

## SCREENING OF SALT TOLERANT WHEAT VARIETIES UNDER SALT STRESS IN REGION KHUIRATTA, DISTRICT KOTLI (AJK) PAKISTAN

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### Abstract

An experimental study was investigated to estimate an interactive effect of environment on the morphological, biochemical and phytochemical characters of *Triticum aestivum* viz; Aas-2011, Bakhtawar, Chakwal, Faisalabad-2008 and Galaxy lines in region Khuiratta (District Kotli) having different microclimatic conditions. The results conferred that among all the five wheat varieties the maximum plants height was (73.20cm) in Chakwal and (85.60cm) in Aas-2011 without stress salinity and it was (60.32cm) in galaxy and (70.30cm) in Faisalabad-2008 under stress salinity. The peduncle length was (65cm) in Bakhtawar and (85cm) in Galaxy without using salt. The peduncle length was (50cm) in Bakhtawar and (75cm) in Faisalabad-2008 after salt while, spike length was (4.30cm) in Bakhtawar and (9.5cm) in Aas-2011 before applying salt and after applying salts the spike length was (3cm) in Bakhtawar and (7.5cm) in Aas-2011. Number of spike/spikelet's before applying salt was (10.60) in Bakhtawar and (50.60) in Aas-2011. Number of spike per spikelet was (5.50) in Bakhtawar and (45.50) in Aas-2011 stress salinity. The number of grains/spike was (9.33) in Bakhtawar and (48) in Aas-2011 before applying salts while it was (6.40) in Bakhtawar and (38) in Aas-2011 respectively after applying salt. Flag leaf weight was (0.15gm) in Bakhtawar and (0.37gm) in Galaxy before using salts. Whereas flag leaf weight was (0.09gm) in Bakhtawar and (0.30gm) in Galaxy after applying salts. The leaf area was (8.40cm) in Bakhtawar and (9.50cm) in Chakwal without stress salinity, it was (6.30cm) in Bakhtawar and (7.80cm) in Chakwal under stress salinity. Similarly, grain yield (1.05gm) was in Bakhtawar and (2.30gm) in Aas-2011 without using salts, while it was (1gm) in Bakhtawar and (2.10gm) in Aas-2011 under stress salinity. The soil parameters analyzed included saturation percentage (36%), pH (6.8), E.C(0.40ms), organic matter (0.80), K(90ppm), P(8ppm), respectively. To evaluate the genetic diversity in the seed endosperm of *Triticum aestivum* for the nutritional grading by using SDS-PAGE. Five bands were attained by using 10 to 180kDa (Asbastas/SM0661), among which the band B, F and A with molecular weights 180kDa, 95kDa and 43kDa respectively were common in five wheat varieties but other band, show little variations. Wheat grouped in some cluster analysis which showed their initiation from same parental line so these are genetically less variance.

**Key words:** Salinity tolerance, Wheat, Organic matter, SDS-PAGE.

### Introduction

The world's more suitable eating grain cereal grass crop is wheat which belongs to family Gramineae (Poaceae). The researchers observed these days that the nutrient rich type of young grass is wheat in the cereal grass family, this is much wealthier in the levels of minerals, proteins and vitamins as compared to grain products of mature cereal plant or as compared to seed kernel (Trigar *et al.*, 2011). Wheat imparts a major function in terms of economic stability and food safety of country. We have to enhance the grain yields for a fast-increasing population in Pakistan with all forthcoming abiotic and biotic factors. Pakistan is anticipated to miss the wheat production target of 25.6 million tons in the current crop year as farmers have planted the staple crop over a lower area because of water scarcity amid drop in fertiliser consumption.

The country is likely to produce 24.8 million tons of wheat in the current crop year, which is a five-year low, if farmers manage to take per-acre production to the level of 2,885 kg they achieved in the previous year, according to the State Bank of Pakistan's (SBP) second quarterly report on the state of economy and there is still chances of improvement.

SDS-PAGE is a valuable tool and a biochemical technique which is extensively used for distinguishing the wheat cultivars of actual crop species because it is a modest and active system for interpretation of genetic structure of crop (Jha & Ohri, 1996).

District Kotli is a bordered area which is bordered by Bhimber and Mirpur, it is bordered on western side by Rawalpindi and Mirpur. Moreover it is surrounded by Sudhonooti and Poonch on northern side and on east it is bordered by Rajori. It is centered at longitude '73° 53 5.316" East latitude 33° 32 9.925" North. There is subtropical and humid ecological zone in district Kotli with average annual rain fall of 92.5mm. The hottest months are June and July of the year with minimum temperature of about 19.7 and 17.9°C and maximum temperature 37.3 and 34.3°C respectively (Muhammad *et al.*, 2012).

Objective of this study comprised of (i) to assess the variations in wheat varieties by applying salts growing in Region Khuiratta District Kotli on the basis of morphological traits (ii) To measure the result of the soil on yield of wheat and (iii) to evaluate the biochemical characterization of wheat genotype by using SDS-PAGE.

## Materials and Method

**Sample of plant:** From Agriculture Research Centre, the seeds of wheat varieties were collected for sowing in region Khairatta District Kotli AJK. Five seed varieties of wheat cultivars including Aas-2011, Faisalabad-2008, Galaxy, Bakhtawar and Chakwal were attained. The samples were stored in labelled glass bottles for further research work.

**Field experiment:** By using furrows on line seeds were sown with particular length on the basis of randomized complete block design which has applications at locations.

**Evaluation of morphological traits:** About nine morphological characters of the plants were selected at arbitrary as follows Plant height (cm), flowering time (maturity days), length of peduncle(cm), length of spike (cm), grains per spike (gm), Spikelet'/spike(cm), grain yield(gm) weight of flag leaf (gm), area of leaf (cm<sup>2</sup>) (Saleem *et al.*, 2008; Zarkati *et al.*, 2010).

**Observations and recordings:** Crop attributing traits and remarks on produce were documented. To trace explanations haphazard plants were assigned in each plant by adopting the methods of (Shashikala, 1996) the characters were studied to mark out the remarks which are as follows:

**Biochemical analysis (SDS-PAGE):** By using Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) the variation in seed storage protein was analyzed (Damania *et al.*, 1983) to characterize the wheat cultivars and scrutinize the genetic diversity (Shuaib *et al.*, 2007).

**Sample of plant:** Seeds of five wheat cultivars including, Faisalabad-2008, Galaxy, Bakhtawar, Chakwal and Aas-2011 were crushed into powder and 0.1g of each sample was taken in 1.5 ml micro tube.

**Extraction of protein:** Each micro tube was filled with 400 protein extraction buffer. This protein Extraction buffer contained Tris-1+cl 0.05M (pH:8), Urea 30 percent, SDS (sodium dodecyl sulfate) 0.02%, 2-mercaptoethanol. After this at 100°C sample was boiled for 3 minutes and it was kept over height at 40°C then it was centrifuged at 13000rpm for 10min. The supernatant consist of liquefied protein which was needy for further experiment.

**Preparation of the separation and stacking gel:** 40 ml of separation gel solution was added in a sidearm vacuum flask and mixed with the help of magnetic stirrer. A vacuum was applied to the flask for several minutes. TEMED and (APS) Ammonium per sulfate were added and swirled the flask to mix. The methodology of Anjum *et al.*, 2005, was followed.

**Loading and running the gel:** Combs were removed gradually from the gel. Individually well was cleaned with distilled water and wisely upturned casting stand to trench wells. Each well was filled with tank buffer by using Hamilton syringe under layer the sample in each well. A spinner was added to lower buffer chamber. The chamber was placed on a magnetic stirrer. When lower bubble is circulated the temperature of the buffer left over unchanged. The methodology of Anjum *et al.*, 2005 was followed.

**Staining of gel:** When the process of electrophoresis completed the gel was shifted to tray consisting of ethanol, acetic acid solution, and coomassie brilliant blue.

**De-staining of gel:** The De-staining solution contain (methanol acetic acid and distilled water) was used for de-staining and kept until the color of the background disappear. Distilled water was used to rinsed the gel. After that the picture was taken with scanning of gel (Bietz & Wall, 1972; Shuaib *et al.*, 2010).

**Data analysis:** The data matrix was designed by recording the banding patterns of proteins on gel. Complete linkage method was used to analyse Hierarchical cluster by measuring Euclidian distance measured for assessment the occurrence of protein storage in seed endosperm of wheat varieties, STATISTICS computer software (MATLAB) was used to analyzed the data (Shuaib *et al.*, 2007).

## Results and Discussion

An experimental work on field containing five varieties of *Triticum aestivum* growing in region Khairatta District Kotli Azad Jammu and Kashmir, and was propagated with all agronomics practices in different complete block design. The determination of present work was screening of salt tolerated wheat varieties under salt stress among five wheat genotypes viz; Faisalabad 2008, Aas-2011, Galaxy, Chakwal and Bakhtawar for morphological traits, soil analysis, phytochemical analysis (SDS-PAGE).

The analysis of data conversed that substantial differences were there in five investigated genotypes of wheat cultivars for nine morphological traits. The data analysis for dendrogram across the Khairatta illustrated the significant variations in plant height among wheat genotypes. Variable performance of height of wheat cultivars was found collaborated with environmental factors. The height of plant without salinity depicting the mean values for plant heights is shown in Table 1. The mean values for plants height without salinity between 73.20cm-85.60cm in selected wheat cultivars. The maximum wheat values were reported in Aas-2011 (85.60cm) followed by Faisalabad-2008 (80.32cm), Galaxy (76.35cm), Bakhtawar (75.30cm) and then Chakwal (73.20). The mean values for plants height under salinity ranges from 60.32cm-75.50cm in various wheat cultivars. The maximum wheat values were reported in Aas-2011 line (75.50cm) followed by Faisalabad-2008 (70.30cm), Bakhtawar (65.30cm), Chakwal (65.18cm), and then Galaxy (60.32cm).

Table 1. Morphological characters of five *Triticum aestivum* cultivars.

	Aas-2011		Faisalabad-2008		Galaxy		Bakhtawar		Chakwal	
	WOSS	USS	WOSS	USS	WOSS	USS	WOSS	USS	WOSS	USS
	Plant height (cm)	85.60 ± 0.85	75.50 ± 0.60	80.32 ± 0.88	70.30 ± 0.80	76.35 ± 0.90	60.32 ± 0.60	75.30 ± 0.88	65.30 ± 0.70	73.20 ± 0.85
Peduncle length (cm)	75.30 ± 0.66	70.00 ± 0.40	80.60 ± 0.88	75.60 ± 0.70	85.00 ± 0.57	70.00 ± 0.40	65.00 ± 0.99	50.00 ± 0.80	65.00 ± 0.99	50.00 ± 0.80
Number of spikes	50.60 ± 0.33	45.50 ± 0.30	39.60 ± 0.34	35.40 ± 0.20	35.70 ± 0.88	31.60 ± 0.80	10.60 ± 0.70	5.50 ± 0.50	30.60 ± 0.88	25.00 ± 0.60
Number of grains	48.00 ± 0.57	32.00 ± 0.59	35.00 ± 0.88	30.50 ± 0.90	30.09 ± 0.90	25.40 ± 0.95	9.33 ± 0.80	6.40 ± 0.85	34.00 ± 0.90	20.00 ± 0.95
Grain yield (gm)	2.30 ± 0.21	2.10 ± 0.10	1.90 ± 0.18	1.00 ± 0.15	1.42 ± 0.38	1.15 ± 0.30	1.05 ± 0.09	1.00 ± 0.09	1.80 ± 0.10	1.50 ± 0.05
Flag leaf weight (gm)	0.30 ± 0.06	0.25 ± 0.01	0.31 ± 0.04	0.27 ± 0.02	0.37 ± 0.08	0.30 ± 0.05	0.15 ± 0.03	0.09 ± 0.01	0.25 ± 0.01	0.20 ± 0.01
Leaf area (cm)	9.05 ± 0.90	7.05 ± 0.95	9.20 ± 0.60	7.30 ± 0.70	9.30 ± 0.70	7.40 ± 0.72	8.40 ± 0.60	6.30 ± 0.65	9.50 ± 0.80	7.80 ± 0.90
Spike length(cm)	9.50 ± 0.11	7.50 ± 0.02	8.00 ± 0.10	7.00 ± 0.05	7.00 ± 0.15	6.00 ± 0.15	4.30 ± 0.88	3.00 ± 0.70	8.00 ± 0.30	6.00 ± 0.30

Key: WOSS: Without stress salinity; USS: Under stress salinity

The peduncle length without salinity showed variation in mean values of different wheat cultivars varies from 65.00cm-85.00cm. The highest mean value for the peduncle length showed by Galaxy (85.00cm), then Faisalabad-2008 (80.60cm), followed by Aas-2011 (75.30cm), Bakhtawar and Chakwal (65.00cm) showed same values. The peduncle length under salinity showed variation for mean values in different wheat cultivars different from 50.00cm to 75.60cm. The maximum value for the peduncle length was recorded by Faisalabad-2008 (75.60cm), followed by Aas-2010 (70.00cm), Galaxy (70.00cm), then Bakhtawar and Chakwal (50.00cm) showed same values.

Significantly different mean values of spike length were evidenced from variety to variety as shown in (Table 1). Spike length value without salinity ranges between 4.30cm-9.50cm. Largest spike length value was documented for Aas-2011 (9.50cm) proceeded by Faisalabad-2008 (8.00cm) and Chakwal (8.00cm), Galaxy (7.00cm), Bakhtawar (4.30cm). The maximum values for the spike length under salinity ranges from 3.00cm-7.50cm. Aas-2011 (7.50cm) displayed largest spike length proceeded by the following Faisalabad-2008 (7.00cm) and Chakwal (6.00cm), Galaxy (6.00cm) and Bakhtawar (3.00cm).

The mean value for number of spikes without salt salinity ranges from 10.60-50.60. The maximum value was recorded in Aas-2011 (50.60) while other varieties conferred mean values as Faisalabad-2008 (39.60), Galaxy (35.70), Chakwal (30.60) and Bakhtawar (10.60) had minimum mean value for given parameter. Number of grains in wheat cultivars under study illustrates a greater variation in mean values ranging from 9.33-48.00. Without salinity maximum grain yield value was witnessed in Aas-2011 (48.00), proceeded by Faisalabad-2008 (35.00), Chakwal (34.00), Galaxy (30.00) and Bakhtawar (9.33). Moreover under salinity maximum grain yield value was recorded in Aas-2011 (32.00), chased by Faisalabad-2008 (30.50), Galaxy (25.00), Chakwal (20.00), Bakhtawar (6.40).

Greater variations among wheat cultivars were recorded for the mean values of grain yield without salinity between 1.05-2.30. Aas-2011 (2.30) had exhibited the minimum mean value for the given morphological trait and Faisalabad-2008 (1.90) whereas other values recorded were Chakwal (1.80), Galaxy (1.42) and Bakhtawar (1.05) (Table 1).

Mean values of the flag leaf weight without stress salinity for selected wheat genotypes varies between 0.15-0.37gm. Highest value was witnessed in Galaxy (0.37) followed by Faisalabad-2008 (0.31), Aas-2011 (0.30), Chakwal (0.25) and Bakhtawar (0.15). The widest leaf area without stress salinity was observed in genotype Chakwal (9.50) and Bakhtawar (8.40) with smallest leaf area as displayed in Table 1 of leaf area.

**Days to maturity:** Days to maturity ranges from 160-165 days as recorded in Table of days to maturity and Figure of days to maturity. Dissemination of currently investigated five different cultivars of wheat requires 165 days to reach maturity grown in District Kotli (Khairatta). Cultivars Aas-2011 and Faisalabad-2008 necessitate 165 days to become mature and their spike turned golden. While Galaxy and Chakwal required 164 days to get mature while Bakhtawar had taken 160 days to mature as shown in (Table 2).

**Table 2. Days to maturity of All Genotypes in Khuiratta (Kotli).**

S. No.	Genotype	District Kotli
1.	Aas-2011	165
2.	Faisalabad-2008	165
3.	Galaxy	164
4.	Bakhtawar	160
5.	Chakwal	164

**Cluster analysis:** The data analysis was used to obtain similarity coefficient matrix of 9 morphological traits of five wheat varieties grown in District Kotli. Similarity coefficient of similarity between (1.00-0.975). Genetic diversity among Faisalabad and Chakwal was highest with 0.975 value referring as genetically similar lines. The dendrogram from unweighted pair group method with arithmetic average (UPGMA) and from similarity coefficient matrix was calculated from nine morphological traits in 5 wheat genotypes. The Cluster-I includes Bakhtawar and cluster II includes two subcluster one having Galaxy and Aas with the bootstrap value of 0.987 while other subcluster have Faisalabad and Chakwal with the bootstrap value of 0.975 exhibiting highest similarity as shown in figure 1. Wheat cultivars are less divergent genetically, originated from same parental line and grouped in same cluster (Nisar *et al.*, 2010). In this dendrogram Bakhtawar line was divergent while rest of the lines were assumed from similar genetic source.

**SDS-PAGE:** SDS-PAGE is used to study the low and high molecular weight glutenin sub-units as a tool to assess the characterization and genetic diversity to separate wheat cultivars. The SDS-PAGE was used to test genotypes which evaluates the morphological traits through molecular characterization to reveal the true picture of the storage protein profile. To look over the genetic diversity among wheat cultivars by SDS-PAGE of storage seed protein to ensure the molecular weight of glutelin. Electrophorogram of different wheat varieties was recorded by patterns of protein banding as

in SDS-PAGE as shown in figure 2. Standard bands of marker 10-180kDa (Asbastas/SM 0661) was used to calculate the relative percentage of each band and five bands were attained.

Usually the highest mean value for the plant height was seen in Galaxy (85.60) without the application of salt but, with the application of salt the plant height decreases and maximum plant height under salinity was Galaxy (75.50cm) showed unexpected decrease in plant height. The current research work depicted the additional view on different wheat varieties, which showed diversity on the basis of application of salt, SDS-PAGE and phytochemical analysis. (Saqib *et al.*, 2012) indicated that wheat can be considered as glycophytes and due to soil salinity, it generally shows limited growth and development.

It was depicted from the dendrogram that three main clusters showed variations among each other. The cluster -I comprised of Faisalabad (for change) line grown in Khuiratta trend to which was a singleton assumed that all lines (B, G, F, A, C) originated from this line respectively. While on the other hand cluster- I again sub-grouped into two sub-groups. The first sub-group comprised of (4,5) which depicted that all these lines are genetically similar as shown in figure 3. All of the wheat cultivars produced different spike lengths due to the salinity. The maximum spike length was recorded in (9.5cm) Aas-2011 and minimum (4.30cm) without salt application but under salinity the maximum value recorded in (7.50cm) Aas-2011 and minimum value (3.0cm) Bakhtawar. The present work indicated that extreme numbers of grains per spike (48) were formed in Aas-2011. The smallest number of grains per spike (9.33) was noted in Bakhtawar without applying salt. The maximum number of grains per spike under salt stress (32) Aas-2011, and the smallest number of grains per spike were verified (6.4) Bakhtawar. Environmental factors like rainfall, humidity and temperature variation considerably affect grain number per spike, moreover these results are comparable with the Altindal, 2019; Memon *et al.*, 2020.

### Similarity

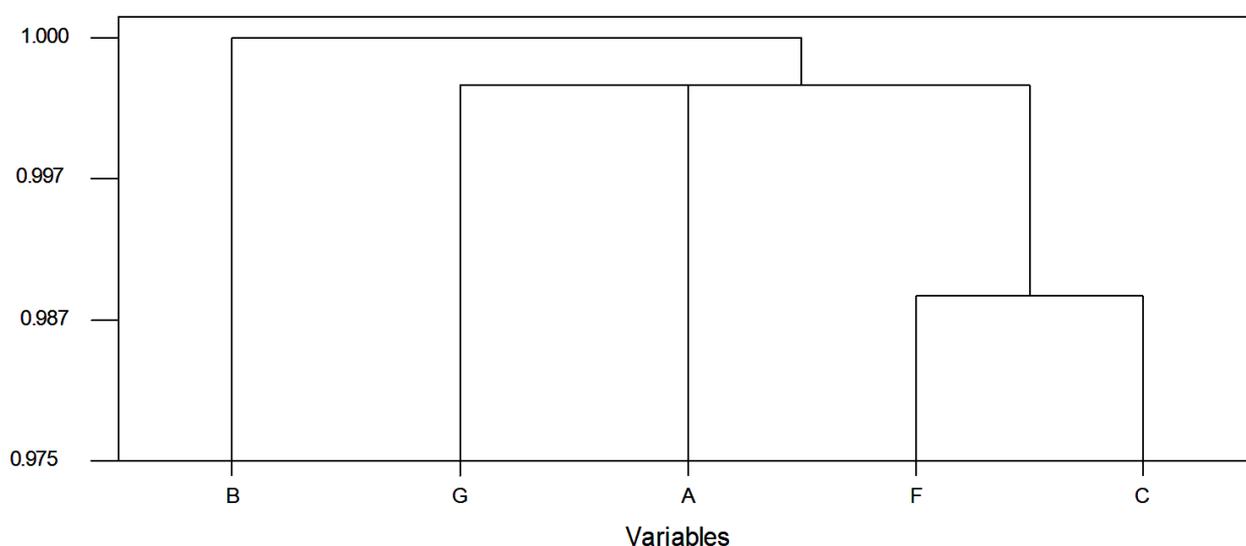


Fig. 1. Dendrogram based on morphological Traits.

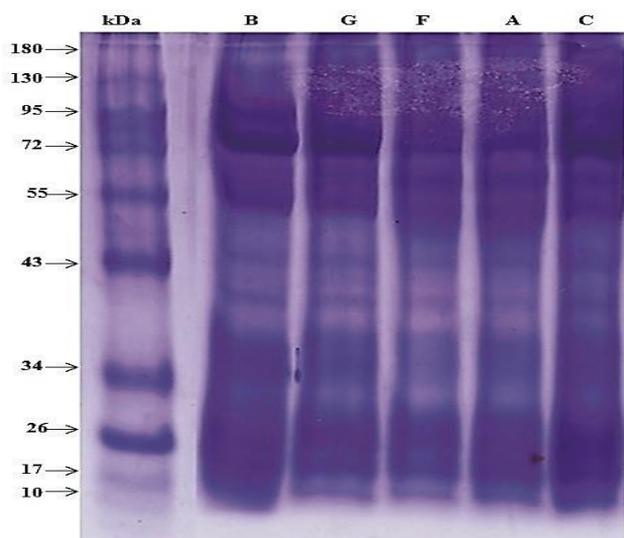


Fig. 2. SDS-PAGE of Seed Storage Protein of all selected wheat varieties.

**Table 3. Similarity index of *Triticum aestivum* based upon SDS-PAGE.**

B	G	F	A	C	
B	1.00				
G	0.86513	1.00			
F	0.90985	0.95245	1.00		
A	0.85127	0.767	0.84156	1.00	
C	0.65545	0.70	0.72474	0.75873	1.00

Key words: B: Bakhtawar, G: Galaxy, F: Faisalabad-2008, A: Aas-2011, C: Chakwal

Different performance was detected concerning leaf area of plant for the wheat cultivars. Widest leaf area was found in genotype Chakwal (9.50cm), while

smallest leaf area was observed in genotype Bakhtawar (8.40cm) these recordings were observed without the application of salts and recordings under salinity, widest leaf area was found in genotype Chakwal (7.80cm) and smallest leaf area was observed in Bakhtawar (6.30cm). These results are in harmony with the findings Ahamed *et al.*, (2010) illustrating negative impact on the growth of wheat on exposure to high temperatures. Moreover high temperature in reproductive stage reduces leaf area due to leaf senescence (Amin *et al.*, 2005; Wrigley *et al.*, 2006).

Days required by wheat cultivars to reach maturity differ from 165 (Aas-2011), 165 (Faisalabad-2008), 164 Galaxy, 164 Chakwal and Bakhtawar took 160 days to mature. Among all five varieties of wheat Bakhtawar took more days to mature. (Inamullah *et al.*, 2007) reported that some environmental factors such as temperature and drain fall effect a variety of traits, which in turn influenced by the enlargement and growth of crop, so, in this way the yield of cultivar is also affected. The present work represents that methanolic, ethylacetate and chloroform extracts of the five wheat varieties, including Aas-2011, Faisalabad-2008, Galaxy, Bakhtawar, Chakwal. Wheat showed positive results for steroids and tannins. In the present study marker with molecular weight of 10-180KDA was applied. Protein bands of B and F were most frequent with molecular weight of 180 kDa and 95 kDa representing HMW bands while the protein bands of low molecular weight were (A and C) with molecular weight of 17 kDa and 10kDa respectively. The morphological characters and SDS-PAGE were not predominant match in the result (Martnez *et al.*, 2005). Underlying reasons of squat correlation of morphological markers with genetic markers is the presence of fact that in artificial selection molecular markers are less imperiled than morphological traits.

Similarity

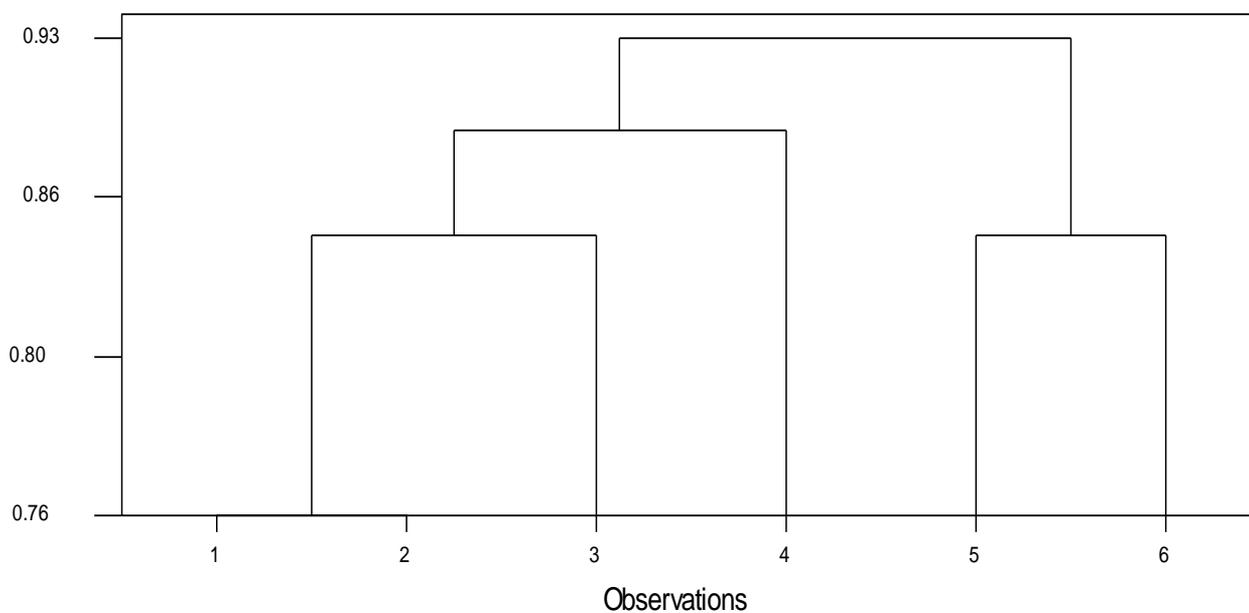


Fig. 3. Dendrogram based upon SDS-PAGE.

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