

Morphologic and anatomic features of new breeding lines of cucumber var. Borszczagowski

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Abstract

Four growth type mutants 2-75, W-SK, W-19, W-97 obtained from seeds of *Cucumis sativus* var. Borszczagowski treated with ethylene imine were studied. These mutants represented two types of growth: intensive (2-75) and dwarf (W-SK, W-19, W-97). The exuberant growth of mutant 2-75 was the result of internode elongation, intensive production of lateral shoots, enlargement of leaf and number of vascular bundles (11 as compared with 9 in the remaining mutants). The dwarfness of W-SK, W-19, W-97 mutants was caused by strong shoot reduction resulting from decreased length and number of internodes, limiting of lateral shoot production, smaller and darker leaf blade. The mutants produced fruits which differed in shape, dimension and colouring. Moreover, the fruits of mutant 2-75 were characterized by the presence of empty chambers in the endocarp.

Key words: cucumber, growth mutants, shoot, leaf, fruit

INTRODUCTION

A cucumber is one of the most universally grown vegetables in Poland, chiefly due to its breeding possibilities both in the field and under different covers (greenhouse, foil tunnel). However, the reduction in breeding acreage and changes in harvesting methods compel the growers to obtain new lines which are not only fertile, resistant to diseases and unfavourable conditions but also adapted to mechanical harvesting.

An ideal plant designed for mechanical collection ought to be of the bushy, compact type and also be characterized by a number of features especially important for marketing. These features include: suitable fruit length, a green epidermis, few warts, white thorns (which are associated with fruit not turning yellowish), parthenocarpy and flavour values.

Growers have for several years been looking for effective methods of obtaining varieties fulfilling the above requirements. One of such methods is mutation induced by physical and chemical factors. Ethylene imine which evokes a number of changes in plants, is mutagenic agent of exceptional effectivity. Cucumber seeds of Borszczagowski cv. treated with ethylene imine produced a number of mutants including changed fruit shape and size, leaf size, chlorophyll content, fertility of male and female gametes, as well as flower structure and their sex (Kubicki 1978). Growth type mutations produced group characterized by different degrees of main shoot reduction. In addition to W-SK, W-19 and W-97 dwarf forms characterized by strong main shoot reduction, some intensively growing mutants were also obtained, including mutant 2-75.

In spite of the fact that some of the obtained mutants are only of rather theoretical value and are not always useful for agriculture, nevertheless they can be used as material for obtaining further hybrids after their morphological and anatomical features have been determined.

MATERIAL AND METHODS

Control plants of *Cucumis sativus* Borszczagowski cv. and the growth type mutants of 2-75, W-SK, W-19, W-97 were used in this study. These mutants were obtained from seeds of *Cucumis sativus* Borszczagowski cv. treated with a 0.06% aqueous ethylene imine solution. The plants were grown in two vegetative seasons (1983, 1984) at the Experimental Station of SGGW-AR in Warsaw. The length of the main and lateral shoots, internodes as well as the leaf stalks and fruits were measured by means of graph paper. The leaf surface was calculated using a planimeter, the outlines of twenty randomly selected leaves made on cardboard were measured. The anatomical study was carried out on material fixed in Carnoy fixative for leaves and leaf stalks, and in CrAF mixture (weak Navashin fixer) for fruits (Teleżyńska 1975). The chloroplast number per cell of mesophyll was calculated on material fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer and macerated in 0.05 M EDTA in 0.35 M sucrose solution, pH 9.0, at 60°C for 6 h (Possingham and Smith 1972). The cell surface of palisade and spongy parenchyma was determined by planimetry of their outlines made on a Zeiss demonstration attachment set up on a microscope. Samples of fixed fruits were dehydrated and infiltrated with toluene and were embedded in paraffin. Thin sections about 12 µm thick were made. The presence of pectins, lipids, starch and lignin was examined according

to Broda (1974). The observations were performed in a BIOLAR SK-14 microscope. Microphotographs were taken with a Zeiss camera. The results of measurements were analyzed statistically.

RESULTS

Comparative analysis of shoot morphology of the examined forms permitted 2 types of growth to be distinguished. The first one is represented by the Borszczagowski var. and mutant 2-75 showing intensive growth, the second type W-SK, W-19, W-97 dwarf mutants characterized by strong reduction in the main shoot.

The extensive growth of 2-75 mutant was the result of internode elongation and enhanced production of numerous and long lateral shoots of the 1st and sometimes 2nd line (Table 1, Fig. 1). The characteristic feature of W-SK, W-19, W-97 dwarf mutants was strong reduction in the main shoot which was the result of a 2-3 fold shortening of the internodes in comparison to the intensively growing mutant. However, weak spreading of the W-SK mutant was additionally caused by reduction in internode number which was expressed by the production of scarce and very short lateral shoots or complete inhibition of their formation.

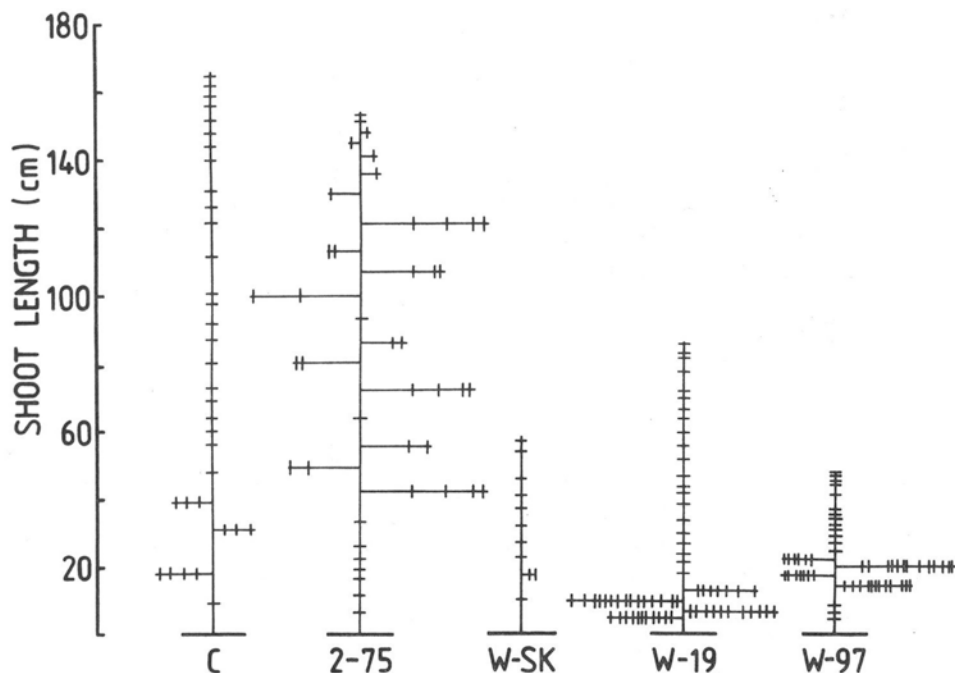


Fig. 1. Shoot type scheme of cucumber growth type mutants. C — initial form Borszczagowski var. Shoot lines denote the nodes

Table 1

Shoot characterization of cucumber (*Cucumis sativus*) growth type mutants. C — Borszczagowski var.

Mutant	Main Shoot			Lateral Shoots				
	main shoot length, cm	internode number	internode length, cm	lateral shoot general length, cm	lateral shoot number	1 shoot length, cm	internode number	internode length, cm
C	162.8±1.2	27.0±1.7	6.0±0.6	58.4± 3.1	3.3±0.7	17.7±3.1	2.8±0.9	5.7±0.9
2-75	153.7±3.6	21.3±0.6	7.3±0.1	252.6±19.8	11.4±0.5	20.9±0.9	25.7±1.8	8.9±0.2
W-SK	53.3±1.5	12.7±0.3	4.4±0.2	6.3± 0.9	1.8±0.2	2.7±0.3	4.7±0.7	1.0±0.5
W-19	82.8±6.3	28.3±1.3	2.9±0.2	89.6± 2.6	4.0±0.5	22.4±2.5	37.0±2.9	2.9±0.2
W-97	47.6±15.9	22.4±2.2	2.2±0.2	58.0± 1.3	4.0±0.2	14.5±1.1	26.0±4.8	2.2±0.1

Table 2

Leaf blade characterization of cucumber (*Cucumis sativus*) growth type mutants. C— Borszczagowski var. (tissue thickness, μm — in brackets)

Mutant	Leaf			Epidermis cell surface, μm^2	Aperture index, %	Palisade parenchyma cell surface, μm^2	Spongy parenchyma cell surface, μm^2
	blade surface, cm^2	stalk length, cm	thickness, μm				
C	372.0±9.5	21.0±0.8	372.7±0.0	897.6±23.6 (69.6±0.0)	31.8±2.2	1866.2±48.8 (135.6±0.0)	1991.1±54.6 (167.5±0.0)
2-75	317.2±12.7	21.4±0.5	284.0±0.0	782.3±25.3 (59.0±0.0)	43.0±1.5	1402.8±29.7 (95.0±0.0)	1400.3±33.6 (125.0±0.0)
W-SK	170.6±9.4	17.2±0.7	254.0±0.0	723.4±0.0 (42.0±0.0)	45.9±1.4	1794.2±36.2 (88.0±0.0)	1744.8±47.0 (124.0±0.0)
W-19	224.1±7.3	16.9±0.8	217.9±4.3	759.9±21.5 (37.6±2.8)	37.4±1.6	1541.9±39.2 (78.7±2.4)	1469.0±37.5 (105.1±3.9)
W-97	198.4±9.5	15.2±1.0	168.4±1.7	798.2±23.9 (30.0±2.1)	34.8±1.3	1094.1±30.1 (60.5±1.1)	1213.0±34.7 (77.9±2.0)

The examined dwarf mutants (W-SK, W-19, W-97) revealed a self-completing type of main shoot growth. As a consequence of strong internode shortening near the blocked growth apex, leaves were situated very close to each other making a horizontal rosette. Leaf surface enlargement and light green colouring of leaves in 2-75 mutant were accompanied by an increase in thickness of its particular layers (Table 2). Simultaneously, the diminution in cell surfaces of palisade and spongy parenchyma and reduction in chloroplast number in both types of parenchyma were observed (Table 2, Fig. 2).

The leaf blade surface of dwarf mutants was nearly 1.5 times smaller than that in the intensively growing 2-75 mutant and somewhat more that in the initial material of the Borszczagowski var. (Table 2). Reduction in leaf surface was parallel with thickness diminution of its particular layers (Table 2).

The dark green colouring of W-SK, W-19 mutant leaves especially in case of W-97 was caused by an increase in chloroplast number per cell of mesophyll, in spite of surface reduction in these cells.

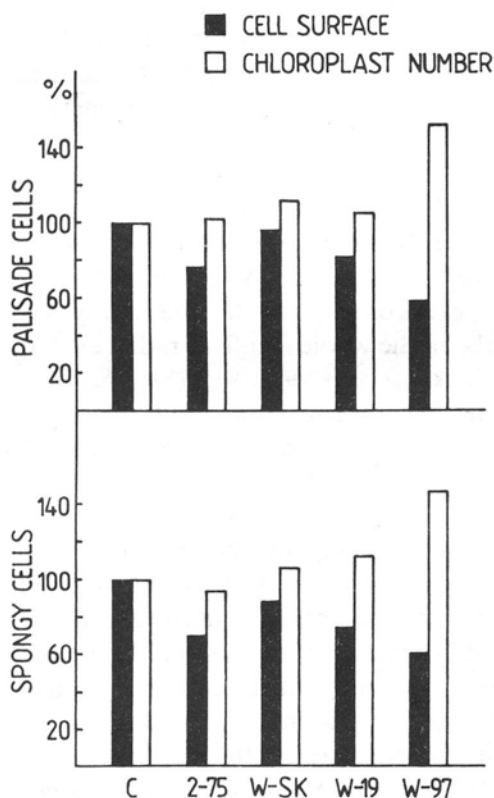


Fig. 2. The dependence between palisade and spongy parenchyma cell surface and chloroplast number (the palisade and spongy parenchyma cell surface and chloroplast number of Borszczagowski var. were taken as 100%)

The leaves of 2-75 mutant grew on the long stalks which contained 11 bicollateral vascular bundles whereas the leaf stalks of dwarf mutants were usually about 4-6 cm shorter, and similarly as the stalks of the Borszczagowski var., contained 9 vascular bundles. The leaf stalks of the examined mutants also revealed a different distribution of colenchyma than the initial form, the Borszczagowski var. (Fig. 3).

The investigated mutants produced fruits which differed in shape, dimension and colouring (Fig. 4). Brown fruits covered with a network of longitudinal and transversal cracks, oval-shaped and large in diameter, characteristic of the Borszczagowski var. were set in the intensively growing 2-75 mutant (Fig. 4).

Anatomical analysis revealed that under strongly cracked fruit, in the epidermis of the 2-75 mutant, as in Borszczagowski var., some layers of cells with strongly lignified walls occurred. The dwarf mutants produced green (W-19) or yellowish-green fruits (W-SK, W-97) and with a smooth or slightly cracked surface.

In the early developmental stages the fruits of dwarf mutants were longer (Fig. 5), though their diameter was similar to that in the strongly growing form (Fig. 5). About the 22nd day of growth the fruits of the initial form, Borszczagowski, were longer (16.7 cm) than fruits produced by the intensively growing 2-75 mutant — only 7.5 cm (Fig. 5). The length of dwarf mutant ripe fruit ranged from 9.2 cm to 11.8 cm, but their diameter was smaller than that in the initial form (Borszczagowski var.) and 2-75 mutant (Figs. 4, 5).

The changes in epidermis shape and size of the examined mutants took place during fruit growth (Fig. 6). Isodiametric, ovary epidermis cells in mature fruit elongated in the radial direction, so that their length and diameter increased 3-4 times (in comparison to the ovary epidermis cells). The appearance of cuticula on the whole length of radial epidermis walls along with the increase in its thickness was observed (Fig. 6c).

Empty air chambers were found in mature fruits of the examined mutants. In case of 2-75 mutant, a single, extensive empty chamber was situated in the centre of the endocarp (Fig. 4), whereas in W-19, W-97 dwarf mutants, small empty chambers were observed near the placenta.

In the early developmental stages of the fruit, the "suture" of fused carpels was made of regular, pentagonal cells of similar size. The cell walls of the "suture" cells contained considerable amounts of pectins, whereas significant amounts of starch grains were characteristic inside the cells near the placenta. However, during fruit ripening, their contents decreased. The structural changes of cells forming the "suture" were doubtlessly the cause of empty chamber formation in the endocarp centre of the 2-75 mutant. As early as in 2 day-old fruit of the 2-75 mutant, part of the cells forming the "suture" became distinctly larger than the others (Fig. 7a). Gradually those cells lost their regular shape and their walls underwent hydrolysis (Fig. 7c). It became difficult to identify the place of carpel fusion in the centre of the pericarp (Fig. 7d). The

C

2-75

W-SK
W-19
W-97

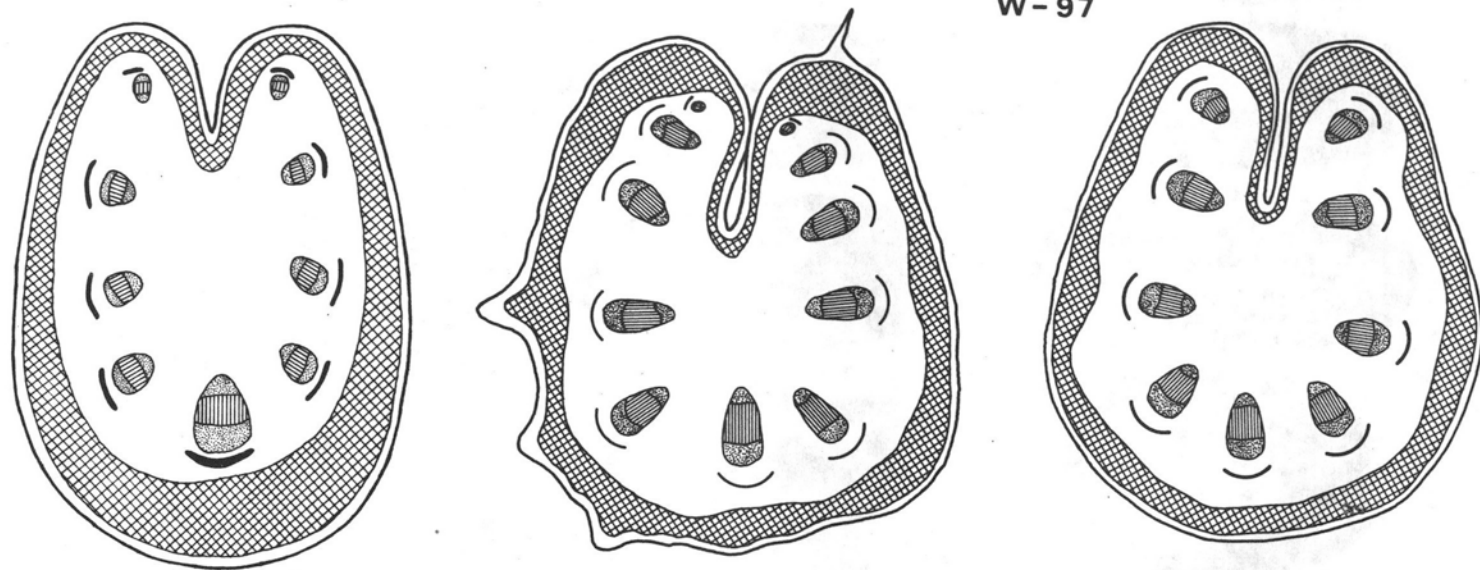


Fig. 3. Transverse section of leaf stalk of cucumber growth type mutants. C — Borszczagowski var. Shaded area — colenchyma

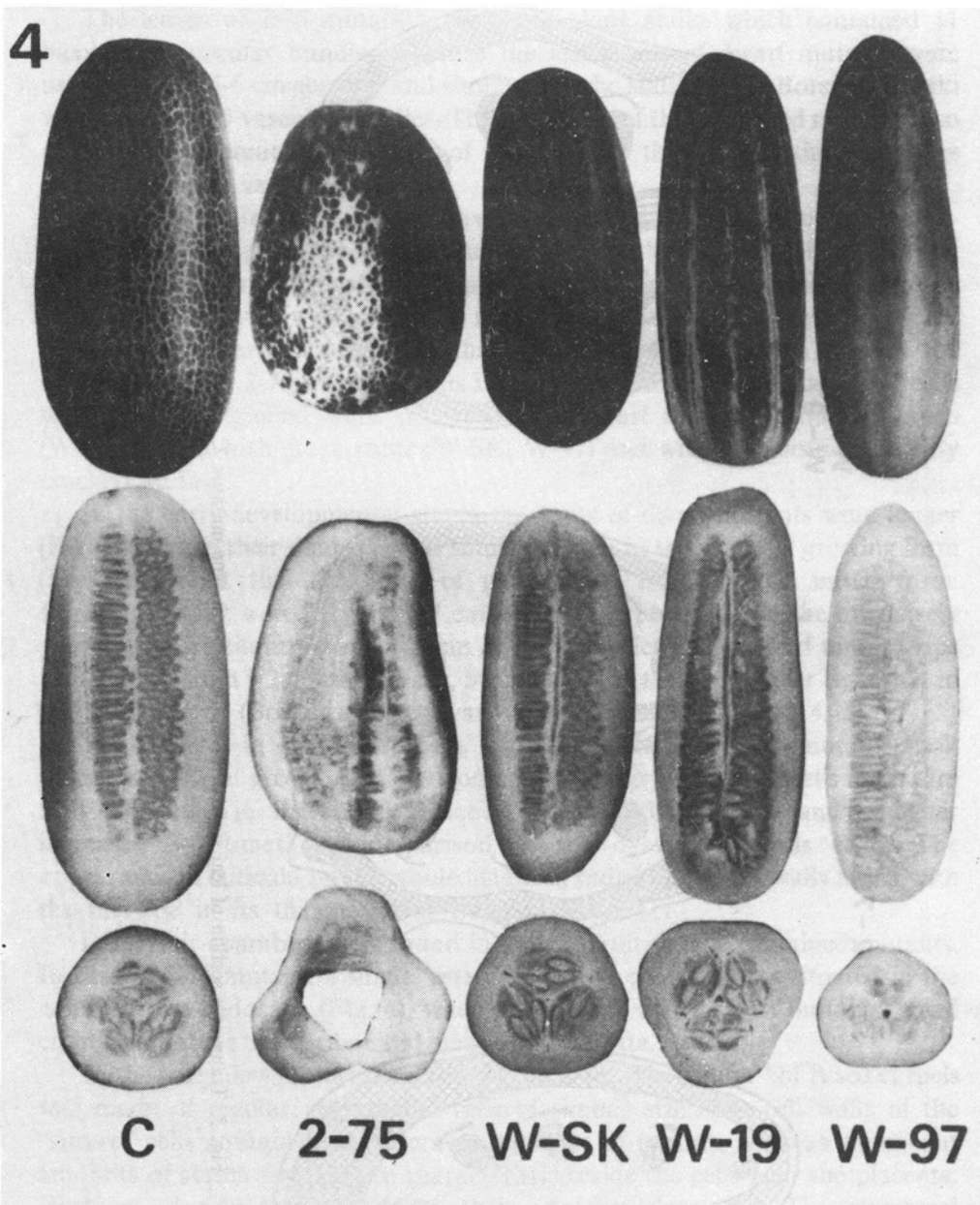


Fig. 4. The mature fruit of cucumber growth type mutants (the appearance, longitudinal and transverse sections). C — Borszczagowski var. Scale 1:2

empty chamber, microscopic in size at first (Fig. 7b), became so large in the mature fruit of the 2-75 mutant, that it came into contact with the exocarp (Fig. 4).

However, the considerably smaller empty chambers occurring in W-19, W-97 dwarf mutants near the placenta, were the result of partial decay of endocarp tissue which surrounded the mature seeds.

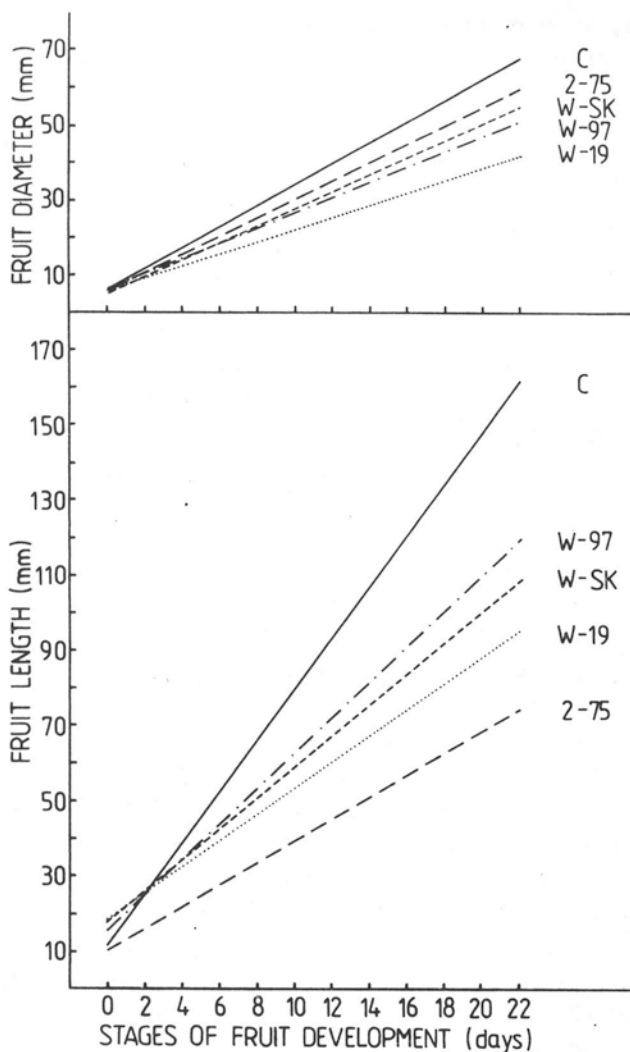


Fig. 5. Changes in fruit length and diameter of cucumber growth type mutants during successive developmental stages. C — Borszczagowski var. Point "O" — anthesis

DISCUSSION

Dwarf mutants are not rare in the plant kingdom. Many of them had been described and used in agriculture as, e.g. the bushy form of paprika (Tal et al. 1974).

However, in 1976 Kauffman and Lower in great detail examined and described cucumber dwarf mutations. A new type of growth was defined as compact. This form is phenotypically characterized by remarkably short internodes, strong bushing, weakly formed tentacles, small flowers and minute seeds.

For about 20 years investigations in Poland on cucumber mutations have been carried out by Professor Kubicki. From the data in his report (1979), it is concluded that the breeding conditions (field, foil tunnel) can actually influence the internode length, bushing power and whole plant luxuriance. For instance, W-SK mutant grown in a foil tunnel in 1976 demonstrated stronger internode reduction than under field conditions (Kubicki 1976).

The authors of the present work had the material cultivated in different harvesting places at their disposal. The unfavourable atmospheric conditions in succeeding vegetative seasons, infections and necrosis of plants attacked by powdery mildew were the main reasons of difficulties in harvesting under similar conditions. Nevertheless, the repeatable data in our possession permit us to state that the dwarfness of W-SK, W-19 and W-97 mutants results from strong internode reduction in the main and lateral shoot. Particularly, the weak growth of W-SK mutant is caused by internode number and lateral shoot reduction. The leaf blade surface reduction and leaf stalk length abridgement occurred as an additional effect in dwarf mutants. Besides, the dwarf mutants are characterized by dark-green leaf colouring. This is caused by the increase in chloroplast number per one cell of mesophyll in spite of the decrease in cell size, which is particularly visible in the W-97 mutant.

The Borszczagowski var. which constitutes the initial form for all of the examined mutants is the representative of greenhouse varieties. The main shoot of this variety attains a length of about 2 m, whereas the main shoot length in some intensively growing greenhouse varieties can reach 4-5 m (Lityński 1969). Furthermore, they can produce lateral shoots of IIIrd and IVth line and even further lines (Borna 1973).

Only 2-75 out of all the examined mutants showed as intensive growth as Borszczagowski var. plants. Moreover, 2-75 mutant produces numerous and very long lateral shoots of Ist and sometimes IInd line which had been observed in neither the Borszczagowski var. nor in dwarf mutants.

The intensively growing form (2-75 mutant) is characterized by a somewhat smaller leaf blade than that in the initial form (Borszczagowski var.) and the leaf stalk length is similar to that in Borszczagowski var. However, in the 2-75

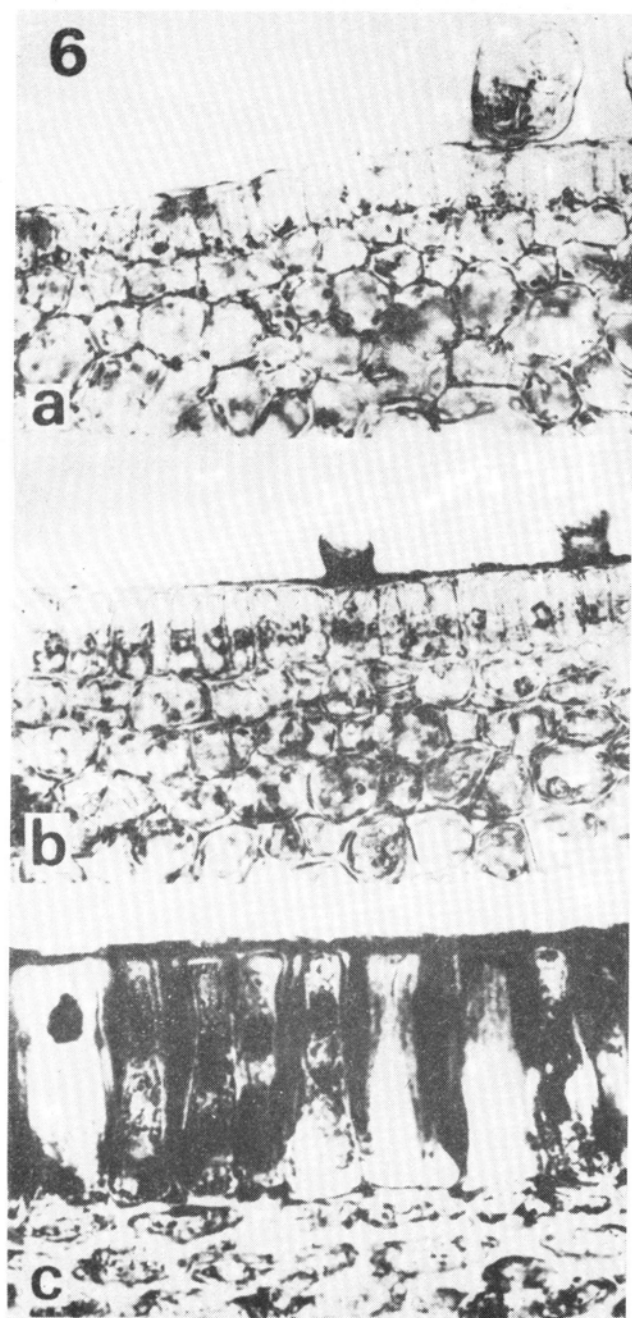


Fig. 6. Transverse section of fruit of cucumber growth type mutants. a — 3-day-old fruit.
b — 4-day-old fruit, c — mature fruit, 1100 \times

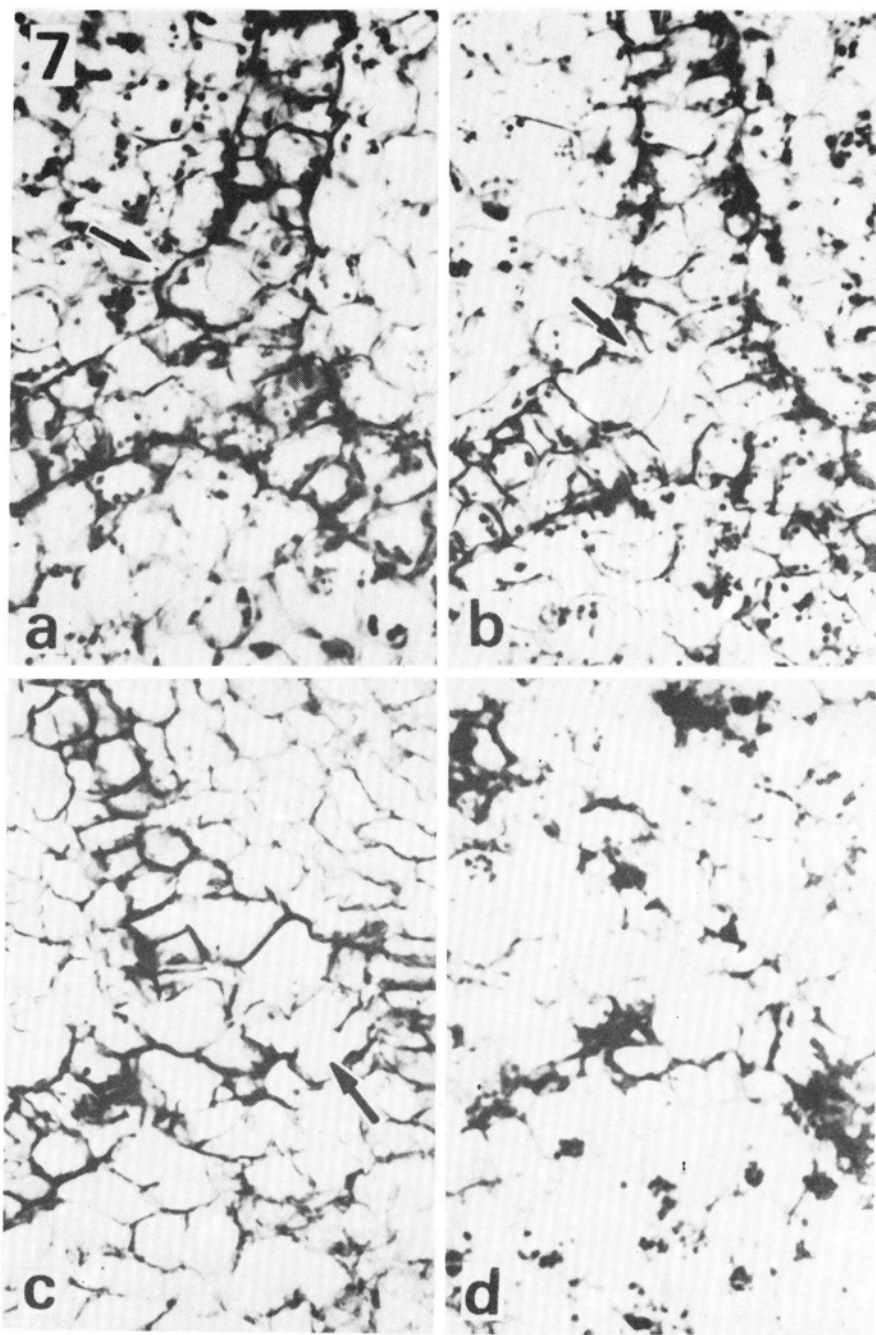


Fig. 7. The early stages of empty chamber formation in the fruit centre of 2-75 mutant (transverse section, PAS). a — 2-day-old fruit — noticeable larger size of cells in the "suture" of fused carpels (arrow); b — 3-day-old fruit — small empty chamber in the place of carpel fusion (arrow); c — 4-day-old fruit — the degraded walls of cells forming the carpel "suture" (arrow); d — 6-day-old fruit — the place of carpel fusion in the centre is difficult to identify, part of the cells is disintegrated. 1100 \times

mutant the number of vascular bundles increase in comparison to dwarf forms and Borszczagowski var.

The examined mutants produce 2 types of fruits which differ from each other in colouring, shape and size. Brown fruits with a strongly cracked epidermis, typical of intensively growing 2-75 mutant, are also characteristic of Borszczagowski var. However, the dwarf mutants produce green or yellowish-green smooth or somewhat cracked fruits.

Fruit growth is determined by 2 processes: cell division and cell size increase. Intensive cell proliferation takes place during bud development until anthesis, and is continued to a lesser extent after fruit setting (Mapelli et al. 1978, Esau 1979). For example, the apple ovary during anthesis contains about 2 mln cells, the mature fruit at the collecting season possesses as many as 40 mln cells. Twenty one divisions before and only 4.5 after anthesis are required to attain such a cell number (Coombe 1976). After achieving the suitable cell number, fruit growth is achieved by enlargement in cell size and inter-cellular spaces. In case of cucumber fruit, cell size increase is difficult. On the basis of Lauk's unpublished data it appears that the exocarp cells adjacent to the endocarp attain the largest sizes, so that their surface increases about 9 times during fruit development, whereas the cells under the epidermis increase only 3 times.

During fruit maturing, besides the size increase, colour changing, also observed was the appearance of empty chambers in 2-75 mutant fruit.

The empty air spaces have been found in fruits of several plants, e.g. in melons, pumpkin and cucumber (Esau 1979). Szymański (1976) observed similar empty chambers in strawberry fruit. They appeared about 20 days after pollination.

The harvesting conditions can influence their formation. Low rainfall and high air temperature during the vegetative season intensify the tendency for air chambers to form in cucumber fruit (Osińska 1950). Intensified N, P, K mineral fertilization caused a similar effect (Elkner 1979). Up to the present, there is still no univocal explanation for the appearance of empty chambers.

Załęska (1964) thinks that the formation of empty chambers in cucumber fruit is caused by separation of previously fused carpels. It is accompanied by destruction of endocarp tissues situated near vascular bundles. As a result of these changes, the empty chamber is formed on the length of the fruit or its part (Elkner 1979).

Next, investigations on cucumber growth-type mutants proved that the excessive cell size increase on parts of fused carpels and then the disintegration of the walls of these cells are the immediate causes of the formation of empty chambers. As the fruit develops, the size of this air chamber increases.

The dwarf mutants possess more advantageous features from the point of view of usefulness for mechanical collection and for market production. The

compact type of plants, the proper fruit shape, size and colouring are valuable merits of W-SK, W-19, W-97 mutants.

An especially essential feature of dwarf mutants is self complicting growth type, ensuring almost simultaneous fruit maturation on the lateral shoot, which is very desirable in the case of mechanical collection. However, strong reduction in lateral shoot number in the W-SK mutant involves the restriction of female flower production, which causes depression of plant productivity. Because of this only W-19 and W-97 dwarf mutants seem to be good material for the further breeding. After increasing female flower number, seed vitality, plant productivity they can become valuable material for market production.

The only essential feature of the strongly growing 2-75 mutant is the intensive production of lateral shoots on which female flowers are mainly formed. However, both fruit shape, size and colouring and the presence of empty chamber are undesirable features. In spite of this, the 2-75 mutant can obtain new profitable features after its hybridization with other forms.

REFERENCES

- Borna Z., 1973. Szczegółowa uprawa warzyw. PWRiL, Warszawa.
- Broda B., 1974. Metody cytochemii roślinnej. PZWŁ, Warszawa.
- Coombe B. G., 1976. The development of fleshy fruits. *Ann. Rev. Plant Physiol.* 24: 507-528.
- Elkner K., 1979. Wpływ odmiany i czynników agrotechnicznych na jakość ogórków przeznaczonych do kwaszenia. Ph. D. Thesis, Inst. Warzywn., Skierniewice.
- Esau K., 1979. Anatomy of seed plants. Wiley J. and Sons, New York. pp. 429-454.
- Kauffman C. S., Lower R. L., 1976. Inheritance of an extreme dwarf plant type in the cucumber. *J. Amer. Soc. Hort. Sci.* 101: 150-151.
- Kubicki B., 1976. Genetyczne podstawy hodowli wysokoproduktywnych odmian ogórków przystosowanych do mechanicznego zbioru. *Mater. Inst. Warzywn., Skierniewice.*
- Kubicki B., 1978. Możliwości wykorzystania indukowanych mutacji typu wzrostu ogórka w hodowli odmian przystosowanych do mechanicznego zbioru. *Pr. Naukowe U. Śl., Mutageniza Roślin Wyższych*, 222: 170-176.
- Kubicki B., 1979. Recesywna żeńskość ogórków i możliwości jej wykorzystania w hodowli mieszańców heterozygnych. *HRAiN* 23: 357-370.
- Lityński M., 1969. Ogórki w gruncie. PWRiL, Warszawa.
- Mapelli S., Frova C., Torti G., Soressi G. P., 1978. Relationship between set, development and activities of growth regulators in tomato fruits. *Plant Cell Physiol.* 19: 1281-1288.
- Osińska J., 1950. Ogórki kiszane. PWT, Warszawa.
- Possingham J. V., Smith J. W., 1972. Factor affecting chloroplasts replication in spinach. *J. Exp. Bot.* 21: 1051-1059.
- Szymański J., 1976. Badania anatomiczne i autoradiograficzne młodych stadiów rozwojowych dna kwiatowego truskawki *Fragaria grandiflora* Ehrh. Ph. D. Thesis. ZCCR-IFC. UŁ, Łódź.
- Tal M., Witzum A., Shifriss C., 1974. Abnormal stomatal behaviour and leaf anatomy in *Capsicum annuum* scabrous diminutive a wilted mutant of pepper. *Ann. Bot.* 38: 983-988.
- Teleżyńska J., 1975. Porównanie dynamiki rozwoju zapłodnionych owoców pomidora z rozwojem owoców powstałych w wyniku zastosowania kwasu β -naftoksyoctowego. Ph. D Thesis. SGGW-AR, Warszawa.
- Załęska Z., 1964. Badania anatomiczne nad owocnią ogórka *Cucumis sativus* L. *Acta Agrobot.* 16: 87-104.

*Cechy morfologiczne i anatomiczne nowych linii hodowlanych ogórka odmiany
Borszczagowski*

Streszczenie

Obiektem badań były 4 mutanty typu wzrostu (2-75, W-SK, W-19, W-97) uzyskane z nasion *Cucumis sativus* var. Borszczagowski traktowanych etylenoiminą. Mutanty te reprezentują dwa typy wzrostu: intensywny (2-75) oraz karłowaty (W-SK, W-19, W-97). Bujny wzrost mutantu 2-75 jest wynikiem wydłużenia międzywęźli, wzmożonej produkcji pędów bocznych, zwiększenia blaszki liściowej oraz większej liczby wiązek przewodzących (11, podczas gdy pozostałe mutanty wytwarzają 9). Karłowatość mutantów W-SK, W-19, W-97 jest spowodowana silną redukcją pędu w wyniku zmniejszenia liczby i długości międzywęźli, ograniczeniem produkcji pędów bocznych, mniejszą blaszką liściową. Badane mutanty wytwarzają owoce różniące się kształtem, wielkością oraz barwą. Ponadto, owoce mutantu 2-75 charakteryzuje obecność pustych powietrznych komórek w centrum endokarpu.