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Marker aided selection for aroma in F2 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 dey lamani were made followed by phenotypic selection for desirable individual plants in F2 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 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.

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 dey lamani were done in 2007. F1 seeds of these crosses were grown in field to obtain F2 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).

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

Table 2. Phenotypic and genotypic screening of F$_2$ plants in segregating populations of rice.

<table>
<thead>
<tr>
<th>Population</th>
<th>Total plants</th>
<th>Number of phenotypically superior plants</th>
<th>Number of homozygous aromatic plants (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sang tarom × Neda</td>
<td>200</td>
<td>50</td>
<td>7 (14.00)</td>
</tr>
<tr>
<td>Sang tarom × Nemat</td>
<td>200</td>
<td>52</td>
<td>10 (19.23)</td>
</tr>
<tr>
<td>Tarom deylamani × Neda</td>
<td>200</td>
<td>39</td>
<td>4 (10.26)</td>
</tr>
<tr>
<td>Tarom deylamani × Nemat</td>
<td>200</td>
<td>46</td>
<td>9 (19.56)</td>
</tr>
</tbody>
</table>

**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).

**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.

**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...
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 assisted 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 F2 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, \textit{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.

ACKNOWLEDGEMENT

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REFERENCES


