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AN EFFICIENT TECHNIQUE FOR SCREENING WHEAT (*TRITICUM AESTIVUM* L.) GERMPLASM FOR DROUGHT TOLERANCE

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Abstract

Effective screening techniques for drought resistance would be beneficial in wheat breeding programme. Higher water requirement and increasing labour costs are the major problems of field and laboratory screening techniques. An attempt to improve the economic and rapid screening was made in the present study. The investigation was mainly restricted to seedling response of 100 wheat varieties/lines. Wheat seedlings were counted to measure the following seedling traits; emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, percent seedling recovery and desiccation tolerance index. These traits when pooled together could discriminate between drought tolerant and susceptible genotypes.

Introduction

Increasing food demand and declining water availability are the major threats to world food security. It is obviously true that present and future wheat food security will squarely depend on water scarce environments. The global water crisis is a severe threat for sustainable agriculture, particularly in most of the Asian countries where irrigated agriculture accounts for 90% of total diverted fresh water (Huaqi *et al*. 2002). Farmers and researchers are striving hard to find means to decrease water use in wheat production and to increase its use efficiently.

In our crop improvement programmes, the most threatning problem is the shortage of water at the seedling stage, mid season water stress, terminal stress or a combination of any two or three. 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 vigour, growth rate, mean emergence time and desication tolerance affect the yield of a crop (Crosbie *et al*., 1980).

Emergence percentage among all seedling traits which 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. The survival was the next important seedling trait (Chang & Loresto, 1986; Farooq *et al.*, 2006). Survival after desiccation was the most important and suitable technique for screening large population (Winter *et al*., 1988).

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 greenhouse in the Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan. One hundred varieties/lines of wheat were collected from National Cereal Breeding programmes and International research organizations. The layout used was complete randomized design with three replications. The seeds were sown in 18x9 cm polythene bags filled with measured quantity of normal field soil (450 g/bag). 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 essential to examine only those traits which could be visually and easily recognized due to large number of genotypes used. The following seven traits were measured:-

Emergence percentage (%): Counting was started immediately when first seedling emerged in any bag from then to onwards measurements were made daily at 1700 h. The number of visible seedlings was recorded. The measurement continued until there was no further increase and was calculated according to the formula derived by Smith & Millet (1964).

$$E\% = \frac{\text{Total number of seedling emerged 18 DAP}}{\text{Total number of seeds grown}} \times 100$$

DAP = Days after planting

Emergence index (EI): It is the estimate of emergence rate of seedlings and was calculated as described in Association of Official Seed Analysis (1983).

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

Emergence rate index (ERI): Emergence rate index for each treatment and replication was calculated as emergence index divided by emergence percentage.

$$ERI = \frac{\text{Emergence index}}{\text{Emergence percentage}}$$

Energy of emergence (EE): Energy of emergence was computed according to the method as delineated 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 according to the equation of Ellis and Roberts (1981) as under:

$$MET = \frac{\Sigma Dn}{\Sigma n}$$

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

Desiccation tolerance index: The plants were well watered until 2-3 leaf stage which is considered proper stage for seedling evaluation as suggested by ISTA (Anon., 1997). Then the water was withheld due to which most of seedlings died. Then the plants were rewatered and survival was counted after regrowth in each replication. The number of live seedlings and at the same time number of dead seedlings were counted daily following different researchers (O'Toole *et al*., 1978; Younis *et al*., 1963). Desiccation tolerance index was calculated according to Peacock *et al*., (1990).

$$\text{Desiccation tolerance index} = \frac{\text{Final number of dead seedlings}}{\text{Final emergence number}}$$

Percent seedling recovery: It is the measure of percent recovery or re-growth of seedlings after desiccation and is calculated by the formula as given elsewhere Blum *et al*., 1980; Peacock *et al*., 1990).

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

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

Results

Analysis of variance was performed for all seven traits. According to the results differences among genotypes were highly significant for most of the traits indicating high variability among genotypes (Table 1).

Among the hundred genotypes the emergence percentage ranged between 46% to 100%. Maximum value of emergence index was obtained by the genotypes Chakwal-86 is (7.620) while minimum (2.470) in PBW-222. 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 60 to 100% and 0.076 to 0.047, respectively as shown in Table 3.

Maximum mean emergence time (2.79) was recorded for genotype UAF-8126, while minimum 1.52) in genotype Pasban-90. Desiccation gave very interesting results. Only those genotypes survived which had good emergence and low Desiccation tolerance index. Percent seedling recovery was observed in the genotype Nesser (93.33%) while in genotype Dirk (0.00%). Twenty-five genotypes showed zero percent recovery.

The Cluster analysis is a recent computerized multivariate technique, which differentiates various items by analyzing data similarities. There are different methods for cluster classification such as average linkage methods, centeroid methods, complete linkage methods and ward`s method etc.

The cluster analysis based on average linkage method (between groups) was performed in the present study. The wheat genotypes were classified into four groups which are presented in Table 2 Fig. 1.

Table 1. Mean squares of seedling traits in wheat genotypes.

S.O.V.	d.f.	E%	EI	ERI	EE	MET	DTI	PSR
Replication	2	234.33**	2.75*	0.00	184.33	0.099	0.001	8.16
Genotypes	99	391.58**	3.19**	0.00	303.96**	0.196**	0.347**	3484.64**
Error	198	75.07	0.68	0.00	90.06	0.122	0.009	89.58

*Significant at p<0.05, **Significant at p<0.01, NS= Non-significant.
Where
E % = Emergence percentage
EE = Energy of emergence, MET Mean emergence time
DTI = Desiccation tolerance index and
PSR = Percent seedling recovery

Table 2. Cluster analysis grouping of 100 wheat genotypes.

Group 1	Group 2	Group 3	Group 4
1, 3, 4, 6, 9, 11, 12, 13, 14, 15, 16, 17, 22, 23, 27, 28, 30, 33, 36, 38, 39, 60, 61, 62, 65, 66, 67, 69, 71, 72, 73, 83, 92	2, 7, 8, 10, 18, 19, 20, 21, 24, 25, 26, 29, 31, 32, 34, 35, 37, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 63, 64, 68, 70, 74, 75, 76, 78, 79, 80, 81, 82, 84, 85, 86, 87, 88, 89, 90, 91, 93, 94, 95, 96, 97, 98, 99, 100	5	58, 59

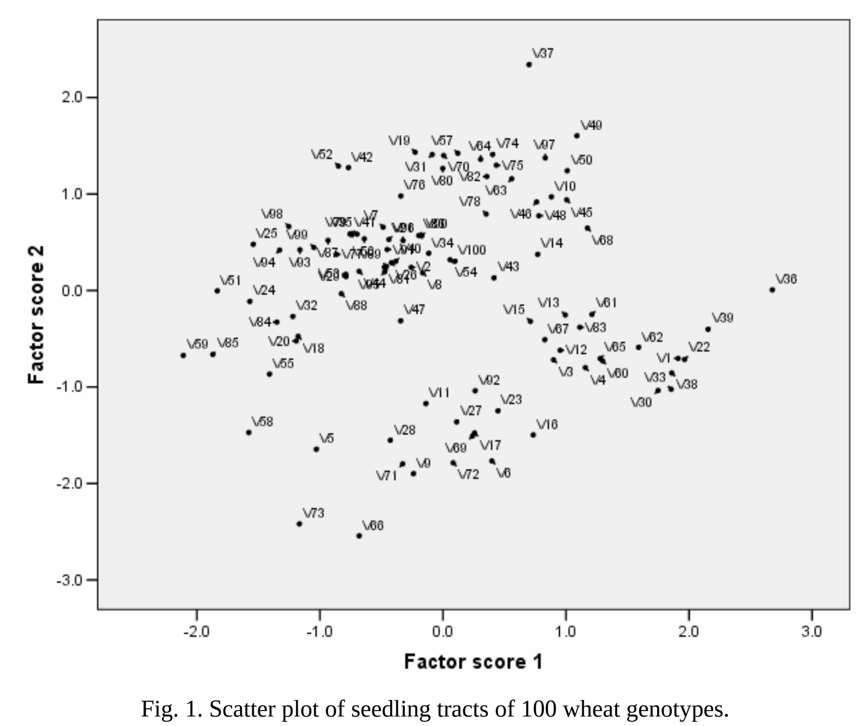

Fig. 1. Scatter plot of seedling tracts of 100 wheat genotypes.

Table 3. Mean values of emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and percent seedling recovery.

Genotypes	Emergence % age	Emergence index	Emergence rate index	Energy of emergence % age	Mean emergence time days	Desiccation tolerance index	Percent seedling recovery
V_1.GA-2002	100.0	6.917	0.6933	46.67	1.800	0.1333	86.67
V_2.C-271	90.00	5.097	0.5767	23.33	2.140	0.8963	10.37
V_3.C-250	76.67	5.333	0.06800	36.67	1.873	0.1800	82.01
V_4.WL-711	86.67	5.807	0.06633	40.00	1.970	0.1897	81.02
V_5. C- 518	86.67	4.360	0.0500	16.67	2.720	0.6167	38.33
V_6.PARI-73	90.00	5.233	0.05800	30.00	2.297	0.1787	82.13
V_7. SA-42	76.67	4.750	0.06233	30.00	2.157	1.000	0.0000
V_8. C-217	80.00	4.973	0.06300	30.00	2.070	0.7950	20.50
V_9. T-96725	70.00	4.307	0.06067	23.33	2.320	0.1390	86.11
V_{10}.SHAHKAR	96.67	6.317	0.6567	40.00	1.963	0.8630	13.70
V_{11}.YEKORA	76.67	4.530	0.05800	23.33	2.140	0.3470	65.28
V_{12}.C-273	80.00	5.473	0.06733	33.33	1.777	0.203	79.96
V_{13}.SA-75	83.33	5.723	0.06800	40.00	1.903	0.3563	64.35
V_{14}.POROWAR	73.33	5.583	0.06900	36.67	1.767	0.5483	45.17
V_{15}.C-591	66.67	5.277	0.06700	30.00	1.807	0.3420	65.81
V_{16}.PAK-81	83.33	5.677	0.06333	36.67	2.160	0.1787	82.13
V_{17}.MANTHAR-03	86.67	50.10	0.06100	30.00	2.213	0.2370	76.31
V_{18}.ROHTAS-90	96.67	4.197	0.05800	20.00	2.753	0.8750	12.50
V_{19}.SANDAL-73	80.00	4.557	0.06867	30.00	1.850	0.100	0.000
V_{20}.SARHAD-82	66.67	4.247	0.05100	16.67	2.477	0.9260	7.407
V_{21}.UFAO-2001	53.33	5.000	0.05800	26.67	2.147	0.9630	0.000
V_{22}.KOHISTAN-97	90.00	6.953	0.07722	50.00	1.797	0.1037	89.63
V_{23}.LYALPUR-73	80.00	4.723	0.06000	30.00	1.843	0.1787	84.26
V_{24}.PUNJAB-76	70.00	3.583	0.05367	16.67	2.343	1.000	0.0000
V_{25}.CHENAB-76	70.00	3.087	0.05667	16.67	2.210	1.000	0.0000
V_{26}.C-228	96.67	4.553	0.06467	30.00	2.063	0.8133	18.65
V_{27}.BARANI-70	70.00	4.807	0.06100	30.00	2.227	0.2467	67.92
V_{28}.ARZ	76.67	4.000	0.05733	23.33	2.330	0.2820	71.82
V_{29}.INDUS-79	100.0	4.163	0.05933	23.33	2.213	0.8630	13.69
V_{30}.NESSER	90.00	6.633	0.06900	46.67	1.953	0.6667	93.33
V_{31}.DIRK	73.33	4.777	0.06833	33.33	1.900	1.000	0.000
V_{32}.CHENAB-70	100.0	4.137	0.05733	20.00	2.393	0.9580	4.167
V_{33}.DHARWERDRY	82.00	6.817	0.8313	46.67	1.933	0.06667	93.33
V_{34}.PAVON-76	10.00	5.333	0.05933	30.00	2.180	0.9333	6.667
V_{35}.SHALIMAR-88	96.67	4.443	0.06067	26.67	2.180	1.000	0.0000
V_{36}.CHAKWAL-86	80.00	7.620	0.07633	60.00	1.700	0.1333	86.67
V_{37}.PASBAN-90	73.33	5.387	0.07667	40.00	1.523	1.000	0.000
V_{38}.INOULAB-91	86.37	6.873	0.07926	50.00	1.967	0.1333	86.67
V_{39}.BAKHAR-02	96.67	7.220	0.07467	53.33	1.660	0.1370	86.30
V_{40}.MAXIPAK-65	73.33	4.807	0.06000	26.67	2.127	0.8843	11.57
V_{41}.PUNJAB-90	86.67	4.387	0.06000	23.33	2.143	1.000	0.0000
V_{42}.WADNAK-85	83.33	3.693	0.0633	23.33	1.933	1.000	0.0000
V_{43}.UAF-9252	86.67	6.037	0.06267	40.00	2.237	0.7960	17.04
V_{44}.BWP-79	80.00	4.333	0.05933	23.33	2.167	0.8690	13.10
V_{45}.UAF-9021	80.00	6.167	0.07133	43.33	1.767	0.7270	27.31
V_{46}.UAF-9247	86.67	5.833	0.07000	40.00	1.803	0.7547	24.54
V_{47}.UAF-9244	56.67	4.860	0.05600	26.67	2.380	0.7733	22.68
V_{48}.UAF-9316	53.33	5.000	0.06667	43.33	2.043	0.7797	18.70
V_{49}.UAF-9258	90.00	6.527	0.07267	46.67	1.697	0.9333	6.667
V_{50}.UAF-9267	90.00	6.277	0.07267	43.33	1.693	0.8083	19.17

Table 3 (Cont'd.).

Genotypes	Emergence % age	Emergence index	Emergence rate index	Energy of emergence % age	Mean emergence time days	Desiccation tolerance index	Percent seedling recovery
V_{51}.CIMMYT-117	86.67	3.110	0.05467	13.33	2.243	1.0000	0.0000
V_{52}.CIMMYT-122	56.67	3.553	0.06667	23.33	1.933	1.0000	0.0000
V_{53}.CIMMYT-124	53.33	4.193	0.05700	20.00	2.170	0.8750	12.50
V_{54}.CIMMYT-125	73.33	5.540	0.06133	33.33	2.160	0.8593	10.37
V_{55}.CIMMYT-127	90.00	3.917	0.04767	16.67	2.670	0.8843	11.57
V_{56}.CIMMYT-130	80.00	4.557	0.5900	26.67	2.217	1.000	0.0000
V_{57}.CIMMYT-156	76.67	5.167	0.6767	26.67	1.873	1.000	0.0000
V_{58}.MH-97	76.67	2.973	0.05333	33.33	2.400	0.5223	47.78
V_{59}. PBW-222	56.67	2.470	0.05333	13.33	2.350	0.8000	20.00
V_{60}.AS-2002	4636	6.177	0.0633	10.00	2.070	0.2517	74.82
V_{61}.AUQAB-2000	93.33	5.913	0.06600	43.33	1.820	0.3397	66.02
V_{62}.SH-2002	90.00	6.583	0.06833	40.00	1.957	0.2407	75.93
V_{63}.FD-85	96.67	5.580	0.07033	36.67	1.737	0.8307	16.93
V_{64}.PUNJAB-96	80.00	5.193	0.07200	36.67	1.753	0.9027	9.723
V_{65}.IQBAL-2000	73.33	5.887	0.07067	43.33	1.887	0.1620	83.80
V_{66}.RAWAL-87	83.33	3.917	0.05367	20.00	2.450	0.1310	86.90
V_{67}.CHENAB-2000	73.33	5.080	0.07233	36.67	1.733	0.1867	81.35
V_{68}.LU-26	93.33	6.443	0.06933	46.67	1.923	0.6817	31.85
V_{69}.FD-83	76.67	4.807	0.06267	30.00	2.097	0.1667	83.33
V_{70}.PARWAZ-94	73.00	4.970	0.06767	33.33	1.903	0.1667	0.0000
V_{71}.PUNJAB-85	70.00	4.027	0.05833	23.33	2.310	1.0000	81.94
V_{72}.BARANI-83	76.67	4.567	0.05967	26.67	2.197	0.1807	87.50
V_{73}.PITIC-62	70.00	3.473	0.04800	16.67	2.663	0.1250	71.63
V_{74}.UAF-6500	80.00	5.500	0.06967	36.67	1.807	0.9520	4.763
V_{75}.UAF-4943	76.67	5.417	0.07067	36.67	1.737	0.8750	12.50
V_{76}.UAF-8121	70.00	5.080	0.07067	30.00	2.270	1.000	0.000
V_{77}.UAF-8053	73.33	4.177	0.07300	20.00	2.183	0.9583	4.167
V_{78}.UAF-7086-1	80.00	5.330	0.05800	36.67	1.997	0.8057	19.44
V_{79}.UAF-6142	70.00	4.343	0.6667	30.00	2.247	1.000	0.000
V_{80}.UAF-8073	76.67	5.000	0.06300	33.33	2.000	1.000	0.000
V_{81}.UAF-5039	80.00	4.510	0.06500	30.00	2.117	0.8213	17.86
V_{82}.WATAN-92	70.00	5.387	0.06400	40.00	1.933	0.8750	8.333
V_{83}.KOHINUR-83	70.00	5.693	0.0700	40.00	1.770	0.2467	75.33
V_{84}.UAF-7028	80.00	3.833	0.7067	16.67	2.290	0.9027	9.723
V_{85}.UAF-8126	66.67	3.360	0.05433	16.67	2.797	1.000	0.000
V_{86}.UAF-7012	66.67	5.057	0.04833	33.33	2.093	0.9260	7.407
V_{87}.UAF-4072	80.00	4.080	0.06367	26.67	2.263	1.000	0.000
V_{88}.UAF-6544-6	80.00	3.973	0.6100	23.33	2.210	0.6260	7.407
V_{89}.UAF-9233	73.33	4.790	0.05933	30.00	2.307	0.9167	0.000
V_{90}.UAF-9242	90.00	5.110	0.5933	33.33	2.080	0.9213	20.83
V_{91}.NOORI	63.33	4.443	0.5967	30.00	2.073	0.8783	8.333
V_{92}.UAF-4770	60.00	5.317	0.6367	33.33	2.323	0.4437	7.870
V_{93}.UAF-6529-11	63.33	3.747	0.6033	23.33	2.310	1.000	12.17
V_{94}.UAF-8031-1	60.00	3.473	0.5933	20.00	2.277	1.000	55.65
V_{95}.UAF-9189	80.00	4.667	0.5967	26.67	2.223	0.8843	0.0000
V_{96}.C-250	76.67	4.777	0.5767	30.00	2.150	0.9523	0.0000
V_{97}.UAF-9259	86.67	6.510	0.5900	43.33	1.887	0.9333	11.57
V_{98}.UAF-8177	56.67	3.447	0.06000	20.00	2.077	1.000	4.763
V_{99}.UAF-6039-4	66.67	3.943	0.05800	23.33	2.277	1.000	6.667
V_{100}.BLUE SILVER	86.67	5.473	0.06333	36.67	2.153	0.8057	0.0000

Discussion

Earlier and more uniform germination and emergence was observed in some genotypes. Higher the emergence percentage, emergence index and energy of emergence and lower mean emergence time indicated earlier and rapid germination. These finding support the earlier work on Canola (*Brassica compestris*) by Zheng *et al*., (1994), wheat (*Triticum aestivum* L.) by Nayyar *et al*., (1995) and rice (*Oryza sativa*) by Basra *et al*., (2003). Among one hundred genotypes 33 genotypes exhibited emergence percentage (44.36-100), having emergence index ranging (5.33-7.62) and energy of emergence ranging (36.67-60.00). These genotypes also exhibited lower mean emergence time ranging (1.66-2.32) days and desiccation tolerance index ranging (0.066-1.00). Percent seedling recovery measures the re-growth percentage .The genotypes Nesser and Dharwar Dry exhibited maximum re-growth (93.33%). These genotypes showed lower desiccation tolerance index and higher percent seedling recovery. These results are supported by the early findings of Milthorpe (1950). Twenty-five genotypes totally failed to re-growth, they permanently died.

Average linkage method showed cluster of 33 genotypes of group 1. The points closest to each other are gathered in one cluster because distance between them is small as compared to others. These genotypes in group 1 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. Different researchers have used cluster analysis to group different wheat genotypes based on various characteristics and found similarities of wheat genotypes with a group (Ahmad, 2001, Mahmood, 2004) Cluster (group 1) consist of 33 genotypes (Table 2) GA-2002, C-250, WL-711, Pari-73, T-96725, Yecora, C-273, SA-75, Pothowar, C-591, Pak-81. Manthar-2003, Kohistan-87, Lyallpur-73, Barani-70 ARZ, Nesser, Dharwar Dry, Chakwal-86, Inqlab-91, Bakhar-2002, AS-2002, Uqab-2000, SH-2002, Iqbal-2000, Rawal-87, Chenab-2000, Faisalabad-83, Punjab-85, Barani-83, Pitic-62, Kohinoor-83 and 4770.These genotypes could be used in further breeding programmes for drought resistance. This method i.e., survival after desiccation proved an efficient and precise while other methods developed for physiological morphological studies are inefficient to screen large plant population.Similar findings had been reported by Winter *et al*., (1988).

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