Journal of Plant Molecular Breeding (JPMB)
Vol. 2/No. 2/ December 2014/ 43-55

Marker assisted selection for the improvement of Sarjoo-52 for drought tolerance by introgression of MQTL1.1 from the source Nagina–22

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Received: August 2014 Accepted: December 2014

Abstract

Literatures have reported that a lot of drought related genes were cloned and individual gene showed positive effects under controlled stress experiments, but were not much effective in the field. Although, the progresses by conventional breeding approaches were achievable as some drought varieties have been released to the farmers in the recent years but this is not adequate to cope up with the future demand of high yield for rice, as drought seems to spread to more regions and seasons. Therefore, marker assisted selection came into lime light for accelerating and giving pace to plant breeding.From the cross (Sarjoo-52× Nagina- 22) × Sarjoo- 52, plants were selected on the basis of presence of gene $MQTL_{1.1}$ responsible for the drought tolerance. These lines have been subjected to further breeding and trial tests. Agronomic performances and physiological behavior of these lines are also under track. The results showed that the variety Sarjoo 52 could be efficiently converted to a drought tolerant variety in a backcross generation followed by selfing and selection, involving a time of two to three years. Polymorphic markers for foreground and background selection were identified for the high yielding variety to develop a wider range of drought tolerant variety to meet the needs of farmers in the drought-prone regions. This approach demonstrates the effective use of marker assisted selection for a major QTL in a molecular breeding program.

Keywords: Background Selection, Drought, Foreground Selection, Marker Assisted Selection, Oryza Sativa, QTLs.

Introduction

More than three billion people in the world depends upon rice (*Oryza sativa* L.) and most of them comes from Asia. Surprisingly, ninety percent of world's total rice production is cultivated and consumed in Asia. Rice is a crop which is being cultivated under diverse eco-logies, from irrigated to rainfed upland to rainfed lowland to deep water. Irrigated rice accounts for 55% of world area and about 75% of total rice production. Rainfed lowland represents about 25% of total rice area, accounting for 17% of world rice production. Upland rice covers 13% of the world rice area and accounts for 4% of global rice production. Rice being a water loving

crop, is more prone to drought as compared to other cereal crops which shows better adaptation to lesser water availability.

Studies on the plant response to water stress are becoming increasingly important, as most of climatic change scenarios suggest an increase in aridity in many areas of the globe (Petit *et al*., 1989). On a global basis, drought in conjunction with high temperature and radiation,is known to be the most important environmental constraints to plant survival and to crop productivity. As irrigation water is not adequate as per crop requirement, the possible solutions to improve field productivity are i) environment control development i.e. improve plant living environment to fit the needs of crop, this includes technologies which reduce soil and water loss, decrease soil water evaporation, increase and maximize the use of soil water storage, collect non cultivate field run offs and use them as irrigation supplement. ii) Approach of biological water saving i.e. modify plant to adapt the dry environment; this includes genetic modification of plant, physiological regulation and application of crop complementary effort.As a matter of fact, management practices can contribute to increase yield in moisture stress environments but major progress will be realized through genetic improvement and therefore through plant breeding and molecular breeding, it would be better to develop drought tolerant varieties than to irrigate drylands.

Despite the importance of drought as a constraint, little effort has been devoted to developing drought-tolerant rice cultivars. In drought years, high yielding varieties inflict high yield losses, leading to a sudden decline in the country's rice production. Farmers of drought-prone areas require varieties that provide them with high yield in years of good rainfall and sustainable good yield in years with drought. The earlier approach of improving grain yield under drought through selection on secondary traits such as root architecture, leaf water potential, panicle water potential, osmotic adjustment, and relative water content (Fukai *et al*., 1999; Price and Courtois, 1999; Jongdee *et al*., 2002; Pantuwan *et al*., 2002) did not yield the expected results to improve yield under drought. Breeders and physiologists practiced selection for secondary traits as several earlier studies reported low selection efficiency for direct selection for grain yield under drought stress (Rosielle and Hamblin, 1981; Blum, 1988; Edmeades *et al*., 1989). Similarly, at the molecular level, initial efforts in rice were devoted to mapping of QTLs for secondary drought-related traits such as root morphology and osmotic adjustment (Yadav*et al*. 1997; Kamoshita *et al*. 2002; Babu *et al*. 2003). Because QTLs for secondary traits are not linked to direct yield increase under drought, marker-assisted selection for such QTLs has not been successfully used to improve yield under drought stress in rice.

Upto now, a lot of drought related genes were cloned and individual gene showed positive effects under controlled stress experiments, but were not effective in the field. However, the progresses by conventional breeding approaches were

achievable as some drought varieties have been released to the farmers in the recent years. Although, this is not adequate to cope up with the future demand of high yield for rice, as drought seems to spread to more regions and seasons across the country. Therefore, marker assisted selection came into lime light for accelerating and giving pace to plant breeding.

Mapping studies are performed to detect linkage of a molecular marker to a gene affecting a trait of interest. It then becomes possible to select for the desirable allele of those genes based on marker genotype rather than, or in addition to, field phenotype (Jongdee *et al*., 2002). This technique, known as marker assisted selection (MAS), is theoretically more reliable than selection based solely on phenotype, as a marker tightly liked to the desirable gene would represent selection with a heritability of near unity for that specific gene (Bernardo, 2002). Marker-assisted selection may be useful to improve traits that are either controlled by a few genes or where phenotypic evaluation is difficult/costly to perform. The relative difficulty associated with drought-resistance phenotyping suggests that there is scope for the use of MAS in breeding for drought resistance (Bernardo, 2002).

The effectiveness of MAS depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch *et al*. 1999a; Frisch and Melchinger 2005). MAS has previously been used in rice breeding to incorporate the bacterial blight resistance gene *Xa21* (Chen *et al*. 2000) and *waxy* gene (Zhou *et al*. 2003) into elite varieties.

The identification of QTLs with a major effect on grain yield raises a new hope of improving grain yield under drought through marker assisted breeding. The availability of the major effect QTL for drought tolerance, a theoretical framework for marker assisted selection and the existence of intolerant varieties that are widely accepted by farmers provides an opportunity to develop cultivars that would be suitable for larger areas of drought prone rice (Mackill, 2006).

Considering the above aspects, the present study was proposed to exploit the gene action and variability through marker assisted selection with the objective of isolating promising high yielding drought tolerant recombinants through conventional and marker assisted selection (MAS) approaches.

Materials and method

The present investigation was conducted during three seasons i.e. 2010, 2011 and 2012 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi and off season, 2010- 2011 at Central Rice Research Institute (C.R.R.I.) Cuttack, Odisha.Sarjoo –52, derived from T(N)1 ×Kashi was notified in 1982 for general cultivation. It takes approximately 130-133 days after sowing DAS to harvest. Sarjoo–52 is an irrigated, semi dwarf (98cm) and erect type. Grains are long, bold, abdominal white present (AWP) and white. It is moderately resistant to bacterial leaf blight BLB. It is reported

to yield 50-60 q/ha. It is mainly grown in Uttar Pradesh. Nagina 22, a selection from Rajbhog was used as donar parent. Nagina–22 was notified in 1978. It takes around 85-102 days. Grains are short, bold and white. The variety is susceptible to blast, BLB and resistant to drought. It gives yield of about 20-25 Q/ha and grown well in Uttar Pradesh as upland crop.

The seeds of drought tolerant variety (Nagina-22) and drought susceptible variety (Sarjoo–52) were sown in raised nursery beds in the last week of June, 2010 at the Research Farm, Banaras Hindu University. Twenty one days old seedlings were transplanted in the well puddled field at a spacing of 30 × 15 cm between row to row and plant to plant, respectively with row length of 3 m in a crossing block on three different dates at the interval of seven days in three replications. Standard agronomic practices were followed to raise good crop. Five rows of Nagina-22 (donar) were transplanted in separate blocks on different dates at the interval of 7 days to synchronize the flowering for making the crosses.

Half of the F_1 seeds of the cross with their parents were transplanted at the Research Farm at Central Rice Research Institute (C.R.R.I.), Cuttack, at the spacing of 30 × 15 cm between row to row and plant to plant, respectively with a row length of 3.0m in three replications.

Screening of the F_1s was done at different growth stages i.e., seedling stage and vegetative stage. Depending upon the screening test, backcross with drought susceptible parents was done. Seeds from thebackcross (BC_1) and F_1 plants were harvested separately. Marker validation and hybrid confirmation were done before commencing marker assisted selection.F_1 plant progeny, along with the parents were grown to raise F_2 generation. Compact family randomized block design with three replications was followed. Drought susceptible and drought tolerant plants were screened on the basis of leaf rolling and were harvested separately. Fresh crosses were also made to get F_1 seeds.

BC_1F_1 along with the parents were also grown in compact family randomized block design in three replications for phenotypic study.20 plants in parents F_1s and 50 plants in F_2 per replication were selected for MAS. Heterozygous plants selected on the basis of marker assisted selection were allowed to self to produce BC_1F_2 generation.BC_1F_2 plants were again subjected to second round of marker assisted selection.

Young leaves were collected from 20-25 days old seedlings and immediately stored in -20°C till further processing. The DNA was extracted following CTAB extraction method (Doyle and Doyle, 1987). Polymerase chain reaction was performed to selectively amplify *in vitro* a specific segment of the total genomic DNA to a billion fold (Mullis *et al.,* 1986). The most essential requirement of PCR is the availability of a pair of short (typically 20-25bp nucleotides) primers having sequence complementary to either end of the target DNA segment (called template DNA) to be synthesized in large amount. The components of the PCR reaction were first added in a sterilized 1.5ml microcentifuge tube and

then mixed thoroughly by vortexing. To each PCR tubes (0.2ml), 14 µl of reaction mixture was distributed, and finally template DNA of individual rice genotpyes was added. The tubes containing reaction mixture were placed in the wells of the thermal cycler block (Eppendorf Thermo–cycler, USA) and amplification reaction was carried out with the thermalcycler programme. 40 cycles of denaturation, annealing and extension was programmed for the study. The amplified DNA fragments generated through SSR primers were resolved through electrophoresis in 2.5% agarose gel prepared in TAE [242g Tris–base; 57.1ml glacial acetic acid and 100ml 0.5 M EDTA (pH 8.0) bring final volume to 1000ml] buffer. Ethidium bromide solution at a final concentration of 0.03 ng/µl was added to the agarose solution.

For electrophoresis, 15µl of the PCR product was mixed with 2µl of 6x loading dye (0.25% bromophenol blue in 30% glycerol) and loaded in the slot of the agarose gel. In order to determine the molecular size of the amplified products, each gel was also loaded with 6 µl of 50 bp DNA size marker (Fermentas, USA). Gel electrophoresis was performed at a constant voltage of 65V for about 3.5 hours. Finally, the gels were visualized under a UV light source in a gel documentation system (Gel Doc^{TM} XR +, BIO RAD, USA) and the images of amplification products were captured and stored in a computer for further analysis and future use.

19 SSR markers linked to the QTLs for drought tolerance on various linkage groups were used for foreground selection to select the individuals presumably having the donor allele. Particular target (drought tolerance QTL) was flanked by these markers. The tighter the markers are linked to the QTL, the greater the chance that the QTL mapped between a pair of flanking marker has indeed been transferred. Therefore, phenotypic testing of final products of the MAS exercise needs to be performed in order to confirm the transfer of drought tolerance QTL. At the same time selected markers unlinked to drought tolerance have been used to select those individuals with minimal linkage drag (background selection).

Results:

Parental polymorphism survey

Initially, parental polymorphism survey was performed among parental genotypes, Sarjoo–52 (drought susceptible) and Nagina-22 (drought tolerant). On the basis of parental polymorphism survey, 19 SSR markers were used to validate for the drought tolerance in the back-cross population. Flanking markers RM 212- RM 3825, produced reproducible and polymorphic bands. These markers clearly distinguished drought susceptible and tolerant parents. In rice, hundreds of microsatellite markers were developed which are publicly available and are being used for MAS, gene tagging, mapping and phylogenetic studies.

Hybrid confirmation in F_1's

Oryza sativa is basically a self-pollinated crop, with limited degree of outcrossing (< 0.5%). The factors limiting the receptivity of rice flowers to outcrossing include a short style and stigma (1.5 to 4

mm in combined length), short anthers, limited pollen viability and brief period between opening of florets and release of pollen (between 30 seconds and 9 minutes) (Morishima, 1984; Oka, 1988). It therefore became essential to confirm the true type hybrid condition in the F_1s. SSR markers RM 212- RM 3825 (linked with $MQTL_{1.1}$) which are co - dominant in nature, gave promising results. Heterozygous plants were selected for further generation advancement and backcrossing.

Production of BC_1F_1 generation

Five plants in F_1 generation of the cross Sarjoo-52 × Nagina-22 were back crossed with the recurrent parent Sarjoo-52 to produce around 250 seeds at Agricultural farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Marker assisted foreground selection in BC_1F_1 and production of BC_1F_2 generation

BC_1F_1 plants were screened for the presence of *$MQTL_{1.1}$* with the linked and validated SSR markers RM 212–RM 3825, respectively from the cross (Sarjoo-52 × Nagina-22) × Sarjoo-52. SSR markers used in the study are co-dominant in nature therefore, in BC_1F_1 population, two types of banding patterns were amplified i.e., homozygous susceptible type and heterozygous types. The segregation of BC_1F_1 plants into drought tolerant and susceptible can be seen clearly in the representative gel picture of screening of 107 BC_1F_1 plants for *$MQTL_{1.1}$* with linked molecular marker RM 212–RM 3825 (Figure1).

Production of BC_1F_2 generation

Since, flanking markers were used in the study, emphasis was given on the selection of only those plants which exhibited heterozygous banding pattern for both the markers i.e. RM 212–RM 3825. Thus plant number SA-C-5, SA–C–7, SA–C–24, SA–C–25, SA–C–31, SA–C–39 and SA–C-65 from the cross (Sarjoo-52 × Nagina-22) × Sarjoo-52 were selected. Based on agronomic performance and drought related traits, three top performers were selected and allowed to self to produce BC_1F_2 seeds.

Marker assisted foreground and background selection in BC_1F_2 generation and production of BC_1F_3 seeds

Foreground selection: Selected BC_1F_2 plants from the cross (Sarjoo-52 × Nagina-22) × Sarjoo-52 was screened for *$MQTl_{1.1}$* with the linked and validated markers (Figure 2).

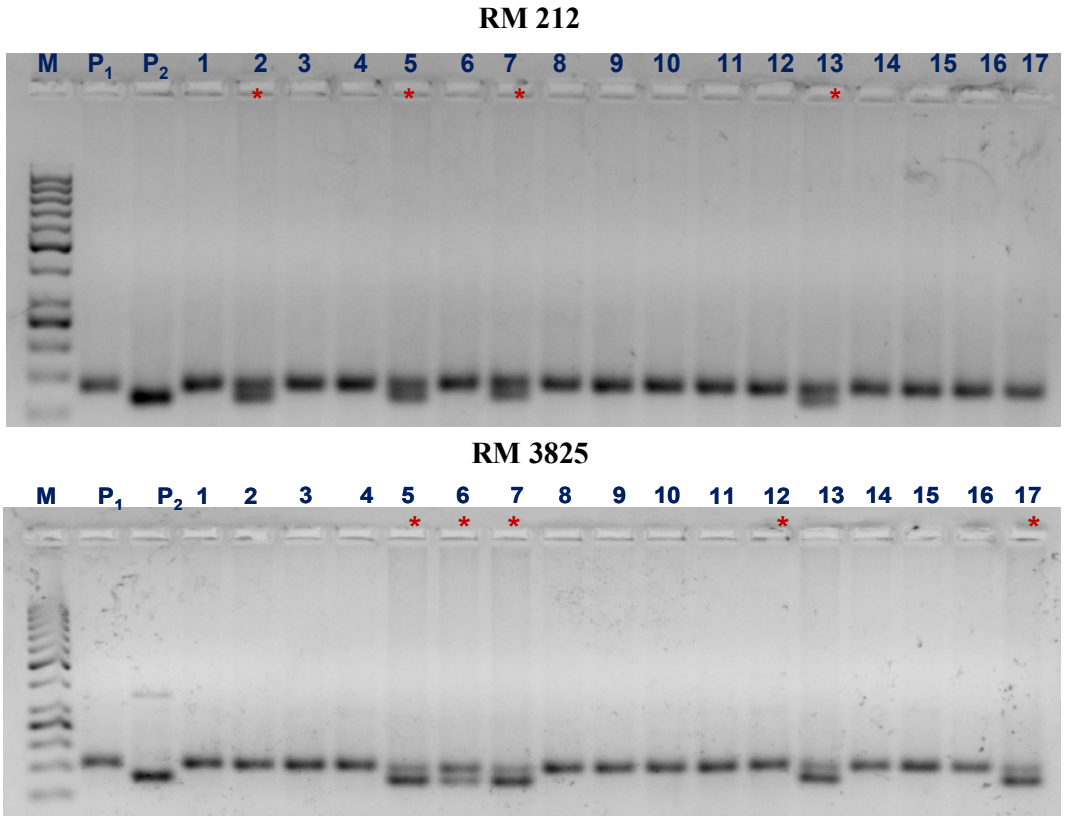

Figure 1. Representative gel picture of foreground selection for MQTL1.1 in BC1F1 generation of (Sarjoo-52×Nagina–22) × Sarjoo-52).

Background selection:

The gene positive plants in BC_1F_2 generation were taken for background selection with polymorphic primers (Figure 3). After, background selection in BC_1F_2, 70 loci became homozygous out of 73 polymorphic loci between Sarjoo–52 and Nagina-22+MQTL1.1 in plant no. SA–C–10–23 of the cross (Sarjoo–52 × Nagina-22) × Sarjoo–52. Maximum genome recovery in BC_1F_2 with *MQTL*$_{1.1}$ was about 83.5%.

Discussion

The present study indicated that MAS strategy is an effective means of utilizing QTLs with large effects in rice breeding programs. Sarjoo 52 is an elite Indian rice variety that was first introduced in India way back in 1982 and still widely grown in many areas of the country due to it stable yield, aromatic and good quality. Hence, Sarjoo 52 was selected as a recipient parent. Marker assisted foreground selection was proposed by Tanksley (1983) and investigated in the context of introgression of tolerant genes by Melchinger (1990). If in BC_1 generation more than one individual satisfying the strongest condition is found, selection between them can be performed on the basis of analysis of other marker loci (located either on the carrier or on non carrier chromosome) to determine the most desirable individual

for producing BC_2 (Tanksley *et al*., 1989).

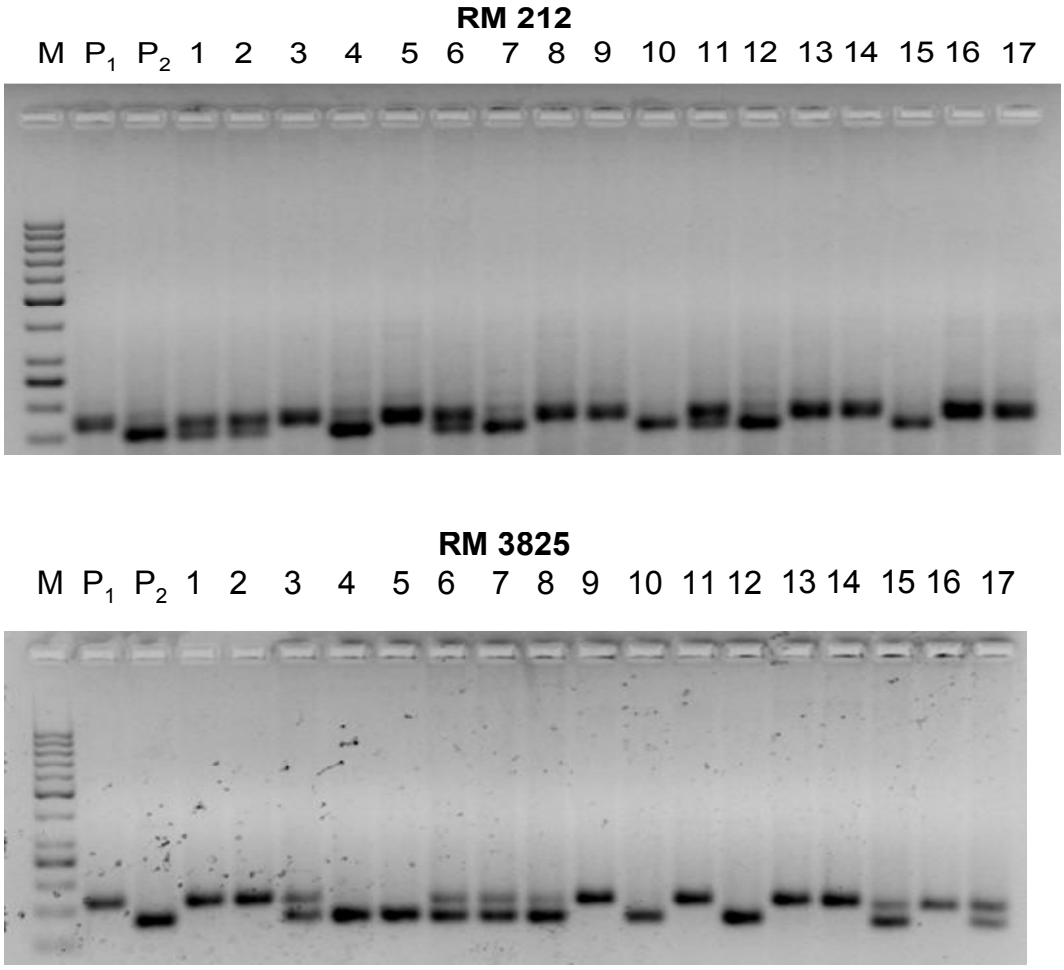

Figure 2. Representative gel picture of foreground selection for MQTL1.1 in BC1F2 generation of (Sarjoo-52 × Nagina–22) × Sarjoo 52).

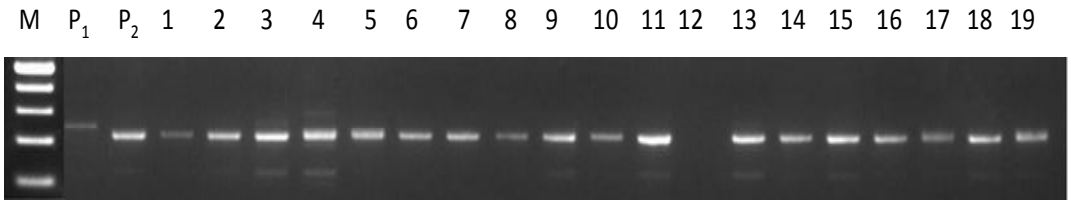

Figure 3. Representative gel picture of background selection in BC_1F_2 of (Sarjoo–52× Nagina-22) × Sarjoo–52 using polymorphic markers.

The success of marker assisted backcross breeding (MAB) depends upon several factors, including the distance between the closest markers and the target gene, the number of target genes to be transferred, the genetic base of the trait, the

number of individuals that can be analyzed and the genetic background in which the target gene has to be transferred, the type of molecular marker (s) used and available technical facilities (Weeden *et al*., 1992; Francia *et al*., 2005). Identification of molecular markers that should co- segregate or be closely linked with the desired trait (if possible, physically located beside or within genes of interest) is a critical step for the success of MAB. The most favourable case for MAB is when the molecular marker is located directly within the gene of interest (direct markers). MAB conducted using direct markers is called gene assisted selection (Dekkers, 2003). Alternatively, the marker is genetically linked to the trait of interest. Before a breeder can utilize linkage–based associations between a trait and markers, the associations have to be assessed with a certain degree of accuracy so that marker genotypes can be used as indicators or predictors of trait genotypes and phenotypes.

Table 1. Details of SSR primers associated with drought QTL *MQTL*$_{1.1}$.

QTL	Flanking markers	LG	Forward sequence	Reverse sequence	R^2	Tm	Reference
Meta QTL	RM 212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG	12.1	55℃	Salunke *et al*., 2011
	RM 3825		AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG		60℃	Salunke *et al*., 2011

The lower the genetic distance between the marker and the gene, the more reliable is the application of the marker in MAS. However only in few cases will the selected marker allele be separated from the desired trait due to a recombination event (appearance of false positives). The presence of a tight linkage between desirable trait(s) and a molecular marker(s) may be useful in MAS to increase gain from selection. Based on the studies by Lee (1995) and Ribaut *et al.* (2002), it could be generalized that whenever a target gene is introduced for the first time from either wild or unadapted germplasm, flanking markers as close as 2cM is considered an ideal option, while in the transfer of the same target gene in subsequent phases from elite into elite lines, positioning the flanking markers near by might be effective in reducing the required size of the backcross population.

MAS have generated a good deal of expectations, which in some cases has leaded to over optimism and in others to disappointment because many of the expectations have not yet been realized. Although documentation is limited the current impact of MAS on products delivered to farmers seems to be small. New developments and improvements in marker technology, the integration of functional genomics with QTL mapping and the availability of more high density maps are the other factors that will greatly affect the efficiency and

effectiveness of QTL mapping and MAS in the future. The development of high density maps that incorporate new marker types, such as single nucleotide polymorphism (SNPs) and expressed sequence tags (EST) will provide researchers with a great arsenal of tools for QTL mapping and MAS.

Conclusion

Cross (Sarjoo–52 × Nagina-22) × Sarjoo–52 exhibited polymorphism for the presence of a single gene for drought tolerance i.e. $MQTL_{1.1}$. The selected lines have been subjected to further breeding and trial tests. Agronomic performances and physiological behavior of these lines are also under track. The results showed that the variety Sarjoo-52 could be efficiently converted to a drought tolerant variety in a backcross generation followed by selfing and selection, involving a time of two to three years. Polymorphic markers for foreground and background selection were identified for the high yielding variety to develop a wider range of drought tolerant variety to meet the needs of farmers in the drought-prone regions. This approach demonstrates the effective use of marker assisted selection for a major QTL in a molecular breeding program. The obtained results suggest that MAS should be applied not only for the target segment but also for the background selection. This study could have a good impact in rice breeding and it is applicable for the introduction of important agronomic traits into the genomes of popular rice cultivars.

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انتخاب به کمک نشانگر به منظور بهبود رقم Sarjoo-52 برای مقاومت به خشکی با استفاده از ژن MQTL1.1 از رقم Nagina-22

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چکیده

گزارش‌های زیادی مبنی بر کلون ژن‌های مربوط به خشکی وجود دارند. اگرچه بسیاری از آنها مؤید اثرات مثبت در شرایط آزمایشگاهی بوده اما در سطح مزرعه اطلاعات زیادی در اختیار نمی‌باشد. اگرچه بوسیله اهداف اصلاحی سنتی در سال‌های اخیر در بعضی از ارقام خشکی، پیشرفت‌هایی در سطح مزرعه بدست آمده است، اما این دستاوردها نمی‌تواند بدلیل گسترش خشکی در بعضی از فصول و در بعضی از مناطق، کافی باشد و نتایجی در عملکرد آتی در برنج داشته باشد. بنابراین، انتخاب به کمک نشانگر ، مسیر را به منظور شتاب و تسریع اصلاح نباتات هموار نموده است. از تلاقی (Sarjoo-52 χ Nagina-22) χ (Sarjoo- 52)، گیاهانی براساس وجود ژن MQTL1 که مسئول مقاومت به خشکی است انتخاب شدند. این لاین‌ها در معرض آزمون‌های آزمایش و اصلاح مجدد قرار گرفتند. عملکرد زراعی و رفتارهای فیزیولوژیکی این لاین‌ها در حال بررسی هستند. نتایج نشان دادند که ارقام Sarjoo 52 می‌تواند به طور موثری به رقم مقاوم به خشکی تبدیل گردد. نتاج حاصل از تلاقی برگشتی و انتخاب آن‌ها در بازه‌ی زمانی ۲ تا ۳ سال رخ داد. نشانگرهای چند شکلی به کار گرفته شده برای پس و پیش انتخاب به منظور انتخاب رقم پر محصول و توسعه طیف وسیع‌تری از رقم مقاوم به خشکی برای پاسخگویی به نیازهای کشاورزان در مناطق مستعد خشکسالی شناسایی شدند. این رویکرد حاکی از استفاده موثر از انتخاب به کمک نشانگر برای یک QTL کمی در برنامه اصلاح مولکولی است.

کلمات کلیدی: برنج، پس انتخاب، پیش انتخاب، خشکی، نشانگرهای انتخاب، QTL.