Theor Appl Genet (1994) 88:914–916

M. Lapidot · D. Bar-Zvi · V. Kagan-Zur · Y. Mizrahi

Identification of the double genome donor in spontaneous triploid tomato plants by RFLP analysis

Received: 25 October 1993 / Accepted: 24 January 1994

Abstract Independent spontaneous triploid tomato plants (*Lycopersicon esculentum* Mill.) were collected among diploid hybrids growing in commercial greenhouses. Ploidy levels were verified by counting chromosomes, and the donor of the double genome dose was determined by restriction fragment length polymorphism (RFLP) analysis. The TG101 probe, which is tightly linked to the *Tm-2*^{a} locus, revealed different restriction patterns between TMV-resistant and TMV-susceptible parent lines. The parent donor which provided two genomes to the triploid was identified by comparing the relative intensity of alleles in the triploid with that in the diploid. The results indicate that both parents can serve as a double genome donor.

Key words *Lycopersicon esculentum* Mill. · Triploid Restriction fragment length polymorphism (RFLP)

Introduction

Although triploid plants can readily be obtained by crossing tetraploids and diploids in crops such as watermelon (*Citrulus lanatus* Thunb.) (Kihara 1951) and lettuce (*Lactuca sativa* L.) (Eenink 1980), attempts to obtain tomato triploid plants by this technique have met with failure. The stumbling block in tomato, as in other crops like alfalfa (*Medicago sativa* L.) (Veronesi et al. 1986) and potato (*Solanum tuberosum* L.) (Hanneman and Pelquin 1968), seems to be failure of normal endosperm development (Cooper and Brink 1945; Contolini and Hughes 1989) due to chromosome imbalance (Johnston et al. 1980). However, triploids have been found to arise spontaneously among diploid tomato plants, at a frequency of 1:3 000 (Mizrahi and Kagan-Zur, unpublished).

The accepted assumption is that the female parent is the double genome donor in spontaneous triploid plants. This assumption is supported by research on a number of different species (Geraci 1981; Chyi and Weeden 1984; Chen and Palmer 1985). In those cases where a degenerating fertilized egg is replaced by an endosperm cell (Muniyamma 1977), the resulting triploid plant has a double chromosome complement derived from the female parent. Triploids can also originate from the fertilization of an unreduced gamete of a diploid parent (Harlan and DeWet 1975). This mechanism is consonant with donation from either parent but, like the problematic cross between tetraploids and diploids, it may result in production of an unbalanced endosperm.

In previous studies, in which two spontaneous tomato triploids belonging to two different cultivars (FC-121, BR-54) were examined, we found, somewhat to our surprise, that the male parent was the double genome donor (Meir 1989; Kagan-Zur et al. 1991). A similar conclusion was reached by Dempsey (1961) through statistical analysis of F_1 progeny of a spontaneous tomato triploid crossed with diploid sister plants.

The purpose of the present study was to determine whether the male parent is the sole source of spontaneous triploid production in tomato plants. To this end, we examined independent spontaneous triploids collected from several different cultivars of tomato. Each of the triploids was a hybrid obtained by crossing parents differing in resistance to TMV (Tobacco Mosaic Virus). The double genome donor parent was identified using the restriction fragment length polymorphism (RFLP) marker TG101, whose DNA sequence is tightly linked to *Tm-2*^{a} (Young et al. 1988).

Communicated by M. Koornneef

M. Lapidot (✉) · D. Bar-Zvi · Y. Mizrahi
Department of Life Sciences,
Ben-Gurion University of the Negev,
P. O. Box 1025, Beer-Sheva 84110, Israel

V. Kagan-Zur · Y. Mizrahi
The Institutes for Applied Research,
Ben-Gurion University of the Negev,
P. O. Box 1025, Beer-Sheva 84110, Israel

Table 1 Verified independent triploids of commercial cultivars, along with parents' response to TMV infection. Letters A–G are codes representing the different breeding lines (+, resistance to TMV; –, susceptibility to TMV)

Cultivar	Male line	Female line	No. of triploids
FC-121	C^+	B^-	4
BR-135	F^-	D^+	4
FC-111	A^+	B^-	1
R-144	E^-	G^+	1

Table 2 Intensity of signals in autoradiograms obtained by RFLP analysis of *Eco*RV-restricted DNA of cultivar FC-121, as determined by a densitometric scan. Values represent % of each band from the total of each lane

Band size	Source	2x	3x #1	3x #2	3x #3	3x #4
a=8.5 kbp	Male	44	26	71	28	30
b=4.5 kbp	Female	56	74	29	72	70
a/b		0.8	0.4	2.4	0.4	0.4

Materials and methods

Plant material and ploidy identification

Vigorous sterile tomato (*Lycopersicon esculentum* Mill) plants suspected of being triploids were collected among four different cultivars in commercial greenhouses (Table 1). Both parent lines and the diploid hybrids of the different cultivars were grown from seeds kindly provided by N. Kedar (Faculty of Agriculture, The Hebrew University of Jerusalem). All plants were further propagated through cuttings.

Ploidy levels were verified by root-tip chromosome counting. The staining was performed with 1% aceto-orcein according to Darlington and La Cour (1962). Only plants having 3x=36 chromosomes were used in the triploid group.

DNA isolation and Southern-blot analysis

Total DNA was extracted from 1 g of fresh young leaf tissue from individual plants as described by Dellaporta et al. (1983). Approximately 30 µg of genomic DNA was digested with *Eco*RV or *Hin*dIII (U.S. Biochemicals or Promega Biotech). Each sample was fractionated on 0.7% agarose gels. Southern blotting onto GeneScreen plus (New England Nuclear), hybridization and autoradiography were as described by Sambrook et al. (1989). The TG101 probe (obtained from Dr. Zamir, The Hebrew University of Jerusalem, by permission of Prof. Tanksley, Cornell University) had been radiolabeled with [α-^{32}P] dATP (Amersham) by the random priming method (Sambrook et al. 1989). Hybridization signals were quantified from the autoradiograms with a Molecular Dynamics Personal Scanning Densitometer.

Table 3 Summary of spontaneous independent triploids of different cultivars according to the parental origin of their double chromosome dose

Cultivar	Male origin	Female origin
FC-121	1	3
BR-135	4	–
FC-111	–	1
R-144	1	–
Total	6	4

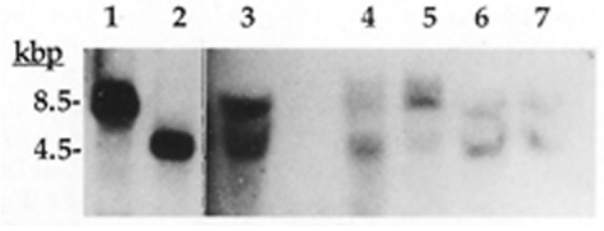

Fig. 1 An autoradiogram obtained by RFLP analysis of the FC-121 cultivar family. *Eco*RV-restricted genomic DNA of male (*lane 1*), female (*lane 2*), diploid hybrid (*lane 3*) and four triploids (*lanes 4–7*) was separated on an agarose gel, transferred to a nylon membrane and probed with radiolabelled TG101

Results

Genomic DNA isolated from both parents of each hybrid, from the diploid hybrid, and from the different independent triploids of each cultivar (verified by chromosome counts) was digested with *Eco*RV or *Hin*dIII, fractionated by electrophoresis on an agarose gel, and probed with the radiolabeled DNA clone TG101. The probe labeled a 8.5-kbp *Eco*RV fragment in the male parent of the FC-121 hybrid and a 4.5-kbp *Eco*RV fragment in the female parent of this hybrid (Fig. 1, lanes 1–2); the same fragments were labeled in both the diploid and the triploid progenies of these plants (Fig. 1, lanes 3–7). The labeling intensities of the male- and female-origin bands of these progenies relative to the total intensity for each lane were recorded. Male/female ratios were then calculated and the ratio obtained for each triploid was compared with the corresponding ratio for the diploid. In three of the FC-121 triploids examined the relative intensity of the lower-molecular-weight band was stronger, while in the fourth triploid that of the higher-molecular-weight band was stronger (Table 2).

Since *Hin*dIII produced clearer results in the case of cultivars BR-135, FC-111 and R-144, it was used in preference to *Eco*RV to digest the genomic DNA of these cultivars and of their triploid progenies and parent plants. The *Hin*dIII restriction fragments labeled by the TG101 probe in the male and female parents of BR-135, FC-111 and R-144 were respectively 4.0 kbp/8.0 kbp, 8.0 kbp/4.5 kbp, and 4.5 kbp/8.5 bkp. All triploids collected from cultivar BR-135 exhibited stronger intensity of the lower-molecular-weight band (data not shown); so did the triploid from cultivar FC-111. However, the triploid collected from cultivar R-144 displayed stronger intensity of the higher-molecular-weight band.

The parental origin of the double chromosome dose of the various triploids examined is summarized in Table 3.

Discussion

Based on the relative labeling intensities of male- and female-origin RFLP bands and corresponding male/female ratios established for each of the diploid and triploid progenies in this study, it seems that either parent – male or female – can donate a double chromosome dose to produce a triploid. In cultivar FC-121, for example, the ratio between male and female bands was 0.8 in the diploid versus 0.4 or 2.4 in the triploid progeny, indicating double dosage of the female or male genome, respectively (Table 2). This finding clearly demonstrates that, contrary to the suggestion put forward by Kagan-Zur et al. (1991), the male parent is not exclusively responsible for the emergence of spontaneous triploids in tomato plants.

While in cultivar FC-121 we found evidence that either parent can contribute to triploid formation (Fig. 1, Table 3), in cultivar BR-135 the emergence of triploids was due to male donation in all four instances studied (Table 3).

Male double donation seems to be frequent in tomato, as indicated by the present study and supported by other evidence (Dempsey 1961; Kagan-Zur et al. 1991). But most of the reports of spontaneous triploids in other species present evidence of female donation (Finch and Bennet 1979; Dwivedi et al. 1989), and the rare reports which mention the male origin of triploids indicate that such events are infrequent (e.g., in apple, Manganaris and Alston 1989).

The most common mechanism of induction of spontaneous triploids is generally thought to be fertilization of a reduced gamete by an unreduced gamete (Harlan and DeWet 1975), in which either parent may contribute the double chromosome dose. A bias has been shown towards the formation of triploids by female donation, e.g., in corn and barley (Rhoads and Dempsey 1966; Finch and Bennet 1979). Another mechanism of female donation is when an endosperm cell takes the place of a failed embryo (Muniyamma 1977). Male origination of triploid tomato plants by a true diploid pollen should cause chromosome imbalance in the endosperm (Johnston et al. 1980), ending in embryo abortion. We therefore suggested in an earlier work (Kagan-Zur et al. 1991) that male double donation probably proceeds by one of two different routes: (1) fertilization of the egg by the two generative nuclei, while the vegetative nucleus (which usually disintegrates) merges with the pre-endosperm female diploid nucleus to form a normally triploid endosperm; or (2) duplication of the haploid generative nucleus due to failure of a mitotic division just before merger with the egg and ensuing fertilization.

Since the ability to produce triploid seeds is probably a genetic trait (Geraci 1981), the incidence of tomato triploids originating from one or the other sex may be expected to differ between cultivars. Our results cannot exclude the possibility that genetic differences between tomato cultivars influence their capacity to induce triploidy via one or the other parent.

Acknowledgements The authors wish to thank the Moriah Fund for partial support of this work.

References

Chen LP, Palmer RG (1985) Cytological studies of triploids and their progeny from male-sterile (ms1) soybean. Theor Appl Genet 71:400–407

Chyi YS, Weeden NF (1984) Relative isozymes band intensities permit identification of the 2n gamete parent of triploid apple cultivars. Hort Sci 19:818–819

Contolini CS, Hughes KW (1989) Reciprocal differences in intraspecific crosses of tobacco result from embryo death. Am J Bot 76:6–13

Cooper DC, Brink RA (1945) Seed collapse following matings between diploid and tetraploid races of *Lycopersicon pimpinellifolium*. Genetics 30:376–401

Darlington CD, La Cour LF (1962) The handling of chromosomes. George Allen and Unwin Ltd., London

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Dempsey WH (1961) Origin of a spontaneous triploid. TGC Report, Part I: 10, p 11

Dwivedi NK, Suryanarayana N, Sikdar AK, Susheelamma BN, Jolly MS (1989) Cytomorphological studies in triploid mulberry evolved by diploidization of female gamete cells. Cytologia 54:13–19

Eenink AH (1980) Plant characteristics for distinction of diploids, triploids and tetraploids in lettuce. Scient Hort 12:109–115

Finch RA, Bennet MD (1979) Action of triploid inducer (tri) on meiosis in barley (*Hordeum vulgare* L.). Heredity 43:87–93

Geraci G (1981) Chromosome number in *citrus* and some aspects of embryogenesis. Genet Agr 35:165–176

Hanneman RE, Pelquin SJ (1968) Ploidy levels of progeny from diploid-tetraploid crosses in the potato. Am Potato J 45:255–261

Harlan JR, DeWet JMJ (1975) The origins of polyploidy. Bot Rev 41:361–383

Johnston SA, Den Nijs TPN, Peloquin SJ, Hanneman RE (1980) The significance of genetic balance to endosperm development in interspecific crosses. Theor Appl Genet 57:5–9

Kagan-Zur V, Mizrahi Y, Zamir D, Navot N (1991) A tomato triploid hybrid whose double genome parent is the male. J Amer Soc Hort Sci 116:342–345

Kihara H (1951) Triploid watermelons. Proc Am Soc Hort Sci 58:217–230

Manganaris AG, Alston FH (1989) Glutamate oxaloacetate transaminase isoenzymes in apple cultivars and rootstocks. J Hort Sci 64:9–15

Meir A (1989) Influence of the ploidy levels on the ripening process in tomato fruits *Lycopersicon esculentum* Mill. MSc thesis (Hebrew), Ben-Gurion University of the Negev, Beer Sheva, Israel

Muniyamma M (1977) Triploid embryos from endosperm in vivo. Ann Bot 41:1077–1079

Rhoads MW, Dempsey YE (1966) Induction of chromosome doubling at meiosis by the *elongate* gene in maize. Genetics 54:505–522

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Veronesi F, Mariani A, Bingham ET (1986) Unreduced gametes in diploid *Medicago* and their importance in alfalfa breeding. Theor Appl Genet 72:37–41

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2^a gene in tomato. Genetics 120: 579–585