

Using molecular markers SSR to search for wild introgressions from a relative tetraploid species in the diploid *Oryza sativa* L.

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INTRODUCTION

The genus *Oryza* consists of two cultivated rice species (*O. sativa*, and *O. glaberrima*) and about 20 related species (Vaughan *et al.* 2003), which represent a source of new alleles for improving the yield, quality and stress resistance of cultivated rice. Genome differentiation at the diploid and tetraploid levels has been considerable, which makes difficult the transfer of alleles of interest to cultivated rice by crossing. There are ten different genomes in *Oryza* genus, AA, BB, CC, EE, FF, GG, BBCC, CCDD, HHJJ and one unknown. Some interspecific crosses have been achieved, but mainly with diploids AA species due its close relation with *O. sativa* (Brar and Kush, 1997). Reports of diploid - tetraploid crosses did not use molecular markers to prove introgressions nor evaluated cytogenetic relationships (Mariam *et al.*, 1996; Multani *et al.*, 2003). In this work, we obtained diploid individuals from a cross between the cultivated rice *O. sativa* (AA genome) and the wild tetraploid *O. latifolia* (CCDD genome), evaluated cytogenetic process to recover fertility, and showed introgression from tetraploid to diploid rice by using SSRs markers.

MATERIALS AND METHODS

Figure 1 shows the crossing scheme used in this study. 80 SSRs markers out of 313 proved in *O. latifolia* were polymorphic and used to follow introgressions into *O. sativa* (figure 3). A total of 28 BC₂ and BC₃ individuals were obtained and evaluated using the polymorphic SSRs distributed throughout the whole genome. Molecular data were analyzed with the CSSL Finder software (Lorieux, 2006) to look for wild chromosomal segments introgressed into *O. sativa*

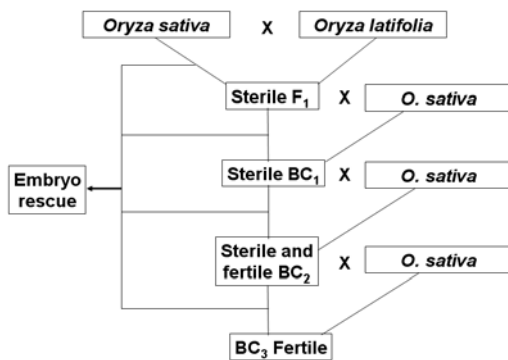


Fig 1. Schematic representation of crosses and backcrosses. For the first cross *O. latifolia* was used as pollen donor. In backcrosses *O. sativa* was used as pollen donor. In all generation till BC₃ embryo rescue was used to overcome sterility barriers.

RESULTS AND DISCUSSION

Triploid sterile F₁ hybrids between an accession of allotetraploid species *Oryza latifolia* (2n=48) and the diploid *O. sativa* (2n=24) were obtained by embryo rescue. After three backcrosses to *O. sativa* fertile progenies were obtained (figure 2). Cytogenetical evaluations in all generations showed some pairings between the *O. latifolia* and *O. sativa* chromosomes in F₁, and a trend to complete diploidy in later generations. Additional chromosomes were observed in twenty-one BC₂ and BC₃ individuals (eleven individuals with one, and ten with 2), but seven of them had not additional chromosomes. SSRs showed clearly the additional chromosomes in some plants (figure 4 and 5); however, in some individuals, presence of additional chromosomes was not clear possibly due to several null alleles. Wild alleles were detected in chromosomes 1,2,3,5,6,8,9,10,11, and 12 in some progenies, which would suggest chromosomal segments introgressed by recombination, maybe in F₁, since some wild alleles, as RM220 and RM236 markers, were present in almost all BC₂ and BC₃ individuals (figure 5).

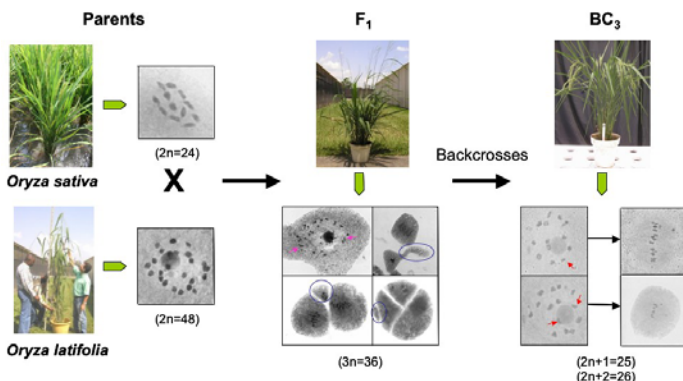


Fig 2. These two species are very different in terms of morphological and cytogenetic characteristics. A cross between diploid and tetraploid species generates triploid F₁ with aberrant behavior due to chromosomal incompatibility; however, homologue recombination was observed. By backcrossing to *O. sativa* it was possible to eliminate wild chromosomes and recover normal fertility, but with wild introgressions.

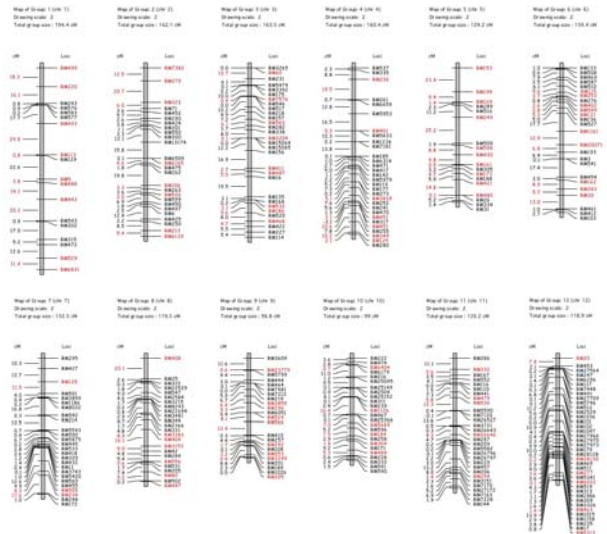


Fig 3. Rice genetic map showing the position of 313 SSR markers. Only markers in red amplified in *O. latifolia*.

Plants 12, 16, 20 and 25 carry an additional chromosome, probably chromosome 8, but also there is a wild allele of the chromosome 11 in these plants, which would suggest a translocation.



Fig 4. Molecular marker RM402 showing wild introgressions obtained from the cross *O. latifolia* X *O. sativa* in BC₂ and BC₃ plants.

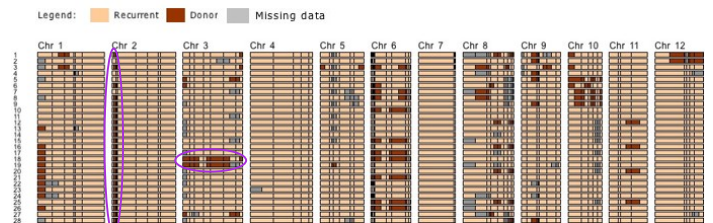


Fig 5. Ninety SSRs amplified in *O. latifolia*, of which 80 were used to assess chromosomal segments of *O. latifolia* in BC₂ and BC₃ individuals. This figure generated by CSSL Finder programme (Lorieux, 2006) shows chromosomes of 28 plants with wild chromosomal segments (red bars). In most cases, introgressions are present as small pieces in almost all plants as in chromosome 2, while in others there are entire chromosomes as chromosome 3 in individuals 18 and 19.

CONCLUSIONS

- Use of wild allotetraploids of genus *Oryza* in cultivar rice improvement, by means of interspecific crosses is possible.
- Evaluation by SSRs demonstrated that pairing between homeologous chromosomes may produce recombination.
- Three backcrosses towards *O. sativa* were required to recover diploidy, and therefore, fertile individuals with wild introgressions.
- Additional work using other accessions and more markers would give more information about recombination capacity between these species and their evolutive relationships.

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