stress Tolerance Indices.

3.2. Stress Tolerance Indices. The combined analysis of variance across the three locations revealed highly significant genotype variations and genotype-by-environment interactions for all the stress indices except for yield stability index (YSI), tolerance index (TOL), and relative reduction of yield due to stress (RRS) (Table S7). Similar to these results, [40] found significant winter wheat cultivar variations and cultivar by location interaction for stress tolerance indicators like MP, GMP, and STI, but not TOL. In contrast to our findings, they reported no significant effects of genotype and genotype by location interaction on SSI. Furthermore, unlike the present findings [41], we observed significant effects of wheat cultivar and cultivar-by-environment interaction on YSI under waterlogging stress. This disparity could be attributed to differences in the test genotypes and the environmental conditions under which the experiments were carried out.

The present results demonstrated that durum wheat genotypes with higher grain yields under optimum N had greater SSI and MP values, whereas those with higher grain yields under low N had larger STI, YI, and GMP values. Under both N conditions, high-yielding genotypes, such as 101, 140, 155, 10, and 27, also had higher SSI, STI, YSI, MP, YI, and GMP values (Table S3). According to [42], higher GMP and YSI values have been used as selection criteria for identifying nitrogen stress-tolerant cultivars with high grain yield potential under limited N supply. Similarly, [38] used stress indices as selection criteria to identify promising and poor-performing wheat cultivars for low-N tolerance [43], which identified N stress-tolerant durum wheat genotypes under normal and stress conditions using TOL, SSI, GMP, and YSI. However, [40] proposed using STI in conjunction with GMP and MP to screen cultivars. Accordingly, in this study, genotypes such as 155, 101, and 27 were the most promising of the 200 durum wheat genotypes evaluated for low-N tolerance. Moreover, these genotypes gave higher grain yields under optimum N conditions, indicating that they are also the most responsive ones.

3.3. Screening of Genotypes for Low N-Tolerance and N-Responsiveness. The screening of the 200 durum wheat genotypes for low N-tolerance and N-responsiveness was made based on absolute and relative values of grain yield, biomass yield, and NDVI values, as presented follows.

3.3.1. Screening of Genotypes Based on Grain Yields. According to [30], categorization of nutrient response efficiencies of crop genotypes classified 58 genotypes (29%) as highly N-efficient and responsive, 30 genotypes (15%) as efficient and nonresponsive, 42 genotypes (21%) as inefficient and responsive, and 70 genotypes (35%) as inefficient and nonresponsive to N application (Figure 1). All the tested genotypes yielded more under optimum than low N conditions, and this can be attributed to the genetic variabilities of the genotypes and the deficiency of nutrients, particularly N, necessary for plant growth and development. Taking absolute grain yield as a screening parameter, genotypes 155, 101, 154, 196, 105, 140, 30, 147, 105, 84, 157,

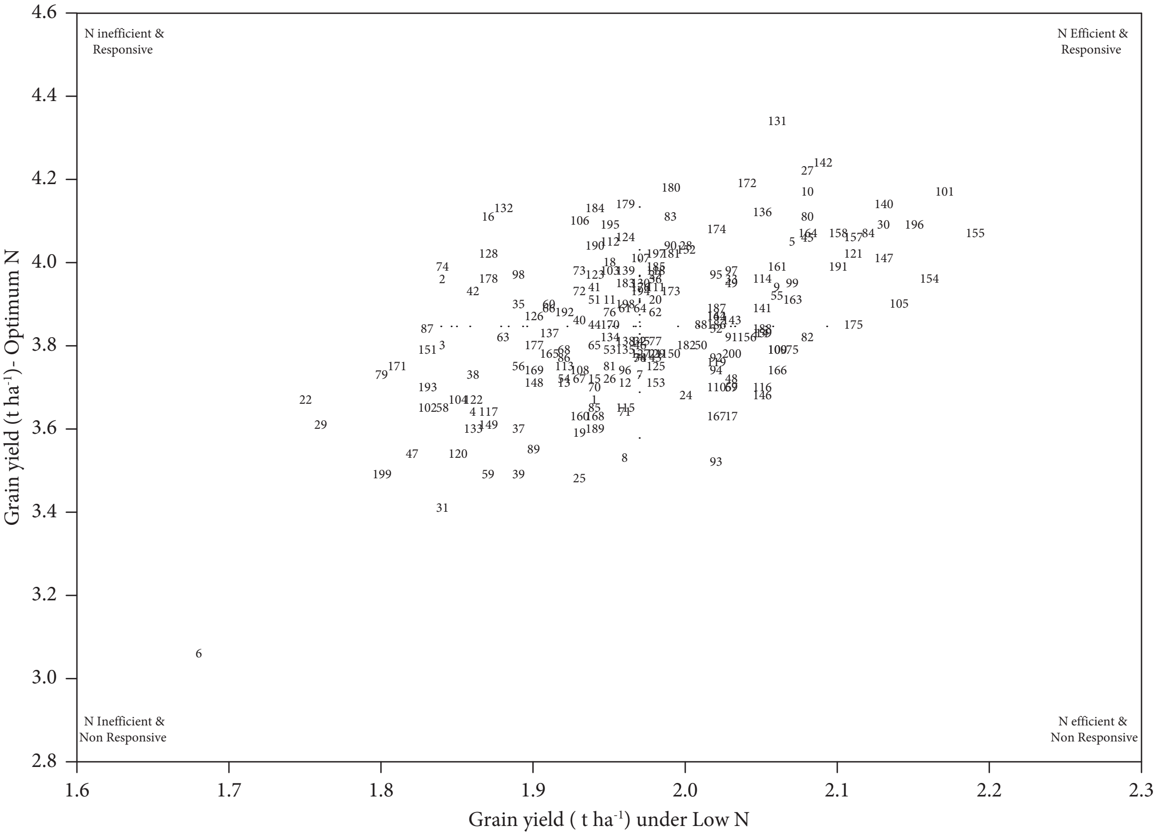

Figure 1: Categorization of durum wheat genotypes into N-efficiency and N-responsive groups based on grain yield. Horizontal and vertical broken lines depict mean grain yield under low and optimum N conditions, respectively.

Table 4: Summary of durum wheat genotypes classified using multiple criteria for low-N tolerance.

Parameters	N-efficient genotypes selected														
Absolute grain yield	155	101	196	154	140	30	147	105	84	157	121	175	158	191	142
	27	10	80	164	45	82	5	99	163	131	161	9	55	100	75
	166	172	136	114	141	188	159	14	116	146	174	97	33	49	95
	143	91	156	200	48	57	69	110	17	167					
Absolute biomass yield	27	80	84	43	57	49	70	45	33	9	54	82	146	28	60
	61	157	76	114	48	69	42	101	164	24	142	50	75	196	17
	147	158	156	155	163	55	153	121	154	73	23	131	46	52	64
	67	34	30	140	105	100	166	13	62	32					
Absolute NDVI values	45	9	84	80	196	43	35	48	55	33	49	191	154	23	67
	66	75	153	64	57	83	73	11	53	40	136	70	166	50	24
	46	164	179	140	71	17	4	62	8	26	27	36	16	42	68
	82	61	28	69	51	92	174	38	168	163					
Relative grain yield	175	100	167	14	17	146	57	168	166	105	55	191	200	110	94
	82	25	121	188	116	160	163	8	109	182	39	154	150	159	155
	93	24	91	157	75	48	88	71	119	45	153	156	141	59	196
	85	147	127	143	189	158	27	92	120	140					
Relative biomass yield	200	146	100	188	57	39	14	168	110	70	167	94	157	8	59
	191	17	121	85	158	175	109	160	105	166	156	55	154	150	71
	189	192	61	25	116	76	5	143	120	153	155	54	82	91	92
	182	60	21	78	77	161	187	159	141	185					
Relative NDVI values	200	39	57	9	160	146	17	166	165	186	164	70	121	85	110
	4	64	55	51	86	71	191	199	29	45	78	154	91	104	140
	88	127	182	136	153	33	14	100	179	196	31	7	192	80	75
	26	130	43	49	103	6	92	35	18	48					
Selected genotypes based on common criteria	55	166	17	75	82	57	154	196	100	191	146	48			

Genotypes are chosen if they appear in five or more different traits.

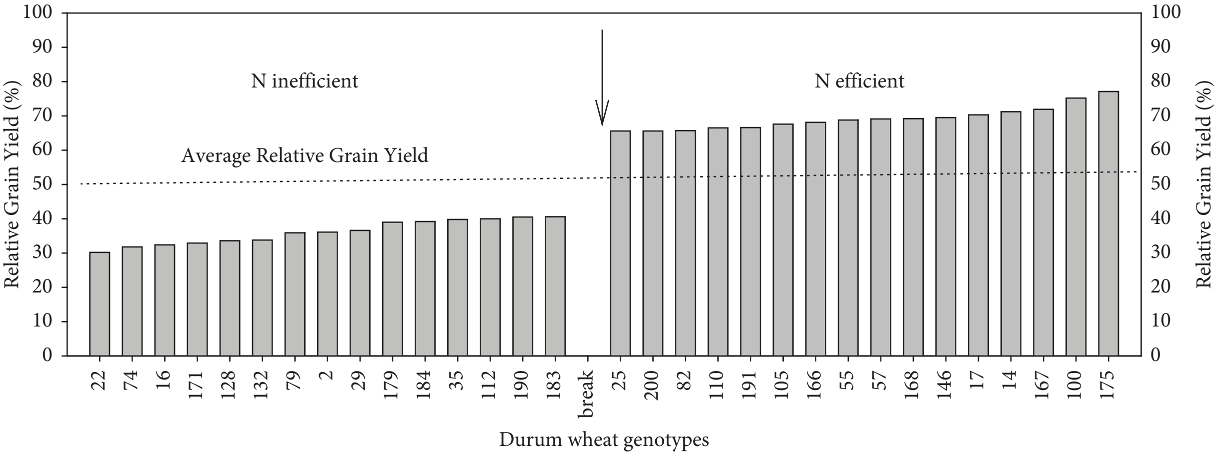

Figure 2: Categorization of N-efficient and inefficient genotypes based on relative grain yield (only the 15 most extreme genotypes from each efficient and inefficient category were mentioned in the abovementioned figure due to limited space to accommodate all; the break is the demarcation between N-efficient and inefficient genotypes).

121, 158, 191, 142, 27, 10, 80, 164, and 45 were found to be the most desirable genotypes because they were grouped as efficient and responsive to N and produced higher grain yield under both N deficiency and sufficiency. On the other hand, genotypes 6, 22, 29, 79, 171, 199, 151, 193, 102, 47, and 3 were considered as being among the most inefficient and nonresponsive to N application (Figure 1 and Table 4) because they produced lower grain yield under both optimum and low N conditions. Similar to these results, [10, 44, 45] used grain yield to categorize diverse wheat genotypes as efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive to N, zinc, and manganese, respectively.

Relative GY also varied significantly among durum wheat genotypes, ranging from 30.2% for genotype 22 to 77.1% for genotype 175. Genotype 175 had the highest relative grain yield, followed by genotypes 100, 164, 14, and 17, while genotype 22 had the lowest relative grain yield, followed by genotypes 74, 16, 171, and 128 (Figure 2). Based on relative grain yield, 48.5% and 51.5% of the total genotypes evaluated were classified as N-efficient and inefficient, respectively. Relative yield has been used as a parameter for genotype ranking in several studies, including that of [46] in wheat, [47] in potato, and [48] in barley.

3.3.2. Screening of Genotypes Based on Biomass Yields.

Based on the data presented in Figure 3, of the 200 durum wheat genotypes evaluated, 70 (35%), 26 (13%), 28 (14%), and 76 (38%), were considered efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive, respectively. The genotypes 27, 80, 84, 43, 57, 49, 70, 45, 33, 9, 54, and 82 had higher biomass yields under low N conditions compared to genotypes 6, 102, 151, 193, 2, 126, 22, 87, 104, 31, and 3, which had lower biomass yields (Figure 3 and Table 4), possibly due to variation in N uptake and utilization. Therefore, genotypes with high biomass yield under low N condition can be considered as low N tolerant and those with lower biomass yield are grouped as low N sensitive genotypes when biomass yield is regarded as a selection parameter for N efficiency. These top-performing genotypes also gave greater biomass yields under optimum N conditions, indicating that they were among the most responsive. The results showed that the majority of Ethiopian landraces produced higher biomass yields under low N conditions than genotypes obtained from other sources, which could be attributed to the 'genotypes' high biomass production capacity and possibly high N uptake efficiencies under low N conditions. Similarly, [10] screened twelve bread and durum wheat cultivars for N efficiency, considering total above ground biomass yield as a categorization criterion. [49] also classified ten wheat genotypes as efficient, responsive, inefficient, or nonresponsive for phosphorus use efficiency based on total dry matter biomass yield. The current results indicate the need to consider both BY and GY to categorize durum wheat genotypes for N-efficiency and N-responsiveness, with due emphasis given to GY. This was because most genotypes characterized as N-efficient and N-responsive based on BY alone did not similarly give a higher grain yield under both optimum and low N conditions. In this regard, [10] suggested relying more on GY than BY as the main criteria for the categorization of wheat genotypes for N-efficiency and/or N-responsiveness.

The relative BY varied greatly among durum wheat genotypes, with 50% of the total genotypes classified as N-efficient and the remaining 50% classified as N inefficient, indicating the presence of variability among the tested materials. As a result, genotypes 200, 146, 100, 188, 57, 39, 14, 168, 110, 70, and 167 produced the highest BY yield and performed best under both N conditions (Figure 4 and Table 4). Genotypes 22, 16, 74, 79, 11, 183, 132, and 184, on the other hand, were among durum wheat genotypes with a relative BY of less than 45%. Similarly, based on relative dry matter yield [50], we grouped durum wheat genotypes as acid soil-tolerant and intolerant.

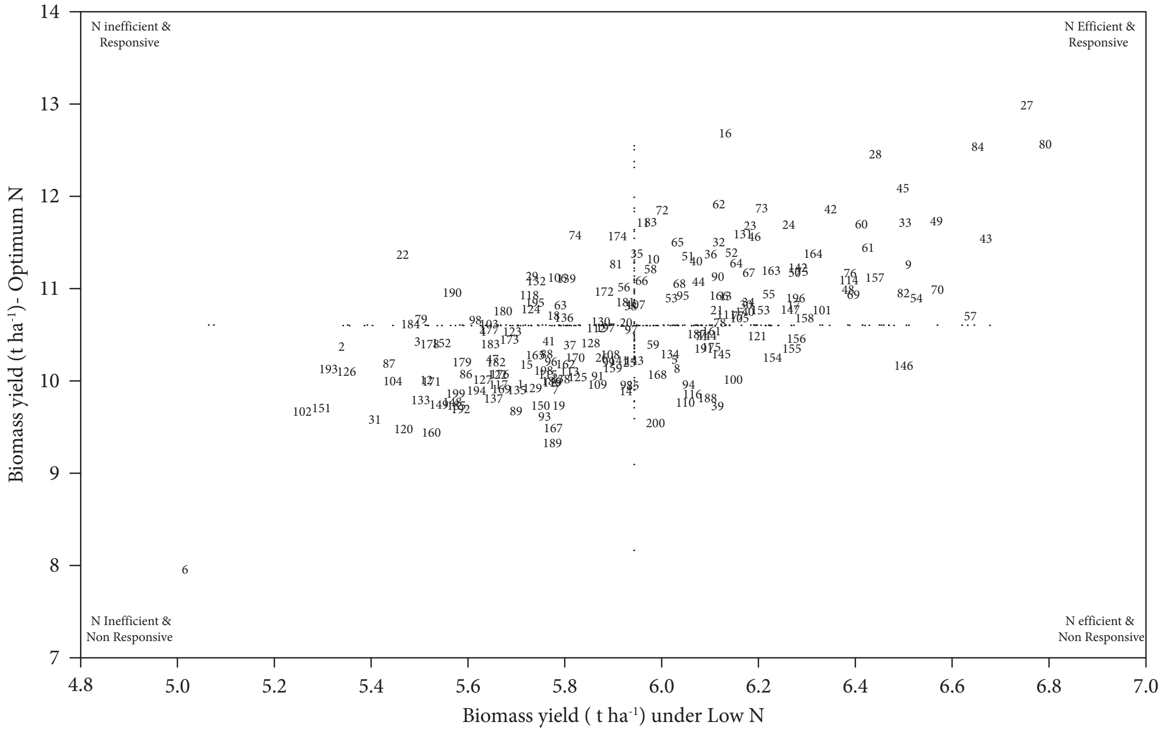

Figure 3: Categorization of N-efficient and N-responsive durum wheat genotypes based on above-ground total biomass yield. Horizontal and vertical broken lines depict the mean biomass yield under low and optimum N conditions, respectively.

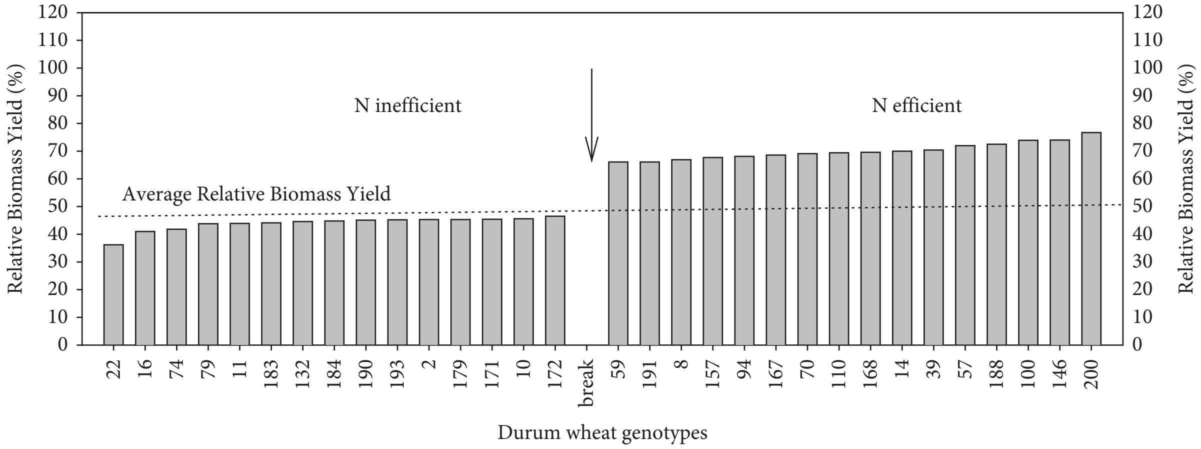

Figure 4: Categorization of N-efficient and inefficient genotypes based on relative biomass yield (only the 15 most extreme genotypes from each efficient and inefficient category were mentioned in the abovementioned figure due to limited space to accommodate all; the break is the demarcation between N-efficient and inefficient genotypes).

3.3.3. Screening of Genotypes Based on NDVI Readings. The use of NDVI readings allows for quick and accurate crop tracking of N status and yield estimation in crops [51]. According to the findings of this study, durum wheat genotypes differed greatly based on NDVI readings. Based on this criterion, about 31.5, 13, 16, and 39.5% of the total genotypes evaluated were classified as efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive to N fertilization, respectively (Figure 5). The highest NDVI readings were found in genotypes 45, 9, 84, 80, 196, 43, 35, 48, 55, 191, and 49, while the lowest readings were recorded in genotypes 193, 151, 143, 3, 134, 188, 117, 120, and 93 under low N conditions. These variations could be attributed to differences in N uptake and utilization efficiencies and genetic variability for the response to N applications among durum wheat genotypes [10]. The potential of using NDVI readings as a tool to distinguish and identify superior wheat genotypes grown under dry land and irrigated conditions was demonstrated by the authors of [52].

The relative NDVI values also varied significantly among durum wheat genotypes grown under optimum and low-N

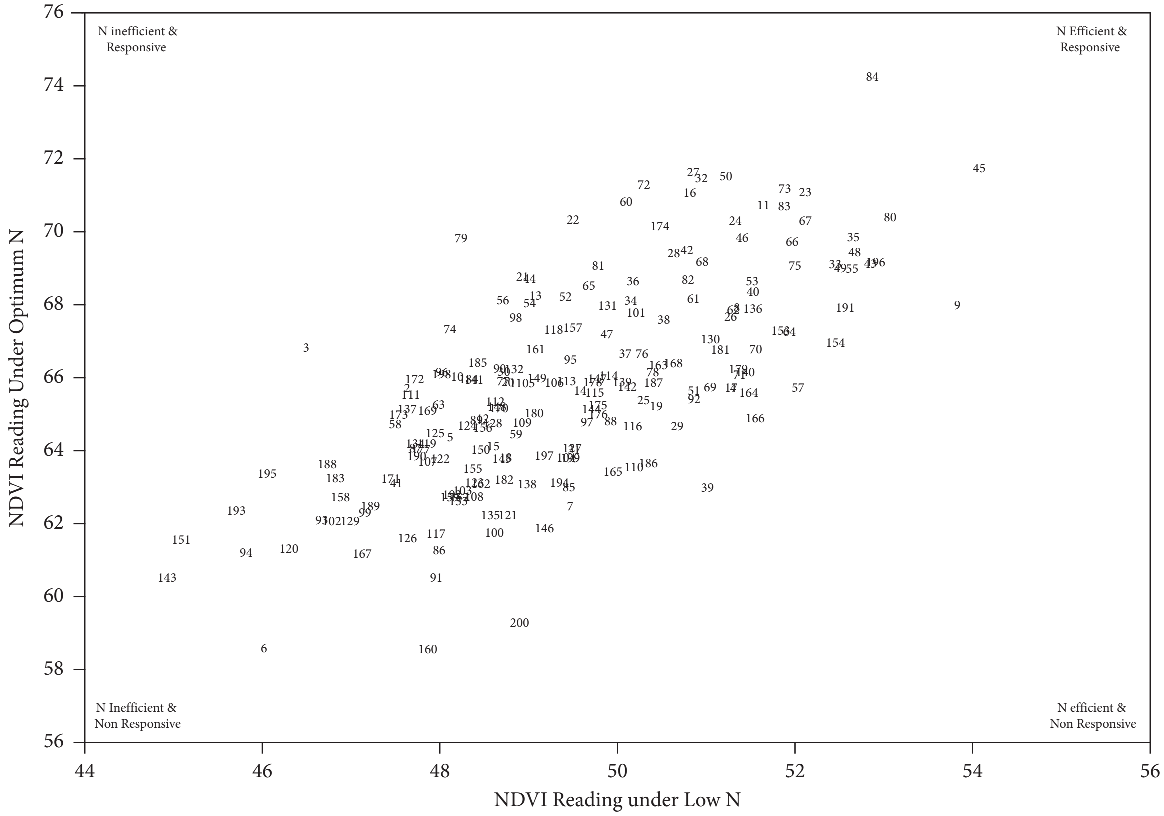

Figure 5: Categorization of N-efficient and N-responsive durum wheat genotypes based on NDVI reading. Horizontal and vertical broken lines depict mean NDVI values under low and optimum N conditions, respectively.

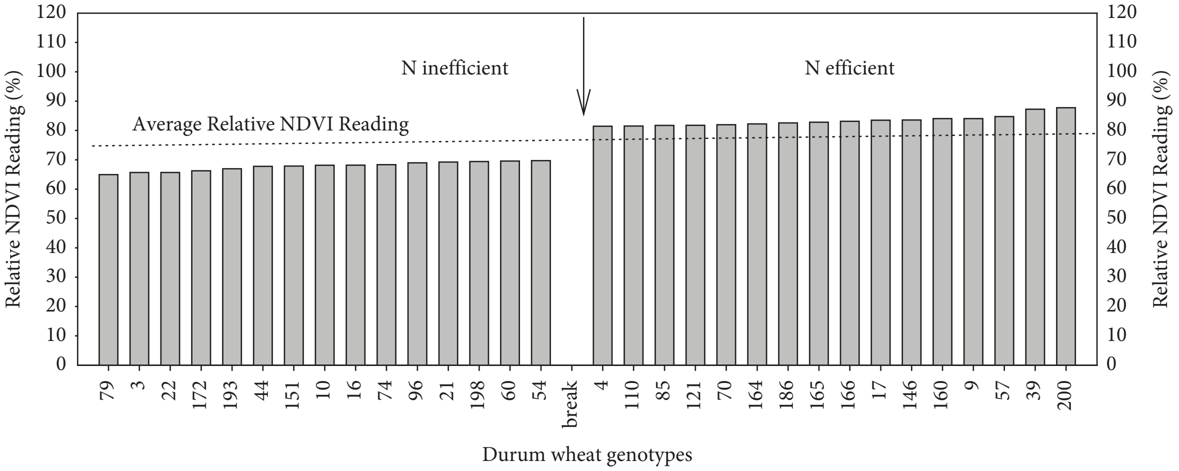

Figure 6: Categorization of N-efficient and inefficient genotypes based on relative NDVI reading (only the 15 most extreme genotypes from each efficient and inefficient category were mentioned in the abovementioned figure due to limited space to accommodate all; the break is the demarcation between N-efficient and inefficient genotypes).

environments. The genotype with the highest relative NDVI value was 200, followed by 39, 57, 9, 160, 146, 17, and 166. On the other hand, the genotype with the lowest relative NDVI value was 79, followed by 3, 22, 172, 193, 44, 151, and 10 (Figure 6 and Table 4). In this study, 46.5% of the genotypes were N-efficient, while the remaining 53.5% were N-inefficient (Figure 6).

Generally, the screening procedure for N-efficient and N-responsive durum wheat genotypes in the current study is summarized in Table 4. Results presented in Table 4 show that the use of multiple criteria is more reliable in selecting N-efficient and N-responsive durum wheat genotypes than using single or few criteria. Biomass yield and NDVI reading were chosen as selection criteria over other agronomic traits

Table 5: Description of the best-performing durum wheat genotypes.

Genotypes code	Genotypes	Origin	Names/pedigree	Performance for key traits
55	Land race	EBI	226958	AGY, ABY, ANDVI, RGY, RBY, and RNDVI
166	CD15DZELT/off/1516/2015	CIMMYT	JUPARE C 2001*2/IM/5/K0FA/4/DUKEM1//PATKA-7/YAZI-1/3/PATKA-7/YAZI-1/6/ALAS/. . .	AGY, ABY, ANDVI, RGY, RBY, and RNDVI
17	FIGSDRYWET108	ICARDA	IRNS294/ID-98797	AGY, ABY, ANDVI, RGY, RBY, and RNDVI
57	Land race	EBI	222415	AGY, ABY, ANDVI, RGY, RBY, and RNDVI
154	CD15DZELT/off/1032/2015	CIMMYT	JUPARE C 2001*2/KHAPLI/5/M0HAWK/4/DUKEM-1//PATKA-7/YAZI-1/3/PATKA-7YAZI-1/11/. . .	AGY, ABY, ANDVI, RGY, RBY, and RNDVI
75	Land race	EBI	236974-1	AGY, ABY, ANDVI, RGY, and RNDVI
82	Land race	EBI	231585	AGY, ABY, ANDVI, RGY, and RBY
196	Breeding pipeline, DWNL p#21	DZARC	Icasyr-1/3/Gcn//Sti/Mrb3	AGY, ANDVI, RGY, and RNDVI
100	CDSS09B00190T-099Y-036M-18Y-0M	CIMMYT	RBC/HUALITA/5/MOHAWK/3/GUANAY//TILO-1/LOTUS-4/4/ARMENT//SRN-3/NIGRIS-4/3/. . .	AGY, ABY, RGY, RBY, and RNDVI
191	Breeding pipeline, DWNL p#13	DZARC	JUPARE C 2001*2/IM/5/K0FA/4/DUKEM-1//PATKA-7/YAZI-1/3/PATKA-7/YAZI-1/6/ALAS/. . .	AGY, ANDVI, RGY, RBY, and RNDVI
146	CD15DZELT/off/745/2015	CIMMYT	CND0/VEE//PLATA-8/3/6*PLATA-11/6/PLATA-8/4/GARZA/AFN//CRA/3/GTA/5/RASC0N/9/. . .	AGY, ABY, RGY, RBY, and RNDVI
48	Land race	EBI	222191	AGY, ABY, ANDVI, RGY, and RNDVI

AGY = absolute grain yield; ABY = absolute biomass yield; ANDVI = absolute normalized difference vegetative index; RGY = relative grain yield; RBY = relative biomass yield; RNDVI = relative normalized difference vegetative index; EBI = Ethiopian biodiversity institute; DZARC = Debre Zeit Agricultural Research center.

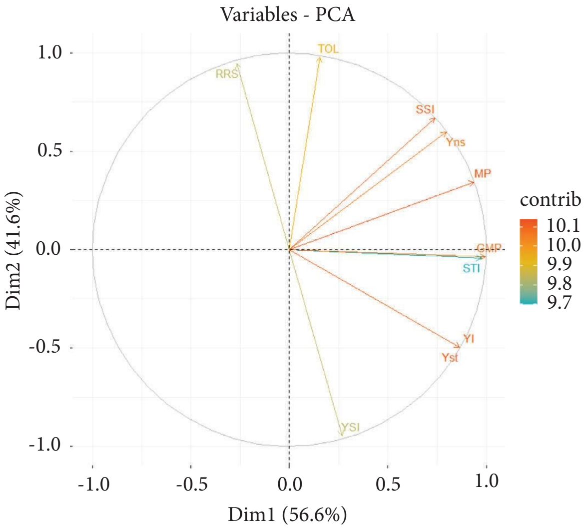

Figure 7: Phenotypic correlations between grain yield and stress screening indices among the 200 durum wheat genotypes evaluated under optimum and low N conditions at three locations.

because they demonstrated a moderately significant correlation with grain yield under optimum and low N conditions. The description of the best-performing genotypes based on the findings of this study, including their pedigree, origin, and performance for key traits, is shown in Table 5.

3.4. Relationships among Parameters Evaluated

3.4.1. Grain Yield versus Stress Screening Indices. The results of regression analyses for grain yield (GY) under optimum and low N conditions against stress indices revealed that the relationships vary in strength and significance levels (Figure 7 and Table S4). There were strong positive correlations between GY and SSI ($r = 0.99^{**}$), GMP ($r = 0.81^{**}$), and STI ($r = 0.77^{**}$) but only a moderate correlation with YI ($r = 0.55^{**}$), and no correlation with MP, TOL, and RRS under optimum N conditions (Figure 7 and Table S4). A weak but significant negative correlation ($r = -0.083^{**}$) was observed between GY and YSI. Positive and significant correlations of GY were found with YI ($r = 0.99^{**}$), GMP ($r = 0.93^{**}$), STI ($r = 0.92^{**}$), and MP ($r = 0.74^{**}$) under low N conditions. Moderately significant and positive correlations of GY were found with YSI ($r = 0.57^{**}$) and SSI ($r = 0.49^{*}$). GY and RRS had a significant negative correlation ($r = -0.21^{**}$). TOL ($r = -0.57$) was negatively and nonsignificantly correlated with GY under the low N condition (Figure 7 and Table S4). These results generally revealed that the strongest correlations were found between GY and SSI and between GY and YI under optimum and low N growth conditions, respectively, indicating that selection based on these indices under both N conditions could be more effective.

The stress screening indices GMP, STI, YI, and SSI showed similar correlation trends with GY under both optimum and low N conditions (Figure 7); thus, either one or multiples of these traits can be used to select low-N-tolerant durum wheat genotypes. In accordance with our findings, [53] reported that STI, GMP, and MP were the stress indices of choice for identifying low-N-tolerant wheat cultivars. In line with our findings, the correlation of grain yield with most of the stress indices under normal and stressed conditions was reported by [54, 55] in durum wheat for drought tolerance, [56] in spring wheat for heat stress tolerance, [57] in maize for drought tolerance, and [38, 40] in wheat for low-N tolerance.

Additionally, the SSI screening index had strong and significant positive correlations with MP ($r = 0.95^{**}$), GMP ($r = 0.77^{**}$), TOL ($r = 0.74^{**}$), and STI ($r = 0.73^{**}$) (Figure 7 and Table S4). It had a moderate and significant correlation with YI ($r = 0.49^{**}$). STI depicted significant positive correlations with GMP ($r = 0.97^{**}$), YI ($r = 0.92^{**}$), and MP ($r = 0.90^{**}$). A negative but significant correlation was observed between YSI and RRS ($r = -0.97^{**}$), and it is moderately correlated with TOL ($r = -0.58^{**}$) and YI (0.57^{**}). MP had significant correlations with GMP ($r = 0.93^{**}$) and YI (0.74^{**}). TOL was moderately correlated with RRS ($r = 0.57^{**}$), while the correlation between RRS and YI ($r = -0.57^{**}$) was moderate but negative. The YI was highly correlated to GMP ($r = 0.92^{**}$) (Figure 7 and Table S4).

3.4.2. Grain Yield versus Yield Components. Correlation coefficients were also estimated for grain yield against phenological, yield components, and physiological traits under both optimum and low N conditions (Figure 8 and Table S5). Under optimum N condition, there were moderately significant and positive correlations between GY and BY ($r = 0.56^{**}$), HI ($r = 0.51^{**}$), and NDVI values ($r = 0.32^{**}$) (Figure 8 and Table S5). GY had a significant but weak correlation with NSPS ($r = 0.18^{**}$). BY showed a significant positive correlation with NDVI values ($r = 0.80^{**}$), and moderately significant positive correlations with DM, PH, NFT, SL, and SPS, but it showed significant negative correlations with NSPS and HI. Similarly, NDVI exhibited a moderate to highly significant positive correlations with all traits studied except NSPS and HI (Figure 8). The strong correlation of NDVI with BY and GY shows a significant agronomic and biological relationship between these traits, as NDVI can be used to predict the BY and N status of crops in the field, as indicated by [58].

Similar correlation trends with that under optimum N were observed under low N conditions, as well. GY correlated significantly with HI ($r = 0.67^{**}$), BY ($r = 0.63^{**}$) and NDVI values ($r = 0.33^{**}$) (Figure 9 and Table S5). Both BY and NDVI had significant positive correlations with DH, DM, PH, NFT, SL, and SPS but a negative association with NSPS (Figure 9). In this study, all phenological and yield component traits were positively and significantly correlated to each other except NSPS, which had a negative correlation with all traits except GY and HI under both optimum and low N conditions (Figures 8 and 9; Table S5). Generally, the relationship between grain yield and BY, HI, and NDVI under low N conditions is slightly higher than under high N conditions. In agreement with our results, [34] reported a significant and positive correlation between GY and NDVI values under high and low N conditions. Similar association

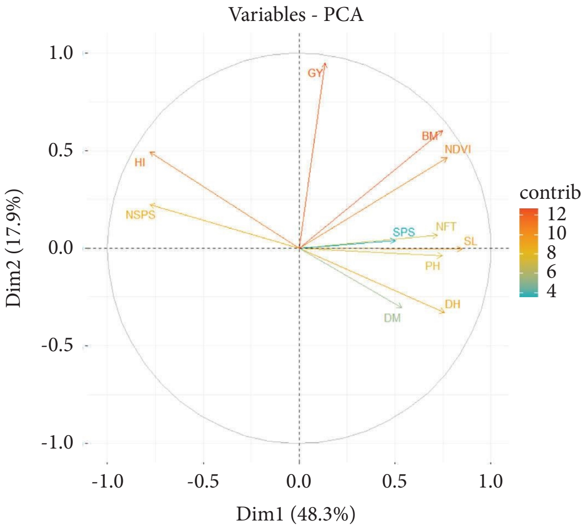

Figure 8: Phenotypic correlations between grain yield and phenological, yield components, and physiological traits of 200 durum wheat genotypes evaluated under optimum N conditions at three locations.

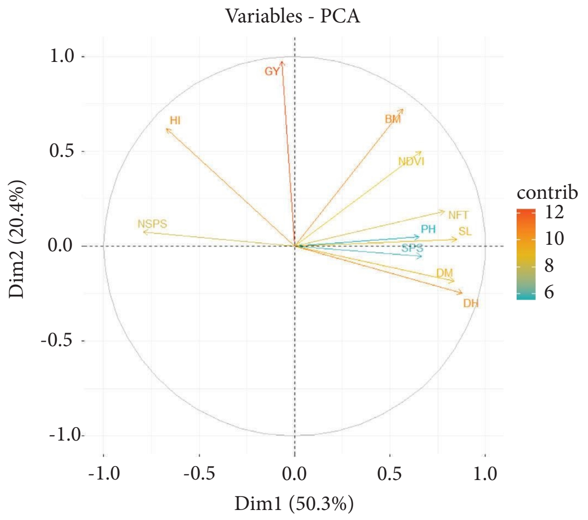

Figure 9: Phenotypic correlations among phenological, yield and its components, and physiological traits of 200 durum wheat genotypes tested under low N conditions at three locations.

trends with our results for PH, BY, and NDVI with GY under high and low N conditions were also reported by [38]. A negative association between GY and PH and a significant positive correlation between GY and HI were reported by [13] in wheat grown under contrasting N treatments in south-eastern Europe. [5] also found strong and positive correlations between GY and BY, HI, NSPS, and NFT in bread wheat tested at four different N levels, but the latter two traits did not show such strong and significant correlations in our study. This, might be due to the variations in the test genotypes and environmental conditions. In contrast to our findings, [59] observed positive and significant correlations of GY with PH, SL, NFT, and NSPS in wheat grown under slow-releasing N fertilizer, which could also be attributed to genotypic variations. They also found positive and significant correlations between GY and BY, which agrees with our findings.

4. Conclusions

This study examined the low-nitrogen tolerance and responsiveness to N application of two hundred durum wheat genotypes at three locations in the central highlands of Ethiopia under both optimum and low nitrogen conditions. The results indicated significant variation for the studied quantitative traits and stress indices among durum wheat genotypes under optimum and low N conditions, and significant genotype by environment interaction effects under both low N for quantitative traits. Based on grain yield, the top high-yielding genotypes under optimum N were 131, 172, 10, 142, 179, 101, 180, 27, 16, 132, 83, 84, and 155, which were responsive to N application. However, genotypes 155, 121, 175, 27, 196, 191, 105, 14, 100, 55, 101, 157, and 140 were among the high yielder genotypes under low N conditions. Thus, genotypes that produced high yields under low N conditions can be used as parents in the durum wheat breeding program.

In this study, the average reduction in GY under low N conditions *versus* optimum N conditions was 48.03 percent across genotypes and three locations, while only about 17 percent of the genotypes tested performed well (GY reduction <40%) under low N conditions. In terms of GY reduction under low N, genotype 175 had the lowest reduction percentage (22.5%), while genotype 22 had the maximum reduction (70.4%). The high yielder genotypes 101, 140, 155, 10, and 27 had higher SSI, STI, YSI, MP, YI, and GMP values under both N conditions, indicating that these stress indices could be used as selection parameters for genotype screening. On average, absolute GY, BY, and NDVI readings categorized 32, 14, 17, and 37% of the tested durum wheat genotypes as efficient and responsive, efficient and nonresponsive, inefficient and responsive, and inefficient and nonresponsive, respectively, while relative yields of these traits were less stringent in grouping the genotypes as efficient and inefficient. Using multivariate evaluation such as absolute and relative grain yield, biomass yield, NDVI reading, and stress tolerance inducements (SSI, STI, YI, and GMP) as a selection criterion, genotypes showing superior performance were 55, 166, 17, 75, 82, 57, 154, 196, 100, 191, 146, 48, 155, 101, 10, 27, and 140.

Additionally, our findings demonstrated that the stress screening indices GMP, STI, YI, and SSI had significant and positive strong correlations with grain yield under both high and low N conditions; hence, these indices can be utilized for the selection of low-N-tolerant durum wheat genotypes. Among the agronomic and physiological traits, BY, HI, and NDVI were moderately correlated with grain yield under both N conditions, despite the fact that these traits are slightly higher under low N than high N conditions. In general, genotype evaluation based on GY, BY, NDVI, and stress tolerance indices such as GMP, STI, YI, and SSI can be useful in wheat improvement to track better-performing genotypes under different N conditions. Furthermore, the durum wheat genotypes distinguished and identified as low-N-tolerant in our study could be exploited as parental parents for developing N-efficient durum wheat varieties.

Data Availability

The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

T. G, T. B., K. A., and F. A. conceptualized the study. The methodology was performed by T. G., F. A., T. B., K. A., and N. G. Data curation was done by T. G. and T. B. Formal analysis and software were carried out by T. G., T. B., and K. A. The funding acquisition was done by T.G. Supervision was carried out by T. G., F. A., T. B., K. A., B. A., and N. G. T. G. wrote the original draft and performed visualization. Writing, review, and editing were done by T. G., F. A., T. B., K. A., B. A., and N. G. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

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Supplementary Materials

The following are available online as supplementary materials: Table S1: Description of durum wheat genotypes used for the experiment; Table S2: BLUP values for grain yield under optimum and low N conditions and stress screening induces; Table S3: Mean grain and biomass yield of genotypes under optimum and low N conditions and reduction due to N stress; Table S4: Correlation coefficient upper diagonal (P values for significance level) and lower diagonal (correlation values) for grain yield and stress screening induces; Table S5: Phenotypic correlation coefficient of 200 durum wheat genotypes' phenological, yield and its components, and physiological traits under optimum N (upper diagonal) and low N (lower diagonal) conditions; Table S6: Combined analysis of variance for grain yields, yield components and other traits in 200 durum wheat genotypes grown under optimum and low N conditions; Table S7: Summary of the ANOVA for grain yield and stress indices under optimum and low N conditions susceptibility. (*Supplementary Materials*)

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