



Thesis abstracts

Evaluation of genetic resources for hybrid rice (*Oryza sativa* L.) production

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Rice (*Oryza sativa* L.) is one of the most important food crops in the world, consumed daily by more than half of the human population. The narrow genetic base of rice breeding programs and the inadequate use of the species genetic variability are some of the main factors limiting increments in grain yield. The exploitation of hybrid vigor in rice has the potential to overcome the current yield *plateaus* observed in most breeding programs. This is limited by an inherent difficulty to obtain and test new hybrid combinations, due to the autogamous habit of the species and the male sterile system currently exploited. The present study used molecular markers to evaluate rice genetic resources (accessions, lines and varieties) conserved by the Brazilian Rice Germplasm Bank as well as breeding lines of the Cytoplasmic Male Sterility (CMS) Hybrid Rice Program developed by EMBRAPA. The study was divided into three major sections: (a) subspecific classification of a reference collection of rice germplasm using molecular markers, (b) development of fluorescent-based microsatellite (SSR) multiplex marker systems for genetic analysis of rice germplasm and (c) correlation analysis of heterosis and genetic distance in a cytoplasmic male sterile based hybrid rice program.

Both RAPD and SSR markers were used to evaluate 108 accessions of irrigated and upland rice representing the genetic basis of rice breeding in Brazil ("reference collection – RC"), including the parental lines of recurrent selection populations such as CNA-5, as well as two *O. glumaepatula* accessions and one *O. rufipogon* accession. Cluster analysis based on 114 RAPD markers and 13 SSR loci detected strong genetic structuring of the RC accessions in two groups corresponding to the subspecies *indica* (36% of the accessions) and *japonica* (64% of the accessions). The data based on RAPD and SSR polymorphic markers was contrasted with morphological, botanical and crop system information. Results indicated that the use of four *indica/japonica*-specific RAPD markers could reliably classify a rice accession at the subspecific level, providing a useful tool for fast analysis of large collections. The data also indicated that some accessions are probably derived from *indica* x *japonica* crosses (I/J). The four *indica/japonica*-specific RAPD markers were used to fingerprint 267 lines of the CMS hybrid program. The data indicated that nine lines were *japonica*, 104 were *indica* and 158 a possible intercross (I/J) between the two

subspecies. It was interesting to notice that several maintainers have a genetic background (I/J) that differs from that observed in the male sterile lines (*indica*). Polymorphism between male sterile lines and their current maintainers at 11 SSR loci detected with the two multiplex systems confirmed these differences, as mentioned below.

Thirteen SSR markers previously mapped in the rice chromosomes were characterized and selected according to the number of alleles, allelic size range and polymorphism. Two fluorescent-based SSR multiplex marker systems were developed for genetic analysis of rice germplasm that allow fast and reliable DNA typing at 11 independent hypervariable loci by automated DNA sequencers. Allelic frequencies at the 11 loci were computed after genotyping the 108 *O. sativa* RC accessions and can now be used as a reference database for accurate genetic analysis in rice. The multiplex systems were tested on real cases of hybrid identification and variety identity, validating their use in the analysis of genetic relationships in rice, such as required for paternity testing of hybrid progeny, analysis of sample contamination and variety protection. Polymorphism at these loci was also used to estimate genetic distances between four male-sterile lines of the CMS hybrid program and their maintainers. The data indicated that some of the maintainer lines are not as near isogenic to the male sterile lines as previously assumed by the breeding program.

The correlation analysis of heterosis and genetic distance in a cytoplasmic male sterile based hybrid rice program was based on molecular polymorphism data. Pairwise genetic distances based on 98 RAPD polymorphic markers were estimated for 267 lines of the program, distributed in four male-sterile lines, 43 maintainers and 220 restorer lines. The restorer lines were classified in three categories according to their similarity levels (high, intermediate and low) in relation to the four male-sterile lines. A total of 70 hybrids were generated on the three levels of genetic similarity between male-sterile and restorer lines. The hybrids were evaluated for yield and yield-component traits in a replicated experiment in the field and a correlation study between genetic distance and hybrid vigor was developed. The newly constructed hybrids presented higher yield than the varieties used as control, reaching up to 9,8 t/ha. Significant differences between hybrids derived from *indica* x *japonica* and *indica* x *indica* crosses were detected for some traits. Correlation between genetic distance and heterosis was observed for some specific genotype combinations. However, the data did not allow the detection of a clear relationship between genetic distance and heterosis in the parental crosses selected based on marker polymorphism.

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