Theor Appl Genet (1987) 75:105–108

A comparison of shoot regeneration from protoplasts and leaf discs of different genotypes of the cultivated tomato

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Received June 25, 1987; Accepted August 10, 1987
Communicated by G. Wenzel

Summary. Shoot regeneration from leaf discs and leaf mesophyll protoplasts of 11 genotypes of *Lycopersicon esculentum* (the cultivated tomato), were compared. In both regeneration procedures genotypic differences were observed between inbred lines, and also between F1 hybrids and their parental lines. In the tested hybrid genotypes no heterosis effect with respect to shoot regeneration capacity was observed. A correlation between shoot regeneration from leaf discs and from leaf mesophyll protoplasts was apparent in the tested genotypes. This suggests that using the described procedure, shoot regeneration from leaf discs can be usef for rapid pre-screening for regeneration capacity from protoplasts of tomato genotypes.

Key words: Tomato – Protoplasts – Leaf disc – Shoot regeneration – Inheritance

Introduction

Genetic manipulation of crop plants requires in most instances efficient procedures for plant regeneration from explants or protoplasts. Plant regeneration of the tomato (*Lycopersicon esculentum* Mill.) is known to be genotype dependent (Zelcer et al. 1984). Although plant regeneration from tomato protoplasts is no longer limited to a small number of genotypes, a rapid development of microcalli and shoots has only been obtained when specific preconditioning procedures were applied in combination with a culture medium, suitable for the genotype or for the type of donor tissue (Shahin 1985; Niedz et al. 1985; Tan et al. 1987). This is in contrast to shoot regeneration from leaf disc explants, which is relatively easy (Padmanabhan et al. 1974; Kartha et al. 1976; Tal et al. 1977), and less elaborate and specific than the protoplast system.

So far, no comparison has been made between shoot regeneration capacity from leaf discs and from protoplasts of the same genotypes of *L. esculentum*. The aim of this research was to study the variation in shoot regenerating capacity of eleven tomato genotypes, and to determine whether a correlation can be found between the two types of shoot regeneration and how the shoot regeneration capacity of some genotypes is inherited. This knowledge may help for the rapid selection of genotype with different shoot regenerating capacity of protoplasts.

Materials and methods

Plant material

The tomato hybrid cultivars Abunda, Bellina, their parental lines, and the cultivar Moneymaker were obtained from Rijk Zwaan (De Lier); the cultivar Lukulls (a true breeding line) was provided by Royal Sluis, (Enkhuizen) and the hybrid cultivar Bonabel and its parent lines by Nunhems Seeds (Haelen), the Netherlands.

Plant growth conditions

Seeds of 11 genotypes were germinated and grown in the greenhouse (18°–25 °C). For protoplast isolation plants were taken at the three leaf stage (5–6 weeks). They were pretreated according to the procedure as described earlier for the isolation of tomato protoplasts (Tan et al. 1987). For shoot regeneration from leaf discs, plants were grown in the greenhouse until the eleven leaf stage.

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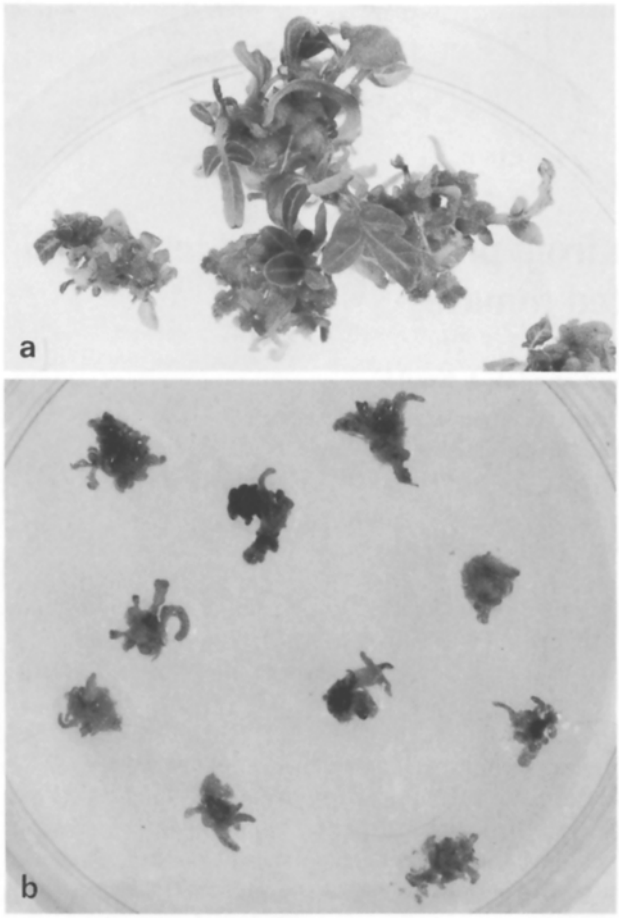

Fig. 1. Shoot formation from leaf discs (a) and from protoplast microcalli (b) of the tomato cultivar Moneymaker

Leaf explant culture

Leaf explants were taken from the 9th leaf of 16 plants of each genotype. These leaves were surface sterilized for 4 min in a solution containing 2% (w/v) hypochlorite followed by three rinses in sterile water. Leaf discs, 6 mm in diameter, were cut out with a cork borer and placed on a shoot inducing medium consisting of MS medium (Murashige and Skoog 1962), supplemented with 0.02 mg/l Indoleacetic acid (IAA), 2.0 mg/l zeatin and 2% (w/v) sucrose and solidified with 0.7% (w/v) agar. Per genotype 50–80 leaf discs were tested. The explants were cultured in 100 mm × 15 mm plastic petri dishes and maintained at 25 + /–1 °C under a 16 h photoperiod (cool-white fluorescent tubes supplying 11 W/m²). The petri dishes were placed on trays according to a randomized block design. Young shoots were counted and removed from the leaf disc when they were 0.5 cm–2.0 cm in height. At time intervals up to 10 weeks the total number of shoots per leaf disc was counted.

Protoplast isolation

In three independent experiments protoplasts of the 11 genotypes were isolated, cultured, and regenerated according to the described procedure for tomato protoplasts (Tan et al. 1987). Freshly isolated protoplasts were cultured in liquid LCM medium at a density of 1×10^5 protoplasts/ml. The plating efficiency was expressed as the percentage of the originally plated protoplasts undergoing cell division 14 days after protoplast isolation. A fraction of the resulting microcalli (approximately 100) was used for a subsequent analysis of shoot regenerating capacity. Shoot regeneration frequency was determined after four months in culture, and was defined as the percentage of shoot regenerating microcalli, which produced at least one shoot.

Results

Plant regeneration from leaf discs

Various concentrations of IAA, NAA, BA and zeatin were tested in the shoot inducing medium to determine the optimum combination for shoot induction. The best results for shoot development on leaf discs were obtained with a combination of IAA (0.02 mg/l) and zeatin (2 mg/l). The sucrose concentration was also important for shoot regeneration. One and three percent (w/v) sucrose resulted in a lower number of shoots than 2% sucrose (data not shown). In all genotypes the highest frequency of shoot formation was obtained from explants originating from the basal area of the leaf, near the veins. The first shoots were observed after 4 weeks (Fig. 1 a). When they were 1 cm–2 cm in height, they were removed. This promoted the development of other shoot primordia, possibly by reducing the apical dominance.

Effect of genotype on the regeneration capacity of leaf discs

In the 11 tested genotypes significant genotypic differences in shoot regeneration response of tomato leaf disc explants were observed (Table 1). The female parent lines of Bellina and Bonabel as well as the true breeding cultivar Moneymaker showed a higher regeneration capacity than the other tested *L. esculentum* genotypes. The male parent lines of Abunda, Bellina and Bonabel had a very low regeneration capacity. The shoot regeneration response of the F_1 hybrids Bellina and Bonabel were intermediate with respect to their parents ($P < 0.05$), whereas F_1 hybrid Abunda was not. This is illustrated for the Bellina genotypes in Fig. 2.

Effect of genotype on shoot regeneration from protoplasts

The data presented in Table 2 is based on three independent experiments. Protoplasts of all tested tomato genotypes divided at least a few times within 10 days. The percentage of protoplasts which showed sustained cell division varied between 5% and 38%, depending on the genotype. The male parents of the hybrids Bellina and Abunda, and Abunda F_1, showed the lowest rates of cell division. Protoplasts of the male

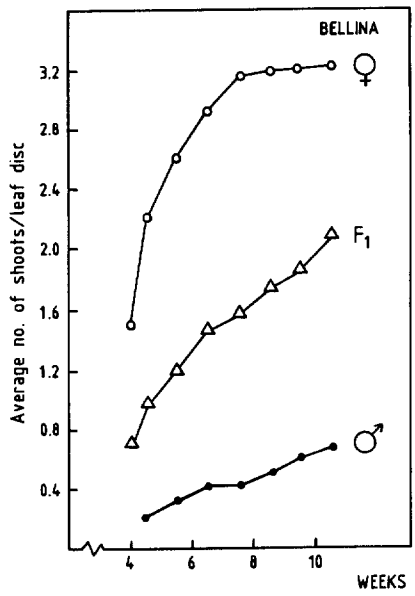

Fig. 2. Comparison of shoot forming capacity of leaf discs of the parental lines and the F_1 hybrid cultivar Bellina

parent of Bellina and Abunda only divided when plated at a relatively high density (2.10^5/ml). Regeneration of shoots from these genotypes was very poor when compared to the hybrids and their female parents (Table 2). The F_1 hybrid Bellina showed a shoot regeneration capacity which equals the shoot regeneration frequency of the female parent, suggesting a dominant or maternal inheritance in this specific combination. Figure 1 b shows an example of shoot regeneration from protoplasts of the cultivar Moneymaker.

Inheritance of shoot regeneration capacity of leaf discs and protoplasts

The shoot regeneration capacity of F_1 hybrids was not significantly higher than that of the parents. Therefore, no heterosis with respect to shoot regeneration was found in the tested genotypes, neither in leaf discs nor in protoplast-derived calli. Furthermore, the results indicate that the genotype dependent shoot regeneration in both systems is correlated. Genotypes with low level of shoot regeneration from leaf discs also showed low level of shoot regeneration from protoplasts (Fig. 3.). A significant correlation (Spearman rank correlation $\varrho = 0.78$, $P < 0.01$) was found between both types of regeneration in the tested tomato genotypes. However, there are genotypes which do not obey this general rule. For instance Bonabel ♀ and Bonabel F_1 showed a high (25%–30%) regeneration frequency from leaf discs, whereas the shoot regeneration from protoplasts was low (4% and 9%). Nevertheless in these cases protoplast regeneration did occur. On the other hand, genotypes with a high shoot regeneration frequency from protoplasts never showed a low shoot regeneration frequency from leaf discs.

Table 1. Shoot regeneration from leaf discs of eleven tomato genotypes

Genotype	No. of leaf discs	No. of shoots	No. of shoots per leaf disc after 8.5 weeks[1]
Moneymaker	67	204	3.0 ± 0.37^{f}
Lukullus	67	132	2.1 ± 0.26^{e}
Bellina ♂	61	42	0.6 ± 0.08^{b}
Bellina ♀	57	181	3.2 ± 0.24^{f}
Bellina F_1	68	128	2.1 ± 0.25^{e}
Bonabel ♂	70	82	1.2 ± 0.14^{c}
Bonabel ♀	80	204	3.0 ± 0.34^{f}
Bonabel F_1	55	134	2.5 ± 0.34^{e}
Abunda ♂	69	19	0.3 ± 0.04^{a}
Abunda ♀	72	146	1.6 ± 0.19^{d}
Abunda F_1	78	107	1.4 ± 0.16^{d}

[1] Two means which do not have the same letter differ significantly from each other ($P = 0.05$)

Table 2. Plating efficiency and % shoot regeneration from protoplasts of eleven tomato genotypes

Genotype	P.E.[1]	No. of calli tested	% shoot formation[2]
Moneymaker	35	210	27.0 ± 2.74^{d}
Lukullus	30	109	10.7 ± 1.15^{b}
Bellina ♂	8	98	3.3 ± 2.52^{b}
Bellina ♀	33	104	18.8 ± 3.99^{c}
Bellina F_1	31	113	18.4 ± 2.70^{c}
Bonabel ♂	10	93	4.5 ± 2.53^{b}
Bonabel ♀	38	146	9.3 ± 3.77^{b}
Bonabel F_1	23	112	4.0 ± 1.41^{b}
Abunda ♂	5	80	0.7 ± 0.15^{a}
Abunda ♀	14	102	4.3 ± 2.08^{b}
Abunda F_1	5	98	2.6 ± 1.15^{b}

[1] P.E. = plating efficiency (no. of dividing protoplasts/no. of plated protoplasts) × 100%

[2] % shoot formation = no. of calli which give at least one shoot/total no. of calli; two percentages which do not have the same letter differ significantly ($P = 0.05$)

Discussion

In this paper we showed that genotypic variation exists for shoot regeneration from leaf discs and protoplasts of *L. esculentum*. The variation found may indicate that several genes are involved in the regeneration process. The occurrence of genotypic differences in shoot regeneration capacity is a phenomenon which has been

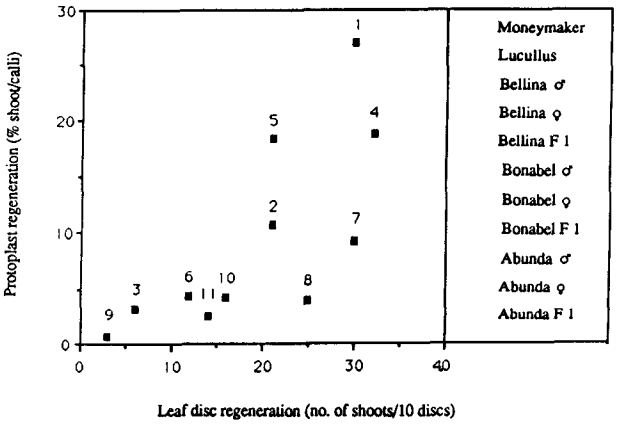

Fig. 3. Relationship between shoot regeneration from tomato leaf discs and protoplasts

described for various plant species. In tomato, such differences between genotypes have been found when hypocotyl explants were used (Zelcer et al. 1984). Although shoot-forming capacity has been found to have a high heritability ($h^2 = 0.98$; Frankenberger et al. 1981), exogenous factors (which influence the physiological state of the donor plants), as well as endogenous factors (such as hormone metabolism), play an important role in determining the final regeneration response of a given cultivar. Interactions between genotype and hormones have been observed in *Nicotiana* (Paulet and Nitsch 1963), *Brassica* (Buiatti et al. 1974) and *L. esculentum* (Behki and Lesley 1980). Ohki et al. (1978) showed for tomato that besides the genotype-hormone interactions, the type of explants and also the developmental stage of the tissue have an effect on subsequent shoot regeneration. This implies that when other types of explants or hormones are used, other responses of the tested genotypes can be expected. Considering these variables, we designed our experiments in such a way that the same type of tissue was chosen (namely leaf), from which direct or indirect (through protoplasts) shoot regeneration has been induced on a medium which contained the same auxin and cytokinin combination for both shoot regeneration systems. The results indicate that, in general, there is a significant correlation between shoot regeneration from leaf discs and protoplasts. With this method it is possible to rapidly screen many genotypes and eliminate those genotypes with a low shoot regeneration response from leaf discs, because they will most probably not be able to give a good shoot regeneration response from protoplast. In conclusion, it can be stated that the efficiency of shoot regeneration from leaf discs of the tested tomato genotypes is indicative for shoot regeneration from protoplasts.

As shoot induction from leaf disc explants is less complicated and faster to achieve than that from protoplasts, this approach offers a rapid and less laborious screening method to identify genotypes with good shoot regeneration capacity, which is an important step in the development of methods aimed at the genetic manipulation of plants.

Acknowledgements. The authors wish to thank Dr. M. Koornneef and Dr. J. J. M. Dons for their comments, and G. Pet and M. G. Nootebos for their excellent technical assistance. This work is part of the research program of the Netherlands Foundation for Biological Research (BION), and was made possible by financial support from the Netherlands Technology Foundation (STW) and from the committee for Energy Consumption of the Dutch Ministry of Agriculture and Fisheries.

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