Floral biology and isoenzymatic variability in *Ocimum selloi* Benth (Biologia floral e variabilidade isoenzimática em *Ocimum selloi* Benth)

*Cláudio Lúcio Fernandes Amaral**

The floral biology and the reproductive mechanisms as well as the inter- and intrapopulational isoenzymatic variability of *Ocimum selloi* Benth was studied. Floral activity was found to have three phases: in the first one, pre-anthesis, pollination occurs; in the second one, anthesis, floral bud opening and subsequently the asychronic stamen externalization occur, and in the third phase, post-anthesis, ovule fecundation occurs. Since the flower was closed at pollination time, it was considered to be cleistogamic. Fecundation occurred in the post-anthesis phase, when the flower was already opened. Thus, low rates of cross fertilization are possible, as a flower from one plant may receive pollen from another plant by means of pollinating insects. Even if cleistogamy does not occur, the ovules are still effectively fertilized because in the post-anthesis phase the stigma still remains receptive to cross fertilization. The enzymatic systems alcohol dehydrogenase, esterase, acid phosphatase, glutamate dehydrogenase, glutamate oxaloacetate transaminase, isocitrate dehydrogenase, leucine aminopeptidase, malate dehydrogenase, peroxidase and shikmic dehydrogenase, were tested. They were adequate for identifying, characterizing and differentiating populations and their respective individuals.

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Cytogenetic studies of fish (Pimelodidae and Rhamdiidae) from the Tibagi River basin/Paraná (Estudos citogenéticos em peixes das famílias Pimelodidae e Rhamdiidae da bacia do rio Tibagi/PR)

*Ana Cláudia Swarça**

Cytogenetic studies were made of Pimelodidae (*Pimelodus maculatus*, *Pinirampus pirinampu*) and Rhamdiidae (*Pimelodella* sp., *Pimelodella* aff. *gracilis*) from the Tibagi River basin in the State of Paraná. *P. maculatus* had 2n = 56 chromosomes (20m + 20sm + 10st + 6a), *P. pirinampu* had 2n = 50 (26m + 12sm + 2st + 10a), *Pimelodella* sp. had 2n = 46 (34m + 12sm) and *P.* aff. *gracilis* 2n = 52 (24m + 18sm + 4st + 6a). Nucleolus organizer regions (NOR) were observed in a pair of chromosomes in the telomeric region through impregnation by $AgNO_3$ in all specimens. In *Pimelodus maculatus* an NOR was located in the long arm of a pair of subtelocentric chromosome. In *P. pirinampu*, *Pimelodella* sp. and *P.* aff. *gracilis* an NOR was located in the short arm of a pair of subtelocentric, metacentric and submetacentric chromosomes, respectively. In *P. pirinampu* size variations in the NOR among the paired chromosomes were observed, probably due to genic amplification in this region. Staining through chromomycin A_3 showed the NOR regions in all specimens. In *P. pirinampu*, besides the nucleolar chromosome pair, fluorescent markings were observed in the telomeric and centromeric regions of other chromosomes which seemed to correspond to the distribution patterns of the constitutive heterochromatin. In this species several chromosomes with strongly heterochromatic telomeric and centromeric regions were observed with C banding. The constitutive heterochromatin in *P. maculatus* was weakly visualized in telomeric and/or centromeric regions of several chromosomes. A restriction enzyme *Alu*I was used in these two specimens of pimelodideos and the pattern of reaction observed corresponded to the heterochromatin constitutive distribution. *Pimelodella* sp. and *P.* aff. *gracilis* had a small quantity of heterochromatin in the majority of their chromosomes, but in these two specimens a strongly heterochromatic chromosome pair was observed in the telomeric region mainly in *P.* aff. *gracilis*.

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BiP-storage protein interactions and isolation of BiP cDNA and BiP genomic clones from soybean [*Glycine max* (L.) Merrill] (Determinação da interação BiP ("binding protein")-proteína de reserva e isolamento de clones de cDNA e genômicos de BiP da soja [*Glycine max* (L.) Merrill])

*José Edson Fontes Figueiredo**

The endoplasmic reticulum (ER) luminal binding protein (BiP) is thought to be a key mediator of folding and assembly of *de novo* synthesized secretory proteins. A maize (*Zea mays* L.) BiP antibody was used to identify its homolog from soybean [*Glycine max* (L.) Merril]. BiP accumulation in developing soybean seeds seems to be coordinated with the onset of active storage protein synthesis. A co-immunoprecipitation assay was used to detect soybean BiP:β-conglycinin interactions. Either a maize BiP antibody or a β-conglycinin antibody co-immunoprecipitated with the reciprocal protein from the whole seed protein extract enzymatically depleted of adenosine 5'-triphosphate (ATP), while an unrelated antibody failed to immunoprecipitate either one. BiP:β-conglycinin complexes were completely dissociated by ATP addition, a diagnostic feature of molecular chaperone-mediated interaction. However, a very small fraction of β-conglycinin was found to be associated with BiP in the whole cell protein extracts from immature seeds. These results indicate a transient association between BiP and β-conglycinin subunits and suggest its involvement in the biosynthetic transport pathway of storage proteins to protein bodies.

A BiP cDNA clone (cUFVBiP1) was isolated from a seed expression library and molecular analysis of the soybean BiP gene family was performed. cUFVBiP1 encodes a pre-protein of 668-amino acid residues and Mr 85.729 kDa. After processing, a mature protein of 641 amino acids (Mr 70,790) and isoelectric point 5.08 displayed several features shared by Hsp70 proteins and specifically by BiP protein: the ATP binding domain (sequence LGIETVGGV), the conserved peptide TVIGIDLGTTYSC found in all members of the Hsp70 family which are stress induced, a putative calmodulin-binding domain (NRALGKLRREAERAKRALSSQ), and the hydrophobic ER N-terminus signal peptide and the tetrapeptide HDEL at the C-terminus, which is responsible for the ER retention and retrieval of the protein. Amino acid comparison with the encoded products of other previously sequenced soybean BiP cDNAs (sBiPA, sBiPB, sBiPC) (Kalinski *et al.*, 1995) showed 86, 92 and 96% identity, respectively. At the nucleotide level, the high conservation of the soybean BiP genes was lower within the 5' and 3' untranslated regions. While the 5' and 3' untranslated regions of sBiPA, sBiPB, and sBiPC were quite dissimilar, these regions were more conserved between sBiPB and cUFVBiP1.

In order to gain more insight into the complexity of the soybean BiP gene family, an extensive DNA gel blot analysis of Doko and IAC 100 varieties was performed using restriction endonucleases-digested leaf DNA and the cUFVBiP1 clone as a probe. A very similar hybridization pattern for all restriction enzymes was observed between these two varieties. Several high molecular bands were observed even when restriction endonucleases that do not digest cUFVBiP1 were used. Some of these bands probably are the result of hybridization between clone cUFVBiP1 with other members of the highly conserved *hsp*70 gene family.

The cUFVBiP1 clone was used to screen a lambda EMBL3 genomic library constructed with leaf DNA from soybean cv Roonocke. Two clones were isolated and sequenced. The nucleotide sequence identity between these clones was very high (>98%) and shared the same structural organization, with intron and exons displaying the same size and localization. Moreover, these two clones also showed high identity with the genomic spinach BiP gene at the nucleotide sequence level (89%) and the structural organization (introns and exons).

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Beta-galactosidase activity and characterization of geneticin-resistant *Kluyveromyces lactis* mutants (Atividade de beta-galactosidase e caracterização de mutantes de *Kluyveromyces lactis* resistentes à geneticina)

*Eriana Serpa Barreto**

Geneticin-resistant *Kluyveromyces lactis* mutants were isolated and characterized with the purpose of isolating a β-galactosidase-secreting strain. The mutagenic agent used was ultraviolet light and geneticin resistance was tested over a concentration range of 0.5 to 1.0 mg/ml. Seven-minute radiation resulted in 1 to 5% survival of the population. Five thousand geneticin-resistant mutant colonies were tested. None of these showed β-galactosidase extracellular activity. However, one mutant resistant to 1.0 mg/ml geneticin presented a high amount of extracellular protein, 1.75 times more than the wild type *K. lactis*. No differences among mutants and wild type cells were observed in the assimilation of six carbon sources: sucrose, lactose, galactose, mannitol, raffinose and cellobiose. Resistance to geneticin did not affect the growth kinetics of *K. lactis* in ultrafiltered cheese