

Development of methodologies for *in vitro* multiplication, plant regeneration, and genetic transformation of naranjilla (lulo)

V. Segovia (SB2) Z. Lentini (SB2)

A large number of fruits of Andean origin have great potential to become premium products for local and export markets with a high economic return for the farmers. Naranjilla (*Solanum quitoense*) is among these fruits. This species is native from Colombia and Ecuador, and it is normally cultivated between 700 and 2000 meters above sea level. Some of the main attributes of this fruit includes its high level of vitamin C, and the sub-shrubby perennial growth amenable for cultivation in hillsides and inter-cropping, aiding soil conservation practices. Recently in Colombia, naranjilla changed from being a fruit of local fresh consumption to become an important industrial fruit for juice and yogurt products, increasing its market value. A major constraint for the rapid adoption of naranjilla by the local farmers is the limited availability of elite germplasm free of pathogens. The high level of trait segregation restrains its multiplication through seeds. Rapid multiplication of quality planting materials is of paramount importance. One of the main objectives of this project is to develop a protocol for *in vitro* propagation of naranjilla with application for conservation and rapid multiplication of clones free of pathogens. The expected results include the mass multiplication of elite clones that then can be distributed to farmers. Since breeding for this species is almost non-existing, parallelly to the *in vitro* propagation effort, it will be important to develop plant regeneration and transformation systems to aid the development of germplasm. Last year it was reported preliminary results on developing a plant regeneration protocol. This year it is presented the advancement in establishing a system for maintenance of the *in vitro* germplasm collection, and the progress made identifying factors to increase the plant regeneration efficiency from elite naranjilla materials.

Materials and Methods

High quality and elite clones provided by the Andean Fruit Center (Centro Frutícola Andino – CEFA) and Corpoica La Selva are used. This collection includes naranjilla with or without thorns commonly grown by farmers. Various media were tested for *in vitro* maintenance of the clones. Statistical experimental design was used to determine the optimal plant tissue and medium for an increase efficiency in plant regeneration. Regenerated plants were taken to the greenhouse and the field to evaluate plant growth and development to maturity. Plants initially grown *in vitro* and then in soil, were taken back to the *in vitro* system to establish a protocol for renewing the *in vitro* collection.

Results and Discussion

In Vitro Propagation

Last year it was reported that plant develops healthier and faster *in vitro* when cultured onto ½ MS medium supplemented with ANA 0.02 mg/l, BAP 0.04 mg/l, and GA₃ 0.05 mg/l, and agar 4.5 g/l, in contrast to the regular micro-propagation medium used by CEFA or Corpoica La Selva (SB2 Annual Report 2000). However, after 10 months of sub-culturing

naranjilla *in vitro* plants in this medium, most of the plants showed white stripes on leaves, thick stems, and slow development. Reason why other media were tested again. For this purpose, it was evaluated media commonly used for long term maintenance of *in vitro* germplasm of other *Solaneace* species. Of the medium tested, a medium containing MS basal salts and vitamins, and supplemented with calcium pantothenic acid 2.5 mg/l and gelrite 3.5 g/l (Hussey and Stacey, 1981) used for potato, also showed to be the optimal for naranjilla (medium A). On this medium, *in vitro* shoots start showing root proliferation two weeks earlier respect to medium ½ MS medium. Fully expanded new leaves were present at one month after subculture on medium A, whereas it took about 6 weeks on ½ MS medium (Figure 1). Moreover, new leaves developed on medium A were completely green although the starting materials had leaves with white stripes. The number of leaves with white stripes increased as plants were sub-cultured on ½ MS medium. By sub-culturing plants on medium A contained in jars rather than in test tubes, it is possible to obtain a large number of explants to conduct series of experiments for optimizing plant regeneration with various replicates every month. Medium A also seems to be a more appropriate for a long term *in vitro* maintenance of the germplasm collection since plants are healthier, develop faster, and differentiate normal green looking leaves.

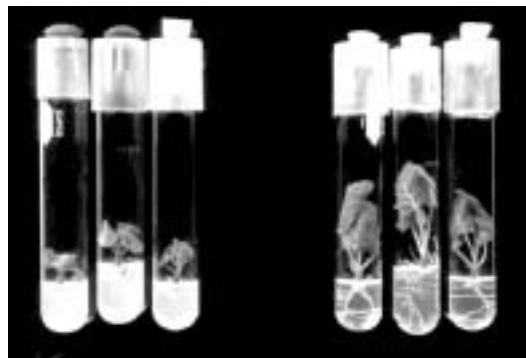


Figure 1. In vitro development of naranjilla plants in ½ MS medium (left) and medium A (right) one mothe after subculture

Plant Regeneration

A randomized block design of four replicates each of 15 experimental units was used to determine the best medium composition and explant to induce a direct plant regeneration in naranjilla. A non-parametric chi-square analysis indicated that petioles from the first and second node showed 17 times and about four times more plant regeneration that the corresponding leaves from thorny and non-thorny clones respectively. (Figure 2 and 3). A significant higher response was also noted on medium originally develop for plant regeneration on tomato (Ultzen et al, 1995), consisting on MS salts, B5 vitamins, supplemented with sucrose 10 g/l, glucose 10 g/l, gelrite 1.5 g/l, zeatine 2 mg/l and IAA 0.02 mg/l (Figure 2A). On this medium petioles from thorny genotypes showed three fold increase in plant regeneration respect to a medium reported for naranjila (Hendrix et al., 1987) composed by MS salts and vitamins and supplemented with sucrose 30 g/l, agar 7 g/l, IAA 0.01 mg/l, kinetin 5 mg/l, or with a modification consisting on gelrite 2

g/l and BAP 2 mg/l (modification suggested by Dr Richard Litz, University of Florida, laboratory which Hendrix work was conducted)(Figure 2B). Non-thorny genotypes did not regenerate any plant on medium developed by Hendrix (Figure 2B).

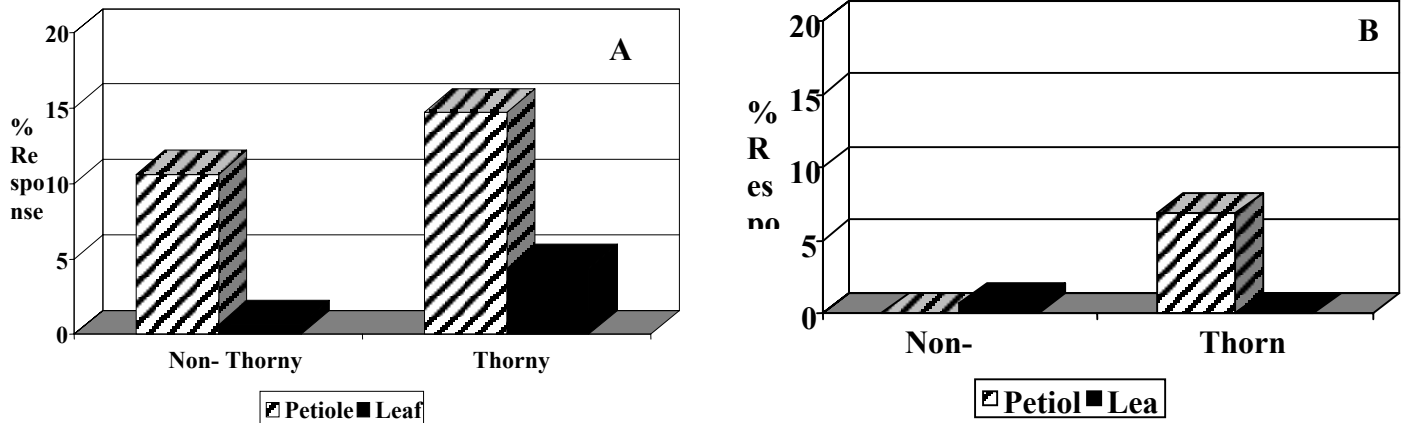


Figure 2.- Plant regeneration from petiole or leaf explants of genotypes with or without thorns using medium previously developed for tomato (A) or reported by Hendrix for naranjilla (B).



Figure 3.- Multiple shoot formation from petiole of naranjilla

Plant Evaluations in the Greenhouse and the Field

A methodology was established to transfer *in vitro* material (from the *in vitro* germplasm collection and regenerated plants) to the greenhouse and thereafter, to the field. The first regenerated plants evaluated shown a normal plant growth and development to maturity in the greenhouse and in the field. Fruit formation is being evaluated in the field only, since temperatures in the greenhouse at CIAT headquarters is too high for naranjilla fructification. The field selected is located at 1,700 m.s.n.m. and with a mean temperature of 22 C, ideal for induction of fruit formation of naranjilla. The field plot is located in a farm (La Casona) at Dapa about 20 min from Cali, where there is a naranjilla production by farmers.

Future plans

- Establish a cyclic culture from greenhouse to *in vitro*, to completely renew the existing germplasm *in vitro* collection and propagate it in medium A
- Evaluate other factors affecting plant regeneration response to increase it at about 50%
- Develop a genetic transformation protocol
- Complete evaluation of regenerated plants in the field and compare the growth and development with *in vitro* propagated and seed derived materials

References

- Hendrix R., R. Litz, and B. Kirchoff. 1987. *In vitro* organogenesis and plant regeneration from leaves of *Solanum candidum*, *S. quitoense* (naranjilla) and *S. sessiliflorum*. Plant Cell Tissue and Organ Culture. 11: 67-73.
- Hussey G. and N.J. Stacey. 1984. Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum* L.). Ann.Bot. 53: 565-578.
- Ultzen T., J. Gielen, F. Venema, A. Westerbroek, P. Haan, M. Tan, A. Schram, M. Grinsven, and R. Goldbach. 1995. Resistance to tomato spotted wilt virus in transgenic tomato hybrids. Euphytica. 85: 159-168.

