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Research Article

Agro-morphological and molecular assessment of advanced wheat breeding lines for grain yield, quality and rust resistance

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Abstract

The genetic gain for yield in wheat and other cereals has been known to be stagnating in recent years. Breeding wheat cultivars with increased genetic potential can contribute to meet at least half of the desired production increases. In this context we have developed some advanced wheat lines in our breeding program, which aims to utilize the untapped variability. We assessed a set of 60 diverse lines along with four commercial check varieties for various agro-morphological traits, grain quality and yield based and also the adaptability using molecular markers. Significant genotypic differences were observed for most of the grain quality and agro-morphological traits recorded, including grain yield. We could identify lines which have significantly higher grain yield. The diversity analysis carried out using the DNA markers could explain the variability for different traits in this set. This set of genotypes is thus a very useful source as far as enhancing the yield levels. The lines developed through the conventional breeding program were based on crosses between elite breeding lines and specific donors and hence might resulted in higher yield potential.

Keywords: Wheat, advance breeding lines, cluster analysis, grain quality, stripe rust

1. Introduction

Wheat (*Triticum* spp) is a significant stable crop with worldwide production over 600 million tones globally per year (Asseng *et al.,* 2011). As per research report of 2017-18, the total world harvest of wheat was about 760.3 million tons (http://faostat.fao.org/). Wheat is an ancient food crop of India as revealed from archeological evidences. Today, India is the second largest producer of wheat in the world and it constitutes the major staple food after rice of the Indian people. During 2018-19, India produced a record breaking 101.20 million tonnes of wheat thus maintaining its position as the second largest producer of wheat in the World (Anonymous, 2019). This national production is reflected in increased yields per hectare that went from around 0.6 t/ha in 1950 to 3.5 t/ha in 2018-19. Although increased wheat area played its role in increased wheat production in the initial years of the Green Revolution it appears to have stabilized in recent times. The increased yield levels are mainly attributed to the improved semi dwarf wheats, which were released from the direct introductions from CIMMYT, crosses of exotic and local cultivars, spring and winter cross derivatives and the synthetic derivatives (Halford, 2006; Pingali, 2012).

The genetic gain for yield in wheat and other cereals has been known to be stagnating in recent years (Graybosch and Peterson, 2010; Fischer and Edmeades, 2010; Brisson *et al.,* 2010; Grassini *et al.,* 2013). Also various biotic and abiotic stresses are threatening to bring down the yield levels. The recently released wheat varieties are prone to the attack of new aggressive strains/races of yellow rust especially in Northern India. Wheat rusts have always posed a serious concern to wheat crop in this region. Unless tackled effectively, the threat of emerging new races of rusts could result in severe yield losses threatening national food security (Chaves *et al.,* 2013). On the abiotic stress front terminal as well as early heat stress has been a concern in recent years and the wheat scenario in the country is aggravated due to higher temperature regime during crop growth period especially at grain filling. Wheat grain quality remains an area of concern as no specific breeding efforts are carried out to improve end use quality.

Breeding wheat cultivars with increased genetic potential can contribute to meet at least half of the desired production increases. Non conventional sources can be used in pre-breeding programs for broadening the gene pool for different yield component traits. Development of synthetics and utilization of winter wheats can pave way for wheat genotypes having high yielding ability. Hybrid wheat can also be assessed as an option for breeding for high yield potential. In this context we have developed some advanced wheat lines in our breeding program, which aims to utilize the untapped variability available in the germplasm and also through international collaborations. The genotypes developed after hybridization with diverse sources for yield attributing traits were subjected to rigorous screening against wheat rusts particularly stripe and leaf at various stages of development. In this study we evaluated these lines along with commercially grown wheat varieties, for yield, disease resistance and grain quality. Also we tried to assess the diversity of these lines based on some known molecular markers which were known to be associated with stripe rust resistance, photoperiod sensitivity and vernalization response.

2. Materials and Methods

Plant Materials: A set of 60 advanced wheat breeding lines and four check varieties namely, DBW90, HD2967, HD3086 and WH1105 were used. Experiment was conducted during 2016-17 in main cropping season at Indian Institute of Wheat and Barley Research, Karnal, India (29.68° N, 76.99° E).The experiment was laid out in 8x8 lattices with a plot of 6rows of 6m length and 20 cm apart. The genotypes were evaluated for agronomic traits such as, heading days (HD), maturity days (DM), plant height (PH), number of tillers per meter, grain per spike, biomass, harvest index, 1000 grains weight and grain yield per plot.

2.1 Evaluation for stripe and leaf rusts: For adult plant resistance evaluation, stripe and leaf rusts were artificially created on the set of genotypes using a mixture of most prevalent races of both stripe (78S84, 46S119, 111S68) and leaf (77-5, 77-9, 104-3) rusts. The artificial epiphytotics were created using standard procedures on two different sets. The rust severity was recorded following the modified Cobb scale (Peterson et al. 1948). The diseases severity was assessed as resistant (free or 0 value), moderately resistant (upto10S), moderately susceptible (upto 40S) and susceptible (more than 40S). Seedling resistance test was carried out for stripe rust using two most virulent and prominent pathotypes of *Puccinia striiformis f sp tritici* (46S1149 and 110S119). Infection types were recorded two weeks after inoculation (Stakman *et al.,* 1962) with some modifications.

2.2 Assessment of grain quality: The quality parameters measured on the harvested seeds of each genotype were: Grain appearance score, Hectolitre weight / test weight (Mishra, 1998), Sedimentation value (Axford *et al.,*1979), Phenol score, Protein and Moisture content (using whole grain analyzer Infratec 1241supplied by M/S Foss Analytical AB, Sweden). Micronutrient content for Iron (Fe) and Zinc (Zn) was estimated using, an X-ray inflorescence machine (XRF) Oxford Instruments X-Supreme 8000 (Paltridge *et al.,* 2012).

2.3 Molecular marker analysis: For molecular marker analysis, fresh leaf material (100mg) of each genotype was cut and collected in 1.5ml eppendorf tube, to extract genomic DNA according to Riede and Anderson (1996). Finally the DNA was dissolved in 150μl of 1XTE buffer and was incubated at room temperature for overnight. A total of 23 DNA based markers (*URIC/LN2-F, xbarc8, STS-7/8, xgwm389, xgwm413, xbarc349, sun106, ppdB1a.2, vrnB1a, ZCCT-1, ppdB1a.1(b), ppdB1a(b), ppdA1a.3, ppdA1a.1/2, ppdA1null, vrnA1(a), vrnA1(b), vrnB3, ppdB1a(a), ppdD1b.3, ppdD1null, ppdD1a, ppdD1a.1*) already known to be associated with stripe rust resistance, photoperiod response and vernalization requirement were used to study the overall diversity present in this set of genotypes for these traits.

Polymerase chain reaction (PCR) was performed using a Thermal Cycler Life Eco (Bioer) PCR system. PCR products were separated by 2% agarose gel and were stained with Ethidium bromide.

2.4 Data analysis: Analysis of variance (ANOVA) was conducted to evaluate the variance and significance among genotypes for different traits. The grand mean, standard of error, and coefficient of variation were calculated as indicators of trait variability. The clustering analysis was performed with DARwin software ver 5.0 (Perrier and Jacquemoud-Collet, 2006). The allelic classes were used to calculate pairwise genetic distances with Nei's genetic distance matrix.

Table 1a: Analysis of variance (ANOVA) for the test lines and check varieties on various agro-morphological and yield traits

Source of variation	DF	F-Value								
		Heading Days	Maturity Days	Height (cm)	Tiller/m	Grain/Spike	1000 gr. Wt.	Biomass (g/plot)	Harvest index	Yield (g/plot)
Replications	1	8.65E-01	2.33E-01	1.31E-01	7.33E+00	3.20E+00	4.59E+00	8.36E+00	8.51E-01	2.16E+00
Treatments	63	4.29E+00	5.03E+00	3.63E+00	1.77E+00	1.75E+00	2.20E+00	1.51E+00	2.20E+00	2.41E+00
Error	63	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00

Table 1b: Analysis of variance (ANOVA) for the test lines and check varieties on various grain quality traits

Source of variation	DF	F-Value							
		Grain Appearance Score	Fe (ppm)	Zn (ppm)	Protein (%)	Moisture (%)	Test weight (Kg/L)	Sedimentation value (cc)	Phenol score
Replications	1	4.12E+00	6.51E+00	1.38E+00	3.63E+01	4.14E+01	2.49E+00	4.72E+00	3.31E+01
Treatments	63	2.38E+00	1.45E+00	1.24E+00	1.58E+00	1.84E+00	3.72E+00	8.40E+00	7.10E+00
Error	63	1.00E+01	1.00E+01	1.00E+01	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00

3. Results and Discussion

Analysis of variance showed that significant genotypic differences were observed for most of the grain quality and agro-morphological traits recorded, including grain yield (Table 1a & b). Many genotypes were either comparable to the check varieties or over performing for all the traits studied. Some of the genotypes evaluated in this study showed significantly superior plot yields than the check varieties, at 5% level of significance.

3.1. Evaluation for agro-morphological traits including grain yield: The mean performance of the genotypes and checks for different traits (table 2) is discussed below:

3.1.1. Days to heading (HD): The heading days ranged from 79 (G14) to 102 (G26, G36) days with an average of 93 days in the test lines. Among the checks, HD 3086 flowered in 89 days whereas HD 2967 took 95 days. Among the genotypes tested, the lines flowered early are G14 (79 days), G16 (85 days) and G18 (85 days). As many as 25 genotypes flowered earlier than the average days to flowering observed in the set.

3.1.2. Days to maturity (DM): The number of days required for the genotypes to attain maturity ranged from 130 (G15) to 143 (G11, G12, G36, G42) days with an average maturity of 136 days. More over among the checks, HD 3086 matured in 129.5 days whereas HD 2967 matured in 135.5 days. Hence we were able to identify genotypes with a maturity period more than the checks and thus these may get more degree days which may lead to enhanced yield.

3.1.3. Plant height (PH): The plant height in the set of genotypes ranged from 83cm (G40) to 113cm (G25) with an average height of 99cm. Among the checks the height was 96 cm in HD 3086 and 104cm in HD 2967. The preference of wheat genotypes with more height among the farmers is due to more biomass and straw yield requirements. However taller genotypes tend to lodge and hence a balance of height is required in the genotypes to be successful in commercial cultivation. 27 genotypes had plant height below the average value of the trial.

3.1.4. Number of tillers per meter: The number of productive tillers is one of the post important traits that determine the overall yield of wheat plant. The tiller number per meter in the set of genotypes ranged from 64 (G38) to 130 (G31) with a mean of 96. Among the checks, WH 1105 had highest tillers per meter (119) and DBW 90 had a value of 79.

3.1.5. Number of grains per spike: The number of grains per spike is another important trait that contributes to the overall yield. Among the test genotypes, the range of grain number per spike was observed from 46 grains (G14, G56) to 79 grains (G34) with an average of 58 grains per spike. Among checks, the number of grains per spike ranged from 46.5 in HD2967 to 66 in WH1105.

3.1.6. 1000 grains weight: The low estimate of 1000 grains weight was recorded as 30g (G48) and the maximum of 46g (G4) among the set of genotypes. HD 3086 had a mean 1000 grains weight of 43g and HD 2967 recorded a value of 39g. 32 genotypes had 1000 grains weight above average.

Table 2: Mean performance of the test genotypes and check varieties for different grain quality and agro-morphological traits

SN	Genotype	HD	DM	PH (cm)	Tiller /m	GPS	1000 gw (g)	BM (kg)	HI	Yield (kg)	GAS	Fe (ppm)	Zn (ppm)	Protein (%)	Moisture (%)	TW (kg/l)	SV (cc)	PS
1	G1	97	138	101	114	51	40	6.3	0.302	1.9	3.0	41.5	33.1	10.3	9.2	79.1	63.0	2.5
2	G2	94	133	98	115	52	38	5.1	0.316	1.6	2.0	42.2	31.6	9.0	8.7	78.1	59.0	2.3
3	G3	91	135	92	125	57	39	5.4	0.302	1.6	2.5	45.2	35.3	10.1	9.0	81.2	60.5	3.7
4	G4	95	132	101	91	59	46	5.7	0.331	1.8	3.0	46.0	33.1	9.5	9.0	81.0	61.5	3.5
5	G5	98	138	102	119	62	38	6.3	0.309	1.9	3.0	46.4	34.2	9.3	8.7	79.8	67.5	3.3
6	G6	97	139	98	94	52	35	4.9	0.254	1.2	2.0	39.6	36.8	10.3	9.2	72.6	45.0	3.4
7	G7	95	135	103	116	50	40	6.0	0.306	1.8	3.0	44.4	33.4	10.1	9.1	80.3	41.5	3.5
8	G8	94	134	105	110	59	42	6.4	0.315	2.0	3.0	42.3	29.0	8.9	8.7	82.6	53.0	3.4
9	G9	99	138	100	95	57	38	5.7	0.289	1.6	2.5	38.8	30.9	9.5	9.4	79.9	47.0	3.4
10	G10	91	137	105	81	67	37	5.8	0.296	1.7	2.0	42.2	34.0	9.3	8.8	79.5	55.5	3.8
11	G11	98	143	100	69	51	39	5.6	0.269	1.5	2.5	40.7	33.6	9.9	9.4	81.7	54.5	3.5
12	G12	95	143	97	101	53	34	6.9	0.235	1.6	2.0	42.0	33.2	9.8	9.8	78.6	36.0	3.0
13	G13	88	137	107	109	55	36	6.1	0.266	1.6	2.5	43.0	30.5	10.0	8.7	82.5	38.0	3.3
14	G14	79	137	102	86	46	37	6.1	0.306	1.8	3.0	42.2	31.9	9.5	9.2	80.9	49.0	3.5
15	G15	89	130	99	95	59	43	5.7	0.331	1.8	4.0	45.1	34.1	9.5	9.3	82.9	39.0	3.6
16	G16	85	132	99	83	70	44	5.5	0.350	1.9	3.0	46.1	34.6	10.2	9.2	81.2	48.0	3.5
17	G17	89	138	96	83	60	40	5.1	0.299	1.5	3.5	41.4	33.3	10.1	9.5	78.4	65.0	3.5
18	G18	85	133	97	101	71	40	5.0	0.361	1.7	3.5	40.9	32.2	10.3	9.3	80.9	67.0	3.0
19	G19	95	136	100	111	54	41	6.0	0.338	2.0	3.0	44.9	37.0	9.8	8.8	79.9	40.5	3.8
20	G20	92	134	101	92	65	41	5.7	0.296	1.6	3.5	47.6	31.2	8.1	9.0	81.2	43.0	3.3
21	G21	88	131	98	110	60	45	5.6	0.388	2.1	4.0	46.6	31.0	8.8	9.1	81.7	65.0	3.4
22	G22	89	134	93	79	65	43	5.2	0.337	1.7	2.0	45.0	33.5	9.1	9.4	82.8	53.0	3.5
23	G23	94	137	104	78	59	38	5.8	0.289	1.6	3.0	44.9	33.7	9.6	9.4	83.4	57.0	4.1
24	G24	97	136	106	83	62	40	6.0	0.293	1.7	2.5	47.4	31.2	8.8	9.1	79.1	58.0	2.5
25	G25	99	136	113	103	62	38	5.4	0.251	1.3	3.0	46.5	36.1	10.5	9.0	79.0	46.5	2.8
26	G26	102	143	101	108	61	38	6.5	0.212	1.3	2.0	45.0	37.0	9.8	10.4	78.8	51.0	2.0
27	G27	95	137	108	106	49	41	5.8	0.277	1.6	3.0	48.6	28.4	10.1	9.8	78.3	64.5	4.0
28	G28	91	135	96	92	57	42	6.3	0.334	2.1	3.0	44.0	31.0	9.7	8.8	80.3	42.5	3.5
29	G29	93	136	98	100	72	39	6.4	0.291	1.8	3.0	49.4	34.4	10.3	8.8	81.4	50.0	3.3
30	G30	94	133	108	94	64	34	6.3	0.257	1.6	2.0	41.8	32.7	10.6	8.9	78.1	46.0	3.3
31	G31	92	136	107	130	47	40	5.7	0.294	1.5	3.5	45.7	32.8	12.5	9.2	79.9	65.5	2.5
32	G32	88	135	99	114	57	44	5.8	0.354	2.0	4.0	49.5	31.1	10.9	9.1	79.2	59.0	3.1
33	G33	94	135	85	86	68	35	4.7	0.327	1.5	2.5	42.2	29.2	9.8	8.9	78.0	52.0	3.1

34	G34	95	133	107	86	79	40	5.7	0.325	1.8	3.0	52.1	40.9	10.0	9.0	81.5	63.5	2.8
35	G35	92	136	99	86	58	45	5.0	0.411	2.0	3.0	48.0	30.6	8.7	8.9	80.0	47.0	3.2
36	G36	102	143	107	86	61	35	4.9	0.316	1.5	3.0	45.0	35.2	10.3	9.5	79.5	51.5	3.6
37	G37	93	137	100	90	60	38	5.8	0.318	1.8	2.0	42.9	30.7	8.9	8.9	79.8	48.0	3.5
38	G38	94	135	99	64	58	40	5.2	0.306	1.6	3.5	43.4	28.9	8.9	9.2	81.7	46.5	3.4
39	G39	98	140	101	81	76	38	5.1	0.299	1.5	2.5	44.1	33.1	9.9	9.1	78.8	54.5	3.4
40	G40	100	138	83	96	57	37	4.5	0.378	1.7	2.5	39.6	32.0	8.8	8.4	79.4	44.5	3.2
41	G41	91	133	96	85	57	40	5.2	0.344	1.8	2.5	42.5	30.1	9.3	9.1	80.6	70.0	3.8
42	G42	95	143	89	95	54	36	5.5	0.274	1.5	2.0	41.5	31.0	8.8	9.1	80.6	53.5	3.2
43	G43	91	134	98	86	55	43	5.4	0.373	2.0	2.5	42.8	32.6	9.3	9.0	80.1	39.0	2.6
44	G44	98	137	94	90	59	38	5.2	0.340	1.7	2.5	44.6	37.1	10.3	9.5	79.6	67.0	3.5
45	G45	98	137	101	104	61	41	5.6	0.325	1.8	4.0	46.1	28.0	10.4	9.2	80.7	47.0	3.4
46	G46	93	132	100	98	65	37	5.7	0.332	1.8	2.5	42.5	30.7	8.8	8.9	80.3	61.0	3.7
47	G47	95	131	101	94	64	40	5.9	0.284	1.6	2.5	52.4	33.1	9.6	8.9	77.5	59.5	3.3
48	G48	90	141	88	124	55	30	5.7	0.288	1.6	2.0	45.0	32.1	10.7	8.9	82.6	49.0	2.2
49	G49	95	141	89	108	55	35	5.6	0.271	1.5	2.0	45.2	32.6	10.8	8.9	79.3	52.5	2.7
50	G50	93	134	104	117	55	35	5.9	0.301	1.7	2.5	43.7	31.5	10.4	9.1	79.1	64.0	2.8
51	G51	98	135	97	84	57	37	4.6	0.230	1.0	2.0	38.0	33.1	10.4	9.2	73.4	44.0	2.4
52	G52	91	135	104	89	65	31	4.9	0.235	1.1	1.5	41.6	35.4	10.3	8.9	74.3	49.0	2.0
53	G53	100	141	104	82	54	42	5.5	0.309	1.7	3.0	47.3	33.4	9.7	9.2	79.1	59.5	2.1
54	G54	93	136	101	80	53	41	5.8	0.324	1.8	2.5	41.9	31.6	9.2	8.9	81.5	63.0	3.5
55	G55	98	137	99	83	70	34	5.7	0.287	1.6	2.0	44.8	33.6	9.7	8.9	78.5	60.0	2.8
56	G56	94	133	100	98	46	41	5.8	0.332	1.9	3.0	41.9	29.7	8.7	8.8	80.4	39.5	3.4
57	G57	94	135	91	99	55	39	5.9	0.292	1.7	3.0	43.8	29.9	9.2	9.2	81.2	59.0	3.0
58	G58	94	136	95	92	57	33	5.8	0.305	1.7	3.0	47.0	30.2	9.1	8.9	78.8	50.0	3.3
59	G59	96	138	102	91	50	40	6.0	0.333	2.0	2.0	45.1	31.9	10.2	8.9	82.1	36.0	3.1
60	G60	93	135	107	103	67	34	6.3	0.248	1.5	2.0	46.7	30.7	10.6	9.0	78.4	66.0	2.9
61	DBW90	91	131	101	119	48	40	5.7	0.323	1.8	3.5	52.8	33.7	10.1	8.8	81.2	56.0	3.5
62	HD2967	95	136	104	105	47	39	5.4	0.301	1.6	3.5	44.9	32.5	10.0	9.0	79.5	62.0	3.5
63	HD3086	89	130	96	104	50	43	5.8	0.350	2.0	4.0	46.2	28.6	9.4	9.0	81.3	58.0	3.4
64	WH1105	89	132	97	79	66	40	5.5	0.374	2.0	3.0	46.4	35.0	9.9	9.0	81.1	63.0	3.5
Grand mean		93.4	135.7	99.3	96.2	58.3	38.6	5.6	0.308	1.7	2.8	44.5	32.6	9.7	9.1	79.9	53.6	3.2
CV (5%)		3.0	1.5	4.2	15.6	13.3	8.2	0.9	0.121	1.1	20.5	8.1	9.5	8.3	3.5	1.9	8.3	16.4
SE		2.8	2.1	4.1	15.0	7.8	3.2	0.5	0.037	0.2	0.6	3.6	3.1	0.8	0.3	1.5	4.4	0.5

GAS = Grain Appearance Score, TW = Test Weight, SV = Sedimentation Value, PS = Phenol Score

3.1.7. Biomass per plot: The range of the biomass observed in the set of genotypes was 4.5 kg (G40) to 6.9 kg (G12) whereas the mean biomass was recorded to be 5.6 kg per plot. Out of 4 checks, maximum biomass was recorded in HD 3086 (5.8 kg) whereas the minimum (5.4 kg) in HD 2967. The low biomass observed in the mega cultivar HD 2967 was due to high incidence of stripe rust.

3.1.8. Grain yield per plot: The values of grain yield per plot ranged from 1.0 kg (G51) to 2.1 kg (G21, G28) in the set of genotypes. The average grain yield per plot was observed to be 1.7 kg. Further among the checks, maximum yield (2.0 kg) was reported in WH 1105, whereas HD 2967 had the minimum (1.6 kg) grain yield. Several genotypes (G1, G4, G5, G7, G8, G14, G15, G16, G18, G19, G21, G22, G24, G28, G29, G32, G34, G35, G37, G41, G43, G44, G45, G46, G50, G54, G56, G58 and G59) had grain yield per plot above average.

3.1.9. Harvest index: Harvest index defines the efficiency by which a genotype is able to convert its biomass into the economic grain yield. A maximum harvest index of 0.41 (G35) and a minimum of 0.21 (G26) was observed in the set of genotypes. The average harvest index of the trial was 0.31. Among the checks, WH 1105 (0.37) and HD 2967 (0.30) had the highest and lowest values for harvest index. 27 genotypes had harvest index above average.

Based on the morphological data, the elite lines such as G11, G12, G14, G18, G21, G24, G34, G35, G46, G58 recorded values in desirable direction for early heading, early maturity, high yield, harvest index, 1000 grain weight etc. These genotypes may be used as parents in breeding program. Agro-morphological traits such as days to heading, plant height, number of grains per spike and 1000 kernel weight are known to have a significant and positive relationship with grain yield (Tahmasebi *et al.,* 2013; Eivazi *et al.,* 2017). Information on diversity and relationship among the agro-morphological traits will be helpful to breeders in constructing their breeding populations or lines and implementing selection strategies.

3.2. Evaluation for grain quality traits: The mean performance of the genotypes and checks for different traits (table 2) is discussed below:

3.2.1. Grain appearance score (GAS): GAS defines overall quality of the wheat grain. Grains amber in colour with a shining luster are preferred and hence given a higher score on a scale of 1 to 5. In the set of genotypes the score ranged from 1.5 (G52) to 4 (G15, G21, G32, G45) with a mean of 3. Among checks HD 3086 had a good grain appearance score of 4.0.

3.2.2. Protein and moisture content: The mean value of protein content for genotypes ranged from 8.1 % (G20) to 12.5 % (G31) with a trial average of 10.0 %. Among checks, DBW 90 had the highest protein content of 10.12 %. The moisture content ranged from 8.4 % (G40) to 10.4 % (G26) in the genotypes. Maximum moisture content of 9.04 % was reported in check variety HD 2967. Many genotypes had high protein content of more than 10.0 %.

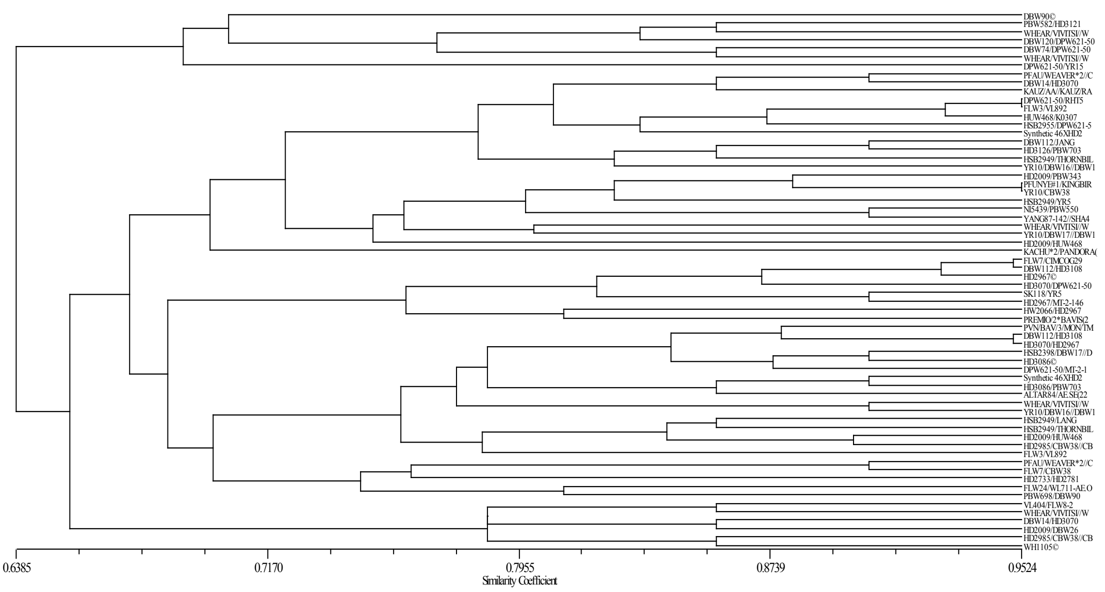

Fig 1: The graphical representations of the dissimilarity among studied 64 genotypes based on neighbor-joining method was generated by DARWIN 5 software.

Table 3: Genotypes and their pedigree details in each cluster as derived through DNA marker analysis

Clusters	Genotype Number	Pedigree details
Group A		
Cluster A1	G16	PFUNY#1/KINGBIRD #1
	G23	HD3070/DPW621-50
	G56	WHEAR/VIVITSI//WHEAR/DBW17//DBW16
Cluster A2	G17	KACHU*2/PANDORA
	G25	Synthetic46/HD2932
	G26	PFAU/WEAVER*2//CHAPIO/DBW146
	G28	WHEAR/VIVITSI//WHEAR/DBW17//DBW17
	G29	YR10/DBW16//DBW16
	G30	FLW24/WL711-AE.OVATA/CS PHL 3*WL711
	G31	HD2733/HD2781
	G35	PREMIO/2*BAVIS
	G36	YR10/DBW17//DBW17
	G37	HD2009/HUW468
	G40	HSB2398/DBW17//DBW17
	G41	DPW621-50/YR15
	G42	HSB2949/THORNBILL
	G43	WHEAR/VIVITSI//WHEAR/DBW17//DBW17
	G45	DBW112/HD3108
	G50	HD2985/CBW38//CBW38
	G51	FLW3/VL892
	G52	Synthetic-46/HD2932
	G54	NI5439/PBW550
	G55	VL404/FLW8-2
	G59	YANG 87-142//SHA4/CHIL/3/TNMU/DBW16
	G60	HD2985/CBW38//CBW38
	DBW90	Check
	HD3086	Check
Group B		
Cluster B1	G10	HD3126/PBW703
	G21	HD2967/PBW703
	G24	HW2066/HD2967
	G33	HD3086/PBW703
	G39	DBW120/DPW621-50
	G49	HSB2949/THORNBILL
	G53	YR10/CBW38
	G58	HD2009/DBW26
Cluster B2	G2	KAUZ/AA//KAUZ/RAJ3765
	G3	DBW14/HD3070
	G4	DPW621-50/Rht5
	G5	HD2009/PBW343
	G6	FLW3/VL892
	G7	HUW468/K0307
	G8	HSB2955/DPW621-50
	G14	YR10/DBW16//DBW16
	G15	WHEAR/VIVITSI//WHEAR/DBW17//DBW17
	G20	DBW74/DPW621-50
	G38	DBW112/HD3108
	G44	DPW621-50/PBW702
	G46	HD2009/HUW468
	G47	HD3070/HD2967
	WH1105	Check
Group C		
Cluster C1	G1	PFAU/WEAVER*2//CHAPIO/HD2009
	G12	SK118/YR5
	G13	HSB2949/YR5
Cluster C2	G9	DBW112/JANG
	G11	FLW7/CIMCOG29
	G18	PBW582/HD3121
	G19	WHEAR/VIVITSI//WHEAR/DBW17//DBW16
	G22	PVN/BAV/3/MON/TMU/ALD/PVN/4/VEE#5/SARA/DBW16//DBBW16
	G27	FLW7/CBW38
	G34	ALTAR84/AE.SE(224)//2*YACO/3/MILAN/CBW38//CBW38
	G48	HSB2949/LANG
	G57	DBW14/HD3070
	HD2967	Check
Group D		
Cluster D1	G32	PBW698/DBW90

3.2.3. Test weight (hectoliter weight): The range of the hectoliter weight recorded in the genotypes was 72.6kg/hl (G6) to 83.4kg/hl (G23). The mean hectoliter weight was 80kg/hl. Among checks, maximum hectoliter was recorded in genotype HD 3086 (81.3kg/hl).

3.2.4. Sedimentation value: The sedimentation value ranged from 36cc (G12, G59) to 70cc (G41) in the genotypes with an average of 54cc. The maximum sedimentation value of 63cc was observed in the check variety WH1105.

3.2.5. Phenol score: Phenol score gives an overall estimate of the grain quality and its suitability for specific end product. The phenol score ranged from 2.0 (G26, G52) to 4.1 (G23) in the set of genotypes with an average value of 3.0. Among check varieties HD 3086 had a minimum phenol score of 3.4.

3.2.6. Micronutrient content: The micronutrient content of the wheat grain defines industrial quality of the produce and is also important as far as nutritional quality of wheat is considered. Grain Iron (Fe) and Zinc (Zn) contents of the genotypes were estimated to determine the nutritional quality. The mean Iron content observed in the set was 44.0 ppm with a range of 38.0 ppm (G51) to 52.4 ppm (G47) in the set of genotypes. Among checks DBW 90 had a high value of 52.8 ppm Iron content. Zinc content in the set was 33.0 ppm with a range of 28.0 ppm (G45) to 40.9 ppm (G34). Among the checks, a maximum Zn content of 34.95 ppm was reported WH 1105. Wide variation for iron and Zinc has been reported by several authors in different studies (Graham *et al.,* 1999; Morgounov*et al.,* 2007; Rawat *et al.,* 2008), but the availability of micronutrients in modern day varieties is partial.

3.3. Evaluation for wheat stripe and leaf rusts: The set of genotypes and the check varieties were evaluated for resistance to stripe and leaf rusts at both seedling and adult plant stages to determine the level of resistance (data not given).

3.3.1. Adult plant resistance (APR): For evaluation of APR against stripe and leaf rusts, artificially created epiphytotics were created separately at Karnal using a mixture of most prevalent and virulent races of the pathogen. Based on this screening it was observed that, for stripe rust, most of the advanced lines and the check varieties were resistant. Among the genotypes, nine lines (G2, G6, G10, G17, G22, G33, G44, G51 & G52) and the variety HD 2967 had a reaction value of more than 40S and hence were susceptible. Whereas, for leaf rust only one genotype (G15), was susceptible with a high score of more than 40S.

3.3.2. Race specific seedling resistance test (SRT): SRT was carried out in the set for stripe rust with two most virulent and prevalent pathotypes, 46S119 and 110S119. In case of 46S119 trait, all the genotypes including checks showed resistance reaction. Only one line (G20) has found to be highly susceptible. Whereas for the most virulent pathotype 110S119, seventeen lines (G1, G6, G9, G14, G15, G17, G19, G22, G24, G32, G33, G38, G39, G45, G51, G53 & G59) and the check variety HD 2967 were found to be highly susceptible.

3.4. Diversity analysis using molecular markers: To assess the diversity of these lines based on some known molecular markers which were known to be associated with stripe rust resistance, photoperiod sensitivity and vernalization response. The dendrogram generated (Fig 1) clearly indicates the clustering pattern of genotypes along with the checks. Based on DNA marker based diversity, the set was divided in to four major groups. First group included 27 genotypes, second group had 23 genotypes, third group had 13 genotypes and there was only one genotype in fourth group. The first three groups further had two major clusters each. Table 3 shows the list of genotypes in each cluster along with their pedigree details. The check varieties classified themselves into three different groups, with DBW 90 and HD 3086 being in one group. The genotype with G32 did not classify into any major group and thus was only constituent of the fourth group.

Evaluation of a set of advance wheat breeding lines along with the commercially grown wheat varieties for different agro-morphological and grain quality traits has led to identifications of lines which do not only have significantly higher grain yield but are also superior in various traits studied. The diversity analysis carried out using the DNA markers could explain the variability for different traits in this set. This set of genotypes is thus a very useful source as far as enhancing the yield levels. The lines developed through the conventional breeding program were based on crosses between elite breeding lines and specific donors and hence may have resulted in higher yield potential.

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