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Poster Abstract - B.42

CONSTRUCTION OF COLLECTIONS OF MUTANTS IN *MEDICAGO TRUNCATULA.* I. REVERSE GENETICS STRATEGY: TILLING

E. PIANO*, L. SCARAMELLI*, G. BRUSCHI*, M. CARELLI*, C. SCOTTI*, L. LANFALONI***, A. PORCEDDU**, S. ARCIONI**

*) Institute of Forage Crops – C.R.A., V.le Piacenza 29, 26900 Lodi, Italy
**) Institute of Plant Genetics – CNR, Research Division Perugia, Via della Madonna Alta 130, 06128 Perugia, Italy
***) Dip. Biol. Cell and Amb. Facoltà SSMMFFNN, Università Perugia

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The construction of collections of mutants in *Medicago truncatula* has been realized in the frame of the MIUR National Project 'FIRB – Post Genomica di leguminose foraggere' in order to i) build up a key-tool, based in Italy, for reverse genetic studies in the model species *M. truncatula* , ii) provide potential allelic series of point mutations in plant regions of interest and iii) get the collection available for high-throughput screening for mutation discovery by means of TILLING technique (Mc Callum *et al.*, 2000; Colbert *et al.*, 2001; Bradley *et al.*, 2003).

Medicago truncatula seeds from genotype 2HA10-9-3 were treated with 0.15% EMS and the M1 generation, formed by about 2500 plants, was grown to produce the M2 generation; M2 seeds were collected on 2281 M1 individuals together with 65 tester plants derived from treatment with 0% EMS.

The effectiveness of the EMS treatment (maximum of mutant induction and minimal fertility depression) was verified by controlling the fertility of M1 generation and the percentage of M2 mutant seedlings in comparison with testers. The percentage of fertile plants (96%) was not significantly different from that of the testers (99%), while the average number of seeds per pod resulted significantly lower in M1 (2.53 ± 0.06) than in tester plants (5.56 ± 0.42). A sample of 567 M2 and 5 tester families (20 seedlings/family) were used for phenotypic screening of 14-days old seedlings: arrested or delayed embrios accounted for 18.4% on average in M2 generation and 1% in the tester families, while seedlings with abnormal pigmentation (albino, chlorotic, pale green) showed a frequency of 1.6 and 0% respectively. These data, in agreement with those reported in the literature (Penmetsa *et al.*, 2000) , confirmed the effectiveness of the mutagenizing treatment.

The M2 generation (1658 families, represented by 1-5 plants/family) together with 34 tester families is at present grown and phenotypically screened at Lodi and Perugia; classes of mutants for plant architecture, growth habit, shape and dimension of leaves, leaf pigmentation and floral morphology have been identified and documented. As for plant physiology, the presence of condensed tannins in the leaves and pollen viability have been screened on M2 generation. DNA extraction from the whole of M2 families has been currently performing together with M3 seed collection.

M2 DNA and M3 generation will constitute the basis for PCR-based screening to identify point mutations in regions of interest by means of tilling technique and to study their expression in plants.