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In vitro culture of *Cucumis sativus* L.

7. Genes controlling plant regeneration

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Summary. The ability to regenerate plants from leaf explants has been tested for three highly inbred cucumber lines (B, G, S), their reciprocal hybrids, F_2 and BC_1 generations. The lines differed from each other in their regenerating ability, which was expressed by the percentage of explants regenerating embryoidal callus and mean number of plantlets per plant. Thus, the lines could be classified as frequently (B), intermediately (G) or occasionally regenerating ones (S). There were no reciprocal cross differences in the regeneration. It was found that the intermediately and intensively regenerating lines contain two pairs of dominant genes responsible for plant regeneration, characterized by complementary and probably additive interaction. The frequently regenerating line differed from the intermediately regenerating in the effect of one gene. It is supposed that the above-mentioned genes belong to three different loci. The ability to regenerate plants from leaf explants had high heritability.

Key words: Genetic control of regeneration – *Cucumis sativus*

Introduction

The effect of genotypes on the results of culture is observable in both monocotyledons and dicotyledons. It was proven that a genotype could control various stages of in vitro culture, namely: anther callus development (Miah et al. 1985; Rines 1983), intensive callus growth (Sharma et al. 1980), induction of callus (Nesticky et al. 1983; Tomes and Smith 1985) embryoid development (Brown and Atanassov 1985), plant regeneration ability (Sharma et al. 1980; Abe and Futsufara 1984; Kurtz and Lineberger 1983) and plant regeneration in long-term culture (Malmberg 1979; Locky 1983).

It was discovered for *Medicago sativa* that as a result of cyclic selection, genes responsible for plant regeneration were cumulated. This caused a considerable increase in the number of regenerated plants (Bingham et al. 1975). Due to gene complementation and segregation, more advanced developmental stages of embryoids can be obtained in anther culture of potato (Jacobsen and Sopory 1978). In a number of studies, the cultivar differences were analyzed by comparing hybrids with their parents (Ohki et al. 1978; Frankenberger et al. 1981; Charmet and Bernard 1984; Nesticky et al. 1983; Tomes and Smith 1985). Only a few papers have proposed in detail the mode of inheritance of regeneration ability using F_1, F_2 and BC_1 generations (Reisch and Bingham 1980; Broda 1984; Koornneef et al. 1987).

It was shown that cucumber lines and varieties differ from one another in their plant regeneration ability using hypocotyls and cotyledons (Wehner and Locy 1981) and leaf explants as well (Malepszy and Nadolska-Orczyk 1983). This paper contains analysis of inheritance of plant regeneration ability. On the basis of the observation of F_1, F_2 and BC_1 generations, we suggest the mode of interaction among genes controlling this trait.

Materials and methods

Three highly inbred lines (I_{11}), their reciprocal hybrids and F_2 generation were used for the experiments. The parents used in the crosses were: S – obtained from Skierniewicki female, parthenocarpic greenhouse variety, B – developed from Borszcza-

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** To whom correspondence should be addressed gowski field monoecious variety and G – weekly female line GY-3 from our department collection, which was given to us by Dr. J. Peterson, Madison, Wisconsin, USA in 1974. The choice of lines was made after testing a considerable number of varieties and lines.

The growing conditions of donor plants and conditions of in vitro culture were described in a previous paper (Nadolska-Orczyk and Malepszy 1984). Explants (0.5 x 0.5 cm) were collected from the first two seedling leaves without margins. Culture medium was based on Murashige and Skoog salts and vitamins (1962) enriched with: 0.8 mg/l 2,4-D and 2iP, 30 g/l sucrose, 250 mg/l peptone from lactalbumin L/ELG and 6 g/l agar. The medium used for plantlet regeneration was without growth regulators.

The number of plants and explants from each plant is presented in Table 1. The explants gave the callus lines, which were evaluated with regard to the ability to develop embryoidal callus with embryoidal structures. This was expresed by the percentage of explants developing this callus per one plant.

The plants in F_2 and BC_1 generations were classified into three groups (classes) for each genotype screened: class I – frequently formed, class II – intermediately and class III – occasionally formed embryoidal callus (Tables 3–5). The second analyzed trait was the mean number of plantlets developed between the 8th and 14th week of culture from ten explants (callus line) per one plant. Using this trait, plants were divided into two classes: class I - frequently and class II – intermediately developed plantlets (Table 6). The results of experiments presented in Tables 2–4 were obtained at different times.

Inheritance coefficients in a wide sense were calculated according to Simmonds (1981). The conformity of the segregations observed with the expected ones was verified by the $\varkappa^2$ test for significance level 0.05.

Results

The regeneration system from leaf explants of cucumber

The regeneration system can be divided into three stages. The first stage consists in development of compact primary callus, from which characteristic gel-like callus is formed. Such primary callus is initiated from meristematic cells surrounding the vascular bundles (Nadolska-Orczyk and Malepszy 1987). In the second stage, gel-like callus is transformed into embryoidal callus, from which embryoidal structures are developed. Embryoidal structures developed into plantlets (stage three) on medium without growth regulators (Nadolska-Orczyk and Malepszy 1984). Plant development can be observed between the 8th and the 14th week of culture.

Ability to develop embryoidal callus

Cucumber inbred lines differed in their ability to develop embryoidal callus on the same medium. Line B was always the best responding, G showed intermediate and S showed the least response or was not responsive (forming this type of callus not more often than one callus line from one plant – Tables 2, 3 and 4). F_1 hybrids obtained after crossing B × S or G × S showed lower ability to develop embryoidal callus than B or G, respectively. In the F_1 generation, the crossing direction had no effect on this trait (Table 2).

Table 1. The number of plants and explants taken for the analysis of percentage of explants developing embryoidal callus in three experiments

Combination	No. of plants	No. of explants from each plant
Experiment I[a]		
Parents	6 plants per parent	12
F_1 hybrids	6 plants per hybrid	12
Experiment II[a]		
Parents	60 plants per parent	25
F_2 generations	60 plants per F_2 generation	25
Experiment III[a]		
Parents	20 plants per parent	12
BC_1	20 plants per BC_1 generation	12

[a] Experiments I, II, III, were performed in the same culture conditions but at different times

Table 2. The ability of inbred lines and their F_1 hybrids to develop embryoidal callus and to regenerate plantlets (Experiment 1)

Lines and their hybrids	% of explants developing embryoidalcallus	No. of plantlets per plant	
		Range	Mean
B	75	316–1,514	916
G	68	193– 617	394
S	0	0	0
B × G	72.5	107–1,408	542
G × B	74	263–1,059	577
S × G	5	55– 204	193
G × S	10	48– 55	184
S × B	7.5	57– 169	94
B × S	no seedling seed		

The three classes of ability to develop embryoidal callus in F_2 and BC_1 generations were distinguished according to the characteristic ranges for each of the three parents (Tables 3 and 4). Segregation of the trait in the $F_{2\,(B \times G)}$ generation approximated the theoretical ratio of 9:6:1. This suggests that the investigated trait is inherited by two dominant genes that show complementary interaction. Additionally, it was noticed that two segregants exceeded the parents' values. The more intensive callus formation in these cases can be caused by an addition effect of dominant alleles. In $F_{2\,(S \times B)}$ and $F_{2\,(S \times G)}$ generations, the ratio observed is very close to the theoretical ratio of 9:7, which indicates the complementary interaction between two dominant genes.

On the basis of the results obtained, it is possible to propose the following parental genotypes: (1) line B – R_1 R_1 R_2 R_2, assuming the complementary interaction of genes: (2) line G – $R_1 R_1 R_3 R_3$, assuming the complementary interaction between R_1 and R_3. The interaction of R_1 and R_3 alleles gives a lower callus formation effect than the interaction of R_1 and R_2 alleles. For the above combinations the additive effect of dominant alleles probably occurred: (3) line S – $r_1 r_1 r_2 r_2 r_3 r_3$. The absence of dominant genes causes the lack of development of embryoidal callus or only occasional callus formation. On the basis of the above-presented genotypes, it is possible to conclude that regeneration depends on the presence of at least two different dominant alleles, namely R_1 and R_2 or R_3. Genotypes obtained in the F_2 and BC_1 generations were classified into the three classes:

class I – $R_1 R_1 R_2 R_2 R_3 R_3$; $R_1 R_1 R_2 R_2 R_3 -$; $R_1 R_1 R_2 - R_3 R_3$; $R_1 R_1 R_2 R_2 - -$ and $R_1 R_1 R_2 - R_3 -$;

class II – $R_1 R_1 - - R_3 R_3$; $R_1 R_1 R_2 - - -$; $R_1 R_1 - - R_3 -$; $R_1 - R_2 R_2 - -$; $R_1 - - - R_3 R_3$; $R_1 - R_2 - - -$; $R_1 - - - R_3 -$;

class III – $R_1 R_1 - - - -$; $- - R_2 R_2 - -$; $R_1 - - - - -$; $- - - - R_3 -$ (Tables 3–6)

The deviation of observed ratio from the theoretical one is not significant for $\varkappa^2_{0.05}$ so there is no reason to reject the model of inheritance presented above (Table 5). The broad sense heritabilities estimated from this study range from 0.77 for B × G to 0.43 for S × G crosses.

Table 3. The percentage of explants developing embryoidal callus in parental plants and F_2 generations (Experiment II)

Combination	% of explants developing embryoidal callus		Classes (%)
	Range	Mean	
B	34–81	62	
G	9–49	26	I >41.5
S	0– 5	0.7	
$F_{2(B \times G)}$	7–93	44.7	II 6–41
$F_{2(S \times B)}$	0–63	15.2	
$F_{2(S \times G)}$	0–41	9.4	III 0– 5

Table 4. The percentage of explants developing embryoidal callus in parental plants and BC_1 generations (Experiment III)

Combination	% of explants developing embryoidal callus		Classes (%)
	Range	Mean	
G	0–67	24.2	II>6
S	0	0	
(S × G) × G	0–30	8.9	III 0–5
(S × G) × S	0–25	4	

Number of regenerated plantlets per plant

The genotypes which developed a high percentage of explants with embryoidal callus also appeared to develop the greatest number of plantlets per plant. Boundary values of mean number of plantlets in classes were established similarly as for the previous trait (Table 6). A comparison of data indicates that in the F_1 generation the number of regenerated plantlets was usually a mean of two parental forms (Table 2). The cross direction had no effect on those values. The observed values appeared to be very close to the expected ones (Table 6). This confirms that the number of regenerated plants is controlled by the same genes as those responsible for the ability to develop embryoidal callus or that the genes responsible for both traits are strongly linked.

Table 5. Observed and expected segregation ratios in F_2 and BC_1 generations of the ability to develop embryoidal callus (in three classes)

Combinations	No. of plants in classes						χ^2	*P*
	Observed			Expected				
	I	II	III	I	II	III		
$F_{2(B \times G)}$	36	21	3	37.5	18.75	3.75	0.16	0.9–0.8
	(a)		(24)			(22.5)	(0.42)	(0.7–0.8)
$F_{2(S \times B)}$	5	31	24	3.75	30	26.25	0.64	0.8–0.7
$F_{2(S \times G)}$		29	31		33.75	26.25	1.63	0.3–0.2
(S × G) × G		13	7		20	0	(b)	
(S × G) × S		12	28		10	30	0.53	0.5–0.3

(a) After the summing up of II and III classes

(b) Chi-square undefined due to division by zero

Table 6. Observed and expected segregation ratios of the mean number of plantlets per plant in frequently regenerating (I) and intermediately regenerating (II) classes (mean from ten explants per one plant)

Combinations	Range	Mean	No. of plants in classes			
			Expected		Observed	
			I	II	I	II
Experiment II						
B	181–370	274	–	–	–	–
G	88–135	109	–	–	–	–
S	0–21	2	–	–	–	–
$F_{2(B \times G)}$	102–441	219	10	5	10	6
$F_{2(S \times B)}$	23–327	106.6	1	8	1	8
$F_{2(S \times G)}$	41–224	106.7	0	9	3	7
Experiment III						
G	21–313	138	–	–	–	–
S	0	0	–	–	–	–
(S × G) × G	9–394	152	0	1	1	5
(S × G) × S	31–273	132.5	0	4	0	6

Discussion

Among various factors influencing in vitro response, genotype is one of the most important. Many articles reveal the influence of genotype on the basis of diallel analysis (Ohki et al. 1978; Frankenberger et al. 1981; Charmet and Bernard 1984; Nesticky et al. 1983; Tomes and Smith 1985). In most of them, the authors found predominantly additive gene action for callus and plant development. As presented in this article, plant regeneration from cucumber leaf explants was controlled by complementary interaction of dominant genes and probably additive allele interaction. Because of the different method of analysis and different units used to quantify culture response, it is difficult to compare accurately the results from these various studies with the results obtained in this work. There are only a few papers presenting complete models of inheritance using F_1, F_2 and BC_1 generations (Reisch and Bingham 1980; Broda 1984; Koornneef et al. 1987).

In one of the experiments described by Broda (1984), segregation ratios were determined on the basis of three phenotypes, easy to identify qualitiatively: (1) lack of callus formation, (2) callus growth without regeneration, (3) callus growth and regeneration. After detailed analyses of parents. F_1, F_2 and BC_1 generations, it was concluded that plant regeneration from hypocotyl and ovary-derived callus was phenotypically expressed by triple recessive homozygotes, that model, proposed for *Trifolium pratense* L., is considerably different from the one presented in this paper. Another paper (Reisch and Bingham 1980) presents the type of inheritance of bud formation from callus cultures of *Medicago sativa*. In this work segregation of phenotypes expressed by a percentage of regenerated buds was made according to variability classes. Similar to the experiments with cucumber, classes were determined on the basis of parental phenotypes. There are two points in which these two inheritance models are similar, namely: (1) a dominant gene is necessary to obtain regeneration and one of the alleles more intensively controls regeneration: (2) regeneration is the most frequent in the presence of two different dominant genes. In the article of Koornneef et al. (1987), the genetics of regeneration and callus growth from leaf explants have been studied in selfed and backcross progenies of selected hybrid plants of *Lycopersicon peruvianum* and *L. esculentum*. Similar to the case of cucumber, regeneration ability from leaf explants was controlled by two dominant genes from *L. peruvianum* and there was no cytoplasmic inheritance of investigated traits. A similar effect of gene interaction was described for cucumber sex inheritance by Kubicki (1965). According to his research, line B contains ffMM sex genes, line G – FFmm and line S – FFMM. Some researchers pointed to a certain interdependence between genes responsible for these traits (Litz and Conover 1983). Nevertheless, comparison between the results presented by Kubicki (1965) and in this paper indicates an independent inheritance of sex and regeneration ability.

Foroughi-Wehr et al. (1982) and Lazar et al. (1984) concluded that two characters – the frequency of anthers giving callus or embryos and the frequency of calli which gave green plants – were separately inherited. No correlation was observed between plant regeneration and callus growth rate from immature embryos of numerous *Zea mays* genotypes (Duncan et al. 1985) and from leaf explants of *Lycopersicon peruvianum* and *L. esculentum* (Koornneef et al. 1987). Cucumber plant regeneration is positively correlated with the induction of embryoidal callus by a great number of explants. This means that varieties and lines which are able to form the embryoidal callus in most explants are at the same time characterized by a considerable number of regenerated plantlets per plant. The conformability between expected and observed segregation ratios confirms the hypothesis that both traits, i.e., percentage of explants regenerating embryoidal callus (which have formed embryoidal structures) and mean number of plantlets per plant, are controlled by the same or strongly linked genes.

Ability of response in tissue culture is dependent on both heritable and non-heritable components. As was shown by Dunwell et al. (1987), the major part of variation in the production of microspore-derived callus was due to differences between the spikes from a single plant. Koornneef et al. (1987) proved that callus growth as well as shoot regeneration from leaf explants had high heritabilities (0.47–0.87). The broad sense heritability of embryogenesis for triticale (Charmet and Bernard 1984) and wheat (Lazar et al. 1984) was 0.66 and 0.6–0.7, respectively. For cucumber it was relatively high too (0.77–0.43), but expression of these traits was also affected by the physiological state of the donor plants as well as by the timing of experiments. This was indicated by wide ranges of investigated traits within one genotype in spite of identical experimental conditions and highly inbred lines (I_{11}). The timing of experiments shifted the ranges of the results towards higher or lower values. But in all cases B was the best regenerating, G – the intermediately and S the occasionally regenerating line. Because of this variability, the ranges of three classes were established for each experiment separately.

The simple model of inheritance as well as the dominance of the characters tested above would facilitate breeding for favourable tissue culture traits of cucumber by means of crossing into the lines previously deprived of them. An experiment of this type has been done on tomato (Koornneef et al. 1987). The authors obtained better regenerating genotypes by transferring regeneration capacity from *Lycopersicon peruvianum* into *L. esculentum* by classical breeding. Since line B is the best regenerating breeding line, transferring the traits is not connected with introduction of undesired characters. Furthermore, the plant material differed in its regenerating ability and, analyzed in detail, would be the basis for further physiological and biochemical studies.

References

Abe T, Futsufara Y (1984) Varietal difference of plant regeneration from root callus tissue in rice. Jpn J Breed 34:147–155

Bingham ET, Hurley LV, Kaatz DM, Saunders JW (1975) Breeding alfalfa which regenerates from callus tissue in culture. Crop Sci 15:719–721

Broda Z (1984) Wegetatywna propagacja koniczyny czerwonej (*Trifolium pratense* L.) poprzez kultury tkankowe ze szczegolnym uwzglednieniem genetycznego uwarunkowania zdolnosci do regeneracji z kalusa. Rocz Akad Roln Poznaniu. Rozpr Nauk Zes 140:5–40

Brown DCW, Atanassov A (1985) Role of genetic background in somatic embryogenesis in *Medicago*. Plant Cell Tiss Org Cult 4:111–122

Charmet G, Bernard S (1984) Diallel analysis of androgenetic plant production in hexaploid Triticale (*X. triticosecale*, Wittmack). Theor Appl Genet 69:55–61

Duncan DR, Williams ME, Zehr BE, Widholm JM (1985) The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta 165:322–332

Dunwell JM, Francis RJ, Powell W (1987) Anther culture of *Hordeum vulgare* L.: a genetic study of microspore callus production and differentiation. Theor Appl Genet 74:60–64

Foroughi-Wehr B, Friedt W, Wenzel G (1982) On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. Theor Appl Genet 62:233–239

Frankenberger EA, Hasegawa PM, Tigchelaar EC (1981) Diallel analysis of shoot-forming capacity among selected tomato genotypes. Z Pflanzenphysiol 102:233–242

Jacobsen E, Sopory SK (1978) The influence and possible recombination of genotypes on the production of microspore embryoids in anther culture of *Solanum tuberosum* and dihaploid hybrids. Theor Appl Genet 52:119–123

Koornneef M, Hanhart CJ, Martinelli L (1987) A genetic analysis of cell culture traits in tomato. Theor Appl Genet 74: 633–641

Kubicki B (1965) Investigations in the sex determination in cucumbers (*Cucumis sativus* L.). II. The effects of polyploidy on sex expression in monoecious and gynoecious cucumbers. Genet Pol 6:251–265

Kurtz SM, Lineberger RD (1983) Genotypic differences in morphogenic capacity of cultured leaf explants of tomato. J Am Soc Hortic Sci 108:710–714

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures. Theor Appl Genet 68:131–134

Litz RE, Conover RA (1983) High-frequency somatic embryogenesis from Carica suspension cultures. Ann Bot 51:683–686

Locky RD (1983) Callus formation and organogenesis by explants of six *Lycopersicon* species. Can J Bot 61:1072–1079

Malepszy S, Nadolska-Orczyk A (1983) In vitro culture of *Cucumis sativus* L. I. Regeneration of plantlets from callus formed by leaf explants. Z Pflanzenphysiol 11:273–276

Malmberg RL (1979) Regeneration of whole plants from callus culture of diverse genetic lines of *Pisum sativum* L. Planta 146:243–244

Miah MAA, Earle ED, Khush GS (1985) Inheritance of callus formation ability in anther cultures of rice, *Oryza sativa* L. Theor Appl Genet 70:113–116

Murashige T, Skoog FW (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 51:473–497

Nadolska-Orczyk A, Malepszy S (1984) Cucumber plant regeneration from leaf explants – selected characteristics. Bull Pol Acad Sci 32:425–428

Nadolska-Orczyk A, Malepszy S (1987) In vitro culture of *Cucumis sativus* L. VI. Histological analysis of leaf explants cultured on media with 2.4-D or 2.4.5-T. Acta Soc Bot Pol 56:55–60

Nesticky M, Novak FJ, Piovarci A, Dolezelova M (1983) Genetic analysis of callus growth of maize (*Zea mays* L.) in vitro. Z Pflanzenzuecht 91:322–328

Ohki S, Bigot C, Mousseau J (1978) Analysis of shoot-forming capacity in vitro in two lines of tomato (*Lycopersicon esculentum* Mill.) and their hybrids. Plant Cell Physiol 19:27–42

Reisch B, Bingham ET (1980) The genetic control of bud formation from callus cultures of diploid alfalfa. Plant Sci Lett 20:71–77

Rines HW (1983) Oat anther culture: genotype effects on callus initiation and the production of a haploid plant. Crop Sci 23:268–272

Sharma GS, Bello LL, Sapra VT (1980) Genotypic differences in organogenesis from callus of ten triticale lines. Euphytica 29:751–754

Simmonds NW (1981) Heritability. In: Simmonds NW (ed) Principles of crop improvement. Longman, London, pp 91–95

Tomes DT, Smith OS (1985) The effect of parental genotype on initiation of embryogenetic callus from elite maize (*Zea mays* L.) germplasm. Theor Appl Genet 70:505–509

Wehner TC, Locy RD (1981) In vitro adventitious shoot and root formation of cultivars and lines of *Cucumis sativus* L. Hort Sci 16:759–760