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Yield stability evaluation of wheat (*Triticum aestivum* L.) cultivated on different environments of district Poonch (AJK) Pakistan based upon water-related parameters

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Key words: Genotype × Environment Interaction; Stability analysis; Relative water content.

Abstract

Stability of wheat is a key concern in rain fed areas which are influenced by different environmental issues. In rain fed areas to reduce the environmental consequences on wheat production, the best strategy is growing adapted varieties with yield stability. For this purpose, ten wheat (*Triticum aestivum* L.) genotypes were grown at three different locations *viz* Arja, Rawalakot and Hajira of District Poonch Azad Kashmir to study the stability and performance of different genotypes of wheat based upon water related attributes during 2010-11 and 2011-12 according to Randomized Complete Block Design (RCBD).Water related attributes such as cell membrane thermo-stability (CMTS), relative water content (RWC) and stomatal behavior was calculated from flag leaves to check the association of these water related parameters with biological yield of the crop. The genotypes which have more thermostable and that hold optimum relative water content and more stomatal size and frequency were more biological yield as compared to other genotypes. So the Saleem-2000 was the best genotype followed by Wafaq-2001 according to these water related parameters. To check the stability of yield the major parameter of stability was calculated through coefficient of determination Ri^2, mean yield(μ) and regression coefficient (b_i). From these stability parameters the genotypes which have above 90 percent coefficient of determination Ri^2, regression coefficient closest to one and high mean yield was most stable as compared to all other genotypes. According to these stability parameters Saleem-2000 was most stable followed by Wafaq-2001 in these three locations of District Poonch (AJK) Pakistan.

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Introduction

Wheat (*Triticum aestivum* L.) has third position in the world amongst cereals. In Pakistan it is widely adapted and most important cereal crop because food security of country is based on it (Amir *et al*., 2013). Wheat plays a key role in the economic stability of our country because the nation has to bear import bill of worth billions of rupees annually to meet food requirements of its population. Most of the people living in Pakistan are using it as a main ingredient of their daily diet. Wheat as the principal food grain produced, occupies a leading position in the cropping patterns of Pakistan. It is cultivated on an area of 9,039 thousand hectares with 25,286 thousand tons production (GOP, 2014). It is grown both in irrigated and rain fed condition, where wheat shares around 36 percent of the total crop area of Pakistan and 20 percent of the total wheat crop in rain-fed areas and 80 percent confined to irrigated conditions. (Inamullah *et al*., 1999). In Pakistan, grain yield of wheat crop never exceeded 2.5 tons per hectare, whereas managing methodologies permit farmers of the world to produce10 tons per hectare. The reasons for low yield in Pakistan include poor planning and land preparation, use of low yielding wheat varieties, use of low quality seed, inappropriate use of fertilizers, water logging and salinity, insect infestation and poor wheat management (Qureshi and Bhatti, 2001). The demand of wheat is globally increasing at the rate of 2% annually.

Stability of wheat is a key concern in rain fed areas which are exaggerated by different environmental issues. In rain fed areas to reduce the environmental consequences on wheat production, the best strategy is growing adapted varieties with yield stability. To develop suitable varieties, evaluation of better genotypes is a serious issue in breeding programs, for the reason that the large numbers of genotypes have need to be assessed across locations over many years. So studying the different genotypes response under diverse conditions may significantly surge their productivity performance and potential (Kang, 2002).

Relative water content (RWC) of leaf is a very important feature that is directly associated with the water contents of soil (Sarker *et al*., 1999). RWC is showed the volume of the cell which is closely reflecting the balance between rate of transpiration and supply of water to the leaf (Farquhar and Richards, 1984). This uses the plant ability for recovery from stress and therefore affects the stability of yield (Lilley and Ludlow, 1996).

Cell membrane thermos-stability (CMTS) has used to select and analyze the genotypes of wheat for thermal tolerance against high temperature. In this method electrolyte dispersion is measured from leaf by heat induced leakage of this cell membrane (Saadalla *et al*., 1990). Electrical conductivity has used as monitor in this method to check the stability of membraneto identified the tolerant genotype of wheat and also inother crops (Blum, 1988). When tissues are subjected to high temperature, the electrical conductivity of tissues is also increased that subsequently leakage the solute, which finally damages the leaf cell membrane. So the Cell membrane thermo-stability (CMTS) is the effective screening approaches of wheat genotypes in contradiction of physiological based stresses.

Stomata are basically specialized epidermal cells of leaf that regularize the exchange of water and gases among plant and atmosphere (Bergmann, 2004). Reduction of water loss in demand to maximizing the photosynthetic process the size of stomatal pore is modulated. The maximum exchange of gas thus contains the regulation of size,number, opening and closing ability of stomata (Nadeau and Sack, 2002).

After the comprehensive study of literature, the current study was planned to evaluate the stability and performance of different genotypes of wheat on different locations of District Poonch (AJ&k) Pakistan and recognized the suitable genotypes by using the water related parameters for this area.

Material and methods

Ten wheat genotypes *viz* Chakwal-97, Chakwal-86, Marwat-J01, Haider-2000, Saleem-2000, Auqab-2000, GA-2001, Wafaq-2001, AS-2003 and NARC-2010 were grown on three different locations of District Poonch Azad Jammu and Kashmir. This experiment was conducted on the basis of Randomized Complete Block Design for two consecutive years 2010-11 and 2011-12 with three replications. All these water related parameters were analyzed in the laboratory Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad.

Relative Water Content (RWC) of leaves

For RWC, leaves samples were obtained from 5 randomly selected flag leaves from each replicate. The leaves were covered with polythene bag after excision. Fresh weight was recorded in the laboratory by using electronic balance. After that these leaves were immersed in distilled water in a beaker for 24 hours. After 24 hours when leaves were fully turgid weighed again and noted the reading. These leaves were then dried in oven at 70°C for 72hrs. Then again calculated the dry weight of these oven dried leaves. RWC of flag leaves was calculated by the method of Weatherley (1950) by using the formula,

RWC= [(fresh weight- dry weight) / (saturated weight- dry weight)] × 100

Cell Membrane Thermo-stability (CMT)

CMT was calculated by following the method of Ibrahim and Quick (2001). For CMT estimation leaf samples were collected from 5 randomly selected plants from each replicate. For the removal of any adherent these samples were then washed with de-ionized water. Then these leaves samples were cut into ten discs of 10mm and again washed twice with de-ionized water. These leaves discs were then put into the test tubes with 10ml of de-ionized water. These test tubes were then put into water bath for 30min at 50°C. After this treatment, these tubes were held at room temperature overnight. After that the electrical conductivity (C1) was measured of these test tubes with electrical conductivity (EC) meter and noted the reading. Now autoclaved these test tubes at 100°C and 0.10 Mpa for ten minutes and their electrical conductivity (C2) was also recorded. MTS was expressed in percentage units. MTS was calculated by using the formula,

MTS = (1-C1/C2) × 100

Where C1= conductivity reading after the heat treatment.

C2= conductivity reading after the autoclaving.

Stomatal Behavior

By following the method of Wang and Clarke (1993) stomatal frequency, stomatal size and stomatal area was studied. By using the formula stomatal area (SA) was calculated in mm^2,

SA= SF× SL× SW

Biological Yield (BY) (Kg/ha^{-1})

Biological yield was calculated by harvesting the plants and then converted into Kg/ha^{-1}.

Statistical Analysis

Statistical work was done by using computer package MSTATC. On the basis of factorial experiment analysis of variance (ANOVA) was performed. Least significant difference (LSD) was performed by methods of Steel *et al.* (1997). By following the method of Finlay and Wilkinson (1963) and Eberhart and Russell (1966) stability analysis was done.

Results and discussions

Relative Water Content (RWC %)

The analysis of variance (ANOVA), results of RWC during 2010-11 and 2011-12 showed non-significant ($P<0.05$) difference between years and locations (Fig. 1) in both of years and showed significant ($P<0.05$) difference with in these three locations. In 2010-11 highest RWC was observed in Rawalakot (96.583%), non-significantly followed by that of Arja (91.660%) and Hajira (86.303). In Hajira lowest RWC was observed. During 2011-12 same situation was recorded. Maximum relative water content was observed in Saleem-2000 in all these three locations both of year which is significantly followed by NARC-

2010, Wafaq-2001 and Chakwal-86.Lowest water content was observed in Auqab-2000 in both of years.

The most important indicator of water status of plant is the Relative water content (RWC) as compared to further attributes of water potential of leaf (Jongdee *et al.*, 2002).RWC in normal range, in turgid condition 98% RWC, 40%RWC for severely desiccated leaves. Most crop species RWC around 60-70% at wilting with some exceptions. RWC closely reveal the stability between available water of plant and rate of transpiration. This showed the ability of plant to recover from different environmental pressures and as a result stability of yield is affected (Lilley and Ludlow, 1996). According to Lugojan and Ciulca (2011), the genotype which holds the optimum water capacity is improved yield or provided more stability to the yield. According to present results Saleem-2000 showed maximum water holding capacity in in these locations which is followed by NARC-2010, Wafaq-2001 and Chakwal-86 (Fig. 1) both of the years. The results of these RWC are according to the earlier findings of (Siddique *et al.*, 2000; Khakwani *et al.,* 2012; Huang *et al.*, 2013).

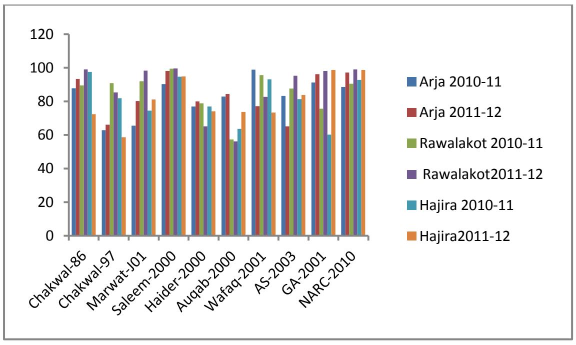

Fig. 1. Relative Water Contents (Percentage) studied in leaves of wheat genotypes grown at different locations of District Poonch.

Cell Membrane Thermo-stability (CMT %)

Cell membrane thermo-stability (CMTS) is method of measuring the electrical conductivity of leaf tissue which is exposed to high temperature in an aqueous phase. The results showed highly significant ($P<0.05$) difference in CMTS during 2010-11 and 2011-12 (Fig. 2). Very highly significant difference was observed in these three locations both of the years. Highest mean of CMTS was observed in 2011-12 as compared to 2010-11. In 2010-11 maximum CMTS was observed in Hajira (46.780%), non- significantly followed by that of Arja (45.033%). Minimum CMTS was recorded in Rawalakot (20.818%). During 2011-12 (Fig.2) maximum CMTS was noted in Arja (48.830%), significantly different from other two locations. Minimum CMTS was also recorded in Rawalakot (26.970%). Comparison of these three locations within two years showed very highly significant difference noted in both of the years. Maximum CMTS was observed in Hajira (46.780%) during 2010-11 and in Arja (48.830%) during 2011-12, while minimum CMTS was studied in Rawalakot both of the years.

CMTs is the effective screening procedure to determine the physiological based pressures(Ibrahim and Quick, 2001). In Pakistan late sowing is at high risk of loss of productivity of wheat crops. When the temperature is increase 1°C during the period of grain filling, yield is approximately 4% reduced (Dupont *et al.*, 2006). Therefore, need of time that a policy should be made to develop genotypes which are tolerant against adverse effects of these environmental changes.

For this purpose we can avail a chance to selecting superior parents and their hybrids for improving the genotypes that are proficient to producing yield of high quality. Yield is increased significantly when genotypes is more cell membrane thermostable. Cell membrane thermos-stability is greatly correlated with yield of a crop (Fokar *et al.*, 1998). Biological yield of wheat is increased in those genotypes which is more thermostable. In this experiment the Saleem-2000 showed optimum CMTs in both years which is non-significantly followed Haider-2000 and Wafaq-2001 (Fig. 2). These results are according with the results of (Fokar *et al.*, 1998; Assad and Paulsen, 2002; Dias and Brüggemann, 2010).

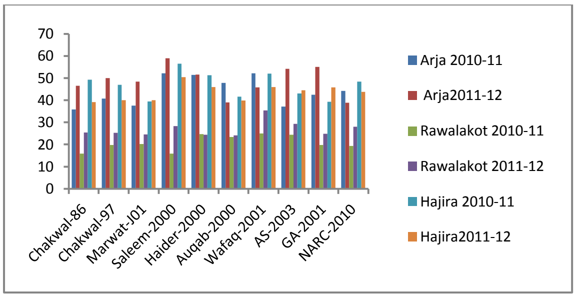

Fig. 2. Cell membrane thermo-stability (Percentage) studied in leaves of wheat genotypes grown at different locations of District Poonch.

Stomatal behavior

Stomatal frequency showed non-significant difference in both of year (Fig.3). High stomatal frequency was observed in Saleem-2000 which is non significantly followed by NARC-2010, Wafaq-2003 and Aquab-2000 but more stomatal size was also noted in Saleem-2000 (Fig.4). Stomatal size showed highly significant differences between locations and years. High stomatal size was recorded in 2010-11 as compared to 2011-12 but the location Rawalakot showed more stomatal size as compared to other locations (Fig.4).

Stomatal area also showed significant difference. More stomatal area was recorded in GA-2001 in Arja and Hajira as compared to Rawalakot which is non significantly followed by Saleem-2000 (Fig.5) while the lowest stomatal area was observed in Wafaq-2001 in all these locations both of years.

Stomatal behavior play an vital role in improving the yield and components of yield (Teare *et al*., 1971)**.** The changes in physiology and anatomy of genotypes for instance stomatal density, frequency, leaf water content (LWC) and stomatal conductance have been main criteria to assessed the yield progress because plant physiologists and breeders continuously select the superior expression of these attributes to keep optimum yield of crops in rain-fed environment (Blum, 1988; Khokhar and da Silva, 2012). Stomata are basically specialized epidermal cells of leaf which regulate the exchange of water and CO^2 between atmosphere and plant (Bergmann, 2004). To lower the water loss and greater the photosynthetic activity, size of stomatal pore is modified (Bergmann, 2004). To optimize the exchange of gases, a regulatory mechanism is required to control the stomatal opening and closing and also modification in stomatal size and number. (Nadeau and Sack, 2002). In water deficit conditions the stomatal density and frequency is required to minimized for achieving the tolerant genotypes (Tanzarella *et al*., 1984). Baodi *et al*. (2008) Reported the relationship between many physio-biochemical parameters and water use efficiency (WUE) of leaf and also suggested that rate of transpiration, rate of photosynthesis and stomatal behavior was the most vital leaf WUE attributes in wheat under rain-fed conditions.

Yousufazi *et al* (2009) studied the positive and significant association between stomatal behavior, yield and yield components and concluded that the occurrence of positive relationships among stomatal behavior, yield component and yield is very helpful designed for screening high yielding wheat genotypes, because counting the stomata is greatly easier as compared to selection of high yielding genotypes of wheat in field for long time. Furthermore new cross combination be able to established among high and low frequency of stomata in flag leaves that the more stomatal frequency provides a new selection principle for plant breeder to screening the high yielding genotypes of wheat (Rajendra *et al*., 1978; Yousufzai *et al*., 2009). The key processes which reduced the yield of a crop are photosynthesis and growth that directly linked to the behavior of stomata Kramer and Boyer, (1995). Stability of yield and production of crop can be improved by selecting the rain fed genotypes. The reason is that the rain fed genotypes succeeded well in dry conditions, with minimum reduction in behavior of stomata. Same results was reported byAhmadi and Siosemardeh (2005).

In present experimental work Saleem-2000 showed more stomatal frequency (Fig.3) and stomatal size (Fig.4) which is positively contributed to increase the biological yield of wheat (Fig. 6) significantly followed by Wafaq-2001. Our results are according with previous finding of (Khan *et al.*, 2003; Bahar *et al.*, 2008, 2009).

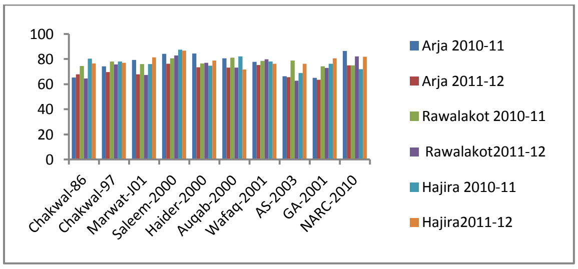

Fig. 3. Stomatal frequency (Percentage) studied in flag leaves of wheat genotypes grown at different locations of District Poonch.

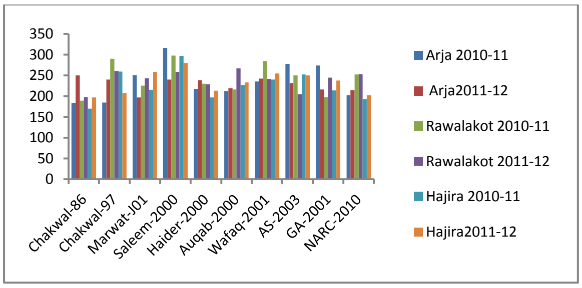

Fig. 4. Stomatal Size (µm²) studied in leaves of wheat genotypes grown in different locations of District Poonch.

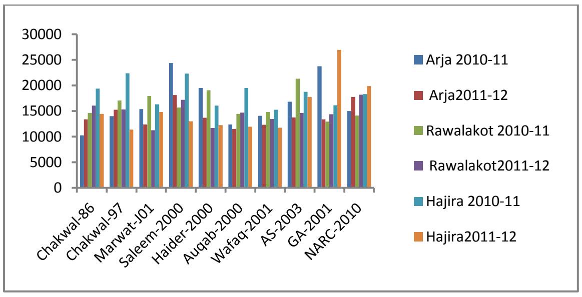

Fig. 5. Stomatal Area (mm²) studied in leaves of wheat genotypes grown at different locations of District Poonch.

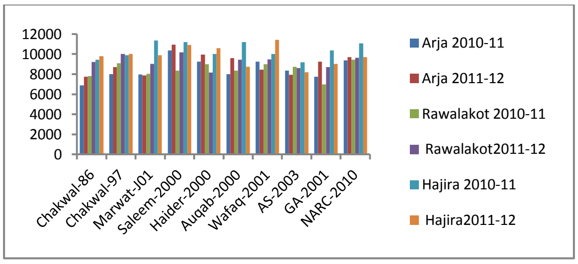

Fig. 6. Biological Yield (Kg/ha^{-1}) studied in wheat genotypes grown at different locations of District Poonch.

Stability Analysis for Water Related Parameters

The stability of yield is the main objective of plant breeder that is considered equally important for production of yield itself. Basically yield stability is used to measure the different crop varieties under various environments in contrast to other varieties. Stability of yield is a special case of genotype environment interaction that is defined the degree in which different genotypes act upon consistently across the diverse environmental conditions. The G×E of crop yield is affected by two major factors that is the degree of similarity among the tested environment and the genetic diversity of genotypes in growing environments.

Stability of yield is vital feature of multi-environment experiment and need of plant breeder are some stability statics that provide a reliable technique for stability of yield. A lot of them are analyzed by Lin *et al.* (1986).

Finlay and Wilkinson (1963), indicated that the slop showed the stability as well as adaptability of a region. If a slop of genotypes is greater than one, then its mean that genotypes adapted particularly high yielding environments and also show sensitivity against changing environments. But the genotypes which have slop less than one showed no sensitivity against changing environments and better adapted the low yielding environments.

Another stability method was developed by Eberhart and Russell (1966), which is performed in relative trials set going on changing environments. This method is used to calculating the linear regression (b_i) and deviation from regression (S^2d_i) of every genotype in relation to the environment. On the bases of this method, the genotypes which have linear regression equal to one and $S^2d_{i=0}$ is more stable. The genotype with linear regression greater than one better respond to harsh environmental conditions, on the other hand the genotypes which are equal to one better adapted the high yielding environments.

Stability measurements for water related parameters were studied according to the method of Eberhart and Russell (1966) Finlay and Wilkinson (1963). This method is suggested that a genotype is stable if it have high mean and its regression coefficient (b_i) is equal to one or near to one across a wide range of environments. If the genotype have a (b_i)>1 is considered to be adapted a poor or harsh environment and genotype have a (b_i) <1is special adaptations for favorable or high yielding environment.

Stability analysis for water related parameter was calculated in present study (Table 01) by following the method of Eberhart and Russell (1966), Finlay and Wilkinson (1963). We calculating the regression coefficient (b_i) separately for both of years, for relative water content ranged between67.853-113.863 and 32.403- 45.490 for cell membrane thermo-stability.

Table 1. Stability Analysis for Water related parameters.

Parameters	μ		b_i		R_i^2	
Years	201011	2011-12	2010-11	2011-12	2010-11	2011-12
Relative Water Content (RWC) (percentage)						
Chakwal-86	102.253	91.525	-1.292	-4.069	0.896	0.957
Chakwal-97	78.450	69.950	1.378	1.103	0.879	0.983
Marwat-J01	77.337	86.473	1.253	1.094	0.544	0.812
Saleem-2000	105.019	103.766	1.001	0.804	0.999	0.926
Haider-2000	77.487	72.958	0.226	-1.167	0.897	0.478
Auqab-2000	67.853	71.350	-1.068	-2.274	0.125	0.496
Wafaq-2001	113.863	81.273	0.901	0.999	0.131	0.237
AS-2003	99.703	77.657	1.523	1.001	0.978	0.999
GA-2001	75.597	108.270	1.366	0.667	0.150	0.077
NARC-2010	109.217	104.840	0.214	0.557	0.803	0.924
Cell Membrane Thermo-stability (CMT) (percentage)						
Chakwal-86	33.637	37.050	1.186	0.804	0.800	0.911
Chakwal-97	35.813	38.447	1.002	0.916	0.928	0.875
Marwat-J01	32.403	37.643	0.928	0.913	0.982	0.915
Saleem-2000	41.530	45.490	1.737	1.217	0.978	0.952
Haider-200	42.477	44.502	1.213	0.999	0.998	0.032
Uqab-2000	37.617	35.860	0.987	0.982	0.962	0.0692
Wafaq-2001	43.023	42.400	1.001	0.977	0.998	0.995
AS-2003	34.890	42.640	0.699	0.930	0.870	0.888
GA-2001	33.860	41.923	0.966	0.821	0.994	0.938
NARC-2010	37.330	36.917	1.218	0.592	0.966	0.870
Stomatal Frequency (Percentage)						
Chakwal-86	73.37	69.58	0.724	0.895	0.934	0.860
Chakwal-97	76.71	74.04	1.049	0.732	0.988	0.690
Marwat-J01	77.08	72.21	1.001	0.938	1.000	0.895
Saleem-2000	84.00	81.34	1.00	0.907	0.940	0.981
Haider-2000	78.48	76.32	0.915	0.940	0.970	0.907
Auqab-2000	81.17	72.67	0.874	1.202	0.973	0.811
Wafaq-2001	78.20	77.46	1.086	1.101	0.986	0.981
AS-2003	71.29	68.12	1.063	1.070	0.876	0.993
GA-2001	71.77	72.31	0.882	1.382	0.799	0.905
NARC-2010	77.75	79.85	1.065	0.926	0.996	0.889

Parameters	μ		b_i		R_i^2	
Years	201011	2011-12	2010-11	2011-12	2010-11	2011-12
Stomatal size(µm²)						
Chakwal-86	180.9	214.9	0.758	0.814	0.893	0.875
Chakwal-97	244.4	235.9	0.729	0.762	0.709	1.000
Marwat-J01	215.0	232.5	0.650	0.518	0.985	0.780
Saleem-2000	303.3	259.2	0.968	0.941	0.990	0.934
Haider-2000	214.5	226.4	0.996	1.001	0.989	0.920
Auqab-2000	218.2	239.4	1.444	1.115	0.991	0.883
Wafaq-2001	221.3	245.8	0.903	0.952	0.974	0.995
AS-2003	259.8	228.5	0.877	1.322	0.982	0.702
GA-2001	228.4	218.9	0.719	1.345	0.983	0.827
NARC-2010	215.5	223.0	0.624	0.795	0.936	0.889
Stomatal area (mm²)						
Chakwal-86	14739	145844	0.072	0.396	0.033	0.020
Chakwal-97	17790	13962	0.311	-0.698	0.063	0.078
Marwat-J01	16529	12788	-0.319	0.717	0.032	0.897
Saleem-2000	20770	16085	0.978	0.990	0.907	0.945
Haider-2000	18181	12505	1.873	1.236	0.902	0.912
Auqab-2000	15407	12675	0.494	0.828	0.246	0.905
Wafaq-2001	14701	12490	1.001	0.937	0.989	0.900
AS-2003	18924	15337	0.914	0.823	0.348	0.993
GA-2001	17124	18210	0.628	1.617	0.904	0.530
NARC-2010	15782	18590	2.153	0.662	0.814	0.809
Biological Yield kg/ha⁻²						
Chakwal-86	8030	8902	2.148	1.285	0.805	0.430
Chakwal-97	8985	9572	1.336	0.599	0.580	0.188
Marwat-J01	9113	8923	3.576	0.414	0.889	0.589
Saleem-2000	9958	10669	2.109	0.448	0.610	0.305
Haider-2000	9410	9560	0.959	1.768	0.981	0.894
Auqab-2000	9486	9247	0.257	-0.819	0.984	0.936
Wafaq-2001	9666	9777	1.232	2.526	0.140	0.818
AS-2003	7486	8249	0.261	0.160	0.511	0.065
GA-2001	7571	8985	0.762	0.111	0.586	0.049
NARC-2010	8760	9677	0.661	0.048	0.695	0.568

μ: Varietal Mean

b_i: Regression Coefficient (Slope)

R_i^2: Coefficient of Determination

On the bases of relative water content Wafaq-2001 showed closest to one regression coefficient both of years and highest mean was also noted in Wafaq-2001 in one year and GA-2001 in next year. According to cell membrane thermos-stability Chakwal-97, Marwat-J01, Auqab-2000 and Wafaq-2001 had close to one regression coefficient both of years but the highest mean was observed in Wafaq-2001one year and in next year Saleem-2000 showed highest mean.

By following the procedure of Finlay and Wilkinson (1963)Saleem-2000 have highest mean in stomatal size and stomatal frequency during both of years (Table 01) however highest mean of stomatal area was observed in genotype saleem-2000 during 2010-

11(20770.9) and NARC-2010 in 2011-12 (18590.6). According to stomatal frequency in this experiment the Saleem-2000, Haider-2000 and Marwat-J01, would be stable for the reason that its regression coefficient was close to one during this two years. On the bases of stomatal size Wafaq-2001, NARC-2010 and Haider-2000, have stable because its regression coefficient during both of the years was also close to one. According to stomatal area Saleem-2000 and Wafaq-2001 was the stable genotypes. The other genotypes that have a greater than one regression coefficient adapted the poor or harsh conditions and the genotypes which have regression coefficient lower than one adapted the high yielding or favorable conditions.

Coefficient of determination (Ri^2) is an another stability parameter that was discussed by Petersen (1989) is also evaluated in this study (Table 01). Basically this stability parameter is used to calculate the proportion of variation or mean yield of genotypes. According to this water related parameters Saleem-2000, Wafaq-2001 and Haider-2000 have coefficient of determinations almost 90% in these two years and come across to be stable on the bases of this stability parameter. Other genotypes in one year also showed coefficient of determination 90% but not in others.

Conclusion

The results of present study showed that higher relative water content (RWC), stomatal behavior and cell membrane thermo-stability (CMTs) with optimum biological yield is helpful screening methods for stability of yield. Because RWC is a key indicator of water status of plant as compare to other water potential parameters. The genotypes that have the ability to hold optimum RWC increased the crop yield. Biological yield of a crop also increase by selecting the CMTs lines. Similarly stomatal behavior also contributed positivity to enhancing the yield of a crop. So the water related parameters of this study confer the significant and positive association with biological yield which can be considered in wheat breeding programme to improve the yield of a crop in future.

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