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GENETIC SCREENING OF WHEAT USING MULTIVARIATE ANALYSIS UNDER MOISTURE STRESS

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Abstract

Drought is a major environmental constraint to wheat (*Triticum aestivum* L.) productivity worldwide. Screening the drought tolerance of novel wheat genotypes is an important mitigation strategy. To achieve this, an experiment was carried out to investigate 26 wheat genotypes in randomized complete block design (RCBD) with three replications and two treatments water stress (T1) and well-watered (T2). Analysis of variance depicted highly significant differences among genotypes and treatments for all the observed morphological, physiological and biochemical traits viz., flag leaf area, plant height, spike length, grains weight per plant, straw weight per plant, thousand kernels weight, tillers per plant, glycine-betaine, potassium content, nitrate reductase activity, osmotic potential, proline content, relative water content, total chlorophyll content and total soluble sugars. Under drought conditions, thousand kernels weight positively correlated with flag leaf area, spike length, tillers per plant, grains weight per plant, glycine-betaine, potassium content, nitrate reductase activity, proline content, relative water content and total chlorophyll content. Genetic distances grouped all the wheat genotypes into two different clusters. In water stress conditions, cluster I consisted susceptible wheat genotypes, while cluster number II contained all the tolerant wheat genotypes. Principal components 1, 2 and 3 revealed the respective variability of 54.25, 12.33 and 8.6 under water stress. In this study, stress tolerance index (STI) and drought susceptibility index (DSI) expressed wheat genotypes IBWSN-1025, IBWSN-1144, IBWSN-1150, DH-12/7, DH-12/31 and MASR-64 as drought tolerant. These wheat genotypes bestow drought tolerance and might contribute to circumvent food security issues. The findings of this study will be recommended to the local farmers regarding grain yield improvements.

Key words: Moisture stress, Agronomic traits, Osmoregulation, Proline, Chlorophyll.

Introduction

Wheat is an important cereal crop cultivated as a source of staple food. This crop meets 20% requirement of the proteins and 21% of the food calories for more than 4.5 billion people belonging to 94 countries of the world (Chowdhury *et al*., 2021). Pakistan ranks at seventh among the leading wheat producing countries of the world (FAO, 2020). Wheat is an indispensable part of the Pakistan's agriculture sector with the additions of 7.8% in the agriculture value and 1.8% to the total GDP (Anon., 2021). However, water stress is a leading constraint to the crop production worldwide (Li *et al*., 2021; Mir *et al*, 2012; Hossain *et al*., 2012). Indus Basin Irrigation System (IBIS) is the world's largest contiguous irrigation system irrigating almost 70% of the cultivated area in Pakistan (Janjua *et al*., 2021). Conversely, annual precipitation below the average consequences into the failure of this large irrigation system to provide the optimum water for the agriculture lands located along the Kotri downstream of Sindh (Memon *et al*., 2022). In this context, developing high grain-yielding drought tolerant wheat genotypes by assessing their genetic diversity and performance under various stress conditions is important (Mondal *et al*., 2015).

Water stress can be defined as the depletion of soil water necessary for the normal growth of the plants. Water deficiency limits the water absorption of the plants along with decreased uptake of macro and micronutrients. Consequently, plants express early senescence, stunted growth, reduced size of the leaves, florets sterility and decrease in number of grains. In addition, water stress disrupts water use efficiency (Aroca, 2012), changes morphology and metabolism (Khakwani *et al*., 2012), decreases chlorophyll content, photosynthetic activity and relative water content in the plants. All such changes culminate into major grain yield losses (Turner *et al*., 2014). However, the extent of the yield losses depends upon the severity of the drought and the stage of occurrence (Khakwani *et al*., 2011) and the level of tolerance a wheat genotype owes.

Wheat is hexaploid with an intricated and complex genome. Grain yield and drought tolerance are polygenic traits. Thus, breeding wheat for grain yield increase and drought tolerance is a major challenge. To reach a decision about tolerance and susceptibility, a wheat genotype must undergo various experimental strategies. This involves the efforts in transferring various combinations of genes to a genotype with desired traits and evaluation of the newly evolved genetic material *in vitro* and *in vivo* using grain yield and drought tolerance traits. The molecular level identification of drought associated genes in the genetic material is comparatively expensive and less accessible for all the researchers than the screening the genotypes for agronomic, physiological and biochemical traits. Obviously, the variations in phenotypic traits and the internal mechanisms under drought stress are ultimately genotypic expressions of the genetic material. Hence, investigating the newly evolved genetic material for their acclimations under water stress through changes in phenotypic, physiological and biochemical traits is important. Drought adaptive phenotyping for yield and yield-associated morpho-physiological traits in plants is required in selecting the drought tolerant breeding material (Passioura, 2012; Monneveux *et al*., 2012). Targeting the relative high grain yield together with drought adaptive traits is necessary in the selection of drought tolerant wheat genotypes. This strategy is highly effective and enables the researchers in the selection of widely adapted drought tolerant wheat genotypes bestowed with high grain yield.

Drought tolerance is a complex trait and involves the complexity and interaction of various plant growth affecting molecular, physiological and biochemical mechanisms (Zhu, 2002). Plants trigger various physiological and biochemical mechanisms at different stages of plant development to confer drought tolerance in plants (Rampino *et al*., 2006; Arora *et al*., 2002). Drought adaptive mechanisms ensure decreased plant damage and mitigate grain yield losses under water stress. On the other hand, susceptibility of a plant to a stressed environment is cumulative effect of morpho-physiological, phenological, biochemical and molecular traits encoded by genetic factors (Grzesiak *et al*., 2019).

Osmoregulation is a life associated important homeostatic process in which salts and water content of the cell are maintained to steady state. This mechanism is critical for water absorption in the plants. Water uptake in plants is a result of water potential gradient between the cell cytoplasm and its rhizosphere (Kosová *et al*., 2014). Under drought, plants face extreme difficulty in obtaining water from rhizosphere to carry out their normal biological mechanisms (Ali *et al*., 2020). To circumvent this problem, plants synthesize osmotically active compounds to maintain the osmoregulation of cells (Wang *et al*., 2019). Osmotic adjustment is a common process in the plants under drought that helps the water uptake in the plants from soil to the root cells through accumulation of osmotically active biochemical compounds in the plants (Nawaz *et al*., 2014). Osmotically active compounds include sugar alcohols, calcium, organic acids, soluble sugars, glycine-betaine, proline, potassium, ammonium compounds and chlorides (Farooq *et al*., 2009). Under moisture stress conditions, this process maintains the cell turgor that allows the cell enlargement and plant growth, stomata to minimally partially opened and to continue CO_2 assimilation (Alves & Setter, 2004).

Drought tolerance indexes differentiate potential drought tolerant wheat genotypes from drought susceptible genotypes (Clarke *et al*., 1992; Mitra, 2001). Stress tolerance index (STI), drought susceptibility index (DSI) and geometric mean productivity (GMP) are best indicators for the selection of genotypes under stress and control conditions (Golbashy *et al*., 2010). The objectives of this study were: (a) evaluation of the newly evolved wheat genotypes for drought tolerance using morphological, physiological and biochemical traits, (b) to establish the correlation between drought tolerance and various observed traits of the wheat genotypes and (c) selection of the drought-tolerant wheat genotypes based on drought tolerance indexes.

Material and Methods

Plant material: In this experiment, 26 wheat genotypes comprised of 19 advanced wheat lines and 7 commercial wheat varieties were assessed. Wheat genotype included IBWSN-1010, IBWSN-1025, IBWSN-1142, IBWSN-1132, IBWSN-1144, IBWSN-1148, IBWSN-1149, IBWSN-1150, IBWSN-1156, IBWSN-1157, DH-9/1, DH-9/6, DH-12/7, DH-12/31, MASR-08, MASR-22, MASR-64, ESW-9525, MSH-14, while commercial check varieties consisted Khirman, Chakwal-86, NIA-Saarang (drought-tolerant checks), NIA-Amber, NIA-Sarsabz (high grain yielding widely adapted), TD-1 and Benazir (drought susceptible check varieties). All the wheat genotypes were collected from Nuclear Institute of Agriculture (NIA), Tando Jam.

Plant growth conditions and data collection: Experiment was carried out in the rain exclusion shelter of plant growth facilities, Plant Physiology Division at Nuclear Institute of Agriculture, Tando Jam, Pakistan. Seeds were sown in RCBD design with three replications and two treatments water-stress (T1) and well-watered. Plants were grown under drought stress (T1) with single irrigations, while four irrigations were applied to the well-watered treatment. Meteorological data during cropping season were collected from the Regional Agromet Center, Tando Jam located along the experimental field. Data were collected on 15 agronomic, physiological and biochemical traits variability among 26 wheat genotypes under water stressed and well-watered conditions. Fully expanded flag leaf samples were collected at the heading stage of the plants showing 80% of the main spike emergence. Leaf area (cm^2) of the wheat genotypes was obtained using LICOR leaf area meter (LI3100-C, Nebraska, USA). Plant physiological and biochemical traits including proline content, total soluble sugar, total chlorophyll content, osmotic potential and glycine-betaine were indicated according to the research protocols of Bates, (1973), Riazi *et al*., (1985), Lichtenthaler, (1987), Ashraf *et. al*., (1992) and grieve & Grattan, (1983), respectively. Nitrate reductase activity of the wheat samples was recorded according to Ramarao *et al*., (1983). Potassium content was obtained following Ansari & Flowers, (1986). Relative water content in the fully expanded leaves of wheat genotypes was assessed according to Barrs & Weatherley, (1962). Leaves were collected and weighed immediately to record fresh weight. The leaves were then soaked in distilled water for 24 h to record the turgid weight of the leaves. The samples were oven-dried at 70°C for 24 h to record the dry mass. The dry mass of the samples was recorded using a high precision balance and calculated. Agronomic data for plant height, spikes per plant, grain yield per plant, spike length, straw weight per plant, thousand kernels weight and tillers per plant were recorded after harvesting the crop.

Statistical analysis

Data were statistically analyzed for analysis of variance (ANOVA), Duncan's multiple range test (DMRT), Pearson's correlation, hierarchical clustering for cluster dendrogram with centroid linkage using squared Euclidean distance and Principal component analysis (PCA) using SPSS 20.0 statistical software (Armonk, NY: IBM Corp). Drought tolerance indexes were calculated using grain yield per plant means according to the (Fernandez, 1992), (Fischer & Maurer, 1978), (Rosielle & Hamblin, 1981) and (Farshadfar *et al*., 2013) as under:

- Stress tolerance index (STI)
  STI = (Yd × Yc)/(xc)
- Drought Susceptibility Index (DSI)
  DSI = (1 − Yd⁄Yc) ⁄ (1 − xd⁄xc)
- Mean Productivity (MP)
  MPI = (Yc + Yd)/2
  - Geometric mean productivity (GMP)
  - GMP = √Yc × Yd
  - Harmonic Mean (HM)
  HMI = 2 (Yc × Yd)/ (Yc + Yd)
  - Tolerance (TOL)
  TOL = Yc – Yd

where Y; grain yield per plant, d; water stress (T1), c; well-watered and x; overall mean.

Results

Meteorological conditions: Maximum and minimum temperatures during crop growing season are provided in (Table 1). The highest temperature during the crop growth period was recorded during February (25.4°C) and April (39.0°C), while lowest in April (20.9°C) and during March (17.1°C). The lowest humidity, 43.5% was observed during April and highest 59.2% during January.

Analysis of variance (ANOVA): The mean performance of the wheat genotypes for observed morphological, physiological and biochemical traits is summarized in (Table 2). Pooled analysis of variance (ANOVA) indicated significant variability (p<0.01) among wheat genotypes for all the observed traits under two treatments. Interaction for genotype and treatments (G × T) was highly significant for glycine-betaine, nitrate reductase activity, osmotic potential, proline content, total chlorophyll content, flag leaf area, plant height, spike length and straw weight per plant under two treatments (Table 3). Coefficient of variation ranged from 8.0-70.1% under water stress. The highest coefficient of variation in water stress was recorded for proline content (70.1%) followed by total chlorophyll content (55.8%) and tillers per plant (27.9%). (Fig. 1) presents the agronomic, physiological and biochemical traits variability of the wheat genotypes under water stress (T1) and control conditions (T2).

Table 1. Mean ± standard error of the environmental conditions during the evaluation of 26 wheat genotypes under water stress and well-watered conditions.

Months	Total Rain (m.m)	Min. Temp. (°C)	Max. Temp. (°C)	Relative Humidity (%)	Sunshine (Hours)	Evaporation (m.m/day)
Nov.	0 ± 0	14.5 ± 0.39	30.3 ± 0.41	48.4 ± 1.39	8.63 ± 0.10	4.41 ± 0.14
Dec.	0 ± 0	8.71 ± 0.44	25.8 ± 0.46	58.0 ± 1.05	8.6 ± 0.11	3.93 ± 0.81
Jan.	0 ± 0	10.3 ± 0.34	25.4 ± 0.40	59.2 ± 1.15	8.05 ± 0.32	2.2 ± 0.12
Feb.	0 ± 0	9.24 ± 0.34	28.4 ± 0.63	44.1 ± 0.89	9.1 ± 0.09	3.38 ± 0.15
Mar.	0 ± 0	17.1 ± 0.41	34.1 ± 0.51	47.0 ± 1.59	9.11 ± 0.24	5.04 ± 0.15
Apr.	0 ± 0	20.9 ± 0.27	39.0 ± 0.46	43.5 ± 1.17	9.43 ± 0.40	5.98 ± 0.16
May	0 ± 0	25 ± 0.27	41.1 ± 0.41	49.7 ± 1.16	10.7 ± 0.13	7.26 ± 0.10

Table 2. Means performance, standard deviation (SD), coefficient of variation (CV) and mean range for morpho-physiological and biochemical traits of 26 wheat genotypes under water stress (T1) and control conditions (T2).

Traits	Water stress (T1)			Well-watered (T2)		
	Overall mean ± SD	C.V.	Mean range	Overall mean ± SD	C.V.	Mean range
Flag leaf area (cm^2)	29.3 ± 4.29	14.7	22.3-38.4	38.6 ± 4.08	10.6	32.4-47.2
Plant height (cm)	62.7 ± 5.00	8.0	46.7-69.7	82.1 ± 8.76	10.7	52.7-93.7
Spike length (cm)	10.2 ± 1.54	15.1	7.53-13.0	12.8 ± 1.14	8.9	10.9-14.7
Grain yield per plant (g)	15.9 ± 2.27	14.3	12.2-19.9	20.6 ± 1.67	8.11	17.8-23.6
Straw weight (g./ plant)	10.6 ± 2.17	20.5	6.2-14.5	28.5 ± 7.10	24.9	13.3-41.1
Thousand kernels weight (g)	32.0 ± 5.13	16.0	21.7-39.4	42.5 ± 2.76	6.50	35.7-46.2
Tillers per plant	7.15 ± 1.99	27.9	4.00-11.0	9.77 ± 1.63	16.7	7.00-13.0
Glycine-betaine (μ mol^{-1} fresh weight)	39.5 ± 10.5	26.4	19.6-56.4	15.6 ± 4.09	26.3	9.80-23.2
Potassium content (mg. g^{-1})	3.00 ± 0.34	11.5	2.27-3.69	2.58 ± 0.32	12.4	2.17-3.18
Nitrate reductase activity (μ mol^{-1} fresh wt. hr^{-1})	0.15 ± 0.03	22.6	0.09-0.20	0.23 ± 0.05	21.4	0.14-0.36
Osmotic potential (-Mpa)	1.15 ± 0.12	10.2	0.86-1.36	0.71 ± 0.09	12.6	0.56-0.86
Proline content (μ mol^{-1} fresh weight)	53.5 ± 37.5	70.1	7.80-95.1	10.9 ± 6.2	56.5	4.62-28.0
Relative water content (%)	55.0 ± 9.11	16.6	34.7-68.9	75.5 ± 5.25	7.0	60.9-85.5
Total chlorophyll content (mg/g fresh wt.)	0.42 ± 0.24	55.8	0.10-0.95	0.74 ± 0.22	29.4	0.26-0.99
Total soluble sugars (μ mol^{-1} fresh weight)	1.10 ± 0.24	21.4	0.80-1.55	0.89 ± 0.12	13.7	0.69-1.18

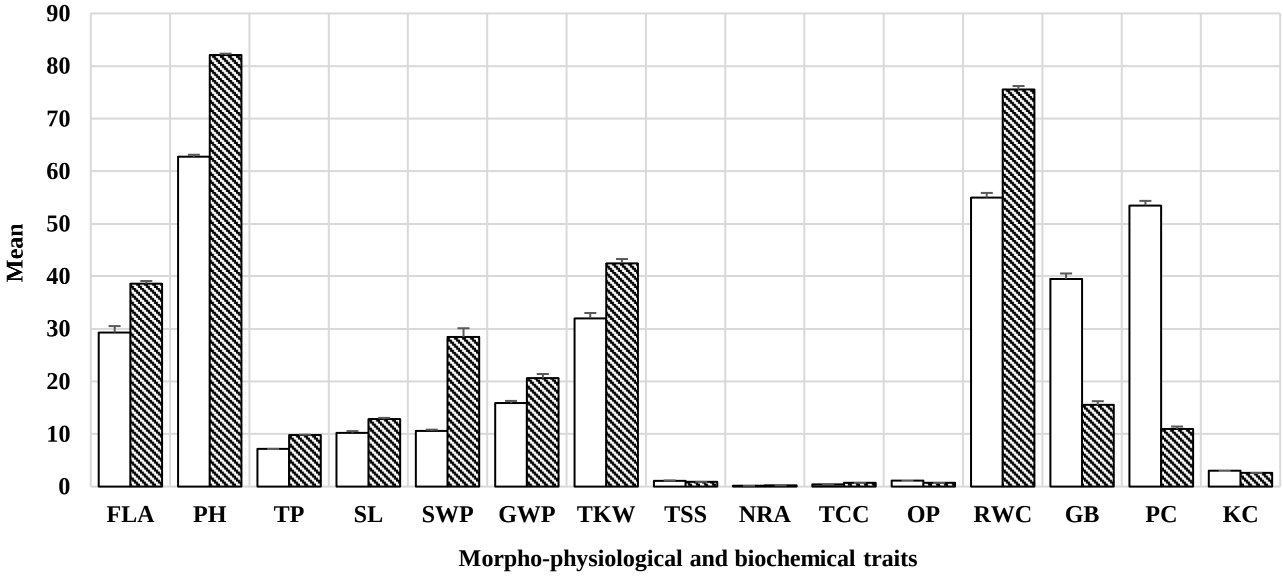

Fig. 1. Mean performance of 26 wheat genotypes for morph-physiological and biochemical traits under moisture stress and well-watered conditions.

Agronomic traits: Agronomic traits of the wheat genotypes were highly affected under water stress. Water stress expressed -42.0% reduction in the overall means for flag leaf area of the plants under water stress. Flag leaf area means were in the range of 2.97-38.4 and 2.47-47.2 cm^2 under water stress and well-watered, respectively. Under water stress, IBWSN-1144 and IBWSN-1149 expressed excelled flag leaf area means of 38.4 and 35.3 cm^2, whereas IBWSN-1157 and NIA-Sarsabz had the lowest flag leaf area means (22.3 and 22.4). Plant height ranged from 46.7-69.7 cm in water stress and from 52.7-93.7 cm under well-watered treatment. Results exhibited a reduction of -23.6% in the plant height of the wheat genotypes under water stress conditions. Under water stress, MASR-22 and Khirman attained the tallest height (69.7 and 69.7 cm), whereas TD-1 and IBWSN-1025 had lowest plant height (46.7 and 57.3 cm). Results revealed a decrease of 20.3% for spike length among the test entries in water stress. Spike length means ranged between 7.53 and 13.0 cm in water stress and from 10.9-14.7 cm^2 in well-watered conditions. Wheat genotypes ESW-9525 and NIA-Amber possessed the highest means of 13.0 and 12.3 cm for spike length under water stress, however, all the contesting lines had the highest spike length means than drought susceptible check TD-1 (7.53). Straw weight per plant values were in the range between 6.2 and 14.5 g and 13.3 and 41.1 g, respectively under water stress and well-watered regimes. Under water stress, straw weight per plant means of wheat lines expressed reduction of -62.8% as compared to well-watered treatment. DH-12/7 (14.5 g), ESW-9525 (14.3 g) and MASR-22 (14.2 g) produced the highest straw weight per plant in water stress. Results revealed the reduction of -23.3% in grain yield per plant under water stress.

Grain yield per plant values ranged from 12.2 to 19.9 g and from 17.8 to 23.6 g under water stress and control conditions. Results depicted excelled grain yield per plant in NIA-Saarang (19.9 g) and Khirman (19.2 g) as compared to all test entries under water stress. Nine test entries had excelled means for grain yield per plant than drought susceptible check TD-1 (15.2 g). Meanwhile, decreased grain yield per plant in water stress was indicated among the wheat genotypes DH-9/6 (12.2 g) and MASR-22 (12.3 g). Water stress decreased the grain yield per plant overall values by -24.7%. Means for thousand kernels weight ranged 21.7-39.4 g under water stress and 35.7-46.2 g under control conditions. Water stress decreased thousand kernels weight of the plants by -24.7%. IBWSN-1010 (37.8 g) had excelled means for thousand kernels weight under water stress as compared to other wheat genotypes including Khirman (37.7 g) and Chakwal (37.3 g). Water stress reduced the number of tillers per plant of wheat genotypes by -26.8% as compared to well-watered conditions. The means range for tillers per plant of the wheat genotypes under water stress and control conditions were recorded 4.00-11.0 and 7.00-13.0, respectively. IBWSN-1025, IBWSN-1042, DH-12/7 and DH-12/31 had the highest number of tillers per plant than drought susceptible check variety TD-1.

Plant physiological and biochemical traits: Water stress increased overall means (62.0%) for osmotic potential. Significantly the increased osmotic potential means under moisture stress were observed in Khirman, Chakwal-86 and MASR-64 (1.36, 1.33 and 1.32, respectively), whereas high yielding check variety Benazir (1.06), DH-9/6 (1.06), MASR-22 (0.98) and IBWSN-1010 (0.91) had the lowest osmotic potential means. Under drought stress, increased relative water content means were assigned to ESW-9525 (68.9), Chakwal-86 (67.6), Khirman (67.1), NIA-Amber (66.6) and NIA-Saarang (66.2) followed by wheat genotypes DH-12/7 (64.2) and DH-12/31 (63.2), while decreased relative water content was observed in MASR-08 (47.6), MASR-22 (45.1), MSH-14 (44.5), Benazir (43.3), NIA-Sarsabz (40.5) and T.D-1 (34.7).

Under highly stressed conditions, wheat genotypes Khirman, ESW-9525, NIA-Amber and DH-12/7 attained the highest means of 56.4, 54.9, 54.7 and 51.8, respectively for glycine-betaine, whereas the lowest glycine-betaine values were observed among drought susceptible wheat genotypes Benazir and T.D-1 (20.5 and 19.6). Under control conditions, wheat genotypes DH-9/6, DH-9/1 and IBWSN-1156 had maximum (31.0, 35.5 and 36.5, respectively) glycine-betaine, besides, IBWSN-1042 and drought susceptible check variety Benazir possessed the lowest means (10.3 and 20.5) for glycine-betaine. Increased accumulation of K^+ content under drought conditions was observed among IBWSN-1148 (3.31) and IBWSN-1132 (3.29) than drought-tolerant check Chakwal-86 (3.27), whereas lowest values for K^+ were observed among the wheat genotypes IBWSN-1149 and IBWSN-1010 with the means of 2.50 and 2.27.

Water stress reduced nitrate reductase activity among the wheat genotypes compared to the well-watered treatment. Maximum values for nitrate reductase activity under water stress conditions were among the drought-tolerant wheat genotypes NIA-Saarang (0.2), ESW-9525 (0.2), IBWSN-1148 (0.2), MASR-64 (0.19), IBWSN-1042 (0.19) and IBWSN-1156 (0.19), meanwhile, the lowest values were observed among IBWSN-1010 (0.11), DH-9/1 (0.1) and IBWSN-1149 (0.09). Highly significant and increased proline content (89.6, 89.3 and 86.2) was produced in DH-12/7, DH-12/31 and MASR-64, respectively, following three check varieties NIA-Saarang (90.5), NIA-Amber (90.0) and Khirman (77.8), besides, MASR-08, MASR-22, MSH-14, NIA-Sarsabz, Benazir and T.D-1 synthesized the decreased proline content under water stress condition ranged from 7.80 to 10.0.

Water stress significantly decreased total chlorophyll content in the plants. Here in this study, we found wheat genotype DH-12/7 with increased total chlorophyll content (0.71) synthesis following three check varieties NIA-Saarang (0.95), Chakwal-86 (0.80) and NIA-Amber (0.74), while the lowest values for total chlorophyll content than drought susceptible check T.D-1 (0.13) were observed in MSH-14 and DH-9/6 with the means of (0.12 and 0.10). Results showed an increase of total soluble sugar under water stress as compared to the control. Commercial wheat varieties NIA-Amber and NIA-Saarang along with MASR-64 exhibited significantly the highest total soluble sugar (1.55, 1.52 and 1.52) under water stress conditions, while lowest (0.80) in MASR-08 and IBWSN-1010. In the young vegetative stage of growth and development, increased carbohydrate synthesis can be advantageous towards water stress tolerance.

Traits association: Pearson's correlations among the morphological and physio-biochemical traits of wheat genotypes under moisture stress and control conditions are presented in (Table 4). Thousand kernels weight expressed strong positive correlation with flag leaf area (r = 0.57**), spike length (r = 0.67**), tillers per plant (r = 0.68**), grains weight per plant (r = 0.82**), glycine-betaine (r = 0.75**), potassium content (r = 0.60**), proline content (r = 0.90**), relative water content (r = 0.79**) and total chlorophyll content (r = 0.71**) under moisture stress conditions. Grains weight per plant had positive correlation with spike length (r = 0.77**), tillers per plant (r = 0.84**), thousand kernels weight (r = 0.82**), glycine-betaine (r = 0.81**), potassium content (r = 0.75**), osmotic potential (r = 0.52**), proline content (r = 0.84**), relative water content (r = 0.81**) and total chlorophyll content (r = 0.66**) under moisture stress conditions. Significant positive correlations of plant height under water-limited environment existed with spike length (r = 0.53**), glycine-betaine (r = 0.52**), relative water content (r = 0.56**) and total chlorophyll content (r = 0.52**).

Cluster association: The dendrogram depicted the grouping of 26 wheat genotypes into two clusters in water-stress and control conditions. In moisture-stressed conditions, clusters I and II consisted of 14 and 12 wheat genotypes, respectively (Fig. 2). Cluster I have comprised of the susceptible advanced lines and drought susceptible cultivars including NIA-Sarsabz, Benazir, IBWSN-1157, DH-9/1, IBWSN-1149, MASR-22, DH-9/7, MSH-14, MASR-08, IBWSN-1156 and TD-1. Meanwhile, cluster II contained the drought-tolerant wheat cultivars and advanced wheat genotypes bestowed with the highest means for morpho-physiological and biochemical traits viz., ESW-9525, NIA-Saarang, NIA-amber, Khirman, IBWSN-1010, IBWSN-1025, IBWSN-1150, DH-12/31, Chakwal-86, IBWSN-1142, IBWSN-1132, IBWSN-1148, IBWSN-1144 and MASR-64. In control conditions, clusters I and II contained 10 and 16 wheat genotypes, respectively. Cluster I consisted of IBWSN-1156, IBWSN-1157, DH-9/1, MSH-14, TD-1, MASR-22, Benazir, MASR-08, DH-9/6 and NIA-Sarsabz, while the rest of wheat genotypes ESW-9525, NIA-Sarang, NIA-Amber, DH-12/7, Chakwal-86, Khirman, IBWSN-1025, DH-12/31, IBWSN-1148, IBWSN-1150, IBWSN-1042, IBWSN-1010, IBWSN-1149, IBWSN-1132, MASR-64 and IBWSN-1144 were included in cluster II.

Drought indices: Wheat genotypes exhibited highly significant variability for drought indices (Table 5). Nine test entries possessed the drought susceptibility index (DSI) lowest than 1. Wheat genotypes IBWSN-1144 (0.75), IBWSN-1150 (0.55), DH-12/7 (0.72), DH-12/31 (0.73), MASR-64 (0.66) had the lowest DSI than drought-tolerant check Chakwal-86 (0.84) and high yielding variety NIA-Saarang 0.69. Results showed ten advanced lines with the highest stress-tolerant index (%) than drought susceptible check TD-1 (14.4). The stress tolerance index (%) was highest in IBWSN-1025 (19.5) and DH-12/31 (19.5) than drought-tolerant check Chakwal-86 (17.8). Test entries IBWSN-1025, IBWSN-1042 and DH-12/31expressed the respective highest 19.6, 19.1 and 19.8 harmonic mean index than drought-tolerant check Chakwal-86. Highest stress index was reported among the wheat varieties IBWSN-1042 (0.83), IBWSN-1144 (0.83), IBWSN-1148 (0.83), IBWSN-1150 (0.87), DH-12/7 (0.83), DH-12/31 (0.83), ESW-9525 (0.90) highest than drought-tolerant check Khirman (0.83).

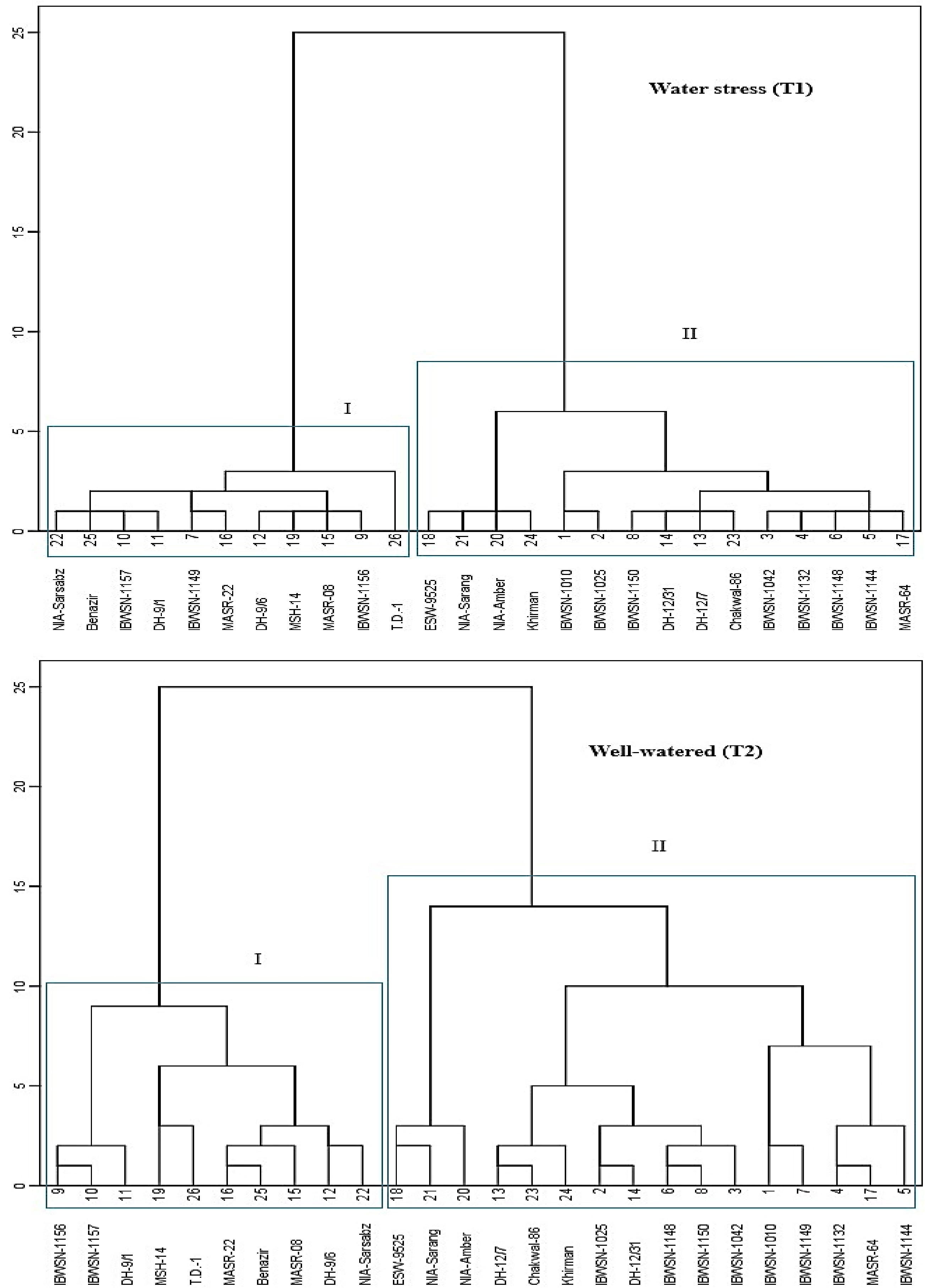

Fig. 2. Hierarchical clustering of 26 wheat genotypes for morpho-physiological and biochemical traits under water stress (T1) and control conditions (T2).

Table 3. Means square from combined analysis of variance (ANOVA) for observed morpho-physiological and biochemical traits of 26 wheat genotypes evaluated under water stress (T1) and control conditions (T2).

Source of variation	Df	FLA	PH	SL	GWP	SWP	TKW	TP	GB	KC	NRA	OP	PC	RWC	TCC	TSS
Genotypes (G)	25	82.2**	236.3**	10.4**	21.1**	127.5**	87.8**	19.3**	220.8**	0.57**	0.01**	0.03**	2673.3**	242.4**	0.22**	0.17**
Treatments (T)	1	3375.5**	14616.0**	263.4**	875.1**	12476.0**	4255.5**	266.8**	22405.7**	6.99**	0.25**	7.61**	70578.5**	16471.0**	3.90**	1.71**
Replications (R)	2	105.0	11.5	4.16	57.3	127.7	125.1	0.25	103.0	0.03	0.001	0.0003	72.4	18.7	0.03	0.31
G × T interactions	25	23.5**	69.2**	0.69**	2.70^{ns}	37.9**	14.3^{ns}	0.61^{ns}	157.6**	0.1^{ns}	0.006**	0.035**	1658.0**	89.6^{ns}	0.09**	0.04^{ns}
Error	104	11.4	17.7	0.80	2.69	12.7	9.08	1.58	9.4	0.062	0.0007	0.0004	14.2	55.6	0.001	0.047
Total	155															

* Significant at $p<0.05$, ** Highly significant ** $p<0.01$. FLA; Flag leaf area, PH; Plant height, SL; Spike length, GWP; Grain weight per plant, SWP; Straw weight per plant, TKW; Thousand kernels weight, TP; Tillers per plant, GB; Glycine-betaine, KC; Potassium content, NRA; Nitrate reductase activity, OP; Osmotic potential, PC; Proline content, RWC; Relative water content, TCC; Total chlorophyll content and TSS; Total soluble sugars

Table 4. Pearson's correlation among the observed physiological and biochemical traits under water stress (upper diagonal) and well-watered conditions (lower diagonal).

Traits	FLA	PH	SL	SWP	TP	GWP	TKW	GB	KC	NRA	OP	PC	RWC	TCC	TSS
FLA	1	0.07	0.47*	0.33	0.34	0.37	0.57**	0.52	0.35	0.33	0.13	0.55**	0.51**	0.38	-0.09
PH	-0.18	1	0.53**	0.49**	0.19	0.32	0.30	0.52**	0.09	0.06	0.03	0.36	0.56**	0.52**	-0.03
SL	-0.21	0.20	1	0.41*	0.70**	0.77**	0.67**	0.89**	0.56**	0.41*	0.39*	0.72**	0.88**	0.61**	0.21
SWP	0.23	0.18	0.46*	1	0.39*	0.37	0.18	0.38	0.22	0.04	0.13	0.20	0.36	0.35	0.16
TP	-0.28	-0.37	0.56**	0.32	1	0.84**	0.68**	0.64**	0.75**	0.37	0.43*	0.61**	0.67**	0.60**	0.55**
GWP	-0.38	-0.09	0.52**	0.24	0.67**	1	0.82**	0.81**	0.75**	0.50*	0.52**	0.84**	0.81**	0.66**	0.38
TKW	-0.02	-0.28	0.42*	0.03	0.57**	0.45*	1	0.75**	0.60**	0.40*	0.36	0.90**	0.79**	0.71**	0.19
GB	0.15	0.15	0.13	0.14	0.09	0.18	0.10	1	0.50**	0.37	0.43*	0.85**	0.97**	0.64**	0.08
KC	-0.19	-0.24	0.29	0.01	0.23	0.25	0.23	0.00	1	0.70**	0.64**	0.66**	0.51**	0.55**	0.46*
NRA	0.26	0.10	-0.61**	-0.36	-0.70**	-0.52**	-0.50**	-0.02	-0.38	1	0.57**	0.54**	0.40*	0.53**	0.25
OP	0.09	0.28	-0.35	0.05	-0.27	-0.21	-0.55**	0.11	-0.45*	0.40*	1	0.47*	0.40*	0.35	0.25
PC	-0.27	-0.25	0.51**	0.29	0.57**	0.64**	0.50**	0.06	0.62**	-0.49*	-0.48*	1	0.85**	0.70**	0.07
RWC	0.05	0.12	0.51**	0.40*	0.18	0.40*	0.26	0.34	0.11	-0.20	-0.33	0.30	1	0.73**	0.11
TCC	-0.04	0.51**	0.01	0.12	-0.12	-0.17	-0.19	0.31	-0.13	0.25	0.16	0.01	0.19	1	0.23
TSS	-0.24	-0.18	0.16	0.06	0.39	0.37	0.11	0.22	0.36	-0.12	0.22	0.40*	-0.14	0.07	1

* Significant ($p<0.05$). **Highly significant ($p<0.01$) 2 tailed. FLA; Flag leaf area, PH; Plant height, SL; Spike length, SWP; Straw weight per plant, GYP; Grain yield per plant, TKW; Thousand kernels weight, TP; Tillers per plant, PC; Proline content, RWC; Relative water content, GB; Glycine-betaine, TSS; Total soluble sugars, NRA; Nitrate reductase activity, TCC; Total chlorophyll content, OP; Osmotic potential and KC; Potassium content

Table 5. Drought indices of 26 wheat genotypes using grain yield per plant under water stress and well-watered conditions.

Genotypes	DSI	STI	MPI	HMI	TOL	GMP	PD
IBWSN-1010	1.10	14.5	17.6	17.0	5.60	17.3	-39.3
IBWSN-1025	0.90	19.1	19.9	19.6	4.53	19.8	-25.7
IBWSN-1042	0.84	17.9	19.3	19.1	3.97	19.2	-23.5
IBWSN-1132	0.85	15.0	17.7	17.4	3.90	17.5	-26.8
IBWSN-1144	0.75	14.3	17.3	17.1	3.17	17.2	-20.4
IBWSN-1148	0.81	15.5	18.0	17.7	3.40	17.9	-23.3
IBWSN-1149	1.10	11.4	15.5	15.1	4.57	15.3	-34.4
IBWSN-1150	0.55	17.3	19.1	18.6	3.60	18.9	-24.2
IBWSN-1156	1.50	15.2	18.1	17.3	7.40	17.7	-51.6
IBWSN-1157	1.11	15.0	17.8	17.4	5.27	17.6	-35.0
DH-9/1	1.59	15.3	18.2	17.3	8.13	17.7	-57.4
DH-9/6	1.37	10.6	15.1	14.5	5.70	14.8	-46.1
DH-12/7	0.72	17.4	19.0	18.9	3.43	18.9	-19.9
DH-12/31	0.73	19.1	19.9	19.8	3.53	19.8	-19.6
MASR-08	1.48	12.8	16.5	15.9	6.73	16.2	-51.4
MASR-22	1.53	11.2	15.5	14.9	6.50	15.2	-53.1
MASR-64	0.66	15.4	18.0	17.6	3.80	17.8	-24.2
ESW-9525	0.34	22.0	21.5	21.0	3.63	21.2	-18.3
MSH-14	1.39	12.7	16.5	15.8	6.33	16.2	-47.5
NIA-Amber	0.76	20.7	20.7	20.5	3.87	20.6	-20.8
NIA-Sarang	0.69	22.8	21.7	21.6	3.73	21.7	-18.8
NIA-Sarsabz	1.18	12.6	16.3	15.9	5.13	16.1	-37.2
Chakwal-86	0.84	17.8	19.2	19.0	4.10	19.1	-24.1
Khirman	0.74	21.8	21.3	21.1	4.13	21.2	-21.8
Benazir	1.07	13.0	16.5	16.2	4.60	16.3	-32.2
T.D-1	1.07	14.4	17.5	17.0	4.57	17.2	-35.2

DSI; Drought susceptibility index, STI; Stress tolerance index, MPI; Mean productivity index, HMI; Harmonic mean index, TI; Tolerance index, GMP; Geometric mean productivity, PD; Percent decrease

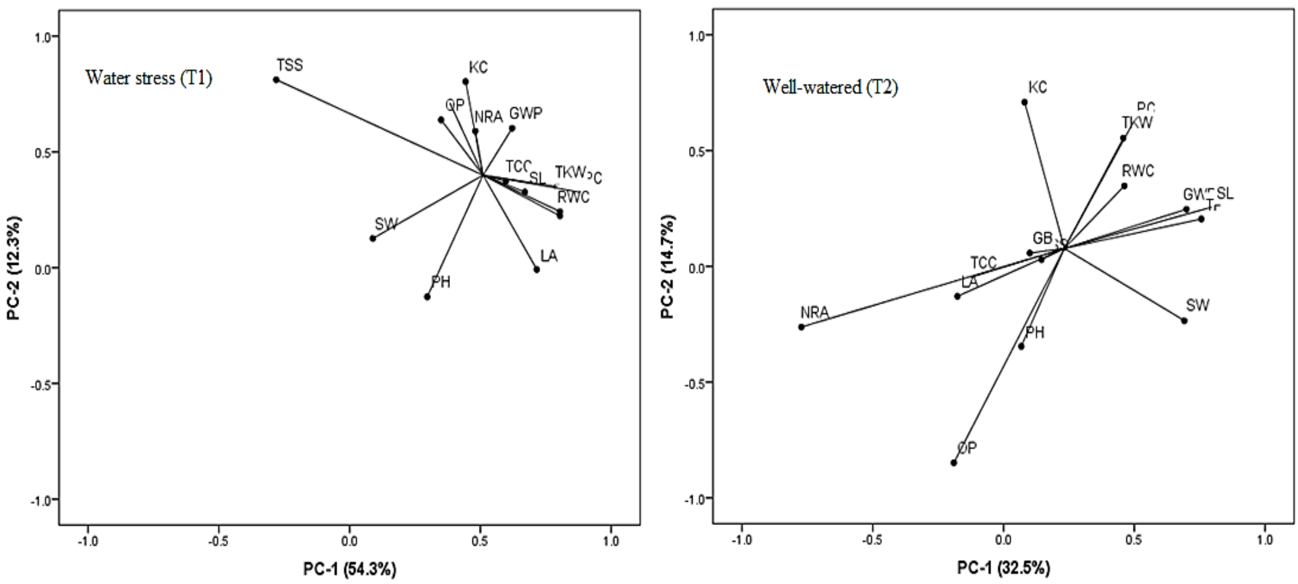

Fig. 3. Principal component analysis of observed morpho-physiological traits of 26 wheat genotypes under water stress (T1) and well-watered control conditions.

Table 6. Principal component analysis of 26 wheat genotypes for morpho-physiological and biochemical traits under moisture stress and well-watered conditions

Treatments	Axes	LA	PH	SL	SW	TP	GWP	TKW	GB	KC	NRA	OP	PC	RWC	TCC	TSS	Eigen value	Proportion of variance (%)	Cumulative proportion (%)
Water stress (T1)	1	0.550	0.454	0.872	0.422	0.816	0.923	0.860	0.897	0.767	0.591	0.558	0.901	0.914	0.798	0.309	8.138	54.25	54.25
	2	-0.209	-0.615	-0.203	-0.352	0.235	0.112	-0.043	-0.285	0.503	0.463	0.474	-0.065	-0.282	-0.084	0.572	1.849	12.33	66.58
	3	-0.409	0.353	0.076	0.545	0.294	0.083	-0.215	-0.081	0.011	-0.259	-0.086	-0.305	-0.060	0.052	0.613	1.290	8.60	75.18
Well-watered (T2)	1	-0.176	0.068	0.797	0.692	0.756	0.699	0.458	0.100	0.081	-0.773	-0.190	0.496	0.462	-0.127	0.145	4.872	32.48	32.48
	2	-0.129	-0.346	0.256	-0.235	0.205	0.247	0.554	0.057	0.709	-0.262	-0.848	0.622	0.348	-0.048	0.029	2.210	14.73	47.22
	3	-0.141	0.760	0.233	0.194	-0.265	-0.014	-0.233	0.485	-0.028	0.199	0.117	0.020	0.463	0.816	-0.007	1.637	10.91	58.13

LA; Leaf area, PH; Plant height, SL; Spike length, SW; Straw weight, TP; Tillers per plant, GWP; Grains weight per plant, TKW; Thousand kernels weight, GB; Glycine-betaine, KC; Potassium content, NRA; Nitrate reductase activity, OP; Osmotic potential, PC; Proline content, RWC; Relative water content, TCC; Total chlorophyll content and TSS; Total soluble sugars

Principal component analysis (PCA): The principal component analysis is used to explain the relative contribution of the traits to the genetic diversity of genotypes. Principal component analysis (PCA) reduces the dimensionality of large data set of variables by constructing new smaller variables called principal components through a linear combination of initial variables. Principal components explain a large amount of variance through the lines by capturing the data information. Under moisture stress and well-watered conditions, the cumulative variance by the first three-axis was 75.18 % and 58.13%, respectively (Table 6). Under moisture stress, the respective variability of 54.25%, 12.33% and 8.6% were revealed by principal components 1, 2 and 3, respectively (Fig. 3). Meanwhile, 32.48%, 14.73%, 10.91% variation was accounted to the principal component 1, 2 and 3, respectively. In moisture stress conditions, the first principal component. Three traits including grains weight per plant, relative water content and proline content expressed positive highest contribution of 0.923%, 0.914% and 0.901%, respectively to the total variation of the first principal component. Under well-watered conditions, the first principal component total variation had the highest positive contribution glycine-betaine (1.00) and had the highest negative contribution from glycine-betaine (1.0).

Discussion

Climate change has increased the intensity of drought spells resulting into major grain yield losses among the cereal crops. Development of drought tolerant wheat genotypes accompanied with grain yield increase is critically important (Serraj *et al*., 2011). Plant breeding programs are focusing on the development of drought tolerant wheat genotypes with grain yield increase. Assessment of such newly evolved wheat genotypes has necessity in the selection of drought tolerant genetic material. This study aimed to investigate the genotypic differences among 26 wheat genotypes for their acclimation to water stress conditions using 15 drought tolerance associated agronomic, physiological and biochemical traits viz. flag leaf area, plant height, spike length, straw weight per plant, grain yield per plant, thousand kernels weight, tillers per plant, proline content, relative water content, glycine-betaine, total soluble sugars, nitrate reductase activity, total chlorophyll content, osmotic potential and potassium content. Additionally, grain yield of the plants under water stressed and well-watered treatments was subjected to drought tolerance indexes to determine the tolerance and susceptibility of the wheat genotypes. ANOVA showed highly significant genotypic variability among the test entries for all the observed traits under two treatments. Meanwhile, genotype × treatment interaction was also significant for most of the traits. Results are contemporary with the findings of Mwadzingeni *et al*., (2016). Genotypic variability for the traits provides a choice of selection between well-performing high grain yielding and drought susceptible wheat genotypes and is highly needed in the breeding programs.

Water stress triggers some of the important internal mechanisms leading to various phenotypic changes in the plants. In this study, drought stress significantly reduced the values of plant growth and allied agronomic traits including flag leaf area, plant height, spike length, straw weight per plant, spikes per plant, grain yield per plant and thousand kernels weight. Nitrate reductase activity, relative water content and total chlorophyll content of wheat genotypes were also decreased in the plants under drought stress. On the contrary, water stress increased osmolytes accumulation in wheat genotypes including glycine-betaine, potassium content, osmotic potential, proline content and total soluble sugars. Results indicated that the grain weight of the plants under moisture stress conditions had excelled means due to the accumulation of various osmolytes. We observed strong positive correlation of thousand kernels weight of the plants with flag leaf area, spike length, tillers per plant, grains weight per plant, glycine-betaine, potassium content, proline content, relative water content and total chlorophyll content under moisture stress conditions. This suggests that in water-limited conditions, the accumulation of osmolytes in the plants such as proline content, potassium content and glycine-betaine increase the weight of the kernel by maintaining the osmotic gradient across the roots. Under drought conditions reduced leaf area of the plants is considered a drought adaptive water-saving trait. Flag leaves are developed very close to the spikes, hence considered as the initial source of assimilation for grain filling adding to the ultimate grain yield gains and remains green for a longer period than the rest of the plant leaves (Khaliq *et al*., 2004). Leaf area and photosynthesis are reduced under drought stress conditions by accelerating chlorophyll degradation that leads to senescence increase (del Pozo *et al*., 2016). Findings from this work showed significant reduction in PH of the wheat genotypes grown under water stress conditions as compared to the plants grown under well-watered conditions. This research expressed that plant height can contribute to water supply under harsh water-scarce environments thus increasing the relative water content and total chlorophyll content.

Osmotic adjustment is the acclimation of the plants to facilitate osmoregulation under water stress conditions. This mechanism due to osmolytes accumulation is considered as an adaptation to improve drought tolerance in the plants (Shao *et al*., 2005). Osmolyte accumulation mitigates dehydration conditions in the plants and provides structural integrity to the membranes (Loutfy *et al*., 2012). Abid *et al*., (2016) and Seher *et al*., (2015) reported osmotic adjustment in the plants under water stress through accumulation of osmolytes such as proline, free amino acids and increased enzymatic and non-enzymatic antioxidant activities. In this experiment, a significant increase in the osmotic potential of the wheat genotypes was recorded under moisture stress conditions due to accumulation of various types of osmolytes in the plants. As mentioned earlier, grain yield is a polygenic trait and is under control of various cumulative intrinsic and extrinsic traits. However, results in this study showed that wheat genotypes with increased osmotic adjustment had also increased grain yield potential.

Water stress resulted in decrease of Nitrate reductase activity (NRA) among the wheat genotypes as compared to the control (Table 2). Nitrate reductase enzyme converts nitrate to nitrite, a critical step in protein formation. We observed an increase of total soluble sugars in wheat genotypes under water stress conditions. Drought increases the accumulation of both mono- and disaccharide soluble sugars and sugar alcohols such as fructans, myo-inositol and mannitol (Williamson *et al*., 2002). It has been widely reported that plants accumulate soluble carbohydrates as a response to water stress (Zhang *et al*., 2009). Increased carbohydrates concentration in the plants under drought decreases water potential, prevents oxidative damage and maintains the structure of proteins and membranes under moderate dehydration during the water stress period (Hoekstra *et al*., 2001). In the young vegetative stage of growth of plants, an increased carbohydrate synthesis can be advantageous towards water stress tolerance. This study showed a positive role of proline content in the grain yield increase of plants. Proline is one of the amino acids osmolyte playing an important role in stabilizing the cellular membranes and proteins (Errabii *et al*., 2006). Plants accumulate proline as one of the common osmolytes under water stress conditions (Marcińska *et al*., 2013). Total soluble sugars and proline have a key role in water stress tolerance (Johari-Pireivatlou *et al*., 2010).

The findings of the present study showed an increase of glycine-betaine means under water stress treatment. Glycine-betaine maintains the osmotic potential inside the cell, regulates cytoplasmic pH and stabilizes cell membrane structures in the wheat under drought. Huseynova *et al*., (2016), and Guo *et al*., (2018) reported that proline and glycine-betaine (quaternary ammonium compound) are often accumulated under drought stress conditions. Both compounds play an osmo-protective role in balancing the cell turgidity (Marček *et al*., 2019). Potassium (K^+) performs various functions in the plants under water stress conditions including osmoregulation, upholding turgor pressure, stomatal regulation, reducing transpiration, protein biosynthesis and charge-balanced (Ahmad *et al*., 2018). We observed an increase in the potassium content of the plants under a limited water environment. Potassium (K^+) regulates the opening and closing of the stomata in the plants. K^+ affects osmoregulation in the plants and is critical in the adaptation and avoidance in the drought and its deficiency reduces drought tolerance (Tränkner *et al*., 2018). Water stress decreased the relative water content in the plants under water stress as compared to the well-watered treatment. Drought stress decreases relative water content in the plants (Geravandi *et al*., 2011). Results agree with the findings of (Siddique *et al*., 2000), who reported a significant decrease in the relative water content in the wheat genotypes under drought stress conditions. According to Tatar & Gevrek, (2008), drought stress decreases biological weight and relative water content in the plants, whereas proline content increases.

Drought indices are widely used to indicate the drought tolerance of the wheat genotypes. Arifuzzaman *et al*., (2020) reported that wheat genotypes with the drought susceptible index (DSI) value less than 1 could be and be stable under water stress and well-watered conditions and recommended in the breeding programs. We observed nine test entries possessing the drought susceptibility index (DSI) lowest than 1. Wheat genotypes included IBWSN-1144, IBWSN-1150, DH-12/7, DH-12/31 and MASR-64 had the lowest DSI than drought-tolerant check Chakwal-86 (0.84) and high yielding variety NIA-Saarang. Wheat genotypes with the lowest drought susceptibility index showed the highest means for morpho-physiological and biochemical traits. IBWSN-1025, IBWSN-1042, IBWSN-1132, IBWSN-1144 and IBWSN-1148 exhibited increased grain yield per plant, thousand kernels weight, glycine-betaine, potassium content, proline content, relative water content and total soluble sugar under water stress conditions. Under water-stress conditions, IBWSN-1010 bestowed with highest thousand kernels weight. Among other contenting lines IBWSN-1148 depicted increased potassium content, nitrate reductase activity and spike length. IBWSN-1132 showed highest potassium content. IBWSN-1144 depicted highest osmotic potential. DH-12/7 had highest glycine-betaine, proline content, relative water content, total chlorophyll content and straw weight. Wheat genotype DH-12/31 expressed increased glycine-betaine and proline content, spike length and grain yield per plant. MASR-64 possessed the highest nitrate reductase activity, osmotic potential, proline content and total soluble sugars. Test entries also showed increased means for tolerance indexes showing tolerance under water stress. In this study, we selected ten test entries IBWSN-1010, IBWSN-1025, IBWSN-1042, IBWSN-1132, IBWSN-1144, IBWSN-1148, IBWSN-1150, DH-12/7, DH-12/31 and MASR-64 as drought tolerant bestowed with increased means for drought adaptive traits. Selected wheat genotypes can be used in further conventional breeding programs regarding drought tolerance.

Conclusion

Agronomic, physiological and biochemical traits play an important role in the drought-tolerance and grain yield increase among wheat genotypes under water-limited environments. Genotype × environment interaction has profound effects drought tolerance. Hence, such studies should be carried out to further investigate the grain yield stability and drought tolerance of the selected wheat genotypes. Based on the findings, drought tolerant and high grain yielding wheat genotypes could be recommended to further breeding programs. This will help in the release of the drought tolerant wheat genotypes to benefit the local small farmers for increased grain yield and reasonable income.

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