Micro-MsK: a tomato genotype with miniature size, short life cycle, and improved in vitro shoot regeneration

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Abstract

The in vitro regeneration ability of tomato (Lycopersicon esculentum Mill.) remains the main limiting factor for efficient genetic transformation. However, competence for in vitro regeneration can be transferred from wild Lycopersicon species to cultivated tomato. The high regeneration capacity presented by the MsK genotype, derived from L. peruvianum, was attributed to the dominant loci Rg-1 and Rg-2. In the present work, we have transferred the high organogenetic competence from the MsK genotype to the cultivar Micro-Tom, which presents miniature size (8-cm tall) and a rapid life cycle (75 days from seed to fruit ripening). We obtained stable and non-segregating F6 ‘Micro-MsK’ plants, which were expressively superior to Micro-Tom in regeneration capacity, when hypocotyls but not cotyledons were used as explants. In addition to the regeneration ability, the obtained genotype presented a delayed leaf senescence and a reduced apical dominance. The development of ‘Micro-MsK’ genotype will enable the application of insertional mutagenesis in functional genomics of tomato.

Keywords: Micro-Tom; Regeneration competence; Rg-1 locus

1. Introduction

Using the same rationale underlying the Arabidopsis success, i.e., small size and short life cycle, Meissner et al. [1] proposed the tomato (Lycopersicon esculentum Mill.) cultivar Micro-Tom as a model system for tomato genetics. This miniature cultivar, originally bred for home gardening purposes [2], presents a very small size (8 cm) and thus, it can be grown at high density, yielding mature fruits within 70–90 days from sowing [1,3].

Another important requisite for a plant model system is the easiness for genetic transformation. Plant transformation permits checking gene function through overexpression [4] or silencing by antisense [5]. Furthermore, easiness for transgenic production would be highly desirable in functional genomics, such as transposon and T-DNA tagging [6]. This requisite was accomplished in Arabidopsis by using a highly efficient in vitro regeneration-dependent protocol [7] or by in planta transformation methods (infiltration and floral dip), which do not require plant tissue culture and regeneration [8]. Although, in planta transformation methods are still not available for tomato, it is possible to improve its efficiency for in vitro transformation [9,10].

The in vitro regeneration ability of tomato remains the main limiting factor for efficient genetic transformation [11]. However, competence for in vitro regeneration can be transferred from wild Lycopersicon species to cultivated tomato [12,13]. The high regeneration capacity presented by the MsK genotype, derived from L. peruvianum [12], was attributed to two loci, named Rg-1 and Rg-2 [14]. Apparently, regeneration from root explants and callus is controlled by the same loci, although the behavior of some MsK lines suggested that one dominant allele at one locus can be sufficient for shoot regeneration from root explants [15]. Conversely,
for callus regeneration, dominant alleles must be present at the two loci [14]. The locus necessary for shoot formation from root explants (Rg-1) was mapped on chromosome 3 near the r locus that confers a yellow color to the fruit [15].

In this report, we have described a tomato genotype that combines the small size and rapid life cycle of the cultivar Micro-Tom with the high regeneration capacity from the MsK genotype. This genotype was allowed to inbreed for six generations and the resulting plants presented a homogeneous phenotype, with a greater regeneration ability than the conventional Micro-Tom. In addition to the regeneration ability, the obtained genotype presented a delayed leaf senescence and a reduced apical dominance that have also been discussed. The genotype presented in this work will be of great usefulness in exploring functional genomics in tomato by insertion mutagenesis [16] and will also facilitate production and maintenance of transgenic lines used in gene expression studies.

2. Material and methods

2.1. Plant material

The cultivars Micro-Tom and MsK genotype were kindly provided by Dr. A. Levy (Weizmann Institute of Science, Israel) and Dr. M. Koornneef (Wageningen Agricultural University, The Netherlands), respectively.

2.2. Breeding and cultivation

The two genotypes were crossed by conventional methods [17]. Micro-Tom was used as a female parent since it has recessive markers (dwarf gene) not present in MsK. Seeds from both genotypes were sown in boxes containing a mixture (by volume) of 1:1 commercial pot mix (Plantmax HT Eucatex, Brazil) and vermiculite, supplemented with 1 g of NPK 10:10:10 and 4 g lime/L of mixture. When the first true leaves were observed, seedlings were transplanted to 150 mL (Micro-Tom) or 10 L (MsK) pots with the same pot mix. True leaves were formed. Cotyledons were sectioned in the mid-veins. Hypocotyls and roots were sectioned to obtain 0.5–1 cm long explants. Explants were transferred to fresh MS media supplemented with 5 μM 6-benzylaminopurine (BAP) and 2% sucrose. The petri dishes were sealed with PVC film. All the cultures were maintained at 25 °C under fluorescent light (irradiance of 55 μmol m⁻² s⁻¹) at a 16 h light/8 h dark cycle. After 15–30 days, when shoot buds were visible, cultures were scored as regenerating.

2.4. Statistical analysis

The percentage of regenerated explants (cotyledons and hypocotyls) was evaluated in 150 explants (10 petri dishes with 15 explants each) for each genotype and explant type (n = 10). The number of shoots per explants was scored in 20 random chosen explants for each genotype and type of explant (n = 20). From these parameters, means and standard errors were estimated. Student's t-test was performed for means comparisons.

3. Results and discussion

3.1. The genetic basis of the Micro-Tom phenotype

The F1 plants grown from seeds collected from Micro-Tom plants had a normal size (Fig. 1). This result confirmed that the dwarfism presented by Micro-Tom is recessive [1]. Other crosses performed in our laboratory revealed that, when one of the parental genotypes used in crosses with Micro-Tom has the dwarf mutation, the resulting F1 plants continued to show the dwarf phenotype. Therefore, one of the mutations that contributed to the development of the Micro-Tom phenotype seems to be the dwarf gene. Unfortunately, the available Micro-Tom pedigree [2] does not provide information about the mutations that make this cultivar. It is interesting to note that the dwarf mutation was associated to decreased brassinosteroid synthesis [18], which suggests that Micro-Tom might have reduced levels of this hormone.

Four F1 plants were allowed to self-pollinate producing F2 seeds which were sown and grown at high density in nursery boxes. The first screening for miniature size was performed 14 days after sowing. It was observed that the hypocotyl length was a good indicator of the dwarfism in Micro-Tom. Thirty one out of 861 seedlings were selected based on hypocotyl length. These figures represent a 1:27 proportion (or a 1/28 ratio), which may be attributed to the requirement of two recessive and two dominant genes (1/4 × 1/4 × 3/4 × 3/4) to produce the Micro-Tom phenotype. As expected, about 1/4 (seven) of the 31 selected dwarf plants presented yellow fruit at maturity (Figs. 1 and 2A). The yellow fruit phenotype indicated the presence of the r allele linked to the Rg-1 locus in the chromosome 3 of tomato [15]. Seeds derived from four of these seven selected plants
Fig. 1. Breeding for regeneration improvement. The genotype MsK has a recessive yellow fruit phenotype that is linked to the Rg-1 locus. Among the genes that contribute to the Micro-Tom phenotype is the recessive mutation dwarf (d). F1 plants show a dominant phenotype which combines characteristics from the two parents (tall plants with red fruits). F2 plants were screened for dwarf size and afterwards for yellow fruit phenotype. The selected plants produced F3 seeds which were used to confirm the presence of the Rg-1 locus by means of in vitro regeneration of their root explants. The F3 generation was allowed to self-pollinate (denoted by the 'X' symbol) until the F6 generation, giving rise to a very homogeneous and non-segregating genotype named Micro-MsK.

(F3 seeds) were sown in vitro and their root explants were tested for their capacity in regenerating shoots (Fig. 2B). Although the Rg-1 allele is dominant, the presence of the recessive yellow fruit phenotype has indicated that the selected plants have been homozygous for the Rg-1 locus. One F3 r/r, Rg-1/Rg-1 plant was allowed to self-pollinate and this procedure was repeated until F6, producing a homogeneous and non-segregating genotype, which we named ‘Micro-MsK’. Limited quantities of seed from Micro-MsK are available to interested researchers.

3.2. The Micro-MsK phenotype

After we had obtained stable and non-segregating F6 plants, we observed that they presented some interesting features. Micro-MsK plants have reduced fertility, but seed production for in vitro use was not compromised. This low fertility has already been described for the MsK parent [14], and this could be attributed to the presence of deleterious genes that were dragged from L. peruvianum together with the Rg-1 locus. The proper linkage between r locus (yellow fruit) and Rg-1, both derived from L. peruvianum, may limit some applications of Micro-MsK, especially those concerning the study of carotenoid biosynthesis. All Micro-MsK plants tended to form more lateral shoots than Micro-Tom, and they also exhibited a stay-green aspect, with a delayed leaf senescence (Fig. 2A). Both reduction of apical dominance and delayed leaf senescence are features expected in
plants with alterations in their endogenous cytokinin/auxin ratio [19]. Since, a high endogenous cytokinin/auxin ratio is also related to shoot regeneration capacity [20], an important question is whether the Rg-1 locus is linked to alterations in the endogenous hormonal balance. Boiten et al. [21] measured endogenous cytokinins in tomato genotypes harboring the Rg-1 allele and concluded that this locus does not affect the cytokinin metabolism. We are currently introgressing the Rg-1 locus into the Micro-Tom cultivar to create near-isogenic lines (NILs). Such NILs will enable the test of whether the reduced senescence and apical dominance presented by 'Micro-MsK' could be attributed to the Rg-1 locus or, alternatively, to other linked loci that were derived from L. peruvianum. Also, the development of NILs could be a useful tool to re-evaluate the impact of the Rg-1 locus on the endogenous hormonal balance and sensitivity.

3.3. The high regeneration capacity of Micro-MsK hypocotyls

Cotyledon are the most used explant for tomato in vitro regeneration and transformation [9,11,10]. We tested different types of explants from Micro-MsK and Micro-Tom. No significant differences were found for a percentage of regenerating explants when comparing cotyledon explants from the two genotypes 15 days after culturing (Fig. 3A). However, the percentage of regenerating Micro-MsK hypocotyls explants was significantly (P < 0.001) higher than those from Micro-Tom (Figs. 2C and 3A). About 73% of the hypocotyls from Micro-MsK regenerated into shoots in comparison to 12% of the Micro-Tom under the same conditions (Fig. 3A). When the number of shoots formed per explant were compared, the best response was observed in the genotype Micro-MsK for both types of explants (Fig. 3B). The average shoot number formed per regenerating hypocotyls was scored to be 5–6 for Micro-MsK and 2–3 for the Micro-Tom explants (hypocotyls and cotyledons). Considering the best explant from Micro-MsK (73% of regenerating hypocotyls forming 5.5 shoots) and Micro-Tom (37% of regenerating cotyledons forming 2.5 shoots), we can conclude that the regeneration capacity of Micro-MsK could be four times (300%) higher than that of Micro-Tom. The observation that Micro-MsK genotype is superior to Micro-Tom in terms of regeneration capacity when the explants are hypocotyls but not cotyledons, may provide a hypothesis in order to understand the physiological function of the Rg-1 locus. The presence of Rg-1 allele seems to be sufficient for high regeneration from hypocotyls explants (Fig. 2C) and root explants (Fig. 2B) but not from cotyledons (Fig. 3) or callus [15]. Since hypocotyls and root explants have pre-existing meristematic cells, while cotyledons and callus do not have, the Rg-1 allele appears to act in meristems, but it may not be sufficient to enhance the induction of new meristems. The observation that the shoot formation in MsK roots is in the pericycle [22], a meristematic tissue, corroborates this hypothesis. However, since Micro-MsK is not near-isogenic to Micro-Tom, these assumptions need to be tested before confirmation. Previous experiments in our laboratory indicated that the presence of Rg-2 locus is necessary for high regeneration from cotyledon explants (unpublished results). Therefore, the Micro-MsK probably lacks the Rg-2 locus.

3.4. Concluding remarks

Tomato is a candidate plant for complete genome sequencing, as it has been recently proposed [23]. As in Arabidopsis, the available tomato sequences will demand functional genomic tools, such as insertional mutagenesis. Recently, Mathews et al. [16] developed a high-throughput T-DNA insertional mutagenesis program in Micro-Tom using activation-tagging to identify genes that regulate metabolic pathways. Micro-Tom has also been used for insertional mutagenesis based on transposon tagging [3]. Since insertional mutagenesis is highly dependent on regeneration ability, the Micro-MsK genotype that we have presented here will be useful for such applications. Although we have not demonstrated here the transformability of the Micro-MsK, this genotype is being successfully used...
in our laboratory for transformation with Agrobacterium rhizogenes and more recently with Agrobacterium tumefaciens. Finally, as Micro-Tom and Micro-MsK have a very rapid life cycle, it will be easy to introgress the Rg-1 locus, by means of six backcrosses with the recurrent Micro-Tom parental, and create near-isogenic lines. Such lines will allow to understand the Rg-1 function and certainly will gain insights on the genetic and physiological base of in vitro regeneration competence.

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References