Marker-assisted Forward Breeding Approach to Simultaneously Introgress Multiple Value-added Traits While Improving Agronomic Performance

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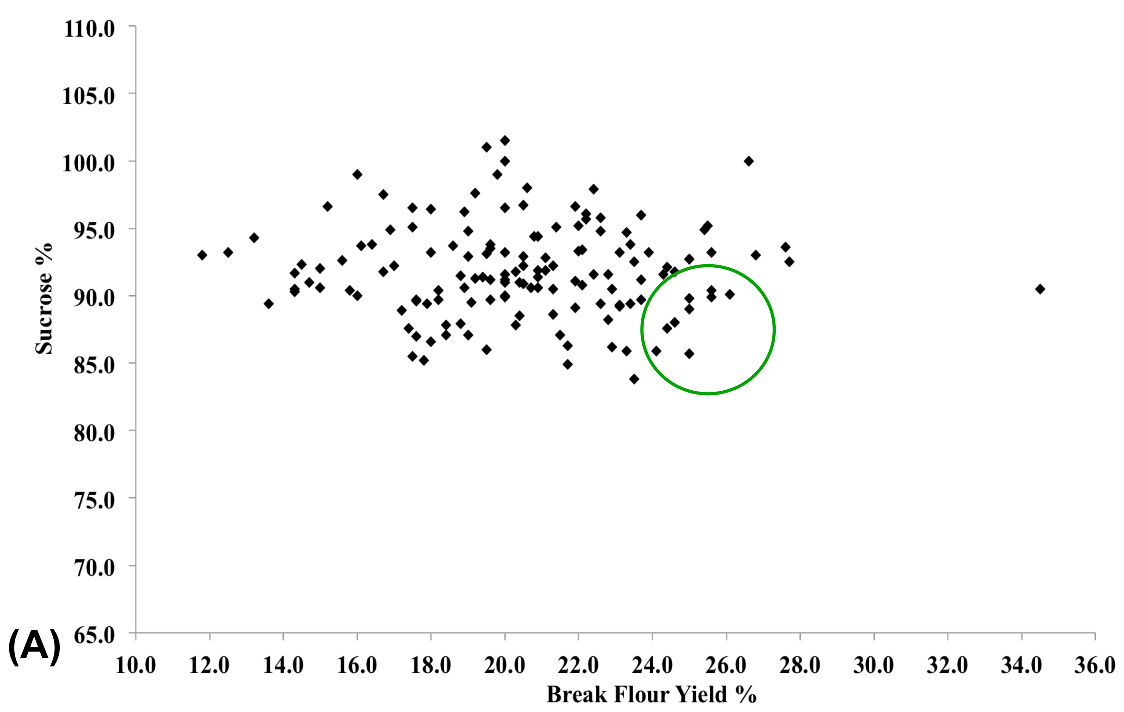
Introduction and Objectives

A combined approach of marker-assisted background selection (MABS) and forward breeding was used to transfer value added traits & improved agronomic performance of a variety. Two-mutations in *ALS* (acetolactate synthase) gene located on chromosomes 6B and 6D providing imidazolinone (IMI) herbicide tolerance were transferred to a popular soft white winter cv. Eltan. Four steps MABS approach was used to transfer two-gene Clearfield traits and recovered recipient parent genome >96% as Eltan in two backcrosses. Phenotypic selections were exercised in greenhouse conditions to select superior agronomic traits. Grain quality parameters were tested using single kernel characterization system (SKCS). Micro solvent retention capacity (SRC) test was applied to select desired flour quality using single plant seed. Two selected lines carrying two-gene Clearfield trait with highest recurrent parent genome (RPG) and superior agronomic and quality performance were released as soft white winter wheat varieties (Curiosity CL + and Mela CL+).

Materials and Methods

An Australian HRS wheat line 'CL618' that carry two mutant alleles of the *ALS* gene that provide resistance to wheat against imidazolinone group of herbicides 'imazamox'. The recurrent parent was a SWW wheat variety Eltan. Large number of backcross (BC$_1$ & BC$_2$) plants were phenotypically screened using herbicide spray in greenhouse conditions. Resistant plants were used for marker assisted selection. Foreground selection was carried out using allele specific markers to select resistant plants for two-gene Clearfield (Fig. 1B). MABS was carried out using SSR markers as described in Randhawa et al (2009) (Fig. 3).



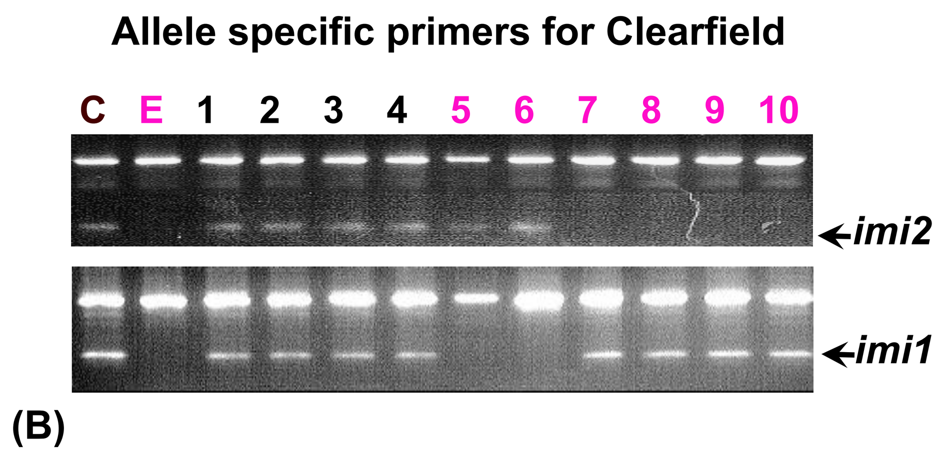


Figure 1. (A) Binary plot distribution of two quality parameters (% sucrose and % break flour yield) of Solvent Retention Capacity (SRC) test estimated from Clearfield backcross population, (B). Foreground selection for *imi1* and *imi2* gene using allele specific markers.

Results

Eltan × CL618 (Two-gene IMI line with D+B) Ist Backcross

- Phenotypic screening of 1600 BC$_1$F$_1$ plants for resistance by herbicide spray
- 378 plants selected which were survived after herbicide spray with rate of 12 oz/A
- Employed foreground selection to confirm for D+B gene and background selection for both carrier chromosomes (6D & 6B) of the RP using SSR markers
- Seven plants (carrying D+B gene) with maximum similarity of carrier chromosome background of the RP were used to make 1400 BC$_2$F$_1$ plants.

Eltan × BC$_1$F$_1$ 2nd Backcross

- Phenotypic screening of 1400 BC$_2$F$_1$ plants for resistance by herbicide spray
- 364 plants selected which were survived after herbicide spray with rate of 20 oz/A
- Confirm for both D+B IMI-gene, fix both carrier chromosome & background selection for 19 non-carrier chromosome with 3 markers from each arm
- Selected 17 resistant plants (with D+B gene) with maximum similarity of the RPG

BC$_2$F$_2$ Selfing

- Approximately 1700 seed were planted (~96 seed from each selected plant) and phenotypic screening were conducted by herbicide spray with rate of 16 oz/A
- 332 BC$_2$F$_2$ vigorous progenies were selected by phenotypic selections in the greenhouse and employed background selection using whole genome (~100) SSRs as well as tested for quality parameters (by SKCS and SRC)
- Finally, 12 plants (with D+B gene) were selected with maximum RPG and increased seed for conducting field trials

Figure 2. Schematic representation of transferring two-gene (*IMI1* and *IMI2*) into 'Eltan, using marker-assisted forward breeding approach.

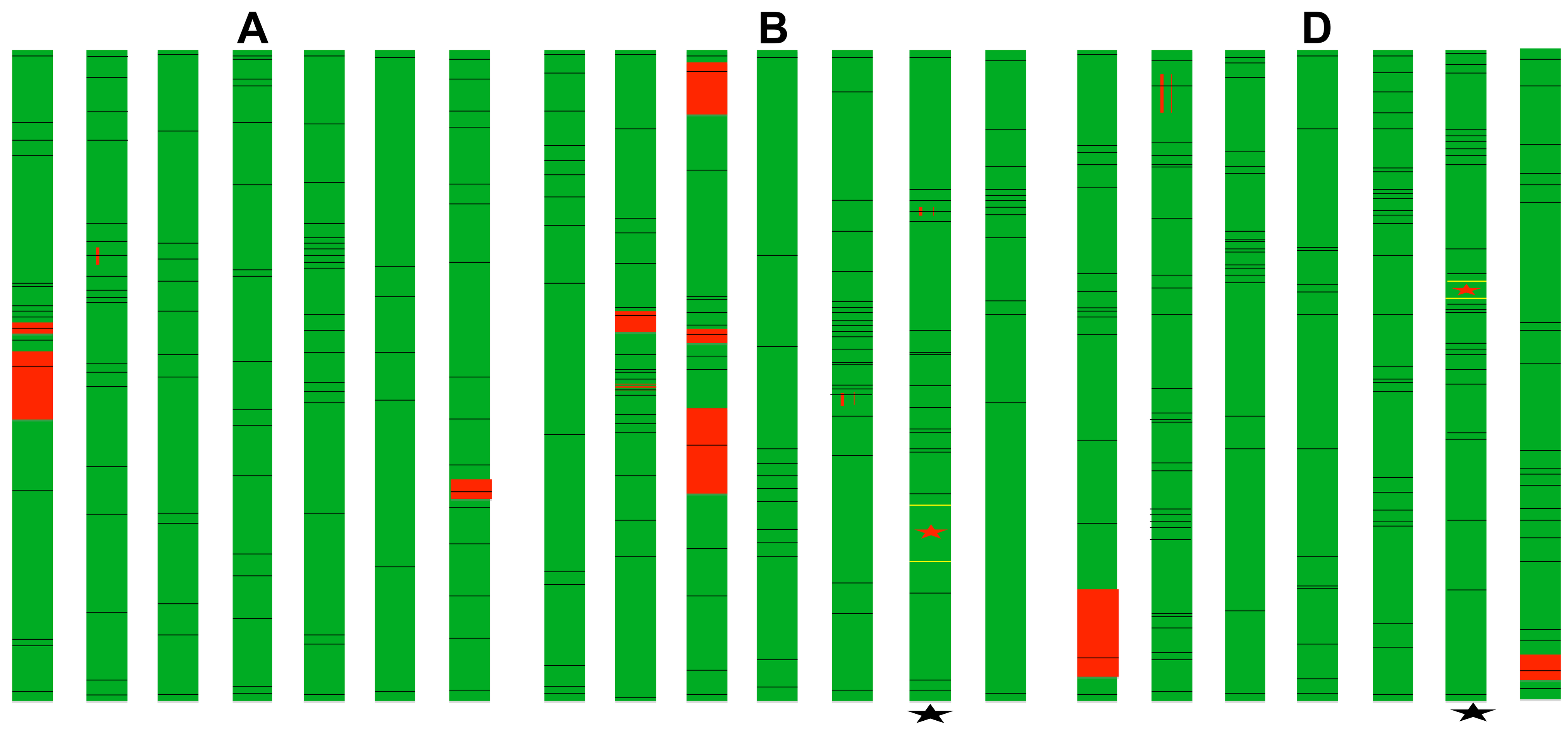


Figure 3. Graphical representations, showing parental derivation with green bars representing the homozygous recipient genotype alleles, red bars indicate the marker allele homozygous for donor genotype, and heterozygous loci were marked with half green and half red bars. Black star represents the carrier chromosome, with red star corresponding to the approximate position of the target genes for Clearfield on the carrier chromosomes 6B and 6D and black lines denote relative position of the SSR markers used for the background selection.

Table 1. Major characteristics of Curiosity CL+ & Mela CL+.

Characteristics	Curiosity CL+	Mela CL+
Imidazolinone Tolerance	Two-gene	Two-gene
Yield Potential	Excellent	Excellent
Test Weight	High than recipient	High than recipient
Stripe Rust Resistance	Better than recipient	Better than recipient
Snow Mold	Similar to better than recipient	Similar to better than recipient
Winter Hardiness	Similar to recipient	Similar to recipient
Cephalosporium Stripe	Similar to recipient	Similar to recipient
Strawbreaker Foot Rot	Similar to recipient	Similar to recipient
Cookie Diameter	Very Good	Very Good
Sponge Cake Performance	Superior than recipient	Superior than recipient

Figure 4. Field screening of Curiosity CL+ and wild type Eltan under spray trial.

Conclusion

Progeny of selected 12 plants was field evaluated under single and multi-location trials with and without spray. Genotypically the lines showed >96% recovery of the recurrent parent genome. MABS saved significant time for transferring two-gene mutations & enhanced the percentage of recipient parent genome recovery in two backcross cycles. Two recovered lines were released as two-gene Clearfield wheat varieties (Curiosity CL+ and Mela CL+) in soft white common winter market class.

Acknowledgement

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References

Randhawa HS, Mutti JS, Kidwell K, Morris CF, Chen, XM, and Gill KS (2009). Rapid introgression of single genes into popular cultivars using marker-assisted background selection. PLoS One Vol 4 (6) e-5752.