## SSR MARKER POLYMORPHISM IN RECURRENT SELECTION POPULATIONS CG3 AND CNA6

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**INTRODUCTION**: Recurrent selection is a breeding program in which selection and recombination are done cyclically and continuously. In this scheme, the expectation is that the population mean advance in the direction of the selection applied, and that genetic variability is not markedly reduced, in a way that continuous gains can be achieved. However, some loss of variability is a natural consequence of selection, thus it should be monitored. Information provided by molecular markers allows monitoring the variability remaining in the population (Courtois et al., 2005). The objective of this study was to quantify the allele diversity in the upland rice recurrent selection populations CG3 and CNA6, through the analysis of 16 unlinked SSR markers.

MATERIAL AND METHODS: The populations used in this study are part of the upland rice pre-breeding program at Embrapa Rice and Beans. CNA6 is a wide genetic base population recombined by means of a male-sterile gene (Chatel and Guimarães, 1995), whereas CG3 is a elite germplasm-based population recombined manually, in a partial diallel scheme. Both populations were submitted to two cycles of recurrent selection based on evaluation of S0:2 families. However, CNA6 was previously submitted to two cycles of mass selection. DNA was extracted from 96 families each population, by bulking leaf tissue from 6 plants per family, such that both alleles could be detected with high probability. The generation used was F1:4 in the case of CG3, and F1:3 in the case of CNA6. Sixteen fluorescent-labeled SSR markers were analyzed, however one marker failed in the population CG3. Alleles were detected in a DNA sequencer ABI3700 (Applied Biosystems). Total alleles correspond to all fragment sizes detected in the sequencer. Common alleles are those found in at least 5 families, out of the 96 analyzed. Effective allele number is the theoretical number of equally-frequent alleles that would result in the same frequency of heterozygosity in a random mating population (Ne= $1/\Sigma p_i^2$ ; Hartl and Clark, 1997).

**RESULTS AND DISCUSSION**: The average number of alleles detected in CG3 and CNA6 was 7.6 and 11.4, respectively (Table 1). Those numbers represent an

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intermediate to high genetic diversity, compared to other studies (Lu et al.,2005; Xu et al. 2005). CNA6 was synthesized from many landraces (Morais et al. 1997), whereas CG3 derives from elite material. The wider genetic base of CNA6 was reflected in the presence of more rare alleles. The highest number of alleles detected in CG3 was 13 alleles at RM224. In CNA6, seven out of the 16 markers tested detected more than that number, and two markers (OG106 and RM335) detected as many as 18 alleles in the 96 families. Besides the wider diversity among parents used in the synthesis of CNA6, recombination by male-sterility in the field creates opportunities for gene inflow from neighboring plots. CG3 is recombined manually, thus only new mutations or planned introgressions can increase allele diversity. On the other hand, the numbers of common alleles are very similar between the populations, and the number of effective alleles in CG3 is higher than in CNA6 (Figure 1). Those data indicate that allele frequencies in CG3 are more uniform, and a surprising consequence of this observation is that the expected heterozygosity in CG3 is slightly higher, despite its narrower genetic base compared to CNA6. Besides preventing stranger pollen inflow, manual crossing offers the possibility of recombining the population in a balanced scheme, which also contributes to prevent genetic drift.

Marker		CG3		_		CNA6	
	Total	Common	Effective		Total	Common	Effective
OG05	6	2	1.28		10	3	1.29
OG101	-	-	-		14	4	3.25
OG106	11	5	4.52		18	5	4.59
OG44	11	7	4.71		4	2	1.61
OG61	8	4	3.91		16	5	3.51
OG81	7	4	2.35		10	5	3.89
OS19	6	3	2.01		7	5	3.75
RM224	13	5	4.49		6	5	2.74
RM248	10	3	1.99		7	2	1.17
RM252	5	2	1.97		7	4	2.94
RM259	9	3	1.31		16	4	2.08
RM263	4	3	2.42		11	3	2.15
RM335	9	5	3.13		18	3	2.90
RM418	5	3	2.23		15	4	1.85
RM420	5	2	1.87		8	2	1.53
RM475	5	2	1.45		16	3	1.45
Average	7.6	3.5	2.64		11.4	3.7	2.54

Table 1: Number of total, common and effective alleles detected in the populations CG3 and CNA6 on 16 unlinked SSR markers.

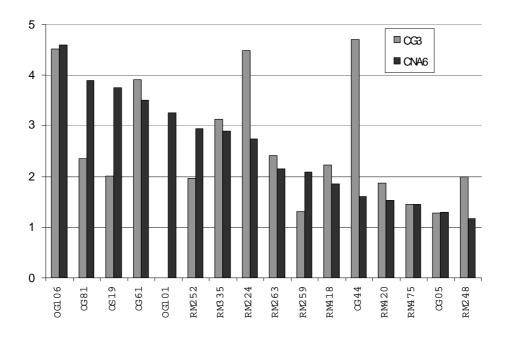


Figure 1. Comparison of the effective number of alleles detected in each population, on 16 SSR loci. Marker OG101 was genotyped on CNA6 only.

**CONCLUSIONS:** This study demonstrated that the recurrent selection populations CG3 and CNA6 have relatively high genetic diversity, which is favorable for recurrent selection. Additionally, the study provided evidences that allele diversity is more uniformly distributed in CG3 than in CNA6, which may be related to the differences in the group of parents used for synthesis, and the fact that CG3 is manually recombined, whereas CNA6 is randomly recombined with help of a male-sterility gene.

## **REFERENCES:**

CHATEL, M., E.P., GUIMARÃES. 1995. Seleccion Recurrente con Androesterilidad en Arroz CIRAD/CIAT, Cali, Colômbia.

COURTOIS, B., D. FILLOUX, N. AHMADI, J.-L. NOYER, C. BILLOT, E.P. GUIMARÃES. 2005. Using molecular markers in rice population improvement through recurrent selection, p. 52-74, In E. P. Guimarães, ed. Population Improvement: A Way of Exploiting the Rice Genetic Resources of Latin America. FAO, Rome.

HEDRICK, P.W. 2005. Genetics of Populations. 3 ed. Jones and Bartlett, Sudbury. Hartl, D., and A. Clark. 1997. **Principles of Population Genetics** Sinauer, Sunderland.

LU, H., M.A. REDUS, J.R. COBURN, J.N. RUTGER, S.R. MCCOUCH, AND T.H. TAI. 2005. Population structure and breeding patterns of 145 US rice cultivars based on SSR marker analysis. **Crop Science** 45:66-76.

MORAIS, O.P., E.M. CASTRO, E.P. SANT'ANA. 1997. Selección recurrente en arroz de secano em Brasil, p. 99 - 115, In E. P. Guimarães, ed. Selección Recurrente en Arroz. CIAT, Cali, Colombia.

XU, Y.B., H. BEACHELL, S.R. MCCOUCH. 2004. A marker-based approach to broadening the genetic base of rice in the USA. **Crop Science** 44:1947-1959.