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Full Length Research Paper

Marker aided selection for aroma in F_2 populations of rice

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Marker aided selection (MAS) 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 two high yielding cultivars, Neda and Nemat with local aromatic varieties Sang tarom and Tarom deylamani were made followed by phenotypic selection for desirable individual plants in F_2 populations. Then, marker aided selection was applied to genotypic selection of aromatic plants using allele specific amplification (ASA) marker in these populations. Plants homozygous for aroma gene with good agronomic performance were identified. These plants can be used to develop new aromatic rice lines in the near future in Iran.

Key words: Rice, phenotypic selection, marker aided selection, aroma.

INTRODUCTION

Rice is the most important food crop, providing the staple food for nearly half of the global population, especially in Asia, Africa and Latin America (FAO, 2004). The aromatic rice is preferred over non-aromatic rice due to special occasions and for export, and thus they command a higher market price. Aroma in rice has been associated with increased levels of 2-acetyl-1-pyrroline (2AP) (Widjaja et al., 1996; Yoshihashi, 2002). There are some traditional methods used to assist breeders in selecting aromatic rice cultivars. These methods are based on tasting individual grains, chemical methods and gas chromatography, however, evaluation of aroma using these methods is time consuming, difficult and unreliable. Recently, molecular markers have been developed for the selection of aromatic plants in rice; they have the advantages of being inexpensive, simple, rapid, requiring small amounts of tissue and highly reliable (Bradbury et al., 2005b). Genetic studies on the inheritance of aroma in rice revealed that a recessive nuclear gene controls aroma in rice (Dong et al., 2000). Molecular marker that is closely linked to the aroma gene can be used to facilitate early selection for the presence or absence of aroma, and to identify the nature of the locus (homozygous or heterozygous condition), it may also be useful for the rapid incorporation of the aroma into breeding lines.

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. The use of cost-effective DNA markers for important agronomic traits provides opportunities for breeders todevelop 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.

The aim of this study was to screen the aroma gene in individual F2 plants with superior agronomic attributes which are derived from the crosses between high yielding varieties with local high quality ones.

MATERIALS AND METHODS

Plant materials and phenotypic evaluations

The crosses between high yielding rice cultivars namely, Neda and Nemat with high quality local varieties Sang tarom and Tarom deylamani were done in 2007. F_1 seeds of these crosses were grown in field to obtain F_2 seeds in 2008. Subsequently, 4 segregating populations were planted in cropping season of 2009 at research farm of Sari University of Agriculture. Cultural practices were those practiced traditionally in the region. Data on important agronomic traits like plant height, tiller number, panicle length, filled grains/panicle and 1000-grain weight were recorded on selected

Table 1. List of primers used for aroma genotyping in rice (Bradbury et al., 2005b).

Primer name	Primer sequence
External sense primer (ESP)	TTg TTT ggA gCT TgC TgA Tg
Internal fragrant antisense primer (IFAP)	CAT Agg AgC AgC TgA AAT ATA TAC C
Internal non-fragrant sense primer (INSP)	CTg gTA AAA AgA TTA Tgg CTT CA
External antisense primer (EAP)	AgT gCT TTA CAA AgT CCC gC

Table 2. Phenotypic and genotypic screening of F_2 plants in segregating populations of rice.

Population	Total plants	Number of phenotypically superior plants	Number of homozygous aromatic plants (percent)
Sang tarom × Neda	200	50	7 (14.00)
Sang tarom × Nemat	200	52	10 (19.23)
Tarom deylamani × Neda	200	39	4 (10.26)
Tarom deylamani × Nemat	200	46	9 (19.56)

superior individual F_2 plants.

PCR analysis for aroma

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. (2005b). 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 $MgCl_2$, 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.

RESULTS

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. So, effective phenotypic screening will be less expensive for selection in large populations.

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. Number of superior selected F_2 plants in four populations each with 200 plants is presented in Table 2.

Molecular screening for aroma

With the aim of producing new aromatic elite lines, genotyping was carried out using a PCR marker as described by Bradbury et al. (2005b). As shown in Figure 1, external primers, ESP and EAP produced expected fragment of approximately 580 bp as a positive control in all genotypes. Internal primers, INSP and IFAP produced two bands of 355 and 257 bp when paired with external primers, ESP and EAP. IFAP primer has been designed specifically for detecting aromatic genotype and INSP primer has been designed specifically for identifying non-aromatic genotype. Accordingly, ESP and IFAP primer pair amplified a 257 bp band showing the marker for fragrant genotype (Figure 2). Results show that 7, 10, 4 and 9 out of 50, 52, 39 and 46 superior plants were homozygous aromatic in Sang tarom × Neda, Sang tarom × Nemat, Tarom deylamani × Neda and Tarom deylamani × Nemat F_2 populations, respectively (Table 2).

DISCUSSION

Classical plant breeding is based on phenotypic selection of superior genotypes within segregating populations obtained from crosses. Phenotyping procedure for aroma is often carried out by plant breeders to select aromatic plants in segregating generations. However, the objective evaluation of fragrance using this method is labor intensive and varies from individual to individual and the ability to distinguish between fragrant and non-fragrant samples diminishes with each successive analysis due to saturation of sensory organ and/or physical abrasions to the tongue (Singh et al., 2011). MAS is an approach that has been developed to avoid the problems related with conventional plant breeding, changing the selection criteria from selection of phenotypes to selection of genes

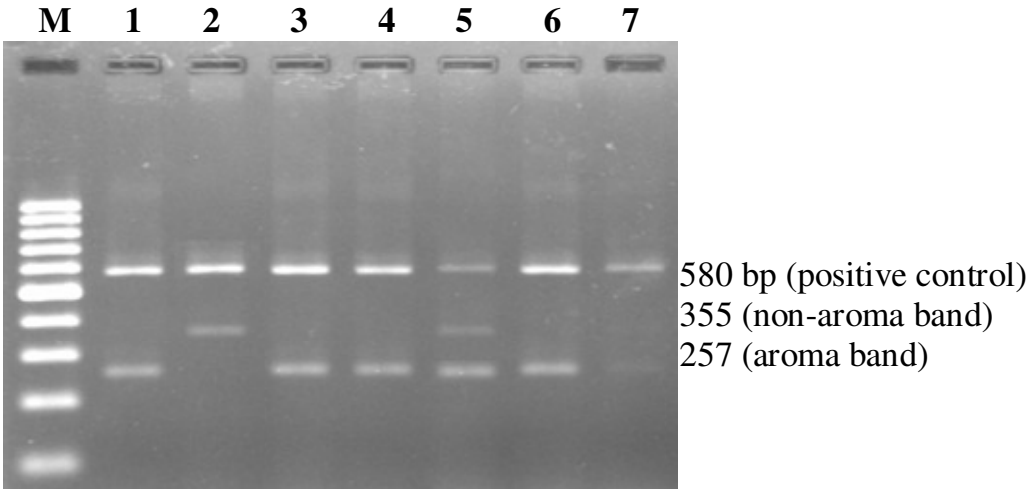

Figure 1. PCR analysis of some selected F_2 plants in Sang tarom (aromatic) x Neda (non-aromatic) population for presence of aroma gene. Lanes 1, 3, 4, 6 and 7 are homozygous aromatic plants; lane 2 is non aromatic; lane 5 is heterozygous genotype and M is 100 bp ladder.

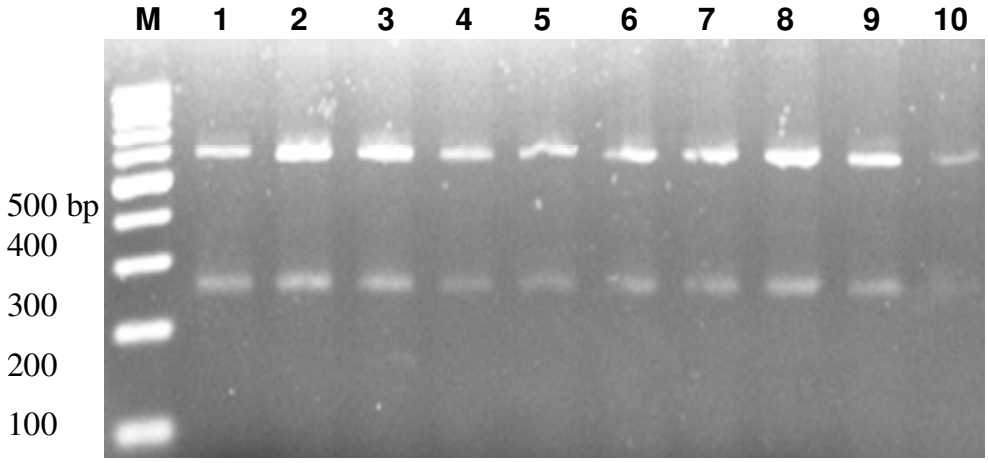

Figure 2. Homozygous aromatic genotypes in Sang tarom (aromatic) x Nemat (non-aromatic) population (lanes 1 to 10) detected by PCR analysis using aroma marker. Lane M is 100 bp ladder.

which increases the efficiency of variety development (Francia et al., 2005).

Using 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 was 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.

In this study for quality improvement, four segregating F_2 populations were developed from crosses between high yielding varieties with aromatic local ones. In these populations, superior individual plants were selected based on their desirable morphological characteristics such as early maturity, panicle length, etc. In this way, stringent phenotypic selection reduced the population size for further PCR analyses. Then, we focused on their molecular screening through identifying plants carrying aroma gene at homozygous status using PCR-based

marker. This base marker aided selection showed that 14, 19.23, 10.26 and 19.56% of phenotypically superior plants were homozygous for aroma status in Sang tarom × Neda, Sang tarom × Nemat, Tarom deylamani × Neda and Tarom deylamani × Nemat populations, respectively (Table 2).

A single allele, *badh2.1*, is common in fragrant rice varieties (Bradbury et al., 2005a; Bourgis et al., 2008; Fitzgerald et al., 2008), including the widely recognized Basmati and Jasmine types (Kovach et al., 2009). In this study, the ASA marker of this allele revealed high efficacy in genotyping of aroma in varieties Sang tarom and Tarom deylamani. Also, this marker is applicable in other aromatic varieties like Domsiah, Sang jo, Hasani and Hashemi (data not shown).

Important agronomic characteristics of selected aromatic plants were recorded. Further analyses are underway for these plants on subsequent generations through pedigree breeding method. These plants can be used to develop new aromatic rice lines through classical and molecular breeding in the near future in Iran.

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