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RESEARCH ARTICLE

Quality evaluation of near isogenic lines of wheat developed through marker assisted backcross breeding for grain softness

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Abstract

A set of 16 Near Isogenic Lines (NILs) developed from a cross, Barham x DBW14 for grain softness gene (*Pina-D1aPinbD1a*) combined with *Lr37-Yr17-Sr38* segment was evaluated for different quality traits. Rheological test of flour and baking test with sugar-snap cookies were also performed. The Hardness Index of the NILs ranged between 13.86 and 47.13. The flour of the NILs had relatively lower proportion of particles >150 µm than in the classes of 0 to ≤ 55 µm and > 55 to ≤ 105 µm. Starch damage of all the NILs was significantly lower than the hard grain parent DBW14 (4.54%) and the popular variety HD2967 (7.49%). All the 16 NILs showed optimum Solvent Retention Capacity of NaSRC ≤ 64% and SucroseSRC ≤ 89%. The farinograms of all the NILs were typical of weak gluten with low water absorption capacity. The alveographs showed an Extensibility ratio (P/L) between 0.17 and 0.36, and much lower Deformation Energy (46-94 J) for the NILs. The spread factor of the cookies made with the flour of the NILs varied between 7.90 and 12.77, while that ofthe soft grain parent was 9.44. The findings suggest that the NILs developed in the Indian wheat variety DBW14 may be suitable for production of flour for making cakes, cookies, muffins, etc. These NILs, after evaluation in varietal trials and release for cultivation, can have a strong domestic and export potential. Thus, marker assisted selection is a useful tool for the targeted quality breeding.

Keywords: Near isogenic lines, MAS, soft wheat, quality breeding, quality traits

Introduction

Wheat is grown in nearly every region of the world due to its high adaptability over a range of agro-climatic conditions. Wheat represents an important source of food and income for millions of farmers. It is, therefore, the most traded commodity in the world. Grain endosperm texture and the content and quality of protein are among the most important parameters that determine the price of premium wheat in international trade. Additionally, each end-use requires a specific quality of protein. Protein content in cultivated wheat generally ranges from 8 to 16%. Nearly 80% of the total grain protein is present in the endosperm as storage protein. The unique ability of polypeptides in endosperm to form a viscoelastic network (called gluten) allows for the production of a large number of end-products. Hard wheat with relatively higher protein and strong gluten is most suitable for bread, pizza, and similar products. In contrast, soft wheat with lower protein and weak gluten is preferred for biscuits, cookies, cakes, etc. (Bushuk 1998). There is relatively less information on the critical parameters for flatbreads production; generally, hard wheat with moderate protein content and weak gluten is considered optimum (Rasheed et al. 2014). In many countries, a newly developed cultivar needs to have approved quality characteristics before it is registered as a commercial cultivar (Bushuk 1998). However, the case has been different in India. While it has made great strides in wheat production becoming the second largest producer

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of wheat in the world, any improvement in end-use quality of wheat in the country has been neglected. India is now self-sufficient and has comfortable buffer stocks of nearly all food grains.

There has been a rapid growth in the food industry in India. Biscuit is a major product accounting for 80% of the total bakery products in India and is undoubtedly the largest among all other food industries (https://www.magazinebbm.com/english/world-biscuit-market-and-trends/). There is a large variety of biscuits ranging from glucose biscuits, high sugar and high fat cookies, wafers, crackers etc. produced in the Indian bakeries. India is, today, the second-largest producer of biscuits in the world next to USA. The international prices of soft wheat have been rising over the last few years having almost doubled in a short period primarily due to reduced availability of such wheat (https://apps.fas.usda.gov/psdonline/circulars/grain.pdf, Retrieved: 10/03/2021). Soft wheat varieties are not yet cultivated in India. Therefore, it is important to explore development of soft wheat considering its demand and supply scenario both domestically and internationally.

Grain texture is under genetic control and more than 70% of the variation in grain hardness results due to the action of two extremely closely linked genes *PinaD1* and *PinbD1* that code for puroindoline polypeptides called as PINA and PINB proteins, respectively (Morris et al. 2019). The pair of these genes was transferred from Australian soft wheat variety Barham into Indian hard wheat variety DBW14, which possesses weak and extensible gluten (Rai et al. 2019a). A number of near isogenic lines (NILs) with *PinaD1aPinbD1a* were produced and evaluated for their grain hardness and yield (Kumar et al. 2021). Detailed quality analysis of these NILs has been carried out in the present study to judge their suitability and selection of best lines for end-products.

Materials and methods

Materials

Sixteen NILs developed from the cross, DBW14 x Barham through marker-assisted backcross breeding carrying *PinaD1a* and rust resistance linked genes, *Lr37-Yr18-Sr38* were used in this experiment. The donor Barham, is a soft wheat variety carries wild type alleles of both the genes *PinaD1* and *PinbD1* (*PinaD1aPinbD1a).* The Indian wheat variety DBW14 possesses hard grains and has null allele of *PinaD1* and wild type allele of *PinbD1* (*PinaD1bPinbD1a).* This variety does not have rust resistance gene *Lr37* and was used as the recurrent parent. A popular variety of India HD2967 which is hard grained and possesses the HMW-GS subunit 5+10 was also evaluated for its quality traits along with the NILs and their parents.

Analysis of quality traits

To standardize the moisture contents of the grains, the flour samples of NILs and parents (5g) were weighed and placed in a ceramic cup and placed in a hot air oven and heated at 130°C for one hour. The samples were cooled to room temperature and the residue was weighed (AACC method 44-15A, 2000).

A total of twelve quality traits covering the analysis of quality traits, functional characteristics, rheological tests and baking evaluation were studied subsequently.

Grain Hardness Index (GHI) of NILs and parents was recorded as per Kumar et al. (2021); Flour protein content (FPC) of NILs and parents was analysed using a Diode Array NIR Analysis System 7200 (Perten Instruments, Sweden). Protein data were recorded at 14% moisture basis (Mb); Particle Size Distribution of the NILs and parents was determined by using S3500 Tubotrac Particle size analyser (Microtrac Inc. USA).

The tempered grain was milled in the Quadrumat Senior Mill (Brabender, Germany) following AACC method 26-31 (2000). Milling fractions, such as flour, bran and fine bran were weighed, Extraction rate (ER) or flour recovery was calculated as per the following formula.

ER % = (weight of the Flour ÷ weight of the flour + weight of the bran fractions) X 100

SDS-Sedimentation test of flour was conducted using sodium dodecyl sulphate (SDS) sedimentation volume of flour samples was estimated according to the method of Axford (1979) and cited in Preston et al. (1982). Gluten parameters in terms of Glutomatic System (Perten, Sweden) and AACC method 38-12A (2000) were used to determine gluten parameters in the flour samples. Solvent Retention Capacity (SRC) such as the tests water SRC%, sucrose SRC%, lactic acid SRC% and Na_2CO_3 SRC% were conducted as per AACC approved method 56-11 (AACC, International 2000) and as described by Katyal et al. (2019).

Starch Damage (SD) analysis of the NILs and parents was carried using the MegaZyme Kit as per the instructions of the supplier (Megazyme, Ireland). This kit was validated by Gibson et al. (1992) and is accepted as AACC method 76-30A (2000). The starch pasting properties of the NILs and parent flours were evaluated with Rapid Visco-analyser (RVA-4, Newport Scientific, Australia) using standard profile I.

Dough mixing properties were analysed using Farinograph (BrabenderOHG, Germany) according to AACC method 54-21 (2000). The alveograph parameters were determined on Alveoconsistograph (Chopin Inc, France) using the AACC method 54-30 (2000), whereas the baking evaluation of biscuit was performed using the AACC method 10-50D (2000).

Statistical analysis

The analysis of variance (ANOVA) was performed using the

SAS 9.4 software (SAS/STAT R, SAS Institute Inc., NC, USA). Comparison of the least square mean was performed using Fisher's Least Significant Difference (LSD) test.

Results

Grain harvest index (GHI) and flour protein content (FPC)

Results of GHI and FPC are presented in Table 1. GHI of NILs ranged between 13.86 (NIL-14) and 47.13 (NIL-4) and that of the donor parent Barham was 29.98. The GHI of the other parent DBW14 was 81.76 and that of the widely cultivated wheat variety HD2967 was 85.86. The FPC of the NILs varied between 10.25% (NIL-14) and 12.22% (NIL-2) compared to 12.41% in DBW14, 10.97% in Barham and 12.6% in HD2967. Grain appearance of one of the NILs, and both the parents is shown in Fig. 1a.

Gluten parameters

SDS-Sedimentation volume (SDS-SV) of the tested NILs varied between 38ml (NIL-14) and 49.5ml (NIL-15), while for DBW14, Barham and HD2967, it was 39.0, 64.5 and 45.5ml respectively. Values of Wet Gluten Content (WGC), Dry Gluten Content (DGC), Gluten Index (GI) and water binding are depicted in Table 1. Dry gluten discs of the parents and one NIL are shown in Fig. 1b. WGC of the NILs varied between 24.51% (NIL-12) and 30.27% (NIL-8), and was 30.62% and 29.43% for DBW14 and Barham respectively. DGC ranged between 7.65% (NIL-10) and 9.5% (NIL-9), which is similar to DBW14; while Barham had DGC of 9.4%. GI values ranged from 32.66 (NIL-14) to 60.59 (NIL-15) and were observed to be 40.2 and 73.4 for DBW14 and Barham. Water binding ranged between 17.05% (NIL-10) and 21.1% (NIL-8) while DBW14 and Barham recorded values of 21.25% and 20.35%, respectively.

Flour extraction rate (ER %) and particle size distribution

The ER of the NILs and their parents is depicted in Table 1 and the calculation of ER is given in Supplementary Table S1. The milling recovery of NILs varied between 61.70% (NIL-16) and 71.01% (NIL-4) and DBW14 showed ER of 70.05%. Particle size analyser distributed the particles into three fractions: size 0 to ≤ 55 µm (fraction 1), > 55 µm to ≤ 105 µm (fraction 2), > 105 µm (fraction 3). The percentage of flour particles falling in these three classes is depicted in Table 2. The range of 47.67 µm (NIL-5) to 74.21 µm (NIL-10) was obtained for fraction 1, 15.2 µm (NIL-6) to 22.29 µm (NIL-8) for fraction 2 and 9.38 µm (NIL-11) to 22.31 µm (NIL-8) was obtained for fraction 3 amongst the NILs. Values of fraction 1, 2 and 3 for DBW14 were 58.39, 20.73, 20.51 µm respectively, for Barham were 59.81, 22.67, 17.56 µm and that for HD2967 were 47.43, 32.1, 20.39 µm.

Table 1. ER, GHI, FPC, SDS-SV and Gluten parameters of NILs and their parents

NIL Number	ER%	GHI	FPC (14% Mb)	SDS-SV flour (ml)	Wet Gluten Content (WGC) (%)	Dry Gluten Content (DGC) (%)	Gluten Index (GI)
NIL-1	60.09^{j}	15.76^{m}	10.92^{j}	40.00^{hi}	28.63^{h}	8.80^{cde}	40.69^{j}
NIL-2	66.38^{b}	20.05^{j}	12.22^{c}	41.00^{fgh}	28.02^{i}	8.65^{def}	51.08^{f}
NIL-3	64.74^{defg}	21.42^{i}	11.35^{g}	40.00^{hi}	29.26^{fg}	9.46^{b}	45.50^{g}
NIL-4	65.10^{cdef}	47.13^{c}	11.05^{i}	42.00^{ef}	29.67^{d}	9.35^{b}	41.49^{i}
NIL-5	64.71^{efg}	19.37^{jk}	11.22^{h}	40.50^{gh}	28.12^{i}	8.45^{ef}	41.58^{i}
NIL-6	65.21^{cde}	18.86^{k}	11.15^{h}	44.00^{d}	28.69^{h}	9.20^{bc}	38.22^{o}
NIL-7	64.67^{efg}	23.25^{h}	11.15^{h}	42.50^{e}	29.54^{de}	9.35^{b}	39.69^{n}
NIL-8	64.64^{fg}	26.82^{e}	11.18^{h}	41.50^{efg}	30.27^{c}	9.20^{bc}	40.34^{k}
NIL-9	66.64^{b}	24.55^{g}	11.71^{e}	44.00^{d}	30.24^{c}	9.50^{b}	39.84^{r}
NIL-10	63.58^{h}	20.32^{j}	10.91^{j}	40.00^{hi}	24.89^{m}	7.65^{g}	40.06^{m}
NIL-11	64.41^{g}	19.89^{j}	11.65^{e}	49.00^{b}	27.56^{j}	8.70^{cde}	56.24^{e}
NIL-12	66.36^{b}	25.72^{f}	11.43^{gf}	40.00^{hi}	24.51^{n}	8.35^{ef}	35.25^{p}
NIL-13	65.38^{c}	21.37^{i}	10.90^{j}	42.00^{ef}	25.37^{l}	8.15^{fg}	42.38^{h}
NIL-14	61.70^{i}	13.86^{n}	10.25^{k}	38.00^{j}	27.37^{j}	8.55^{ef}	32.66^{q}
NIL-15	65.28^{cd}	17.39^{l}	11.92^{d}	49.50^{b}	29.16^{g}	9.15^{bcd}	60.59^{c}
NIL-16	61.60^{i}	25.97^{ef}	11.44^{f}	44.50^{cd}	26.16^{k}	8.45^{ef}	58.81^{d}
HD2967	70.27^{a}	85.86^{a}	12.64^{a}	52.50^{a}	36.52^{a}	14.50^{a}	68.08^{b}
Barham	66.29^{b}	29.98^{d}	10.97^{ji}	37.50^{j}	29.43^{ef}	9.40^{b}	40.38^{k}
DBW14	70.19^{a}	81.76^{b}	12.41^{b}	52.00^{a}	30.62^{b}	9.50^{b}	40.20^{l}

ER=Extraction rate; GHI=Grain hardness index; FPC= Flour protein content; SDS-SV =SDS-Sedimentation value. Means with a similar superscript in a column do not differ significantly between NILs (p ≤ 0.05). The grain hardness data is reproduced here from our earlier paper (Kumar et al. 2021).

Table 2. Particle size distribution and solvent retention capacity (SRC) of NILs and their parents

NIL Number	Particle Size Distribution			SRC			
	0 to ≤ 55 (µm)	>55 to ≤ 105 (µm)	>105 (µm)	Water SRC. (%)	50% Sucrose SRC (%)	5% Lactic Acid SRC (%)	5% Na_2CO_3 (%)
NIL-1	61.57^{m}	19.93^{ef}	18.48^{d}	48.19^{o}	79.12^{r}	79.22^{o}	56.51^{p}
NIL-2	67.32^{h}	18.10^{h}	14.57^{h}	48.3^{n}	81.63^{o}	80.70^{n}	60.52^{l}
NIL-3	66.14^{j}	17.49^{i}	16.18^{f}	47.82^{p}	79.78^{q}	87.48^{l}	56.68^{p}
NIL-4	60.52^{n}	20.65^{d}	18.56^{d}	53.59^{c}	84.60^{i}	97.66^{d}	60.87^{j}
NIL-5	62.57^{l}	20.09^{e}	17.32^{e}	50.59^{l}	82.82^{l}	85.85^{m}	56.73^{p}
NIL-6	68.05^{f}	17.21^{ij}	14.57^{h}	50.17^{m}	82.66^{m}	95.10^{g}	59.59^{o}
NIL-7	67.53^{g}	18.37^{h}	14.09^{i}	52.05^{h}	85.48^{g}	95.84^{e}	60.23^{m}
NIL-8	55.40^{q}	22.29^{c}	22.30^{b}	53.21^{d}	88.47^{d}	99.50^{c}	62.83^{f}
NIL-9	69.57^{d}	17.10^{j}	13.63^{j}	50.64^{l}	86.30^{e}	97.75^{d}	61.48^{i}
NIL-10	74.21^{a}	16.53^{k}	9.24^{l}	52.2^{g}	86.28^{e}	90.20^{j}	60.65^{k}
NIL-11	72.49^{c}	18.31^{h}	9.30^{l}	51.55^{k}	83.13^{k}	91.03^{i}	61.60^{h}
NIL-12	66.76^{i}	19.64^{fg}	13.58^{j}	51.81^{j}	83.53^{j}	91.47^{i}	61.84^{g}
NIL-13	66.73^{i}	18.12^{h}	15.19^{g}	53.27^{d}	82.33^{n}	94.88^{g}	63.10^{d}
NIL-14	72.94^{b}	16.48^{k}	10.63^{k}	51.93^{i}	88.64^{c}	88.60^{k}	63.29^{c}
NIL-15	65.08^{k}	19.58^{g}	15.47^{g}	52.53^{e}	86.05^{f}	99.69^{c}	60.05^{n}
NIL-16	68.91^{e}	16.55^{k}	14.50^{h}	52.16^{g}	85.28^{h}	91.32^{i}	63.23^{c}
HD2967	47.43^{r}	32.10^{a}	20.39^{c}	68.14^{a}	95.80^{a}	124.64^{a}	82.28^{a}
Barham	59.81^{o}	22.67^{b}	17.56^{e}	52.39^{f}	81.44^{p}	95.46^{f}	62.94^{e}
DBW14	47.29^{p}	30.30^{d}	22.41^{a}	62.77^{b}	94.41^{b}	93.95^{h}	77.25^{b}

Means with a similar superscript in a column do not differ significantly between NILs ($p \leq 0.05$)

Solvent retention capacity (SRC) with different solvents

Values of water based SRC (WSRC), 50% sucrose based SRC (SuSRC), 5% lactic acid based SRC (LASRC) and 5% Na_2CO_3 based SRC are listed in Table 2. WSRC% ranged between 47.82 (NIL-3) and 88.64 (NIL-14), 50% SuSRC% ranged between 79.12 (NIL-1) and 53.59 (NIL-4), 50% LASRC% ranged between 79.22 (NIL-1) and 103.89 (NIL-11) while 5% Na_2CO_3 ranged between 56.51 (NIL-1) and 63.29 (NIL-14). NIL-1 has the lowest SRC value of 50% sucrose, 5% Lactic Acid, 5% Na_2CO_3 SRC and second lowest for water SRC. DBW14 and Barham had the values 62.77, 94.41, 93.95, 77.25 and 52.39, 81.44, 95.46, 62.25 respectively for WSRC%, 50% SuSRC %, 50% LASRC% and 5% Na_2CO_3%.

Starch damage and starch pasting properties

Starch damage ranged between 2.07% (NIL-14) and 3.65% (NIL-4) for NILs and 2.97% for Barham. However, Starch damageof the recurrent parent DBW14 was found to be 4.54% and for popular hard wheat variety HD2967 as 7.49% (Table 3). The RVA (Rapid Visco Analyser) parameters showed a wide range between 2911.5 (NIL-2) to 3846.5 (NIL-13) for pasting PV ($_{c}$P), 2231.5 (NIL-8) and 2723.5 (NIL-14) for Through1, 485 (NIL-9) and 1303 (NIL-13). Similarly the range recorded for BDV ($_{c}$P) was 3460 (NIL-8) and 4297.5 (NIL-14), for FV ($_{c}$P), 1228.5 (NIL-8) and 1574 (NIL-14) for SBV ($_{c}$P), 6.36 min (NIL-16) and 6.60 min (NIL-9) for Peak time, 70.27°C (NIL-13) and 84.77°C (NIL-1) for PT.

The value for pasting PV ($_{c}$P) for the recurrent parent DBW14 was 3046.5, that for donor Barham was 3463.5 and for the popular variety HD2967 was 3225.5. The value of DBW14, Barham and HD2967 for Trough1 was 2418, 2495 and 2525.5 respectively; for BDV ($_{c}$P) it was 628.5, 968.5 and 700 respectively and for FV ($_{c}$P) it was 3787, 3673 and 3816 respectively. SBV ($_{c}$P) was found to be 1369, 1184.5 and 1287.5 for DBW14, Barham and HD2967 respectively; Peak time was recorded as 6.47 min, 6.5min and 6.4 min for DBW14, Barham and HD2967 respectively; and PT as 66.57°C, 68.92°C and 66.52°C, respectively.

Farinographic and alveographic indices

The Farinograph parameters (WA, DDT, DOS, Stability and FQN) and Alveograph parameters (P/L ratio, G, W, Ie) of different NILs and parents are shown in Table 4 and Farinograms are depicted in Fig. 2 and Fig. 3. Farinograms of other NILs are shown in Supplementary figures S1 and S2. WA (14% Mb) varied between 50.8% (NIL-10) and 54.2% (NIL-14) while for DBW14, Barham and HD2967 it was 63.1%, 52.8% and 64.5% respectively. DDT varied between 0.7 min (NIL-16) and 1.9 min (NIL-9 & 11) amongst the NILs while that

Table 3. Starch damage and viscometric parameters for flour of NILs, parents and HD2967

NIL Number	Starch damage (%)	Viscometric parameters through Rapid Visco Analyser						
		PV (cP)	Trough 1	BDV (cP)	FV (cP)	SBV (cP)	Peak Time (min)	PT (^{0}C)
NIL-1	2.26	3099.50^{efg}	2350.50^{cde}	749.00^{b}	3805.50^{cdefgh}	1455.00^{bcde}	6.43^{cde}	84.77^{a}
NIL-2	2.47	2911.50^{g}	2384.50^{cde}	527.00^{b}	3780.50^{defgh}	1396.00^{ef}	6.53^{ab}	84.75^{a}
NIL-3	2.36	3096.50^{efg}	2453.50^{bcde}	643.00^{b}	3890.50^{bcdefg}	1437.00^{cde}	6.53^{ab}	83.60^{ab}
NIL-4	3.65	3020.00^{efg}	2414.00^{cde}	606.00^{b}	3930.00^{bcdef}	1516.00^{ab}	6.47^{bcd}	76.70^{cd}
NIL-5	2.31	3030.50^{efg}	2469.00^{bcd}	561.50^{b}	3961.00^{bcde}	1492.00^{bc}	6.50^{bc}	83.97^{ab}
NIL-6	2.32	3261.50^{cde}	2536.50^{abcd}	725.00^{b}	3939.00^{bcdef}	1402.50^{def}	6.53^{ab}	78.72^{bc}
NIL-7	2.56	3147.00^{efg}	2552.50^{abc}	594.50^{b}	4013.00^{bcd}	1460.50^{bcd}	6.53^{ab}	84.00^{ab}
NIL-8	2.69	3276.00^{cde}	2231.50^{e}	1044.50^{b}	3460.00^{i}	1228.50^{ij}	6.47^{bcd}	70.62^{e}
NIL-9	2.48	3031.50^{efg}	2546.50^{abcd}	485.00^{b}	3959.00^{bcde}	1412.50^{def}	6.60^{a}	84.37^{a}
NIL-10	2.35	3269.50^{cde}	2640.00^{ab}	629.50^{b}	4129.00^{ab}	1489.00^{bc}	6.53ab	83.57^{ab}
NIL-11	2.08	3543.00^{b}	2396.50^{cde}	1146.50^{b}	3701.50^{fghi}	1305.00^{gh}	6.47^{bcd}	71.82^{de}
NIL-12	2.34	3076.50^{efg}	2478.00^{bcd}	598.50^{b}	4047.00^{abc}	1569.00^{a}	6.47^{bcd}	83.57^{ab}
NIL-13	2.28	3846.50^{a}	2543.50^{abcd}	1303.00^{b}	3796.50^{cdefgh}	1253.00^{hi}	6.40^{de}	70.27^{e}
NIL-14	2.07	3522.00^{bc}	2723.50^{a}	798.50^{b}	4297.50^{a}	1574.00^{a}	6.47^{bcd}	81.90^{abc}
NIL-15	2.19	2983.00^{fg}	2337.50^{cde}	645.50^{b}	3729.50^{efgh}	1392.00^{ef}	6.47^{bcd}	84.30^{ab}
NIL-16	2.35	3413.00^{bcd}	2327.00^{de}	1086.00^{b}	3573.50^{hi}	1246.50^{hi}	6.36^{e}	71.07^{e}
HD2967	7.49	3225.50^{def}	2525.50^{abcd}	700.00^{b}	3813.00^{cdefgh}	1287.50^{hi}	6.40^{de}	66.52^{e}
Barham	2.97	3463.50^{bcd}	2495.00^{bcd}	5468.50^{a}	3673.00^{hi}	1184.50^{j}	6.50^{bc}	68.92^{e}
DBW14	4.54	3046.50^{efg}	2418.00^{bcde}	628.50^{b}	3787^{defgh}	1369^{fg}	6.47^{bcd}	66.57^{e}

PV= Peak viscosity; BDV= Breakdown viscosity; FV = Final viscosity; SBV = Setback viscosity; PT = Pasting temperature. Means with a similar superscript in a column do not differ significantly between NILs ($p \leq 0.05$)

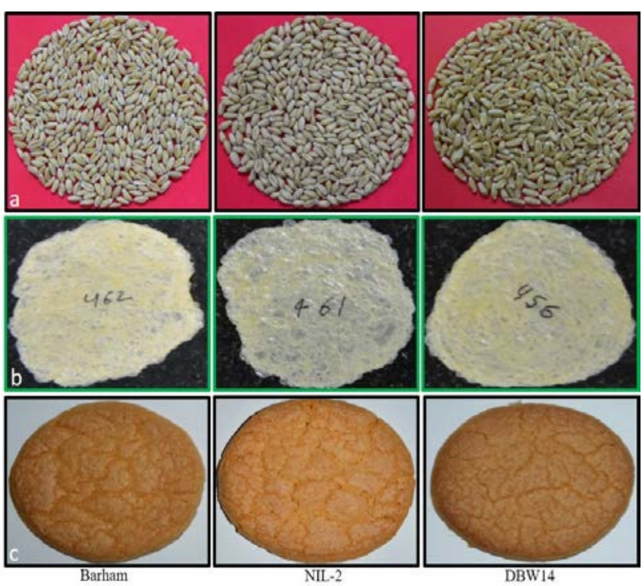

Fig. 1. Visual comparison of NIL-2 with donor Barham and recurrent parent DBW14, (a) Grains, (b) Dry gluten of sample number 462 (Barham), 461 (NIL-2) and 456 (DBW14), and (c) Biscuit.

for DBW14, Barham, and HD2976 it was 1.9, 1.5, and 6.2 min respectively. DOS of different NILs varied between 114 FU (NIL-11) and 206 FU (NIL-14) while for DBW14, Barham and HD2967 it was 149 FU, 120 FU and 22 FU respectively. Dough

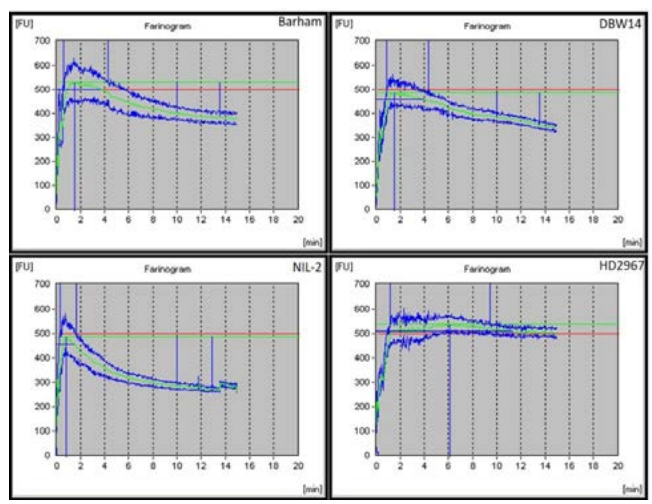

Fig. 2. Schematic presentation of Farinograph curve of NIL-2, its parents and popular Indian variety HD2967

Stability in NILs varied between 1.3 min (NIL-14) and 2.8 min (NIL-9) while that in DBW14, Barham and HD2967 was observed as 3.4, 3.7 and 8.3 min. The FQN of the NILs was observed to be in the range of 15 (NIL-2 & 14) and 29 (NIL-8, 9 & 15), that for the two parents, DBW14 and Barham, it was 30 and 37 respectively. FQN of the popular variety HD2967 was observed to be 113.

The Alveographic parameters were also recorded. Deformation Energy (W) of NILs ranged between 46 J (NIL-14)

Table 4. Farinographic, alveographic and quality parameters of biscuits made from flour of NILs along with the parents Barham, DBW14 and a popular Indian variety HD2967

NIL No.	Farinograph indices					Alveograph indices					Biscuit parameters				
	WA %	DDT (min)	Stability (min)	DOS (FU)	FQN	P (mm)	L (mm)	P/L	W (J)	Ie	Diameter (mm)*	Thickness (mm)	Spread factor	Hardness/ Force (N)	Fracturability (mm)
NIL-1	52.9	1.2	1.8	177	19	23	95	0.24	49	32.9	84.07^{ef}	8.40^{efg}	10.01^{ghi}	71.23^{de}	6.43^{c}
NIL-2	52.2	0.9	1.4	201	15	21	109	0.19	49	33.5	85.96^{cdef}	7.50^{ij}	11.44^{bcd}	44.95^{h}	5.53^{d}
NIL-3	51.7	0.9	2.0	167	22	20	118	0.17	53	37.2	90.18^{abc}	7.70^{hij}	12.05^{ab}	68.10^{ef}	4.59^{fg}
NIL-4	54.1	1.2	2.3	147	25	32	117	0.27	89	40.7	73.86^{g}	9.30^{bc}	7.90^{k}	65.90^{f}	5.39^{de}
NIL-5	51.4	1.4	2.0	152	23	25	111	0.23	63	37.4	93.92^{a}	7.30^{j}	12.77^{a}	71.55^{de}	4.53^{fg}
NIL-6	51.7	1.4	2.5	156	26	27	132	0.20	78	39.2	83.85^{ef}	8.30^{efg}	10.07^{ghi}	64.08^{f}	4.56^{fg}
NIL-7	53.4	1.3	2.2	172	22	25	111	0.23	64	37.9	87.24^{bcdef}	7.90^{ghi}	10.96^{def}	57.00^{g}	3.41^{h}
NIL-8	52.7	1.4	2.5	123	29	28	111	0.25	84	45.3	84.08^{def}	9.40^{b}	9.04^{j}	83.90^{b}	6.46^{c}
NIL-9	52.1	1.5	2.8	131	29	27	90	0.30	65	40.7	83.51^{ef}	8.60^{def}	9.63^{hij}	84.66^{a}	6.82^{b}
NIL-10	50.8	1.4	2.0	174	18	25	104	0.24	66	40.2	85.24^{cdef}	9.00^{bcd}	9.43^{ij}	81.20^{c}	4.66^{fg}
NIL-11	51.9	1.5	2.5	114	28	29	96	0.30	94	55.4	87.08^{bcdef}	7.70^{hij}	11.18^{cde}	64.30^{f}	4.56^{fg}
NIL-12	52.2	1.4	2.5	160	25	29	99	0.29	78	43.1	88.58^{bcde}	8.50^{defg}	10.40^{efgh}	57.17^{g}	5.70^{d}
NIL-13	51.0	0.9	2.2	171	22	23	95	0.24	55	38.2	91.26^{ab}	7.60^{hij}	11.86^{bc}	84.05^{a}	4.35^{g}
NIL-14	54.2	1.0	1.3	206	15	25	69	0.36	46	32.6	89.16^{abcd}	8.70^{def}	10.13^{fghi}	57.33^{g}	5.69^{d}
NIL-15	52.6	1.5	2.8	128	29	25	88	0.28	67	47.0	85.50^{cdef}	8.40^{defg}	10.04^{ghi}	56.20^{g}	5.53^{d}
NIL-16	52.0	0.7	2.5	179	20	27	97	0.28	59	41.6	87.08^{bcdef}	8.20^{fgh}	10.63^{defg}	76.15^{d}	4.42^{fg}
HD2967	64.5	6.2	8.3	22	113	97	59	1.64	221	55.0	74.94^{g}	10.20^{a}	7.29^{k}	83.71^{b}	7.91^{a}
Barham	52.8	1.5	3.7	120	37	31	112	0.28	94	45.9	83.31^{f}	8.80^{cde}	9.44^{ij}	75.43^{d}	5.15^{e}
DBW14	63.1	1.9	3.4	149	30	49	79	0.62	105	37.7	76.33^{g}	10.50^{a}	7.30^{k}	65.82^{f}	4.75^{f}

WA = Water absorption; DDT= Dough development time; DOS= Degree of softening; FQN= Farinograph quality number; P : Dough strength, L: Extensibility, G:Swelling index, W: Deformation energy and Ie: Elasticity index. Mean with a similar superscript in a column do not differ significantly between NILs ($p \le 0.05$)

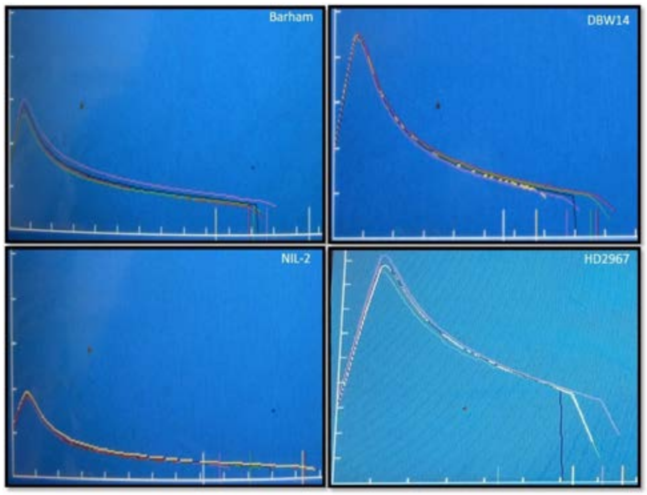

Fig. 3. Schematic presentation of Alveograph curve of NIL-2 and their parents along with popular Indian variety HD2967

and 94 J (NIL-11), it was 94 J for Barham and 105 J for DBW14 while it was 221 J for HD2967. P/L ratio ranged between 0.17 (NIL-3) and 0.36 (NIL-14) while for DBW14, Barham and HD2967 it was 0.62, 0.28, 1.64 respectively. Swelling Index (G) for NILs varied between 21.1 cm^3 (NIL-9) and31.7 cm^3 (NIL-1), it was 19.8 cm^3 for DBW14, 23.6 cm^3 for Barham while it was 17.1 cm^3 for HD2967. Value of Ie for NILs was ranged between 32.6 (NIL-14) and 55.4 (NIL-11) while for DBW14, Barham and HD2967 it was recorded as 37.7, 45.9, and 55 respectively.

Baking evaluation of biscuits

Biscuits made from the NILs and parents are depicted in Fig. 1c. Mean diameter of biscuits in the NILs varied between 73.86 mm (NIL-4) and 93.92 mm (NIL-5). Soft wheat Barham, and hard wheats DBW14, and HD2967 recorded mean diameters of 83.31 mm,76.33 mm and 74.94 mm respectively. Biscuit thickness in NILs varied between 7.3 mm (NIL-5) and 9.4 mm (NIL-8), while Barham, DBW14 and HD2967 recorded 8.8 mm, 10.5mm, and 10.2 mmrespectively. Spread Factor varied between 7.90 (NIL-4) and 12.77 (NIL-5) while it was 9.44, 7.30 and 7.29 for Barham, DBW14 and HD2967 respectively. Hardness on texture analyser among NILs varied between 44.95N (NIL-2) and 118.9N (NIL-8) while DBW14, Barhamand HD2967 recorded 65.82N, 75.43N, 73.71N respectively. Fracturability amongst NILs varied between 3.4 mm (NIL-7) and 6.82 mm (NIL-9) while that for Barham, DBW14, and HD2967 it was 5.15 mm, 4.75 mm and 7.91 mm respectively.

Discussion

A detailed analysis of 12 important quality parameters has been carried out in wheat lines developed in a breeding programme where the parent to be improved for its grain texture was chosen to be a popular and old Indian wheat variety DBW14 (Rai et al. 2019a) and the trait for soft grain texture suitable for soft wheat products such as cakes and cookies was transferred from the donor parent Barham which is an Australian variety. Marker assisted backcross breeding procedure was adopted to develop 16 derivatives (Near Isogenic Lines or NILs) which had the genes *PinaD1aPinbD1a* besides a leaf rust resistance gene *Lr37b*. The details of the breeding procedure can be found in Kumar et al. (2021).

Hard wheat requires greater force during milling and produces higher mean particle size than soft wheat (Morris. 2019). The mean particle size for most biscuit flours is around 50µm, with less than 10% of the biscuit flour consisting of particle size of more than 130µm (Manley, 2000). In the present study also, the soft NILs all showed the highest proportion (>60% except in NIL-8) of particles of size smaller than 55µm while the hard wheat lines DBW14 (parent) and HD2967 (new popular variety) had only 47% of this fraction.

Hard wheat also suffers greater starch damage than the soft wheat and the extent of starch damage is directly proportional to Grain Hardness (Williams and McEwin 1967). Leonet al. (2006) also found a highly significant positive correlation between GH and starch damage. In the present study, the hard grained parent DBW14 and *cv.* HD2967 showed much higher starch damage (4.54% and 7.49% respectively) than Barham (2.97%) and the NILsranging between 2.07% and 3.65%. The soft grained NILs and soft grained donor Barham also showed lower water absorption than hard grained DBW14 and HD2967. Hydration takes less time in soft wheat flour due to smaller particle size and it absorbs less water due to less amount of starch damage compared to hard wheat flour (Roman et al. 2002). This leads to cookie dough stiffness, decreased cookie diameter and biscuit lower spread. Extraction Rate of flours of the NILs was significantly lower than the hard wheats DBW14 and HD2967 and closer to the donor soft wheat parent Barham. Reduction in flour extraction rate expectations by up to 2% have been reported for soft wheat under similar conditions of milling as hard wheat primarily because endosperm of soft wheat adheres more strongly to bran during milling (https://www.world-grain.com/articles/11991-milling-ops-finding-the-sweet-spot; Retrieved: 30th April, 2019) .A few NILs showed much lower ER than the donor parent of grain softness Barham, this may impact the commercial exploitation of such genotypes. FPC of the NILs ranged between that of the hard and the soft parents. Cookie and cake flours with FPC ≤ 10% are desired in baking industry (Hoseney et al. 1994). However, it has been shown that the functional quality of a flour has greater influence than FPC. High Molecular Weight Glutenin Subunit (HMW-GS) and Low Molecular Weight Glutenin Subunit (LMW-GS) proteins constitute the glutenin fraction of gluten and may vary between different cultivars. The genes (*Glu-1* and *Glu-3*) responsible for the production of HMW and LMW proteins are multi-allelic and are present on the long and short arms of chromosome 1 of wheat genome respectively.

Our earlier studies (Rai et al.2019a) had identified DBW14 and Barham to be carrying the alleles *GluA1b*, *GluB1u* and *GluD1a*.*GluD1a*codes for the HMW-GS 2+12 and is known to impart weakness property to the gluten (Payne, 1987). The gluten parameters, allelic combination for LMW-GS and rheology of these two cultivars also indicated a weak and extensible gluten. Gluten parameters in the NILs also confirmed that both the parents have weak gluten. The WG and DG of the NILs in the present study were either similar or lower to that of the parents. GI however, varied much more among the NILs and ranged between 32.66 and 60.59. The sedimentation value of the NILs also indicates moderate to weak gluten in them. Weak flour proteins do not form a continuous gluten matrix when flour is mixed with water due to smaller quantity and basic quality characteristics of gluten proteins in weak flour (Al-Dmoor, 2013). Dough with such characteristics extends more when subjected to higher temperature in the baking and more numbers of biscuits are obtained from a given mass of dough (Edmund et al. 2008).

Solvent retention capacity (SRC) is a solvation test for flours that uses a large excess of solvent and is based on the swelling behaviour of polymer networks in diagnostic solvents. Individual diagnostic solvents are used to identify which functional flour component is responsible for a given variation in flour-swelling behaviour (Kweonet al. 2011). The four standard solvents of the SRC method are water, 5% LA in water, 5% Na_2CO_3 in water, and 50% Sucrose. The following pattern of all four SRC values has been suggested for a gold-standard cookie flour: water SRC, ≤51%; LA SRC, ≥87%; Na_2CO_3SRC, ≤64%; and SucSRC, ≤89% (Kweon 1 et al. 2014). The SRC values for the NILs and donor soft wheat parent Barham analysed in this study are commensurate with the expectation of the values of SRC for cookie flour. SRC of hard grained DBW14 and HD2967 do not meet the requirements of the cookie flour based on SRC results. There was a lot of variability for almost all the parameters amongst the NILs and these were significantly different from both the parents. Starch pasting properties do not appear to be significantly correlated with biscuit parameters as indicated in previous studies (Moiraghi et al. 2019). Rheology of the flour of the NILs was also analysed in this study. Farinograph is used to estimate the water absorption of flours, the relative mixing time, the stability to overmixing and rheological properties of the dough during mixing. The hard wheats HD2967 and DBW14 had much higher water absorption (WA) as compared to the soft wheat Barham and all the soft NILs of DBW14. It is reported that doughs having higher water absorption capacity produce an extensive gluten structure and result in harder cookies (Labuschagne et al. 1996). Variation in flours WA has been reported to be due to differences in damaged starch content (Jukic et al. 2019) though several other factors may be responsible for variation within a class (Sapirstein et al. 2018). The indices of the NILs in comparison to parents show that all the NILs maintained weak to very weak flour property. Dough development time and stability in the NILs were low and the degree of softening was much lower than even the soft parent Barham indicating that the genes for this trait are contributed by both the parents. The Farinograph Quality Number of the NILs was also lower than both the parents. Thus, the NILs with their grain softness and weak flour are highly suitable for cake and cookie purposes.

In addition to weak gluten, flours with good stretchability or extensibility are desired for confectionary. In fact, dough extensibility is a favoured character for most wheat-based products (Pena et al. 2008). Williams 1 et al. (1988) classified dough alveograph energy values as W= 0-50J for very weak, 50-100 J for weak, 100-200 J for medium, 200-300 J for medium-strong, 300-400 J for strong, and over 400 J for very strong. Usually, W values are lower than 60J for the cookie flours (Bordes et al. 2008). Wheat flour relevant for confectionary products, should exhibit a P/L value lower than 0.50 (Rai et al. 2019a). In the present study, NILs produced ideal values of most parameters of Alveograph. The alveograph energy W between 55 to 80, P/L between 0.17 to 0.36, Ie between 33 to 55 and thus qualify for flours highly suited for cake, biscuits and other similar products. The Spread factor (SF) determined as the ratio of diameter and thickness varied among the NILs between 7.9 and 12.05. NILs 2, 3, 5 and 13 had the highest SF. These NILs also had lower grain hardness than the soft donor. The positive correlation between grain hardness and spread factor has been reported by several workers (Miller and Hoseney 1997). The higher the SF, better is the output per unit flour weight for the baker (Ahmad et al. 2017). Therefore, a higher SF is a highly desirable trait for baking industry. The cookie snap test also confirmed the suitability of these NILs for confectionary purpose though NIL-2 appeared to be the best in this regard. All the NILs were evaluated for grain yield in the earlier study by us (Kumar et al. 2021). The yield of NIL-13 is significantly lower than NIL-2 and 3.

Authors' contribution

Conceptualization of research (AMS, TPJK); Designing of the experiments (AMS, TPJK, AR, AKA); Contribution of experimental materials (SKS); Execution of field/lab experiments and data collection (NDR, KB); Analysis of data and interpretation (AMS, TPJK, NB); Preparation of the manuscript (TPJK, AMS).

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Supplementary materials

Supplementary Table S1 and Supplementary Figures S1 to S2 are presented.

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Supplementary Table S1. Extraction rate (%) of NILs along with parents and HD2967

NIL No.	Grains wt. for grinding (gm)	Moisture (%)	Amount of water Added (ml)	Weight Before milling (gm)	Weight of Bran (gm)	Weight of Fine bran (gm)	Weight of flour (gm)	Total weight of flour (gm)	ER %
NIL-1	1500.00	10.32	47	1540.30	442.00	21.00	910.30	1373.3	60.69
NIL-2	1500.00	10.47	44	1523.70	476.00	29.20	996.90	1502.1	66.46
NIL-3	1500.00	10.47	44	1516.90	491.30	43.40	974.80	1509.5	64.99
NIL-4	1500.00	10.10	50	1552.40	453.20	68.50	1065.20	1586.9	71.01
NIL-5	1500.00	10.50	44	1525.90	519.00	41.30	974.00	1534.3	64.93
NIL-6	1500.00	10.53	44	1547.20	514.00	48.80	978.30	1541.1	65.22
NIL-7	1500.00	10.30	47	1550.70	498.00	43.90	972.60	1514.5	64.84
NIL-8	1500.00	10.63	42	1546.60	534.70	43.30	971.70	1549.7	64.78
NIL-9	1500.00	10.50	44	1545.60	496.20	44.90	1004.80	1545.9	66.99
NIL-10	1500.00	10.67	41	1543.50	516.30	45.10	955.10	1516.5	63.67
NIL-11	1500.00	10.69	41	1545.10	484.50	56.00	965.00	1505.5	64.33
NIL-12	1500.00	10.67	41	1540.40	493.10	38.10	996.50	1527.7	66.43
NIL-13	1500.00	10.52	44	1546.40	527.70	51.10	983.50	1562.3	65.57
NIL-14	1500.00	10.51	44	1541.80	516.90	58.90	926.50	1502.3	61.77
NIL-15	1500.00	10.36	46	1547.60	527.80	41.30	979.00	1548.1	65.27
NIL-16	1500.00	10.94	32	1537.60	560.00	29.00	925.50	1514.5	61.70
HD2967	1500.00	9.82	55	1633.50	453.00	130.30	1050.70	1634	70.05
Barham	1500.00	9.28	55	1584.60	494.50	48.00	1025.70	1568.2	68.38
DBW14	1500.00	9.82	55	1633.50	453.00	130.30	1050.70	1634	70.05

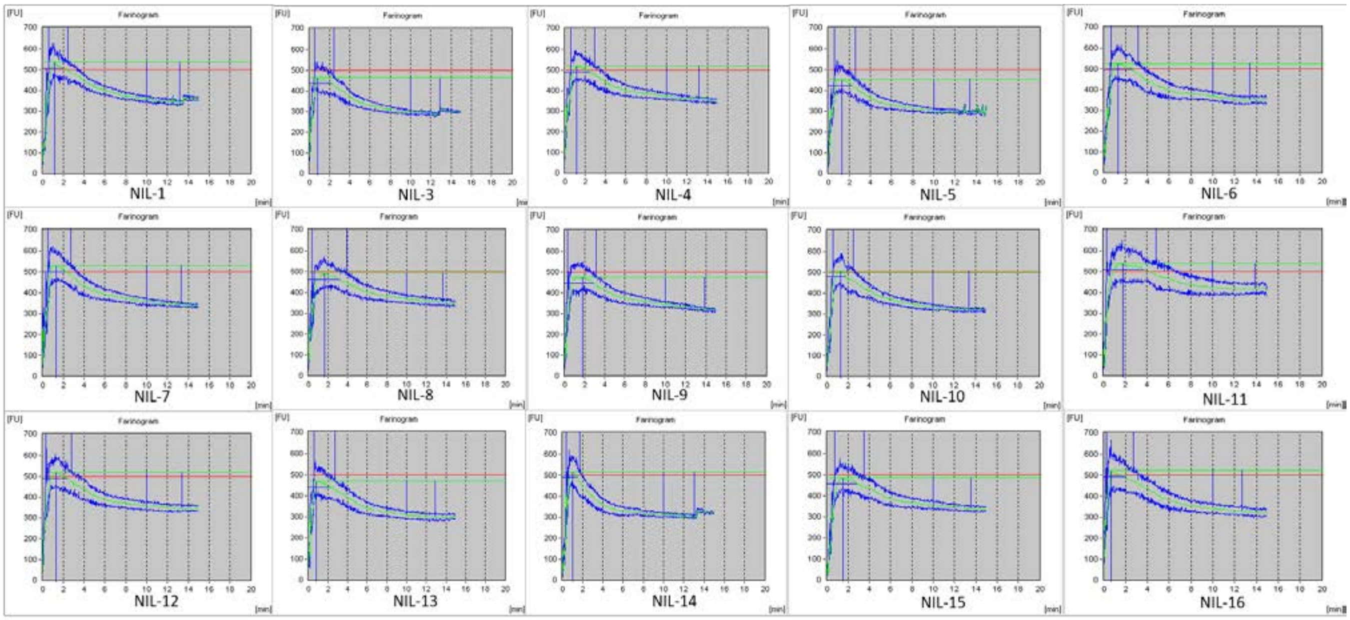

Fig. S1: Schematic presentation of Farinographic parameters of fifteen NILs flour samples

Supplementary Fig. S1. **Schematic presentation of farinogram of the flour of NILs (NIL-2 is presented in the results)**

Fig. S2: Schematic presentation of Alveograpg parameters of fifteen NILs flour samples

Supplementary Fig. S2. **Schematic presentation of alveograph of the flour of NILs (NIL- 2 is presented in the results)**