Solanum pennellii LA716 as a Source of Genes Improving In Vitro Organogenesis in Cultivated Tomato

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Abstract

In the present work we are reporting the high in vitro regeneration capacity of the tomato related wild species *S. pennellii* LA716, which enabled us to used a collection of 50 introgression lines (ILs), each containing small chromosomal segments of LA716 introgressed and mapped into the cultivar 'M82'. We found a high shoot regeneration capacity for IL3-2, IL6-1, IL7-1 (7-2, 7-3), IL 8-3 (8-2), IL-9-1 (9-2) and IL10-2 (10-3), when 12-days-old cotyledon explants were cultivated in MS medium containing 5.0 µM BAP. This means that *S. pennellii* probably presents superior alleles for in vitro regeneration in such chromosomal segments. Since ILs 3-2, 7-1, 8-3, and 10-2 also presented enhanced root formation in MS medium containing 0.4 µM NAA, they may represent novel alleles controlling the competence to assume different cell fates, rather than the induction of a specific organ. The alleles discovered here will provide for the characterization and isolation of important genes for plant development studies and biotechnological applications.

INTRODUCTION

Despite the wide use of adventitious organ formation for biotechnological purposes, the genetic basis of this capacity remains largely unknown. Tomato (*Solanum lycopersicum* L.) presents many characteristics of a suitable genetic model: it is an autogamous diploid species with a small genome (950 Mb) distributed in 12 chromosomes, and it has a saturated genetic linkage map (http://solgenomics.net/) with numerous markers associated with traits of great economic and biological importance, as well as, a plethora of well characterized mutants (http://tgrc.ucdavis.edu/). Besides induced mutations, natural genetic variation can be found in the wild *Solanum* species section *Lycopersicon*, most of which are inter-fertile and amenable for crossing with cultivated tomato (Stevens and Rick, 1986). The observation of new phenotypes and identification of novel alleles coming from wild species is facilitated by the use of introgression lines (ILs), which are permanent mapping resource populations (Eshed and Zamir, 1994). Once identified, the specific effect of a given natural genetic variation can be efficiently studied by constructing nearly isogenic lines (NILs) that differ only at a single quantitative trait loci (QTL) region or Mendelian gene (Paran and Zamir, 2003).

Tomato has been proven to be an excellent model for the study of natural genetic variations controlling the in vitro regeneration capacity. Among the wild tomato related species, *S. peruvianum* and its sibling *S. chilense* are considered highly organogenetic (Koornneef et al., 1987; Peres et al., 2001). Studying the genetic basis of organogenetic competence in *S. peruvianum*, Koornneef et al. (1987) found that this character was associated with two major dominant alleles (named *Rg1* and *Rg2*). The *Rg1* was further mapped to chromosome 3, close to the *yellow fresh* (*r*) locus (Koornneef et al., 1993). The recessive *r* allele represents a loss of function in the chromoplast-specific phytoene synthase (PSY) gene (Fray and Grierson, 1993), conferring yellow color to fruits when introgressed into the *S. lycopersicum* background (Koornneef et al., 1993). It was suggested that other green fruited species harboring the *r* allele may also have versions of the *Rg1* allele conferring high organogenetic capacity (Peres et al., 2001). The presence of the *r* allele in the green fruited species *S. peruvianum* creates the opportunity to use it as a

morphological marker for the introgression of *Rg1* into cultivated tomato. Using this procedure, the *Rg1* allele from *S. peruvianum* was further transferred to the cultivar 'MT' (Lima et al., 2004), creating a genotype that is been put forward as a tool for genetic transformation of MT model system (Pino et al., 2010).

In the present work we are reporting the high in vitro regeneration capacity of the green fruited species *S. pennellii* LA716, which enabled us to used a collection of 50 ILs, each containing small chromosomal segments of *S. pennellii* LA716 introgressed and mapped into the cultivar 'M82', to search for natural genetic variation controlling in vitro organ formation capacity.

MATERIALS AND METHODS

The tomato (*Solanum lycopersicum* L.) cultivar 'M82', the wild species *S. pennellii* LA716, and the collection of 50 introgression lines (ILs) derived from the cross 'M82' × 'LA716' (Eshed and Zamir, 1994) were provided by Dr. Roger Chetelat at The C.M. Rick Tomato Genetics Resource Center (http://tgrc.ucdavis.edu/). Seeds from 'M82', 'LA716' and the introgression lines (ILs) were surface-sterilized by shaking in 100 ml of 30% (v/v) commercial bleach (2.7% sodium hypochloride) plus two drops of commercial detergent, for 15 min, followed by three rinses with sterile water. The seeds were then germinated on half strength MS salts; half strength B5 vitamins; 15 g L^{-1} sucrose and 6 g L^{-1} agar (Merck, Darmstadt, Germany). The medium pH was adjusted to 5.8 before autoclaving. Approximately 40 seeds were sown per flask containing 30 ml of medium. Cultures were sealed with PVC and incubated at 25±1°C in the dark for 4 d, followed by 8 d under 16-h photoperiod provided by a 40 W cool white fluorescent tube (c.a. 45 μmol PAR m^{-2} s^{-1}). Cotyledons were isolated from 12-day-old seedlings. The distal and proximal tips were removed, and the cotyledons were divided transversally in two or three pieces. Explants were placed with the abaxial side down immediately after isolation, with 15 explants per petri dish (90×15 mm), using 6 plates per treatment. Explants were placed onto semi solid Shoot Inducing Medium (SIM), composed by MS salts with B5 vitamins, 30 g L^{-1} sucrose, 6 g L^{-1} agar, 5 μM BAP (Sigma, St. Louis, USA), or Root Inducer Medium (RIM), which has the same composition of SIM, except for the replacement of BAP with 0.4 μM NAA (Sigma, St Louis, USA). Plates were sealed with PVC and maintained under 16 h photoperiod at 25±1°C for 3 weeks.

RESULTS

Taking into account that some tomato related green fruit wild species are high regenerating in vitro (Koornneef et al., 1987; Peres et al., 2001), in the present work we tested such capacity for *S. pennellii*, a green fruited species. The improved in vitro shoot formation capacity of *S. pennellii* is evident when compared to the cultivar 'M82' in 12-day-old cotyledons explants cultivated in 5.0 μM BAP (Fig. 1A-B). We were able to observe a considerable variation in the capacity to form shoot in cotyledon explants cultured in vitro in a population of ILs harboring small segments of *S. pennellii* introgressed into the cultivar 'M82' (Fig. 1C). Among the genotypes observed, the ILs 2-1, 3-1, 6-3 and 7-5 presented the lowest formation of shoots in vitro (Fig. 1C). Such ILs may represent loci where *S. pennellii* alleles are inferior to that of 'M82' for regeneration capacity. These transgressive phenotypes may be the product of epistatic interactions into the 'M82' background, or the effect of *S. pennellii* alleles per se (DeVicente and Tanksley, 1993). On the other hand, the ILs 3-2, 6-1, 7-1 (and 7-2), 8-3 (and 8-2), 9-1 (and 9-2) and 10-2 (and 10-3) are likely representing superior alleles present in *S. pennellii* controlling the capacity to form shoots in vitro. Using the concept of bin mapping created for this same population of ILs (Liu et al., 2003), it was possible to classify the chromosomal regions most probable to harbor the alleles for high shoot formation capacity into bins 3C, 6A, 7H, 8F, 9DE and 10F (Fig. 2).

In independent experiments, we had confirmed the high frequency of explants forming shoots in ILs 3-2, 6-1, 7-1, 8-3, 9-1 and 10-2, which presented a regeneration capacity not statistically different from that of the high regenerating parental *S. pennellii*

(Fig. 3A). We further tested the capacity of the select ILs to form roots in RIM (0.4 μM NAA). The high root formation capacity of ILs 3-2, 8-3, 10-2 and, in a minor extend, of 7-1 (Fig. 3B), indicates that the alleles presented in these ILs are controlling the formation of both shoot and roots. On the other hand, the ILs 6-1 and 9-1 seems to be specifically improving shoot formation (Fig. 3A), but not root formation (Fig. 3B).

DISCUSSION

In the present work we identified six chromosomal segments whose alleles from the tomato relative wild species *S. pennellii* improve organogenesis in vitro. These QTL (Paran and Zamir, 2003) for in vitro regeneration capacity, were named, after Liu et al. (2003), as *RG3C*, *RG6A*, *RG7H*, *RG8F*, *RG9DE* and *RG10F*. Studying the genetic basis of in vitro high organogenetic capacity in *S. peruvianum*, Koornneef et al. (1987) found that this character was associated with two major dominant alleles (named *Rg1* and *Rg2*). The *Rg1* was further mapped to chromosome 3, close to the *yellow fresh* (*r*) locus (Koornneef et al., 1993). The *RG3C* described here is likely to be the *S. pennellii* equivalent to the *Rg1* from *S. peruvianum*, since they, and the *r* locus, all map in the same bin 3C (Fig. 2; Koornneef et al., 1993). It might be also that some one of the other loci described here corresponds to the *Rg2* (Koornneef et al., 1987), although we do not have information about the map position of *Rg2* for comparison.

The loci *RG3C*, *RG7H*, *RG8F* and *RG10F* described here as controlling high in vitro shoot formation capacity also enhanced root formation in adequate medium. The other two loci, *RG6A* and *RG9DE*, seem to be specific for shoot formation capacity. Christianson and Warnick (1988) divided the process of organogenesis in vitro in the following steps: 1) dedifferentiation, 2) acquisition of competence, 3) induction, 4) determination, 5) differentiation and 6) formation of the organ. In this division, the step of acquisition of competence is a general process necessary for both shoot and root formation, whereas the induction requires specific auxin-to-cytokinin balances leading to shoot or root formation (Skoog and Miller, 1957), but not both organs. Based on this concept, it is proposed that *RG3C*, *RG7H*, *RG8F* and *RG10F* are probably affecting the step of acquisition of competence and that *RG6A* and *RG9DE* are affecting the step of shoot induction.

Regardless the genetic identity of the loci described here, the usefulness of genes improving in vitro regeneration capacity is evident not only for tomato but for most crop species. Although *Agrobacterium*-mediated plant transformation has long been established for tomato (Fillati et al., 1987), this procedure is being continuously improved, with key contribution of alleles enhancing in vitro regeneration (Pino et al., 2010). Moreover, until present date, the important breeding tool of double haploid production (Forster and Thomas, 2005), and thus reverse breeding (Dirks et al., 2009), is not available in tomato. The main barrier for haploid production in tomato stands in the low regeneration of anthers when cultured in different media (Seguí-Simarro et al., 2011). Since some of the alleles described here are likely to be controlling the capacity to assume different cell fates (competence), they may be useful to improve different regeneration systems.

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Figures

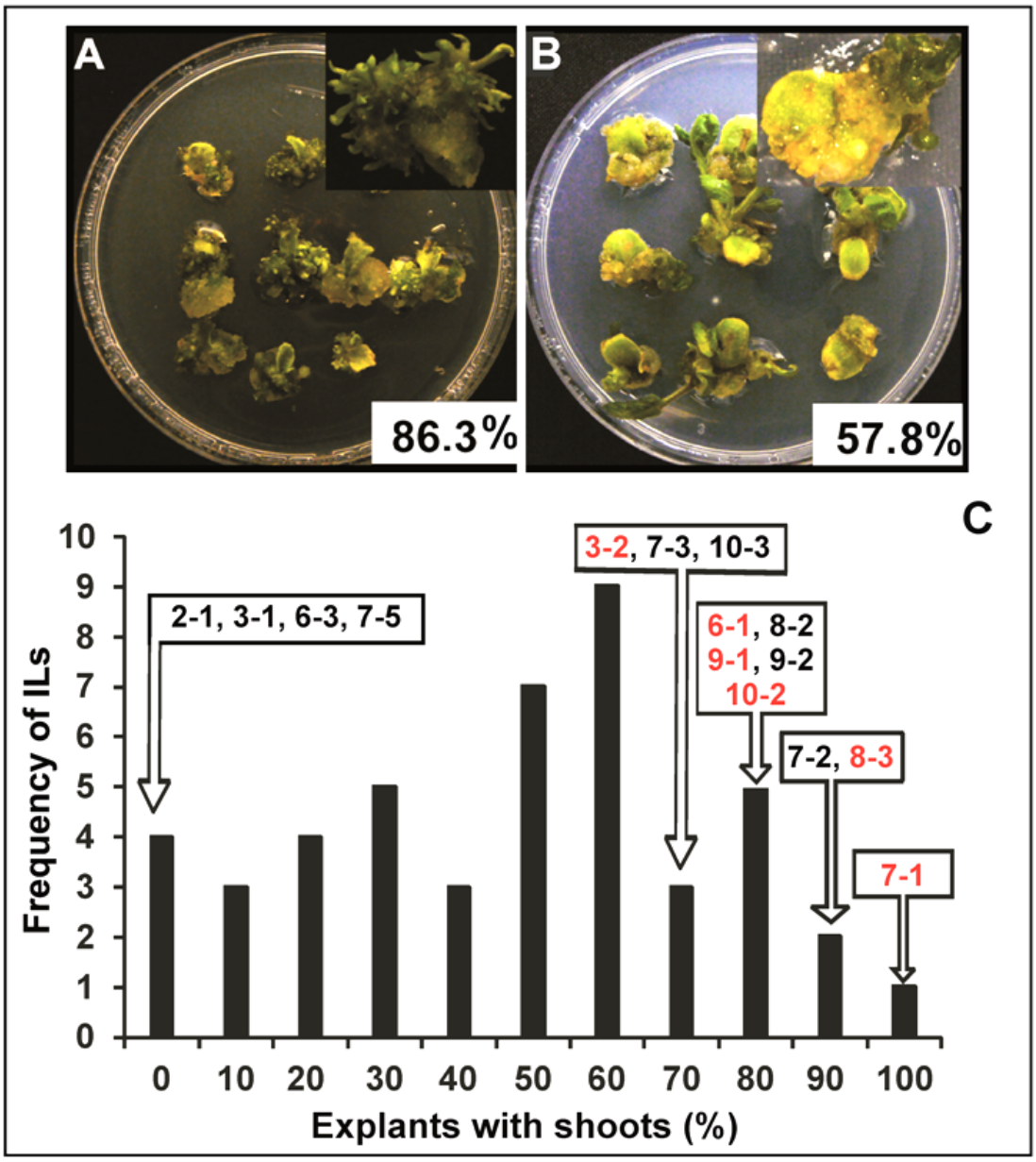

Fig. 1. Tomato chromosomal regions controlling in vitro shoot formation. (A) Shoot regeneration capacity of the wild species *S. pennellii* LA716 and the 'M82' (B). (C) Distribution of the shoot regeneration capacity of tomato introgression lines (IL) representing small chromosomal segments of LA716 introgressed into 'M82'. In A-B the inserted numbers represent the % of explants forming shoots. In C, the numbers in boxes represent IL identification denoted by the chromosome and the segment, respectively. 12-day-old (from sowing) cotyledon explants were cultivated in MS medium plus 5.0 µM BAP for 21 days.

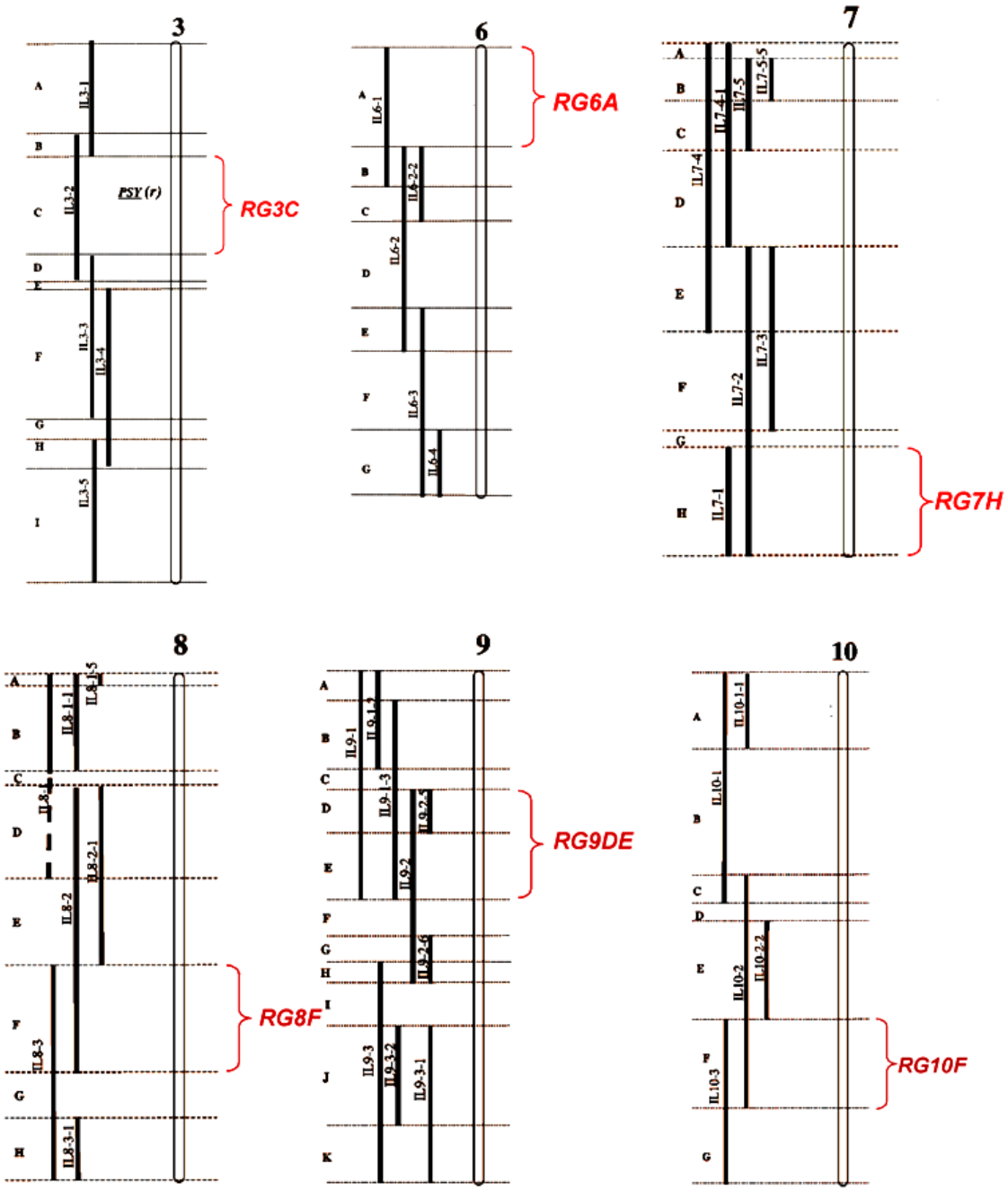

Fig. 2. Bin mapping (after Liu et al., 2003) of chromosomal segments improving shoot formation in vitro. The phytoene synthase (PSY) gene is depicted in the bin C of chromosome 3.

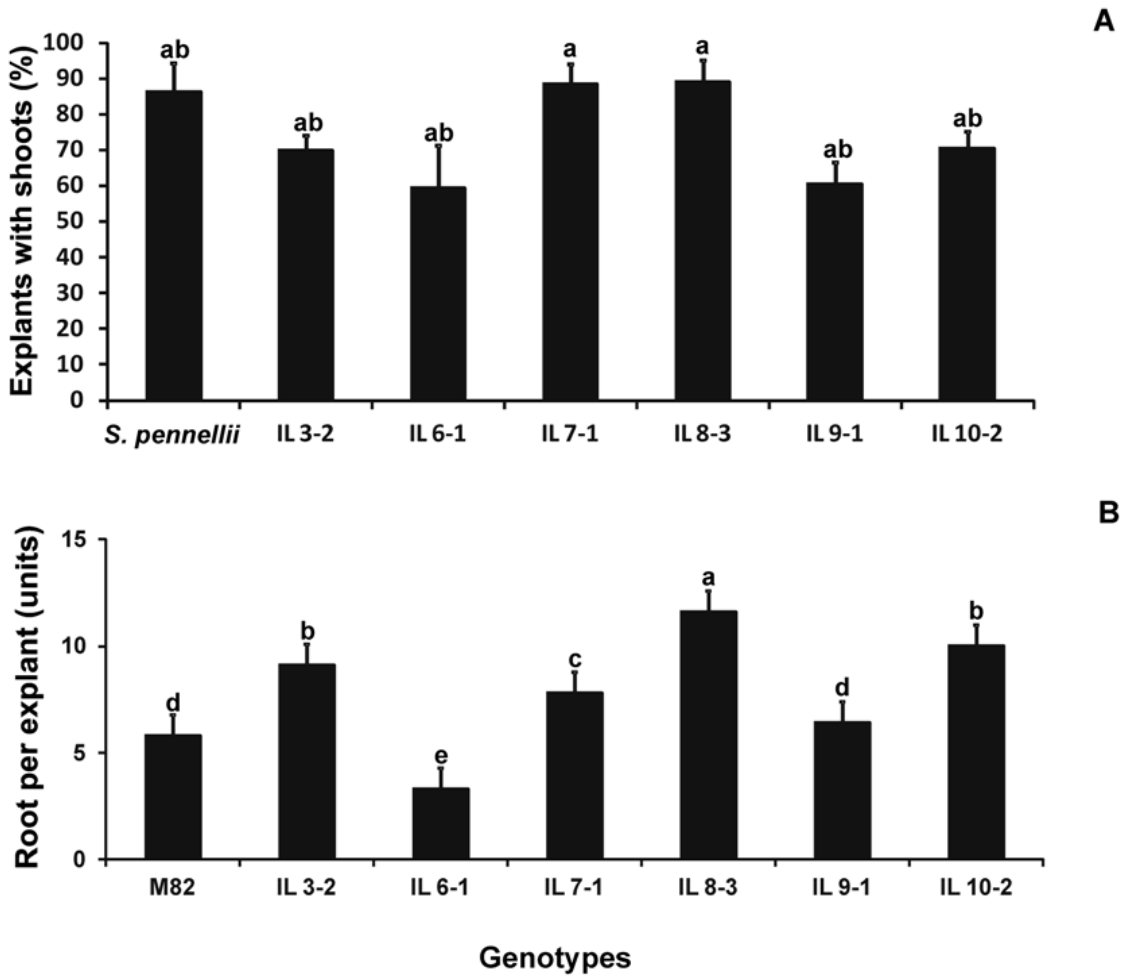

Fig. 3. Shoot and root regeneration capacity of selected introgression lines (ILs). (A) Shoot formation in cotyledonary explants from *S. pennellii* LA716 and selected ILs cultivated in SIM. (B) Root formation in cotyledonary explants from 'M82' and selected ILs cultivate in RIM. 12-day-old cotyledon explants were cultivated in MS medium plus 5.0 μM BAP (SIM) or 0.4 μM NAA (RIM) for 21 days. Different letters indicate significant differences at P≤0,05 (unpaired Student's t-test) (n=6 petri dishes each containing 15 cotyledons).