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IDENTIFICATION OF WHEAT MUTANTS WITH IMPROVED DROUGHT TOLERANCE AND GRAIN YIELD POTENTIAL USING BIPLOT ANALYSIS

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Abstract

Water shortage resulting from climate change has become one of the major threats to agricultural production globally. Performance of 68 mutants (M_4) of wheat (*Triticum aestivum* L.), 35 of Bhittai and 33 of Kiran-95 varieties, induced by gamma-rays were evaluated, along with wild-types and a drought-resistant check, under three irrigation treatments, viz., T-1(no irrigation), T-2 (two irrigations) and T-3 (four irrigations). Drought susceptibility index (DSI) and relative yield (RY) values were used to describe yield stability and yield potential of wheat genotypes. BM-14, BM-15, KM-26, and KM-27 had the lowest DSI (.3, .18, -0.1,0.27) and higher relative yield under stress (.88, 1.00,1.00,1.00), than all other entries and check varieties; indicated better performance under water-deficit stress. A significant positive association was observed between biomass and grain yield in both the mutant populations of Bhittai (r=0.61) and Kiran-95(r=0.83) under T-1. However, a significantly negative relationship was observed in DSI with grain yield (r=-0.70; r=-0.68), biological yield (r=-0.30; r=-0.67), and harvest index (r=-0.66; r=-0.29) in Bhittai and Kiran mutants, respectively. Principal component analysis (PCA) identified seven of Bhittai and 11of Kiran-95 mutants for both water-stress and irrigated conditions. Furthermore, mutants with low DSI and high RY could be employed to breed varieties with both high yield stability and high yield potential (DSI<1 and RY>mean RY) for drought-prone areas.

Key words: Bread wheat, Drought susceptibility index, Induced mutation, Water stress.

Introduction

Wheat (*Triticum aestivum* L.) serves as a principal diet for over 35% of the world population and is the major cereal crop of Pakistan. It contributes to nearly 20% of both calories and protein requirements for humans in more than 94 developing countries globally (Braun *et al.*, 2010). In Pakistan, about 19% area is rainfed. In irrigated areas, water scarcity is more prevalent and damaging, especially in wheat-producing zones (Khakwani *et al.*, 2012), and its intensity is projected to increase under changing climate in coming decades in most of the wheat-producing countries worldwide (Lobell *et al.*, 2008; Trenberth *et al.*, 2014). Moreover, developing countries have been projected to suffer 29 to 34% of wheat yield loss by 2050 because of these changes. (Hellin *et al.*, 2012).The area under water shortage has increased by 50% to 200% because of the worldwide occurrence of agricultural drought during the 21st century (Zhao & Dai, 2017). An approximate water reduction of 40% resulted in as much as a 21% yield decrease in wheat (Daryanto *et al.*, 2016).

Crop plants often face multiple abiotic stresses, such as water scarcity, flooding, increasing temperatures, and soil salinity during their life cycle. In the current scenario of climate change, wheat production is most prone to drought stress in semi-arid zones of the world (Fleury, 2010; Khakwani *et al.*, 2011). Water-stress tolerance is a complex trait and is polygenic in nature (Serba & Yadav, 2016; Sallam *et al.*, 2019), which negatively influences plant growth and grain yield. Improving tolerance to water stress has long been a prime objective in most of the wheat-breeding programs. Therefore, from time to time, significant work has been done in bread wheat to characterize and study the traits influencing grain yield subjected to environments with the scarcity of irrigation water. (Kiliç &Yağbasanlar 2010; Amiri*et al.*, 2013; Ahmad *et al.*, 2015; Zhang *et al.*, 2018).

The availability of genetic divergence is the mainstay of crop improvement programs to preserve and harness economically important traits. It provides the basis for the selection of superior combinations, endowed with the potential to face the environmental stresses and fast-changing pathogen races. Genetic divergence in common wheat has been greatly reduced by the replacement of traditional wheat varieties (landraces) with large-scale cultivation of uniform, high-yielding, semi-dwarf varieties. For improved sustainability, these varieties should be replaced with new superior varieties (Shiferaw *et al.*, 2013). To create variation, there are several molecular and conventional hybridization techniques available. However, these techniques and approaches are time-consuming, need special skills, and are tedious and costly. Hence, there are other promising ways to induce genetic variation in crop plants, i.e., the use of chemical or physical mutagens (Kharkwal & Shu, 2009; Yang *et al.*, 2014; Çelik & Atak, 2017; Bano *et al.*, 2017). Through the use of mutation breeding techniques, >3000 mutants of major crops have been commercialized (Tanaka *et al.*, 2010; Pathirana, 2011) and 286 wheat mutant varieties have been developed globally (Kharkwal, 2018). Among the available mutagens, the gamma source of radiation is the most efficient physical mutagen used by the breeders to create a variation for use in crop improvement programs. Hence, our objectives were to evaluate and select the gamma-irradiated bread wheat mutants possessing improved yield potential, stability and other yield-related traits under water-deficit conditions.

Materials and Methods

The wheat variety Bhittai, developed in 2004, possesses high yield potential (7300 $kgha^{-1}$), has long spikes and amber grain color, and is suitable for early sowing in Pakistan (Baloch *et al.*, 2014; Anon., 2016);

whereas, Kiran-95 was developed through the use of chemical mutagen sodium azide (NaN_3) in the year 1996, with the yield potential of 6200 kg/ha and moderate plant height, and is suitable for both early- and late-sowing systems (Anonymous, 2016; 2019). To improve these varieties (Bhittai and Kiran-95) with respect to yield and associated traits in stress environments, seeds of each variety were irradiated with different doses (100, 150, 200, and 250 Gy) of gamma (γ) rays. Irradiated seeds were space planted using the dibbler method. Five grains perspike from each plant of M_1 generation were taken and planted individually to grow the M_2 generation. Fifty putative mutant plants from both mutant groups (Bhittai and Kiran-95), with superior traits, were collected in M_2, and individual plant to progeny rows were planted to raise the M_3 generation. In the M_3 generation, superior plants with desirable traits, viz., fertility, early in heading days, short stature (semi-dwarf type plant height), more tillers and grain yield per plant, were promoted to M_4 generation. Selected M_4 mutant plants were tested under different irrigation regimes for their response to water stress. The studies on water stress were conducted during the crop year 2013-14 at NIA (Nuclear Institute of Agriculture) Research Farm in Tando Jam, Pakistan.

The materials consisted of 68 M_4 mutants of Bhittai (35) and Kiran-95 (33) wheat varieties (Supplementary Table 1), along with wild-type and drought-tolerant check viz., Margalla-99 (Sial *et al.,* 2009). Bhittai wild-type was used as a check for Bhitai-mutants and Kiran-95 for Kiran-mutants, Margalla-99 was used as a drought-tolerant check. The experiment with three replications was set in a randomized complete block design (RCBD). The sowing of wheat was done using a single row, hand drilled on 15 November 2013. The experimental area of soil was a sandy-loam type. Four rows of each genotype were planted, each row 2 m long and 30 cm apart; net plot size = 2.4 m^2. The Nitrogen (46%) was applied as urea @ 130 kg/ha and phosphorus using Diammonium phosphate (DAP containingP2O5 46% and N 18%) @ 90 kg/ha. All of the DAP was utilized at the time of sowing, while nitrogen fertilizer was used in three portions following the irrigation schedule.

Supplementary Table 1. Details of the material studied under this work are given as follows.

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	WT-1	20	BM -19	1	WT-1	19	KM-18
2	BM-1	21	BM -20	2	KM-1	20	KM-19
3	BM-2	22	BM -21	3	KM -2	21	KM-20
4	BM -3	23	BM -22	4	KM -3	22	KM-21
5	BM -4	24	BM -23	5	KM -4	23	KM-22
6	BM -5	25	BM -24	6	KM -5	24	KM-23
7	BM -6	26	BM -25	7	KM -6	25	KM-24
8	BM -7	27	BM -26	8	KM -7	26	KM-25
9	BM -8	28	BM -27	9	KM -8	27	KM-26
10	BM -9	29	BM -28	10	KM -9	28	KM-27
11	BM -10	30	BM -29	11	KM-10	29	KM-28
12	BM -11	31	BM -30	12	KM-11	30	KM-29
13	BM -12	32	BM -31	13	KM-12	31	KM-30
14	BM -13	33	BM -32	14	KM-13	32	KM-31
15	BM-14	34	BM -33	15	KM-14	33	KM-32
16	BM -15	35	BM -34	16	KM-15	34	KM-33
17	BM -16	36	BM -35	17	KM-16	35	WT-2
18	BM -17	37	WT-2	18	KM-17		
19	BM -18						

Note= Mutants of Bhittai are designated as Bhit (1-35) and of Kiran-95 as Kir (1-33), Bhittai wild type is given on number 1 and Marghalla-99 on 37 and of Kiran-95 on 1 and Marghalla-99 on 35. WT=Wild-type control

The mutants were evaluated under three irrigation treatments, i.e., treatment-1 (T-1; no-irrigation), treatment-2 (T-2; two irrigations: 1st at three-leaf stage (Zadoks-13) and 2nd at tillering (Zadoks-21) and treatment-3 (T-3; four irrigations: 1st at three-leaf stage (Zadoks-13); 2nd at tillering (Zadoks-21); 3rd at booting (Zadoks-41), 4th at grain filling (Zadoks-71). The T-3 with four irrigations served as a control. The three irrigation treatments translated as follows: T-1 = 15% field capacity, T-2 = 45% field capacity (stress conditions) and T-3 = 75% field capacity (control). Field capacity was measured according to Estefan *et al.,* (2013). There was no rainfall during the crop season.

After maturity, five plants from each replicate were selected randomly and harvested from the two central rows to collect the data on yield and other agro-morphictraits,*viz*., days to heading (DH); counted as days from sowing date to 50% spike emergence on the tillers in each plot, plant height (PH); in centimeters, from the base of the plant to the tip f the spike, excluding awns and spike length (SL); measured in centimeters, as the distance from the basal rachis to the tip of the spike, without awns. Data on the spikelets per spike (SPS), the numbers of grains per spike (GPS) and yield per the main spike (g) were noted from the harvested main tillers of the five randomly selected plants. Thousand-grain weight (g/1000 grains) was analyzed from randomly sampled 1000 kernels after harvest using a top-loading digital balance. Data on biological and grain yield were determined from two middle rows in g/ per uni area; later was transformed to yield per hectare (kg/ha). Harvest index was validated by following the equation:

$$\text{Harvest index (\%)} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Drought Susceptibility Index (DSI)

The yield stability (DSI) and yield potential (RY) were obtained from mean grain yield. The DSI (Drought Susceptibility Index) was calculated to quantify the relative water stress tolerance in wheat genotypes using grain yield according to the formula of Fischer & Maurer (1978).

$$DSI = [(1\text{-}Yd)/Yw]/D$$

where Yd=mean yield under drought, Yw =mean yield under well-watered conditions, and D = environmental stress intensity = 1- (all genotype mean yield under water stress /mean yield of all genotypes under well-watered conditions). However, relative yield under water stress/ yield for control condition were calculated as the yield of a given genotype under water scarcity divided by the yield of the highest yielding genotype in the population.

Statistical computing software, statistix version 8.1(https://statistix.informer.com/8.1/), was used to compute the combined ANOVA; means were separated by Tukey's HSD test. To quantify the extent of the association among the agromorphic traits along with DSI, correlation coefficients (r) were calculated separately for stressed and normal treatment using the statistix (8.1) software. Biplots were developed from the first two principal components (PC1 and PC2) using a multivariate statistical package (MSVP).

Results and Discussion

Mean squares for various traits of wheat mutants showed highly significant differences ($p \leq 0.01$) attributable to the three irrigation treatments and mutant genotypes. Additionally, a highly significant ($p \leq 0.01$) genotype by treatment interaction effect was observed for all the traits under study (Table 1). The significant genotype x treatment interaction suggests the impact of the various water stresses over the performance of genotypes. It provides better opportunities to select suitable genotypes for water-stressed environments.

Phenological changes in mutants: The results are based on the mean values of pooled genotypes (Table 2). Water stress caused a significant decrease in the number of days from sowing to heading, plant height, spike length, grains per spike, main spike yield, and grain yield in both Bhittai and Kiran-95 mutants in T-1 as compared to T2. The effect of water stress led to early heading (84.77 days) relative to the check (87.41 days) in the case of BhittaiinT-1 (no-irrigation). The Kiran-95 mutants had early heading (79.74 days) inT-1, which might be attributable to accelerated plant growth when subjected to stress. However, Kiran-95 mutants in T-3 (full-irrigation) had late heading (87.63 days). Blum (2010) and Khakwani *et al*., (2012) stated that early heading might contribute to plant escape from stress, and reportedly, a drought at this stage reduced grain yield by16.8% (Zhang *et al*., 2018). The height of both mutant populations (Bhittai and Kiran-95) was reduced in T-1 as compared to T-2 and T-3. Similarly, reduced plant height in response to water stress has also been revealed in other studies (Khan & Naqvi, 2011; Li *et al*., 2011; Bowman, 2015; Maqbool *et al*., 2015). Insufficient water availability reduces plant height; in that case, taller genotypes (to a certain extent) could be helpful in producing more biomass and assimilates to feed developing grains, consequently increasing grain yield (Lopes *et al*., 2014). The spike length in Bhittai mutants (12.67) was increased as compared to wild-type (12.45) in T-1, T-2(13.32) and T-3 (13.39). Results showed significant differences between mutants and wild-type for the spike length in T-1 and T-2 but not in T-3. The Kiran-95 mutants also showed decreased spike length in T-1 (13.20) in relation to T-2 (14.50) and T-3 (14.32). Sokoto & Singh (2013) indicated that decreased photosynthesis and translocation of photosynthates resulted in retarded spike growth under water stress. Bhittai-mutants produced significantly more spikelets per spike in T-1(20.87) and T-2 (22.04) as compared to the wild-type. Similarly, Kiran-95 mutants also produced significantly more spikelets per spike (20.04) as compared to the wild-type (19.02) in T-1. However, the number of spikelets per spike was decreased in T-1 as compared to T-2 and T-3. Although, Kiliç & Yağbasanlar (2010) found no significant effect of drought stress on the number of spikelets per spike. In T-1, Bhittai mutants produced a significantly higher number of grains (70.22) than the wild-type (66.40). Similarly, Kiran-95 mutants showed a significantly higher number of grains per spike than its wild-type check, in all three treatments. Overall, no-irrigation (T-1) showed a reduced number of grains per spike as compared to two (T-2) and four irrigations (T-3) in both mutant groups under study. Water stress imposed after heading has been shown to decrease grain yield because of the reduced number of grains per spike, that is one of the main yield contributing traits in cereals (Mori & Inagaki, 2012; Mohammadi, 2018). Prior research has indicated that water stress at a particular stage affects a specific trait (Sokoto & Singh, 2013), reduced spike length under water stress (Mujtaba *et al*., 2018), and at the reproductive stage, it significantly affects seed setting because of male and female sterility caused by water stress (Dolferus *et al*., 2011; Powell *et al*., 2012; Barber *et al*., 2015; Bogale & Tesfaye, 2016; Onyemaobi *et al*., 2017).

Bhittai mutants had significantly higher grain yield per the main spike (2.33 g), (2.36 g), and (2.61 g) as compared to wild-type in T-1, T-2, and T-3, respectively. Further, Kiran-95 mutants also had increased main spike yield compared with the check variety in T-1, T-2, and T-3. Overall, results showed lower main spike yield under deficit irrigation (T-1) in comparison to T-2 and control (T-3) in both mutants and wild-type genotypes. However, some mutants produced a comparatively higher main spike yield than check varieties in T-1. Reduced main spike yield might be attributable to shriveling of grains as a result of reduced time for seed formation under water stress. The impacts of low water on grain yield performance of Bhittai and Kiran-95 and their respective M4 mutants are given in (Tables 3 and Table 4), respectively. The BM-15mutant produced the highest yield in T-1 (3438 kg/ha) and T-3 (3583 kg/ha), with lower DSI value and highest relative yield under stress and control, whereas BM-18 produced highest yield in T-2 (4236 kg/ha) in contrast to the wild-type. While in Kiran-95 mutants, the highest yield among mutants in comparison to wild-type was produced by KM-27 and KM-28 (3229 kg/ha) in T-1, KM-29 (4514 kg/ha) in T-2, and KM-23 (4558 kg/ha) in T-3 (Table 4). The drought susceptibility index of Bhittai mutants for yield ranged from -0.03 to 3.2 and of Kiran-95 mutants from -0.1 to 1.73 (Table3 and Table 4, respectively). Twenty Bhittai mutants (BM-3, BM-5, BM-6, BM-7, BM-8, BM-10, BM-11, BM-12, BM-13, BM-14, BM-15, BM-16, BM- 17, BM-29, BM-30, BM-32, BM-33, BM-34, BM-35 and BM-36), considered as water-stress tolerant having DSI value <1were selected whereas others with DSI >1 were rejected. However, mutants from Kiran-95-based population,viz., KM-2, KM-3, KM-6, KM-9, KM-11, KM-12, KM-15, KM-21, KM-24, KM-25, KM-26, KM-27, KM-28, KM-29, KM-30, KM-31 and KM-33 were relatively water-stress tolerant (DSI value <1), as only a slight reduction in yield was recorded in these mutants under no-irrigation as compared to the control treatment. Moreover, under stress conditions Kiran-95 mutants, KM-27, and KM-28 produced the highest grain yield and low DSI values than all other contesting lines and check varieties, also showed low DSI values were, thereby selected for further evaluation.

When pooled data were analyzed, Bhittai mutants had comparatively higher grain yield (2196 kg/ha), (3086 kg/ha) and (2907 kg/ha) than its wild-type variety Bhittai in T-1 (1958 kg/ha), T-2 (2578 kg/ha) and T-3 (2594 kg/ha), respectively (Table 2). The higher yield in T-1 might be attributable to early heading (84.77 days) relative to the check and in T-3 attributable to the increase in the number of grains per spike (81.10) and comparatively better spike yield (2. 61g). Likewise, Kiran-95 mutants also had significantly higher grain yield in T-1 (2336 kg/ha), T-2 (3777 kg/ha) and T-3 (3602 kg/ha) than wild-type genotypes. In T-1 and T-3, moderate plant height might be the reason for increased yield, whereas, in T-2, longer spikes, more spikelets per spike, and comparatively better spike yield might have contributed to increased yield in mutants. The mean RY values in response to water stress and full irrigation ranged from 0.79 to 0.64 for Bhittai mutants, and from 0.80 to 0.70 for Kiran-95 mutants (Tables 3 and 4). Among the Bhittai mutants, a total of 17 entries (BM-2, BM-3, BM-5, BM-6, BM- 7, BM-8, BM-10, BM-11, BM-12, BM-13, BM-14, BM-15, BM-16, BM-17, BM-27, BM-28, and BM-36) were comparatively high yielding (RY> mean RY) under water-deficit conditions, whereas BM-4, BM-9, BM-18, BM-19, BM-20, BM-21, BM-22, BM-23, BM-24, 25, BM-26, BM-29, BM-30, BM-31, BM-32, BM-33, BM-34, BM-35 and wild-type (1 Bhittai& 37 Margalla-99) were relatively low yielding under water stress.

Correlation analysis of traits under no-irrigation and four irrigations condition: Correlation coefficients between grain yield and main yield constituents, underwater stress (no irrigation), and control (four irrigations) settings, of Bhittai and Kiran-95mutants, are presented in Table 5and Table 6, respectively. In Bhittai mutants, under stress (no irrigation) conditions, grain yield showed a strong positive correlation with biomass ($r=0.60^{***}$) and harvest index ($r=0.82^{***}$), whereas positive but weak correlation with grains per spike ($r=0.34^{***}$) and main spike yield ($r=0.21^{*}$), whereas grain yield had a highly significant negative ($r=-0.70^{***}$) correlation with DSI (Table 5 upper diagonal). Further, a positive correlation was also found between spike length and DSI ($r=0.30^{**}$). The positive correlation of biological yield with grain yield suggested that grain yield was increased because of improved biomass yield, as it might have helped to accumulate more photosynthates to developing grain. The correlation was also determined for the control (T-3) of Bhittai mutants (Table 5lower diagonal). Correlation analysis revealed significant positive correlations of grain yield with main spike yield ($r=0.76^{***}$), and harvest index ($r=0.92^{***}$).

Likewise, in Kiran-95 mutants under stress (no-irrigation), grain yield exhibited a strong positive correlation with biomass ($r=0.83^{***}$) and harvest index ($r=0.62^{***}$), whereas it had a negative correlation with DSI ($r=-0.68^{***}$) (Table 6, upper diagonal DSI exhibited a significantly negative association to biomass yield ($r=-0.67^{***}$), and grain yield (r=-0.68). The number of grains per spike showed positively significant association with the main spike yield ($r=0.67^{***}$), whereas the association was negative with the height of the plant ($r=-0.34^{**}$). The number of grains plays a pivotal role in improving yield, specifically under conditions of low water availability (Dencic*et al.*, 2000; Slafer*et al.*, 2005; Li *et al.*, 2012). Under normal irrigation, grain yield showed a strong positive correlation with biomass yield ($r=0.69^{***}$) and harvest index ($r=0.75^{***}$), (Table 6 lower diagonal). Moreover, significant relationships of biomass were also recorded with some other traits. These results indicated that adequate irrigation water is crucial for growth and crop productivity, whereas biomass is a vital trait for selection under water stress. Therefore, a significant positive association of grain yield with biomass and harvest index could be helpful in selecting drought-tolerant genotypes.

Table 1. The joint ANOVA for various quantitative traits of bread wheat (Bhittai& Kiran-95) mutants/ genotypes tested under different irrigation levels. [T1= Treatment-1(no irrigation); T-2= Treatment-2 (Two irrigations); T-3= Treatment -3 (full irrigations)].

Variety	Source	DF	DH	PH	SL	SPS	GPS	MSY	Bykgha^{-1}	Gykgha^{-1}	HI (%)
	Replications	2	3.5^{ns}	4.5^{ns}	0.15^{ns}	3.1^{ns}	12.5^{ns}	0.06^{ns}	1815976^{ns}	2066312^{ns}	133.6^{ns}
	Genotypes (G)	36	14.8**	781.9**	1.6**	6.0**	895.7**	0.6**	5181260**	14820605**	66.87**
	Treatments (T)	2	1942.9**	3198.3**	14.6**	32.1**	3818.8**	0.03^{ns}	2.728E+08**	2.464E+07**	405.1**
Bhittai	G* T	72	12.1**	77.0**	0.65	2.02**	190.2**	0.28**	2604631**	66406**	37.65**
	Error	220	0.4	14.7	0.05	0.2	12.6	0.02	276011	51083.3	3.45
	Total	332									
	CV (%)		0.74	4.26	1.66	2.13	4.80	6.52	5.24	8.35	6.91
	Rep	2	6.07^{ns}	1.37^{ns}	0.55^{ns}	3.4^{ns}	289.2^{ns}	0.004^{ns}	556884^{ns}	378966^{ns}	25.1^{ns}
	Genotypes (G)	34	3.95**	534.5**	1.4**	3.1**	224.98**	0.16**	5537539**	4635068**	35.3**
	Treatments (T)	2	1736.5**	1236.0**	31.1**	66.4**	590.1**	5.3**	1.135E+09**	3.825E+09**	1084.8**
Kiran-95	G * T	68	2.12**	52.9**	1.13**	3.3**	208.0**	0.12**	4082011**	3001871**	28.9**
	Error	208	0.56	24.1	0.03	0.3	25.9	0.03	174422	151969	6.84
	Total	314									
	CV (%)		0.89	5.35	1.24	2.79	7.8	7.2	6.96	4.25	7.84

**Significant at 1% level of probability; ns, non-significant. DH (days to heading), PH (plant height in cm), SL (spike length in cm), SPS (spikelets per spike), GPS (grains per spike), MSY (main spike yield in grams), BY (biomass yield in kg ha-1), GY (grain yield kg ha^{-1}) and HI (harvest index %); CV (coefficient of variation).

Table 2. Mean summary of quantitative traits of gamma-irradiated mutants (M_4) bread wheat tested under different irrigation levels. T1= Treatment-1 (no irrigation); T-2= Treatment-2 (Two irrigations); T-3= Treatment -3 (full irrigations).

Variety	Treatments	Genotypes	DH	PH	SL	SPS	GPS	MSY	GY	YI (%)
Bhittai	T-1	M(35)	84.77**	84.00^{ns}	12.67*	20.87*	70.22*	2.33*	2196**	10.8
		WT(2)	87.41	84.00	12.45	20.15	66.40	1.72	1958	
	T-2	M(35)	91.95^{ns}	93.23^{ns}	13.75*	22.04*	71.83*	2.36**	3086*	16.5
		WT (2)	91.42	93.27	13.32	20.67	71.10	1.77	2578	
	T-3	M(35)	91.58**	93.40^{ns}	13.39^{ns}	21.34^{ns}	81.10*	2. 61*	2907*	10.76
		WT (2)	89.66	94.74	13.52	21.08	73.27	1.82	2594	
Kiran-95	T-1	M(33)	79.74^{ns}	88.09*	13.20^{ns}	20.04*	70.74*	2.15*	2336*	13.4
		WT (2)	79.00	82.00	13.19	19.02	63.64	1.92	2023	
	T-2	M(33)	87.63^{ns}	93.74^{ns}	14.50*	22.3^{ns}	71.90*	2.79*	3777*	14.5
		WT(2)	83.83	92.37	13.30	21.2	67.29	2.25	3229	
	T-3	M(33)	84.44*	95.30**	14.32^{ns}	21.10^{ns}	71.35*	2.39*	3602*	7.2
		WT (2)	87.40	88.33	13.78	20.20	65.00	2.10	3344	

**Significant at 1%;*significant at 5%; ^{ns}, non-significant. DH= days to heading; PH= plant height in cm; SL= spike length in cm; SPS= spikelets per spike; GPS=grains per spike; MSY= main spike yield in grams; GY= grain yield kgha^{-1}; YI=yield increase of mutants over wild-type checks in percent); M = mutants; WT = wild type

Table 3. Mean performance for grain yield (kgha^{-1}) of M_4Bhittai mutants and effect of irrigation treatments.
(T1= Treatment-1(no irrigation); T-2= Treatment-2 (Two irrigations); T-3= Treatment -3 (full irrigations) on drought susceptibility index and yield potential.

S#	Gen.	T-1	T-2	T-3	DSI	RYW	RYS	S#	Gen.	T-1	T-2	T-3	DSI	RYW	RYS
1.	WT-1	1450 o-q	3306 a-k	2750 c-h	2.11	0.77	0.45	20	BM -19	1542 n-q	3625 a-h	2278 g-j	1.44	0.64	0.40
2.	BM-1	2181 g-l	2472 i-m	3167 a-f	1.39	0.88	0.65	21	BM -20	1762 l-q	3847 a-e	3500 ab	2.22	0.98	0.48
3.	BM-2	2642 c-h	2194 k-m	2695 c-j	0.09	0.75	0.82	22	BM -21	1354 pq	2695 f-m	2708 c-i	2.23	0.76	0.41
4.	BM-3	1975 i-n	2153 lm	2750 c-h	1.26	0.77	0.53	23	BM -22	1250 q	2958 c-m	2570 f-j	2.29	0.72	0.35
5.	BM-4	2219 f-k	2042 m	2250 g-j	0.06	0.63	0.71	24	BM -23	1542 n-q	2931 c-m	2750 c-h	1.96	0.77	0.47
6.	BM-5	2873 b-d	2639 g-m	3153 a-f	0.40	0.88	0.76	25	BM -24	1658 l-q	3055 b-m	2611 e-j	1.63	0.73	0.48
7.	BM-6	2728 b-f	3819 a-f	3389 a-c	0.87	0.95	0.94	26	BM -25	1600 m-q	4000 a-c	3306 a-e	2.31	0.92	0.49
8.	BM-7	2443 d-i	2500 h-m	2764 c-h	0.52	0.77	0.71	27	BM -26	2346 e-j	3958 a-d	3361 a-c	1.35	0.94	0.66
9.	BM-8	1975 i-n	3583 a-i	2875 a-g	1.40	0.80	0.59	28	BM -27	2079 i-m	4028 a-c	3333 a-d	1.68	0.93	0.65
10.	BM-9	2703 c-g	2847 d-m	2847 b-g	0.23	0.79	0.76	29	BM -28	2209 f-k	2569 h-m	2014 ij	-0.43	0.56	0.68
11.	BM-10	2143 h-l	3375 a-j	2597 e-j	0.78	0.72	0.65	30	BM -29	2113 i-m	3000 c-m	2569 f-j	0.79	0.72	0.58
12.	BM-11	2829 b-e	2514 h-m	3403 a-c	0.75	0.95	0.88	31	BM -30	1979 i-n	2417 j-m	2944 a-g	1.47	0.82	0.53
13.	BM-12	2436 d-i	3264 a-l	3097 a-f	0.95	0.86	0.76	32	BM -31	1762 k-q	2111 m	2125 h-j	0.76	0.59	0.48
14.	BM-13	3121 a-c	3611 a-h	3347 a-d	0.30	0.93	0.88	33	BM -32	2246 f-k	1945 m	2292 g-j	0.09	0.64	0.61
15.	BM-14	3438 a	4166 ab	3583 a	0.18	1.00	1.00	34	BM -33	2125 h-l	3959 a-d	2111 h-j	-0.03	0.59	0.61
16.	BM-15	3241 ab	3764 a-g	3195 a-f	-0.06	0.89	0.94	35	BM -34	1870 j-p	2139 lm	1986 j	0.26	0.55	0.56
17.	BM-16	2847 b-e	2278 j-m	3125 a-f	0.40	0.87	0.82	36	BM -35	2428 d-i	3333 a-j	2764 c-h	0.54	0.77	0.67
18.	BM-17	2000 i-n	4236 a	2791 b-h	1.27	0.78	0.59	37	WT-2	1958 i-o	2778 e-m	2639 d-j	1.15	0.74	0.47
19.	BM-18	1942 i-o	3778 a-f	2708 c-i	3.20	0.76	0.53		GM	2189.4	3078.1	2820.2		0.79	0.64
									SD	130.68	282.15	176.65			
									HSD (5%)	524.5	1132.5	709.0			
									F-value	33.12	12.35	12.25			

Note: Means followed by different letters within a column for each trial have significant differences at the level of Tukey's, HSD test, $p<0.05$, DSI= Drought Susceptibility Index, RYW = Relative yield under well-watered conditions (control); RYS = relative yield under stress (zero irrigation); WT: wild-type; BM= Bhittai mutant; GM= Grand mean: SD= standard deviation, HSD= honestly significant difference; F-value from genotypes

Table 4. Mean values of grain yield (kgha^{-1}) of M_4Kiran-95 mutants under different irrigation levels.
(T1= Treatment-1(no irrigation); T-2= Treatment-2 (Two irrigations); T-3= Treatment -3 (full irrigations).

S#	Gen	T-1	T-2	T-3	DSI	RYW	RYS	S#	Gen	T-1	T-2	T-3	DSI	RYW	RYS
1.	WT-1	2063 e-i	2847 d	3486 c-f	1.12	0.77	0.64	19	KM-18	1354 k	3750 a-d	3667 b-f	1.73	0.81	0.42
2.	KM-1	2500 b-f	2944 d	3333 ef	0.68	0.74	0.77	20	KM-19	1458 jk	3750 a-d	3403 c-f	1.56	0.75	0.45
3.	KM-2	2083 e-i	3236 b-d	3264 ef	0.99	0.72	0.65	21	KM-20	2396 b-g	3819 a-d	3097 fg	0.62	0.69	0.74
4.	KM-3	1979 f-j	3000 cd	3681 a-f	1.26	0.82	0.61	22	KM-21	2500 b-f	3750 ad	4208 a-d	1.11	0.93	0.77
5.	KM-4	1771 h-k	3750 b-d	3375 d-f	1.30	0.75	0.55	23	KM-22	2500 b-f	3889 a-d	4558 a	1.22	1.00	0.77
6.	KM-5	2500 b-f	3972a-d	3125 e-g	0.55	0.69	0.77	24	KM-23	2292 c-h	2847 d	2208 h	-0.10	0.49	0.71
7.	KM-6	1875 g-k	4305 ab	3681 a-f	1.34	0.82	0.58	25	KM-24	2188 d-i	2847 d	2333 gh	0.17	0.52	0.68
8.	KM-7	2188 d-i	4028a-d	3945 a-e	1.22	0.87	0.68	26	KM-25	2604 b-e	4375 ab	3264 ef	0.55	0.72	0.81
9.	KM-8	2917 ab	4278 ab	3889 a-f	0.68	0.86	0.90	27	KM-26	3229 a	4278 ab	3111 e-g	-0.10	0.69	1.00
10.	KM-9	2188 d-i	4361 ab	3722 a-f	1.13	0.82	0.68	28	KM-27	3229 a	3819 a-d	3583 c-f	0.27	0.79	1.00
11.	KM-10	2708 a-d	4236 ab	4236 a-c	0.99	0.94	0.84	29	KM-28	2813 a-c	4514 a	4181 a-d	0.90	0.93	0.87
12.	KM-11	2604 b-e	3681a-d	4236 a-c	1.05	0.94	0.81	30	KM-29	2396 b-g	3750 a-d	3306 ef	0.75	0.73	0.74
13.	KM-12	2083 e-i	4167 a-c	3680 a-f	1.19	0.82	0.65	31	KM-30	2917 ab	3264 b-d	3278 ef	0.30	0.73	0.90
14.	KM-13	1771 h-k	3958 b-d	3569 c-f	1.38	0.79	0.55	32	KM-31	2042 e-j	4375 ab	3778 a-f	1.26	0.84	0.63
15.	KM-14	2708 a-d	3472 b-d	4222 a-c	0.98	0.94	0.84	33	KM-32	2292 c-h	3889 a-d	3444 c-f	0.92	0.76	0.71
16.	KM-15	1979 f-j	3958 b-d	3750 a-f	1.29	0.83	0.61	34	KM-33	1979 f-j	3889 a-d	3750 a-f	1.29	0.83	0.61
17.	KM-16	1646 i-k	3819 b-d	4458 ab	1.73	0.99	0.51	35	WT-2	2083 e-i	3403 a-d	3569 c-f	1.14	0.79	0.65
18.	KM-17	2083 e-i	3611 b-d	3583 c-f	1.15	0.79	0.65		GM	2283.3	3766.7	3594.8		0.80	0.71
									SD	146.7	301.3	210.7			
									HSD (5%)	586.0	1203.4	841.35			
									F-value	18.76***	4.97***	11.5***			

Note: Means followed by different letters within a column for each treatment have significant differences at the level of Tukey's, HSD test, $p<0.05$, DSI= Drought Susceptibility Index, RYW = relative yield under well-watered conditions (control); RYS = relative yield under stress (zero irrigation); WT: wild-type KM= Kiran mutant; GM= Grand mean: SD= standard deviation, HSD=honestly significant difference; F-value from genotypes

Table 5. The correlation coefficient (r) of *Bhittai mutants* on various quantitative traits. Upper diagonal showing the correlation under water stress (no irrigation) and lower diagonal for well-watered (control) condition.

Variables	DH	PH(cm)	SL (cm)	SPS	GPS	MSY (g)	BY(kg ha^{-1})	GY(kg ha^{-1})	HI (%)	DSI (GY)
DH	1	0.36^{***}	-0.39^{***}	-0.12^{ns}	0.48^{***}	0.50^{***}	-0.07^{ns}	-0.03^{ns}	0.01^{ns}	0.11^{ns}
PH	0.06^{ns}	1	0.20^{*}	-0.13^{ns}	0.27^{**}	0.24^{*}	-0.12^{ns}	0.11^{ns}	0.26^{**}	0.06^{ns}
SL	0.25^{**}	0.26^{**}	1	0.42^{***}	-0.33^{***}	-0.30^{**}	-0.11^{ns}	-0.08^{ns}	-0.02^{ns}	0.30^{**}
SPS	0.20^{*}	-0.09^{ns}	0.24^{*}	1	-0.07^{ns}	0.02^{ns}	-0.22^{*}	-0.09^{ns}	0.06^{ns}	-0.16^{ns}
GPS	0.07^{ns}	0.41^{***}	0.23^{*}	0.02^{ns}	1	0.82^{***}	0.14^{ns}	0.34^{***}	0.34^{***}	-0.06^{ns}
MSY	-0.07^{ns}	-0.42^{***}	-0.24^{*}	0.07^{ns}	0.25^{**}	1	0.03^{ns}	0.21^{*}	0.26^{**}	-0.06^{ns}
BY(kgha^{-1})	0.37^{***}	0.18ns	0.10^{ns}	-0.04^{ns}	0.20^{*}	-0.18^{ns}	1	0.61^{***}	0.04^{ns}	-0.30^{**}
GY(kg ha^{-1})	0.31^{**}	0.21^{*}	0.23^{*}	-0.01^{ns}	0.22^{*}	0.78^{***}	0.27^{**}	1	0.82^{***}	-0.70^{***}
HI (%)	0.19^{*}	0.19^{*}	0.27^{**}	0.01^{ns}	0.19^{*}	-0.28^{**}	0.48^{***}	0.92^{***}	1	-0.66^{***}

Note: *P<0.05 ** p<0.01. ***p<0.001. DH (days to heading), PH (plant height in cm), SL (spike length in cm), SPS (spikelets per spike), GPS (grains per spike), MSY (main spike yield in grams), BY (biomass yield in kg ha^{-1}), GY (grain yield kgha^{-1}); HI (harvest index %) and DSI (drought susceptibility index)

Table 6. The correlation coefficient (r) of *Kiran mutants* on various quantitative traits. Upper diagonal showing the correlation under water stress (no irrigation), and lower diagonal under control conditions (full-irrigation).

Variables	DH	PH(cm)	SL (cm)	SPS	GPS	MSY (g)	BY (kg ha^{-1})	GY (kg ha^{-1})	HI (%)	DSI (GY)
DH	1	0.12^{ns}	-0.19^{*}	-0.195^{*}	-0.07^{ns}	-0.07^{ns}	-0.05^{ns}	-0.12^{ns}	-0.12^{ns}	-0.05^{ns}
PH(cm)	0.37^{***}	1	0.40^{***}	0.25^{*}	-0.34^{***}	-0.27^{**}	0.17^{ns}	0.10^{ns}	-0.06^{ns}	-0.02^{ns}
SL (cm)	-0.11^{ns}	-0.21^{*}	1	0.70^{***}	-0.13^{ns}	-0.06^{ns}	-0.02^{ns}	-0.01^{ns}	0.01^{ns}	0.20^{*}
SPS	-0.09^{ns}	-0.18^{ns}	0.43^{***}	1	-0.03^{ns}	0.09^{ns}	-0.02^{ns}	0.04^{ns}	0.14^{ns}	0.18^{ns}
GPS	0.05^{ns}	0.07^{ns}	-0.02^{ns}	0.24^{*}	1	0.67^{***}	-0.22^{*}	-0.15^{ns}	0.04^{ns}	0.26^{**}
MSY (g)	-0.10^{ns}	-0.02^{ns}	0.15^{ns}	0.26^{**}	0.72^{***}	1	0.03^{ns}	0.11^{ns}	0.17^{ns}	-0.05^{ns}
BY(kgha^{-1})	0.11^{ns}	0.10^{ns}	0.13^{ns}	-0.08^{ns}	0.10^{ns}	0.15^{ns}	1	0.83^{***}	0.09^{ns}	-0.67^{***}
GY(kg ha^{-1})	0.14^{ns}	0.08^{ns}	0.14^{ns}	-0.12^{ns}	0.17^{ns}	0.19^{ns}	0.69^{***}	1	0.62^{***}	-0.68^{***}
HI (%)	0.03^{ns}	0.05^{ns}	0.04^{ns}	0.01^{ns}	-0.02^{ns}	0.03^{ns}	0.76^{***}	0.75^{***}	1	-0.29^{**}

Note: *P<0.05 ** p<0.01. ***p<0.001.DH (days to heading), PH (plant height in cm), SL (spike length in cm), SPS (spikelets per spike), GPS (grains per spike), MSY (main spike yield in grams), BY (biomass yield in kg ha^{-1}), GY (grain yield kgha^{-1}); HI (harvest index %) and DSI (drought susceptibility index)

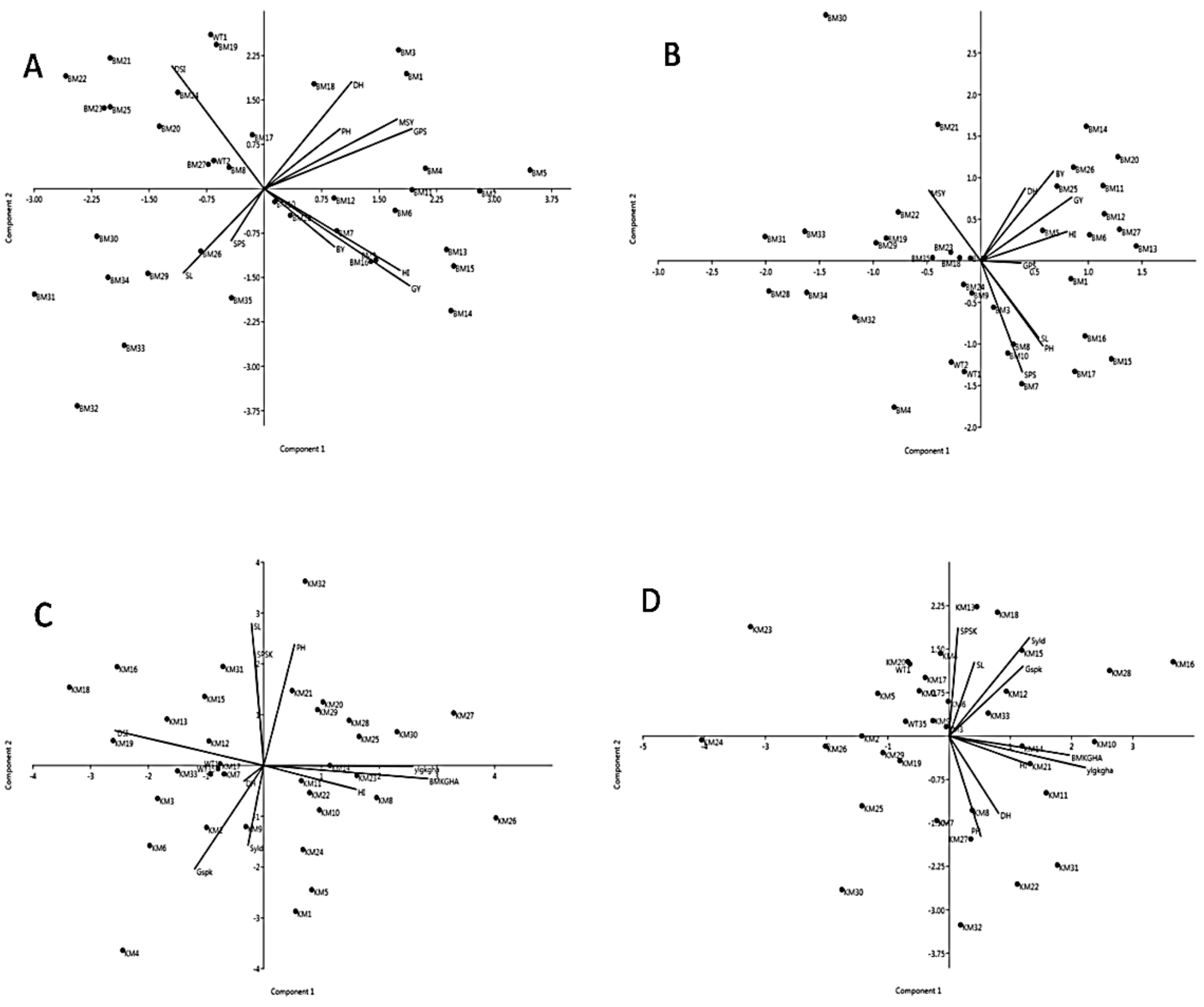

Fig. 1. Biplot diagram derived from the first and second-factor components for Bhittai mutants and Kiran-95 mutants under water stress and non-stress conditions.

Fig. 1 Dispersion of the wheat mutants/genotypes under study according to first and second components, over yield, and other quantitative traits under water- stress and non-stress conditions. (a) PCA biplot for traits and genotypes studied under stress in Bhittai-mutants population (b) PCA biplot for traits and genotypes studied under non-stress treatment in Bhittai-mutants population (c) PCA biplot for traits and genotypes studied under stress in Kiran-mutants population (d) PCA biplot for traits and genotypes studied under non-stress treatment in Kiran-mutants population. Multivariate Statistical Package (MSVP) was used to develop this figure.

Biplot analysis: PC1 showed the significance of MSY, GY, and HI, accounting for 32.27% of the total variability. Hence, these traits proved to be helpful in selecting genotypes for water stress tolerance. The PC2 explained24.1% variation, with more importance placed on DH and DSI, which may be called stress-sensitivity components. Overall, PC1 and PC2 explained56.4% of the variability among the traits associated with these two PCs. This suggests that the best mutants can be selected with increased PC1 and decreased PC2 for drought-prone and normal irrigation systems (Fig. 1a). Genotypes BM-2, BM-6, BM-7, BM-9, BM-10, BM-11, BM-12, BM-13, BM-14, BM-15, BM-16 and BM-28 with higher PC1 and lower PC2 (reduced sensitivity and improved yield) can perform equally well under normalandstressed habitat, and also exhibited higher scores for GY, BY and HI. Genotypes BM-1, BM-3, BM-4, BM-5, and BM-18, having high PC1 and PC2 can perform better under normal environments because of their sensitivity to late-season drought. These genotypes showed high values for DH, PH, GPS, and MSY. Genotypes BM-26, BM-29, BM-30, BM-31, BM-32, BM-33, BM-34, BM-35, BM-36, WT1 and WT2 with reduced PC1 and PC2, showed less sensitivity to stress, scored high values for SPS; hence these would be preferred for drought resistance in, breeding. Mwadzingeni*et al.,* (2016) emphasized the selection of genotypes with higher yield under drought stress on the basis of yield components using PCA analysis. Genotypes BM-8, BM-17, BM-19, BM-20, BM-21, BM-22, BM-23, BM-24, BM-25, BM-26, and BM-27, with lower PC1 and higher PC2, had decreased yield with higher sensitivity to water stress at the end-of-season, and scored higher values for DSI; hence their cultivation is not recommended.

Under a non-stress environment, PC1 showed the importance of ofSL, BM, GY, and HI, explaining 38.1% of the variation (Fig. 1b). However, PC2 accounted for 17.5% variation with more importance laid on DH, MSY, and BY, indicating that these traits were associated with drought sensitivity. Overall, PC1 and PC2 explained 55.6% of the variance for the traits.

PC1 (29.43% of the variance) showed the BM, GY, and HI as productive variables. Therefore, PC1 was related to drought tolerance. The PC2 (21.36% of the variance) placed more importance on PH, SL, and SPS indicated as drought-sensitive components. The first two PCs explained about 50.79% of the total variability. This suggests that the selection of mutants with elevated PC1 and smaller PC2 can be equally recommended for stress and normal conditions (Fig. 1c). Mutants KM-1, KM-5, KM-8, KM-10, KM-11, KM-14, KM-22, KM-23, KM-24 and KM-26 with elevated PC1 and decreased PC2 (less stress-responsive and enhanced yield) can perform better in both stressed and normal conditions, showed elevated scores for BY, Gy and HI. However, genotypes KM-20, KM-21, KM-25, KM-27, KM-28, KM-29, KM-30 and KM-32 depicted high values for PC1 and PC2, therefore, considered suitable for non-stress conditions because of their sensitivity to terminal water stress, with high values for PH. While, genotypes KM-2, KM-3, KM-4, KM-6, KM-7, KM-9, KM-17, KM-33, with reduced PC1 and PC2 showing lesser stress sensitivity c can be recommended in breeding for water stress tolerance, scored high values for GPS and MSY. Genotypes KM-13, KM-14, KM-16, KM-17, KM-19, KM-20, and KM-32 WT1 and WT2 with reduced PC1 and elevated PC2 decreased yield and increased terminal stress sensitivity, hence not proposed for cultivation. However, under normal conditions, PC1 valued GPS, MSY, BM, GY, and HI with 27.1% of total variation (Fig. 1d). While PC2 explained 21.6% variation with more importance placed on SPS and MSY altogether, PC1 and PC2 demonstrate 48.7% of the variation for the studied traits.

Conclusions

The present study suggests that water stress had a negative impact on yield and yield components. The genotype × treatment interaction among the mutants of both populations was highly significant for all the treatments. The results depicted that Kiran-95 mutants produced higher grain yield than Bhittai mutants. It is also noteworthy that selection on the basis of biomass, harvest index (%), optimum plant height, lower DSI value and higher relative yield will be helpful to develop high yielding genotypes for water-stressed environments. Mutants from both groups depicted increased potential for yield and stability under the water-stressed condition and sustained yield over diverse irrigation treatments. Among the Bhittai-mutants population, BM-14 was most stable in yield performance for all the irrigation levels, having the lowest DSI and higher relative yield under stress and normal environment. PCA analysis also suggested BM - 14 for both stressed and non-stressed conditions. Whereas, genotypes KM -26 and KM -27 were found suitable for the stressed environment, having lower DSI and higher relative yield. According to PCA analysis KM-26 is recommended for both stressed and non-stressed, whereas, KM-27 is recommended for well-watered environments. These mutants will be backcrossed with the wild-type (Bhittai/Kiran-95) to get rid of undesirable mutations and will be studied further for varietal evolution with better yield performance under water deficit areas of Sindh province, Pakistan.

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