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Introgression of the high grain protein gene *Gpc-B1* in an elite wheat variety of Indo-Gangetic Plains through marker assisted backcross breeding

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ABSTRACT

Grain protein content (GPC) in wheat has been a major trait of interest for breeders since it has enormous end use potential. In the present study, marker-assisted backcrossing (MABC) was successfully used to improve GPC in wheat cultivar HUW468. The genotype Glu269 was used as the donor parent for introgression of the gene *Gpc-B1* that confers high GPC. In a segregating population, SSR marker Xucw108, with its locus linked to *Gpc-B1* was used for foreground selection to select plants carrying *Gpc-B1*. Background selection, involving 86 polymorphic SSR markers dispersed throughout the genome, was exercised to recover the genome of HUW468. For eliminating linkage drag, markers spanning a 10 cM region around the gene *Gpc-B1* were employed to select lines with a donor segment of the minimum size carrying the gene of interest. Improved lines had significantly higher GPC and displayed 88.4–92.3 per cent of the recurrent parent genome (RPG). For grain yield, selected lines were at par with the recurrent parent HUW468, suggesting that there was no yield penalty. The whole exercise of transfer of *Gpc-B1* and reconstitution of the genome of HUW468 was completed within a period of two and half years (five crop cycles) demonstrating practical utility of MABC for developing high GPC lines in the background of any elite and popular wheat cultivar with relatively higher speed and precision.

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1. Introduction

Wheat (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world with global yield over 700 million tons annually, and providing 20% of the total calorie intake for the world population [1,2]. Wheat is grown in ~128 countries involving all the continents of the world, the top five leading producers being China, India, United States of America, France and Russia [3]. Among cereals involved in cross-border trading, wheat has the highest tonnage, with an estimate value of 135 Mt in 2012/13, with major importing countries being in Asia and Africa [3]. In India, wheat is a staple food for more than 65% of the population with annual production of around 94 Mt [4]. The demand of wheat is expected to keep growing due to steady population increase. The demand for better quality wheat grain will also increase due to increased urbanization [5]. Although, many Indian wheat varieties have been characterized for various end products, these varieties and their traits are spillovers of the routine breeding program for high yield and disease resistance, rather than the product of a systematic quality breeding program [6]. The current challenges for wheat breeding programs around the world are to maintain or improve agronomic performance along with improvement in wheat quality, thus maintaining competitiveness in the increasingly discriminating international market [7].

Wheat is a crop with several end-use products such as pasta, macaroni, biscuit, chapatti and bread. These end-use products differ for their requirements of GPC and the type of wheat. In general, GPC in Indian wheat cultivars is relatively lower than the standards of international market [7]. Under these conditions, either we need to accept wheat with a lower GPC or apply more nitrogen to achieve the desired level of GPC, since some increase in GPC through increased nitrogen application has been documented [8].

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GPC is also an important for bread-making quality, which is known to depend upon both, the content and composition of grain protein [9,10].

According to an estimate of World Health Organization [11], over 3 billion people were deficient in key micronutrients Zn and Fe, and about 160 million children below the age of 5 lack adequate protein, amounting to malnutrition [11–13]; this suggests that not only GPC, but also the content of micronutrients like Zinc (Zn) and Iron (Fe) need to be improved for improving the grain quality of wheat. Progress in breeding for high GPC wheat has been rather slow, because GPC is controlled by a complex genetic system and is also influenced by the environment, thus making it difficult to select effectively for this trait [8,14,15]. However, GPC and grain yield are reported to be negatively correlated [8,14], making it difficult to breed for high GPC without a yield penalty. Although the theoretical basis for this inverse correlation has been debated [16], high GPC cereals are unlikely to be commercially successful without a financial incentive to growers.

Yield is an essential trait for commercial success of a variety, hence developing wheat varieties combining improved grain quality with high grain yield is an important goal in wheat breeding. However specific quality parameters such as protein %, grain hardness, bread loaf volume and biscuit spread factor are getting increased attention due to growing demand for industrial end-products such as bread, biscuit, cake, pasta, etc. Wheat varieties with high GPC (>12%) and micronutrients (Zinc and Iron) are also important for providing nutritionally improved wheat based diet and for enhancing export potential of wheat. In addition, high yielding wheat with superior internal (protein %) and external (grain weight, luster) traits is easy to market and may provide extra cash to poor farmers. In India, although wheat is overwhelmingly consumed in the form of chapatti [7], the demand for other end-products like bread, biscuit, pasta and cakes is growing with expanding urbanization (estimated urban population in 2020 = 550 million) and growing industrialization [5]. Therefore, it is important to combine the high grain yield with better grain quality to meet the twin challenges of nutritionally superior and high quality wheat products [6].

In the recent past, the introgression and pyramiding of major genes/QTL for different traits through marker-assisted selection (MAS) has proved successful in wheat [17–24]. Several RFLP, SSR and CAPS markers were reported to be closely linked with high GPC locus (*Gpc-B1*) on the short arm of chromosome 6B [25–28]. Among these markers, a tightly linked marker at a narrow distance of 0.1 cM within a physical location of a 250 kb, was the SSR marker Xuhw89 for the locus *Gpc-B1* [29]. Since *Gpc B1* has been cloned and characterized, a "gene-specific" marker is also available for this locus [30]. The introgression of *Gpc-B1* has been achieved for improving GPC without yield penalty mostly in the developed countries [8,31], although a report of successful introgression of *Gpc-B1* in 10 elite varieties of India is also available [23].

Conventional breeding program, if supplemented with MAS, can become cost and time-effective [20]. For the last more than 20 years, MAS is being used on a large-scale in several countries including USA, Australia, Canada, and Mexico (CIMMYT). In majority of these MAS programs in wheat, MABC involving backcrossing has been deployed to ensure maximum recovery of the genome and particularly, the carrier chromosome [32]. According to a recent report, more than 60 genes/markers are being deployed for wheat improvement through MAS [33], of which more than 20 traits/genes belong to grain quality like gain hardness, dough strength and swelling volume [34]. Molecular markers for quality traits (protein content, pre-harvest sprouting tolerance, gluten strength and grain weight) are also being increasingly used in Indian wheat breeding program successfully [19,22–24].

The present study was planned to improve GPC through MABC coupled with stringent phenotypic selection into the genetic background of wheat cultivar HUW468, which is a very promising cultivar with good performance under conventional and zero-tillage conditions in the North Eastern Plains Zone (NEPZ) of India [6].

2. Materials and methods

2.1. Plant materials

Plant materials used in the present work included recipient parent HUW468 and a donor parent Glu269 with high GPC procured from Punjab Agriculture University, Ludhiana, Punjab. HUW468 (CPAN-1962//TONI/LIRA'S'/PRL'S'). Glu269 is a high yielding, disease resistant and double dwarf wheat variety released in 1999 for timely sown high fertility irrigated conditions of NEPZ and since then has maintained resistance and popularity among farmers due to its superior agronomic performance. The donor parent Glu269 (DBW16/GluPro//2*DBW16) is a wheat breeding line that is resistant to yellow and brown rusts and is also amenable to late sowings. It also carries higher level of resistance to spot botch relative to all the existing varieties, and has been identified for cultivation in the North Western Plains Zone (NWPZ) of India. Since, it had GluPro as one the parents in its pedigree, it carries *Gpc-B1* providing higher level of GPC (>14%) relative to the recurrent parent HUW468 with only 10% GPC.

2.2. Molecular marker used

Seven GPC linked markers (Xuhw89, QGpc.ccsu-2D.1/2DL, CAPS/ASA/XNor-B2, Xwmc415, Xucw108 and Xucw109) were validated based on published results [26,29,30,35,36]. Out of seven, only one (Xucw108) [30] was selected for foreground selection. Primers were synthesized from Eurofins Genomics India Pvt Ltd., Bangaluru, India. To analyze the recovery of RPG of the segregating backcross progeny during background selection, a total of 744 SSR (simple sequence repeats) markers covering all the 21 chromosomes of wheat were selected to detect polymorphism between HUW468 and Glu269. The primer sequences were obtained from http://wheat.pw.usda.gov/GG2/index.shtml (GrainGenes 2.0: A database for Triticeae and Avena), Somers et al. [37] and Röder et al. [38]. Primers that were polymorphic between parents were used for background selection.

2.3. MABC breeding

2.3.1. DNA isolation, PCR conditions and electrophoresis

DNA isolation of parental genotypes and backcross progenies was carried out from one-month-old plants using a modified CTAB method [39]. The PCR amplification was carried out in a reaction mixture of 20 μL containing 200 μM dNTPs (MBI; Fermentas, Lithuania, USA), 0.75 U Taq DNA polymerase (MBI; Fermentas, Lithuania, USA), 5 pmole of each primer, 20–30 ng template DNA and 10 X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$). PCR cycle consisted of an initial denaturation for 5 min at 94 °C, followed by 40 cycles each with 1 min at 94 °C, 1 min at annealing temperature (which differs for different primers), with a final extension of 7 min at 72 °C. The amplified products were resolved on 2.5% agarose gel for the foreground selection (involving use of gene specific marker Xucw108), and on 10% PAGE (followed by silver staining for visualization) for the background selection (used for RPG recovery).

2.3.2. Breeding scheme

MABC scheme [32] was followed to transfer the *Gpc-B1* gene from Glu269 into the genetic background of HUW468. Recurrent

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