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Review article

Incorporation of the high grain protein gene *Gpc-B1* (Zn and Fe) in high yielding wheat cultivar through marker assisted backcross breeding

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Abstract
Grain protein content in wheat has been a major trait of interest for breeders since it has enormous end use potential. Marker-assisted backcrossing (MABC) was successfully uses to improve GPC in wheat cultivar. The genotype high Gpc-B1 donor parent use for incorporation of the gene Gpc-B1 that confer high GPC. For segregating population, SSR marker Xucw108 generally use foreground selection to select plants carrying Gpc-B1. For background selection generally 86 polymorphic SSR marker use for to recover the recipient parent genome. Eliminating linkage drag, marker spanning 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. They improves lines have significantly higher GPC and near about 88.4 to 92.3 percent of recurrent parent genome recover generally. The whole exercise of transfer of Gpc-B1 and reconstitution of the genome were generally complete within period of two and half years (five crop cycle) demonstrating practical utility of MABC for developing high GPC lines in the background of any elite popular wheat cultivar.

Keywords: *Gpc-B1* (Fe and Zn), wheat, marker assistant selection, SSR maker

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 (Brouns *et al.* 2013) [3]. GPC is also an important for bread-making quality, which is known to depend upon both, the content and composition of grain protein (Avani *et al.* 2014) [9]. According to an estimate of World Health Organization (White *et al.* 2009) [10], 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; this suggests that not only GPC, but also the content of micronutrients like Zinc (Zn) and Iron (Fe) need to be 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 (Simmonds *et al.* 1995) [8]. However, GPC and grain yield are reported to be negatively correlated Simmonds *et al.* 1995) [8], making it difficult to breed for high GPC without a yield penalty. 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 over whelmingly consumed in the form of chapatti (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 (Pena *et al.* 2007) 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. In the recent past, the incorporation and pyramiding of major genes/QTL for different traits through marker-assisted selection (MAS) has proves successful in wheat. 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 (Distelfeld *et al.* 2004) [4]. 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 (Distelfeld *et al.* 2006) [5]. Since *Gpc-B1* has been cloned and characterized, a "gene-specific" marker is also available for this locus. The incorporation of *Gpc- B1* has been achieves for improving GPC without yield penalty mostly in the developed countries, although a report of successful introgression of *Gpc-B1* in 10 elite varieties of India is also available (Kumar, *et al.* 2011) [6]. Conventional breeding programme, if supplement with MAS, can become cost and time-effective. 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 deployes to ensure maximum recovery of the genome and particularly, the carrier chromosome (Randhawa *et al.* 2009) [1]. According to a recent report, more than 60 genes/markers are being deployes for wheat improvement through MAS of which more than 20 traits/genes belong to grain quality like gain hardness, dough strength and swelling volume (William *et al.* 2007) [2]. 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.

Materials and Methods

Depending upon recipient and donar source availability like HUW468-09-131 grain protein content % 16.4, Zinc (ppm) 53.8, Iron (ppm) 54.2 like this sources uses as donar parent and high yielding ability with wide adaptability with resistance to the biotic and abiotic stress resistance recipient parent should be select for developing high *Gpc-B1*.

DNA isolation, PCR conditions and electrophoresis

DNA isolation of parental genotypes and backcross progenies was carry out from twenty five days old plants using a modified CTAB method. The PCR amplification carry out in a reaction mixture of 20 µL containing 200 µM dNTPs, 0.75 U Taq DNA polymerase, 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 ^{0}C, 1 min at annealing temperature (which differs for different primers), with a final extension of 7 min at 72 ^{0}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).

Testing of F_1 hybridity

Testing hybridity of F_1 plants using gene linked marker, true F_1 plants will be cross with the recurrent parent to obtain BC_1F_1seeds. Both foreground and background selections were deploy. Foreground selection for a trait using molecular marker facilitates identification of positive plants for the gene of interest at early plant stage and thus enables a breeder to reduce the population size by around 50% in a backcross breeding programme. Foreground selection for desirable BC_1F_1plants with *Gpc-B1*gene was exercised using gene linked marker. The plants possessing *Gpc-B1* were subjected to further phenotypic selection to identify top plants with desirable recurrent parent phenotype (RPP) for analyzing RPG recovery. Selection of plant with high RPG recovery then backcross to produce BC_2F_1seed. Like BC_1F_1, foreground selection (for *Gpc-B1*), phenotypic selection (for plants having agronomic similarity to recurrent parent) and background selections (for RPG recovery) were again exercise to identify suitable plants for obtaining BC_2F_2 seeds. The selection of BC_2F_2 plants with high RPG recovery were self and advance up to BC_2F_3 using marker assisted pedigree method of selection. The homozygosity of BC_2F_3 families for *Gpc-B1* gene was further confirm by screening of randomly select the plants from each family using Xucw108 associated with *Gpc-B1*.

Estimation of linkage drag Analysis

Incorporation of donor segment on carrier chromosome uses conduct to eliminate linkage drag; this was possible through the use of six additional markers (Xgwm132, Xcfd190, Xgwm193, Xgwm361, Xgwm219 and Xcfd2110) from the10 cM genomic region on either side of *Gpc-B1* marker Xucw108 give.

Evaluation of MABC lines for agronomic performance

The different backcross generations, plants were hand sowing in row with row to row spacing of 22.5 and plant to plant distance of 20 cm, recurrent parent as a check for facilitating morphological evaluation. Use recommended agronomic and fertilizer doses. Zinc not apply. Full doses of K_2O and P_2O_5 were apply at the time of sowing; nitrogen will be supply in split applications, with N at sowing time, N at the first irrigation (21 days after sowing), and N per ha at the second irrigation (45 days after sowing). Data will be record on phenotypic traits in BC_1F_1, BC_2F_1 and BC_2F_2 generations of plants that was positive for *Gpc-B1* gene, as determined through associate marker. In BC_2F_3 generation, data were taken from randomly select plants from each homozygous family for *Gpc-B1* identify in BC_2F_2. Following agronomic traits were uses for recording data on phenotypes: days to maturity (DM), plant height (PH), number of effective tillers/plant (TP), spike length (SL), spikelet number (SN), thousand grain weight (TGW) and grain yield per plant (GY).

Evaluation of MABC lines for protein, zinc and iron content

The seeds of BC_2F_3 lines of having *Gpc-B1* gene were analyzes by Infratec TM 1241 Grain Analyser, Foss, Denmark. A total of 5–7 g sample of clean grain was uses for Zn and Fe analysis, based on X-ray fluorescence (X-Supreme 8000, Oxford Instruments, Oxford, UK). Grain protein content (GPC) was estimate using an Infratec 1241 Grain Analyser (Foss, Hilleroed, Denmark). Protein data will be record at 12% of seed moisture level in (%) unit, while data on Zn and Fe content were taken as particle per molecule (ppm).

Statistical analysis and determination of recurrent parent genome recovery

The data record uses for estimation of progeny means for each replication. The statistical analysis was perform on the basis of progeny means in each replication by the PAST was calculated using the following formula.

$$RPG\% \quad \frac{2\,(R) + (H)}{2N} \times 100$$

where, R = number of marker loci homozygous for recurrent parent allele; H = number of marker loci still remaining heterozygous and N = total number of polymorphic markers use for background analysis. Generation BC_2F_3, the genetic similarity between the recurrent parent and the high GPC incorporation lines was determine through data on morphological features. To check the robustness of the clustering, boot-strap analysis was carrying out. Further graphical genotyping by using GGT software.

Recovery for whole genome

The per cent RPG recovery among the selecting lines generally ranged from 90.7% to 95.4%. Carrier chromosome recovery with the minimum size of donor segment as expecting, donor segments were present in all the improving lines, and a segment carrying the gene *Gpc-B1* was available in all the lines; this segment was not the minimum possible, so that some linkage drag was unavoidable. A screening for recombinants carrying the minimum size of donor fragment was undertake using markers, which were the only polymorphic markers among the some flanking markers that were test for the *Gpc-B1* region. Generally these markers included Xgwm132, Xcfd190, Xgwm193, Xgwm361, Xgwm219 and Xcfd2110. Based on carrier chromosome analysis of lines using these markers, out of some were such, which did not carry any segment other than the small segment carrying *Gpc-B1*.

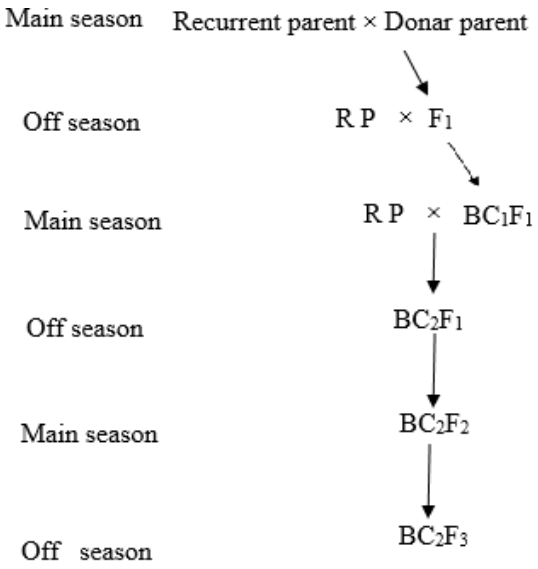

Fig 1: Development of *Gpc- B1* content progeny through foreground and background selection.

Breeding for agronomic and nutritional traits of wheat continues to be important for food security and human health especially in developing countries of south Asia, where demand of wheat is during the last few years, use of molecular tools has grown substantially due to development of high throughput markers that can be used in a cost-effective manner. There are several examples of successful use of MAS for introgression or pyramiding of major genes/QTL for different traits in wheat. There are also examples of introgression of *GpcB1* through MAS for improving GPC without yield penalty but most of these examples are from developed countries.

Linkage drag and its elimination

During incorporation of desirable/target gene (s) in the backcross breeding programme the probability of other closely linked genes getting introgression is quite high, which affects the performance of the end product. This can be significantly reduced by selecting the genomic region of recurrent parent flanking the desirable gene. Carrying the gene *Gpc-B1* was a genotype have poor grain texture and chapatti making quality. Therefore, the only option to get rid of undesirable effects, particularly poor grain texture and chapatti quality, was to minimize linkage drag of the donor parent used for introgressing the gene *Gpc-B1*. For this, were used flanking markers (Xcfd190 and Xgwm193) on the carrier chromosome 6B. These markers were approximately 12 cM apart from each other covering the gene *Gpc-B1*. In a previous study by Kumar *et al*. 2011 [6] flanking markers XNor-B2 (CAPS) and Xgwm193 were used for selection of Gpc-B1 gene to eliminate the risk of losing the gene segment due to lack of closely linked markers. More precise (Xucw108 and Xucw109) markers (within 500 bp region in gene) became available for the transfer of *Gpc-B1*.

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