

DEVELOPING RICE VARIETIES TOLERANT TO ABIOTIC STRESSES THROUGH MARKER ASSISTED BACKCROSSING

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INTRODUCTION

Widespread and persistent rural poverty is a longstanding problem in Asia, particularly where farmers grow rice without irrigation. In these rainfed areas, rice is the dominant crop because it can be easily grown in the wet season. It is the rural population's staple food (up to 60% of energy intake) and the principal source of employment (50–70% of the labor force) and income. Rice yields in rainfed ecosystems remain low (0.5–2.5 t/ha) and unstable primarily due to stresses caused by drought, flooding, and poor soils. These problems will only get worse with looming climate change. In addition to limitations in access to technology and information, marketing infrastructure and seed production institutions are poorly developed.

Tolerance to abiotic stresses has been considered a very difficult trait to breed for. Despite widespread attention by rice breeders to the various stresses, there have been very few successes in terms of new varieties adopted by farmers. This is often attributed to the genetic and physiological complexity of tolerance of these stresses. However, recent scientific discoveries and advances—particularly in genetics, genomics, and crop physiology—have opened up new opportunities to reduce the impact of these stresses that would have been difficult if not impossible as recently as the turn of the century. One of the most promising approaches is the use of molecular markers to identify and select for genes controlling tolerance to these traits.

The present paper will describe the current approach of IRRI for developing stress-tolerant varieties through the use of marker assisted backcrossing (MAB). Submergence tolerance will be used as an example of how this approach is being applied for developing varieties tolerant of abiotic stresses.

THE BACKCROSS APPROACH

The backcrossing approach was proposed by Harlan and Pope (1922) primarily as a method of small grain improvement for traits controlled by single genes such as disease resistance. The seminal paper by Briggs and Knowles (1967) outlined the potential uses of the backcrossing method for both self-pollinated and cross-pollinated crops. One of the major benefits they cited was that “back-cross-derived varieties can be released safely *without the benefit of evaluation*

trials" (emphasis added). In addition, they proposed that the method was also appropriate for quantitatively inherited traits as well as for simply inherited traits.

According to Briggs and Knowles (1967), successful application of backcrossing requires three conditions: (a) existence of a suitable recurrent parent as a recipient of a desirable trait from the donor variety, (b) retention of the target trait through several generations of backcrossing and (c) the ability to reconstitute the recurrent parent genotype with a reasonable number of backcrosses and manageable population sizes. Reconstitution of the recurrent parent genotype would typically require about six backcross generations, assuming population sizes were sufficient to select for the recurrent parent phenotype.

The use of backcrossing has been practiced widely by breeders to develop near isogenic lines for basic studies and to improve inbreds used in hybrid crop breeding. However, it is safe to say that the approach has not generated a large number of varieties that have been successfully grown by farmers. Many breeders are reluctant to use the approach for various reasons, including the lack of a suitable recurrent parent and the laboriousness of the method in terms of number of crosses that need to be made. In addition, as conventionally practiced, the method does not result in a marked reduction of the time to develop a variety. While it should in theory, eliminate the need for extensive evaluation of the backcross-derived variety this has not been applied in practice.

MARKER ASSISTED BACKCROSSING

A more powerful backcrossing approach has been developed through the application of molecular markers such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). One of the early benefits envisioned with the use of molecular markers in plants was their use to accelerate the backcrossing process by reducing the number of backcrosses required to recover the recurrent parent phenotype (Tanksley *et al.* 1989). In addition, markers closely flanking the target gene could be used to select plants that had a crossover close to the gene, greatly reducing the amount of donor genes being introduced along with the target gene ("linkage drag").

The use of this powerful approach for MAB was first reported for rice by Chen *et al.* (2000) introducing resistance to bacterial blight disease into Chinese hybrid parents. It was also described for submergence tolerance using the *Sub1* gene at IRRI (Neeraja *et al.* 2007). The basic approach has been described in more detail in several papers by our group (Mackill *et al.* 2006; Neeraja *et al.* 2007; Collard and Mackill 2008). Using large population sizes (400 or more plants) for the backcross F₁ generations, it is possible to recover the recurrent parent genotype using only two or three backcrosses. This is shown in Fig. 1. The three selection steps (Collard and Mackill 2008) are:

- (i) **Target gene selection.** A marker tightly linked to the target gene, in this case *Sub1*, is used to identify plants in the segregating BC₁F₁ generation

that are heterozygous for the *Sub1* gene. This will be approximately half the population. Plants that do not possess the *Sub1* gene are discarded.

- (ii) **Recombinant selection.** From the plants that possess *Sub1*, markers about 5 cM distance from the gene are used to identify plants that do not have the donor allele at these loci. These are plants that have a recombination event near the *Sub1* gene. The rest of the plants are discarded.
- (iii) **Background selection.** For those plants that were recombinant, markers that are unlinked to the target gene are used to estimate the amount of donor segments they contain. For practical purposes it is usually suitable to use about 2 markers per chromosome arm (i.e. about 48 markers for the entire rice genome) if these are well spaced. Plants having the fewest number of markers are selected to make the next backcross generation.

This approach was used to derive plants that were identical to the mega variety Swarna in two and three backcrosses (Fig. 2). The BC2 version has the introduced genome segment from the donor variety IR49830-7 at the tip of chromosome 9. In this plant there was a recombination event on only one side of the gene. Approximately 6–7 MB of donor DNA was detected in the *Sub1* region. In the BC3 version we obtained a recombinant plant on both sides of *Sub1*. In this version approximately 3 Mb of donor DNA was detected in the *Sub1* region. This represents about 1% of the entire rice genome, meaning that these plants are more than 99% genetically identical with the recurrent parent Swarna. No donor alleles were detected at any of the background markers (Fig. 2a). So far we have not detected any phenotypic differences in the BC2 and BC3 plants. We have now developed six fully converted *Sub1* mega varieties (Table 1).

Table 1. Development of new versions of mega varieties with submergence tolerance (from Septiningsih *et al.* 2008)

Sub1 variety	Donor of <i>Sub1</i>	Generation	IR fixed line designation	<i>Sub1</i> region introgression size (Mb)
Swarna-Sub1	IR49830	BC2	IR05F101	6.5–7.3
		BC3	IR05F102	2.3–3.4
S. Mahsuri-Sub1	IR49830	BC2	IR07F101	6.5–9.2
		BC3	IR07F287	0.8–2.1
IR64-Sub1	IR40931	BC2	IR07F102	6.5–7.8
		BC3	IR07F292	1.1–2.7
TDK1-Sub1	IR40931	BC3	IR07F289	1.5–2.5
BR11-Sub1	IR40931	BC2	IR07F290	0.3–2.6
CR1009-Sub1	IR40931	BC2	IR07F291	2.7–6.4

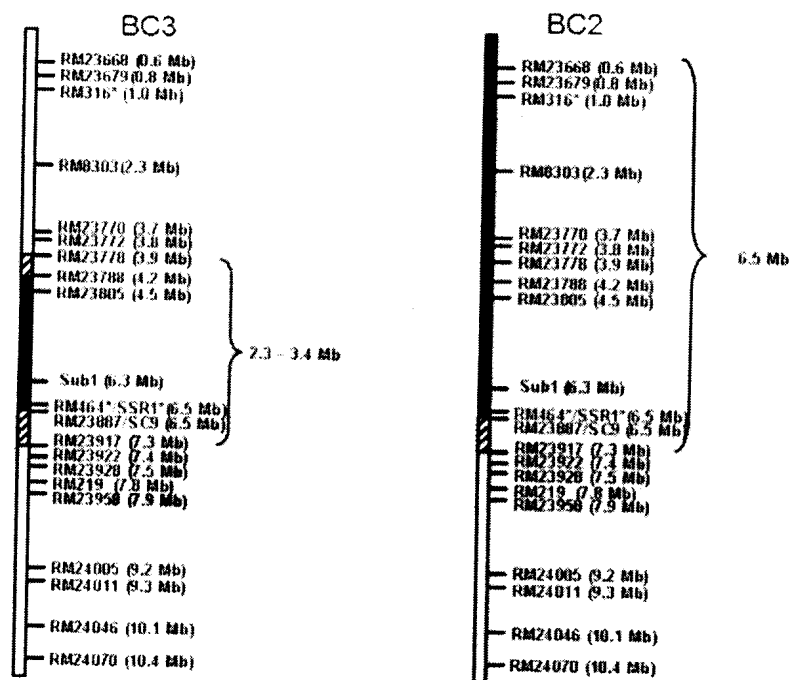


Fig. 2b. Molecular map of rice chromosome 9 in BC3 and BC2 showing the introgression size of chromosome segments from the donor of *Sub1* in Swarna background (Neeraja *et al.* 2007).

EVALUATION OF CONVERTED MEGA VARIETIES

The use of the MAB approach outlined above allows us to rapidly develop Sub1 mega varieties that possess the Sub1 type of submergence tolerance and that are 99% genetically identical to the mega variety used as the recurrent parent. Through the use of large F1 (and BC2F2 or BC3F2) population sizes (400 to 1000 plants) we can derive fully homozygous lines in 2–3 years. Rapid seed increase can occur as the new varieties are quickly evaluated for their resemblance to the recurrent parent and level of submergence tolerance. It has been shown that these varieties behave exactly as we would expect; they have the Sub1 type of submergence tolerance as well as all the other characteristics of the recipient donor variety.

Extensive tests conducted at IRRI show that Swarna-Sub1 has the same agronomic and grain quality characteristics as Swarna, with identical yield potential. The submergence tolerant varieties show two to three-fold yield advantage over the intolerant varieties under prolonged submergence (14–18 days) (Fig. 3). Virtually all of the varieties with Sub1 show strong submergence tolerance under the typical flash flooding conditions (complete submergence up to 18 days followed

Yield advantage of Sub1 under field conditions

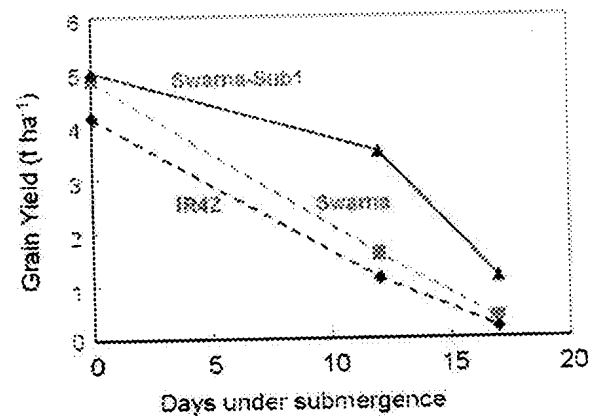


Fig. 3. Yield of Swarna-Sub1 compared with Swarna under different submergence conditions at IRRI (S. Singh and A. M. Ismail, submitted).

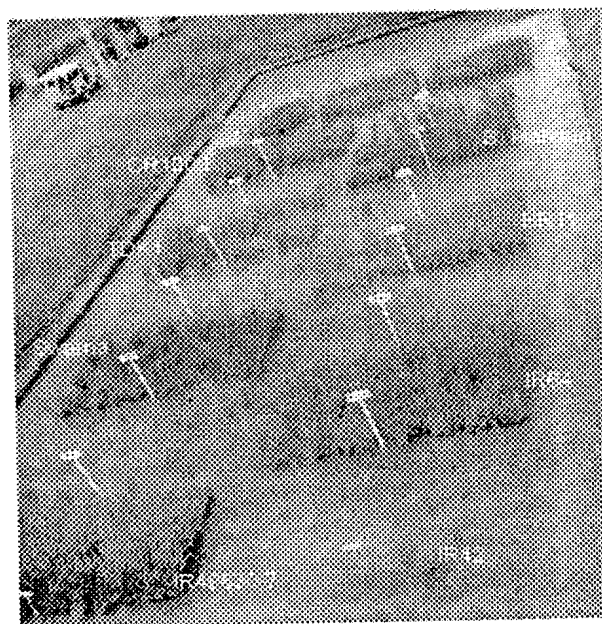


Fig. 4. Performance of submergence tolerant mega varieties after 17 days of complete submergence at IRRI, 2008 DS. In the first tier, the Sub1 donor IR49830-7 (left) is included with the susceptible check IR42 (right). In each subsequent tier, a Sub1 line is paired with its recurrent parent (in alternating sides).

by desubmergence) (Fig. 4). Under more prolonged submergence, the varieties are not tolerant, particularly if the water levels remain high (>20 cm) after flooding, or if there is partial submergence for over 3 weeks (stagnant flooding) if this occurs when the plants are still short. We have developed other varieties that are showing good performance under these conditions.

A major benefit of the MAB strategy is to use the varieties that are already popular with farmers so that the improved variety will be assured of possessing the traits prized by the farmers. This then justifies the added expense of the use of molecular marker technology. In rice, the existence of mega varieties that are widely grown offers the opportunity to use this approach to rapidly upgrade these varieties with new traits (Mackill 2006). As an example, the variety Swarna is widely grown on large areas of eastern India and certain parts of Bangladesh. Many of these areas are submergence-prone and farmers suffer greatly because of flooding. Government and private institutes are now rapidly producing seed for upscaling of Swarna Sub-1 and other Sub1 varieties in these areas. The farmers' reactions have been very positive, and they consider the possession of the properties of Swarna as a real advantage. This means that they do not have to become familiar with handling a completely new variety in order to solve the problem of flooding.

In order for these converted varieties to fulfill their true potential and achieve impact in the rainfed areas, they must be rapidly disseminated to the farmers suffering from the debilitating effects of stresses. This means that government agencies responsible for approving the release, seed production, and certification of varieties should assist in making them rapidly available for the farmers. This should be relatively easy, because the varieties that have been converted are actually modified versions of the varieties already popular with the farmers. As anticipated by Briggs and Knowles, the varieties do not need to be extensively evaluated, but need only to be compared with the original varieties. These varieties, therefore, do not need to enter the normal process for full evaluation, which usually requires at least 3 years of data in multi-location yield trials, superior grain yield and quality to the highest-yielding check. At most, they need to be carefully evaluated for resemblance to the original variety, evaluated for the trait introduced, and tested under PVS approaches for the reaction and feedback from the farmers. Over 2–3 years of these types of evaluation, seed production can be accelerated so that sufficient seed will be available to meet the demand expected from the farmers once the merits of the new lines are apparent.

REFERENCES

- Briggs FN, Knowles PF. 1967. Introduction to plant breeding. Reinhold Pub. Corp. New York.
- Chen S, Lin XH, Xu CG, Zhang QF. 2000. Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Crop Sci* 40: 239–244.
- Collard BCY, Mackill DJ. 2008. Marker-assisted selection: an approach for precision plant breeding in the 21st century. *Phil Trans Royal Soc B Rev* 363: 557–572.
- Harlan HV, Pope MN. 1922. The use and value of back-crosses in small-grain breeding. *J Hered* 13: 319–322.
- Mackill DJ. 2006. Breeding for resistance to abiotic stresses in rice: the value of quantitative trait loci. *In*: Lamkey KR, Lee M (eds). Plant breeding: The Arnel R Hallauer International Symposium. Blackwell Pub, Ames, IA. p. 201–212.
- Mackill DJ, Collard BCY, Neeraja CN, Rodriguez RM, Heuer S, Ismail AM. 2006. QTLs in rice breeding: examples for abiotic stresses. *In*: Brar DS, Mackill DJ, Hardy B (eds). Rice Genetics V. Int. Rice Res. Inst. Manila, Philippines. p. 155–167.
- Neeraja CN, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard BCY, Septiningsih EM, Vergara G, Sanchez D, Xu K, Ismail AM, Mackill DJ. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet* 115: 767–776.
- Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, Ismail AM, Mackill DJ. 2008. Development of submergence tolerant rice cultivars: the *Sub1* locus and beyond. (*submitted*).
- Tanksley SD, Young ND, Paterson AH, Bonierbale MW. 1989. RFLP mapping in plant breeding: New tools for an old science. *Bio/Technology* 7: 257–264.