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#### SEARCH FOR C<sub>4</sub> DEVELOPMENTAL MUTANTS IN *Panicum maximum* Jacq.

Mutant plants are useful tools for studying developmental processes in defined genetic backgrounds by comparing them with their respective wild type forms. In this sense, developmental mutants or mutations involved in the establishment of certain leaf or flower specific traits are of special interest. In particular, the evolution of C<sub>4</sub> photosynthesis from C<sub>3</sub> precursors was accompanied by severe developmental changes in leaf morphology and anatomy. Our search of such mutants was followed by the idea to approach the evolution of the C<sub>4</sub> syndrome from a mutagenic point of view. Variants affecting normal development of the C<sub>4</sub> leaf anatomy may, in fact, represent possible regressive steps in C<sub>4</sub> photosynthesis [1].

Seeds of the C<sub>4</sub> grass *Panicum maximum* Jacq. were mutagenized using ethylmethanesulfonate (EMS) and putative variants were isolated in the M<sub>2</sub> generation by visual inspection. Main selection characteristics were whole plant, leaf morphology and pigmentation, and growth characteristics. The choice of a polyploid species for mutagenesis experiments was based on the need of detecting rare mutants, which are possibly lethal when using a diploid plant species. These variants could be of regulatory nature, affecting both morphology and physiology of C<sub>4</sub> photosynthesis early in leaf development. In total, nearly 100 variants were isolated and grown to maturity. Main isolated variants, which conforms to the prediction mentioned above, were as following: large interveinal space-1 and -3 (*lis1*, *lis3*), abnormal bundle sheath (*abs*), midribless (*mbl*) and variegated leaf -1 (*var1*). The variant *lis1* was a short plant with leaves smaller than the wild type, and had a leaf lamina with a crinkly surface. Photosynthetically, *lis1* indicates a clear regression from the C<sub>4</sub> to the C<sub>3</sub> photosynthesis type, which was correlated in the leaf lamina with an increase in the distance between small veins. The variant *lis3* was not similar phenotypically to *lis1*, but it also had very small leaves and reached a total plant height of maximal 0.6 meter. In leaf sections, it was characterized by an almost lack of the small veins surrounded by four bundle sheath cells. The leaf lamina of the variant *abs* showed several alterations, including doublets of veins, veins without bundle sheath, additional bundle sheath cells outside the veins or large bundle sheath cells participating in two bundle sheaths. Also the distribution of phloem and xylem cells within the bundles were quite altered in the variant compared to the wildtype. The leaves were greener, with a higher than normal chlorophyll content and with longitudinal veins not perfectly straight but following a wavy path on the leaf lamina.

Compared with wild type plants the phenotype of the *mbl* mutant was less erect and had pending leaves because of the absence of the main midrib. In wild type leaves the midrib was represented by an enlargement of the mesophyll parenchyma which included parenchymatous and sclerenchymatous cells. This structure was absent in mutant leaves, only small irregular files of parenchymatous cells were present at the base of the leaf lamina. The florets of this mutant had no carpel but one or two additional stamen.

The variant *var1* had a variegated phenotype with stripes of yellow-green and white tissues alternating the leaf laminae. In yellow-green sectors the chloroplasts were absent only in bundle sheath cells, which supports the hypothesis of different ways of development of bundle sheath and mesophyll cell chloroplasts. The adjacent mesophyll cells were less pigmented than



similar ones present in non-variant sectors. In white sectors, the chloroplasts were absent both in bundle sheath and mesophyll cells. The variant was partially fertile. Seed germination was 30 to 40%, and despite the unknown portion of apomictic seeds, 65 produced white, 20 green and 34 variegated seedlings out of a sample of 119 germinated seeds. Analysis of segregation of these green and variegated plants of the next generation is in progress.

In monocot species, leaves are divided lengthwise by three types of veins: midvein, lateral and small veins. The vascular system is established in a hierarchical fashion as the leaf develops. The midvein is established first in an acropetal direction towards the tip and basipetally into the sheath. Lateral veins develop acropetally in the leaf lamina, while small veins are initiated in this organ basipetally. The mutants in *P. maximum* demonstrate, that the complete process of vein initiation and development is apparently under complex genetic control [2].

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## INDUCED MUTATIONS IN CASTOR

Castor (*Ricinus communis* L.) is an important oilseed crop in India. To create variability mutations were induced in two cultivars 'TMV5' (maturing in 130-140 days) and 'CO1' (perennial type). Gamma rays and diethyl sulphate and ethidium bromide were used for seed treatment. Ten doses, from 100 to 1000 Gy were employed. For chemical mutagenesis five concentrations of mutagens from 10 to 50 mM were tried. No economic mutants could be isolated after treatment with the chemical mutagens. The following economic mutants were identified in the dose 300 Gy of gamma rays.

Annual types from perennial CO 1 castor

CO 1 is a perennial variety (8-10 years) with bold seeds (100 seed weight 90 g) and high oil content (57%). Twenty-one lines were isolated with annual types (160-180 days) with high yield potential as well as bold seeds and high oil content. These mutants, identified in  $M_3$  generation were bred true in subsequent generations up to  $M_8$  generation. Critical evaluation of the mutants in yield evaluation trials is in progress.

Parental lines for development of hybrids

The inflorescence of castor is monoecious type with bottom 30-35% male flowers and top 65-70% female flowers. Four mutants were identified from the variety TMV 5 with higher proportions of female flowers ranging from 80-90%. These mutants in  $M_7$  generation were identified as good combiners in the development of hybrid combinations. The yield of the hybrid combinations are presented in Table 1. Present investigations have clearly shown that there is a great potentiality in improving castor productivity and production by mutation induction.