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Original scientific paper

MARKER AIDED SELECTION (MAS) FOR QUALITATIVE TRAITS IN SEGREGATING F_2 POPULATIONS OF RICE

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The selection and screening of desirable traits at early stages of plant development is possible with the advancement of marker assisted selection (MAS). Marker aided selection can be used for monitoring the presence or absence of genes in breeding populations and can be combined with conventional breeding approaches. In this study, crosses between high-yielding cultivar, Nemat, with 4 local aromatic varieties were made followed by phenotypic selection for desirable individual plants in F_2 populations. Then, MAS applied to genotypic selection of aromatic plants using allele specific amplification (ASA) marker for fragrance (*fgr*) and SSR marker RM190 linked with intermediate amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) in these populations. Molecular screening based on RM190 and fragrance markers showed that 11 lines were homozygous aromatic and 32 lines had desirable cooking quality in segregating F_2 population. Results demonstrated that marker assisted selection in F_2 segregating populations reduced the time and cost effective for accelerating cultivar development in rice. Further analyses are underway on these plants on subsequent generations through pedigree breeding method. These plants promise to develop new aromatic rice lines through classical and molecular breeding in the near future in Iran.

Keywords: aroma, markers, quality, rice, screening

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INTRODUCTION

Rice is one of the most important cereal crops and provides the staple food for about half of the world's population especially for the people in developing countries (MOOSAVI *et al*., 2015). Existence of quality traits in rice has made rice with pleasant aroma and soft tissues to have a higher price in the domestic and foreign market (YI *et al*., 2009). The eating and cooking qualities of rice are heavily emphasized in breeding programs because they determine market values and they are the appealing attributes sought by consumers. Recently, intensive genetic studies have pinpointed the genes that control eating and cooking quality traits (PHING LAU *et al.*, 2013).

The most important chemical composition in aromatic varieties of rice is the 2-acetyl-1-pyrroline (2AP) (LORIEUX *et al*., 1996). Cooking and eating quality of rice is under the influence of some physicochemical properties such as amylose (AC), gel consistency (GC) and gel temperature (GT) (JANGSUTTHIVORAWAT *et al*., 2009). Wx acts as a major gene for AC and SSII acts as a master gene controlling GT. Gelatinization temperature (GT) is another important quality predictor in determining the cooking quality of rice. Low GT rice needs less energy input during cooking than high GT rice. GT in rice is mainly controlled by the starch synthase IIa (SSIIa) gene which is located on chromosome 6 (JIN *et al*., 2010).

Genotyping of markers tightly linked to traits can then rapidly predict the phenotype of a large population often well before phenotype screening at reduced cost. Marker ided selection (MAS) can be used for monitoring the presence or absence of genes in breeding populations and can be combined with conventional breeding approaches (KIANI, 2011). In addition, this approach shortens the varietal development time, and is therefore able to deliver improved rice varieties to farmers within a shorter period of time (PHING LAU *et al*., 2013).

Improvement of grain quality has been the one of the most important objectives of rice breeding, after yield improvement. Grain quality is a complex trait in rice and consisting of amylose content, amylopectin content, amylose: amylopectin ratio, gelatinization temperature, gel consistency, alkali spreading value, kernel length, kernel breadth, length/breadth ratio, kernel length after cooking and aroma are the most important (SUNDARAM *et al.*, 2018).

After the sequencing of the rice genome, the genomic loci underlying these traits are now being discovered and it is known that a few key loci like waxy, granule bound starch synthase, soluble starch synthase, starch branching enzyme, badh2 etc. play a key role in controlling various traits associated with grain quality and markers linked to these loci have been developed earlier. In an association study involving 380 indica rice genotypes, SHOBHA RANI *et al*. (2011) validated the reported molecular markers and established that the markers show varied levels of association for each trait. A functional marker, namedBADEX7-2, targeting a 8-bp indel in the candidate gene for fragrance/aroma trait, i.e., badh2 has been developed. This marker can unequivocally distinguish aromatic rice varieties from non-aromatic ones (SAKTHIVEL *et al*., 2008).

The use of cost-effective DNA markers for important agronomic traits provides opportunities for breeders to develop high-yielding, stress-resistant, and better-quality rice cultivars (JENA and MACKILL, 2008). Therefore, rapid development of new rice cultivars with ideal agronomic properties can be achieved through combination of conventional breeding with marker aided selection.

This study aimed to molecular screening of fragrance gene and intermediate AC, GC and GT status in individual F_2 plants with superior agronomic attributes which derived from the crosses between high-yielding variety with local high quality ones.

MATERIALS AND METHODS

Plant materials

A field experiment was carried out at research farm of Sari University of Agricultural Sciences and Natural Resources during 2014. Four segregating F_2 populations Nemat/Hashemi, Nemat/Sang Tarom, Nemat/Tarom deylamani, and Nemat/Jelodar were developed from crosses between high-yielding rice varietiy Nemat with local landraces (Hashemi, Sang Tarom, Tarom Deylamani and Jelodar). Standard agronomic practices were adopted to ensure crop growth. A hundred superior genotypes were selected from four segregating populations according to superior morphological characteristics. Farm operation was conducted according to local practice.

Phenotypic screening

In classical plant breeding, selection typically involves evaluating a breeding population for one or more traits at field trials. In pedigree breeding method, selection of desirable plants is made at early generations for traits of higher heritability. In each population, phenotypically superior F_2 plants were selected and advanced to molecular assay.

Polymerase Chain Reaction (PCR) Analysis

DNA was extracted from leaves of rice plants as described by DELLAPORTA *et al*. (1983). Aroma genotyping was carried out using allele specific amplification (ASA) method described by BRADBURY *et al.* (2005). PCR was performed using 0.2 µl Taq DNA Polymerase [5 units], 1 µl of genomic DNA 10 ng µl-1, 2.5 µl of 10X buffer, 1 µl of 50 mM MgCl2, 1 µl of dNTPs [5 mM], 2.5 µl of each primer (ESP, IFAP, INSP and EAP – Table 1) [2 µM], in a total volume of 25 µl. Cycling conditions were an initial denaturation of 94°C for 2 min followed by 30 cycles of 5 s at 94°C, 5 s at 58°C, 5 s at 72°C; concluding with a final extension of 72°C for 5 min. PCR products were then fractioned by ethidium bromide-stained %1.5 agarose gel electrophoresis in TAE buffer and photographed. A 100 bp ladder molecular weight standard (Roche) was used to estimate PCR fragment size.

RM190 marker is linked with three cooking characteristics (GT, GC and AC) (JAIRIN *et al*., 2009). PCR conditions for RM190 was 94° C for 4 minutes and 35 cycle of 94 °C for 1 minute, 55 °C for 5 seconds and at the end 72 °C for 7 minutes. PCR products fractioned in 1.5% agarose gel electrophoresis and photographed after staining with 0.5 mg/ml ethidium bromide. A 100 bp ladder molecular weight standard (Roche) was used to estimate PCR fragment size.

RESULTS

Phenotypic screening

Four F_2 populations were developed and stringent phenotypic selection based on phenotypic preference (like early maturity, panicle length, non-shuttering, etc.) was carried out on these populations to obtain agronomical desirable plants and reduce the population size for

further PCR analyses. In each population, 20 phenotypically superior F_2 plants were selected and advanced to molecular assay (Table 2).

Table 1. Molecular markers for screening fragrance and other qualitative genes in rice

Primer name	Primer type	trait	Forward sequence	Reverse sequence	Fragment length (bp)
RM190	SSR	Intermediate AC, GT, GC	GCTACAAATAGCCACCC ACACC	CAACACAAGCAGAGAAGTG AAGC	150
External sense primer (ESP)	SNP	Aroma	TTG TTT GGA GCT TGC TGA TG		577
Internal fragrant antisense primer (IFAP)	SNP		CAT AGG AGC AGC TGA AAT ATA TAC C		257
Internal non-fragrant sense primer (INSP)	SNP		CTG GTA AAA AGA TTA TGG CTT CA		355
External antisense primer (EAP)	SNP		AGT GCT TTA CAA AGT CCC GC		585

Table 2. The results of genotyping using molecular markers in segregating F_2 population

Segregating F_2 population	Total plants	Number of phenotypically superior genotypes	Number of genotypes positive for RM190	Number of homozygous fragrant genotypes
Nemat/Hashemi	100	20	9 (45%)	4 (44.4%)
Nemat/Sang Tarom	100	20	6 (30%)	1 (16.67%)
Nemat/Tarom deylamani	100	20	9 (45%)	4(44.44%)
Nemat/Jelodar	100	20	8 (40%)	2 (25%)

Molecular screening for AC, GT and GC

For monitoring and molecular evaluation of AC, GC and GT, the RM 190 marker was used (Table 1). The use of this marker in the segregating populations had the ability to distinguish the cooking quality lines in studied F_2 populations (Table 2) using Hashemi as standard for allelic detection. Using this marker 9, 6, 9 and 8 lines were positive for intermediate

AC, GT, GC status in populations Nemat/Hashemi, Nemat/Sang Tarom, Nemat/Tarom deylamani, and Nemat/Jelodar, respectively (Figure 1).

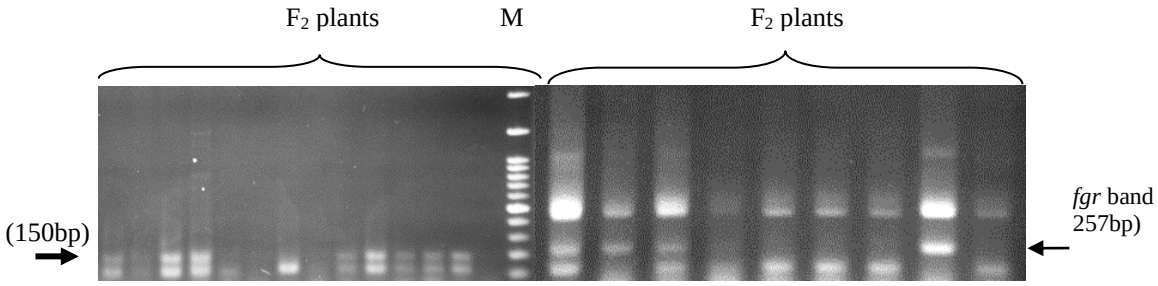

Figure 1. Screening intermediate AC, GC, GT genotypes using RM190 (left) and aromatic genotypes using ASA marker (right) is F_2 poulation of Nemat/Jelodar. M, is 100bp ladder.

Molecular screening for fragrance gene

Hashemi, Tarom Dilmani, Jelodar and Sang Tarom cultivars were used to transfer qualitative traits to Nemat cultivar. In order to evaluate the molecular composition of the fragrance gene, the special marker provided by BRADBURY *et al*. (2005) was used. The results showed that in the population of Hashemi/Nemat, 4 plants had fragrance gene at homozygous status and 3 plants were heterozygous, and the rest of the plants are non-fragrant. In the population of Nemat/Sang tarom, only one plant was homozygous aromatic. The use of this marker at the population of Nemat/Tarom deylamani showed that 4 plants were aromatic and 4 plants were heterozygous and 12 other plants were non-aromatic. In population of Nemat/Jelodar, 2 homozygous plants, 2 plant heterozygous and the rest of the plants were identified as non-aromatic (Figure 1, Table 2).

DISCUSSION

Using marker aided selection (MAS), selection can be carried out at the seedling stage. This may be useful for many traits, but especially for traits that are expressed at later developmental stages. Therefore, undesirable plant genotypes can be quickly eliminated. Also, individual plants can be selected based on their genotype. For most traits, homozygous and heterozygous plants cannot be distinguished by conventional phenotypic screening. These advantages help breeders to accelerate the breeding process (RIBAUT and HOISINGTON, 1998; MORRIS *et al*., 2003).

MAS successfully employed for crop improvement. For example, JIN *et al*. (2010) improved the quality characteristics of II-32B cultivar using marker aided selection and identified 17 aromatic breeding lines. Also, YI *et al*. (2009) improved the quality characteristics of Manawthukha cultivar through marker aided selection using Basmati 370. They identified 12 aromatic BC4F2 lines with desirable agronomical traits. Similarly, KATALANI *et al.* (2012)

identified 2 homozygous aromatic plants and 3 heterozygous aromatic plants in composite cross populations. JIN *et al*. (2010) selected 17 homozygous lines for Wx-(CT) 17, SSIIa-TT, and *fgr* gene according to the marker genotypes. YI *et al.* (2009) successfully introduced both badh2-B and Wx in alleles from Basmati into the Manawthukha genetic background. The identified lines were fragrant and have intermediate AC, GT, GC status.

In this study, four segregating populations were developed through cross between high-yielding and high quality varieties and 100 superior genotypes were selected in terms of morphological characteristics (plant height, earliness etc.). Finally, molecular screening based on RM190 and fragrance markers showed that 11 lines were homozygous aromatic and 32 lines had desirable cooking quality in segregating F_2 population (Table 2). Results demonstrated that marker assisted selection in F_2 segregating populations reduced the time and cost effective for accelerating cultivar development in rice. Further analyses are underway on these plants on subsequent generations through pedigree breeding method. These plants promise to develop new aromatic rice lines through classical and molecular breeding in the near future in Iran.

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MARKER ASISTIRANA SELEKCIJA (MAS) ZA KVALITATIVNA SVOJSTVA U SEGREGIRAJUĆOJ POPULACIJI PIRINČA

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Izvod

Izbor i skrining poželjnih osobina u ranim fazama razvoja biljke moguć je uz unapređivanje marker asistirane selekcije (MAS). Selekcija uz pomoć markera može se koristiti za praćenje prisustva ili odsustva gena u populaciji i može se kombinovati sa konvencionalnim pristupima oplemenjivanja. U ovom istraživanju napravljena su ukrštanja između visokorodnog sorta, Nemat, sa 4 lokalne aromatične sorte praćene fenotipskom selekcijom za poželjne pojedinačne biljke u F2 populaciji. Zatim se MAS primenio na genotipsku selekciju aromatičnih biljaka koristeći marker alel specifičnu amplifikaciju (ASA) za miris (fgr) i SSR marker RM190 povezan sa prelaznim sadržajem amiloze (AC), konzistencijom gela (GC) i temperaturom želatinizacije (GT) kod ovih populacija. Molekularni skrining zasnovan na RM190 i markerima za miris pokazao je da je bilo 11 homozigotnih aromatičnih linija, a 32 linije željenog kvaliteta kuvanja u segregirajućoj F2 populaciji. Rezultati su pokazali da je marker asistirana selekcija u F2 segregirajućim populacijama smanjila vreme i isplativost za ubrzavanje razvoja sorte pirinča. U toku su dalje analize ovih biljaka na sledećim generacijama kroz pedigree oplemenjivački metod. Ove biljke obećavaju da će u bliskoj budućnosti u Iranu razviti nove aromatične linije pirinča kroz klasično i molekularno oplemenjivanje.

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