

the wheat-*Aegilops* homoeologous relationships between the examined *Aegilops* chromosomes. The selection of wheat-*Aegilops* homoeologous recombinations could be successful in later generations.

Molecular cytogenetic evaluation of chromosome instability in *T. aestivum*-*S. cereale* disomic addition lines. The genetic stability of wheat-rye (Chinese Spring-Imperial) disomic addition lines was checked using the Feulgen method and FISH. Feulgen staining detected varying proportions of disomic, monosomic, and telosomic plants among the progenies of the disomic addition lines. The greatest stability was observed for the 7R addition line, whereas the most unstable lines were those with 2R and 4R additions. Chromosome rearrangements also were detected using FISH. Based on the specific hybridization patterns of repetitive DNA probes pSc119.2 and (AAC)5, as well as ribosomal DNA probes (5S and 45S), isochromosomes were identified in the progenies of 1R and 4R addition lines. These results draw attention to the importance of continuous cytological checks on basic genetic materials by using FISH, because this method reveals chromosome rearrangements that could not be detected either with the conventional Feulgen staining technique or with molecular markers.

Selection of U and M genome-specific wheat SSR markers using wheat-*Ae. biuncialis* and wheat-*Ae. geniculata* addition lines. Wheat SSR markers specific to the U and M genomes of *Aegilops* species were selected. A total of 108 wheat SSR markers were successfully tested on *Ae. biuncialis* ($2n = 4x = 28$, $U^bU^bM^bM^b$), on five wheat-*Ae. biuncialis* addition lines (2M^b, 3M^b, 7M^b, 3U^b, and 5U^b) and on a wheat-*Ae. geniculata* (1U^g, 2U^g, 3U^g, 4U^g, 5U^g, 7U^g, 1M^g, 2M^g, 4M^g, 5M^g, 6M^g, and 7M^g) addition series. Among the markers, 86 (79.6%) were amplified in the *Ae. biuncialis* genome. Compared with wheat, polymorphic bands of various lengths were detected in *Ae. biuncialis* for 35 (32.4%) of the wheat microsatellite markers. Three of these (8.6%) exhibited specific PCR products in wheat-*Ae. biuncialis* or wheat-*Ae. geniculata* addition lines. The primers GWM44 and GDM61 gave specific PCR products in the 2M^b and 3M^b wheat-*Ae. biuncialis* addition lines, but not on the 2M^g addition line of *Ae. geniculata*. A specific band was observed on the 7U^g wheat-*Ae. geniculata* addition line using the BARC184 primer. These three markers specific to the U and M genomes are helpful for the identification of 2M^b, 3M^b, and 7U^g chromosome introgressions into wheat.

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ITEMS FROM INDIA

BHABHA ATOMIC RESEARCH CENTRE

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Application of Real-Time PCR in marker-assisted selection for stem rust resistance gene Sr24.

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Introduction. Real-Time PCR (RT-PCR) is a technique mainly used to amplify and simultaneously quantify a targeted DNA molecule (Gibson et al. 1996). Currently, four different chemistries, TaqMan® (Applied Biosystems, Foster City, CA, USA); Molecular Beacons (Newark, New Jersey, USA); Scorpions® (Sigma-Aldrich, St. Louis, MO, USA); and SYBR® Green (Life Technologies, Carlsbad, CA, USA), are available for RT-PCR. All of these chemistries allow

detection of PCR products via the generation of a fluorescent signal. Among the SYBR Green is a fluorogenic dye that exhibits little fluorescence when in solution, but emits a strong fluorescent signal upon binding to double-stranded DNA (Arya et al. 2005).

Real-time PCR can be applied to traditional PCR applications as well as new applications that would have been less effective with traditional PCR. With the ability to collect data in the exponential growth phase, the power of PCR has been expanded into applications such as viral quantitation, quantitation of gene expression, array verification, drug therapy efficacy, DNA damage measurement, quality control and assay validation, pathogen detection, and genotyping. Recently, this technique has been used to develop molecular markers and to evaluate critical aspects for olive oil authentication (Giménez et al. 2010).

This study used RT-PCR as a tool in the marker-assisted selection (MAS) in crop plants in general, and wheat in particular. Screening for stem rust resistance gene *Sr24* by RT-PCR was carried out using primers specific to a SCAR marker.

Materials and methods.

Plant material. The wheat genotypes and segregating lines used in this study are listed in Table 1.

DNA extraction and quantification. DNA was extracted from the leaves of 1 month old wheat seedlings according to Nalini et al. (2004). The DNA was quantified by using fluorimeter (Hoefer DyNA Quant 200).

Polymerase chain reaction. PCR screening used a Realplex4 (Eppendorf, Germany). A SCAR marker (SCS1302609) for the *Sr24/Lr24* gene (Gupta et al. 2006) using specific primers (5' CGCAGGT-TCCAAATACTTTTC 3' and 5' CGCAGGTTC-TACTAATGCAA) were used in a total volume of 25 µl reaction mixture containing 1X PCR buffer (10 mM Tris-HCl (pH-9.0), 1.5 mM MgCl₂, 50 mM KCl, and 0.01% gelatin), 100 µM of each dNTP (Sigma, St. Louis, MO, USA), 0.75 U Taq DNA Polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India), 4.0 picomoles of each primer, 0.4X SYBR green dye (Sigma, St. Louis, MO, USA), and 100 ng of genomic DNA. Amplifications were performed using the following thermal cycling profile: 1 cycle of 94°C for 2 min, 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 7 min.

Analysis of results. The presence or absence of a band in MAS were analyzed in three ways, using a quantification curve, by a melting analysis, and using a +/- assay of the RT-PCR technique. To compare RT-PCR results, PCR products were resolved on 2% agarose gels, stained with ethidium bromide solution (0.5%), and visualized under a UV transilluminator where the presence or absence of bands were scored.

Table 1. Screening of genotypes for presence of stem rust resistance gene *Sr24* using RT-PCR. Cultivars with an * are the F₂ of the cross 'Kalyan Sona/Vaishali' (phenotyping of the individuals by rust inoculations were by Das et al. (2006). *Sr24* gene status: RR = homozygous, Rr = heterozygous). Melting curve samples were scored positive if the melting temperature was 83.9°C. +/- assay samples were scored as positive if the peak was above the threshold line.

Cultivar	<i>Sr24</i> gene status	Scoring for band based on:		
		Ethidium bromide staining followed by PCR	Melting curve	+/- assay
Unnath Kalyan Sona	+	+	+	+
KS-1	+	+	+	+
KS-3	+	+	+	+
Unnath Sonalika	+	+	+	+
FLW-2	+	+	+	+
Kalyan Sona	-	-	-	-
PBW343	-	-	-	-
MACS 2496	-	-	-	-
B-6 (154A)	+	+	+	+
Vaishali	+	+	+	+
Vidisha	+	+	+	+
Agra Local	-	-	-	-
163B*	+(RR)	+	+	+
163C*	+(Rr)	+	+	+
164A*	+(RR)	+	+	+
164B*	+(Rr)	+	+	+

Results and discussion. The protocol parameters were optimized. We observed that samples with DNA concentration of 100 ng and a primer concentration of 4.0 picomoles (each) gave well resolved peaks. Thermal cycling conditions were similar to that used in the Master Cycle Gradient 5300.

Analysis using a quantification curve.

Progress of DNA amplification during PCR could be monitored in real time by measuring the intensity of fluorescent dyes during amplification using quantification curve. A quantification curve is the curve obtained by plotting the increase in fluorescence (Y axis) as the amplification of the target DNA is started (X axis). Carriers increase in fluorescence as the amplification of target DNA started, and non-carriers of *Sr24* gene showed no increase in fluorescence because it lacks the target DNA (Fig. 1). However, using a quantification curve for the analysis needs to be further standardized.

Analysis using a melting curve.

Melting curves were performed at the end of SYBR green quantitative RT-PCR to check for primer-dimer or nonspecific product formation. Using plots of dI/dT against temperature after amplification, the results were analyzed using peaks indicating the T_m (melting temperature) of the amplified products. From the melting curve analysis, we could differentiate between individuals carrying *Sr24* gene and non-carriers (Table 1, p. 21). We also could distinguish homozygous

Sr24 (RR) individuals from heterozygous individuals (Rr) (F_2 of 'Kalyan sona / Vaishali) and also the susceptible parent (rr). The peak height of a heterozygous plant was approximately half that of a homozygous plant (Fig. 2).

Analysis by +/- assay. In RT-PCR, the +/- assay can be used to score the presence or absence of a marker/gene based on the quantification curve, where an increase in the fluorescence unit above a threshold level will be considered positive and below the threshold level will be considered negative. The threshold level also can be manually adjusted and examined for positives and negatives of the *Sr24* gene (Table 1, p. 21).

Conclusions. In MAS, a large number of populations have to be screened using conventional PCR techniques, and requires post-PCR processing, such as resolving in agarose gels, which is time consuming and sometimes may lead to false results due to cross contamination. To overcome these delays and errors, and also to screen a large number of populations, the RT-PCR technique has been used to screen a large number of samples for the presence or absence of a gene of using specific primers. Application of RT-PCR in MAS has not been reported in literature, but the use of this technique for the development of molecular markers has been reported in olive plants by Giménez et al. (2010). We used a specific primer for a SCAR marker reported for stem rust resistance gene *Sr24* (Gupta et al. 2006). The results of phenotypic

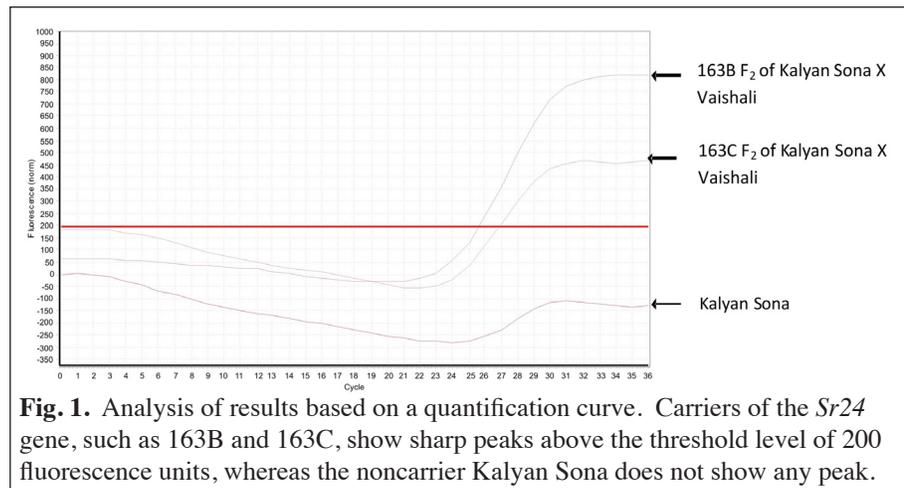


Fig. 1. Analysis of results based on a quantification curve. Carriers of the *Sr24* gene, such as 163B and 163C, show sharp peaks above the threshold level of 200 fluorescence units, whereas the noncarrier Kalyan Sona does not show any peak.

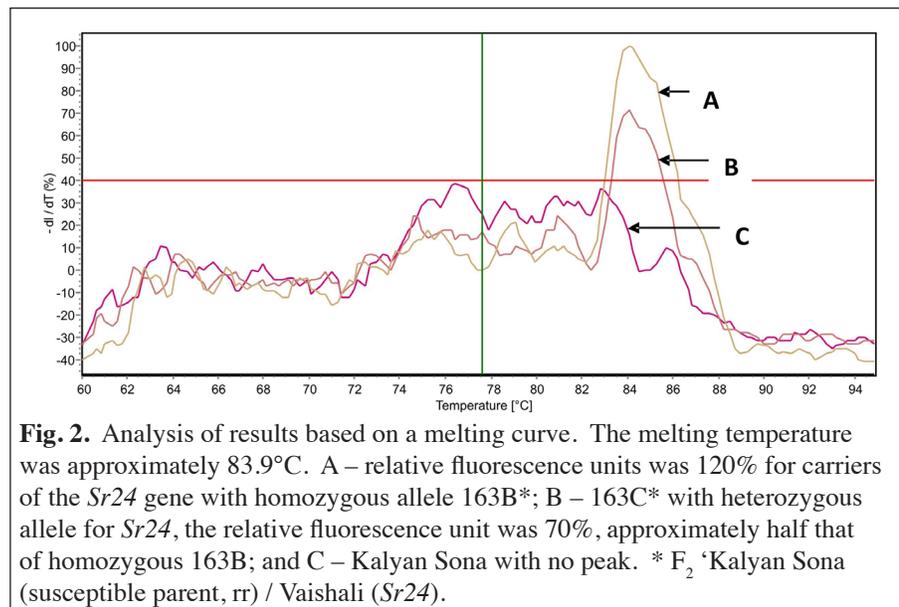


Fig. 2. Analysis of results based on a melting curve. The melting temperature was approximately 83.9°C. A – relative fluorescence units was 120% for carriers of the *Sr24* gene with homozygous allele 163B*; B – 163C* with heterozygous allele for *Sr24*, the relative fluorescence unit was 70%, approximately half that of homozygous 163B; and C – Kalyan Sona with no peak. * F_2 'Kalyan Sona (susceptible parent, rr) / Vaishali (*Sr24*).

and genotypic data of conventional PCR and RT-PCR (melting curve) were compared, and they were found to match exactly, indicating the advantage of using RT-PCR in MAS. This method could avoid post-PCR processing with agarose gel electrophoresis and, thereby, save time. However, the use of a quantification curve for the analysis needs further standardization.

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Threshability in recombinant inbred lines of wheat.

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Recombinant inbred lines of bread wheat arising from two cultivars, Kalyan Sona and Sonalika, were raised in the field at Trombay in 2010–11 season. The lines were sown at two sowing dates corresponding to normal and late sowing. At harvest, one spike each from each RIL was collected at random. Spikelet number and rachis length were recorded. The spikes were threshed by hand and a rating was given, beginning with 1.0 for the softest threshing up to 5.0 for very hard threshing.

Results and discussion. As in earlier years, Kalyan Sona was softer threshing than Sonalika. The RILs showed differences in threshability. Lines softer and harder than the parents were observed. The distribution for threshability of the late sown lines is shown in Table 2. Correlation coefficients were calculated between some of the traits using Microsoft Excel (Table 3).

A significant correlation was found between the threshability ratings for 2009–10 and 2010–11. Although the ratings are based on single spikes and are recorded using a subjective assessment, the correlation showed that the procedure gave repeatable results. Lower ratings were less consistent and the reliability was better for lines with higher ratings. When the ratings were for soft (1.0 to 2.5), intermediate (3.0 and 3.5), and hard (4.0 to 5.0), the frequencies were 52, 62, and 24 (2009–10) and 68, 46, and 24 (2010–11), respectively. Significant correlations for rachis length and spikelets/cm of rachis indicated that the RILs were stabilized for these traits. These RILs could be used to identify loci governing threshability and spike morphology.

Table 2. Scores for threshability in field grown recombinant inbred lines between Kalyan Sona (soft threshing) and Sonalika (hard threshing). Spikes were rated from 1.0 for the softest threshing up to 5.0 for very hard threshing.

Description	Rating	Frequency
Very soft	1.0	17
Intermediate	1.5	00
Kalyan Sona type	2.0	45
Intermediate	2.5	06
Sonalika type	3.0	25
Intermediate	3.5	21
Hard threshing	4.0	15
Intermediate	4.5	02
Very hard threshing	5.0	07

Table 3. Correlation coefficients between selected traits for “Kalyan Sona/Sonalika” recombinant inbred lines (RIL). ** indicates significance at the 1% level.

Trait	Number of RILs	Correlation coefficient
Threshability rating; 2009–10 and 2010–11	138	r = 0.56**
Rachis length; 2009–10 and 2010–11	130	r = 0.54**
Spikelets/cm of rachis; 2009–10 and 2010–11	137	r = 0.58**

During the domestication of bread wheat, selection for the free threshing habit enhanced its suitability for cultivation. Two mutations, *q* to *Q* on chromosome 5A and *Tg* to *tg* on chromosome 2D, mainly are responsible for the free threshing habit of bread wheat. Because threshability is an important trait, many studies have sought to map the loci involved. Jantasuriyarat et al. (2004) analyzed the ITMI mapping population and observed that two QTL that affected threshability were located on chromosomes 2D and 5A. The QTL on 2D probably represented the effect of *Tg*, the gene for tenacious glumes. The QTL on 5A are believed to represent the effect of *Q*. Free threshing-related characteristics were more affected by *Tg* and to a lesser extent by *Q*. Other QTL that were significantly associated with threshability in at least one environment were located on chromosomes 2A, 2B, 6A, 6D, and 7B.

Nalam et al. (2007) analyzed RILs developed by ITMI and a 'Chinese Spring/Chinese Spring 2D' F₂ population and observed that in the ITMI population, two QTL affected threshability and their location coincided with the two QTL affecting glume tenacity. In the 'Chinese Spring/Chinese Spring 2D' F₂ population, the location of QTL that affected glume tenacity coincided with *Tg1*. These results suggest that the effect of *Tg1* and threshability is through the level of attachment of the glumes to the rachilla. In our experiments, we observed that RILs obtained from two free-threshing cultivars showed variation for threshability. The variation was studied using hand threshing. More observations were made during the winter season of 2010–11, which are reported here.

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Current activities.

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Bread wheat cultivars were grown in a replicated experiment and measurements were made on the canopy temperature depression. Another study monitored translocation of reserves from stem to the grain. Analysis of the data is in progress. An RIL population from the intervarietal cross 'Sonalika/Kalyan Sona', a bread wheat RIL population for grain protein content, and early flowering mutant lines in the background of cultivar C306, genotype MP3054, and Hindi62 were carried forward. Grain size and shape mutants of the long grain durum genotype PBNB 1625 and morphological mutants in a bread wheat genotype carrying multiple phenotypic markers were carried forward. The backcross populations carrying sphaerococcum locus in Kalyan Sona background were carried forward. Other genetic stocks, such as an ADH variant (tall and dwarf) and a lax mutant of sphaerococcum type in Kalyan Sona background were carried forward.

Wheat seeds are exposed to soil conditions after sowing, which may include salinity and could affect germination. Seeds of *T. turgidum* subsp. *dicoccum* and *T. aestivum* subsp. *aestivum* were soaked in increasing concentrations (100–500 mM) of NaCl and the germination percent and seedling height were measured. We observed that the germination percent decreased beyond 300 mM; seedling growth was reduced by 40–45% at 100 mM. The aleurone layers of *T. turgidum* subsp. *dicoccum* and *T. aestivum* subsp. *aestivum* were incubated in liquid medium in the presence of different concentrations of NaCl and assayed for amylase stimulation, protein secreted in medium, mitochondrial activity, and weight loss. There was no effect on secreted protein, however, amylase stimulation, respiration, and weight loss were affected by NaCl.

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DIRECTORATE OF WHEAT RESEARCH

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Performance of timely and late-sown cultivars under different sowing times.

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Summary. A field experiment was conducted during winter seasons of 2005–06 to 2006–07 at the Directorate of Wheat Research, Karnal, to evaluate the timely sown and late sown recommended cultivars under normal, late, and very late sowing conditions. A clear picture will be provided as to whether or not timely sown cultivars perform equally good under late and very late sowing conditions. A pooled analysis of two years data revealed a reduction grain yield of 14.4% as sowing was delayed from normal to late sown conditions. Cultivar differences were observed for anthesis, maturity, spike length, grain-filling period, grain production rate, and yield and yield attributing parameters. The interaction between sowing time and cultivars was significant for grain yield. Three timely sown cultivars (PBW 343, HD 2687, and PBW 502) performed better under normal sowing condition whereas the late-sown cultivar UP 2425 produced a maximum grain yield (42.24 q/ha) under late sowing conditions and Raj 3765 produced a maximum grain yield (42.79 q/ha) under very late sowing conditions, which was significantly higher than other cultivars. The resultsshowed that timely sown cultivars did not perform better across the sowing time and that there is a need to develop different cultivars for various sowing conditions.

Introduction. Wheat is the second most important crop after rice in India and in 2008–09 occupied approximately 28×10^6 ha with a production of 78.4×10^6 metric tons. India ranks second in wheat production after China. The area, productivity, and production of wheat have increased 119, 236, and 634%, respectively, since 2005 compared with 1965–66 (base year). Weather is cool and dry in the early part of wheat-growing season (November to February) whereas temperature rises during the grain-filling period (March–April), which is more pronounced in eastern part of Indo-Gangetic plain, resulting in a reduced wheat-growing period. Wheat is grown under different agroclimatic conditions each having variable productivity levels. In India, wheat is generally grown under three sowing conditions, i.e., normal (November sown), late (December sown), and very late sown (January sown) conditions. The normal sown wheat crop is generally preceded by crops such as upland rice, soybean, sorghum, bajra, or even grown after fallow. The late sown wheat crop is generally preceded by crops such as basmati rice, low land rice, cotton, and pigeon pea and very late-sown wheat is grown after toria, pea, potato, and sugarcane ratoon. Delayed wheat sowing (normal to late, mid-November to the first two weeks of December) resulted in a decrease in yield by 15.5, 32.0, 27.6, 32.9, and 26.8 kg/ha/day under NHZ, NWPZ, NEPZ, CZ, and PZ, respectively, for the timely sown cultivars. For the late-sown cultivars, a delay in sowing (late to very late, first two weeks of December to the first two weeks of January) decreased the grain yield by 42.7, 44.8,