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ASSESSMENT OF MORPHO-PHYSIOLOGICAL VARIATIONS AND RAPD POLYMORPHISM OF *AVENA SATIVA* (L) GENOTYPES

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SUMMARY

Twenty three oat (*Avena sativa* L) genotypes were evaluated for their morpho-physiological parameters and correlation with the RAPD data of 12 oligo nucleotide primers. Genetic correlation analysis of 18 morpho-physiological characters showed higher coefficient of variation (CV) for root dry weight (46.918) followed by root: shoot ratio (45.809). The RAPD markers showed a moderate polymorphism (42.10%) out of the total 114 bands generated. The dendrogram showed all 23 genotypes grouped into five clusters. The highest level of similarity was observed among JHO-822 and 2002-3 genotypes (97%). The most divergent genotype was 38-2 among all 23 genotypes. The per cent decrease in root:shoot ratio at flowering compared to vegetative stage is an important parameter in selecting the genotypes for drought resistance characters. The more decrease in per cent of root:shoot ratio in the later stage, i.e. flowering compared to vegetative stage, more resistant to drought (having value 60-70%) and the highest similarity with the 2nd cluster of RAPD with 6 genotypes having the most similar genotype, JHO-2002-5.

***Key words:* *Avena sativa* L., morpho-physiological traits, RAPD polymorphism**

INTRODUCTION

Genetic diversity in inter and intra specific levels in a crop germplasm by evolutionary forces is an important parameter utilized for crop improvement either by selection or applying various breeding methodologies. Information on genetic diversity and relationships within and among crop species and their relatives is essential for the efficient utilization of plant genetic resource collections. Several molecular approaches have been employed to assess genetic diversity and relationships but isozymes and random amplified polymorphic DNA (RAPD) data can be generated faster and with less labour than other methods, such as RFLP (restriction fragment length polymorphism) and the use of the microsatellites. The information obtained through DNA fingerprinting using RAPD and RFLP not only gives a comprehensive picture on diversity and relatedness but also determine efficiency with which each marker can be used in diversity studies.

Oats (*Avena sativa* L) are grown as a multipurpose crop in many parts of world. They are used as grain and forage crop. The crop has wide variability and adaptability, and hence can be grown in diverse agro climatic regions. The cultivated oat is a natural allohexapliod (2n=6x=42) with a genomic constitution of AACCDD. The primary gene pool of genus *Avena* has certain desirable traits such as high protein content, resistance to various biotic and abiotic stresses. Genetic variation within and between populations of crop species is a major interest of plant breeders and population geneticists However, these traits are often polygenic and influenced by environmental conditions. In the present

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investigation attempt has been made to analyze the morpho-physiological character vis-a-vis RAPD polymorphism in 23 *Avena sativa* L. genotypes.

MATERIALS AND METHODS

Twenty three genotypes of oat (*Avena sativa* L.) (38-2, EC-425104, 2002-3, 2000-1, JHO-822, JHO 2202-5, Kent, JHO-992, 99-1, 2002-6, 37-21, 31-13, 38-1, 11-13, 2002-4, 38-6, 2002-1, JHO-851, NGB-8650,851-4, 851-2, 851-12, NGB-6975) were grown in sand culture in the plastic pots. Single plant was grown per pot. Two sets of plants were grown with three replications. Crop was irrigated with Hoagland solution as and when required. One set of crop was harvested at the active tillering stage, *i.e.* at the 35 days after sowing (DAS) and another set was harvested at the flowering stage of the crop, *i.e.* at 65 DAS.

Leaf area (LA): Ten representative leaf blades from plants in each sample were taken at random and their total area was measured by an automatic leaf area meter Li-Cor 3000 (Li-Cor, Lincoln, NE, USA). The leaves were then oven dried and the dry weight of leaf per cm^2 was calculated. The leaf area of the total sample was computed from the total leaf dry weight.

Specific leaf weight (SLW) and specific leaf area (SLA): SLW or leaf thickness was expressed as the dry weight of leaf blade per unit leaf area in $mg.cm^{-2}$ (Yoshida *et al*. 1976). Leaf samples were collected and leaf area was taken by the help of leaf area meter (Li-Cor 3000). Leaves were then kept in the oven at 80 ^{0}C for 48 h or until a constant weight was achieved. The dry weight of the sample was recorded and the specific leaf weight was calculated as leaf dry weight upon leaf area. The specific leaf area was calculated as the leaf area / leaf dry weight and expressed in cm^2g^{-1}.

Total dry matter (TDM): Plant sample were uprooted at scheduled growth stages, washed, separated in to leaves, stem [culms and panicles (excluding roots)] depending on the stage of the plant and dried at 80°C till a constant dry weight was obtained. The dry weights of the individual portions were recorded and the sum of their weight were taken as total dry weight or total dry matter (TDM) expressed in g $plant^{-1}$.

Root dry matter: The plants were uprooted and the roots were cleaned under the tap water to remove the sands attached to the root. After taking the length of the root, the roots were kept for drying in the hot air oven at 80°C for 48 h or until a constant weight was achieved. The final dry weight of the root was recorded.

Estimation of photosynthetic pigments and soluble protein: Chlorophyll a, b, and total chlorophyll contents were determined after extraction in dimethylsulfoxide (DMSO), following non – maceration technique of Hiscox and Israelstam (1979). Total soluble protein in the ground fresh leaf sample was estimated following the method of Lowry *et al.* (1951).

Isolation of genomic DNA: Genomic DNA was isolated following the methodology of Iqabal *et al.* (1997) with suitable modifications. For each genotype, DNA was extracted from 2 g of fresh, young, green leaves of a single plant. It was ground in liquid nitrogen to a fine powder and mixed with CTAB total DNA extraction buffer (CTAB 2 % (w/v), NaCl 1.4 %, tris-HCl 100 mM and EDTA 20 mM and 2-mercaptoethanol 100 mM (added freshly) in a 1:2 ratio w/v) and incubated at 65°C for 1 h with occasional swirling . After incubation, the mixture was emulsified with an equal volume of 24:1(v/v) chloroform-isoamyl alcohol and centrifuged at 5000 g for 15 min, the process was repeated once. The aqueous phase was removed and DNA was precipitated with 0.6 volume of isopropanol by keeping at -20°C for 2-3 h. The DNA pellet was dissolved in 10mM TE buffer (pH 8.0) and kept at 4°C overnight. The nucleic acid solution was treated with RNase and after incubation for 30 min at 37°C, was extracted twice with phenol-chloroform and finally with chloroform. DNA was precipitated in 100% ethanol. After a brief period of air–drying, DNA was suspended in 2 ml TE buffer (pH 8.0). The presence and quality of genomic DNA was confirmed by electrophoresis on a 0.7 % (w/v) agarose gel. The concentration was adjusted to 5ng /ml for use in PCR analysis.

Primers: Twelve random primers namely OPAH-08, OPAE-03, OPX-01, OPB-02, OPT-06, OPAF-13, OPH-15, OPAB-05, OPAK-18, OPN-05, OPAL-11 and OPAE-01 were chosen for the present study. These primers obtained from Operon Technology Inc., CA, USA.

PCR and agarose gel electrophoresis: Each PCR amplification was performed in a final volume of 20 µl of reaction mixture containing 67 mM Tris-HCl (pH8.0), 16.6 mM $(NH_4)_2SO_4$, 0.45%(v/v) BSA, 3.5 mM $MgCl_2$, 150 µM of each of dATP, dCTP, dGTP, dTTP 7.5 pmol (15 ng) primer, 25 ng genomic DNA template and 0.5 unit Taq Polymerase (Bangalore Genei, India) and finally it was overlaid by 20-30 µl light mineral oil. Amplifications were performed on a DNA thermal cycler PTC-200 (MJ Research, USA) with the cycling program consisting of 94°C for 1 min, 37° C for 1 min, and 72°C for 2 min for 40 cycles followed by 41st cycle at 37°C for 1 min and finally at 72°C for 10 min extension. The amplified products were kept at 4°C until loaded on to the gel. Amplification products were separated by electrophoresis on 1.6% agarose gel in 0.5 X TBE buffer (pH 8.0) to which ethidium bromide (0.5µg/ml) was added for the visualisation with UV light. Gels were photographed using a digital camera. PCR reactions were repeated at least twice to establish reproducibility of result under strict control of the reaction conditions.

Statistical analysis and development of dendrogram: An input binary data matrix of 23 *Avena sativa* L genotype were developed by entering the data by assigning 1 to presence or 0 to absence of bands. Only reproducible and unambiguous RAPD fragments were used for scoring. The NTSYS program version 1.60 was used to produce the similarity matrix (Simqual function). Dice similarity coefficient was further processed with SAHN cluster analysis using the un-weighted pair group average method (UPGMA) and tree display was followed for generation of dendrogram (NTSYS tree phenogram). The morphological data was analysed by SPAR-1-Release 1.1 (IASRI-New Delhi)

RESULTS AND DISCUSSION

Correlation analysis of morpho-physiological characters: The mean values for 18 morpho-physiological characters are shown in Table 1. Different clusters were formed on the basis of different morpho-physiological characters. Tall plants were grouped in cluster 2. Genotypes grouped in cluster 5 showed maximum mean value for number of tillers (27.67), total leaf area (4724.15), leaf : stem ratio (1.33), specific leaf area (421.11), leaf area ratio (238.54), leaf weight ratio (0.56), chlorophyll a (1.72) and soluble protein content of leaf (40.08). Genotypes grouped in cluster 3 showed maximum mean value for root dry weight, total shoot weight, root: shoot ratio and chlorophyll b. Maximum distance between clusters was observed between cluster 2 and 5 (9.763) followed by cluster 1 and 2 (9.006), cluster 4 and 5 (8.196) while the minimum distance between cluster was observed between 4 and 6 (2.634), followed by 1 and 5 (3.045) and 2 and 5 (3.635) (Table 2). The average distance between cluster members from cluster centroids indicated that the genotypes in cluster 2 showed maximum diversity (2.574) followed by cluster 3 (2.421) while the minimum distance was observed in cluster 1 (1.125). Correlation analysis is one of the simplest approaches to know the interrelationship of the traits.

Results indicate that at vegetative stage the root growth is higher, hence, root : shoot ratio is more in comparison to flowering stage (Table 3). The per cent decrease in the root: shoot ratio in flowering stage over the vegetative stage is an important parameter in selecting the genotypes for drought resistant characters. Ekanayaka *et al.* (1985) also reported that more decrease in per cent of root : shoot ratio in the later stage, i.e. flowering over the vegetative stage is an indication of more resistant to the drought. The clustering analysis of the percentage decrease in root: shoot ratio at flowering stage over the vegetative stage showed five clusters. Cluster I comprised single genotype, JHO-2002-5 with the highest value (76.92). Cluster II with the value (60-70) comprised of 6 genotypes (851-2, 2002-6, 99-1, JHO-992, 2002-3, JHO-851). Cluster III with the value (50-60) includes 7 genotypes (851-2, 37-2, 31-13, 2002-1, 2002-4, 851-12, 38-2). Cluster IV with the value (40-50) consist of 5 genotypes (NGB-6975, NGB-8650, 11-13, Kent, JHO-822). Cluster V with the value <40 comprised of 4 genotypes (2002-1, 36-6, 38-1, EC-415104). Root and shoots are interdependent. The roots receive photosynthates and growth hormones from shoots and in return furnish water and nutrients to shoots (Taylor and Arkin 1981). After seed germination, plants generally contribute the major portion of the metabolites towards root expansion. For instance, radicle grows faster than the plumule of wheat after germination. However, the trend is reverse at later stages of growth. Root: shoot ratio differs depending on environmental

Table 1. Mean values for various metric traits in genotypes of different clusters

Cluster	Plant height (cm)	Tiller no.	Root length (cm)	Stem dry weight (g)	Root dry weight (g)	Total leaf dry weight (g)	Total leaf area (cm^2)	Total shoot weight (g)	Root : Shoot ratio	Leaf : Stem ratio	Specific leaf area (cm^2)	Specific leaf weight (cm^2/g)	Leaf area ratio	Leaf weight ratio	Chlorophyll a (mg g^{-1} fw)	Chlorophyll b (mg g^{-1} fw)	Total chlorophyll (mg g^{-1} fw)	Soluble protein of leaf (mg g^{-1} fw)
1	50.44± 1.90	26.33± 2.52	23.78± 1.35	7.98± 0.27	5.80± 0.88	13.91± 14.37	3867.30± 180.14	17.84± 0.46	0.31± 0.04	1.25± 0.05	375.31± 11.13	2.73± 0.07	205.73± 5.47	0.55± 0.01	1.35± 0.03	0.32± 0.08	1.67± 0.07	29.15± 7.19
2	98.67± 2.05	14.08± 2.18	26.92± 2.50	11.69± 0.92	8.69± 1.89	93.61± 18.59	1851.60± 189.36	18.28± 1.40	0.49± 0.12	0.59± 0.05	238.96± 21.48	4.31± 0.45	88.33± 7.29	0.37± 0.02	1.88± 0.31	0.44± 0.05	2.33± 0.35	25.68± 12.98
3	59.50± 8.72	22.50± 0.24	22.67± 0.47	10.58± 1.55	16.61± 1.55	78.61± 13.80	3523.41± 674.61	21.03± 0.33	0.82± 0.18	1.01± 0.31	305.38± 7.91	3.30± 0.06	150.91± 26.79	0.49± 0.07	1.24± 0.41	0.45± 0.15	1.69± 0.26	7.84± 0.57
4	89.83± 7.55	14.50± 1.93	23.58± 1.20	10.10± 0.93	8.53± 1.80	75.11± 44.58	2204.79± 209.78	17.14± 1.64	0.50± 0.05	0.73± 0.06	268.56± 5.22	3.75± 0.08	111.81± 5.73	0.42± 0.02	1.45± 0.06	0.33± 0.05	1.79± 0.11	14.88± 11.70
5	44.33± 0.00	27.67± 0.00	22.67± 0.00	8.28± 0.00	7.48± 0.00	7.48± 0.00	4724.15± 0.00	18.99± 0.00	0.39± 0.00	1.33± 0.00	421.11± 0.00	2.40± 0.00	238.54± 0.00	0.56± 0.00	1.72± 0.00	0.40± 0.00	2.11± 0.00	40.08± 0.00
6	82.00± 10.35	14.44± 2.44	24.11± 2.08	10.74± 0.79	7.26± 1.30	74.89± 29.31	2636.91± 336.73	19.78± 1.10	0.36± 0.08	0.85± 0.09	259.42± 31.68	3.92± 0.44	118.29± 13.49	0.46± 0.03	1.62± 0.16	0.37± 0.04	1.99± 0.18	26.19± 9.40
Mean	70.79	19.92	23.96	9.86	9.03	57.27	3134.69	18.84	0.478	0.96	311.46	3.40	152.26	0.475	1.543	0.385	1.93	23.97

Cluster No.	Genotypes
1	JHO-851, 851-4, 851-2
2	Kent, 99-1, 38-1, 2002-1
3	38-2, EC-425104
4	2002-3, 2001-1, JHO-822, NGB-8650
5	851-12
6	JHO2002-5, JHO-992, 2002-6, 37-21, 31-13, 11-13, 2002-4, 38-6, NGB-6975

Table 2. Distances between cluster centroids

Clusters	1	2	3	4	5	6
1	0.000					
2	9.006	0.000				
3	6.803	7.442	0.000			
4	6.488	4.100	5.723	0.000		
5	3.045	9.763	7.381	8.196	0.000	
6	6.106	3.635	5.757	2.634	7.272	0.000

Table 3. Root : shoot ratio of *Avena sativa* genotypes

	Root : shoot (Root weight/shoot weight)		
Genotypes	vegetative	flowering	% decrease
38/2	1.415	0.689	51.307
EC-415104	1.015	0.945	6.897
2002-3	1.184	0.429	63.767
2001-1	0.998	0.476	52.305
JHO-822	1.049	0.546	47.950
JHO-2002-5	0.992	0.229	76.915
Kent	0.947	0.566	40.232
JHO-992	0.964	0.317	67.116
99-1	1.008	0.318	68.452
2002-6	1.099	0.339	69.154
37/21	0.804	0.358	55.473
31/13	0.699	0.316	54.793
38/1	0.894	0.537	39.933
11/13	0.798	0.452	43.358
2002-4	0.788	0.381	51.649
38/6	0.659	0.486	26.252
2002-1	0.568	0.546	3.8732
JHO-851	0.881	0.326	62.997
NGB-8650	0.932	0.535	42.597
851-4	0.861	0.346	59.814
851-2	0.906	0.274	89.757
851-12	0.816	0.391	52.083
NGB-6975	0.704	0.371	47.301

conditions. An estimation of the extent of variation within and between populations of species is useful for analyzing the genetic structure of crop germplasm. Root: shoot ratio seems to be an important parameter in selecting the genotypes for drought resistant characters as more decrease in per cent of root : shoot ratio in the later stage, i.e. flowering over the vegetative is more resistant to drought. Our observation confirms that 85-2 (89.76) showed its drought resistant character in terms of maximum decrease in the root: shoot ratio in flowering over the vegetative stage followed by JHO-2002-5 (76.92) and 2002-6 (69.15)

Genetic Similarity and cluster analysis of RAPD: A total of 114 bands were scored from PCR amplification of genomic DNA from 23 genotypes of *Avena sativa* L. The RAPD markers showed a moderate polymorphism (42.10%), i.e. 48 bands were polymorphic out of the total 114 bands generated (Table 4 and Fig. 1) RAPD data collected from 12 primers were further

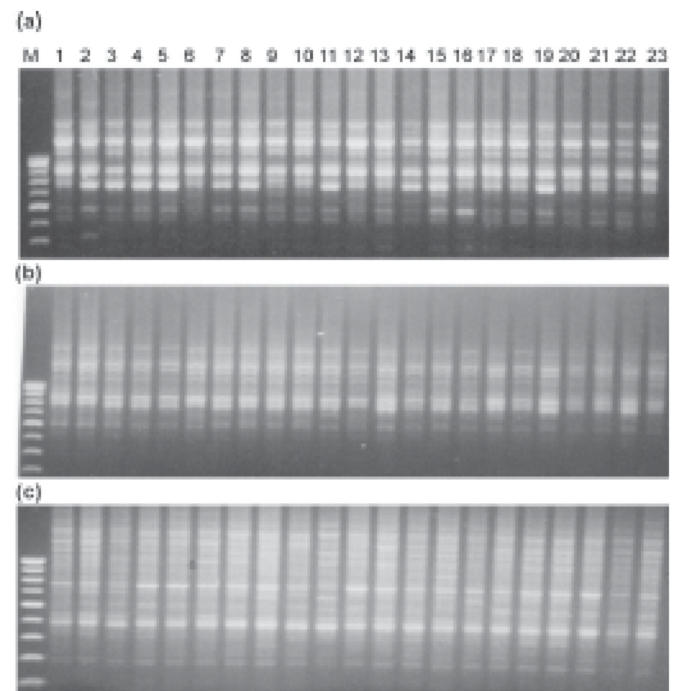

Fig. 1. RAPD profile of oat genotypes by Primer OPB-20 (a), Primer OPAE-03 (b) and OPH-15 (c), M: Molecular marker (100 bp), Lane 1: 38-2, 2: EC-425104, 3: 2002-3, 4: 2000-1, 5: JHO-822, 6: JHO 2202-5, 7: Kent, 8: JHO-992, 9: 99-1, 10: 2002-6, 11: 37-21, 12: 31-13, 13: 38-1, 14: 11-13, 15: 2002-4, 16: 38-6, 17: 2002-1, 18: JHO-851, 19: NGB-8650, 20: 851-4, 21: 851-2, 22: NGB-6975, 23: 851-12.

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Table 4. Sequence and amplified product of 12 random primers used to generate RAPD markers in *Avena sativa* L. genotypes

Primers	Sequence (5'-3')	% of GC	Total no. of bands (T)	Number of base pairs (bp)	Total no. of monomorphic bands (M)	Total no. of polymorphic bands (P)	% of polymorphic (P/T*´100)
OPAH-08	TTCCCGTGCC	70	10	10	3	7	70.00
OPAE-03	CATAGAGCGG	60	15	10	7	8	46.66
OPX-01	CTGGCGACGA	70	07	10	3	4	57.14
OPB-20	GGACCCTTAC	60	08	10	6	2	25
OPT-06	CAAGGGCAGA	60	10	10	10	0	0
OPAF-13	CCGAGGTGAC	70	10	10	6	4	40
OPH-15	AATGCCGCAG	60	16	10	7	9	56.25
OPAB-05	CCCGAAGCGA	70	10	10	5	5	100
OPAK-18	ACCCGGAAAC	60	06	10	4	2	33.33
OPN-05	ACTGAACGCC	60	08	10	6	2	25
OPAL-11	GTCACGTCCT	60	09	10	4	5	55.55
OPAE-01	TGAGGGCCGT	70	05	10	5	0	0
TOTAL			114		66	48	42.1

used to generate dendrogram using DICE coefficient. Dendrogram constructed on the basis of the RAPD markers used to establish the genetic relationships among the 23 *Avena sativa* L. genotypes. All 23 genotypes were grouped in to 5 clusters (Fig. 2). The 1st cluster comprised of 8 genotypes (EC-425104, 99-1, 11-13, 38-6, 2002-4, 2002-3, 2001-1 and JHO-822) followed by 2nd cluster with 6 genotypes (JHO-2002-5, 31-13, Kent, 37-21, NGB-6975 and 851-21) however the 3rd cluster comprised of 4 genotypes (38-1, NGB-8650, 2002-1, JHO-851) and 4th cluster with 3 genotypes (JHO-992, 2002-6 and 38-2).The smallest was the 5th cluster with 2 genotypes (851-4 and 851-2). The highest similarity index was found among JHO-822 and 2002-3 (97%), while the lowest similarity index was found between JHO-992 and 2002-6 (93%) which is similar to 38-2 genotype with the similarity matrix of 90%. Of course, all the genotypes belong to a single species of *Avena sativa* L., therefore, there was not much divergence except 38-2, which was the most divergent genotype among all. The correlation matrix among the

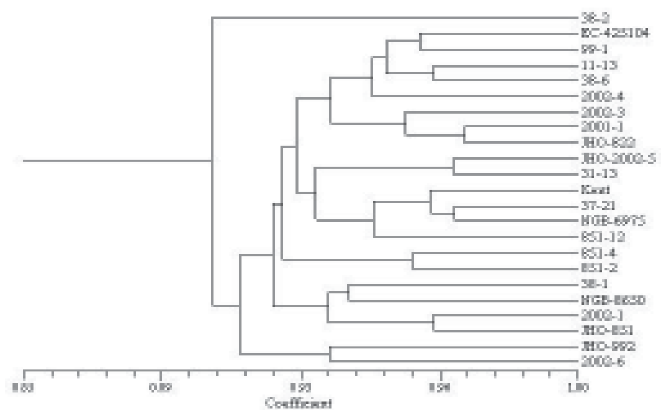

Fig. 2. Dendrogram of the *Avena sativa* L. genotypes constructed using UPGMA based on Dice similarity coefficient

genotypes was depicted in the Table 5. Genetic diversity is the basis for the genetic improvement. Evaluation of Genetic diversity levels among adapted, elite genotypes of *Avena sativa* L can provide predictive estimates of genetic variation among segregating population for pure line cultivar development. Dice similarity coefficient ranged from 0.90 to 0.98 and concentrated mostly

between 0.90 to 0.97. This indicated a rather narrow genetic base of tested cultivars. Genetic similarity obtained in this study may be used for selecting parents for breeding purposes. Clustering of genotypes into five groups showed reasonable variability that may be exploited for yield improvement. This study was in line of Afzal *et al*. (2004) who have conducted a laboratory experiment to asses the genetic diversity of mungbean [*Vigna radiate* (L.)] cultivars using DNA markers. Archak *et al.* (2002) reveals narrowing the genetic base of Indian tomato cultivars by RAPD markers. They studied by using 42 random primers. From their study it was concluded that overall high levels of pair wise similarity (Jaccard's mean=0.825) and low levels of markers diversity (mean=0.165) implied the existence of limited genetic variation in the investigated material. They also suggested that reduction in the genetic diversity among modern tomato cultivars might be attributed to the recent trend towards breeding for similar plant and fruit characteristics.

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