Mutant generation using gamma rays

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In the 1960s-1980s there were numerous attempts for inducing genetic variability by mutagenesis to serve plant breeding objectives in food, feed and ornamental plants. Forage crops were less frequently submitted to these approaches probably because of their frequent high ploïdy status and their lower economic impact in agriculture of this period.
Many mutagenic treatments of this period were using nuclear techniques. Micke 1988 made for pulses a review of such mutagenenesis programs which were conducted on 24 species and in 27 countries. Treatments of dry seeds were usually applied at very diverse irradiation doses ranging from 30 to 400 Gy.

For *Medicago truncatula*, we found only one report in the literature of a gamma rays mutagenesis action. This program used a gamma rays cobalt 60 source and was performed at INRA-URLEG of Dijon France (Sagan et al 1995).

Determination of irradiation dose :
Seeds of line J5 (issued from cv Jemalong) were scarified with abrasive paper and put for imbibition in distilled water in Petri dishes for 24h at 4°C. Seeds were then exposed to a ^{60}Co source which provided different treatment doses of 0, 20, 40, 60, 80 and 100 Gy. Then 450 seeds per treatment were sown in glasshouse on clay-loam soil substrate, inoculated with *Sinorhizobium meliloti* strain 20111and daily watered with a nutrient solution low in nitrate (Sagan et al 1995).

Dose (Gy)	0	20	40	60	80	100
% Plant emergence	78	77	69	63	71	67
Nb of plants with chlorophyll chimera	0	0	0	1	3	3
Nb of pods/plant	44	45	46	45	45	14

In comparison to the control, dose of 100 Gy was the lowest to simultaneously show effects on fertility and chlorophyll mutations.

First results of mutagenesis program :
The dose of 100 Gy was choosen to perform the mutagenesis program (Sagan et al 1995). Same procedure as previously described was followed to produce 350 M1 plants. It resulted in a production of about 57000 M2 seeds. From these seeds about 10 000 M2 plants were grown in glasshouse and screened for chlorophyll and root nodulation traits. On M2 plants, a frequency of 1/300 chlorophyll mutant, 1/2200 nodulation phenotype mutants were found.

From this work at the moment, three mutated genes DMI1, DMI3 and SUNN (Endre et al. 2002, Levy et al. 2004, Schnabel et al 2005) were analysed at molecular level and revealed the nature of mutation event: among the mutants analysed one carries a 14-bp deletion, two carry a 1-bp deletion, two carry a point mutation.

References

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