POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 23/2 (2011): 93-99

DOI: 10.2478/v10245-011-0014-5

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

The assessment of doubled haploid lines obtained in pepper (*Capsicum annuum* L.) anther culture

Dorota Olszewska, Anna Kisiała, Paweł Nowaczyk

Department of Genetics and Plant Biotechnology
University of Technology and Life Sciences in Bydgoszcz
Prof. S. Kaliskiego 7, 85-789 Bydgoszcz, Poland
e-mail: a.kisiala@utp.edu.pl

ABSTRACT

The aim of the research was the analysis of 11 DH-R_2 pepper (*Capsicum annuum* L.) lines, obtained in anther cultures of '(ATZ1 × PO)' F_1, '(ATZ1 × CDT)' F_1 and '(ATZ1 × TG)' F_1 hybrids. To determine the genetic homogeneity of anther-derived lines, the biometrical characteristics of fruits, as well as fruit colour and shape inheritance, were analysed. The biometrical analysis determined the highest phenotype uniformity in androgenic lines of '(ATZ1 × PO)' F_1. Based on the fruit shape and colour variation, it was possible to determine the microspore origin of androgenic diploids obtained in anther cultures of '(ATZ1 × TG)' F_1. Additionally, enzyme analysis of four isozymes (PGM, PGI, IDH and MDH) showed that isocitrate dehydrogenase identified anther-derived diploids of hybrids between 'ATZ1' and 'TG', as well as those between 'ATZ1' and 'CDT', lines.

Key words: androgenesis, biometrical analysis, fruit characters, isozyme markers

Abbreviations:

PGM – phosphoglucomutase
PGI – phosphoglucose isomerase
IDH – isocitrate dehydrogenase
MDH – malate dehydrogenase

INTRODUCTION

Androgenesis *in vitro* is a basic method of obtaining haploid plants and DH (doubled haploid) lines of pepper (Gémesné et al. 2009, Koleva-Godeva et al. 2009, Nowaczyk et al. 2009). Amongst pepper androgenic regenerants, diploids are most frequently the effect of spontaneous doubling of the chromosome number in haploid cells during an embryo's early developmental stages (Vagera 1990). Such fully homozygous DH lines provide valuable breeding material that does not undergo segregation in successive breeding years. Therefore, it is important to determine the origin of diploid regenerants (Munyon et al. 1989).

The genetic homogeneity of pepper anther culture-derived lines can be determined on the basis of a morphological analysis of select plant and fruit characteristics in successive generations (Vagera 1990, Nervo et al. 1995, Supena et al. 2006). A high phenotype uniformity of the plants reflects a high level of homozygosis and the gametic origin of the lines. What is more, the morphological evaluation of DH lines highlights their usefulness for pepper breeding programs and also reflects a differentiation between particular lines. This interline variability comes from a genetic diversity of microspores, which is an effect of random gene segregation in meiosis (Gémesné et al. 2001). Additionally, easily-identifiable fruit characteristics are also very useful

Table 1. The composition of isozyme staining solutions

Enzyme	0.5 M Tris/HCl (ml) pH – 8.0	H_2O (ml)	Substrate (units)	$MgCl_2$ (µl)	NAD (µl)	NAD(P) (µl)	PMS (µl)	MTT (µl)	G6PDH (µl)
PGI	1.7	3.25	1	100	-	100	100	100	0.5
PGM	1.7	3.10	1	100	-	100	100	100	1.0
IDH	1.7	2.80	1	100	-	100	100	100	
MDH	1.7	1.30	2	-	100	-	100	100	

Substrates used in isozyme staining solutions:
– fructose-6-phosphate (PGI)
– glucose-1-phosphate (PGM)
– isocitric acid (IDH)
– sodium malate (MDH)

for the confirmation of microspore origins of pepper androgenic plants, conditioned by single, usually recessive genes, e.g. fruit colour and shape (Smith 1950, Pochard 1977).

Defining genetic homogeneity within plants of particular DH lines and the polymorphism between different lines is also possible using DNA molecular analysis, as well as isozyme markers (Gyulai et al. 2000, Gémesné et al. 2001). Morrison et al. (1986) applied phosphoglucomutase and shikimate dehydrogenase to confirm the androgenic origin of diploid androgenic plants, Munyon et al. (1989) analysed stripe patterns of shikimate dehydrogenase, malate dehydrogenase, phosphoglucomutase and phosphoglucose isomerase, while Dolcet-Sanjuan et al. (1997) used sixteen isozymes, of which isocitrate dehydrogenase turned out to be useful.

The aim of the present paper was the morphological and isozyme analysis of pepper (*C. annuum* L.) DH-R2 lines, obtained in anther cultures of '(ATZ1 × PO)' F_1, '(ATZ1 × CDT)' F_1 and '(ATZ1 × TG)' F_1 hybrids.

MATERIAL AND METHODS

Eleven R_2 generations of the regenerants obtained in anther cultures of '(ATZ1 × PO)' F_1 – 'AP1', 'AP2', 'AP3', 'AP4', 'AP18', '(ATZ1 × CDT)' F_1 – 'AC5', 'AC7', 'AC9', 'AC14' and '(ATZ1 × TG)' F_1 – 'AT4', 'AT6' were used in the study. R_2 generations were derived from seeds obtained as a result of self-pollination of androgenic R_1 regenerants. R_2 plants were grown in plastic tents, following the agrotechnical guidelines for pepper cultivation in Poland.

The biometrical analysis involved 10 plants of each of the tested DH-R_2 generations. Fruit number and total fruit yield per plant were determined and three fruits from each plant were analysed biometrically. The following fruit characters were evaluated according to the principles given in 'Descriptors for *Capsicum* (*Capsicum* ssp.)' (IPGRI 1995): weight of the fruit with stalk, fruit length and width, pericarp weight, pericarp thickness, wet seed weight, number of seeds per fruit, dry weight of pericarp and thousand seed weight. Since the parental forms of F_1 hybrids used as anther-donor plants differed in the fruit shape and colour, these traits were additionally used to help in confirming the microspore origin of the regenerants. The results of the biometrical analyses of plants were statistically verified. The uniformity of each line was defined with the analysis of variance (ANOVA/MANOVA). Standard deviation (SD) and the coefficient of variation (CV) were also determined for the evaluated traits.

The analysed R_2 regenerants were also tested with isozyme markers. The patterns of four enzymes – phosphoglucose isomerase, phosphoglucomutase, isocitrate dehydrogenase and malate dehydrogenase – were studied after isozyme separation by starch gel electrophoresis. Young leaves were macerated in a 100 µl extraction buffer (1.21 g Tris, 0.037 g Na_2EDTA, 0.075 g KCl, 0.203 g $MgCl_2$, 1 ml Triton, 100 ml H_2O; pH – 7.5), with 10 ml of 2-Mercaptoethanol added. Filter paper stripes were soaked in the homogenate and used to load a 14% starch gel with a histidine buffer (10.334 g L-histidines, 1000 ml H_2O; pH – 6.5). The electrophoresis was performed in an electrode buffer (15.14 g Tris, 8.325 g citric acid, 1000 ml H_2O) for 3.5 hours at 200 V. The gels were stained for proteins at 35°C with dying solutions and 5 ml of hot agar (1.5%). The composition of staining mixtures for respective isozymes is given in Table 1.

RESULTS AND DISCUSSION

The use of anther cultures in plant breeding is possible only when a satisfactory efficiency of the

Table 2. Yield and fruit number in R_2 generation of androgenic lines, obtained in anther cultures of pepper hybrids '(ATZ1 × PO)' F_1, '(ATZ1 × CDT)' F_1 and '(ATZ1 × TG)' F_1

Line		Yield (g)	Number of fruit per plant
AP1	mean	608	4
	SD	303	2.59
	CV	49.9	61.3
AP2	mean	873	6
	SD	180	1.92
	CV	20.6	30.2
AP3	mean	984	9
	SD	283	2.45
	CV	28.8	26.2
AP4	mean	922	10
	SD	306	5.16
	CV	33.2	51.6
AP18	mean	1064	10
	SD	323	3.68
	CV	30.4	36.8
AC5	mean	663	7
	SD	251	3.69
	CV	37.9	52.7
AC7	mean	795	7
	SD	255	2.76
	CV	32.1	42.4
AC9	mean	724	6
	SD	301	2.20
	CV	41.6	35.5
AC14	mean	604	6
	SD	192	1.89
	CV	31.8	32.1
AT4	mean	1143	12
	SD	312	2.66
	CV	27.3	22.53
AT6	mean	909	14
	SD	174	4.76
	CV	19.2	34.2

process is attained, and then only if large numbers of doubled haploids lines are produced, which are a valuable source for further breeding processes and molecular studies (Gémesné et al. 2009, Koleva-Godeva et al. 2009). Vagera (1990), Nervo et al. (1995) and Supena et al. (2006) present a biometrical analysis of androgenic pepper lines as one of the methods of verifying the microspore origin of the regenerants. The high phenotype uniformity of the R_2 generation obtained as a result of self-pollination of anther culture-derived diploids reflects their genetic homogeneity. The yielding and fruit number of each of the R_2 generations evaluated in this study are given in Table 2. The researched lines differed considerably in these two traits, and the high values of the coefficient of variation (CV) suggest that the yield and the number of fruit per plant depend significantly on the environmental conditions. The analysis of variance of fruit traits showed that the most phenotype-homogenous were two lines obtained in '(ATZ1 × PO)' F_1 anther culture. Plants of the 'AP1' line did not show significant differences for eight of the tested parameters, while the 'AP2' line was uniform for six of ten analysed fruit traits (Tab. 3). Similarly, the 'AC5' line, derived from '(ATZ1 × CDT)' F_1 microspores, was also morphologically uniform and no significant differences were recorded for five fruit traits (Tab. 4). It is worth mentioning that of all of the analysed plant traits, the most homogenous was weight of wet seeds per fruit – it was quite uniform in the case of six of the 11 tested androgenic lines, while the least useful for phenotype uniformity assessment was dry matter content. The lack of high phenotypic homogeneity was observed especially for two of the lines – 'AT4' and 'AT6' (Tab. 5). This may be the result of environmental variation, which may often modify plant morphology. It suggests that although the importance of biometrical characteristics of anther-derived lines is of great value, as it allows for an objective evaluation of the essential functional characters of plants, which is necessary for the selection of the most interesting materials for breeding programs, the biometrical analysis cannot be the main method for androgenic regenerant microspore origin evaluation. However, especially the total fruit yield, the weight and size of the fruit, as well as the pericarp thickness, appear to be the most important plant characters that should not be omitted while DH lines are being evaluated.

The trait that is not affected by the modifying effect of the environment, and thus can be applied to mark the origin of androgenic pepper plants, is the fruit colour of the regenerants. As for the *Capsicum* genus, this trait is inherited following the principle of full dominance (Smith 1950). F_1 hybrids between red-fruited 'ATZ1' (y^+/y^+, cl^+/cl^+) and yellow-fruited 'PO' and 'TG' lines (y/y, cl^+/cl^+), always result in red fruit (y^+/y, cl^+/cl^+). And so, anther culture-derived diploids with yellow fruit must be formed as a result of the spontaneous diploidisation of haploid embryos, which developed

Table 3. Fruit characteristics of the R_2 generation of androgenic lines, obtained in the anther culture of the '(ATZ1 × PO)' F_1 pepper hybrid

Line		Weight of fruit with stalk (g)	Fruit length (mm)	Fruit width (mm)	Weight of placenta with seeds (g)	Pericarp weight (g)	Pericarp thickness (mm)	Weight of wet seeds (g)	Number of seeds per fruit	Dry matter (%)	Weight of 1000 seeds (g)
	mean	170	111	73.3*	47.7	140	5.32	4.22	262	8.22*	8.52
AP1	SD	31.1	10.7	9.22	11.0	25.3	0.88	1.31	77.9	1.20	1.04
	CV	18.3	9.71	12.6	23.5	18.1	16.5	31.0	29.7	14.7	12.2
	mean	155	117*	69.3	36.0*	135	5.85	2.85	204	7.82*	7.41*
AP2	SD	31.4	12.7	6.62	9.62	27.0	0.92	1.13	83.1	1.51	0.69
	CV	20.2	10.9	9.56	26.7	19.9	15.7	39.7	40.6	19.3	9.34
	mean	128*	110*	66.2*	33.2*	106*	5.32*	4.04*	239*	7.88*	9.48*
AP3	SD	20.5	10.4	5.94	6.82	17.2	0.73	1.25	82.9	1.60	1.12
	CV	16.1	9.49	8.98	20.5	16.2	13.6	31.0	34.7	20.4	11.8
	mean	113*	104*	63.6*	30.6*	93.1*	5.02*	3.90*	224*	8.56*	9.69*
AP4	SD	26.8	15.9	6.83	7.14	23.0	0.82	1.37	83.3	0.73	1.23
	CV	23.8	15.3	10.7	23.4	24.8	16.3	35.1	37.3	8.51	12.7
	mean	126*	108*	68.6*	34.3*	104*	5.27*	4.51	274*	8.61*	8.95*
AP18	SD	14.8	10.0	8.98	4.73	12.9	0.89	1.12	77.6	0.90	0.84
	CV	11.7	9.25	13.1	13.8	12.4	16.9	24.9	28.3	10.4	9.37

*Differences between 10 plants of the given DH line were significant at $\alpha = 0.05$

Table 4. Fruit characteristics of the R_2 generation of androgenic lines, obtained in the anther culture of the '(ATZ1 × CDT)' F_1 pepper hybrid

Line		Weight of fruit with stalk (g)	Fruit length (mm)	Fruit width (mm)	Weight of placenta with seeds (g)	Pericarp weight (g)	Pericarp thickness (mm)	Weight of wet seeds (g)	Number of seeds per fruit	Dry matter (%)	Weight of 1000 seeds (g)
	Mean	114*	138	57.2*	27.6	96.6*	4.86*	3.06	215	7.68*	7.57
AC 5	SD	30.3	15.4	4.80	7.08	28.1	0.64	0.66	55.4	1.80	1.59
	CV	26.7	11.1	8.38	25.7	29.1	13.2	21.7	25.7	23.5	21.1
	Mean	146*	142*	62.0*	33.4	125*	4.79*	3.17	219	8.35*	8.54*
AC 7	SD	36.8	18.5	7.08	10.4	31.1	0.87	1.30	98.0	0.91	1.20
	CV	25.2	13.0	11.4	31.1	24.8	18.3	41.0	44.8	10.9	14.0
	Mean	129*	158*	60.3*	30.2*	110*	4.05*	3.34	223*	8.21*	8.65*
AC 9	SD	26.5	23.2	6.38	8.39	22.0	0.67	1.11	80.9	0.92	1.38
	CV	20.5	14.7	10.6	27.8	19.9	16.5	33.3	36.4	11.2	16.0
	Mean	126*	189*	57.7*	27.0*	109*	4.06*	2.93*	232*	8.29*	6.52*
AC 14	SD	30.3	29.5	7.28	7.06	27.3	0.55	0.94	81.0	1.21	1.52
	CV	24.2	15.6	12.6	26.1	25.0	13.5	32.1	35.0	14.6	23.3

*Explanations: see Table 3

from the microspore with recessive gene *y*. Such plants are then spontaneous diploids, fully homozygous. Moreover, as determined by Pochard (1977), the round-like fruit shape, characteristic for the 'TG' line, is conditioned by dominant allele o^+, while the elongated fruit shape, typical for the 'ATZ1' line, is determined by the recessive form of the gene. F_1 hybrids result in round fruit (o^+/o), whereas segregation occurs in the F_2 generation. Since the parental forms of F_1 hybrids used in this study as anther donor plants differed in fruit colour and shape, these traits were applied as an additional indicator confirming the microspore origin of androgenic regenerants (Figs 1 a-c). There were

Table 5. Fruit characteristics of the R_2 generation of androgenic lines, obtained in the anther culture of the '(ATZ1 × TG)' F_1 pepper hybrid

Line		Weight of fruit with stalk (g)	Fruit length (mm)	Fruit width (mm)	Weight of placenta with seeds (g)	Pericarp weight (g)	Pericarp thickness (mm)	Weight of wet seeds (g)	Number of seeds per fruit	Dry matter (%)	Weight of 1000 seeds (g)
AT4	Mean	118*	58.8*	81.5*	39.5*	94.9*	6.89	4.38*	275*	8.15*	8.95*
	SD	19.3	6.64	4.80	7.32	15.9	0.80	1.17	75.7	1.34	0.69
	CV	16.4	11.3	5.89	18.5	16.8	11.6	26.6	27.6	16.4	7.72
AT6	Mean	85.2*	108*	54.8*	21.2*	71.7*	5.03*	2.25*	182*	8.28*	7.07*
	SD	14.7	12.0	4.66	5.03	12.1	0.60	0.75	52.7	1.10	0.82
	CV	17.3	11.1	8.50	23.7	16.9	11.9	33.5	29.0	13.3	11.6

*Explanations: see Table 3

two yellow-fruited ones amongst the 11 evaluated lines, both derived from anther cultures of '(ATZ1 × TG)' F_1. One of them ('AT6') produced fruit similar in shape to those of the 'ATZ1' line, whereas the second one ('AT4') was more similar to the other hybrid parental form, the 'TG' line (Figs 1 d and e). These results indicate that the 'AT4' as well as the 'AT6' lines developed from the '(ATZ1 × TG)' F_1 haploid microspore, containing recessive alleles.

Additionally, isozyme electrophoresis was used to determine the origin of the analysed androgenic lines derived from F_1 hybrids. The analysis performed for four isozyme patterns demonstrated that parental genotypes 'ATZ1' and 'TG' are homozygous for isocitrate dehydrogenase (one band on the zymograms), and hybrids between these lines are heterozygous – their IDH profile consists of three bands (Fig. 2, lanes 1, 2, 5-8). Because the IDH isozyme profile of 'CDT' line was heterozygous (Fig. 2, lane 3), for a clear interpretation of the zymograms only heterozygous plants of '(ATZ1 × CDT)' F_1 were selected as donor-plants for androgenesis. Thus, this isozyme was found to be useful in defining the genetic uniformity of the progeny of diploid plants obtained in anther cultures of two hybrids, '(ATZ1 × TG)' F_1 (Fig. 2, lanes 13-16) and '(ATZ1 × CDT)' F_1 (Fig. 2, lanes 17-20), as they were homogenous and presented

Figure 1. Fruits of parental forms 'ATZ1' (a) and 'TG' (b) of the hybrid '(ATZ1 × TG)' F_1 (c) and two lines obtained in the '(ATZ1 × TG)' F_1 anther culture: 'AT6' (d) and 'AT4' (e)

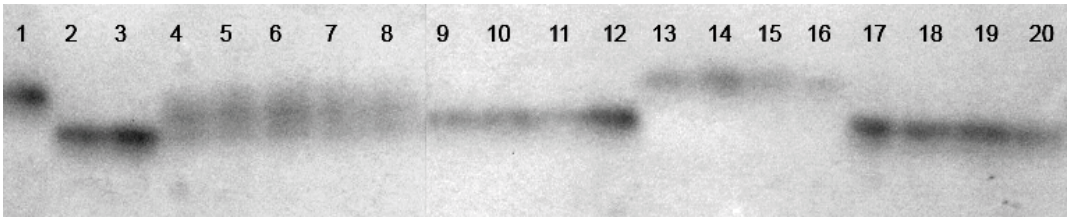

Figure 2. Isocitrate dehydrogenase zymograms from leaf extracts: lane 1 – 'TG', lane 2 – 'ATZ1', lane 3 – 'PO', lane 4 – 'CDT'; lanes 5-8 – '(ATZ1 × TG)' F_1, lanes 9-12 – '(ATZ1 PO)' F_1, lanes 13-20 – doubled haploid lines obtained in anther cultures: 13-16 '(ATZ1 × TG)' F_1 ('AT4'), 17-20 '(ATZ1 × CDT)' F_1 ('AC7')

only one of the bands of their heterozygous donor plants. Isocitrate dehydrogenase was also reported to be effective by Dolcet-Sanjuan et al. (1997). Considering the obtained results, it seems fully justifiable to use biometrical analysis, including fruit colour and shape as the marker traits, in further studies to confirm the microspore origin of the regenerants, especially in the case of anther-derived plants of the hybrid between the red-fruited 'ATZ1' line and the yellow-fruited 'PO' line, since their origin failed to be evaluated using the izozymes researched in this study.

CONCLUSIONS

1. The methods applied in this study are useful for determining the microspore origin of anther culture-derived diploids of *C. annuum* hybrids grown in Poland.
2. The biometrical analysis of R_2 regenerants showed a high phenotype uniformity of three lines obtained in anther cultures of '(ATZ1 × PO)' F_1 and '(ATZ1 × CDT)' F_1 hybrids.
3. Plant marker traits – fruit shape and colour – helped to identify the spontaneously doubled haploids derived from the '(ATZ1 × TG)' F_1 anther culture.
4. Isocitrate dehydrogenase zymograms were useful in confirming the microspore origin of anther-derived diploids of the '(ATZ1 × TG)' F_1 and '(ATZ1 × CDT)' F_1 hybrids.

ACKNOWLEDGEMENTS

The authors are grateful to MSc K. Łączkowska from Kutnowska Hodowla Buraka Cukrowego in Straszków for her assistance in pepper isozyme analysis.

REFERENCES

Dolcet-Sanjuan R., Claveria E., Huerta A., 1997. Androgenesis in *Capsicum annuum* L.: effect of carbohydrate and carbon dioxide enrichment. J. Amer. Soc. Hort. Sci. 122: 468-475.

Gémesné J.A., Kristóf Z., Vági P., Lantos C., Pauk J., 2009. In vitro anther and isolated microspore culture as tools in sweet and spice pepper breeding. Acta Hort. 829: 61-64.

Gémesné J.A., Petus M., Gyulai G., Cséplö M., 2001. Genetic variability of anther donor versus spontaneous doubled haploid descendents and colchicine induced doubled haploid sweet pepper (*Capsicum annuum* L.) lines. Acta Hort. 560: 149-152.

Gyulai G., Gémesné J.A., Sági Z.S., Venczel G., Pintér P., Kristóf Z., Törjék O., Heszky I., Bottka S., Kiss J., Zatykó L., 2000. Doubled haploid development and PCR-analysis of F_1 hybrid derived DH-R_2 paprika (*Capsicum annuum* L.) lines. J. Plant. Physiol. 156: 168-174.

IPGRI, 1995. Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy.

Koleva-Gudeva L., Trajkova F., Dimeska G., Spasenoski M., 2009. Androgenesis efficiency in anther culture of pepper (*Capsicum annuum* L.) Acta Hort. 830: 183-190.

Morrison R.A., Koning R.E., Evans D.A., 1986. Anther culture of an interspecific hybrid of *Capsicum*. J. Plant Physiol. 126: 1-9.

Munyon I.P., Hubstenberger J.F., Phillips C., 1989. Origin of plantlets and callus obtained from chile pepper anther cultures. In Vitro Cell Dev. Biol. 25(3): 293-296.

Nervo G., Ferrari V., Caporali E., 1995. Evaluation of anther culture derived plants of pepper. Proc. IXth EUCARPIA Meeting on Genetics and Breeding of *Capsicum* & Eggplant, 1995 – Budapest, Hungary: 72-75.

Nowaczyk P., Olszewska D., Kisiała A., 2009. Individual reaction of *Capsicum* F_2 hybrid genotypes in anther cultures. Euphytica 168: 225-233.

Pochard E., 1977. Localization of genes in *Capsicum annuum* L. by trisomic analysis. Ann. Amelior. Plantes 27: 255-266.

Smith P.G., 1950. Inheritance of brown and green mature fruit color in peppers. J. Heredity 41: 138-140.

Supena E.D.J., Suharsono S., Jacobsen J., Custers J.B.M., 2006. Successful development of a shed-

microspore culture protocol for doubled haploid production in Indonesian hot pepper (*Capsicum annuum* L.). Plant Cell Rep. 25(1): 1-10.

Vagera, J., 1990. Pepper (*Capsicum* spp.): *In vitro* induction of haploids. In: Biotechnology in agriculture and forestry. Haploids in crop improvement I. Y.P.S. Bajaj (ed.), Springer-Verlag, Berlin, 12: 374-392.

OCENA LINII PODWOJONYCH HAPLOIDÓW OTRZYMANYCH W KULTURACH PYLNIKÓW PAPRYKI (*CAPSICUM ANNUUM* L.)

Streszczenie: Celem badań była charakterystyka pokolenia R_2 jedenastu linii DH papryki (*Capsicum annuum* L.) otrzymanych w kulturach pylników mieszańców: '(ATZ1 × PO)' F_1, '(ATZ1 × CDT)' F_1 i '(ATZ1 × TG)' F_1. Jednorodność genetyczną roślin określono wykorzystując metodę oceny biometrycznej cech użytkowych oraz analizę dziedziczenia barwy i kształtu owoców. Charakterystyka biometryczna wykazała najwyższe wyrównanie fenotypowe w obrębie linii otrzymanych w kulturach pylników mieszańca '(ATZ1 × PO)' F_1. Na podstawie barwy i kształtu owoców możliwe było potwierdzenie mikrosporowego pochodzenia androgenicznych diploidów mieszańca '(ATZ1 × TG)' F_1. Przeprowadzono również analizę izoenzymatyczną, która wykazała, że dehydrogenaza izocytrynianowa (IDH) może być zastosowana do identyfikacji linii DH, pochodzących z mieszańców między liniami 'ATZ1' i 'TG' oraz 'ATZ1' i 'CDT'.

Received March 15, 2010; accepted November 10, 2011