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DETECTION OF GENOTYPIC VARIATION IN RESPONSE TO WATER STRESS AT SEEDLING STAGE IN ESCALATING SELECTION INTENSITY FOR RAPID EVALUATION OF DROUGHT TOLERANCE IN WHEAT BREEDING

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Abstract

A screening of diverse germplasm in field is time consuming and in laboratory is expensive. An easy and economical technique was employed to access the behavior of seventy five diverse wheat genotypes. The experiment was conducted on the basis of germination percentage, emergence rate, emergence rate index, survival after desiccation and seedling recovery with the aim to evaluate best adopted parents. These traits were pooled to differentiate water stress tolerant and susceptible genotypes, by increasing selection intensity and rapid evaluation for drought tolerance which may be used in succeeding research programs.

Introduction

Water stress or water deficit is a predestined and inveterate feature of global agriculture. About one-third of the world's potentially arable land suffers due to water shortage, and most of the crops production is often reduced by drought and diseases (Bray, 1997; Dash & Mohanty, 2001, Ahmad *et al.,* 2010). Being an integral part of plants water plays a pivotal role in the initiation of growth, subsequent maintenance of developmental process throughout the plant's life and ultimately economy of a country (Noorka & Haidery, 2011, Shafi *et al.,* 2012). In Pakistan 25-30% of the total cropped area is under rainfed conditions. It is therefore, a dire need that an effective wheat breeding program would be launched to evolve high yielding and well adapted hybrids/varieties for water deficit conditions (Akram *et al.,* 2002; Akhtar *et al.,* 2008; Noorka & Afzal 2009). However breeding for water stress requires continuous efforts primarily, through the efficient screening techniques and knowledge of genetic mechanism governing heritable parameters. (Noorka *et al.*, 2009, Noorka *et al.,* 2013). Seedling trait is an important aspect of any crop breeding programme, since the final stand of a crop mostly depends on seedling characteristics. Various factors like seed germination, seedling vigor, growth rate, mean emergence time and desiccation tolerance affect the yield of a crop (Noorka & Khaliq, 2007; Crosbie *et al*., 1980). Emergence percentage is the ability of a plant to emerge its aerial parts from the soil (Heydecker, 1960) has been considered a very important component of seedling vigour (Allen & Donnelly, 1965; Basra *et al*., 2003). Poor germination and uneven crop stand are the main constraints of a good crop (Kumari *et al.*, 2000; Siddiqui *et al*., 2008; Noorka *et al.*, 2009). The survival after desiccation was the next important seedling trait after emergence percentage (Chang & Loresto, 1986; Farooq *et al.,* 2006), and most suitable for screening large population (Winter *et al*., 1988; Hameed *et al.,* 2010).

The present study was therefore, aimed to develop appropriate screening technique for large population prior to yield testing. Another objective was to examine the rate of desiccation tolerance in wheat seedling under water stress environment.

Materials and Methods

The experiment was conducted in a temporary green house in the Department of Plant Breeding & Genetics, University College of Agriculture, University of Sargodha, Sargodha, Pakistan. Seventy five varieties/lines of wheat were collected from National Cereal Breeding programmes and International research organizations. The layout used was complete randomized design (CRD) with three replications. The seeds were sown in 18x9 cm polythene bags filled with measured quantity of normal field soil (450 g/bag) as described previously by Noorka & khaliq, (2007). The bags were arranged in iron trays, each genotype comprising five bags per replication. Two seeds of each variety were sown in each bag at uniform depth of 3 cm to ensure full crop stand. In early stages of screening it was assumed essential to examine only those traits which could be visually and easily recognized due to large number of genotypes used in the experiment and the following parameters data was recorded for further analysis.

Emergence Percentage (E%): Data collection began instantly after the emergence of first seedling in any bag from then to onwards measurements were made on daily basis at 1600 h. After eighteen days of sowing, the number of visible seedlings was recorded. Data collection continued until there was no further growth. Than emergence percentage was calculated according to the formula derived by Smith & Millet (1964).

$$E\% = \frac{\text{Total number of seedlings emerged 18 DAS}}{\text{Total number of seedlings grown}} \times 100$$

DAS = Days after Sowing

Emergence Index (EI): It is the estimate of emergence rate of seedlings and was calculated by the formula as delineated in AOSA Association of Official Seed Analysis (Anon., 1983).

$$EI\% = \frac{\text{No. of seeds emerged at first count+ ……+ No of seeds emerged at final count}}{\text{Days of first count+……………+ days of final count}}$$

Emergence Rate Index (ERI): Emergence rate index for each treatment and replication was calculated as follows:

ERI = Emergence index/Emergence percentage

Energy of emergence (EE): Energy of emergence was computed according to the method as outlined by Ruan *et al*., (2002). It is the percentage of emerged seedlings three days after sowing.

Mean emergence time (MET): Mean emergence time was calculated in accordance with the equation of Ellis & Roberts (1981) as under:

$$MET = \Sigma Dn / \Sigma n$$

where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of emergence.

Desiccation tolerance index (DTI): The plants were well watered till 2-3 leaf stage which is considered proper stage for seedling evaluation as suggested by (Anon., 1997). Afterwards the water was withheld as a result of which most of seedlings died. On resumption of irrigation survival was noted on re-growth of plants in each replication. The number of live as well as dead seedlings was counted daily following the work of different researchers (Noorka & Khaliq, 2007; O'Toole *et al*., 1978; Younis *et al*., 1963). Desiccation tolerance index was calculated according to Peacock *et al*., (1990) and (Noorka & Khaliq, 2007).

Desiccation tolerance index = Final number of dead seedlings/Final emergence number

Percent seedling recovery (PSR): It is the measure of percent recovery or re-growth of seedlings after desiccation and is calculated by the formula as used by Peacock *et al*., 1990 and Noorka & Khaliq 2007).

$$\text{Percent seedling recovery} = \frac{\text{Number of plants resuming growth}}{\text{Total number of seedlings}} \times 100$$

Statistical analysis: The data thus obtained was subjected to analysis of variance (Steel *et al*., 1997). The cluster analysis and the principal component analysis were applied as described by Seber (1984), and Brown (1991).

Results and Discussion

Analysis of variance was performed for all 7 traits. Differences among genotypes were highly significant (Table 1).for most of the traits indicating high variability among genotypes

Among the 75 genotypes the emergence percentage ranged between 47% to 100% (Table 2). Maximum value of emergence index was obtained by the genotypes Chakwal-86 which is (7.920) while minimum (2.170) in genotype V07017. A great magnitude of variability was observed in emergence percentage, emergence index and energy of emergence. Earlier and rapid emergence was observed in genotypes which have maximum energy of emergence and emergence rate index ranging from 55 to 100% and 0.083 to 0.043, respectively as shown in Table 2.

Maximum mean emergence time (2.753) was recorded for genotype Rohtas, while minimum (1.532) in genotype Pasban-90. Desiccation gave interesting results, only those genotypes survived which had good emergence and low Desiccation tolerance index. Percent seedling recovery was observed in the genotype Sehar-2006 (92.00%) while minimum percent seedling recovery was observed in genotype Chanab-2000 (0.00%). Sixteen genotypes showed zero percent recovery. The Cluster analysis was performed in the present study to differentiate various items on the basis of similarities present in the data. The wheat genotypes were classified into five groups which are presented in Table 3 and Fig. 1.

Table 1. Mean square of seedling traits.

S.O.V	d.f	E%	EI	ERI	EE	MET	DTI	PSR
Rep	2	214.33**	2.55*	0.00	134.33	0.079	0.001	6.18
Gen	74	497.87**	4.79**	0.00	401.96**	0.196**	0.371**	4384.64**
Error	148	80.07	0.89	0.00	97.06	0.122	0.002	94.58

SOV=Source of variation, Df=Degree of freedom, E%=Emergence percentage, EI=Emergence Index, ERI=Emergence rate index, EE=Energy of emergence, MET=Mean emergence time, DTI=Desiccation tolerance index, PSR=Percent seedling recovery

There is an inverse relation between emergence percentage, emergence index, energy of emergence and mean emergence time. Higher the emergence percentage, emergence index and energy of emergence and lower mean emergence time indicated earlier and rapid germination. These findings support the earlier work on Canola (*Brassica compestris*) by Zheng *et al*., (1994), wheat (*Triticum aestivum* L.) by (Nayyar *et al.*, 1995; Noorka & Khaliq, 2007; Hameed *et al.*, 2010), and on rice (*Oryza sativa*) by Basra *et al.,* (2003). Among seventy five genotypes 25 genotypes exhibited emergence percentage (47.56-100), while emergence index ranging (5.37-7.92) and energy of emergence ranging (36.87-60.00). These genotypes also exhibited lower mean emergence time ranging (1.68-2.43) days and desiccation tolerance index ranging (0.116-1.00). Percent seedling recovery measures the re-growth percentage. The genotypes Sehr-2006 and Kohistan-97 exhibited maximum re-growth (92%). These genotypes showed lower desiccation tolerance index and higher percent seedling recovery. These results are supported by the early findings of Milthorpe (1950). Sixteen genotypes totally failed to re-growth, they permanently died. Group 3 showed the cluster of 25 genotypes. The points closest to each other are assembled in one cluster because distance between them is small as compared to others. These genotypes in group 3 shows maximum emergence percentage, emergence index, emergence rate index, energy of emergence and percent seedling recovery while minimum mean emergence time and desiccation tolerance index respectively. Survival after desiccation proved useful indices for rapid evaluation of water stress response in wheat breeding. Similar findings had been reported by (Winter *et al.,* 1988; Noorka & Khaliq, 2007; Hameed *et al.,* 2010, Muhammad and Hussain 2012).

Different researchers have used cluster analysis to group different wheat genotypes based on diverse characteristics and found similarities of wheat genotypes within a group (Ahmad, 2001; Mahmood, 2004).

Table 2. Mean values of emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percent seedling recovery.

Genotypes	EP	EI	ERI	EOE	MET	DTI	PSR
Lasani 2008	90.00	5.613	0.0760	44.67	1.897	0.7120	87.00
Uqab 2002	93.33	5.913	0.0660	43.33	1.820	0.3397	68.02
Pasban 90	74.00	5.378	0.0767	40.00	1.532	1.000	0.000
Shafaq 2006	82.00	6.734	0.0810	44.33	1.943	0.5672	87.00
Inqlab 91	87.00	6.900	0.0794	50.00	1.967	0.1334	87.00
Sehr 2006	82.00	6.837	0.0834	46.67	1.933	0.6660	92.00
FSD 2008	81.00	6.273	0.0811	44.67	1.834	0.6416	89.00
V02192	80.00	6.167	0.0713	43.33	1.767	0.7270	27.31
V03079	86.67	5.833	0.0700	40.00	1.803	0.7547	23.54
V07194	55.67	4.860	0.0560	26.67	2.380	0.7723	23.00
V03094	54.33	5.100	0.0676	43.23	2.043	0.7797	18.07
V08172	90.00	6.527	0.0726	43.33	1.769	0.8965	7.78
V08181	89.00	6.736	0.0761	43.67	1.693	0.8083	20.00
V07006	80.00	5.500	0.0687	36.67	1.807	0.9520	4.78
V08182	76.67	5.417	0.0706	34.33	1.737	0.8750	13.00
V07080	70.00	5.080	0.0786	31.00	2.270	1.0000	0.00
V07114	72.33	4.177	0.0730	20.00	2.183	0.9586	4.16
V07183	80.00	5.330	0.0580	36.67	1.997	0.8057	20.00
V07160	72.00	4.434	0.6676	30.00	2.000	1.0000	0.00
V08161	76.67	5.000	0.0630	34.33	2.010	1.0000	0.00
V08178	80.00	4.510	0.0650	30.00	2.117	0.8213	18.79
V07079	80.00	3.833	0.7607	17.67	2.290	0.9027	9.72
V08179	66.67	5.067	0.0438	33.33	2.093	0.9260	7.40
V06073	80.00	4.080	0.0636	28.67	2.263	1.0000	0.00
V07068	73.33	4.790	0.0593	31.00	2.307	0.9167	0.00
V07017	56.67	2.170	0.0533	13.33	2.359	0.8000	21.00
V07028	63.33	4.443	0.5964	31.00	2.073	0.8734	9.66
V07029	80.00	4.080	0.0636	26.67	2.263	1.0000	0.00
V06093	60.00	5.317	0.6367	33.33	2.323	0.4437	8.78
V07075	63.33	3.747	0.6033	23.33	2.310	1.0000	0.00
V07080	86.67	6.510	0.5900	43.33	1.887	0.9333	11.57
V07056	66.67	3.417	0.0610	21.00	2.087	1.0000	4.78
V04488	73.33	5.540	0.0613	33.33	2.160	0.8593	10.57

Table 2. (Cont'd.).

Genotypes	EP	EI	ERI	EOE	MET	DTI	PSR
Farid- 2006	100.00	7.213	0.5490	48.98	1.910	0.2413	89.00
SH-2002	90.00	6.593	0.0684	40.00	1.957	0.2407	75.53
Bhakkar- 2002	97.00	7.220	0.0747	54.33	1.670	0.1370	86.50
AS-2002	47.48	6.187	0.0633	10.00	2.070	0.2517	75.62
GA-2002	100.00	7.617	0.5933	47.67	1.800	0.1333	87.60
Ufaq	80.00	4.557	0.5900	26.67	2.217	1.000	0.00
Chanab 2000	70.00	3.087	0.0566	16.67	2.210	1.000	0.00
MH-97	77.07	2.973	0.0521	34.41	2.400	0.5223	48.79
Kohistan 97	91.00	6.953	0.0773	50.00	1.799	0.1047	90.00
Iqbal 2000	74.40	6.887	0.0708	43.43	1.887	0.1620	84.90
Potohar	73.33	5.583	0.0690	36.67	1.767	0.5483	45.17
Kohenoor	70.00	5.798	0.0809	40.00	1.770	0.2467	75.33
D-97	56.76	3.447	0.0600	20.00	2.077	1.0000	5.76
Shahkar 95	97.67	6.317	0.6576	41.00	1.963	0.8731	14.90
Punjab 85	72.00	4.063	0.0538	24.33	2.310	1.0100	82.00
Barani 83	77.00	4.657	0.6967	26.67	2.178	0.1708	88.00
V 46	80.00	4.750	0.0623	30.00	2.070	0.7950	20.50
C-591	90.00	5.097	0.5767	23.33	2.140	0.8963	10.37
C-273	76.67	5.333	0.0680	36.67	1.873	0.1800	82.01
Maxipak- 65	73.40	4.087	0.0610	27.67	2.127	0.8843	12.00
V-03094	70.00	5.387	0.0647	40.00	1.933	0.8750	9.33
Dirk	74.30	4.777	0.0698	34.33	1.900	1.0000	0.00
V-04	50.00	2.312	0.0641	32.33	1.870	1.0000	0.00
Blue silver	87.00	5.473	0.0633	37.87	2.215	0.8057	0.00
D-06623	70.00	3.473	0.0480	16.67	2.663	0.1250	72.00
Chanab 70	73.33	5.080	0.0723	34.67	1.733	0.1867	82.00
Layallpur 73	82.00	4.782	0.0620	31.00	1.894	0.1787	85.00
SA-75	83.33	3.917	0.0536	20.00	2.450	0.131	87.00
SA-42	80.00	3.512	0.0465	33.00	1.958	0.142	78.00
WL-711	85.67	5.807	0.0663	40.00	1.970	0.1860	83.00
Sandal -78	80.00	4.557	0.0686	30.00	1.850	0.1000	0.00
Inqlab-91	87.79	6.573	0.0783	50.00	1.987	0.1313	87.00
Pari- 73	91.00	5.321	0.0580	30.00	2.297	0.1787	82.50
Yakora	75.00	4.590	0.0560	24.00	2.130	0.3470	66.47
Pak-81	84.33	5.786	0.0633	36.87	2.160	0.1787	83.14
Punjab-76	71.00	3.514	0.0563	17.66	2.218	1.0000	0.00
Lu-26	94.76	6.414	0.0679	47.78	1.932	0.6978	32.08
Sahiwal	82.00	6.817	0.0813	46.67	1.933	0.0666	81.00
Punjab 81	86.67	4.387	0.0600	23.33	2.143	1.0000	0.00
Chakwal	81.09	7.920	0.0786	60.00	1.700	0.1333	87.91
Rohtas	97.67	4.187	0.0560	23.00	2.753	0.8709	13.09
FSD-83	77.33	4.970	0.0637	31.00	2.096	0.1676	83.33

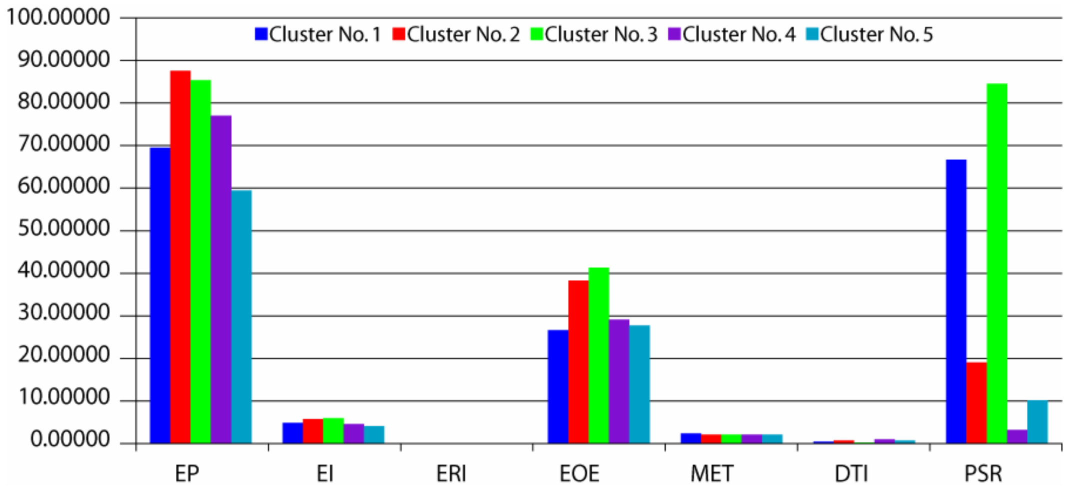

Fig. 1. Graphical representation of mean values of emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percent seedling recovery.

Table 3. Cluster groups obtained in wheat genotypes.

	Cluster No. 1	Cluster No. 2	Cluster No. 3	Cluster No. 4	Cluster No. 5
EP	69.26857	87.49454	85.17080	76.85000	59.34300
EI	4.66671	5.75282	6.04912	4.64691	3.98800
ERI	0.06044	0.16789	0.13556	0.17153	0.22422
EOE	26.58286	38.37364	41.38520	29.14545	27.75500
MET	2.15857	1.97736	1.94584	2.08068	2.16150
DTI	0.43586	0.81669	0.24441	0.91025	0.85951
PSR	66.48286	19.05091	84.53760	3.36046	9.84500

Conclusion

Choice of the parent has pivotal role in any breeding programme. By screening we can select the potential parents for onward selective and beneficial breeding. It is therefore concluded that earlier and more uniform germination and emergence was observed in some genotypes. The genotypes Lasani 2008, Uqab 2002, Shafaq 2006, Inqlab 91, Sehr 2006, FSD 2008, Farid- 2006, SH-2002, Bhakkar-2002, GA-2002, Kohistan 97, Iqbal 2000, Barani 83, C-273, Chanab 70, Layallpur 73, SA-75, SA-42, WL-711, V08180, Pari-73, Pak-81, Sahiwal, Chakwal and FSD-83 proved best genotypes. These genotypes could be used in future breeding programmes for drought tolerance research studies. It is also concluded that by this method, screening a large germplasm in easy and economic way, researchers are in position to differentiate water stress tolerant and susceptible genotypes, by increasing selection intensity and rapid evaluation for drought tolerance which may be used in succeeding research programmes.

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