Application of molecular markers, micro-level tests and interclass hybridizations in improving wheat grain quality

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ABSTRACT

Wide spread use of within class hybridizations has reduced the genetic base of released varieties and restricted the combination of desirable alleles present in hard and soft wheat classes. In the present investigation the utility of combined use of molecular markers, micro-level tests and interclass hybridizations was assessed to improve wheat grain quality. Crosses were made between hard and soft wheats and puroindolines were used as markers for selection of hard and soft genotypes in backcross breeding programme. Microlevel tests were conducted for grain hardness, gluten strength and protein content. Large variations were observed in grain hardness, micro-sedimentation value in back cross populations developed using high yielding genotypes with 1B/1R translocation and soft germplasm lines. Presence of large numbers of transgressive segregants revealed that desirable alleles are present in both hard and soft wheat backgrounds. Data validated the utility of interclass hybridizations, molecular markers and micro-level tests in wheat improvement because such hybridizations have not been explored widely in breeding programmes.

INTRODUCTION

Hard and soft wheats are two distinct classes of wheat, and generally crosses are made within class because of distinct quality goals. For example, hard wheats with strong gluten and high protein content are preferred for bread and chapati making and soft wheat with weak gluten and low protein content for biscuit, cakes, pretzels and white salted noodles (MacRitchie 1980). This has restricted combinations of desirable alleles from both the classes and hence reduced the genetic base (Campbell *et al* 2001). Analysis of large numbers of wheat varieties released in India during the last 100 years showed the predominance of hard wheats with null mutation in puroindoline A (Ram *et al* 2002). This shows that within class (hard) hybridizations had been attempted in majority of crosses in India in wheat breeding. Moreover, during the past two decades wheat breeders have used the short arm of rye chromosome 1R as source of genes for disease and pest resistance and improved agronomic performance (Ram *et al* 2005). However, reduced gluten strength and loaf volume and increased dough stickiness have been reported associated with 1B/1R translocation (Lee *et al* 1995). Therefore, combining desirable alleles using interclass hybridizations would expand genetic bases and improve the chances of wheat improvement for yield and quality traits (Caver 1996, Campbell *et al* 2001 and Breseghello *et al* 2005). This can be accomplished by utilizing the existing knowledge of genetic basis of major grain quality traits such as grain hardness, protein content and gluten strength at molecular level as well as their phenotypic assessment using micro-level tests in early segregating generations.

MATERIALS AND METHODS

Plant materials: A set of 400 genotypes including 280 Indian wheat varieties released during last 100 years were grown at DWR Karnal for consecutive two crop seasons 2005-2006 and 2006-2007 under normal dose of fertilizer and as per recommended agronomic package of practices. BC_2F_4 populations (320 lines) developed using PBW 343 and HD 2687 as recurrent parents and two selected soft wheat germplasm lines HPW 114 and EC 378793 as donor parents were included in the investigation. PBW 343 and HD 2687 are predominant wheat varieties grown in North Western Plains Zones in India and have 1B/1R translocation.

Quality traits: Grain hardness was determined by Perten Model SKCS 4100 Single Kernel Characterization System (Perten Instruments North America Inc, Springfield, IL). Protein contents were estimated by near-infrared reflectance methodology (AACC method 44-16) using Infratec Machine. SDS micro-sedimentation was measured by the modified method of Carter *et al* (1999).

DNA isolation and PCR amplification: DNA was extracted from leaf samples of wheat genotypes and back cross populations using the procedure of Benito *et al* 1993 with slight modification. Amplification of *pinA* and *pinB* was performed as described by Giroux and Morris (1997). 1B/1R translocation was identified using *Glu-B3* specific primers using sequence specific primers by the method of Van Campenhout *et al* (1995).

Statistical analysis: Standard statistical analyses were conducted to assess the variability and heritability of quality traits. Fisher`s t-test was employed to assess the level of significance between the sedimentation value of soft and hard genotypes in two different populations.

RESULTS AND DISCUSSION

With a view to improve wheat quality for soft and hard wheat products, back cross populations were developed using interclass hybridizations. High yielding and widely adapted varieties grown in North Western Plains Zones

of India namely PBW 343 and HD 2687 were used as recurrent parents and germplasm lines identified with soft grain characteristics namely HPW 114 and EC 378793 as donors. The analysis of 280 released varieties for two years exhibited hard texture in majority of lines. This necessitated the identification of soft germplasm lines for use in breeding. The adapted varieties had null mutations in *pinA* while the soft genotypes had wild alleles of both *Pina* and *Pinb*. Our earlier studies also demonstrated the prevalence of null mutation in Indian wheats (Ram *et al* 2002). PCR amplification of *Pina* was used in BC_1F_1 populations to select plants with *Pina* (which was absent in both the recurrent parents) and used them in crossing with respective recurrent parent to develop BC_2F_2 plants which were subsequently advanced to BC_2F_4 populations. The SKCS analysis of grain hardness index in BC_2F_4 derived seeds in both the populations exhibited both soft and hard genotypes, though there were differences in the frequency of hard and soft phenotypes (Fig.1). In PBW 343 X HPW 114 cross, there were larger numbers of lines with hard grain texture in comparison to HD 2687 and EC 378793 cross where large numbers of lines were soft. The PCR amplification of BC_2F4 plants of both the crosses representing soft and hard wheat genotypes showed the presence of *Pina* in all soft genotypes and absent in all the hard genotypes studied. Data further demonstrated that puroindoline genes are the major genes for determining grain hardness. High heritability (H=0.91) was observed for gain hardness among the set of genotypes studied and hence useful in breeding. Though, many transgressive segregants were observed in the present populations towards softer grain texture indicating the role of minor genes.

Micro-sedimentation test exhibited wide range (5.5 to 12.5 ml) in gluten strength in backcross populations and showed normal distribution with transgressive segregants towards lower sedimentation value. Transgressive segregants were more in the population derived from HD2687 X EC378793 cross as compared to PBW343 X HPW114 cross. Transgressive segregants were also observed towards lower protein content in both the populations. The numbers of lines with lower protein content were more in the population derived from HD2687 X EC378793 as compared to PBW343 X HPW114 cross. Though, there was no transgressive segregation towards higher sedimentation value in the population derived by the cross between PBW343 and HPW114, few lines were observed with transgressive segregants towards higher value in the population derived from HD 2687 and EC 378793 cross. There was no significant difference in sedimentation value of hard and soft wheat classes. Thus the combined analysis of hardness index, sedimentation value and grain protein content indicated higher value of the cross HD2687 X EC378793 for the development of soft wheat as compared to the cross PBW343 X HPW114. Since one of the parents in both the crosses had 1B/1R translocation, the large number of transgressive segregants observed towards lower gluten strength may be because of weakening effect of 1B/1R translocation on gluten strength. To identify the number of lines with 1B/1R translocation, BC_2F_2 plants were analyzed using Glu-B3 specific primers. Since primers were specific to Glu-B3 locus, there was no amplification in 1B/1R translocation lines where 1B short arm of wheat is replaced by short arm of rye chromosome. Since recurrent parent had 1B/1R translocation, most of the lines (94 to 100%) in both the back cross populations showed the presence of 1B/1R translocation.

In brief the data demonstrated that interclass can lead to improvement in wheat using molecular markers and micro-level tests. The yield and quality improvement depends upon the favourable alleles present in both the parents. Favourable alleles can be combined by using molecular markers and micro-level tests for kernel hardness, protein content and sedimentation volume in early segregating generations followed by rigorous tests for rheological properties and baking quality in advance generations. Moreover, development of genetic stocks as parent building exercise and simultaneously selection of desirable segregants with better quality and higher yield potential will lead to improvement of wheat.

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Fig. 1**.** Frequency distribution of Hardness Index (HI) in a set of BC_2F_5 grains using HD2687 as recurrent parent and EC 378739 as donor parent. Hardness index values of parents are given in the figure.