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Effect of gamma irradiation on plants in interaction with arbuscular mycorrhizae*

Mihaela Ene, Laura Trandafir, Mioara Alexandru

Abstract. Arbuscular mycorrhizae are endogenous fungi that form symbiotic association with the vast majority of vascular plants. They help to increase the nutrient uptake, especially phosphorus and to exploit a larger area of soil. Also, they improve soil aggregation, contribute to nutrient cycling processes in plants and improve their tolerance of heavy metal contamination or drought, as well as their susceptibility to root pathogens or herbivores. These fungi colonize the root epidermis and outer cortical layers and release bioactive molecules. The transcriptome and the proteome of plants are substantially altered. They have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity, being included in a lot of combination of biofertilizers. In order to study these mechanisms, gamma irradiation was used to create mutants that are defective in their association. Culture under *in vitro* conditions is presented as method for maintaining the useful mutants in pure line.

Key words: tomato • mutant • gamma irradiation • mycorrhiza defective

M. Ene✉, L. Trandafir, M. Alexandru
Radiation Processing Center (IRASM),
Horia Hulubei National Institute of Physics and Nuclear Engineering (IFIN HH),
407 Atomistilor Str., Com. Magurele, Ilfov County,
077125, P. O. Box MG-6, Romania,
Tel.: +00 40 214 042369, Fax: +00 40 214 575331,
E-mail: m_ene@irasm.ro

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Introduction

Mycorrhiza are symbiotic associations that form between the roots of most plant species and a group of fungi, colonizing the cortical tissue. This symbiosis is characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. The mycorrhizal colonization and nutrient exchange (especially phosphorus) is a permissive infection process, ending with the development of new exchange microscopical structures (arbuscules).

Very little information is available about how plant genes facilitate the symbiotic program of colonization and nutrient exchange after the recognition event. That is why isolation of mycorrhizal defective mutants may provide a useful tool for more detailed, molecular level studies about mycorrhizal association physiology or ecology.

We report here the isolation of a tomato mutant, affected in its VA mycorrhizal colonization capacities, after gamma irradiation and culturing under *in vitro* conditions.

Materials and methods

Gamma irradiation was applied to tomato (*Lycopersicum aesculentum* Mill.) in order to obtain mutants

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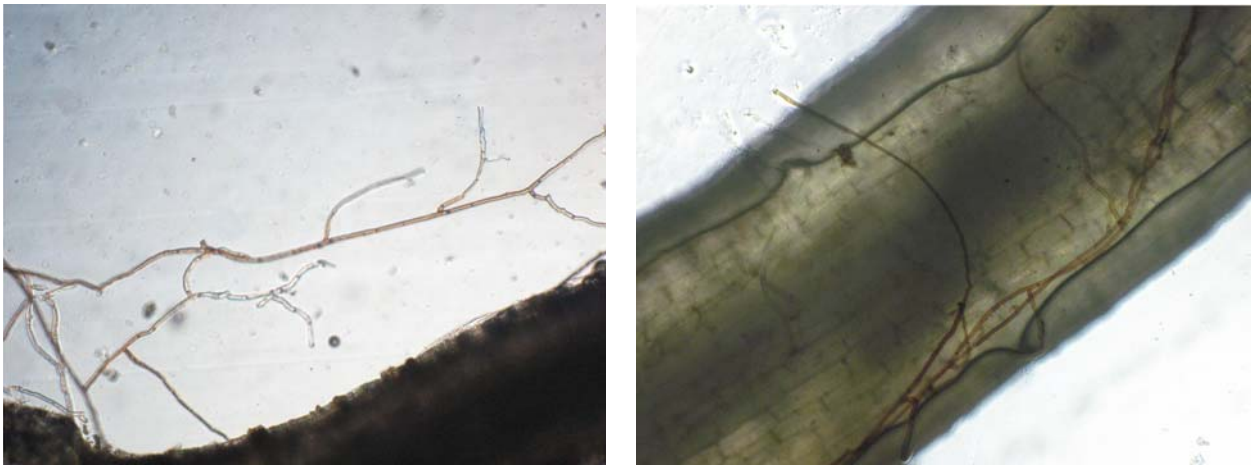

Fig. 1. External mycelium of arbuscular mycorrhizal fungi on a root. No staining.

that are defective in the formation of mycorrhizal symbiosis.

This tool has been widely used previously for plants. For mycorrhizal symbiosis, many mutants are described for legume plants, like *Medicago truncatula* and *Lotus japonicus*. They offer the advantage of *Rhizobium* symbiosis formation simultaneously, since it has been shown that the two symbiosis (mycorrhizal and nitrogen fixation) share common steps and molecules for signaling events. Genetic characterization of the symbiosis began with the identification of nodulation-defective mutants of *Pisum sativum* and *Vicia faba*, that were also non-mycorrhizal [1].

To our knowledge, for *in vitro* cultivation and clonal multiplication, no mutants for mycorrhizal symbiosis in tomato have been reported so far. These conditions offer the advantage of contamination and nutrient control.

Normal mycorrhizal colonization in tomato occurs like the *Arum*-type [7], with external hyphae, appressoria on the root surface, hyphal coils in hypodermal passage cells, intercellular hyphae and intracellular arbuscules, with or without vesicles, depending on the species (Figs. 1 and 2).

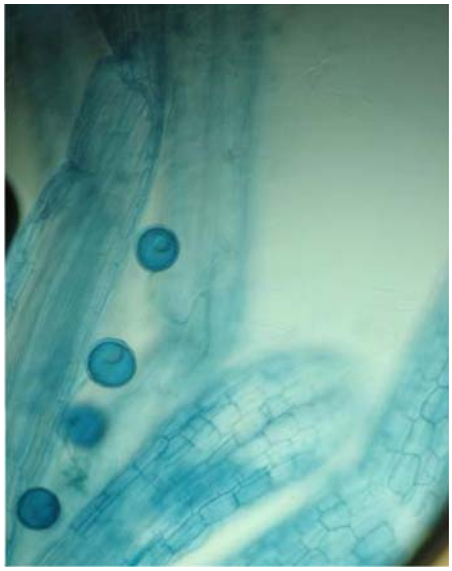

Fig. 2. External spores of a *Glomus* species. Cotton blue staining.

In our experiment, 200 tomato seeds were treated with 4 doses of gamma irradiation, from a ^{60}Co source. 40, 68, 136 and 204 Gy were the absorbed doses, with an error of 10% and a dose rate of 136 Gy/h. After scattering for 2 min in 98% H_2SO_4 solution and rinsed 5 times, tomato seeds were surface disinfected with 3.5% calcium hypochlorite – 10 min, ethanol 70% – 3 min and gentamycin solution (injectable) 1 mg/ml – 3 h. 50 disinfected seeds from each dose were planted on the surface of water-agar medium.

Results and discussion

After 2 weeks, clean, uninfected plantlets were transferred to White medium with macro- and micronutrients, no sucrose (SIGMA W0876) and maintained at 24°C under natural light conditions. Another 1 week was necessary for the plants to adapt and establish a dim root system in the new medium (Fig. 3).

Small blocks of gel containing spores were distributed in the plate along with the liquefied medium, at ~ 40°C. Two MUCL strains were used: *Glomus intraradices* MUCL 43204 and *Gl. clarum* MUCL 46238. When the roots met the spores pool, at least one infection event took place. After 3 weeks, plant roots were examined inside the plate, through the agar, under a Zeiss Imager D1m microscope and also under a Wild

Fig. 3. Mutated tomato plantlets, after 3 weeks of culturing under *in vitro* conditions.

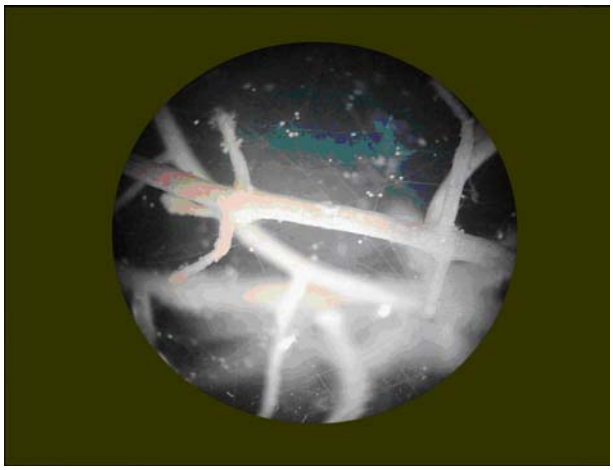

Fig. 4. Image under stereomicroscope of a colonized root in agar plate.

stereomicroscope (Fig. 4). No preparation for microscopy was applied at this point, since a full recovery of the plants was needed.

Three plants that did not present any sign of infection were selected. They have been allowed to develop for another 4 weeks. Clonal multiplication of these lines was applied: shoot peaces with leaves and vegetative tip were cut and transferred to fresh MS medium, with sucrose (Sigma M9274), in bicompartimented plates. Similar blocks of VA mycorrhizae spores were incorporated when distributing the media, except this time shoot fragments from the same mutant line were planted distinctly in the presence of each *Glomus* species. The other half of plate was filled with White medium, without sucrose; the lack of sugar stimulates sporulation in this sector.

As Gao [3] and Morandi [5] reported, the symbiotical behavior may tremendously vary with the fungus strain.

Microscopic examination of the putative mutants

A classical protocol for staining the fungus inside the roots was applied, using either trypan blue [6] or cotton blue [4]. The roots were first observed without squasing, in order to correctly appreciate if the penetration actually occurred.

Inheritance of the mutation

From the 3 mutant lines, only one, exposed to 204 Gy, kept its mycorrhizal defectiveness after the first transfer. Although we are not talking here about the meiotic barrier that tend to exclude the genetic abnormalities, the fact that the meristem generating the roots belonged to the shoot may explain the reverting of the traits for the other two lines.

However, one line remained that maintained its mutational behavior. No sign of symbiosis was still recorded for this line. The spores germinated, but no appressorium formation has been observed. Few poor and antypical infection structures formed, lacking any penetration sign. Trypan blue staining did not reveal any arbuscules or vesicles, neither for *Gl. intraradices* nor for *Gl. clarum*. The culture was maintained for 35 days after inoculation and the phenotype was stable when plants were transferred to fresh culture media. Thus, we exclude the conclusion of very slow colonization, since in normal phenotypes, into nurse pots, the colonization reaches a maximum (plateau) within about 8 days.

Different types of mutations have been described to date: with the formation only of appressoria and no intercellular hyphae developing from them [5] with typical thick lens-shaped appressoria [2]. In case of legumes, in the majority of identified mutations, colonization is blocked at the epidermis, prior to any significant penetration of the root [1].

This behavior with almost no signs of infection is common to the non-host plants; a number of taxons are characteristically non-mycorrhizal (like most of the *Brassicaceae* and *Chenopodiaceae*). On non-host plants roots, spores may germinate, but the penetration event is very rare and physically restricted.

Conclusions

We studied the possibility of using gamma irradiation as a tool for generating mycorrhiza-defective mutants in tomato. The gamma-ray mutagenesis procedure was efficient to produce one tomato mutants that impaired in symbiosis with 2 *Glomus* species (vesicular arbuscular mycorrhizae fungi). The mutation was stable after clonal multiplication from shoots and 2 transfers on fresh culture media. The affected genes are not essential for plant function, reflecting the fact that the symbiosis is not genetically obligate for the plants. The absorbed dose of the selected mutant was 204 Gy, but higher doses are likely to be more efficient in terms of mutation frequency.

In vitro conditions offers highly controlled nutritional and competitor environment, however, complementation tests for the chromosomal localization of the mutation may be difficult, since the plants rarely flower and make fruits. Yet, mutant plants clonally multiplicated remains good models for studying the behavior of non-host plants. Moreover, they can be further accommodated under *ex-vitro* conditions, thus allowing crosses for establishing if the mutation is a mono- or polygenic trait.

Although a lot of mutants have been described among legume plants, with the exception of *M. truncatula*, these species are poorly developed for molecular genetic research and there is no information on the genetic defect responsible for the failure of symbiosis to develop [1]. Thus, tomato is a better model for molecular genetic characterization of such mutants (mapping and cloning the genes), since it has a relatively small number of chromosomes, and a large amount of sequence information is known so far. Furthermore, tomato is easy to grow (e.g. both in pots and *in vitro*) and responsive to well-characterized VA mycorrhizal colonization.

From the experience of Gao *et al*. [3] it is premature to extend our results, concerning the symbiotical incapacity of mutated tomato to all AM fungi, especially because the group in very heterogeneous.

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