

1BL/1RS translocation in durum wheat and its effect on end use quality traits

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Abstract The gluten proteins document the genotypic identity of a wheat variety, in addition to providing valuable clues about its ancestry and technological properties. In this study, an Indian durum wheat genotype B662 was identified to carry 1BL/1RS translocation and characterized further for its effect on end use quality traits. Comparison of the end use quality traits of B662 with five other durum cultivars without 1BL/1RS, showed decreased gluten content, lower swelling index of glutenins and low MSDS-SV indicating that, B662 with 1BL/1RS is not good for pasta making. In F_{2,3} seeds from a durum wheat cross between the 1BL/1RS cultivar B662 and HI8498 without the translocation, the secalin *Sec-1* loci segregated in theoretically expected 3:1 proportion and were inherited as a block of the rye chromosome arm. The analysis of F_{2,3} harvests for the two most important durum wheat quality tests showed that the presence of 1BL/1RS translocation did not alter the grain protein content values, but was associated with significant reduction of micro SDS-sedimentation volume indicating inferior quality, thus limiting the commercial exploitation of durum wheat genotypes with 1BL/1RS translocation. The cautious use of rye translocation in Indian durum wheat breeding is suggested.

Keywords Durum wheat · 1BL/1RS · Protein content · Gluten strength · Quality

Abbreviations

PC Protein content

HMW-GS High molecular weight glutenin subunits
LMW-GS Low molecular weight glutenin subunits
SDS Sodium dodecyl sulphate
MSDS-SV Micro sodium dodecyl sulphate sedimentation volume
PAGE Polyacrylamide gel electrophoresis
NIT Near Infrared Transmittance
SSDSSV Specific SDSSV

Introduction

Translocation 1BL/1RS is one of the most widely utilized sources of alien chromatin in the history of bread wheat (*Triticum aestivum* L.) breeding (Braun et al. 1998). In cultivars with 1BL/1RS translocation, the short arm of the 1B wheat chromosome is replaced by the short arm of the 1R rye (*Secale cereale* L.) chromosome. The translocation has been used extensively in bread wheat improvement programs all over the world as a source of disease and pest resistance as well as for higher grain yield potential (Rajaram et al. 1983; Zeller and Hsam 1984). It also increases above ground biomass, deep root system, canopy water status and abiotic stress tolerance particularly, drought tolerance (Ehdaie et al. 2003; Sharma et al. 2009; Howell et al. 2014).

However, serious defects in bread-making quality such as poor mixing tolerance, superficial dough stickiness and low bread volume have been associated with the presence of the translocation (Dhaliwal et al. 1987; Peña et al. 1990; Lee et al. 1995). The presence of the rye storage proteins secalins encoded by the *Sec-1* locus is associated with dough stickiness while the loss of the wheat low

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molecular weight glutenins (encoded by the *Glu-B3* loci) and possibly the linked gliadins (encoded by the *Gli-B1* loci) results in reduced dough strength (Graybosch et al. 1993). Changes in the balance between monomeric and polymeric proteins in flour have been related to dough stickiness and weakness in translocation lines (Dhaliwal et al. 1987; Graybosch et al. 1990, 1993). Influence of genetic background on the bread-making quality of 1BL/1RS lines has also been demonstrated (Lee et al. 1995; Moreno-Sevilla et al. 1995).

The 1BL/1RS translocation has been introduced into tetraploid wheat lines by crossing the 1BL/1RS bread wheat and durum wheat (Friebe et al. 1987). As expected, these lines showed race-specific resistance against rust due to the *Lr26*, *Sr31* and *Yr9* genes on rye chromosome 1RS. Although the introduction of the 1BL/1RS translocation could improve the agronomic performance under drought-stress conditions, its negative effect on gluten strength could limit the commercial exploitation of durum wheat genotypes with 1BL/1RS translocation (Boggini et al. 1998; Zarco-Hernandez et al. 2005).

The low molecular weight glutenin subunits (LMW-GS) represent about 33 % of the grain protein and nearly 60 % of total glutenins and are essential in determining dough physical properties such as, dough extensibility and gluten strength. Earlier studies have emphasized the importance of LMW-GS in determining the physical dough properties in durum wheats. Two types of LMW-GS, designated LMW-1 (linked to γ -42 gliadin) and LMW-2 (linked to γ -45 gliadin) are related to poor and strong gluten elasticity and to superior and inferior pasta quality respectively in durum wheat. Nieto-Taladriz et al. (1997) showed that LMW-1 and LMW-2 are composed of different LMW glutenin subunits encoded by *Glu-A3*, *Glu-B3* and *Glu-B2* loci and allelic variations at these loci are important for quality. However, difficulties in scoring alleles of the LMW-GS have limited their use in research and hindered the selection for specific LMW-GS alleles in wheat breeding programs. Further improvement in one dimensional SDS-PAGE separation protocols have allowed better discrimination between LMW-GS alleles and opened up the possibility of using them effectively in breeding (Singh et al. 1991; Nieto-Taladriz et al. 1997; D'Ovidio and Masci 2004; Liu et al. 2010).

When several exotic, local and other durum wheat genotypes were analyzed for glutenin and gliadin subunits (Oak et al. 2002; Sai Prasad et al. 2003), one genotype B662 from IARI Regional Station, Indore (Mishra et al. 2001), registered as disease resistant stock for all Indian wheat rusts isolates, interestingly showed only one LMW B glutenin subunit. This indicated that it may be null to *Glu-A3* or *Glu-B3* loci and can be used to understand the inheritance of complex LMW-GS loci in Indian durum

wheats. However, during further studies it was found that B662 is durum wheat with 1BL/1RS translocation and hence does not contain *Glu-B3* coded LMW-GS. In the present article we report the results of the studies on B662 and effect of 1BL/1RS translocation on end use quality traits. There are very few studies on 1BL/1RS containing durum wheat and to our knowledge this is the first report of such study from India.

Materials and methods

Plant material

Seeds of durum wheat (*Triticum turgidum* ssp. *durum*) genotypes PBW34, HI8498, B662, bread wheat (*Triticum aestivum*) Chunmai 18 and F₁ seeds of cross HI8498 × B662 were kindly provided by Dr. HN Pandey (IARI Regional Station, Indore, India). Durum wheat genotypes/cultivars B662, HI8498, MACS3125, WH896, PDW233 and MACS2846 were grown in randomized block design with three replications at the Institute farm. Single F₁ plant harvest (F₂ seeds) of cross HI8498 × B662 was sown with space planting and harvest (F₃ seeds) was used for protein profiling, and measurement of protein content and micro SDS sedimentation volume (MSDS-SV).

Electrophoresis and quality studies

Grain protein content (PC) was measured by NIT (Near Infrared Transmittance) spectroscopy on Infratech Grain Analyser 1241 (Foss, Sweden) and expressed on constant moisture content basis (14 %). Seeds were milled using Tecator Cyclotech mill (Tecator, Hoganas, Sweden) with 1 mm sieve and flour was used for electrophoretic and quality studies. Ethanol (70 %) soluble proteins from harvest of each F₂ plant (20 mg flour) and other durum wheat cultivars were analysed by SDS-PAGE according to Singh et al. (1991). Chinese Spring was used as a standard for glutenins and gliadins. Gliadins were separated using acid PAGE as described in Oak et al. (2002). Micro SDS sedimentation volume (MSDS-SV) was determined as described by Dick and Quick (1983). Specific SDSSV (SSDSSV) was calculated by dividing MSDS-SV by protein content. Gliadins and glutenins were also analyzed by reversed-phase HPLC (RP-HPLC) according to Larroque et al. (2000). Mixographic properties were analysed as described in Oak et al. (2011).

Statistical analysis

All the statistical analyses were done using Excel program (MS Office 2010).

Results and discussion

During the analysis of several exotic, local and other durum wheat genotypes for their glutenin and gliadin subunits, genotype B662 from IARI, Indore, showed only one LMW B glutenin subunit, indicating probable presence of null allele at *Glu-A3* or *Glu-B3* loci and hence its possible use as a suitable background to understand the complex inheritance of different LMW-GS in Indian durum wheats. With this objective, HI8498 with LMW-2 type glutenin subunits was crossed with B662. The glutenin analysis of parental genotypes showed that the B662 is null at *Glu-B3* and *Glu-B2* and contains the LMW-GS subunits coded by *Glu-A3* only (Fig. 1). In the analysis of the gliadin profiles using SDS-PAGE (Fig. 2) and Acid PAGE (Fig. 3), B662 showed null for *Gli-B1*, however, presence of secalins, which are monomeric proteins, coded by 1RS chromosome of rye (Figs. 2, 3) was observed. The presence of secalins was confirmed further by the RP-HPLC of gliadins of B662 (Fig. 4) as well as PCR amplification using *Glu-B3* and *Sec-1* primers (data not shown). To test the inheritance of the 1BL/1RS translocation in B662, parental genotypes Chunmai 18 (bread wheat) and PBW34 (durum wheat) were analyzed for gliadins and glutenins using SDS-PAGE (Figs. 1, 2). Based on the analysis, it was found that the Chunmai 18 carries 1BL/1RS translocation and is the donor of the rye 1RS chromosome to B662 (Figs. 2, 3). Literature search for the confirmation of presence of 1BL/1RS in Chunmai 18 revealed that it is old Chinese bread wheat and does not contain any rye chromosome (personal communication Dr. Sergey Martynov, N.I. Vavilov Institute of Plant Production, St. Petersburg, Russia). From this information, it appears that the genotype

which was used for crossing was not true Chunmai 18. The cross with Chunmai 18 was originally intended to produce triple dwarf durum wheat, by incorporation of *Rht 8* gene from Chunmai 18 (Mishra et al. 2001). However, the resultant genotype B662 also showed very high resistance to stem rusts along with reduced height (triple dwarf, height <50 cm) (Mishra et al. 2001). Therefore, B662 appears to have resulted from unintentional transfer of 1BL/1RS translocation during breeding due to use of wrong genotype as a parent. Due to transfer of 1BL/1RS chromosome, it shows presence of secalins and lacks *Glu-B3* associated glutenins while the *Glu-A3* allele is from its durum parent PBW34. The observed resistance to all rust races prevalent in India may also be due to the presence of rust resistance

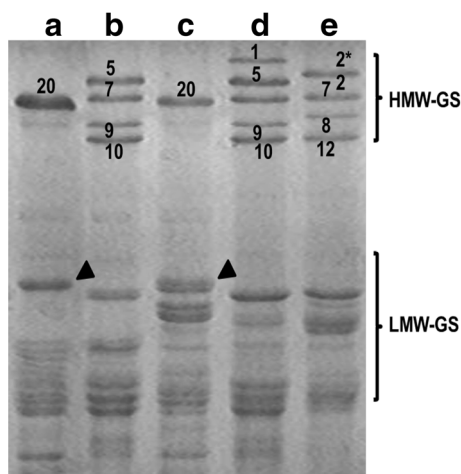


Fig. 1 SDS-PAGE separation of glutenins. **a** B662, **b** Chunmai 18, **c** PBW34, **d** MACS2496, **e** Chinese Spring. The LMW-GS band coded by *Glu-A3* is indicated with *arrow heads* in **a** B662 and **c** PBW34

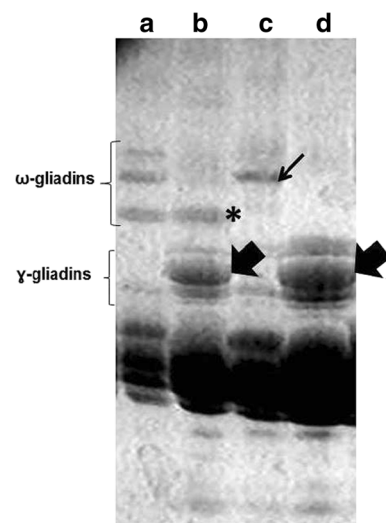
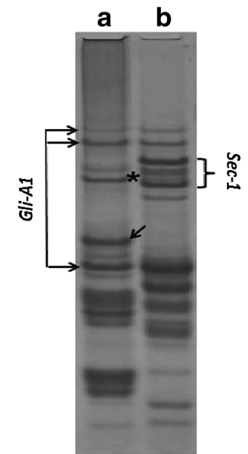


Fig. 2 SDS-PAGE of 70 % ethanol soluble monomeric proteins (gliadins). **a** Chinese Spring, **b** Chunmai 18, **c** PBW34, **d** B662. *Thick arrows* denote the secalins coded by 1RS, *thin arrow* the omega gliadin coded by *Gli-B1* and *asterisk* the omega gliadin coded by *Gli-D1*

Fig. 3 Acid PAGE profiles of gliadins. **a** HI8498, **b** B662. Secalins coded by *Sec-1* loci seen in B662 are indicated by *bracket*. *Arrow* and *asterisk* denote *Gli-B1* coded gamma gliadin 45 and omega gliadin 35 respectively only in HI8498. *Gli-A1* coded bands denoted as a *block* are similar in HI8498 and B662



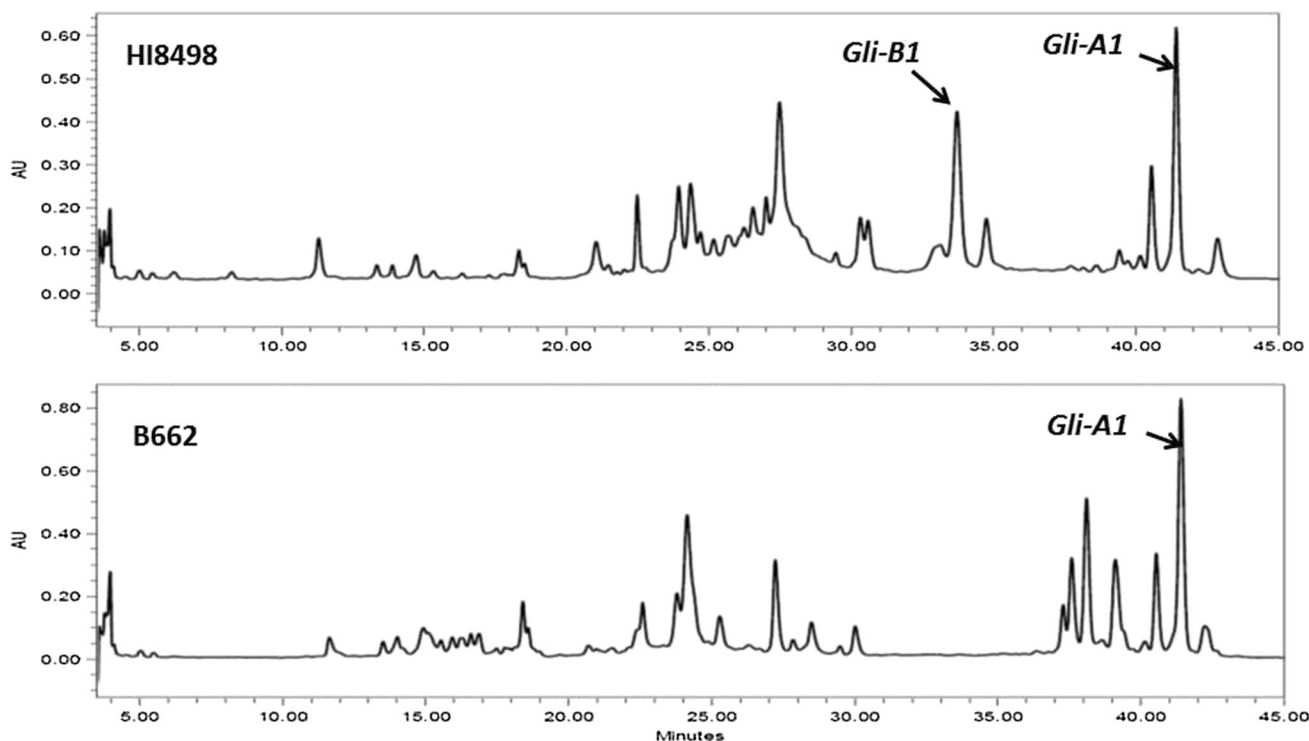


Fig. 4 RP-HPLC profiles of durum wheat gliadins. *Gli-B1* peak is absent in B662

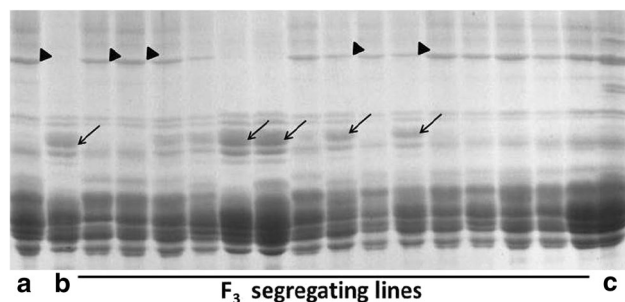


Fig. 5 SDS-PAGE separation of gliadins of $F_{2:3}$ segregating lines. **a** HI8498, **b** B662, **c** Chinese spring. Arrow heads denote *Gli-B1* loci, thin arrows *Sec-1* loci

genes associated with the rye chromosome segment. Since presence of 1BL/1RS translocation is not very common in durum wheat, its effect on end use quality in durum background was studied. Total 105 F_2 progeny harvest (F_3 seeds) derived from HI8498 and B662 cross was analyzed for protein profiles (Fig. 5), gluten strength (SDS-sedimentation) and grain protein content and the results are summarized in Table 1. Theoretically expected 3:1 segregation was observed for secalin *Sec-R1* loci ($\chi^2 = 1.36$, $p = 0.3-0.2$, $df = 1$) and *Gli-B1* loci ($\chi^2 = 1.10$, $p = 0.3-0.2$, $df = 1$), confirming the inheritance of rye chromosome arm translocation as a block. When both *Gli-B1* and *Sec-R1* loci, were considered together, expected

Table 1 Mean values of quality traits of $F_{2:3}$ progeny with homozygous *Gli-B1* and *Gli-R1* loci

Quality traits	Loci compared, mean \pm SD		df	t value
	<i>Gli-B1</i> ^a	<i>Gli-R1</i> ^b		
MSDS-SV (mm)	47.7 \pm 6.18	29.2 \pm 3.52	45	12.7***
PC (%)	12.9 \pm 0.76	12.8 \pm 0.69	45	0.667 ns
SSDSSV	3.71 \pm 0.52	2.28 \pm 0.28	45	11.9***

ns non-significant

Significance was tested using student *t* test, *** $p = 0.0001$

^a Values of 24 homozygous progenies

^b Values of 23 homozygous progenies

1:2:1 segregation was observed ($\chi^2 = 1.94$, $p = 0.2-0.1$, $df = 2$). The data showed that the grain protein content was not affected by presence of rye chromosome (Table 1). However, the gluten strength measured by micro SDS sedimentation volume was significantly reduced. Similar effect of 1BL/1RS chromosome has also been reported in earlier studies on bread wheats and other 1BL/1RS containing durum wheats. Further analysis of B662 and five other durum wheat varieties (without 1BL/1RS) using mixograph analysis and swelling index of glutenins also showed that the B662 is very poor in gluten strength with lower dough stability (Fig. 6, Table 2). This observation was in agreement with the published report on B662 end use quality by Sai Prasad et al. (2006).

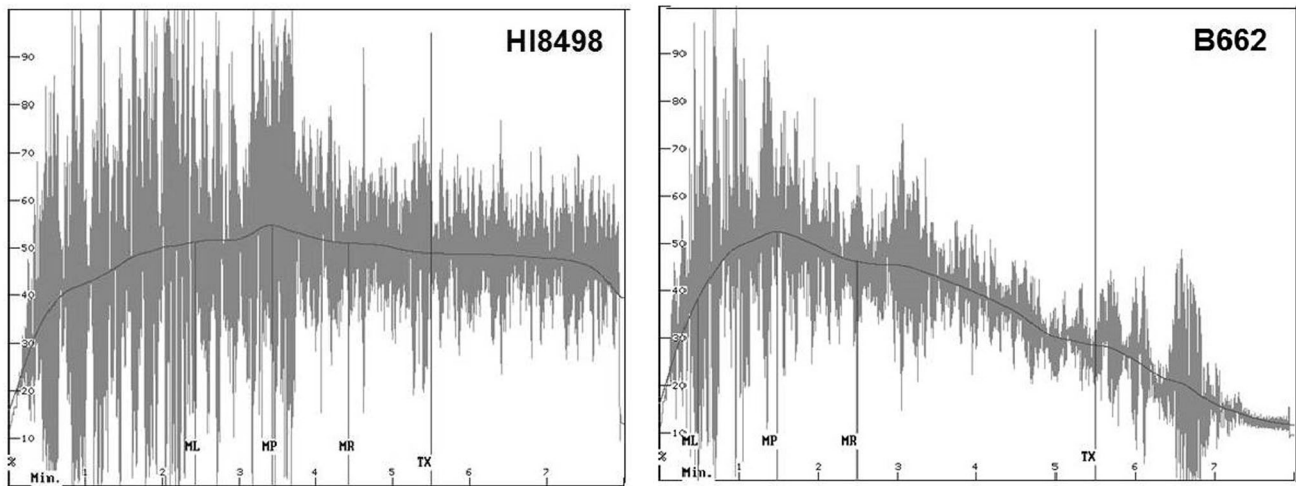


Fig. 6 Dough mixing profiles of HI8498 (*strong*) and B662 (*weak*)

Table 2 Comparisons of B662 and other durum wheat cultivars for gluten strength, swelling index of glutenins and dough mixing properties

Name	MPT (min)	MPV (%)	MPW (%)	MPIT (%*tq/min)	MSDS-SV (mm)	SIG	PC (%)
HI 8498	3.72	56.78	49.96	171.90	44.50	4.5	12.8
MACS 2846	2.66	56.40	45.59	118.93	40.50	3.7	13.6
MACS 3125	5.32	52.34	32.15	246.16	42.50	3.9	12.2
PDW 233	2.83	51.28	52.54	115.38	43.00	4.2	12.1
WH 896	3.92	54.77	55.62	173.04	50.00	4.3	11.8
B 662	1.48	52.45	32.57	81.78	26.00	2.4	12.7
Mean	3.32	54.00	44.74	151.20	41.08	3.8	12.5
LSD (0.05)	0.29	3.24	3.17	8.91	2.90	0.3	0.5
LSD (0.01)	0.41	4.61	4.51	12.67	4.13	0.4	0.7

Bold significance at $P = 0.05$ %, Bold and Italics significance at $P = 0.01$ %

MPT Mixograph peak time in min, MPV mixograph peak value, MPW mixograph peak width, MPIT mixograph work input at peak time, SIG swelling index of glutenins

In conclusion, although B662 is reported to be resistant to all the rust races in India (Mishra et al. 2001, 2005, 2015), it should be used with caution in Indian durum wheat breeding program since all rust resistance genes on 1RS are no longer effective in bread wheat due to evolution of matching rust virulences. The presence of 1BL/1RS greatly reduces the end use quality and hence is not useful for traditional foods or pasta products thus limiting the commercial exploitation of durum wheat genotypes with 1BL/1RS translocation. The poor quality characteristics of durum wheat containing the 1BL/1RS translocation could be improved through three possible genetic approaches without affecting their yield potential. The first approach is deletion of a chromosome segment containing the *Sec-1* locus and addition of loci *Glu-B3a* or *Glu-B3c* alleles/ γ -45, by the centric breakage-fusion manipulation of the translocation, as described in bread wheat (Lukaszewski 2000). Alternatively, *Sec-1* locus can be deleted by

irradiation. The inferior effects can also be reversed or compensated by introduction of *Glu-D1* (5 + 10) locus using 1DL/1AS translocation (Gennaro et al. 2012).

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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