ABSTRACT

Plant regeneration from explants is the starting point to apply technologies such haploid regeneration or genetic transformation. This character shows a wide inter and intra-specific variability. Thus, even within the same species we can find recalcitrant genotypes whose lack or insufficient regeneration ability limits the application of these techniques. In addition, other factors affecting the success of regeneration are: the physiological conditions of the starting material, the components of the culture medium, growth regulators, temperature, light, etc... The lack of information about what factors determine that this process occurs for one or another morphogenetic way (organogenic or embryogenic) and of how many genes are involved, indicates the need for basic research of this process.

The main goal of this work is focused on increasing the knowledge of the genetic basis as well as the localization of QTLs involved in organogenic regeneration way that is predominant in tomato. We have used two mapping populations (F_2, BC_1) obtained by Dr. Gisbert. The F_2 population was obtained from self-pollination of a F_1 plant, resulting from the crossing of a tomato plant (*S. lycopersicum* L.) selected by its limited regenerative capacity (Anl27) and the PE-47 accession of *Solanum pennellii* Correll. with high regeneration capacity. Furthermore, the BC_1 population was obtained from the cross (Anl27 x F_1). Phenotypic and genotypic characterization has been done in clones of each genotype maintained on *in vitro* culture. Using the computer program MapQTL ® we identified six QTL involved in regeneration and located on chromosomes 1, 3, 4, 7 and 8. Five of the QTLs (*SpRg-1*, *Rg-3*, *SpRg-4a*, *SpRg-4b*, *SpRg-7*) come from the wild tomato *S. pennellii* and one (*SlRg-8*) comes from *S. lycopersicum*. The variance percentage explained by each QTL was in the range 7.4-27%, within the common range (6-26%) recorded for the genetic mapping of QTLs associated with *in vitro* regeneration in other crops. *SpRg-1* is most responsible for the morphogenetic response while *SpRg-7* promotes the development of the bud to a complete plant. On the other hand, the QTLs detected in chromosomes 8 (*SlRg-8*) and 4 (*SpRg-4a*, *SpRg-4b*) may contain genes that influence in the formation of buds and its development, respectively. Finally *Rg-3*, located in the middle of chromosome 3 and linked to Acid Invertase gene, is presented as a putative allele of the *Rg* gene detected in *S. peruvianum* (*Rg-1*) and *S. chilense* (Dunal) Reiche. (*Rg-2*).

Type and combination of growth regulators are important to the success of regeneration protocols. In tomato, cytokinins or a combination of these with auxins are the growth regulators most commonly used. Other regulators such as the Ethylene has been little studied and published works show disparate conclusions. In order to study the influence of ethylene in the regeneration many tissue culture experiments were performed using two compounds releasing

ethylene (1-aminocyclopropane-1-carboxylic acid "ACC" and 2-Chloroethylphosphonic acid phosphonic "Ethephon") and two inhibitors of ethylene: AgNO3, which inhibits the action of ethylene; and CoCl2, which inhibits the production of ethylene. The results show that concentration and time of application are two key factors to take into account for the success of the organogenic response. In particular, the application of ethylene inhibitors and its consequent decrease has a negative effect on regeneration in tomato since response is delayed and decreased. Likewise, regenerating plants obtained are reduced in size, and can show vitrification and malformations. On the other hand, ethylene supplementation can improve regeneration. Thus, the number of plants regenerated from explants of *S. pennellii* on ACC supplemented medium doubled the number obtained on control medium. If ACC is applied after the induction of buds, after 10 days, total yield is greater. This result indicates that this compound could be used to improve regeneration in those genotypes which once formed the buds, the development of these to plants is the limiting step. On the other hand, the regenerating plants obtained show good development. Ethylene supplementation does not affect the subsequent growth of the regenerated plants.

Finally we have studied the organogenic capacity of wild tomato species derived from the complex *S. peruvianum* l. sensu lato (s.l.): *Solanum arcanum* Peralta., *Solanum huaylasense* Peralta., *Solanum corneliomulleri* J. F. Macbr. and *Solanum peruvianum* L. sensu stricto (s.s.). These species can be very important on tomato plant breeding for resistance to biotic factors. However, due to hybridization barriers they are not extensively used yet: protoplasts fusion and embryo rescue can be the best way to hybridize. However, it has not been studied the organogenic capacity of the species derived from the Peruvianum complex, which limits the application of these techniques. This work has found intra and interspecific variability in organogenic capacity of wild species from this complex. In general, all *S. corneliomulleri* and *S. huaylasense* tested accessions showed an elevated regenerative capacity and we have identified recalcitrant accessions in *S. arcanum* (LA-2185) and *S. peruvianum* s.s (ECU-106 and CH-20). In this work, a morphological analysis of the leaves has determined that the number of leaflets and dentition can be used to identify *in vitro* species of the complex. However, the leaflet area only allows to identify *S. arcanum* of the rest of the complex species. Finally it has been observed that the accessions whose leaves have greater number of leaflets and higher level of dentition have a greater regeneration reponse.