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Molecular characterization of wheat (*Triticum aestivum* L.) genotypes using PCR-based grain softness markers

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Abstract
This study was undertaken to characterize grain softness traits in a set of 25 wheat (*Triticum aestivum* L.) genotypes, which included 22 mutant lines, along with two known soft-textured varieties, Phule Satwik and Pusa Baker. To achieve this, PCR-based molecular markers specific to the puroindoline genes (Pina-D1 and Pinb-D1) were employed, as these loci are well-established determinants of grain hardness in wheat. The marker analysis confirmed the presence of wild-type alleles associated with soft grain texture in the majority of the mutant lines, as well as in the check soft-textured varieties Phule Satwik and Pusa Baker. These results underscore the existence of valuable genetic variation that directly influences end-use quality traits, particularly baking performance and the production of soft-textured wheat-based products. Furthermore, the study highlights the advantage of molecular marker analysis, for the accurate and efficient identification of grain quality attributes. Such a strategy provides reliable selection indices for wheat breeding programs, enabling the development of cultivars with enhanced processing performance, improved end-use quality, and consumer-preferred traits. In conclusion, the findings demonstrate that molecular characterization using PCR-based markers represents a powerful and resource-efficient tool for advancing quality-oriented wheat improvement programs.

Keywords: Wheat, softness, PinaD1, PinbD1

Introduction
Wheat (*Triticum aestivum* L.) is one of the major cereal crop grown worldwide, serving as a staple food for a large portion of the global population and referred as the "King of Cereals." India, is second-largest producer after China, contributes significantly to global wheat production, highlighting its importance in food security and the economy. The key advantage of wheat over other cereal crops lies in its unique viscoelastic properties of flour, which enable the preparation of variety of food products such as biscuits, breads, cakes, pasta and noodles. These properties depend on the structures and interactions of the grain storage proteins, which together form the 'gluten' protein fraction (Shewry, 2009) [11]. The presence of Pina, Pinb, or both in several wild species with progenitor and non-progenitor genomes other than the D-genome suggests that the Pin genes evolved in their ancestral species before divergence. These genes can be transferred to T.aestivum to develop ultrasoft wheat cultivars with superior properties for biscuits and pastries (Li *et al*., 2020) [5]. High-quality biscuits are often produced using flour from soft wheat due to their low protein levels and damaged starch (Pareyt & Delcour, 2008) [6]. These genotypes typically contain relatively high levels of wild-type PINs, approximately 0.1% on a dry matter basis (Turnbull *et al*., 2002) [12]. Biscuits with dough containing high levels of Prolamin-Inducing Proteins (PIN) are larger, softer, and more porous due to increased air incorporation and gas cell expansion. PIN levels also affect the strength of the biscuit matrix. Low PIN levels require a minimum threshold of lipids or PINs for softening effects, with higher PIN levels having a more significant impact on biscuit quality than lipids (Pauly *et al*., 2013) [7]. The Hardness (Ha) locus on chromosome 5-DS, controls grain softness through two genes puroindoline (Pina and Pinb). Wild alleles of both Pina D1 and Pinb D1 are associated with soft grain texture and mutation in either of puroindolines with hard texture (Sewa Ram *et al*., 2007) [9]. In this present investigation 25 wheat lines were evaluated for grain softness related trait.

Materials and Methods

Seeds of 25 wheat lines containing 22 *Glu-1* mutant lines developed from crossing Naphal and HMW-GS mutants and 2 soft wheat variety obtained from College of Agriculture Pune were used for this study. The genomic DNA was extracted from young leaves of 20 days old seedlings using a modified Cetyltrimethylammonium bromide (CTAB) protocol outlined by Rogers and Bendich (1985) [10]. The quality of DNA was evaluated on 0.8% agarose gel and PCR was performed with primers PinaD1 and PinbD1 at annealing temperature 55 ºC given in table 1.

Results and Discussion

In this study, Genotypes are evaluated for puroindoline genes using primers Pina D1 and Pinb D1. Mutant genotypes were developed using crossing of Naphal and HMW-GS mutants of parent variety contains mutant *PinaD1* and wild *PinbD1* alleles, while NapHal contain both pinaD1 and pinbD1 wild alleles associated with grain softness, as allele-specific primers were not used hence, we can not discriminate wild and mutant puroindoline genes using these primers. The Glu-1 mutants genotypes showed both the pinaD1 and pinbD1 alleles. In variety Phule Satwik, Pusa Baker and mutant genotypes showed amplification for both Pina D1 and Pinb D1 primer showing presence of both puroindoline genes as shown in figure 1 and 2. Wild alleles of both Pina D1 and Pinb D1 are associated with soft grain texture and mutation in either of puroindolines with hard texture (Sewa Ram *et al*., 2007) [9]. Biochemical and molecular studies demonstrated that association of Pina D1 and Pinb D1 (components of friabilin, a 15kD starch bound protein) with grain texture (Giroux MJ & Morris CF (1997) [2], Greenwell P & Schofield JD (1986) [3], Gautier MF *et al*., 1994) [1]. The presence of both puroindoline genes in their wild form in the mutant lines and in Phule satwik and Pusa baker indicates a softer grain texture, which is consistent with the findings of Giroux & Morris (1997) [2], Sewa Ram *et al*. (2002) [8], Greenwell & Schofield (1986) [3], and Gautier *et al*. (1994) [1].

Figures

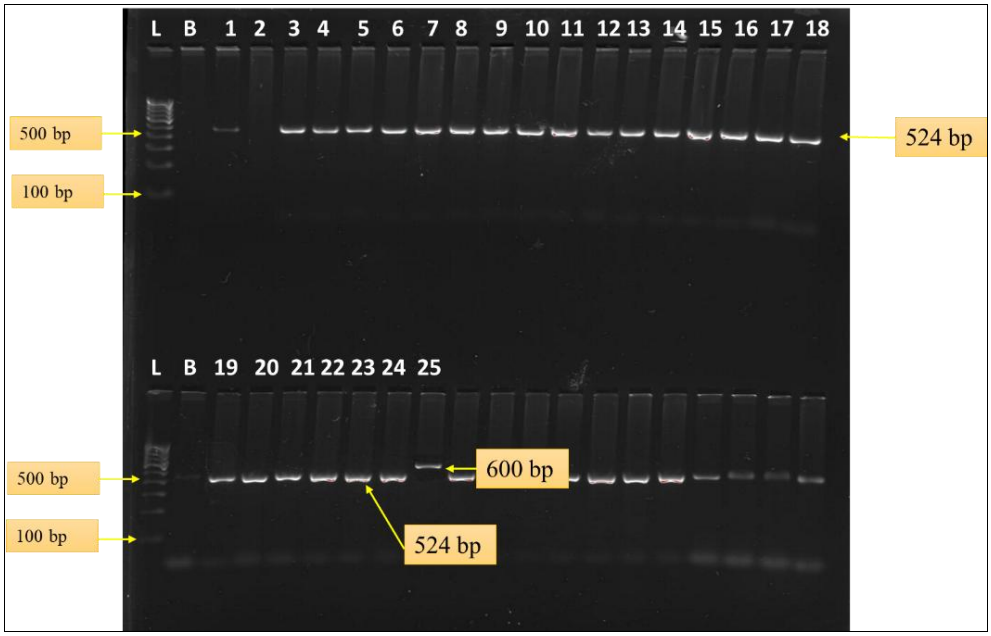

Fig 1: PCR Profile of *Pina D1*

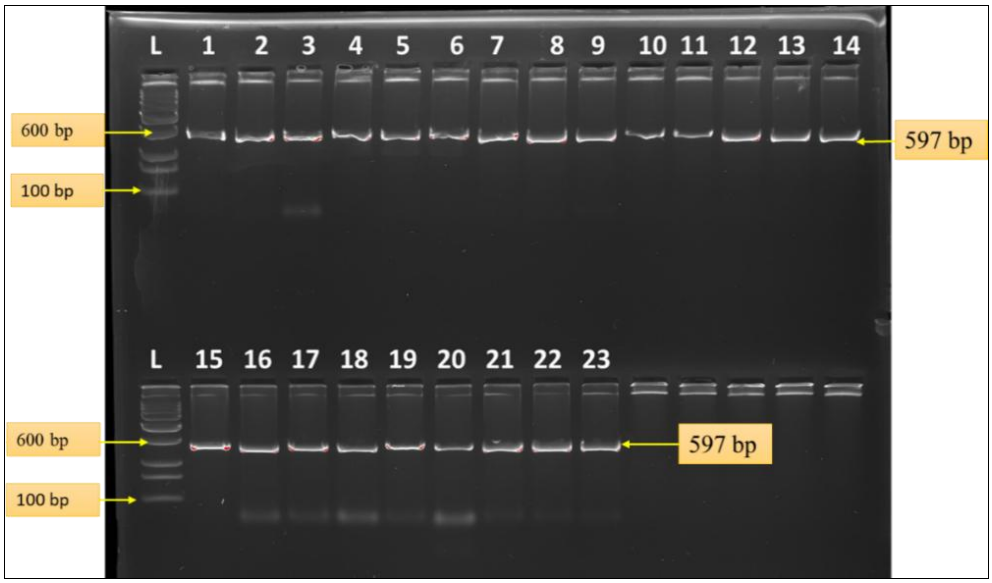

Fig 2: PCR Profile of *Pinb D1*

Table 1: Details of PCR primers used in study

Sr. No.	Primer Name	Sequence (5'-3')	Annealing temperature(°C)	Expected size (bp)	Reference
1.	PinD1 a	F- CATCTATTCATCTCCACCTGC R- GTGACAGTTTATTAGCTAGTC	55	524 bp	Lillemo *et al.,* (2006) [4]
2.	PinD1 b	F-AGCCTCAACCCATCTATTCATC R- CAAGGGTGATTTTATTCATAG	55	597 bp	

Conclusion

PCR-based molecular markers were effectively utilized to characterize the allelic status of Pina-D1 and Pinb-D1 genes, which are the key determinants of grain hardness in wheat (*Triticum aestivum* L.). The analysis confirmed the presence of wild-type alleles for both Pina-D1 and Pinb-D1 in the evaluated wheat genotypes, thereby indicating a soft grain texture. This softness trait is highly desirable, as it is directly associated with improved milling properties, reduced starch damage, and superior baking performance. In the present study, 25 wheat genotypes were assessed, which revealed a wide range of allelic combinations, including diverse mutant lines along with widely cultivated soft wheat varieties such as Phule Satwik and Pusa Baker. These findings highlight the existence of significant genetic variability for grain texture traits, which can be harnessed in wheat breeding programs to develop cultivars suited for different end-use qualities. The integration of PCR-based molecular characterization with traditional breeding tools provides a robust, reliable, and rapid selection index for identifying wheat genotypes with superior grain quality attributes. Specifically, the detection of grain softness alleles through DNA markers eliminates the need for time-consuming and resource-intensive phenotypic screening, thereby accelerating the process of variety development. Overall, this integrated approach of molecular marker-assisted selection not only ensures the accurate identification of soft-textured wheat genotypes but also facilitates the targeted improvement of wheat varieties for enhanced baking performance, end-use quality, and consumer preference. Thus, the utilization of PCR-based grain softness markers represents a powerful strategy for molecular breeding programs aimed at quality enhancement in wheat.

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