

Selection and assessment of bread wheat lines improved for dough strength and rust resistance

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Accepted 17th September 2001

ABSTRACT

The *GluD1-d* encoded subunits 5+10 are known to be associated with higher dough strength of wheat. Aim of this study was to assess selections made in Indian bread wheat background using subunits 5+10 and rust resistance as selection criteria, for quality parameters including dough rheology. From backcrossing experiments involving bread wheats, selections were obtained with subunits 5+10 and moderate rust resistance conferred by *Sr24Lr24*, in the backgrounds of cvs. Kalyansona or Sonalika. Three selections and recurrent parents Kalyansona and Sonalika were assessed for their dough characteristics using Brabender Farinograph. There was improvement in the mixing time, dough stability and mechanical tolerance index in the selections compared to the parents. In the Kalyansona derivatives three selections showed better yield potential.

Key words: *Triticum aestivum*, Glutenin, HMW subunits, dough strength, rust resistance.

INTRODUCTION

Since the first observation that the presence of subunits 5+10 correlates significantly with bread making quality as judged by SDS-sedimentation volume (SDS-SV) test (Payne *et al.* 1981), there have been many reports on intervarietal comparisons confirming association of subunits 5+10 and bread making quality (Moonen *et al.* 1982, 1983; Branlard and Dardevet 1985). The intervarietal comparisons, however, have limitation due to other genetic effects. Although, use of near-isogenic lines is the most convincing way to demonstrate the effect of HMW subunits of glutenin as a primary determinant of dough strength and elasticity (Lawrence *et al.* 1988; Payne and Seeking 1996) use of backcross lines and recombinant inbred lines are also useful material to assess the effect of particular subunit in a comparable genetic background (Moonen and Zeven 1985; Payne *et al.* 1987; Odenbach and Mahgoub 1988; Carrillo *et al.* 1990). Using inbred backcross lines derived from two winter wheat crosses Odenbach and Mahgoub (1988) reported that subunits 2*, 7+9, 7+8, and 5+10 were associated with higher sedimentation values. In closely related lines, loss of subunits 5+10 resulted in poor bread making quality (Payne *et al.* 1988). Positive effect of

subunits 5+10 on the SDS-SV was also observed in selections obtained in backcrossing experiments (Bhagwat and Bhatia 1993). In comparable backgrounds, these selections made only on the basis of presence of subunits 5+10, showed higher SDS-SV than those carrying subunits 2+12. Statistical analysis of data on Canadian cultivars (Ng and Bushuk 1988) indicated that selection on the basis of HMW subunits composition should be useful in breeding programme for screening genotypes for bread-making quality.

Although, selection on the basis of HMW subunits of glutenin is in practice in some countries, in the Indian subcontinent which is a major producer of wheat, it is not yet widely used. There is a need for simultaneous quality improvement along with important criteria like rust resistance. Assessment of quality at every step will ensure that the expected gain is realised. Earlier, we had tested some of the selections for quality parameters like protein content and SDS-SV (Das and Bhagwat 1997). In this paper we report results of experiments using backcross lines of Indian bread wheats, to find out whether the effect of better subunits 5+10 can be combined with rust resistance conferred by *Sr24Lr24* and the improvement due to subunits 5+10 was reflected in gluten properties and in dough properties, measured by using Brabender Farinograph.

MATERIALS AND METHODS

Biological materials

Earlier, cv. Kalyansona (KS) with subunits 2*,

Abbreviations: BC - backcross, B.U. - Brabender Units, E.R. - elastic recovery, HMW - high molecular weight, KS - Kalyansona, SDS-PAGE - SDS polyacrylamide gel electrophoresis, SK - Sonalika, TGW - thousand grain weight, UKS - Unnath Kalyansona, USK - Unnath Sonalika.

17+18, 2+12 was crossed with an early and short culm genetic stock TW-1 having subunits 1, 17+18, 5+10. In a backcrossing programme using KS as recurrent parent and subunits 5+10 as selection criterion, selections were obtained (Bhagwat and Bhatia 1993). One selection true breeding for subunits 5+10 derived after two backcrosses and subsequent selfing was crossed with Unnath Kalyansona (UKS) which was the donor of rust resistance gene *Sr24Lr24* (Kochumadhavan *et al.* 1988). The other cross was between cv. Sonalika (SK) and the genetic stock TW-1. From the F₂ population of this cross, plants were crossed with Unnath Sonalika (USK) which was the donor for rust resistance gene *Sr24Lr24*. The backcross F₂ was grown in field and at one month stage the seedlings were injected with stem rust spore suspension. Sori developed initially on newly emerging leaves. After about ten days rust development on leaves was adequate to compare the reaction. Plants showing smaller round and discrete sori between ten to fifteen days after injection were identified as moderately resistant. Susceptible plants and KS showed larger and longer sori which later fused to form a mat on the leaf surface. Individual plant harvests from resistant plants were analysed on SDS-PAGE to observe the presence or absence of subunits 5+10. Plants with subunits 5+10 were carried forward. The subsequent generations were grown as plant to row progeny. Ten plants from each row were injected with stem rust to identify the rows which were true breeding for resistance. Seedlings in F₄ generation were tested under laboratory conditions for their rust reaction. Individual plant harvests from these rows were analysed on SDS-PAGE to select those true breeding for subunits 5+10. Among F₄ plant harvests, those with poor grain weight per plant were rejected. In the later generations the rows were bulk harvested. The UKS and USK carrying *Sr24Lr24* from *Agropyron elongatum* were obtained after five to seven backcrosses followed by three selfings (Menon and Tomar 2001). As UKS and USK are near-isogenic with KS and SK respectively, the selections used in these experiments may be treated as equivalent of BC₃ and BC₁ selections respectively. In the F₄ generation, selections were grown as three metre rows, and in two subsequent years (1997-98, 1998-99) the selections were grown in a station trial format. There were 5.0m x 1.84m plots of individual entries in randomised block design with three replications. The spacing between rows was 23cm. Seed rate was 100 kg ha⁻¹. Nitrogen was applied at the rate of 120 kg ha⁻¹. All experiments were conducted at the Trombay Field Research Station of Bhabha Atomic

Research Centre, Mumbai. Only one generation per year was raised. The average grain yield (g/m²) was calculated on the basis of three metre line sowing in one year and station trials for two years. The seeds used for the following studies were from station trial experiments done in 1997-98.

Chemical analysis

Grain protein: Determinations of grain protein were made on wholemeal using micro-Kjeldahl procedure using Lab Con Co or KjelPlus apparatus (Pelican Industries Ltd).

SDS-SV: Wholemeal obtained from Udy cyclone mill was used. One gram method described by Dick and Quick (1983) was used.

Gluten extraction and E.R. ratio: One gram wholemeal was mixed with 0.6 ml distilled water for five minutes using a glass rod. Five samples were processed sequentially. The dough balls were washed under a gentle flow of tap water for five minutes to obtain water washed gluten. The gluten ball was pressed between two glass plates under constant weight (4.6 kg) and ten seconds later radii in four places were recorded. The weight was removed and gluten was allowed to contract for about 30 sec. Radii in four places of the contracted gluten were recorded. The ratio of the average expanded radius to average contracted radius gave the Elastic Recovery ratio. Higher ratio indicated higher elasticity. Dry gluten weight was obtained by drying gluten to constant weight at 80°C.

SDS-PAGE: SDS-PAGE was done as described in Bhagwat and Bhatia (1993). Five grain bulks from each replication of the station trial of 1997-98 were used as a sample to check purity and the HMW subunit pattern. Five grain bulks were also used in the previous as well as later year for electrophoresis. The gel measured 17.7 cm x 13.8 cm x 0.7 mm. The running gel contained 0.13% bisacrylamide and 10% acrylamide; the stacking gel contained 0.04% bisacrylamide and 3% acrylamide.

Farinograph studies: For rheological properties, two selections in KS background with best grain yield were used. Selection in SK background although not high yielding was included for comparison. The entries for rheological study were limited by the requirement of large quantity of seed material. The bulked seeds from station trial experiment conducted in 1997-98 were milled and sieved to obtain 60 mesh flour. Brabender Farinograph with 300g mixing bowl was used to determine water absorption capacity (%). Mixing time (min.), dough stability (min.) and mechanical tolerance index (B.U.) were determined by adding,

at a stretch, the amount of water required to obtain optimum (500 Brabender Units) dough consistency. Each sample was analysed in duplicate.

Field observations

Natural leaf rust was observed in some years, usually after flowering. Observations on leaf rust susceptibility (in the recurrent parents) or moderate resistance conferred by *Lr24* were made in years when leaf rust appeared.

RESULTS

Rust reaction

The selections showed true breeding behaviour for moderate stem rust resistance when tested by artificial inoculation in each generation. Plants showing smaller round and discrete sori (Fig-1 A) between ten to fifteen days after injection were identified as moderately resistant. Susceptible plants and KS showed larger and longer sori (Fig-ID) which later fused to form a mat on the leaf surface. Seedlings in the F_4 generation, tested under laboratory conditions also showed differences in the resistant (Fig.-1B) and susceptible (Fig.-1E) reactions, and true breeding plants could be confirmed. Figures 1C and 1F show the enhanced images of resistant and susceptible reactions respectively. Leaf rust resistance was also observed when there was natural leaf rust incidence.

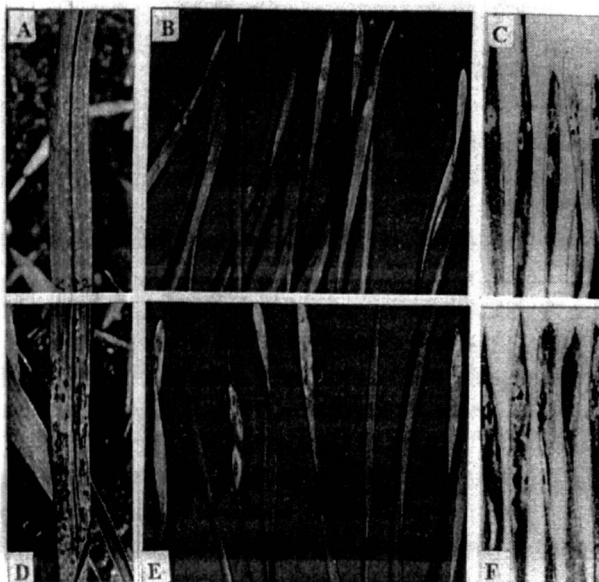


Fig. 1. The rust reaction of moderately resistant selection in Kalyansona background (A) and susceptible Kalyansona (D) in field conditions and progeny of moderately resistant (B) and susceptible (E) plants from segregating population in Sonalika background in laboratory screening. (C) and (F) show enhanced images of moderately resistant and susceptible reaction respectively.

Chemical and dough properties

SDS-PAGE: The selections showed true breeding patterns for subunits 5+10 (Fig.-2A and 2B).

Grain protein: Grain protein ranged from 12.84 in KS control to 15% in two of the selections (Table-1). Four of the six selections were significantly higher than KS. In the second set, SK control was lower than USK and the selection with subunits 5+10. The differences in protein content were found to be variable between years perhaps because they

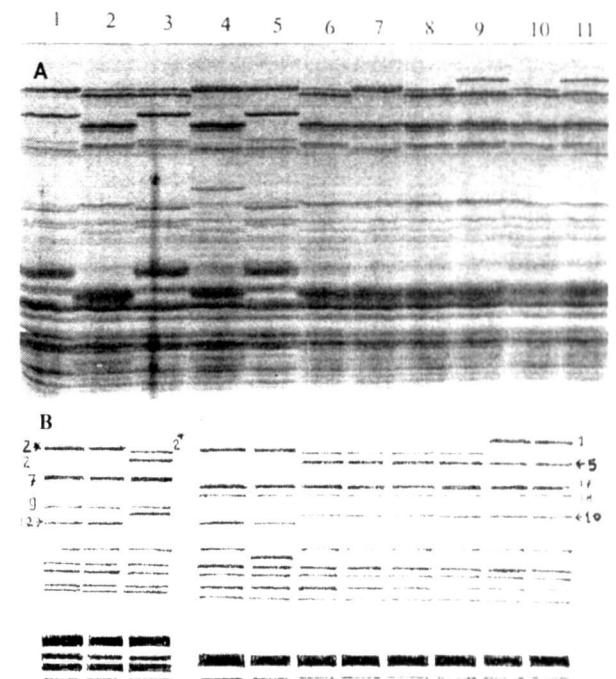


Fig.2 A. Electrophoregram of recurrent parents Kalyansona and Sonalika and their selection with subunits 5+10 resolved on SDS-PAGE. From left to right: SK, 13-7-10, 14-12-16, UKS, USK, 13-27-30, KS, 15-23-24, 13-19-22, 13-11-14, 15-3.

B. Electrophoregram of the recurrent parents Kalyansona, Sonalika and selection with subunits 5+10. From left to right: Sonalika, Unnath Sonalika, Sonalika derivative with subunits 5+10, Kalyansona, Unnath Kalyansona, derivatives of Kalyansona with subunits 2*, 17+18, 5+10, and 1, 17+18, 5+10. (Line diagram of fig 2A with lanes rearranged).

Table 1. Rust reaction and some quality related characteristics of parents and selections.

| Parent/ Selection | HMW subunits | Rust reaction | Grain protein, % | SDS-SV mm | Dry gluten, % | F R ratio |
|-------------------|--------------|---------------|------------------|-----------|---------------|-----------|
| KS | 2 + 12 | S | 12.84 | 78.8 | 13.43 | 1.6675 |
| UKS | 2 + 12 | MR | 13.10 | 75.0 | 13.87 | 1.5886 |
| 13-7-10 | 5 + 10 | MR | 13.06 | 80.3 | 14.18 | 1.7687 |
| 13-27-30 | 5 + 10 | MR | 14.20 | 78.2 | 14.13 | 1.7356 |
| 15-23-24 | 5 + 10 | MR | 13.48 | 77.5 | 13.88 | 1.8633 |
| 13-11-14 | 5 + 10 | MR | 14.95 | 78.0 | 13.95 | 1.7428 |
| 13-19-22 | 5 + 10 | MR | 15.00 | 78.5 | 13.86 | 1.7837 |
| 15-3 | 5 + 10 | MR | 15.00 | 82.0 | 14.90 | 1.8594 |
| SK | 2 + 12 | S | 12.32 | 56.8 | 14.76 | 1.4588 |
| UKS | 2 + 12 | MR | 13.81 | 62.0 | 14.98 | 1.5157 |
| 14-12-16 | 5 + 10 | MR | 14.05 | 80.0 | 15.68 | 1.6013 |
| LSD (5%) | | | 1.17 | 3.7 | 1.05 | 0.184 |

were due to developmental and environmental factors rather than due to genetic factors.

SDS-SV: The selections 13-7-10 and 15-3 showed significantly higher SDS-SV than UKS and numerically higher than KS control in this

experiment. The KS control was higher than expected. Other selections were numerically higher than UKS but not higher than KS control. In the other set, selection 14-12-16 with subunits 5+10 was significantly higher than both SK and USK.

Gluten and E.R. ratios: The association between dry gluten % and protein % was positive but not significant in case of KS and its derivatives. For SK and its derivatives, grain protein % and dry gluten % were in agreement. KS and UKS did not differ significantly in the E.R. ratios. All selections were numerically higher and selections 15-3 and 15-23-24 were significantly higher than KS or UKS. The selection in Sonalika background with subunits 5+10 was numerically higher than SK and USK (Table-1). The selections showed influence of recurrent parent background.

Dough properties: Based on the SDS-SV data and HMW subunit composition and yield data, two selections in KS background and one in SK background were included in the study of dough properties using Farinograph (Table-2). Water absorption of KS (82.2%) was higher than SK (77.5%). Selection 15-23-24 (80.5%) was lower than KS control and the other selection 13-7-10

Table 2. HMW subunits composition, water absorption capacity and dough characteristics of parents and selections

| Sample | HMW Subunits | Water absorptions, % | Mixing time min* | Dough stability min* | Mechanical tolerance index B.U.* |
|----------|---------------|----------------------|------------------|----------------------|----------------------------------|
| KS | 2*,17+18,2+12 | 82.2 | 7.0 | 4.0 | 70 |
| 13-7-10 | 2*,17+18,5+10 | 82.2 | 10.75 | 10.25 | 40 |
| 15-23-24 | 2*,17+18,5+10 | 80.5 | 10.5 | 5.0 | 55 |
| SK | 2*,7+9,2+12 | 77.5 | 4.75 | 0.5 | 120 |
| 14-12-16 | 2*,7+9,5+10 | 79.0 | 8.25 | 2.5 | 55 |

* values are means of two determinations on 1997-98 harvest.

(82.2%). Selection 14-12-16 in SK background was higher than SK control.

Selections in KS background had (1) higher mixing time than KS (Fig.-3 A, B, C), (2) higher dough stability and (3) lesser deterioration after 12 minutes of mechanical abuse. The two selections in KS background themselves showed some difference for dough stability and mechanical tolerance index.

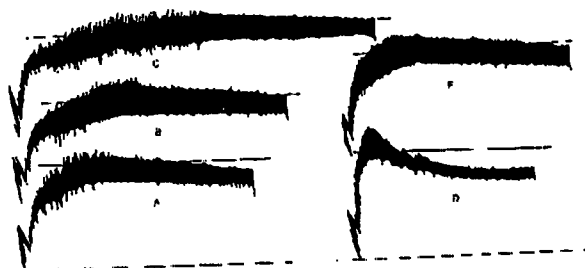


Fig.3. Farinograph tracings of the parents A) Kalyansona, its derivatives B) 15-23-24, C) 13-7-10 and D) Sonalika and its derivatives E) 14-12-16 with subunits 5+10.

The selection in SK background (Fig.-3E) was superior than SK control (Fig.-3D) for all the above mentioned three criteria. The selection in SK background was lower for the three criteria than the selections in the KS background.

Grain yield

The grain weight and grain yield of parents and selections are shown in Table-3. Although the selections were result of backcrossing experiments, differences existed due to less number of backcrosses (equivalent of three and one). The mean thousand grain weights (TGW) for three years given in Table-3 shows that three out of six selections were higher than KS control for TGW. Selection 13-7-10 was significantly higher than KS for grain yield in both years station trials (1997-98 and 1998-99). Selection 15-23-24 and 13-11-14 were significantly higher for grain yield than KS in one year (1997-98). The selection 14-12-16 in the background of SK was lower than SK in all the years. The selections resembled their respective recurrent parents in

Table 3. Grain yield and thousand grain weight (TGW) of parents and selections.

| Parent/Selection | Grain yield*, g m ⁻² | TGW*, g |
|------------------|---------------------------------|---------|
| KS | 235.26 | 31.31 |
| UKS | 220.13 | 32.49 |
| 13-7-10 | 295.53* | 33.11 |
| 13-27-30 | 273.26 | 29.23 |
| 15-23-24 | 315.15** | 33.67 |
| 13-11-14 | 270.36* | 34.77 |
| 13-19-22 | 211.73 | 26.99 |
| 15-3 | 187.73 | 29.91 |
| SK | 223.7 | 42.25 |
| USK | 210.33 | 42.22 |
| 14-12-16 | 168.33 | 34.25 |

* mean of three years, ** mean of two years, † significantly higher than KS in the year 1997-98, * significantly higher than KS in 1997-98 & 98-99.

appearance, flowering and maturity time (data not given).

DISCUSSION

The subunits 5+10 are common in the hexaploid wheats available in India. Among cultivars and experimental stocks, 66% had these subunits (Bhagwat and Bhatia 1988). Analysis of thirty and fifty bread wheat cultivars showed that high Glu-1 scores were frequent and 19 and 12% respectively had subunits 5+10 (Harinder and Payne 1995, Das and Bhagwat 1999). In the entries being tested in the All India Coordinated trial, 41% in the irrigated timely sown category and 37% in the rainfed or late sown category showed the presence of subunits 5+10 (DWR Progress report, 1997-98). The association between subunits 5+10 and better dough strength is well documented. There is, however, need for work on the feasibility and effectiveness of

subunits 5+10 as a selection criterion to improve quality in the Indian context. Conscientious selection for factors contributing to dough strength is needed in the programmes rather than their inclusion by chance. Previously, subunits 5+10 from two sources were transferred in a common background using KS as a recurrent parent and improvement associated with subunits 5+10 was observed in terms of better SDS-SV (Bhagwat and Bhatia 1993). Our previous and current results are in agreement with a large number of previous reports that the subunits 5+10 are associated with higher dough strength.

In these experiments, subunits 5+10 derived from one source (TW-1) were transferred into two different backgrounds KS and SK and a numerical or significant increase in SDS-SV was observed. The enhanced dough strength was also reflected in improvement of mixing time, dough stability and mechanical tolerance index. The presence or absence of subunits 5+10 and some variation in the grain protein % would largely explain the variation in the flour and dough properties. The marked difference in SK and its derivative with subunits 5+10 can also be explained on the basis of presence of subunits 5+10 and higher protein %. Some differences, particularly among the selections can be attributed to differences in the genetic background. Rheological properties are also known to improve with protein content (MacRitchie 1988). The improvement in dough properties was observed in the presence of moderate rust resistance conferred by *Sr24Lr24* indicating that the advantages of these characters could be combined effectively. The segment from *Agropyron elongatum* apart from *Sr24Lr24*, also carries genetic material influencing other traits, such as improvement of resistance to Barley Yellow Dwarf Virus and terminal clubbiness of spikes (Menon and Tomar 2001). Quality can be affected by the genetic manipulation, an extreme example of this is the sticky dough problem associated with the 1B/IR translocation (Dhaliwal *et al.* 1987, Bullrich *et al.* 1998), which also confers disease resistance. The quality traits in presence of subunits 5+10 in this study were unaffected by the incorporation of the segment carrying *Sr24Lr24*. Although, the grain yield and grain weight were not the primary criteria, selections with higher grain yield potential than KS could be observed, which is in contrast to studies by Carillo *et al.* (1990), who reported that in a particular cross high yield potential was strongly associated with the HMW glutenin alleles that are attributed to low bread making quality. Combining grain yield in the selection procedure may result in selection with better yield, better dough strength and rust resistance at least in

certain crosses.

SDS-SV was determined using Dick and Quick (1983) method originally recommended for durum wheats. Although less sensitive, it has an advantage of using 1g sample. It may be used in *T. aestivum* samples where large differences are likely to exist and other confirmatory tests are also being used. SDS-SV of KS control was higher than expected in the year 1997-98. The only difference in KS control and other entries in the year 1997-98 was that KS control was most susceptible to leaf rust which occurred naturally in that year. SK control, which is also susceptible to leaf rust, showed lesser development of leaf rust perhaps due to its different genetic background and earlier maturity. The heavy rust infection may have affected grain properties of KS control. Higher dough mixing strength for leaf rust infected lines compared to rust resistant lines has been reported previously (Dyck and Lukow 1988).

Higher dough mixing time and dough stability in the selections used in this study showed that subunits 5+10 could be used as a selection criterion. However the differences among the selections showed that the extent of improvement will depend upon genetic background. Similar results are reported by Hussain *et al.* (1998) using isolated gluten. The mixing time of subunit combinations 2*, 17+18, 5+10 was longer than that of 2* 7+9, 5+10. This is similar to the result of Lindahl *et al.* (1996) where longest mixing time is reported to be associated with strongest HMW subunit combination. Similar trend is also seen in the case of E.R. ratio values.

In conclusion, the results in this study showed that subunits 5+10 can be effectively combined with moderate rust resistance conferred by *Sr24Lr24* and simultaneous improvement in rust resistance and dough strength as measured by Brabender Farinograph can be obtained. This improvement can also be combined with better yield potential. This approach of purposefully selecting specific subunits and appropriate resistance genes may be helpful in developing selections with higher dough strength specially suitable for specific end use such as bread making.

ACKNOWLEDGEMENT

We thank Dr. D. C. Joshua for suggestions in preparing the manuscript.

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