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Research Article

Assessment of genetic divergence in wheat lines (*Triticum aestivum* L.) involving biochemical and protein markers in rainfed conditions

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Abstract

Wheat (*Triticum aestivum* L.) is the important and strategic cereal crop for the majority of world's populations. It is significant staple food of about two billion people. In next few years, world demand for wheat is expected to be 40 percent higher than that of its level today. Keeping in view the importance of the crop research work was conducted in the laboratory of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot, AJK, Pakistan. The aim of the research was to find out the genetic diversity of different wheat lines. In the experiment, 50 different lines of wheat species (*Triticum aestivum* L.) was used to detect genetic diversity by utilizing Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and biochemical analysis. On the basis of biochemical analysis lines 3111 and 3123 was diverse among 50 lines for antioxidant activity by using DPPH radical and 3135 and 3139 for phenolic contents and for flavonoid 3148 and 3107 was found more promising. Molecular characterization by SDS PAGE showed diversity in three wheat lines 3136, 3138 and 3110. These wheat lines could be our potential lines for future wheat improvement program as they were also promising regarding to the high yields.

Keywords: antioxidant; diversity; flavonoid; phenolic; SDS PAGE

Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant cereal crop and major food crop of Pakistan. Wheat occupies the central position in formulation of agricultural policies as staple diet of the people. In Pakistan, the wheat (*Triticum aestivum* L.) faces the dual hazard of biotic as well as abiotic stresses, which result

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in lower yield. For this information about germplasm diversity for its morpho-physiological traits among elite breeding material is a primary element in plant breeding. However, morphological traits have a number of restrictions, including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects (Autran *et al*., 1995). While protein markers, like seed storage proteins, imitate with more accuracy the genotypes, autonomously from the environmental effects (Bartosova *et al*., 2013). Protein markers are useful tackle in identifying cultivar, registration of new varieties, and classification of crop species. It also helps in studying genetic diversity, thereby improving the efficiency of wheat breeding programs in cultivar development (Berzinova *et al*., 2010; Bietz *et al*., 1972). Proteins are grouped into four classes according to their solubility: albumins, globulins, prolamin and glutelin. Gluten, comprising roughly 78 and 85% of total wheat endosperm protein, is a very large composed mainly of polymeric and proteins known respectively as glutelin and gliadins (Benmoussa *et al*., 2000). Gliadins and glutamines have been briefly studied. Their genetics and biochemistry are relatively well known (Chang 2002, Ehewhbwegerova *et al*., 2009; Feillet *et al*., 2000). Gluten confer elasticity to dough, whereas, gliadins are viscous and give extensibility to dough (Gianibelli *et al*., 2001). Studies showed that two types of subunits were present; the low molecular weight (10,000-70,000 Da) and the high molecular weight Gluten subunits (80,000-130,000 Da) (Ghafoor *et al*., 2009). The high molecular weight (HMW) subunits of wheat gluten are major determinants of the elastic properties of gluten that allow the use of wheat doughs to make bread. The polyacrylamide-gel electrophoresis has been used to show that large size variation exists among LMW and HMW glutelin subunits. It has been recommended that deletions and insertions within the repetitive region are responsible for these variations in length (Hantano, 1998). There are both quantitative and qualitative effects of HMW subunits on the quality of the grain. High Molecular Weight Gluten Subunit (HMW-GS) are encoded at the Glu-1 loci on the long arms of group 1 chromosomes (Glu-A1, Glu-B1 and Glu-D1) (Hammer *et al*., 2001). The polyacrylamide-gel electrophoresis has been used to show that large size variation exists among LMW and HMW glutelin subunits. Bread wheat contain six different HMW-GS but due to the "silencing" of some of the genes, most common wheat cultivars possess three to five HMW-GS. The low molecular weight-gluten subunits (LMW-GS) represents about 33 - 34% of the total seed protein and 60% of total gluten (Ghafoor *et al*., 2009). The LMW-GS are controlled by genes at the Glu-A3, Glu-B3 and Glu-D3 loci. Due to extensive polymorphism, these proteins have been widely used for cultivar identification in hexaploid and tetraploid wheat (Irina *et al*., 2012). Allelic variants differ in the number, mobility and intensity of their components and can be characterized through SDS-PAGE. In-addition to protein source, wheat is significant sources of phytochemical constituent such as antioxidants, phenolics, vitamins, flavonoid other health-beneficial substances for human health (Liu, 2007; Magdalena *et al*., 2002). As this substance scavenge free radicals to protect bio-molecules and also help plants in their adaptation in various environmental conditions (Nakamura *et al*., 2001). The objectives of study were: To study genetic diversity in genotypes of bread wheat lines using seed storage proteins and to evaluate phytochemical properties of wheat bran for preventing disease and promoting health.

Materials and Methods

The experimental material was comprised of 50 wheat lines (*Triticum aestivum* L.). The seeds were used to determined Biochemical and Molecular diversity. Fifty wheat lines were collected from 28th Semi-Arid Wheat Screening Nursery (SAWSN), and CIMMYT (International Wheat and Maize Improvement Centre).

Table 1. List of wheat lines

Sr. No	Lines	Sr. No	Lines
01	3101	26	3126
02	3102	27	3127
03	3103	28	3128
04	3104	29	3129
05	3105	30	3130
06	3106	31	3131
07	3107	32	3132
08	3108	33	3133
09	3109	34	3134
10	3110	35	3135
11	3111	36	3136
12	3112	37	3137
13	3113	38	3138
14	3114	39	3139
15	3115	40	3140
16	3116	41	3141
17	3117	42	3142
18	3118	43	3143
19	3119	44	3144
20	3120	45	3145
21	3121	46	3146
22	3122	47	3147
23	3123	48	3148
24	3124	49	3149
25	3125	50	3150

Biochemical studies

Antioxidant activity (%)

The antioxidant activity of wheat seeds was measured using the stable 1, 1-diphenyl-2-picrylhydrazy (DPPH) radical as described by Payne *et al*. (1984).

Total phenolic compounds (TPC) mg/g

The total phenolic compounds were analyzed using the Folin-Ciocalteau method with some modification (Porto *et al*., 2000).

Total flavonoid mg/100 g

Total flavonoids content was determined by method (Starovicova *et al*., 2003).

Molecular studies

SDS-PAGE technique was used to identify molecular diversity of available lines of *Triticum aestivum* L. Sodium Dodecyl-sulphate (SDS) is an anionic detergent that denatures proteins by wrapping the hydrophobic tail around the polypeptide backbone. For almost all proteins, SDS binds at a ratio of approximately 1.4 g SDS per gram of protein, thus conferring a net negative charge to the polypeptide in proportion to its length.

SDS-PAGE

Preparation of seed samples

Seed flour 10 mg was taken and put into 1.5 ml micro tubes. To extract proteins from flour, 400 µl of the protein extraction buffer was put into the microtube and mixed well by the test tube mixer (vortex). This sample was preserved in a freezer (- 20° C).

Protein extraction buffer

(0.05 M Tris-HCl pH 8.0, 0.2% SDS, 5M Urea, 1% ß-mercaptoethanol)

Solutions for electrophoresis

Solution A

(3.0 M Tris-HCl pH 9.0, 0.4% SDS)

Solution B

(0.493 M Tris-HCl pH 7.0, 0.4% SDS)

Solution C

(30% Acrylamide, Acrylamide/Bis = 30: 0.8) with 10% APS also

Electrode buffer solution

(0.025 M Tris, 0.129 M Glycine, 0.125% SDS)

Staining and destaining solutions were also prepared according to standard protocol.

Preparation of electrophoretic gel

After setting the Electrophoretic apparatus, two types of gel solution were prepared:

1. Separation Gel with 1 mm thickness (For two mini gels) i.e., Separation gel 11.25%.
2. Stacking Gel (For two mini gels i.e., stacking gel 4.5%.

Electrophoresis

Electrophoresis procedure was carried out using slab type SDS-PAGE model: AE-6530M, ATTA Japan, with 11.25% polyacrylamide gel.

Detection of seed protein

Staining and de-staining of separation gel

When blue line reached at the bottom of the gel plates, electric supply was disconnected. Gel plates were taken out from the apparatus and separated by spatula. Stacking gel was removed with the help of same spatula. Separation gel was put in the box which contained staining solution. Box was put on the shaker for two hours. Staining solution was exchanged by de-staining solution and the box was shacked gently almost overnight until the background of the gel disappeared to absorb excess CBB, a piece of Kimwipe was put in the de-staining solution to check absorbance.

Drying of separation gel

Wet filter paper was placed on the plate of gel dryer. Separation gel was carefully placed on the paper and covered with a wrap. It was dried in a drier for about 1.5 hours at 60 °C. When gel sheet was completely dried it was taken out while the pump was still running. All gels were dried with the same manner. The gels were then analyzed and photograph.

Statistical analysis

By means of the cluster analysis, simple statistics as well as the numerical taxonomic techniques was analyzed (Naghavi, 2001) by help of computer software PAST (Picard *et al*., 2005). The cluster analysis was done on the basis of standard distance of k-means and in each cluster the accessions were then analyzed for the basic statistics.

Results and Discussion

Biochemical studies

Antioxidant activity by DPPH assay

The antioxidant activities of these lines were tested at a concentration range of 0.25-5 mg/ml. The maximum antioxidant activity was showed by (1311), (3123), (3137) and (3130) as 30.25±1.61 %, 30.34±1.63 % 29.73±1.54, 29.48±1.19 respectively (Table 2, Figure 1). Whereas, (3117), (3136) and (3148) showed the least antioxidant activity 13.26±0.68, 13.82±0.61 and 13.83±0.60. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Payne *et al*., 1984).

Table 2. Mean values and standard deviation for DPPH activity in 50 wheat lines

S. No.	Lines	Mean	SD	S. No.	Lines	Mean	SD
1	3101	8.26	±1.36	26	3126	7.02	±1.22
2	3102	10.12	±1.11	27	3127	5.18	±0.79
3	3103	20.36	±0.28	28	3128	5.62	±0.67
4	3104	22.8	±0.61	29	3129	9.46	±0.09
5	3105	14.15	±0.56	30	3130	9.66	±1.19
6	3106	24.1	±0.78	31	3131	7.42	±1.36
7	3107	19.21	±0.12	32	3132	8.9	±1.49
8	3108	17.34	±0.13	33	3133	6.54	±0.95
9	3109	6.41	±1.61	34	3134	9.74	±1.71
10	3110	12.29	±0.81	35	3135	7.38	±0.26
11	3111	30.25	±1.61	36	3136	9.32	±0.61
12	3112	17.37	±0.13	37	3137	10.3	±1.54
13	3113	25.15	±0.92	38	3138	8.38	±0.36
14	3114	15.61	±0.63	39	3139	9.806	±1.59
15	3115	22.59	±0.58	40	3140	7.14	±0.90
16	3116	28.42	±1.37	41	3141	6.26	±0.40
17	3117	13.26	±0.68	42	3142	5	±0.16
18	3118	23.64	±0.72	43	3143	8.35	±1.47
19	3119	11.17	±0.96	44	3144	9.28	±1.39
20	3120	10.81	±1.01	45	3145	8.51	±1.17
21	3121	20.19	±0.25	46	3146	10.26	±1.19
22	3122	23.93	±0.76	47	3147	7.22	±0.18
23	3123	30.34	±1.63	48	3148	7.62	±0.60
24	3124	9.25	±1.22	49	3149	8.82	±1.37
25	3125	21.19	±0.39	50	3150	8.62	±0.21

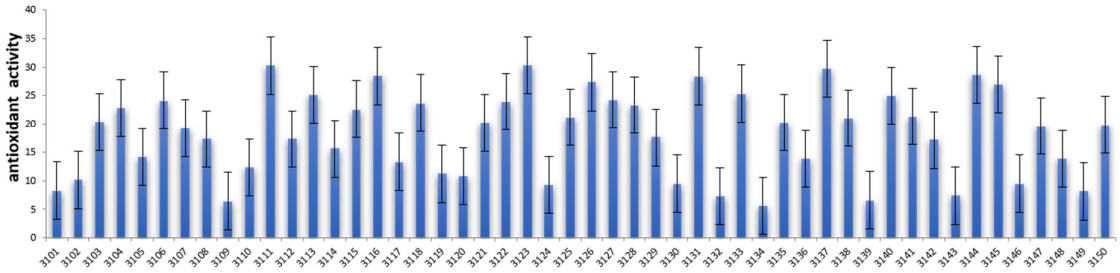

Figure 1. Demonstration of the antioxidant activity % in 50 wheat lines

Total phenolic contents (TPC)

Total phenolic contents measured by Folin-Ciocalteu reagent method ranged from 11.84 to 33.23 mg/g of gallic acid equivalent of aqueous wheat grain extract. Wheat lines (3135), (3139), (3128) and (3136) showed the highest phenolic contents i.e., 33.12±1.93,33.23±1.95, 31.83±1.73, 31.97±1.75 mg GAE/g respectively (Table 3, Figure 2). The lowest phenolic contents were noticed in (3121) and (3134) as 11.84±1.40, 11.84±1.40.

Table 3. Mean values and standard deviation for phenolic contents in 50 wheat lines

S. No.	Lines	Mean	SD	S. No.	Lines	Mean	SD
1	3101	22.54	±0.28	26	3126	17.48	±0.52
2	3102	18.17	±0.41	27	3127	12.76	±1.26
3	3103	17.82	±0.46	28	3128	31.83	±1.73
4	3104	23.57	±0.44	29	3129	14.49	±0.98
5	3105	25	±0.66	30	3130	30.7	±1.56
6	3106	23.57	±0.44	31	3131	19.43	±0.21
7	3107	24.72	±0.62	32	3132	20.24	±0.08
8	3108	23.92	±0.49	33	3133	17.82	±0.46
9	3109	21.73	±0.15	34	3134	11.84	±1.40
10	3110	22.65	±0.29	35	3135	33.12	±1.93
11	3111	28.98	±1.29	36	3136	31.97	±1.75
12	3112	29.32	±1.34	37	3137	13.22	±1.18
13	3113	18.97	±0.28	38	3138	28.4	±1.19
14	3114	21.5	±0.11	39	3139	33.23	±1.95
15	3115	12.99	±1.22	40	3140	29.55	±1.37
16	3116	16.67	±0.64	41	3141	21.39	±0.10
17	3117	16.44	±0.68	42	3142	27.14	±1.00
18	3118	18.89	±0.30	43	3143	12.99	±1.22
19	3119	25.64	±0.76	44	3144	24	±0.51
20	3120	13.11	±1.20	45	3145	13.11	±1.20
21	3121	11.84	±1.40	46	3146	14.95	±0.91
22	3122	15.52	±0.82	47	3147	14.26	±1.02
23	3123	15.87	±0.77	48	3148	26.79	±0.94
24	3124	16.33	±0.70	49	3149	14.37	±1.00
25	3125	13.68	±1.11	50	3150	24.15	±0.53

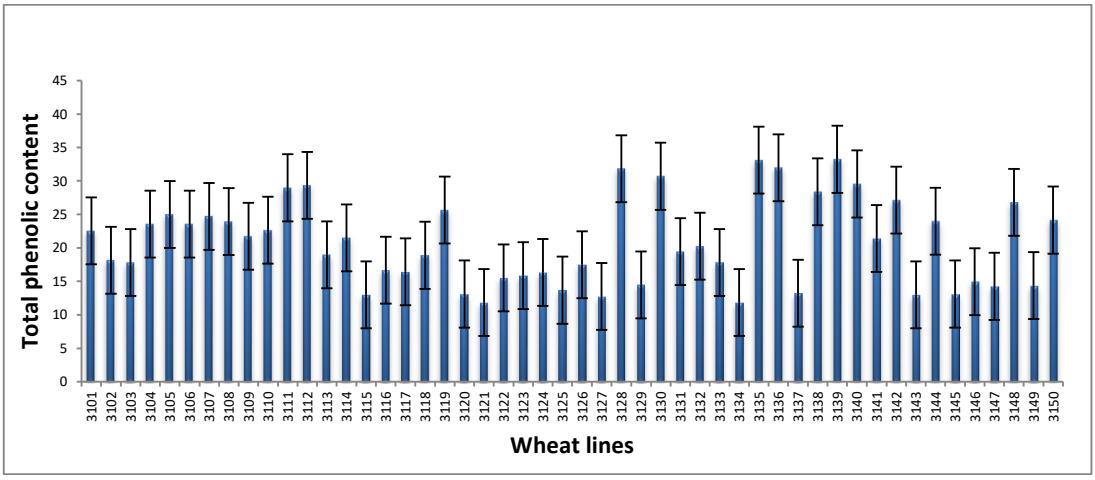

Figure 2. Representation of the mean values of Phenolic contents among 50 wheat lines

Total flavonoid contents

Total flavonoid content was determined by using aluminium chloride and potassium acetate ranged from 2.44 mg/100 g to 15.1 mg/100 g. In (3148) and (3107) showed the highest flavonoid contents i.e., 15.1±1.92 and 15±1.89 mg/100 g. The minimum flavonoid contents were noticed in (3140), (3121), (3113) as 6.13±0.79, 6.24±0.76, 6.66±0.63 respectively (Table 4, Figure 3). The results were in accordance with (Sneath *et al.*, 1973).

Table 4. Mean values and standard deviation for flavonoid contents in 50 wheat lines

S. No.	Lines	Mean	SD	S. No.	Lines	Mean	SD
1	3101	9.27	±0.16	26	3126	3.83	±1.49
2	3102	14	±1.59	27	3127	7.66	±0.33
3	3103	8	±0.23	28	3128	8	±0.23
4	3104	9.92	±0.35	29	3129	8.32	±0.13
5	3105	7	±0.53	30	3130	12.95	±1.27
6	3106	10	±0.38	31	3131	2.44	±1.91
7	3107	15	±1.89	32	3132	10.32	±0.48
8	3108	9	±0.08	33	3133	12.73	±1.21
9	3109	5.36	±1.03	34	3134	4.62	±1.25
10	3110	11.82	±0.93	35	3135	11.38	±0.80
11	3111	14.74	±1.81	36	3136	12.4	±1.11
12	3112	9.12	±0.11	37	3137	7.55	±0.36
13	3113	6.66	±0.63	38	3138	8.17	±0.18
14	3114	3.75	±1.52	39	3139	3.46	±1.60
15	3115	8.61	±0.64	40	3140	6.13	±0.79
16	3116	5.54	±0.97	41	3141	9.85	±0.33
17	3117	10.69	±0.59	42	3142	10.14	±0.42
18	3118	9.41	±0.20	43	3143	10	±0.38
19	3119	9.34	±0.18	44	3144	11.2	±0.74
20	3120	8.28	±0.14	45	3145	9	±0.08
21	3121	6.24	±0.76	46	3146	4.26	±1.36
22	3122	4.53	±1.28	47	3147	13	±1.29
23	3123	5.93	±0.86	48	3148	15.1	±1.92
24	3124	7.4	±0.41	49	3149	14.11	±1.62
25	3125	8.13	±0.19	50	3150	3.22	±1.68

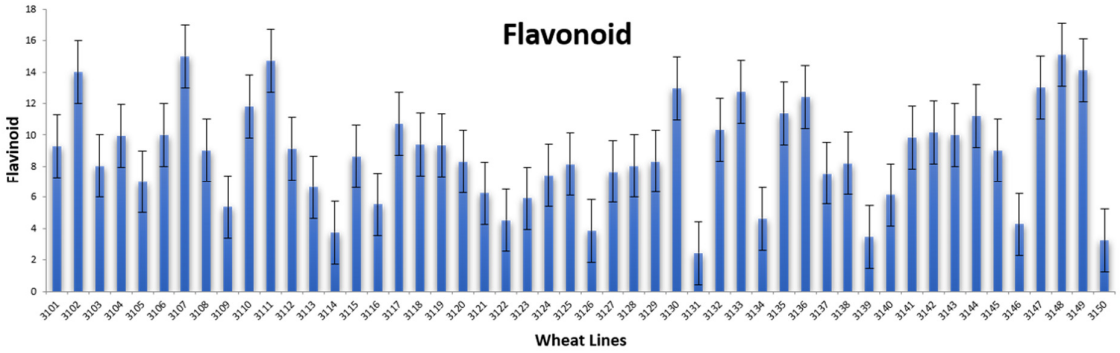

Figure 3. Representation of the flavonoid contents among the 50 wheat lines

Molecular studies

The SDS-PAGE is a practical consistent method because seed storage proteins are largely sovereign of environmental fluctuation. For molecular characterization of wheat lines SDS PAGE has been used because of its reliability and simplicity for protein judgment. Protein profile of different lines is estimated and their distinct banding pattern on running gel has been noted after staining and distaining. The results for SDS-PAGE of 50 wheat lines are shown below in Figure 4 (a, b, c, d, e) and (Table 5).

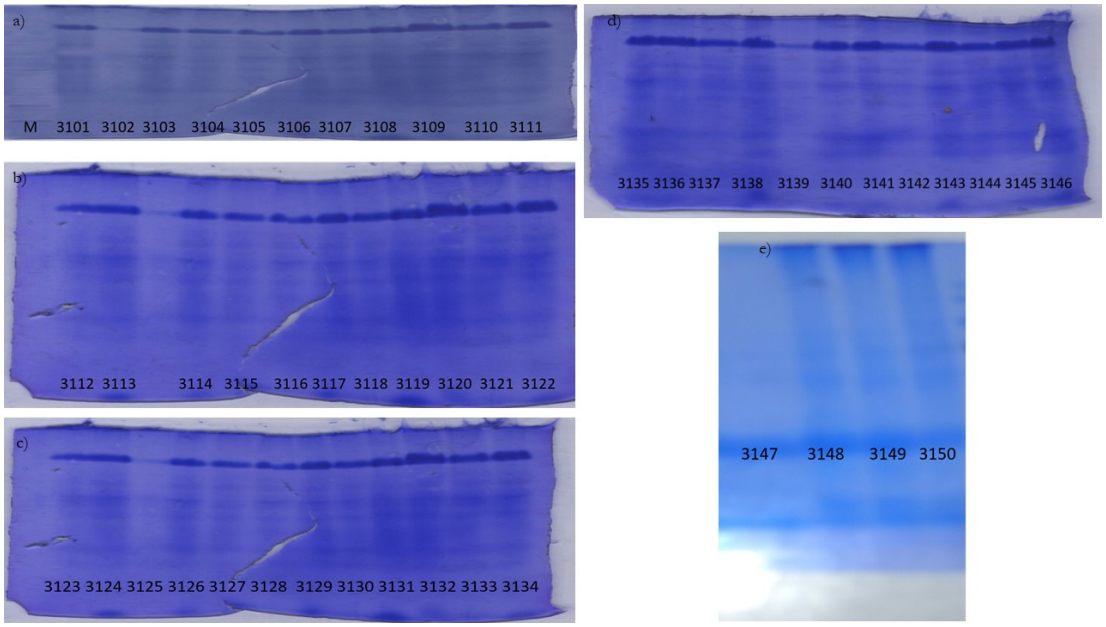

Figure 4. The results for SDS-PAGE of wheat lines
n.b. numbers represent wheat lines

Table 5. Diagrametic sketch showing SDS-PAGE results of fifty wheat lines

Bands	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3101	1	1	0	0	1	0	0	0	0	0	0	0	0	0
3102	1	0	0	1	1	0	1	0	0	0	0	1	0	0
3103	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3104	1	0	0	1	1	0	0	0	0	0	0	1	0	0
3105	1	0	0	1	0	0	0	0	0	0	0	0	0	0
3106	1	0	0	1	1	0	1	0	0	1	0	1	0	0
3107	1	0	0	1	1	0	1	0	0	1	0	1	0	0
3108	1	0	0	1	1	0	1	0	0	1	0	1	0	0
3109	1	0	0	0	0	0	1	1	1	1	0	0	0	0
3110	1	0	0	0	1	0	1	1	1	1	0	1	0	0
3111	1	0	0	1	1	0	0	0	0	1	0	0	0	0
3112	1	0	0	1	1	0	1	0	0	1	0	0	0	0
3113	1	0	1	1	1	0	0	0	0	1	0	1	0	0
3114	1	0	1	0	0	0	0	0	0	0	1	1	0	0
3115	1	0	1	1	0	0	1	0	0	0	0	1	0	0
3116	1	0	1	1	0	0	1	0	0	1	1	1	0	0
3117	1	0	0	0	0	0	0	0	0	0	1	1	0	0
3118	1	0	1	1	1	0	1	0	0	1	0	0	0	0
3119	1	0	1	1	1	0	1	0	0	1	0	0	0	0
3120	1	0	0	1	0	0	0	1	0	0	0	1	0	0
3121	1	0	1	1	1	0	0	0	0	1	0	0	0	0
3122	1	0	1	1	0	0	1	0	0	1	0	0	0	0
3123	1	0	1	1	1	0	1	1	0	1	0	1	0	0
3124	1	0	1	1	1	0	1	1	0	1	0	1	0	0
3125	1	0	1	0	0	1	1	0	1	1	0	0	0	0
3126	1	0	1	0	0	1	0	0	1	0	0	0	0	0
3127	1	0	0	0	0	1	1	0	1	1	0	0	0	0
3128	1	1	1	1	0	1	0	0	1	1	0	0	0	0
3129	1	0	0	0	0	1	0	0	1	1	0	0	0	0
3130	1	0	0	0	0	1	1	0	1	0	0	0	0	0
3131	1	0	0	1	0	1	1	1	1	0	0	0	0	0
3132	1	0	0	0	0	0	0	1	0	1	0	0	0	0
3133	1	0	0	0	0	1	1	0	1	1	0	0	0	0
3134	1	0	0	1	0	1	0	0	1	0	0	0	0	0
3135	1	0	1	0	1	0	1	0	0	1	0	1	0	0
3136	1	0	0	1	1	0	1	0	1	1	1	0	0	0
3137	1	1	1	1	0	1	1	0	1	1	0	0	0	0
3138	0	0	0	0	0	0	1	1	1	0	0	0	0	0
3139	0	0	1	1	1	1	1	0	1	1	0	0	0	0
31409	0	0	1	1	1	1	1	1	0	0	0	0	0	0
3141	0	0	1	1	1	1	1	0	1	1	0	0	0	0
3142	1	0	1	1	1	1	1	1	0	1	0	0	0	0
3143	0	0	1	1	1	1	1	1	1	1	0	0	0	0
3144	1	0	1	1	1	1	1	0	1	1	0	0	0	0
3145	1	0	1	1	1	1	1	1	0	1	0	0	0	0
3146	0	0	0	1	1	1	1	1	0	1	0	0	0	0
3147	0	0	0	1	0	1	1	1	0	0	0	0	0	0
3148	0	0	0	1	0	1	1	1	0	1	0	0	0	0

3149	0	0	0	1	1	1	1	1	0	0	0	0	0	0
3150	0	0	0	1	0	1	1	1	0	0	0	0	0	0

Average linkage distance among 50 wheat lines

The tree diagram based on 50 wheat lines was displayed in (Figure 5, Table 6)**.** The figure indicated five main clusters at linkage distance 2.1. The clusters were named as cluster I, cluster II, cluster III, cluster IV and cluster V. Cluster I is further divided into two sub- clusters i.e., Ia and Ib. Sub-cluster Ia consisted of seventeen lines including 3116, 3115, 3102, 3104, 3106, 3107,3108, 3111, 3112, 3113, 3121, 3118, 3119, 3123, 3122, 3124 and 3135. Among these lines 3113 and 3121 at the close linkage distances. Sub- cluster Ib have only one line 3136 which is an outlier and showed variation. Cluster II is classified into two sub-clusters i.e., IIa and IIb. Sub-cluster IIa consisted of four lines 3131, 3146, 3148 and 3147. Among these lines 3146 and 3148 at the close linkage distance. Sub- cluster IIb have six lines 3139, 3141, 3143, 3144, 3140 and 3142. Cluster III have two sub clusters named as IIIa and IIIb. Sub-cluster IIIa have eight lines 3145, 3125, 3127, 3133, 3129, 3130, 3134 and 3126. Sub-cluster IIIb have two lines i.e., 3128 and 3137 which were at the same linkage distance. Cluster IV is further classified into two sub-clusters i.e., IVa and IVb. Sub-cluster IVa have three lines 3132, 3109 and 3110. Among these lines 3110 and 3109 at the same linkage distance. Sub-cluster IVb have one line 3138 which was an outlier. Cluster V is further divided into two sub-clusters i.e., Va and Vb. Sub-cluster Va have three lines 3103, 3105 and 3120. Among these lines 3103 and 3105 were at the same linkage distance whereas 3120 is an outlier and showed more variation. Sub- cluster Vb have three lines 3114, 3117 and 3101. Among these lines 3114 and 3117 at the close linkage distance. 3101 was an outlier and showed variation (Figure 5; Table 6).

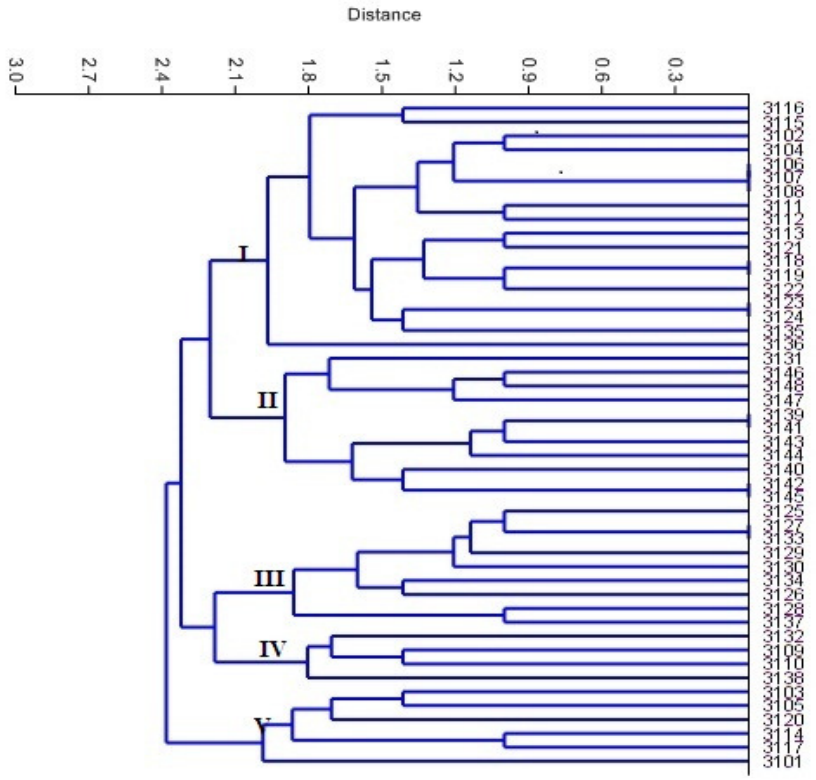

Figure 5. A Dendrogram showing average linkage distance among (50) wheat lines on the basis of SDS-PAGE

Table 6. Members of clusters based on molecular studies

Cluster no.	Members
Cluster I	3116, 3115, 3102, 3104, 3106, 3107, 3108, 3111, 3112, 3113, 3121, 3118, 3119, 3123, 3122, 3124, 3135, 3136
Cluster II	3131, 3146, 3148, 3147, 3139, 3141, 3143, 3144, 3140, 3142
Cluster III	3145, 3125, 3127, 3133, 3129, 3130, 3134, 3126, 3128, 3137
Cluster IV	3132, 3109, 3129, 3110, 3138
Cluster V	3103, 3105, 3120, 3117, 3114, 3101

Conclusions

In the breeding program, biochemical and molecular techniques are used for the detection of genetic variation in wheat cultivars. The biochemical study included antioxidant activity, phenolics and flavonoid contents, while molecular study included SDS- PAGE. The genetic variability of wheat lines was assessed for certain biochemical parameters and significant diversity was found among fifty wheat lines using graphical representation and cluster analysis. For biochemical traits over all lines i.e., 3139, 3111, 3140, 3121, 3127, 3122, 3113, 3144, 3141, 3143, 3117, 3109, 3135, 3108 and 3110 were biochemically superior. For molecular study, Cluster analysis categorized the molecular traits into five major clusters at linkage distance 2.1. On the basis of molecular study four lines, 3136, 3138, 3120 and 3110 showed more diversity among fifty lines. Selected wheat lines might be included in wheat improvement program regarding to breeder's objective. Furthermore, these lines will be helpful to breeders in constructing their breeding materials and implementing selection strategies. These lines were relatively promising in higher yields. Thus, they might be incorporated into broader agricultural practices.

Authors' Contributions

MR conducted study and prepared the manuscript; HT, SFAG, SS helped in data collection. WL supervised the study and provided funding. AR and MFS reviewed and edited the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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