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The use of SDS PAGE to reveal the hybridity of wheat (*Triticum aestivum*) crosses and genetic diversity analysis

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Abstract

An attempt was made to test the hybridity of crosses through SDS PAGE analysis. A total of 31 wheat (*Triticum aestivum*) genotypes were taken for the study out of which 14 genotypes were parental lines (differing in drought tolerance trait) and 17 genotypes were random crosses among the 14 parental lines. Protein bands were scored with their Rf values which ranged from 0.24 to 0.95. A total of 330 bands were obtained. Secondly the genetic diversity was estimated by comparison of the banding pattern in the protein profiles of the genotypes. The bands of Rf values 0.28, 0.32 and 0.60 were present in all the drought tolerant genotypes and were absent in susceptible parents so these could be utilised as markers for drought tolerance trait. Similarity matrix table was made based on the presence and absence of bands. Maximum similarity (0.9041 similarity coefficient) was observed between WH 730 and its cross WH730 X UP 2554 and two crosses WH 730 X UP 2425 & WH 730 X UP 2554. The lowest similarity (0.6575 similarity coefficient) was observed in two cases i.e. between the crosses WH 730 X UP 2338 & HI 385 X UP 2338 and between the crosses HI 385 X UP 2338 & WH730 X UP 2425.

Keywords: SDS PAGE, genetic diversity, wheat, hybridity

Introduction

India is one of the leading countries globally regarding wheat production ranking third, next to European Union and China with an estimated current production of 106.2 million tonnes. Breeding for drought tolerance has always been an important aspect of wheat improvement programme. SDS PAGE i.e. Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis is a technique used to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight as well as higher order protein folding, post translational modifications and other factors). The differences in the protein folding pattern of genotypes are attributed to the differences in the amino acid sequences, which in turn are encoded by the specific DNA sequences. Thus SDS PAGE has the potential to reveal the genetic diversity among the genotypes compared. Sodium dodecyl sulfate (SDS) is an anionic detergent which denatures proteins by “wrapping around” the polypeptide backbone and SDS binds to proteins fairly specifically in a mass ratio of 1.4: 1. In doing so, SDS confers a negative charge to the polypeptide in proportion to its length i.e. the denatured polypeptides become “rods” of negative charge cloud with equal charge or charge densities per unit length. It is usually necessary to reduce disulfide bridges in proteins before they adopt the random coil configuration necessary for separation by size. This is done with 2- mercaptoethanol. In denaturing SDS-PAGE separations therefore, migration is determined not by intrinsic electrical charge of the polypeptide, but by molecular weight.

Materials and Methods

The experimental material used for the study consisted of 14 *Triticum aestivum* lines and 17 crosses. SDS PAGE analysis was done to test the hybridity of crosses and to find out the diversity among these 31 genotypes. The research was carried out at G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India during 2004 – 2008.

Table 1: List of various wheat (*Triticum aestivum*), 2n= 42 genotypes

Cultivars	Parentage	Remarks
Halna	HD 1982 / K 816	Drought tolerant (gene introgressed)
UP 2565	PBW 352 / CPAN 4020	
HI 385 (MUKTA)	HYB 633 // GAZA // PR / PKD 25	Drought tolerant (gene introgressed)
PBW 373	ND / VG 9144 // KAL / BB / 3 / YACO 'S' / 4 / VEE # 5 'S'	
NIAW 34	CNO 79 / PRL "S"	Drought tolerant (gene introgressed)
UP 2425	HD 2320/UP 2263	Drought susceptible
NP 846	NP 760 / RN	Drought tolerant (gene introgressed)
UP 2338	UP 368 / VL 421 / UP 262	Drought susceptible
PBW 175	HD 2160 / WG 1025	Drought tolerant (gene introgressed)
PBN 51	BUC 'S' / FLK 'S'	Drought tolerant (gene introgressed)
UP 2554	SM4 – HSN 24E / CPAN 2099	
UP 2590	Not available	
VL 804	CPAN 3018/CPAN 3004/PBW 65	Drought tolerant
WH 730	CPAN 2092/ Improved Lok - 1	Drought tolerant (gene introgressed)

SDS-PAGE discontinuous system was used to observe the protein banding pattern of the wheat parents and their respective F_1 s. The procedure followed was as per the protocol of Laemmli (1970) with some modifications suggested by Lawrence and Shepherd (1980).

Apparatus and Glasswares

- Vertical slab gel electrophoresis assembly with all accessories (Atto Corporation, Japan)
- Power supply for electrophoresis with adjustable constant current / voltage (200 mA, 500 V) output capacity (Atto Corporation, India)
- A micro centrifuge (Biofuge)
- A platform rocker for shaking the gel for staining and destaining
- Eppendorf tubes for samples
- Plastic trays
- Glass syringes
- Micropipettes with tips
- pH meter
- Thermostat water bath

Sample preparation (total protein extraction)

The total seed storage protein extraction was done according to the method described by Singh *et al.* (1991). One grain (about 30 mg flour) was crushed to fine powdered form and then added to it 400 μ l extraction buffer (pH 6.8) containing 2 per cent β -mercaptoethanol and vortexed briefly for 1 to 2 minutes. The tubes were kept in hot water at 60°C for 30 minutes and cooled to room temperature. 25 μ l dye was added to each tube and the contents were centrifuged for 10 minutes at 10,000 rpm. The supernatant was used for loading the sample in the well of the gel for electrophoresis.

Composition and preparation of gels

The stacking and the separating gels were prepared from the stock solutions by mixing them as given below

Table 2: Composition of stacking and separating gels

Chemicals	Stacking gel (for 2 gels)	Separating gel (for 2 gels)
Gel buffer	4.5 ml	30 ml
Acrybis solution	1.0 ml	20 ml
Distilled water	3.5 ml	10 ml
TEMED	50 μ l	100 μ l
APS (10%)	100 μ l	120 μ l

APS Ammonium Per Sulfate (10%) was added to separating gel mix just before pouring the gel solution between the plates. Separating gel solution was filled up to the 2.5 cm mark from the top of the glass plate. A thin layer of distilled water was made over the gel to seal it off from the air, to get an even surface of gel and to accelerate the polymerization. The water was removed after the gel polymerization. Stacking gel solution was poured between the glass plates using a 5 ml syringe followed by immediate rinsing of the syringe. Comb was carefully and quickly inserted into the stacking gel. Comb was removed after the gel polymerization. The glass cassette was then clamped to the electrophoresis assembly. Electrode buffer was poured in both the tanks. Twenty μ l sample was loaded in each well and the electrodes were connected with the power supply. Initially the instrument was set at 100 V and 26 mA current for 60 minutes. Later on it was increased to 40 mA with constant voltage at 120 V. The composition of different stock solutions was as given below:

(a) Protein extraction buffer (pH 6.8)

Tris	0.75 g
SDS	2.0 g
B-Mercaptoethanol	5.0 ml
Volume made up to 100 ml with distilled water	

(b) 2 X sample buffer (pH 6.8)

Tris	1.51 g
SDS	8.0 g
Glycerol	30 ml
Bromophenol blue	2.0 mg
Volume made up to 100 ml with distilled water.	

(c) 2 X separating gel buffer (pH 8.9)

Tris	45.4 g
SDS	1.0 g
Volume made up to 500 ml with distilled water.	

(d) 2 X stacking gel buffer (pH 6.8)

Tris	6.06 g
SDS	0.4 g
Volume made up to 200 ml with distilled water.	

(e) Stock acrylamide for separating gel (8%)

Acrylamide	75.0 g
Bisacrylamide	0.6 g
Volume made up to 250 ml with distilled water.	

(f) Stock acrylamide for stacking gel (5%)

Acrylamide	15.0 g
Bisacrylamide	0.4 g
Volume made up to 50 ml with distilled water.	

(g) 10 per cent ammonium persulphate

APS	0.5 g
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Distilled water 5.0 ml

Prepared fresh daily before use.

(h) 10 X electrode buffer (pH 8.3)

Tris 30.3 g

Glycine 144.2 g

SDS 10.0 g

Volume made up to 1000 ml with distilled water and for use one part of this solution (10 X) was mixed with 9 parts of water.

(i) Staining solution

Solution A: Coomassie Brilliant Blue 0.25 g in 25 ml of distilled water

Solution B: Trichloroacetic acid 60.0 g

Methanol 180 ml

Glacial acetic acid 60 ml in 720 ml distilled water

Solution A and B were mixed with each other and final volume was adjusted to 1 litre before use and this was stored at room temperature in a dark bottle.

(j) Destaining solution (3% NaCl)

Sodium chloride 30 g

Volume made up to 1 litre with distilled water.

Staining and destaining

Gel was transferred to the staining solution tray after completion of electrophoresis. Added about 200 ml of staining solution, one gel per tray. The gel was left in the staining solution overnight. Next day, the staining solution was drained off and added about 500 ml of 3 per cent NaCl for destaining of the gels as described by Sreeramulu and Singh (1995). It took 6 h to get a clear background. The photographs of gels were taken on gel documentation system.

Analysis of gels

The Rf value was calculated with the help of Alpha Imager, a software for gel documentation.

$Rf = \text{Path travelled by the bands} / \text{Path travelled by the dye front}$.

Pair-wise similarity and cluster analysis were done on the basis of presence and absence of bands. Computer software (NTSYS) was used to perform the similarity matrix analysis using 'UPGMA' with Jaccard's coefficient of similarity. The difference between wheat genotypes and their crosses was detected by the use of SDS-PAGE analysis.

Results

The summary of protein bands with their Rf values in different crosses is provided in the table 3.

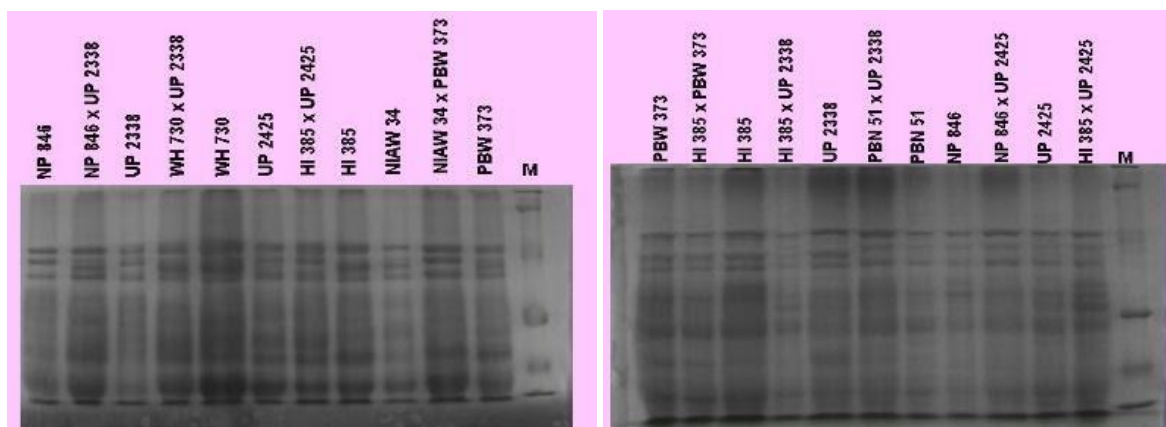


Fig 1: SDS PAGE Electrophoresis of wheat genotypes

Table 3: Summary of protein profiling of wheat genotypes

S. No.	Name of the cross	Bands in female parent (Rf value)	Bands in cross (Rf value)	Bands in male parent (Rf value)	Bands in cross common with both the parents	Bands in cross common with female parent	Bands in cross common with male parent	Unique bands in cross
1.	Halna/UP2565	0.27, 0.28, 0.32, 0.38, 0.41, 0.53, 0.60, 0.64, 0.70, 0.79 and 0.94	0.27, 0.29, 0.33, 0.38, 0.41, 0.60, 0.64, 0.68, 0.73, 0.79 and 0.93	0.28, 0.34, 0.36, 0.41, 0.50, 0.55, 0.59, 0.66, 0.72, 0.79 and 0.93	0.79	0.27, 0.38, 0.41, 0.60, and 0.64	0.93	0.29, 0.33, 0.68 and 0.73
2.	WH 730/UP 2338	0.24, 0.28, 0.32, 0.36, 0.42, 0.48, 0.53, 0.60, 0.68, 0.72, 0.78 and 0.91	0.27, 0.33, 0.34, 0.37, 0.42, 0.55, 0.61, 0.71, 0.78 and 0.91	0.26, 0.29, 0.32, 0.39, 0.42, 0.51, 0.57, 0.63, 0.70, 0.75, 0.79 and 0.93	0.42	0.78 and 0.91	-	0.78 and 0.91 0.78 and 0.91 0.78 and 0.91
3.	NIAW 34/UP 2565	0.28, 0.30, 0.32, 0.42, 0.49, 0.55, 0.60, 0.65, 0.68, 0.77, 0.81, 0.84 and 0.93	0.28, 0.32, 0.41, 0.55, 0.59, 0.64, 0.70, 0.76, 0.81, 0.84, 0.86 and 0.94	0.28, 0.34, 0.36, 0.41, 0.50, 0.55, 0.59, 0.66, 0.72, 0.79, and 0.93	0.28 and 0.55	0.32, 0.81 and 0.84	0.41 and 0.59	0.64, 0.70, 0.76, 0.86 and 0.94
4.	NIAW 34/UP 2590	0.28, 0.30, 0.32, 0.42, 0.49, 0.55, 0.60, 0.65, 0.68, 0.77, 0.81, 0.84 and 0.93	0.28, 0.32, 0.36, 0.42, 0.56, 0.60, 0.65, 0.70, 0.76, 0.80 and 0.94	0.27, 0.29, 0.36, 0.38, 0.41, 0.55, 0.59, 0.64, 0.72, 0.79, 0.89 and 0.93	-	0.28, 0.32, 0.42, 0.60 and 0.65	0.36	0.56, 0.70, 0.76, 0.80 and 0.94
5.	NP 846/UP	0.28, 0.32, 0.41,	0.28, 0.33, 0.36,	0.26, 0.29, 0.32,	-	0.28 and 0.41	0.51	0.33, 0.36,

	2338	0.49, 0.55, 0.60, 0.68, 0.76 and 0.90	0.41, 0.51, 0.61, 0.78 and 0.92	0.39, 0.42, 0.51, 0.57, 0.63, 0.70, 0.75, 0.79 and 0.93				0.61, 0.78 and 0.92
6.	HI 385/UP 2425	0.26, 0.28, 0.32, 0.36, 0.41, 0.50, 0.54, 0.60, 0.68, 0.78 and 0.92	0.26, 0.28, 0.38, 0.41, 0.57, 0.71, 0.79 and 0.91	0.27, 0.32, 0.35, 0.40, 0.59, 0.70, 0.78 and 0.91	-	0.26, 0.28, and 0.41	0.91	0.38, 0.57, 0.71 and 0.79
7.	PBN 51/VL 804	0.28, 0.32, 0.34, 0.42, 0.53, 0.60, 0.66, 0.69, 0.74, 0.81 and 0.91	0.28, 0.32, 0.35, 0.40, 0.42, 0.56, 0.60, 0.65, 0.69, 0.74, 0.81, 0.94	0.28, 0.32, 0.34, 0.39, 0.42, 0.50, 0.60, 0.66, 0.75, 0.80 and 0.93	0.28, 0.32, 0.42 and 0.60	0.69, 0.74 and 0.81	-	0.35, 0.40, 0.56, 0.65 and 0.94
8.	PBN 51/UP 2554	0.66, 0.69, 0.028, 0.32, 0.34, 0.42, 0.53, 0.60, 0.74, 0.81 and 0.91	0.28, 0.30, 0.35, 0.42, 0.53, 0.66, 0.70, 0.82, 0.91 and 0.95	0.29, 0.34, 0.42, 0.53, 0.59, 0.68, 0.70, 0.79, 0.82 and 0.91	0.42, 0.53 and 0.91	0.28 and 0.66	0.70 and 0.82	0.30 and 0.35
9.	WH 730/UP 2425	0.24, 0.28, 0.32, 0.36, 0.42, 0.48, 0.53, 0.60, 0.68, 0.72, 0.78 and 0.91	0.28, 0.35, 0.42, 0.60, 0.68, 0.72, 0.78, 0.82, 0.85 and 0.91	0.27, 0.32, 0.35, 0.40, 0.59, 0.70, 0.78 and 0.91	0.78 and 0.91	0.28, 0.42, 0.60, 0.68 and 0.72	0.35	0.82 and 0.85
10.	WH 730/UP 2554	0.24, 0.28, 0.32, 0.36, 0.42, 0.48, 0.53, 0.60, 0.68, 0.72, 0.78 and 0.91	0.28, 0.32, 0.33, 0.42, 0.53, 0.60, 0.68, 0.78, 0.82, 0.86 and 0.91	0.29, 0.34, 0.42, 0.53, 0.59, 0.68, 0.70, 0.79, 0.82 and 0.91	0.42, 0.53, 0.68 and 0.91	0.28, 0.32, 0.60 and 0.78	0.82	0.33 and 0.86
11.	NIAW 34/PBW 373	0.28, 0.30, 0.32, 0.42, 0.49, 0.55, 0.60, 0.65, 0.68, 0.77, 0.81, 0.84 and 0.93	0.28, 0.30, 0.41, 0.55, 0.62, 0.65, 0.75 and 0.93	0.29, 0.31, 0.37, 0.41, 0.51, 0.62, 0.65, 0.73, 0.76 and 0.93	0.65 and 0.93	0.28, 0.30 and 0.55	0.41 and 0.62	0.75
12.	PBN 51/UP 2338	0.28, 0.32, 0.34, 0.42, 0.53, 0.60, 0.66, 0.69, 0.74, 0.81 and 0.91	0.28, 0.32, 0.34, 0.42, 0.47, 0.58, 0.67, 0.74, 0.82, 0.88, and 0.94	0.26, 0.29, 0.32, 0.39, 0.42, 0.51, 0.57, 0.63, 0.70, 0.75, 0.79 and 0.93	0.32 and 0.42	0.28, 0.34 and 0.74	-	0.47, 0.58, 0.67, 0.82 and 0.88 and 0.94
13.	HI 385/UP 2338	0.26, 0.28, 0.32, 0.36, 0.41, 0.50, 0.54, 0.60, 0.68, 0.78 and 0.92	0.26, 0.30, 0.32, 0.36, 0.38, 0.45, 0.49, 0.51, 0.56, 0.63, 0.77, 0.79, 0.83, 0.89 and 0.94	0.26, 0.29, 0.32, 0.39, 0.42, 0.51, 0.57, 0.63, 0.70, 0.75, 0.79 and 0.93	0.26 and 0.32	0.36	0.51, 0.63 and 0.79	0.30, 0.38, 0.45, 0.49, 0.56, 0.77, 0.83, 0.89 and 0.94
14.	HI 385/PBW 373	0.26, 0.28, 0.32, 0.36, 0.41, 0.50, 0.54, 0.60, 0.68, 0.78 and 0.92	0.28, 0.38, 0.41, 0.54, 0.68, 0.78 and 0.93	0.29, 0.31, 0.37, 0.41, 0.51, 0.62, 0.65, 0.73, 0.76 and 0.93	0.41	0.28, 0.54, 0.68 and 0.78	0.93	0.38
15.	PBW 175/UP 2565	0.26, 0.28, 0.32, 0.36, 0.40, 0.49, 0.60, 0.72, 0.86 and 0.89	0.27, 0.28, 0.33, 0.40, 0.49, 0.53, 0.60, 0.71, 0.86 and 0.89	0.28, 0.34, 0.36, 0.41, 0.50, 0.55, 0.59, 0.66, 0.72, 0.79 and 0.93	0.28	0.40, 0.49, 0.60, 0.86 and 0.89	-	0.27, 0.33, 0.53 and 0.71
16.	PBN 51/UP 2425	0.28, 0.32, 0.34, 0.42, 0.53, 0.60, 0.66, 0.69, 0.74, 0.81 and 0.91	0.26, 0.28, 0.30, 0.33, 0.41, 0.44, 0.50, 0.53, 0.66, 0.74, 0.81 and 0.91	0.27, 0.32, 0.35, 0.40, 0.59, 0.70, 0.78 and 0.91	0.91	0.28, 0.53, 0.66, 0.74 and 0.81	-	0.26, 0.30, 0.33, 0.41, 0.44, and 0.50
17.	NIAW 34/UP 2425	0.28, 0.30, 0.32, 0.42, 0.49, 0.55, 0.60, 0.65, 0.68, 0.77, 0.81, 0.84 and 0.93	0.27, 0.28, 0.32, 0.35, 0.42, 0.49, 0.54, 0.57, 0.65, 0.69, 0.77, 0.84 and 0.93	0.27, 0.32, 0.35, 0.40, 0.59, 0.70, 0.78 and 0.91	0.32	0.28, 0.42, 0.49, 0.65, 0.77, 0.84 and 0.93	0.27 and 0.35	0.54, 0.57 and 0.69

The presence of protein bands with Rf values 0.28, 0.32 and 0.60 in all the drought parents suggests the use of these bands as biochemical marker for the drought tolerance trait. In all the cases except one more bands in the cross were common with the female parent than those of the male parent. In a

cross HI 385 X UP 2338, more bands in the cross were common with the male parent UP 2338 than those of the female parent HI 385. The presence of unique bands in the crosses show the possibilities of recombinations at meiotic cell division.

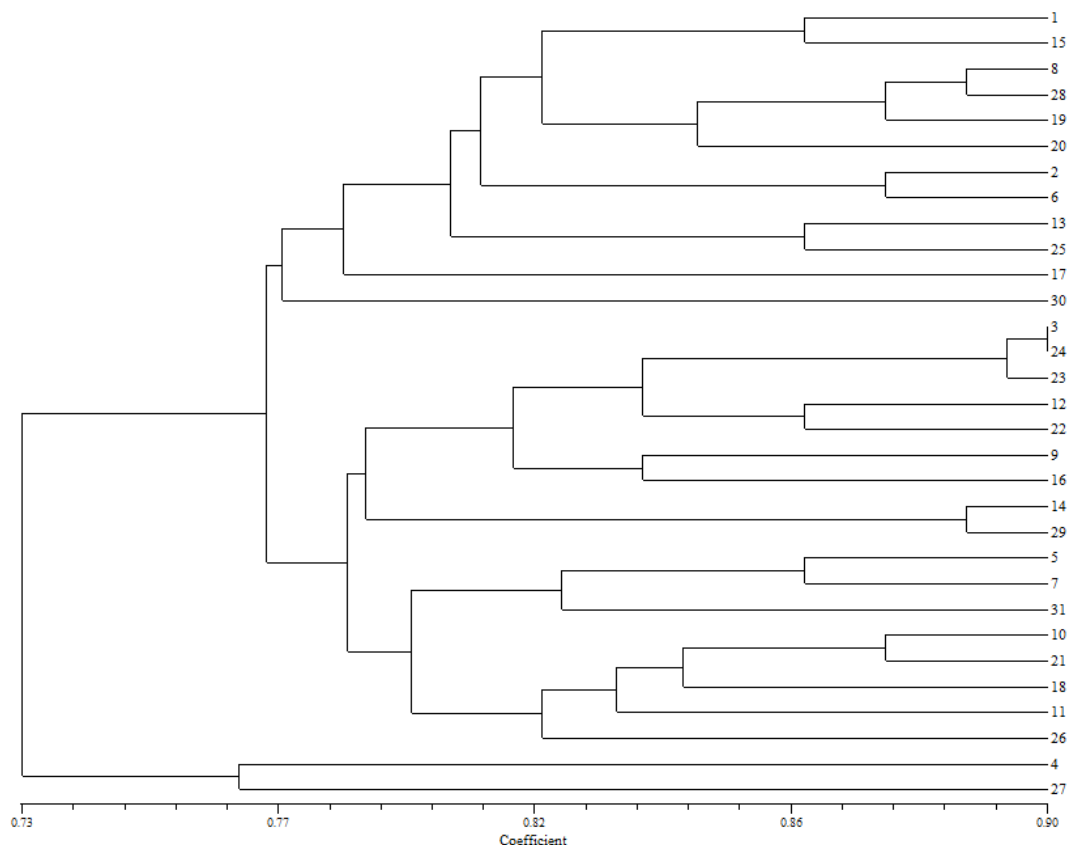


Fig 2: Dendrogram of 31 wheat genotypes (*Triticum aestivum* varieties and the crosses)

List of genotypes in SDS PAGE Dendrogram

1. Halna 2. UP 2565 3. WH730 4. UP 2338 5. NIAW 34 6. UP 2590 7. NP 846 8. HI 385 9. UP 2425 10. PBN 51 11. VL 804 12. UP 2554 13. PBW 373 14. PBW 175 15. Halna X UP 2565 16. WH730 X UP 2338 17. NIAW 34 X UP 2565 18. NIAW 34 X UP 2590 19. NP 846 X UP 2338 20. HI 385 X UP 2425 21. PBN 51 X VL 804 22. PBN 51 X UP 2554 23. WH730 X UP 2425 24. WH 730 X UP 2554 25. NIAW 34 X PBW 373 26. PBN 51 X UP 2338 27. HI 385 X UP 2338 28. HI 385 X PBW 373 29. PBW175 X UP 2565 30. PBN 51 X UP 2425 31. NIAW 34 X UP2425

Dendrogram Cluster Analysis

The dendrogram formed on the basis of similarity matrix values firstly broadly divided the genotypes into two clusters – Group 1 and Group 2. Group 2 had just two genotypes – genotype number 4 and 27 i.e. UP 2338 and HI 385 X UP2338. Rest other 29 genotypes fall under Group 1. It comprised of two sub groups – sub group 1a and sub group 1b. sub group 1a comprised of 2 clusters – sub group 1a cluster1 which had eleven genotypes- genotype numbers 1,

15, 8, 28, 19, 20, 2, 6, 13, 25 and 17 i.e. Halna, Halna X UP 2565, HI 385, HI 385 X PBW 373, NP 846 X UP 2338, HI 385 X UP 2425, UP 2565, UP 2590, PBW 373, NIAW 34 X PBW 373 and NIAW 34 X UP 2565 and sub group 1a cluster2 which had just one genotype- genotype number 30 i.e. PBN 51 X UP 2425.

Sub group 1b comprised of two clusters – sub group 1b cluster1 which had nine genotypes –the genotypes number 3, 24 – WH 730 & WH730 X UP 2554. These genotypes showed maximum similarity with a coefficient of 0.941, genotype number 23 i.e. the cross WH 730 X UP 2425 which again showed maximum similarity with coefficient 0.9041 with genotype number 24 i.e. the cross WH 730 X UP 2554. Other genotypes of sub group 1b cluster1 were genotype numbers 12, 22, 9, 16, 14 and 29 i.e. UP 2554, PBN 51 X UP 2554, UP 2425, WH 730 X UP 2338, PBW 175 and PBW 175 X UP 2565. Sub group 1b cluster2 had eight genotypes – the genotype numbers 5, 7, 31, 10, 21, 18, 11 & 26 i.e. NIAW 34, NP 846, NIAW 34 X UP 2425, PBN 51, PBN 51 X VL 804, NIAW 34 X UP 2590, VL 804 and PBN 51 X UP 2338.

1.0000000

0.7808219 1.0000000

0.7945205 0.7671233 1.0000000

0.7671233 0.7397260 0.7260274 1.0000000

0.7534247 0.7534247 0.7945205 0.7397260 1.0000000

0.8219178 0.8767123 0.7260274 0.7534247 0.7123288 1.0000000

0.8356164 0.8082192 0.8219178 0.7397260 0.8630137 0.7671233 1.0000000

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Fig 3: Similarity matrix of parental lines and the crosses

The similarity matrix table formed by UPGMA method using NTSYS software is given in figure 3. The similarity matrix values ranged from 0.6575 to 0.9041.

The highest similarity matrix value 0.9041 was observed in 2 pairs i.e. between WH730 & its cross WH 730 X UP 2554 and between two crosses WH 730 X UP 2425 & WH 730 X UP 2554.

This was followed by the similarity matrix value 0.8904 in 3 pairs i.e. between WH 730 and its cross WH 730 X UP 2425; between PBW 175 and its cross PBW 175 X UP 2565 and between HI 385 and its cross HI 385 X PBW 373 indicating the obvious similarity between the cross and its mother parent.

The lowest similarity matrix value 0.6575 was observed in 2 pairs i.e. between the crosses WH 730 X UP 2338 and HI 385 X UP 2338; and the crosses HI 385 X UP 2338 and WH 730

X UP 2425 indicating the diversity between two drought tolerant lines WH 730 and HI 385 followed by the similarity matrix value 0.6712 in 4 pairs i.e. between PBN 51 and a cross HI 385 X UP 2338; between VL 804 and a cross HI 385 X UP 2338; between the crosses WH 730 X UP 2554 & HI 385 X UP 2338 and between UP 2590 & a cross PBN 51 X VL 804. It can be inferred that WH 730 and HI 385 are highly diverse drought tolerant lines and they can be utilised in the formation of heterotic hybrids with the introgression of different loci with respect to drought tolerance trait.

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