



Metapontum Agrobios

TILLING in tomato: development of a EMS mutant collection and molecular screening for point mutations



Filomena Carriero (1), Angelo Petrozza (1), Olimpia D'Onofrio (1), Giovanni Sozio (1), Silvia Minoia (2), Florence Piron (2), Abdelhafid Bendahmane (2), Michel Caboche (2), Francesco Cellini (1)

(1) Metapontum Agrobios, S.S. Jonica 106, km 448.2, 75010 Metaponto (MT) Italy
(2) URGV/INRA, 2 rue Gaston Crémieux, 91057 Evry cedex France
E-mail :fcarriero@agrobios.it

INTRODUCTION

We are applying TILLING strategy (1) to tomato for the development of tomato lines carrying agronomic and nutritional traits of interest. In the present work we show results concerning the development and phenotypic characterization of a tomato mutant collection originated by treating tomato seeds (*cv Red Setter*) with two different concentrations (0.7% and 1%) of the chemical mutagen EMS. Furthermore preliminary data of the molecular screening for point mutations of interest will be presented.

RESULTS and DISCUSSION

Roughly 13000 M2 progeny of 6667 EMS-mutagenized M1 tomato plants were grown in open field and scored for their mutant traits. For each mutant phenotype, data and images were recorded and organized in classes and subclasses corresponding to different plant development stages (i.e. seed, seedling) or to different parts of the plant such as leaf morphology or fruits size.

Out of 13000 analysed M2 plants, 5000 resulted to show at least a mutant trait and of these 1834 M2 plants resulted to fall in more than one phenotype class. A high number of the mutations affected cotyledons, leaf morphology, size and habit of the plant. A low number of plants showed mutations affecting morphology and colour of the flower as well as fruit colour. In Figure 1 the percentage of defined phenotype classes and some examples of mutant traits are shown.

M3 seeds were harvested from individual M2 plants. Most of the tomato M2 plants (9000) produced seed number per fruit like control plants. 1650 (12.8%) M2 plants showed total sterility while 2125 M2 plants resulted to be partial sterile since they produced less than 50 seeds per fruit (Figure 2).

All phenotype data are being stored and analyzed with the Microsoft Access 2000 software. Soon after data recording completion, the database, comprising all the mutant information including photographs, will be publicly available. Genomic DNA was extracted from 2000 M2 plants and 1000 M3 plants. For high-throughput screening, we worked on gDNA pooled eightfold. We first begin to screen the *rab11a* gene (ACC. AJ245570), a gene of the GTPase family thought to be involved in the control of protein trafficking within cells (2). We analysed a 407 bp *rab11a* exonic fragment on 1000 plants. The mismatch was cleaved with the endonuclease ENDO I and the detection was performed on denaturing acrylamide gels (Figure 3). In these 1000 plants we found 6 mutations. Therefore, with a target fragment size of 1 kb, we estimate an average of 12.5 mutations per 1000 plants. Ongoing activities are being focused on *rab11* mutant phenotype characterization and on tomato mutant population screening for genes involved in fruit quality, fruit ripening, and pathogen resistance.

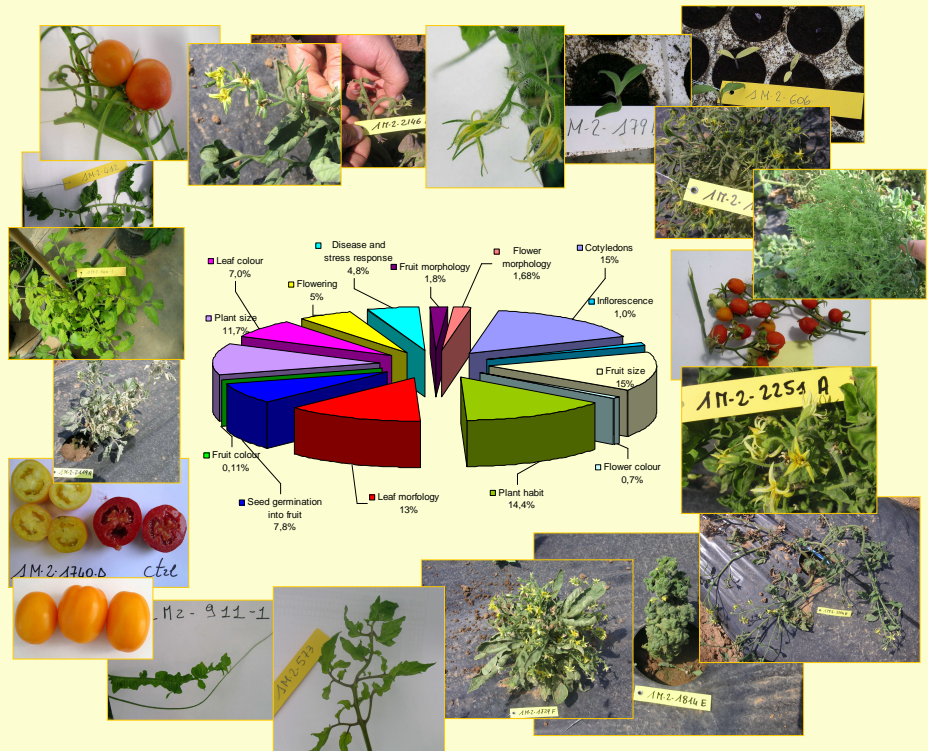


Figure 1. Percentage of defined phenotype classes and examples of tomato mutant traits.

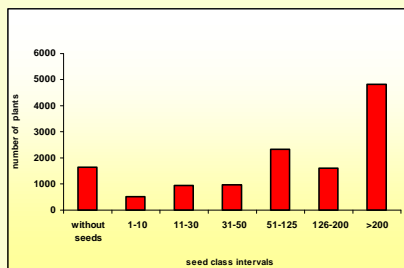


Figure 2. Histogram showing the number of M2 plants that produced M3 seeds falling in our defined seed class intervals.

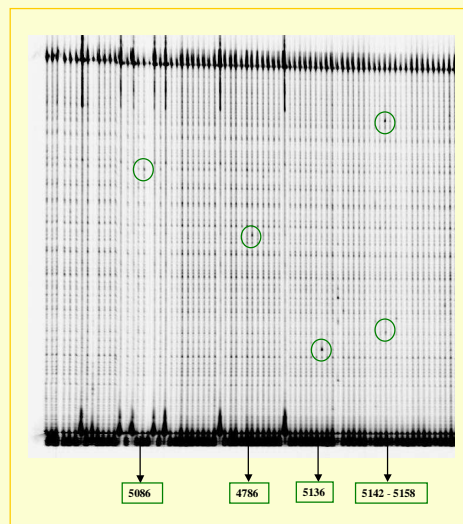


Figure 3. Example of point mutations detection with the *rab11* gene specific primer analysis. The boxed numbers indicate the individual plants having the mutation.

MATERIAL and METHODS

EMS mutagenesis

Tomato seeds (*cv Red Setter*) were treated with two different concentrations (0.7% and 1%) of the chemical mutagen EMS (ethyl methane sulfonate) for 18h at RT with gentle shaking. Then, seeds were extensively washed with H₂O, dried and sown in compost in 96 well seed trays, which allowed an accurate determination of germination frequency.

Plant growth

M1 and M2 tomato plants were either field and greenhouse soil transplanted and grown to maturity for seeds harvest.

DNA extraction and analysis

Genomic DNA was prepared from individual plants by using the "ChargeSwitch gDNA Plant Kit" (Invitrogen). The mismatch was cleaved with the endonuclease ENDO I and the detection was performed on denaturing acrylamide gels by using the Licor 4300 DNA analyzer.

References

- 1) C.M. McCallum, L. Comai, E.A. Greene and S. Henikoff (2000), *Plant Physiology* 123:439-442
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