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GENETIC ANALYSIS OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) TWIN FORMS

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The aim of the study was to determine the origin of additional embryos on the basis of marker characters observed in the phenotype of twin pairs of F_2 and F_3 generations of two hybrid cultivars of tomato (Alonso and Carmello). The marker characters were the presence/absence of the so-called green back, and the type of growth, which can be indeterminate or determinate. Among 112 F_2 twins, differences in marker characters were observed in 24 pairs. In the material derived from Alonso, 9 of 18 observed F_3 progenies did not show segregation in marker characters. Progenies of Carmello had higher variability; only 12 of 28 progeny groups showed no recombinants. Analysis of the F_3 generation enabled further limitation of the sources of additional embryos. Segregation of any marker character excluded the possibility that a particular F_2 twin was formed from a haploid cell of the embryo sac after spontaneous doubling of chromosome number.

Key words: Adventitious embryogenesis, apomictic polyembryony, marker gene, splitting polyembryony.

INTRODUCTION

The great demand for tomato cultivars of high productivity and fruit quality has made it necessary to develop new methods of obtaining such cultivars. Heterosis, which is the basis for breeding the cultivars obtained now, requires a great variety of homozygotic lines, the components of future cultivars. Traditional methods of obtaining the lines are laborious and time-consuming. Haploid forms of plants which, after diploidization, become fully homozygotic organisms are very important and can be attractive lines in breeding. However, there are few plant species characterized by a high frequency of haploids in natural conditions, and the tomato is not one of them. Even in culture in vitro it is very difficult to obtain haploid forms of tomato that grow into fully developed plants.

Additional embryos are believed to originate from different sources. Adventitious polyembryony is regarded as one of them, and is defined as development of an embryo from diploid cells of the nucellus and integuments. In this case the new organism is phenotypically and genotypically identical to the maternal parent. Adventitious polyembryony has been found in many varieties and species of angiosperms, such as *Capsicum annuum* (Nowaczyk, 1987). Splitting polyembryony, resulting from division of a zygote or proembryo into two independent, genotypically and phenotypically identical embryos, has been found in various plant species. Morgan and Rappleye (1950) observed it in *Capsicum frutescens*. Apomictic polyembryony is the outcome of development of a haploid embryo from an unfertilized haploid cell of the female gametophyte, such as a synergid. Morgan and Rappleye (1950) described it in *Capsicum frutescens*. Spontaneous doubling of the chromosome number and further development of the apomictic form of a diploid embryo is possible. The embryo may also be of androgenetic origin, as when a new haploid organism develops from a male gamete after degradation of the egg nucleus. The frequency of occurrence of this type of embryo is extremely low. Campos and Morgan (1958) described it in *Capsicum frutescens*, and Pochard and Vaulx (1979) in *Capsicum annuum*. The marker gene method has to some extent made it possible to determine the origin of additional embryos, or at least to limit the number of possible sources by excluding some of them in a particular situation.

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Polyembryony, the formation of additional embryos, can produce haploids in isolated cases only, and therefore cannot be successfully used in tomato, but it can be a source of diploid twin forms desirable in breeding. The frequency of twins and their fertility can be increased by treatment with growth regulators (Haccius, 1955; Nowaczyk, 1987; Nowaczyk and Nowaczyk, 1999, 2000).

This study examined whether the creation of diploid twins by the above-mentioned means can be observed in tomato. Genetic markers were used to determine the origin of additional embryos, which are useful for breeding diploid forms. The origin of additional embryos was ascertained on the basis of marker characters observed in the phenotype of F_2 and F_3 twin pairs.

MATERIALS AND METHODS

The two Dutch hybrid cultivars of tomato (*Lycopersicon esculentum* Mill.) used in the study, Alonso and Carmello, are characterized by indeterminate growth and large, fleshy fruits with so-called green back. Both features are conditioned by the dominant alleles. The plants were grown in a plastic tunnel. The seeds obtained in this cycle of culture were tested for the presence of polyembryonic forms. They were sown on filter paper moistened with water and placed in a growth chamber at 25–27°C for 14 days. They were regularly examined beginning from the third day after sowing. The germinated seeds were removed after each examination. Two- and three-embryo forms were selected and placed in Petri dishes in a growth chamber. Later they were planted in peat-filled pots and grown in a glasshouse for further observation. Each twin pair was assigned an ordinal number in the order found; the number was unrelated to the form of cultivar from which the pair originated. Well-developed twins of the F_2 generation were planted in a glasshouse 40 cm apart.

The plants were cultivated until one stem was obtained and then were severed above the second raceme. The main aim at this stage of the study was to characterize the twin pairs in respect of marker characters: determinate or indeterminate growth type, and the presence/absence of green back at the fruit base. The presence of green back can be observed in the fruits of raceme I. The growth type is determined from the number of leaves between racemes I and II. According to Tigchelaar (1986), the recessive allele is responsible for the determinate habit of growth, which results in two nodes between inflorescences as opposed to three or more in indeterminate growers. During the vegetative period, the number of flowers, number of set fruits, fruit weight and plant height were determined. The collected fruits were counted and weighed, and the seeds obtained from them were counted, weighed and bagged.

For F_3 generations, the plant pairs differing in marker characters were selected; also selected were two triplets and the pairs with considerable differences in fruit weight. From the seedlings obtained from their seeds, 12 plants representing each of the twins were selected. In two cases there were fewer than 12 plants because few seeds were obtained from the fruits of the selected twin. The planted F_3 generation plants numbered 216 for Alonso and 329 for Carmello offspring. This numerical disparity was due to differences in the numbers of twin pairs differing in marker characters, triplets, and pairs selected according to the criteria described above.

During the vegetative period the derived progeny groups were evaluated for uniformity; their growth type was determined and the presence/absence of green back was noted. To further document differences, the F_2 twins were additionally characterized by yield and mean fruit weight.

TABLE 1. Number of F_2 generation twin pairs of the Alonso and Carmello cultivars

Marker character	Cultivar	
	Alonso	Carmello
p.b. and ind.	38	28
l.b. and ind.	11	6
p.b. and det.	3	8
l.b. and det.	–	2
p.b. and ind. or det.	6	3
l.b. and ind. or det.	1	–
p.b. or l.b. and ind.	4	3
p.b. or l.b. and det.	–	–
p.b. or l.b. and ind. or det.	3	4

det. – determinate growth; ind. – indeterminate growth; p.b. – presence of green back at fruit base; l.b. – lack of green back at fruit base.

RESULTS

Of the 152 twin pairs of the F_2 generation, 120 pairs and two triplets were investigated further in a glasshouse; the rest decayed in various growth stages. All the plants were diploids.

Not all the plants are characterized here. Table 1 presents the twin pairs analyzed in terms of marker characters. In the material derived from the Alonso cultivar, 66 F_2 twin pairs were observed. Of these, 7 pairs differed in growth type; in 6 of these pairs both had green back, and in one of them they both lacked it. Four pairs differed in the presence/

TABLE 2. Characteristics of selected twin pairs of F_2 generation of Alonso cultivar

Number of twin pair	Growth type	Presence or lack of green back	Number of fruits	Total fruit weight (g)	Mean fruit weight (g)
5	ind.	l. b.	15	843	56
5'	det.	p. b.	9	523	58
25	det.	l. b.	7	693	99
25'	ind.	p. b.	12	796	66
27	ind.	l. b.	14	960	69
27'	det.	p. b.	10	791	79
28	ind.	p. b.	9	617	69
28'	ind.	l. b.	9	750	83
61	ind.	l. b.	11	1004	91
61'	ind.	p. b.	11	1026	93
62	ind.	p. b.	13	1261	97
62'	det.	p. b.	16	735	46
63	ind.	p. b.	10	786	79
63'	ind.	l. b.	15	1362	91
67	ind.	l. b.	12	1452	121
67'	ind.	p. b.	13	818	63
122	ind.	p. b.	11	1657	151
122	det.	p. b.	10	1001	100

For abbreviations see Table 1.

TABLE 3. Characteristics of selected twin pairs of F_2 generation of Carmello cultivar

Number of twin pair	Growth type	Presence or lack of green back	Number of fruits	Total fruit weight (g)	Mean fruit weight (g)
32	ind.	p. b.	9	895	99
32'	ind.	p. b.	10	1651	165
42	ind.	p. b.	11	1223	111
42'	det.	l. b.	13	965	74
49	ind.	p. b.	6	489	82
49'	ind.	p. b.	8	980	123
49"	ind.	p. b.	8	855	107
79	ind.	p. b.	11	1092	99
79'	ind.	l. b.	11	1062	97
87	ind.	l. b.	10	519	52
87'	det.	p. b.	9	527	59
90	det.	p. b.	7	323	46
90'	ind.	p. b.	10	696	70
95	ind.	p. b.	9	661	73
95'	det.	p. b.	9	439	49
98	ind.	l. b.	9	689	77
98'	ind.	p. b.	8	941	118
102	ind.	p. b.	11	1167	106
102'	ind.	p. b.	12	778	65
104	ind.	p. b.	9	439	49
104'	ind.	p. b.	8	621	78
104"	ind.	p. b.	4	249	62
112	det.	p. b.	9	1352	150
112'	det.	p. b.	10	855	86
113	ind.	l. b.	12	819	68
113'	ind.	p. b.	12	804	67
128	det.	l. b.	11	905	82
128'	ind.	p. b.	14	1721	123

For abbreviations see Table 1.

absence of green back without differing in growth type (indeterminate). Three pairs differed in the presence/absence of green back as well as in growth type.

In the material from the Carmello cultivar, 54 F_2 twin pairs were investigated. Three pairs differed in growth type while sharing the presence of green back. Another 3 pairs shared indeterminate growth and differed in the other marker character. Four twin pairs differed in growth type and the presence/absence of green back.

The rest of the investigated twins did not differ in marker characters within pairs. However, 38 pairs of the F_2 generation of the Alonso cultivar and 28 pairs of the Carmello cultivar showed marker features characteristic of the F_1 generation: the presence of green back and indeterminate growth. In the remaining twin pairs, different relations between marker characters were noted.

Tables 2 and 3 present the twin pairs selected according to the criteria described above, whose succeeding generation (F_3) was investigated in the next stage of the study. Besides data on marker characters, the yields, numbers of fruits and mean fruit weights are shown in the tables.

In the twin pairs originating from the Alonso cultivar, differences in marker characters were often accompanied by significant differences in other characteristics such as mean fruit weight. Plants nos. 62 and 67 had twice the mean fruit weight of their twin partners, whereas no. 122 had 50% higher mean fruit weight than its partner.

In the F_2 generation of the Carmello cultivar, pairs differing in mean fruit weight generally did not differ in marker characters. In plants nos. 32', 102, and 112, mean fruit weight was 50–90% higher than that in their partners. In pair nos. 128, 128' the differences in mean weight of fruit were accompanied by differences in both marker characters. Two sets of triplets, nos. 49, 49', 49" and nos. 104, 104', 104", did not show differences in marker characters; all of them had indeterminate growth and green back, but they differed in mean fruit weight.

The offspring of twin pairs differing in marker characters were analyzed further in respect of uniformity. The number of recombinants is shown in Tables 4 and 5. In the material derived from the Alonso cultivar, 9 of 18 observed F_3 progenies did not show segregation of marker characters. Among them, progenies nos. 25, 28' and nos. 5, 5' deserve

TABLE 4. Number of recombinants in F_3 progenies of selected twin pairs derived from Alonso cultivar

Number of twin pair (F_2)	Marker characters of offsprings (F_3)			
	ind. and p.b.	det. and p.b.	ind. and l.b.	det. and l.b.
5	-	-	12	-
5'	-	12	-	-
25	-	-	-	12
25'	12	-	-	-
27	-	-	12	-
27'	-	12	-	-
28	9	-	3	-
28'	-	-	12	-
61	-	-	10	2
61'	12	-	-	-
62	12	-	-	-
62'	-	9	-	3
63	11	-	1	-
63'	-	-	8	4
67	-	-	9	3
67'	3	6	1	2
122	10	1	1	-
122'	-	9	-	3

For abbreviations see Table 1.

TABLE 5. Number of recombinants in F_3 progenies of selected twin pairs derived from Carmello cultivar

Number of twin pair (F_2)	Marker characters of offsprings (F_3)			
	ind. and p.b.	det. and p.b.	ind. and l.b.	det. and l.b.
32	12	-	-	-
32'	12	-	-	-
42	8	1	3	-
42'	-	-	-	12
49	12	-	-	-
49'	12	-	-	-
49"	9	-	3	-
79	6	4	2	-
79'	-	-	12	-
87	-	-	12	-
87'	-	12	-	-
90	-	10	-	2
90'	10	-	2	-
95	10	-	2	-
95'	-	8	-	4
98	-	-	6	6
98'	10	-	2	-
102	3	3	2	4
102'	3	5	3	1
104	9	-	3	-
104'	9	-	3	-
104"	8	-	2	-
112	-	12	-	-
112'	-	12	-	-
113	-	-	3	9
113'	11	-	1	-
128	-	-	-	12
128'	12	-	-	-

For abbreviations see Table 1.

special attention: they showed no recombination in marker characters and presented exceptional phenotypic uniformity. In five progenies, nos. 61, 62', 63', 67 and 122', segregations involved a character different from the one that differentiated the parents. Progenies nos. 67' and 122 showed recombination in both growth type and the presence/absence of green back. The percentage share of progenies with recombinants was almost equal to that of progenies without recombinants.

Progenies of the Carmello cultivar had higher within-group variability and a higher percentage of recombinants. Only 12 of 28 progeny groups showed no recombinants. Of these, nos. 87, 87' additionally showed high uniformity. In progenies nos. 42 and 79, the recombinations involved both investigated marker characters. In progenies nos. 90, 90', 95, 95', 98 and 113, segregation involved a character other than the one differentiating their parents.

As mentioned earlier, the study also examined the progeny of twins not differing in marker characters and two sets of triplets derived from the Carmello cultivar. Of 6 progenies, no segregation was found in 4 groups, and nos. 112, 112' showed exceptional uniformity. In progenies nos. 102, 102', recombination involved both tested marker characters. In one of the triplet sets, nos. 49, 49', 49", segregation involved green back only, and only in one group of progeny. In the other triplet set the same character was subject to recombination in all three progeny groups.

DISCUSSION

Two pairs of marker genes determine the presence/absence of green back and determinate/indeterminate growth. The genes that determine the occurrence of green back are located on chromosome pair III. The *U* gene is dominant; it makes green back occur at the fruit base in homozygotic (*UU*) and heterozygotic (*Uu*) configurations. The lack of green back indicates that the recessive *u* gene of this character occurs in a homozygotic configuration (Tigchelaar, 1986). The other pair of marker genes is not linked with the pair described above, and the mechanism of action of this pair is explained in the same way. The *Sp* gene, determining indeterminate growth, is dominant, and the *sp* gene is recessive.

Both studied cultivars were characterized by indeterminate growth and the presence of green back. The occurrence of determinate forms without green back in the F_2 generation indicated that the genes determining these characters occurred in the parental generation. The genetic characterization of this generation is not available as the hybrid components are under patent protection, so it cannot be stated whether one of the parents had both pairs of recessive genes or each parent had only one of the pairs. Both pairs of genes must have been configured as homozygotic in the hybrid components, because the components showed complete uniformity of both characteristics.

As stated earlier, the source of additional embryos can vary. Some suggestions can be made on the basis of observations of the phenotypes and segregation of marker characters in the F_2 and F_3 generations. In the F_1 generation the integuments and nucellus had the *UuSpsp* genotype; haploid elements of the embryo sac could have various gene arrangements in gametes, that is, *Usp*, *Usp*, *uSp*, *usp*, as a result of meiosis.

In the F_2 generation, if the embryo was formed from initial cells of the nucellus or integuments as a result of adventitious embryogenesis, it would have to be of the *UuSpsp* genotype, which means that the mature plant would be characterized by indeterminate growth and its fruits would have green back. If the embryo developed apomictically from embryo sac elements having a reduced number of chromosomes, with spontaneous diploidization preceding embryogenesis, the plants would have homozygotic genotypes within all the pairs of genes. This would exclude any segregation of characters in succeeding generations. Genotypes can differ in embryos developing as a result of fertilization of the egg cell or other element of the embryo sac. From the practical point of view, the formation of additional diploid embryos as a result of diploidization of haploid elements of the embryo sac seems an attractive hypothesis. The development of spontaneous diploids has been observed in studies on experimental androgenesis in *Capsicum* spp. (Morrison et al., 1986) and in Brussel sprout (Kamiński et al., 2005).

In this study, twin pairs were examined in order to determine their origin. The conclusions can be summarized as follows:

1. When both twins had both characters predominant phenotypically, they could have originated from nucellus or integument cells, or from splitting polyembryony. Alternatively, one could have come from the fertilized egg cell, and the other from an apomictically developing element of the embryo sac after spontaneous chromosome doubling. Finally, one could have developed from a fertilized egg cell and the other from a fertilized synergid.
2. When both twins had the same recessive character while the other character was predominant, their origin from nucellus or integument cells should be ruled out. The rest of the mentioned sources are plausible.
3. The occurrence of the same recessive character in both twins while they differ in the other marker character indicates that the twins originated neither from splitting polyembryony nor from nucellus and integument cells.
4. The presence of both recessive marker characters in both twins indicates that there they could not have formed from nucellus or integument cells. Since simultaneous fertilization of an egg cell or synergid having recessive genes by sperm cells with both recessive genes is only a slight possibility, splitting polyembryony is the most probable source of those twins.
5. A difference in one marker character, with the other character being predominant, indicates that the twins did not originate from division of a fertilized egg cell. Nor could twins having a recessive character have come from nucellus and integument cells.
6. The presence of a different recessive character in each of the twins, with the other character being predominant, excludes adventitious and splitting polyembryony as well as apomictic development of the embryo from a haploid element of the embryo sac after spontaneous chromosome doubling as sources of twin formation.

Other sources of additional embryos were eliminated after analysis of the F_3 generation. Segregation of any marker character excluded the possibility that a particular F_2 twin formed from a haploid cell of the embryo sac after spontaneous doubling of the chromosome number. When segregation of one of the marker characters occurred in the F_3 generation derived from a twin with one recessive character, the latter source as well as adventitious polyembryony could be eliminated.

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