Anther culture is being used in cereal crop improvement both as a source of haploids and for inducing the new genetic variability. We studied the possibilities of producing the aneuploids in rice by anther culture. Anthers for callus induction were plated on semi solid media. Callus formation was observed 20 days after plating. The anthers became necrotic prior to callus formation. Tapei 309 exhibited the highest callus induction efficiency (7.8%) followed by IR31917-45-3-2 (7.7%). IR56 produced (1.45%) callus. The F1 hybrids (IR31917-45-3-2 x Oryza australiensis) and IR56 x Oryza brachyantha responded poorly for callus induction (0.033%) and (0.030%) respectively. The 879 calli of IR31917-45-3-2 regenerated into six (0.68%) green plants and 79 (8.9%) albinos. All the green plants were haploids. No green plant was produced from the 112 calli of IR56. Similarly, the calli from IR56 x O. brachyantha did not show any plant regeneration. The calli from the F1 hybrid (IR31917-45-3-2 x O. australiensis) yielded six (20.6%) green and two (6.89%) albino plants. Three of the six plants did not grow after regeneration, while the remaining three plants were used for cytological studies. One plant was aneuploid with 27 chromosomes. Genomic in situ hybridization (GISH) using biotin labeled genomic DNA unequivocally detected 14 chromosomes of O. australiensis and 13 chromosomes from IR31917-45-3-2 in this aneuploid. Similarly, in the other two plants with 24 chromosomes each, the 12 chromosomes of O. australiensis could be discriminated from the 12 chromosomes from IR56.

Key words: Aneuploid, anther culture, Oryza sativa, genomic in situ hybridization (GISH).
development of earliness, increased grain weight, superior grain quality, disease resistance (Zang et al., 1984; Zang, 1989), dwarf plant type and abiotic stresses tolerance have been achieved successfully using anther culture (Alejar et al., 1995).

Anther culture in rice has been improved substantially. However, detail study on wild relatives and their F1 hybrids was extremely limited. Low anther culture response, high percent of albino plantlet regenerations are the principal constraints in establishing anther culture in rice (Roy and Mandal, 2005). We reported the production of haploids, double haploids and aneuploid in rice by using anther culture of wide-crosses. Fluorescence in situ hybridization (FISH) a powerful technique for characterizing anther culture derived progenies of wide-hybrids, plants from wide-hybrids.

**MATERIALS AND METHODS**

**Panicle collection and pre-treatment**

Panicles were collected at booting stage having a distance of 4 to 8 cm between the flag leaf and the leaf below it. These were washed thoroughly with tap water, wrapped in paper towels, moistened with distilled water and subjected to cold treatment in an incubator at 8°C for 8 days. Some of the panicles were used without treatment.

**Callus induction**

The panicles were surface sterilized in 20% chlorox for 20 min. Anthers with pollen grains at mid uni-nucleate to early bi-nucleate stages were plated in the callus induction media. Florets were removed from the panicles and cut at their base. Anthers were transferred to semi-solid medium by tapping cut florets. Three media: MS (Murashige and Skoog, 1962), N6 (Chu et al., 1975) and FJ4, a modified MS medium were used for callus induction.

**Plant regeneration**

All procedures were conducted under aseptic condition using a laminar flow cabinet. Calli were transferred to four modified MS media: M5, M6, SK11 and SK11M and incubated under 16 and 8 h day/night for approximately 30 days. Green plantlets having well developed shoots were transferred to half MS medium without hormones. Roots of the regenerated plantlets were washed with distilled water to remove the calli and medium residues. The regenerated green plantlets were transferred to the phytotron (29 and 21°C) in soil in small pots. Callus induction and plant regeneration efficiency were calculated as reported by Abbasi (1999).

\[
\text{Callus induction (\%) } = \frac{\text{No. of anthers forming callus}}{\text{Total no. of anthers plated}} \times 100
\]

\[
\text{Plant regeneration (\%) } = \frac{\text{No. of calli producing green plants}}{\text{Total no. of calli transferred on regeneration medium}} \times 100
\]

**RESULTS AND DISCUSSION**

**Callus induction**

Anthers for callus induction were plated on semi solid media. Callus formation was observed 20 days after...
plating. The anthers become necrotic prior to callus formation (Figure 1 a and b). Taipei 309 exhibited the highest callus induction efficiency (7.083%), followed by IR31917-45-3-2 which gave the highest response (4.597%). IR56 produced (1.463%) callus. The F1 hybrids (Ir31917-45-3-4 x O. australiensis, IR56 x Oryza brachyanantha) responded poorly to callus induction (0.033%) and (0.03%) (Table 1).

Among the green plants, five plants (14.3%) were fertile. Similarly, 879 calli of IR31917-45-3-2 regenerated into six (0.68%) green and 79 (8.9%) albinos. All the green plants were haploids. No green plants were produced from the 112 calli of IR56. The calli from the F1 hybrid, IR31917-45-3-2 x O. australiensis yielded six (20.6%) green (Figure 1d) and two (6.89%) albino plants. Three of the six plants did not grow after regeneration, while the remaining three plants were used for cytological studies. The calli from IR56 x O. brachyantha did not show any plant regeneration (Table 2).

Cytological characterization of anther culture derived plants

Three anther culture derived plants from the F1 hybrid O. sativa x O. australiensis were used for GISH analysis. One plant had 27 chromosomes whereas, other two plants showed 24 chromosomes. Genomic DNA from O. australiensis was used as a probe. The biotin labeled probe produced uniform labeling pattern over the entire length of all the 14 O. australiensis chromosomes (dark brown) whereas, 13 chromosomes of O. sativa appeared

Figure 1. Anther culture of interspecific crosses between O. sativa and O. ausraraliensis. (A) Anther turned brown before callus induction; (B) same anther as in A producing callus; (C) green plant regenerated; (D) haploid plant (dwarf) and dihaploid (tall).
Table 1. Callus induction from anther culture of wide-cross derivatives of rice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Anthers plated (no)</th>
<th>Callus produced (no)</th>
<th>Callus induction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapei 309</td>
<td>10914</td>
<td>773b</td>
<td>7.083a</td>
</tr>
<tr>
<td>IR31917-45-3-2</td>
<td>28260</td>
<td>1299a</td>
<td>4.597b</td>
</tr>
<tr>
<td><em>O. australiensis</em></td>
<td>3720</td>
<td>0 e</td>
<td>0 e</td>
</tr>
<tr>
<td>IR56</td>
<td>7650</td>
<td>112c</td>
<td>1.463c</td>
</tr>
<tr>
<td>IR31917-45-3-2 x <em>O. australiensis</em></td>
<td>88837</td>
<td>29d</td>
<td>0.033d</td>
</tr>
<tr>
<td>IR56 x <em>O. brachyantha</em></td>
<td>53670</td>
<td>15de</td>
<td>0.030d</td>
</tr>
</tbody>
</table>

Mean followed by same letter do not differ significantly.

Table 2. Plant regeneration from anther derived calli of wide-cross derivatives.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Callus transferred (no)</th>
<th>Plant regeneration</th>
<th>Green (%)</th>
<th>Albino (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapei 309</td>
<td>423</td>
<td>35a</td>
<td>8.30 b</td>
<td>133a</td>
</tr>
<tr>
<td>IR31917-45-3-2</td>
<td>879</td>
<td>06 b</td>
<td>0.68 c</td>
<td>79b</td>
</tr>
<tr>
<td>IR56</td>
<td>112</td>
<td>00 c</td>
<td>0.00 c</td>
<td>04 c</td>
</tr>
<tr>
<td>IR31917-45-3-2 x <em>O. australiensis</em></td>
<td>29</td>
<td>06 b</td>
<td>20.60 a</td>
<td>02cd</td>
</tr>
<tr>
<td>IR56 x <em>O. brachyantha</em></td>
<td>15</td>
<td>00 c</td>
<td>0.00c</td>
<td>00d</td>
</tr>
</tbody>
</table>

Mean followed by same letter do not differ significantly from each other.

light blue after Giemsa staining (Figure 2a).

For FISH analysis when somatic metaphase cells were stained with DAPI, all the 27 chromosomes fluoresced blue under UV light excitation. In the same cell, the *O. australiensis* chromosomes showing the hybridization signal, appeared green under blue light excitation allowing the identification of all the *O. australiensis* chromosomes whereas, *O. sativa* chromosomes appeared blue due to counterstaining with DAPI (Figure 2b).

FISH analysis of the two other plants (2n = 24) was carried out. In a somatic metaphase cell stained with DAPI, all the 24 chromosomes fluoresced blue. After *in situ* hybridization 12 *O. australiensis* chromosomes fluoresced green under blue light excitation due to FITC and unlabeled *O. sativa* chromosomes appeared blue due to counterstaining with DAPI (data not shown). This indicated that, these labeled chromosomes were from *O. australiensis*. Similarly, both the parental chromosomes were clearly discriminated showing labeled *O. australiensis* chromosomes (green) and unlabeled *O. sativa* chromosomes. No restructured chromosome could be identified among 100 cells analyzed through *in situ* hybridization.

Anther culture is being used in rice breeding program both as a source of haploids and new genetic variation. We studied the anther culture ability and chromosomal variation of wide hybrids and their parents using different media formulation. Significant genotypic differences were observed for callus induction. The genotype of the donor plant affects anther culture response (Moieni and Sarrafi, 1995). Nuclear and cytoplasmic control of anther culture has been reported (Ekiz and Konzak, 1991). Similar observations were recorded in this study. No green plants were produced both in IR56 and the hybrid between IR56 and *O. brachyantha*. IR3191745-3-2 has high callus induction and green plant regeneration ability compared with IR56. Similar trends of callus induction and green plant regeneration was observed in their respective hybrids. The low yield of haploid green plants from rice anther culture and albinos are serious problems. Even after culturing 3720 anthers, we did not obtain even a single callus in *O. australiensis* and major portions of the regenerants in other genotypes were albinos. Albino's plants do not contain mature chloroplast and large scale deletion of the plastid genome in the microspore-derived plants of rice has been reported (Jahne and Lorz, 1995). The anther culture media is reported to be a major factor for anther culture response for callus induction and regeneration of plantlets (Fadel and Wenzel, 1990). In this study, MS medium gave the better response for callus induction followed by FJ4 and N6.

Aneuploidy as the chromosomal abnormality has been observed frequently in cell culture. The level of ploidy in our regenerants varied from 2n = 24 to 2n= 27. The chromosomal changes involving multiplication of entire genomes or some chromosomes have been reported in callus of wheat (Wang and Hu, 1985). In this study, the fluorescent GISH revealed 13 chromosomes from *O. sativa* and 14 chromosomes from *O. australiensis* in the regenerant with 27 chromosomes. The preponderance of E genome chromosomes may be related to their predominately heterochromatic nature. Heterochromatin is
known to replicate later than euchromatin, that is why the E genome undergo non-disjunction mechanism by which they multiply. We do not observe any structural abnormality. We have previously reported GISH as a powerful technique for characterizing parental genomes in the wide hybrids (Abbasi et al., 2010), localizing introgression on the chromosomes (Abbasi et al., 2010), detecting genomic affinity (Abbasi et al., 2010) and assessing genomic relationship (Abbasi et al., 2010). Results showed that aneuploidy could be developed by anther culture in rice.

REFERENCES