Forage Biotechnology

Progress in a tropical forage grass – *Brachiaria*

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Introduction

Plant biotechnology involves processes in which genetic information and new techniques are used to complement the objectives of breeding programs. It becomes possible to reach goals that are limited by cross incompatibility overcoming necessity of hybridization techniques. Ultimately, the aim is to increase productivity, reducing losses caused by biotic or abiotic stresses and to diminish production costs. Programs on forage biotechnology involve different techniques such as: tissue culture, used to produce haploids, to rescue embryos, in vitro cloning and keeping germplasm collections; genetic transformation, to produce transgenic forages with characteristics distinct from those attainable by breeding programs and enabling the use of genes available in different

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systems; cloning potentially useful agronomical genes and promoters and guiding breeding programs by the identification of molecular markers. The use of forages is directly associated with cattle production, mainly in countries like Brazil where most of the cattle is raised under pasture conditions. This association is shown comparing the growth of the number of cattle and pasture area during the same period (Zimmer & Rodrigo, 2005). The predominant production system is a simple one based on grazing and relying on native and cultivated pastures, which are grazed at continuous stocking all year round (Carvalho, 2002). Data from Anualpec, (2004 IBGE) show an estimated number of 195.6 million cattle heads in Brazil in a cultivated pasture area estimated around 259 million hectares, 144 million of which with native pastures and 115 million hectares with cultivated pasture with predominance of gramineae from the genus *Brachiaria* (Karia et al. in press).

Biotechnological techniques are expected to contribute to plant breeding in the next years in different areas (Carneiro et al., 2006). Among others, apomixis, cloning through seeds, arises as a challenge. Apomixis could be used to perpetuate hybrids of interest by simple seed propagation. It would permit the high scale production and maintenance of elite genotypes. For farmers this would mean independence in the production of seeds from a hybrid of interest, without the need of frequent acquisition of seeds. For developing countries where the cost of hybrid seeds limits the small farm production, this possibility is of special interest. On the other hand, unlocking apomixis can bring variability to breeding programs. This is an important concern on *Brachiaria* cultures in Brazil where mostly two apomictic varieties are used to cover the massive pasture area (Miles et al., 1996; Carneiro & Dusi, 2004).

Our group is mainly interested in the study of apomixis and the molecular mechanisms involved with this reproductive mode. It is Embrapa's mission to provide feasible solutions for the sustainable development of the Brazilian agribusiness by generating, adapting and transferring knowledge and technology. At Embrapa, the genus *Brachiaria* was identified as a suitable system for studying the basic molecular biology of apomixis as genetic studies have shown that apomixis is a dominant factor controlled by one Mendelian loci (Valle et al., 1994; Valle & Savidan, 1996). Since 1995, our line of research has focused on cellular and molecular analysis of apomixis in order to develop new biotechnological tools for seed production.

These projects refer to fundamental research of the reproductive system of *Brachiaria* plants with sexual and apomictic mode of reproduction. Finding genes associated with apomixis will allow its manipulation in cultivars of interest and unlock the

diversity of apomictic plants. The genes associated with embryo sac and embryo development are most likely responsible for the expression of apomixis or sexuality in plants. Therefore, molecular analysis of ovary development in natural apomicts is an important approach in identifying genes associated with apomixis. The identification of these genes in *Brachiaria* will open the possibility of cloning by seeds and its potential use for seed production in other genera. The acquired knowledge will profit breeding programs of *Brachiaria* as well as other crops.

We have characterized ovary development in sexual and apomictic plants of *Brachiaria* (Valle,1990, Araujo et al 2000; Dusi & Willemse, 1999). Apomictic plants form female gametophytes without meiotic recombination characterized by unreduced embryo sacs of the Panicum-type, with four nuclei while sexual plants have meiotically-derived female gametes, and form a reduced eight-nucleated embryo sac of the Polygonum-type. In *Brachiaria* species, pollen is viable and apomixis is characterized as pseudogamic, i.e. endosperm is resultant of central cell fertilization while embryo develops autonomously (Ngendahayo, 1988; Alves et al., 2001). Comparing to apomictic plants, the seed production of sexual plants of *B.brizantha* is limited by a massive abortion of caryopsis believed to correlate with early inbreeding depression (Araujo et al., 2007). Ovaries cDNA that are differentially expressed between sexual and apomictic plants have been identified primarily through DDRT-PCR (Rodrigues et al., 2003). Among them some are specific to apomictic female reproductive development. In situ hybridization experiments showed significant expression of three sequences in the synergids and it was suggested that they may play a role in the moment of fertilization, and in the autonomous development of unreduced egg cell (Alves et al., 2007). Four cDNA libraries, with 2,976 clones sequenced and 74% of them validated, have been constructed with ovaries in megasporogenesis and megagametogenesis of sexual and apomitic plants. Macroarray analysis indicated differential (stress induce protein, meiotic protein, helicase, cell cycle control 2, chromossome maintenance factor) and constitutive (ubiquitin conjugating enzime, tubulin, actin, gliceraldehyde dehydrogenase, succinil Co-A dehydrogenase) sequences related with ovaries formation that were quantified by q-RT-PCR and in situ hybridization localized. In vitro regeneration methods from meristematic tissues and mature embryos of *Brachiaria* were established allowing development of a transformation protocol using biolistics (Lenis-Manzano, 1998). This method is being improved by the identification of efficient promoters to express target genes in *Brachiaria* (Silveira et al., 2003) in order to allow gain of function studies and by determining tissue culture conditions more favorable

for plant regeneration and transformation (Cabral et al., 2006). Anther culture is being developed to obtain haploid plants, bringing the apomicts to same ploidy of sexuals, in order to evaluate the relationship between apomixis and ploidy and hybridizing diploids for segregation analysis of the apomixis trait. First attempt of in vitro fertilization, aiming to develop a method to avoid the pre fertilization barriers of incompatibility, was successful in introducing the sperm cells into the embryo sac and egg cell.

A population of *B.humidicola* segregating with individuals reproducing by sexuality and apomixis was obtained and RAPD analysis is being performed, to confirm hybrids and genetic mapping is on the way with candidate genes for apomixis of other species and SSR markers. Also, advances towards obtaining new intraspecific lines for breeding programs of *Brachiaria* were achieved with the evidences of the compatibility of *B. brizantha* tetraploid, apomictic plants and artificial sexual tetraploids (Araujo et al., 2005) obtained by colchicine treatment (Pinheiro et al., 2000). This approach will enable the identification of molecular markers associated with the apomictic trait that will be used in for mapped based cloning of the apomixis locus

Together, the output of the research efforts at Embrapa has deepened our understanding of apomixis in *Brachiaria* and the acquired knowledge of this project will open the possibility of controlling or employing apomixis in breeding programs of *Brachiaria* and other crops. Many goals of biotechnology are being achieved due to the progress in plant sciences in the last years. Tools are being developed as complete genome sequences of different species become available, a number of genes controlling different aspects of plant growth and development are identified, methods for production of different transgenic crops described and, molecular markers of many cultures identified. The challenge is beginning to turn into finding better ways to translate the new advances of basic plant science into real successes in agriculture (Delmer, 2005).

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