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Morphological character of plants and fruits quality in cucumber after recurrent regeneration in *in vitro* culture

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ABSTRACT

Borszczagowski line (B), destined to field cultivation and its line derived in the course of recurrent regenerations in *in vitro* culture (BRR) were used in the study. Cucumber plants were grown at 30 × 120 cm, thirty plants on each plot. During vegetation, length measurements of shoots were made and female and male flowers were counted. Fruits were collected several times. Total and marketable yields and fruits quality were estimated. The results of three-year study indicate that cucumber plants regenerated *in vitro* show variability on a molecular level comparing to Borszczagowski line. Multiple regeneration *in vitro* significantly affected both permanent variability of developmental and field parameters and physical and chemical traits of line (BRR) cucumber fruits.

INTRODUCTION

Methods of micro propagation are used to obtain planting material from various plant species.

Many plant species exhibit changes in quality after regeneration *in vitro* (Karp 1991). Differences may be detected in morphological traits (Lyrene 1981, Cassels and Plunkett 1984, Grout et al. 1986) and general morphological nonhomogeneity (Malepszy et al. 1990). In the case of cucumber a regeneration system via somatic embryogenesis from leaf callus is known (Malepszy 1988) as well as direct regeneration from leaf fragments (Burza and Malepszy 1995). After several regeneration cycles from callus via somatic embryogenesis, cucumber (*Cucumis sativus* L.) showed genetically based morphological changes (Nadolska-Orczyk et al. 1989, Pląder et al. 1998).

The aim of the present study was to demonstrate somaclonal variability between plants obtained from monoecious field cultivar Borszczagowski after recurrent regenerations in *in vitro* culture line (BRR), and Borszczagowski line (B) in respect to morphological and physical characters as well as yielding and fruit quality.

MATERIAL AND METHODS

Borszczagowski line (B) destined to field cultivation and its line derived in the course of recurrent regenerations in *in vitro* culture (BRR) were used in the study. The investigation was conducted in 2001 – 2003 on an experimental field of the Department of Vegetable and Medicinal Plants, Faculty of Horticulture and Landscape Architecture in Wilanów. The plants were grown on deep medium-heavy alluvial soil with a high share of silt and the content of organic matter ranging from 1.5 to 2.5%. Each year the rate of particular macro elements was complemented to N – 150 mg dm^{-3}, P – 75 mg dm^{-3}, K – 250 mg dm^{-3}, based on chemical soil analysis performed beforehand.

The experiments were set in a one-factor split-plots with four replicates. The transplants of both lines were obtained from seeds, in the case that line (BRR) after regeneration via somatic embryogenesis from leaf callus repeated four times (according to Malepszy 1998). Plants were planted on May 30, 29 and 28 in consecutive years respectively. Cucumber plants were grown at 30 × 120 cm, thirty plants on each plot. Pest management was applied with accordance to the recommendations of Vegetable Plants Management Program elaborated by the Research Institute of Vegetable Crops in Skierniewice. Climatic conditions, prevailing during the study are shown in Table 1. During vegetation, length measurements of shoots were made and female and male flowers were counted. Fruits were collected several times. Total and marketable yields were estimated.

Table 1. Average temperatures and sum of rainfall for the period May-August in years 2001–2003

Months	10 days	Temperatures (°C) Average for 10 day period			Long term average	Rainfall (mm) Sum for 10 day period			Long term average
		2001	2002	2003		2001	2002	2003	
	1	18.1	18.9	16.1		1.8	0.0	0.8	
May	2	15.6	16.5	13.7	13.7	20.3	9.7	40.7	58.1
	3	13.6	18.0	18.2		17.6	68.5	11.0	
	1	14.5	16.4	20.4		3.8	28.3	1.0	
June	2	15.8	19.4	18.3	16.9	12.1	26.2	19.0	67.8
	3	17.8	18.7	17.6		17.8	4.3	22.5	
	1	20.5	21.3	18.9		36.2	34.9	16.9	
July	2	22.1	22.2	21.2	18.1	44.2	7.6	24.4	66.5
	3	21.5	20.8	23.3		68.2	0.1	71.4	
	1	19.8	21.3	21.8		17.6	64.8	27.1	
August	2	21.6	20.7	20.0	17.6	0.3	29.5	24.9	65.7
	3	18.8	20.7	18.0		17.2	0.8	32.5	
	1	14.7	19.1	13.8		17.2	0.0	20.9	
September	2	13.2	12.3	15.9	13.5	31.9	18.4	18.4	43.1
	3	10.0	10.1	14.6		24.1	12.2	27.9	

The following fruit quality ratios were determined:

- Skin and flash firmness were tested with „Inston" 4303 aparatus. Firmness was defined as the force necessary to break the skin and flash of a fruit with 8 mm diameter cylindrical probe. The results were expressed in Newtons (N).
- Dry matter was determined at 104°C and expressed as percentage share.
- Sugars content was measured with Luffa-Schoorl method. The results are given in g 100 g^{-1}.
- Simple sugars content was determined using HPLC method. The results are given in g 100 g^{-1}.
- Pectins content was determined according to IFU n# 23 method. The results are expressed as the content of galacturonic acid in mg 100 g^{-1}.
- Vitamin C content was determined using a titration method. The results are given in mg of vitamin C per 100 g of fresh matter.
- Cucurbitacins content was determined using a spectrophotometer UV-VIS, at 210 nm wavelength and with model cucurbitacin E solutions. The results are given in mg g^{-1}.
- Potassium and calcium contents were defined using a flame atomic absorption spectrophotometer.
- Phosphorus content was defined with a spectrophotometer at a green filter of 460 nm.

- Magnesium content was defined using a spectrophotometeric method at 540 nm wavelenght.
- Total nitrogen was determined with Kiejdahl method. The results are expressed as the content of protein in %.

Statistical analysis was elaborated using one-way analysis of variance. Differences between variability sources were investigated with Fisher-Snedecor test at $\alpha = 0.05$ significance level. Means were compared of Tukey's test at the probability of P = 95.

RESULTS AND DISCUSSION

The plants obtained after long-term in vitro regeneration may demonstrate some temporal or permanent adverse properties. The phenomenon of variability among regenerated plants had been named somaclonal variability (Larkin and Scowcroft 1981). Somaclonal variability of a durable nature is genetically determined.

Three-year results of the present study indicate that cucumber plants obtained after recurrent in vitro regenerations exhibited some genetically determined somaclonal variability. Plants of BRR line showed higher number of both female and male flowers as compared to Borszczagowski line. Earlier studies of Malepszy et al. (1990), Burza et al. (1996), Ładyżyński et al. (2001) show that plants from seeds after regeneration via somatic embryogenesis from leaf callus repeated four times gave lower yield as compared to Borszczagowski line. The present investigation did not confirm the above results. Total and marketable yields of the plants after recurrent regeneration in *in vitro* culture were significantly higher for line (BRR) as compared to Borszczagowski line (Table 2).

Table 2. Yield and growing parameters of cucumber Borszczagowski line (B) and Borszczagowski line after recurrent regeneration *in vitro* (BRR)

Years of cultivation	Line	Length of sprouts (cm)	Number of female flowers (No. plant^{-1})	Number of male flowers (No. plant^{-1})	Total yield (kg m^{-2})	Marketable yield (kg m^{-2})
2001	B	190 a*	8 b	71 a	7.26 b	4.26 b
	BBR	191 a	10 a	65 b	9.78 a	5.96 a
2002	B	189 a	8 b	74 a	8.36 b	4.13 b
	BBR	190 a	11 a	68 b	10.78 a	6.56 a
2003	B	192 a	9 b	72 a	8.14 b	5.09 b
	BBR	192 a	12 a	64 b	10.45 a	6.11 a
Mean	B	190 a	8 b	72 a	7.92 b	4.49 b
	BBR	191 a	11 a	65 b	10.36 a	6.21 a

*values designated with the same letters within column do not differ significantly at $\alpha = 0.05$

Shape, firmness of flash and skin as well as chemical content are important features of cucumber fruit quality. These are however traits that changed after multiple *in vitro* regeneration. The observation of somaclonal variability among cucumber plants allowed to state that fruits of the regenerated line (BRR) showed both lower firmness skin and flash as compared to Borszczagowski line (Table 3). Regeneration of the plants *in vitro* did not affect the content of dry matter or total sugars in the fruits of the studied objects. On the other hand, higher contents of glucose and fructose were obtained in fruits of line (BRR) in relation to Borszczagowski line (Table 4). In the fruits of the regenerated plants, somaclonal variability in term of the content of vitamin C and pectins was also observed (Table 4).

Table 3. Physical features of cucumber fruit of Borszczagowski line (B) and Borszczagowski line after recurrent regeneration *in vitro* (BBR)

Years of cultivation	Line	Firmness of skin (N)	Firmness of flash (N)
2001	B	86.94 a	55.18 a
	BRR	81.03 b	50.76 b
2002	B	88.45 a	58.29 a
	BRR	79.23 b	51.06 b
2003	B	83.47 b	54.29 a
	BRR	82.13 b	49.13 b
Mean	B	86.28 a	55.92 a
	BRR	80.79 b	50.31 b

*values designated with the same letters within column do not differ significantly at $\alpha = 0.05$

Table 4. Content of chemical composition in cucumber fruit of Borszczagowski line (B) and Borszczagowski line after recurrent regeneration *in vitro* (BRR)

Years of cultivation	Line	Dry Matter (%)	Total sugars (%)	Glucose (%)	Fructose (%)	Vitamin C (mg 100 g^{-1})	Pectin (mg 100 g^{-1})
2001	B	4.06 a	1.81 a	0.62 b	0.77 b	10.49 a	98.2 b
	BRR	4.26 a	1.90 a	0.73 a	0.83 a	8.73 b	104.5 a
2002	B	3.99 a	1.68 a	0.58 b	0.75 b	11.12 a	92.3 b
	BRR	4.01 a	1.75 a	0.70 a	0.89 a	8.01 b	106.8 a
2003	B	4.12 a	1.82 a	0.63 b	0.78 b	10.59 a	96.5 b
	BRR	4.21 a	1.76 a	0.72 a	0.82 a	8.25 b	107.2 a
Mean	B	4.05 a	1.77 a	0.61 b	0.76 b	10.73 a	95.7 b
	BRR	4.16 a	1.80 a	0.71 a	0.84 a	8.33 b	106.2 a

*values designated with the same letters within column do not differ significantly at $\alpha = 0.05$

Fruits of the line (BRR) obtained after recurrent *in vitro* regeneration showed significantly higher content of phosphorus and calcium but lower content of potassium as compared to Borszczagowski line. The content of magnesium in these fruits was at similar level irrespectively the object studied (Table 5). Somaclonal variability in a plant, after recurrent *in vitro* regeneration concerned also the

content of cucurbitacin and the total protein in fruits. Lower rate of cucurbitacin content was observed both in skin and flash of line (BRR) fruits comparing to fruits of Borszczagowski line (Table 6). The content of protein in the fruits was lower in line (BRR) as compared to Borszczagowski line (Table 6).

Table 5. Content of chosen macro-components in cucumber fruit of Borszczagowski line (B) and Borszczagowski line after recurrent regeneration in vitro (BRR)

Years of cultivation	Line	Phosphor (mg 100 g^{-1})	Potassium (mg 100 g^{-1})	Calcium (mg 100 g^{-1})	Magnesium (mg 100 g^{-1})
2001	B	21 b	187 a	32 b	8 a
	BRR	25 a	150 b	38 a	9 a
2002	B	20 b	183 a	30 b	9 a
	BRR	26 a	148 b	35 a	8 a
2003	B	20 b	185 a	31 b	10 a
	BRR	25 a	149 b	37 a	9 a
Mean	B	20 b	185 a	31 b	9 a
	BRR	25 a	149 b	36 a	8 a

*values designated with the same letters within column do not differ significantly at $\alpha = 0.05$

Table 6. Content of cucurbitacin and protein in cucumber fruit of Borszczagowski line (B) and Borszczagowski line after recurrent regeneration *in vitro* (BRR)

Years of cultivation	Line	Cucurbitacin of skin (mg g^{-1})	Cucurbitacin of flash (mg g^{-1})	Protein (% N)
2001	B	26.1 a	16.8 a	0.35 a
	BRR	21.3 b	14.5 b	0.31 b
2002	B	24.8 a	17.4 a	0.36 a
	BRR	20.9 b	15.2 b	0.30 b
2003	B	25.9 a	19.1 a	0.34 a
	BRR	21.4 b	15.8 b	0.32 b
Mean	B	25.6 a	17.7 a	0.35 a
	BRR	21.2 b	15.2 b	0.31 b

*values designated with the same letters within column do not differ significantly at $\alpha = 0.05$

CONCLUSIONS

- Three-year results of the study indicate that cucumber plants after four times regeneration *in vitro* show variability of quality and quantity traits on a molecular level comparing to Borszczagowski line.
- Multiple regeneration *in vitro* significantly affected both permanent variability of developmental and field parameters, as well as the physical and chemical traits of line (BRR) cucumber fruits.

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REFERENCES

BURZA W., MALEPSZY S., 1995. Direct plant regeneration from leaf explants in cucumber (*Cucumis sativus* L.) is free of stable genetic variation. Plant Breeding 114: 341-345.

BURZA W., MALEPSZY S., ROSTEK E., 1996. The effect of simple and recurrent *in vitro* regeneration on a cucumber inbred line under field cultivation. Hort Sci. 28(1-2): 11-13.

CASSELS A.C., PLUNKETT A., 1984. Production and growth analysis of plant from leaf cuttings in African violet (*Saintpaulia ionanta* Wendl.). Sci. Hort. 23: 361-369.

GROUT J.M., READ P.E., WILDUNG D.K., 1986. Influence of tissue culture and leaf bud propagation, growth habit of Northblue blueberry J. Amer. Soc. Hort. Sci. 111: 372-375.

KARP A., 1991. On the current understanding of somaclonal variation. Oxford Surveys of Plant Molecular and Cell Biology 7: 1-58.

LARKIN P.J., SCOWCROFT W.R., 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.

LYRENE P.M., 1981. Juvenility and production of fast rooting cuttings from blueberry shoot cultures. J. Amer. Soc. Hort. Sci. 106: 396-398.

ŁADYŻYŃSKI M., KORZENIEWSKA A., MALEPSZY S., 2001. Recurrent Regeneration through Somatic Embryogenesis Reduces Yield in Cucumber. HortSci. 36(5): 987.

MALEPSZY S., 1988. Cucumber (*Cucumis sativus* L). In: Y.P.S. Bajaj (ed.). Biotechnology in Agriculture and Foresty 6. Crop II. Springer-Verlag, Berlin: 277-293.

MALEPSZY S., MARCINIAK S., NADOLSKA-ORCZYK A., 1990. *In vitro* culture of *Cucumis sativus* L. IX Yielding of regenerated plants. Gartenbauwissenschaft 55(5): 2006-2008.

NADOLSKA-ORCZYK A., MALEPSZY S., BEŁZ S., 1989. Effect of recurrent *in vitro* culture on somaclonal variation. XIIth Eucarpia Congress in Goettingen, Vortrage fur Pflanzenzuchtung 15-II: 26-1.

PLĄDER W., MALEPSZY S., BURZA W., RUSINOWSKI Z., 1998. The relationship between the regeneration system and genetic variability in the cucumber (*Cucumis sativus* L.). Euphytica 103: 9-15.

WPŁYW WIELOKROTNEJ REGENERACJI W KULTURACH *IN VITRO* NA PARAMETRY ROZWOJOWE ROŚLIN I JAKOŚĆ OWOCÓW OGÓRKA LINII BORSZCZAGOWSKI

Streszczenie: Do badań wzięto homozygotyczną linię Borszczagowski (B) przeznaczoną do uprawy w polu i wyprowadzoną z niej linię (BRR) uzyskaną po wielokrotnej regeneracji w kulturach *in vitro*. Ogórki uprawiano w rozstawie 30 × 120 cm. Na jednym poletku rosło 30 roślin. Podczas wegetacji wykonano pomiary długości pędów, liczono kwiaty męskie i żeńskie. Dokonano kilkakrotnych zbiorów owoców. Określono plon ogólny i handlowy, dokonano oceny jakości owoców. Trzyletnie wyniki badań wskazują, że zregenerowane rośliny ogórka w kulturach *in vitro* charakteryzuje zmienność cech na poziomie genetycznym w porównaniu do linii Borszczagowski. Wielokrotna regeneracja w kulturach *in vitro* wpłynęła istotnie zarówno na trwałą zmienność w parametrach rozwojowych i plonowaniu roślin jak również na cechy fizyczne i chemiczne owoców ogórka linii (BRR).

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