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# SPONTANEOUS AND INDUCED POLYEMBRYONY IN PEPPER (*CAPSICUM ANNUUM* L.)[1]

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Summary. In the investigations on the spontaneous polyembryony in pepper some cultivars and their $F_1$ and $F_2$ hybrids were used. Some twin plants observed in the $F_2$ progeny were characterized by a great number of twoembryonic seeds.

The main source of additional embryos was splitting polyembryony. Diploid embryos of the matroclinal origin which accompanied sexual embryos in twin pairs were also observed. That was classified as apomictic polyembryony. It was impossible to state whether they developed as a result of adventitious embryony or whether they were the effect of embryogenesis from the elements of an embryo sac having a reduced number of chromosomes with preceeding spontaneous diploidisation. Possibilities of the formation of twin embryos as a result of fertilisation of the egg cell and other element of the embryo sac were excluded.

In the investigation of the induced polyembryony growth regulators during the blooming of plants were used. Beta-naphtoxyacetic acid (BNOA) and 2,4-dichloricphenoxyacetic acid (2,4 D) increased the frequency of polyembryony.

Haploid forms in twin pairs $n$-$2n$ were obtained only in the material under the influence of growth regulators. 2,4-dichloricphenoxyacetic acid appeared to be the most effective in increasing the frequency of polyembryony and in inducing haploid embryogenesis.

It has been found that polyembryony is genetically determined. The selection of forms with a tendency to produce twoembryonic seeds and the increase of the frequency of polyembryony using growth regulators at a simultaneous induction of haploids in vivo open possibilities for the use of polyembryony in breeding.

In pepper breeding crossing, individual selection and heterosis method are used. Among new possibilities, which can increase the effectiveness of the mentioned methods haploid and polyembryonic breeding deserve special attention. Haploid forms of plants, which can be obtained with their help, constitute a useful material in genetic investigations and first of all in practical breeding (Nei 1963). They can be obtained from in vitro anther cultures. Conditions of these cultures are established for many species of plants (Nitzsche, Wenzel 1977). Doubling of the chromosome number permitted to prepare diploid forms, used directly as cultivars.

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Not long ago it was considered that only reduced parthenogenesis may be a source of haploids in pepper. Studies of Sibi et al. (1979), Dumas de Vaulx et al. (1981) allowed to establish conditions for the induction of androgenesis in vitro. The general principles of potential estimate of the usefulness of haploids in breeding are also known (Pochard, Dumas de Vaulx 1971). The succeses expressed in an adequate efficiency of obtaining haploids does not eliminate considerable expenses connected with this technique. Besides that only some cultivars (Wang et al. 1981) form haploid plants under in vitro conditions.

Presenting, therefore, another method i.e. polyembryonic breeding, twoembryonic seeds should be regarded as a material making possible the obtaining of haploids. As twin forms they sporadically accompany sexual embryos. A possibility of a practical use of diploid twin forms cannot be excluded and their usefulness will depend on their origin. Polyembryony, the phenomenon of the occurrence of two or more embryos in seeds, was the object of the presented investigations. Simple techniques permitting the pursuance of this research or practical application create possibilities of using it at every station dealing with plant breeding, irrespective of its equipment.

The purpose of the present studies, underlining its methodolical character and practical aspect, was an attempt to determine relationships between the frequency of polyembryony and hereditary inclinations in this direction. Cultivars characterized by sufficient combining ability (Nowaczyk 1981), their $F_1$ hybrids and the progeny of these hybrids, derived from twoembryonic seeds were used. Genetic differentiation of the material made it possible to perform an analysis of the origin of additional embryos. The influence of growth regulators used during plant blooming on the frequency of polyembryony and on haploid induction was also estimated.

## MATERIAL AND METHODS

Seeds of five cultivars of sweet pepper and the hybrid material obtained as a result of their crossing were used at the first stage of the studies. These cultivars are as follows:

HPS — a line bred by us. Its plants give long, narrow and thinwalled fruits. They are green and become red when maturing.

Poznańska Słodka — a Polish cultivar. The fruits are bent and coneshaped with thin walls. They are bright-green and then brigth-red at physiological maturity.

Sarga chilli — a Hungarian cultivar. Its plants give cone-shaped, flowing fruits with thin walls. They are dark-green when unripe and dark-red when maturing.

Cuneo — an Italian cultivar. Plants give short, cone-shaped and thick-walled fruits. They are dark-green when unripe and yellowishorange at physiological maturity.

Paradisoni — a Hungarian cultivar. Fruits are round and flat with flowing surface.

Their walls are thick. Unripe fruits are darkgreen and become dark-red when maturing.

Seeds of these cultivars and their hybrids were prepared in 1982. They were taken from plants cultivated under a plastic film and crossed between June 20, 1982 and July 20, 1982. The seeds germinated on a filtering tissue-paper, moistened by the fungicide Benlate, made in the rolls and placed vertically in beakers. The temperature during germinating ranged between 28° — 30°C.

Seeds taken from twin plants, originating from twoembryonic seeds constituted a material for investigations of the frequency of polyembryony in the progeny of these plants.

The vitality of twin plants was presented in percentage in comparison with the number of twin seedlings.

For investigation of the influence of growth regulators on the frequency of polyembryony seeds of three cultivars — Cuneo, Podarok Moldawii and Sarga chilli were used. They were colected from plants grown in a glasshouse. Plants of each cultivar were divided into three groups. One of them was exposed to the action of the growth regulators — beta-naphtoxyacetic acid (BNOA), 2, 4-dichliriophenoxyacetic acid (2,4D), 3-indolylbutric acid (IBA) and alpha-naphtaleneacetic acid (NAA), which were used during blooming of plants, dipping flowers in a 0.005% water solution. The growth substance treatment (hormonisation) perfomed in this way was repeated after two to three days, and in the second group of plants it was preceded by castration of flowers. The last group included the control plants. An analysis of the sowing material for the presence of twoembryonic seeds was made according to the principles shown before.

The origin of additional embryos was determined on the basis of an estimate of morphological characters of twin plant fruits in the $F_1$ generation. This was possible due to a considerable phenotype differentiation of the parental plants.

Twin plants of the $F_2$ generation, making the progeny of the forms from twoembryonic $F_1$ seeds, were subject to the same estimation. A morphological characterization of these plants, which reached physiological maturity under glasshouse conditions, expressed as previously in the terms of data concerning the weight, length, width, coefficient of fruit shape and thickness of fruit walls, was the basis for inferences about the origin of additional embryos.

The plants showing less intensive growth, typical of the forms with a reduced number of chromosomes, were thoroughly studied. There were made macroscope observations of flowers, an estimate of pollen vitality by acetic-carmine staining and some measurment of stomatal cells. A preliminary information obtained in this way was verified by a cytological analysis. Tip roots or developing anthers served as a material when determining the number of chromosomes. Obs rvations were conducted on preparations fixed in the aceticalcohol (1 : 1), stained with acetic-carmine. Plants descending from few seeds of the haploid forms were subjected to observations in 1985.

# RESULTS

## THE FREQUENCY OF THE OCCURRENCE OF TWOEMBRYONIC SEEDS

### Cultivars and $F_1$ hybrids

Data regarding the cultivars present the results obtained from testing of a smaller number of seeds than that of hybrids. That was the yield of a five fold smaller number of plants than that used as maternal form for crossing. Among the germinating seeds two cultivars were not found to have twoembryonic forms. In this connection the frequency of polyembryony for the cultivars Paradisoni and HPS was not determined. In general, the portion of twoembryonic seeds in the parental cultivars was 0.03%. That portion in the $F_1$ seeds was about three fold larger.

Table 1. Frequency of twoembryonic seeds and vitality of twin plants in cultivars and $F_1$ hybrids

| Cultivars and their hybrids | Number of germinating seeds | Twoembryonic seeds | | Mature twin plants | |
|---|---|---|---|---|---|
| | | number | % | number | % |
| HPS | 4691 | 0 | 0 | 0 | 0 |
| Poznańska Słodka | 4857 | 5 | 0.102 | 9 | 90 |
| Sarga chilli | 19 601 | 4 | 0.020 | 4 | 50 |
| Cuneo | 3971 | 3 | 0.075 | 0 | 0 |
| Paradisoni | 6219 | 0 | 0 | 0 | 0 |
| Totally | 39 339 | 12 | 0.030 | 13 | 54 |
| HPS × Cuneo | 27 025 | 30 | 0.111 | 31 | 52 |
| HPS × Paradisoni | 29 611 | 26 | 0.087 | 28 | 54 |
| Poznańska Słodka × Cuneo | 26 998 | 25 | 0.092 | 33 | 66 |
| Poznańska Słodka × Paradisoni | 12 353 | 8 | 0.064 | 12 | 75 |
| Sarga chilli × Cuneo | 12 356 | 7 | 0.057 | 7 | 50 |
| Sarga chilli × Paradisoni | 35 484 | 25 | 0.070 | 40 | 80 |
| Totally | 143 827 | 121 | 0.084 | 152 | 63 |
| Altogether | 182 166 | 133 | 0.073 | 165 | 62 |

Differentiation between individual hybrids was smaller than that of the parental forms. No marked differences were detected when comparing hybrid groups with one common parental form. This concerns groups common to both maternal and paternal cultivars (Table 1).

### The progeny of hybrid twin plants

The portion of seeds with numerous embryos in the progeny of plants from twoembryonic seeds was similar to that observed in $F_1$, obtained as a result of crossing plants from monoembryonic seeds. The $F_2$ hybrid from HPS×Cuneo, distinguishing by the highest frequency of polyembryony, which was twice as high as that of hybrid, and involving another paternal form, should be especially mentioned. Similar dependences were also observed in the hybrids of the cultivars Poznańska Słodka with Cuneo and Paradisoni (Table 2).

Completing the general characterization of hybrids, some data concerning the progeny of twin plants should be presented. Only some of them were found to have numerous embryos. The $F_2$ hybrid of HPS ×Cuneo is worth of being mentioned

Table 2. Frequency of twoembryonic seeds and vitality of obtained $F_2$ plants in the progeny of twin plants

| Symbol of $F_1$ plants | | Number of germinating seeds | Twoembryonic seeds | | Mature twin plants | |
|---|---|---|---|---|---|---|
| | | | number | % | number | % |
| HPS × Cuneo | 11b | 604 | 5 | 0.827 | 10 | 100 |
| | 14a | 607 | 2 | 0.329 | 4 | 100 |
| | 18a | 297 | 2 | 0.673 | 2 | 50 |
| | 20b | 220 | 1 | 0.454 | 2 | 100 |
| other plants[1] | | 4682 | 0 | 0 | 0 | 0 |
| totally | | 6410 | 10 | 0.156 | 18 | 90 |
| HPS × Paradisoni | 3a | 432 | 1 | 0.231 | 2 | 100 |
| | 6b | 197 | 1 | 0.507 | 2 | 100 |
| | 8a | 1465 | 3 | 0.204 | 4 | 67 |
| | 10a | 1145 | 1 | 0.087 | 2 | 100 |
| | 18a | 1677 | 1 | 0.059 | 1 | 50 |
| | 18b | 1290 | 2 | 0.155 | 4 | 100 |
| other plants[1] | | 7461 | 0 | 0 | 0 | 0 |
| totally | | 13 667 | 9 | 0.066 | 15 | 84 |
| Poznańska Słodka × Cuneo | 2a | 304 | 1 | 0.328 | 0 | 0 |
| | 3a | 529 | 2 | 0.337 | 3 | 75 |
| | 4b | 324 | 1 | 0.308 | 2 | 100 |
| | 7b | 1024 | 1 | 0.097 | 2 | 100 |
| | 9b | 737 | 3 | 0.407 | 6 | 100 |
| | 13b | 438 | 3 | 0.684 | 6 | 100 |
| | 15b | 141 | 1 | 0.709 | 2 | 100 |
| other plants[1] | | 6508 | 0 | 0 | 0 | 0 |
| totally | | 10 068 | 12 | 0.119 | 21 | 88 |
| Poznańska Słodka × Paradisoni | 1b | 348 | 1 | 0.287 | 2 | 100 |
| | 3a | 238 | 1 | 0.420 | 0 | 0 |
| | 3b | 394 | 1 | 0.253 | 0 | 0 |
| other plants[1] | | 3421 | 0 | 0 | 0 | 0 |
| totally | | 4601 | 3 | 0.065 | 2 | 33 |
| Sarga chilli × Cuneo | 1a | 924 | 1 | 0.108 | 2 | 100 |
| | 2a | 85 | 1 | 1.176 | 1 | 50 |
| | 2b | 516 | 1 | 0.193 | 2 | 100 |
| | 4a | 445 | 1 | 0.224 | 1 | 50 |
| | 5a | 1044 | 1 | 0.095 | 2 | 100 |
| other plants[1] | | 2490 | 0 | 0 | 0 | 0 |
| totally | | 5504 | 5 | 0.091 | 8 | 80 |
| Sarga chilli × Paradisoni | 5b | 492 | 1 | 0.203 | 0 | 0 |
| | 7a | 393 | 1 | 0.254 | 0 | 0 |
| | 7b | 882 | 1 | 0.113 | 0 | 0 |
| | 9b | 850 | 1 | 0.117 | 2 | 100 |
| | 12b | 109 | 1 | 0.917 | 1 | 50 |
| | 16a | 103 | 1 | 0.970 | 1 | 50 |
| | 19a | 590 | 1 | 0.169 | 1 | 50 |
| | 20a | 362 | 1 | 0.276 | 0 | 0 |
| | 22a | 339 | 1 | 0.294 | 0 | 0 |
| other plants[1] | | 8235 | 0 | 0 | 0 | 0 |
| totally | | 12 355 | 9 | 0.073 | 5 | 27 |
| Altogether | | 52 605 | 48 | 0.091 | 71 | 74 |

[1] plants with monoembryonic seeds only.

again. Seeds of only four plants of this hybrid had additional embryos. The frequency of their occurrence was, however, very high, exceeding 0.8% in one of them. Despite the fact that these seeds constituted hardly one third of the total number of the hybrid seeds, the portion of those containing numerous embryos in relation to all of them, which germinated, was the largest. Other hybrids were also found to have some twoembryonic seeds in progeny of single twin plants.

## The effect of growth regulators

In view of a limited number of germinating seeds of individual cultivars subject to the action of each of the growth regulators, data concerning the frequency of polyembryony were presented as a sum for cultivars and growth regulators (Table 3). It was observed that twoembryonic seeds occurred at a higher frequency in the material from plants treated with growth regulators in the cultivars Cuneo and Sarga chilli. An exceptionally small frequency of this phenomenon in the cultivar Podarok Mołdawii, and lack of twoembryonic seeds in the group of control plants, made impossible an explicit assessment of the results. We may be, however, recognized similar to the data shown previously. Attention should be paid to differentiation between the cultivars. Differences were also observed in the effects of individual growh regulators. The largest portion of twoembryonic seeds was found

Table 3. Frequency of twoembryonic seeds and vitality of twins from plants after treating with growth regulators

| Seed origin | Number of germinating seeds | Twoembryonic seeds | | Mature twin plants | |
|---|---|---|---|---|---|
| | | number | % | number | % |
| **For cultivars** | | | | | |
| Cuneo | | | | | |
| Control | 831 | 3 | 0.361 | 6 | 100 |
| Hormonisation | 2033 | 12 | 0.590 | 20 | 83 |
| Castration and hormonisation | 1354 | 7 | 0.517 | 12 | 86 |
| Podarok Mołdawii | | | | | |
| Control | 840 | 0 | 0 | 0 | 0 |
| Hormonisation | 4043 | 1 | 0.025 | 0 | 0 |
| Castration and hormonisation | 2994 | 2 | 0.067 | 2 | 50 |
| Sarga chilli | | | | | |
| Control | 4347 | 3 | 0.069 | 5 | 83 |
| Hormonisation | 13 266 | 24 | 0.181 | 32 | 67 |
| Castration and hormonisation | 6668 | 12 | 0.181 | 20 | 83 |
| **For growth regulators** | | | | | |
| Beta-naphtoxyacetic acid | | | | | |
| Hormonisation | 5923 | 19 | 0.321 | 31 | 83 |
| Castration and hormonisation | 5219 | 9 | 0.173 | 16 | 89 |
| 2,4 dichloricphenoxyacetic acid | | | | | |
| Hormonisation | 3116 | 10 | 0.262 | 17 | 85 |
| Castration and hormonisation | 808 | 6 | 0.743 | 12 | 100 |
| 3-indolylbutric acid | | | | | |
| Hormonisation | 3421 | 2 | 0.059 | 4 | 100 |
| Castration and hormonisation | 3238 | 6 | 0.185 | 10 | 83 |
| Alpha-naphtaleneacetic acid | | | | | |
| Hormonisation | 6182 | 6 | 0.098 | 10 | 80 |
| Castration and hormonisation | 1746 | 0 | 0 | 0 | 0 |
| Totally | | | | | |
| Hormonisation | 19 342 | 37 | 0.191 | 54 | 73 |
| Castration and hormonisation | 11 016 | 21 | 0.190 | 34 | 81 |
| Control | 6018 | 6 | 0.099 | 11 | 92 |

in the material from the plants treated with the 2, 4-dichloricphenoxyacetic acid and it was over thrice as large as that of the control plants. An increased frequency of polyembryony was also observed under the effect of betanaphtoxyacetic acid. The values characterizing the material from plants treated with the remaining growth regulators were similar to those of the control plants.

The results, characterizing the frequency of polyembryony, presented as a sum for all growth regulators in the material from plants under the influence of hormonisation and castrated before being treated with growth regulators were also similar. A more detailed analysis shows differentiation in the action of growth regulators. Nearly a twofold frequency of this phenomenon was characteristic of seeds from plants treated with beta-naphtoxyacetic acid as compared to that of plants in which hormonisation was preceded by castration. The application of 2, 4-dichloricphenoxyacetic acid to the material prepared in this way gave about threefold larger portion of twoembryonic seeds than the material from the plants subject to hormonisation only.

## PHENOTYPE ANALYSIS OF TWIN PLANTS

### Twin plants from $F_1$ seeds

Twin plants brought to physiological maturity represented different phenotypes. The most numerous were the groups showing the phenotype of hybrids forms. The second one constituting about 9.8% consisted of plants exhibiting similarities to their maternal form, used for crossing. No plants, morphologically similar to the paternal forms, were observed (Table 4).

Table 4. Phenotypical differentiation of twin plants obtained from $F_1$ seeds

| Hybrids | Number of plants representing phenotype | | |
|---|---|---|---|
| | maternal | paternal | hybrid |
| HPS × Cuneo | 3 | 0 | 29 |
| HPS × Paradisoni | 3 | 0 | 26 |
| Poznańska Słodka × Cuneo | 0 | 0 | 33 |
| Poznańska Słodka × Paradisoni | 3 | 0 | 9 |
| Sarga chilli × Cuneo | 0 | 0 | 7 |
| Sarga chilli × Paradisoni | 6 | 0 | 34 |
| Totally | 15 | 0 | 138 |

More attention should be paid to plants representing the phenotype of the maternal cultivar. Their characteristics are given in Table 5 in comparison to the value of some morphological characters of parental cultivars and hybrids forms from monoembryonic seeds.

Three plants of the matroclinal origin obtained from hybrid twoembryonic seeds of (HPS ×Cuneo) $F_1$ originated from three pairs, in which twin forms died at the seedlings phase. Two of them were accreted in the hypocotil part.

In the second hybrid, (HPS ×Paradisoni) $F_1$, two plants originated from the pairs where twin seedlings died; they were not accreted. The third one designated 5b was from the pair where the twin partner showed characters of a hybrid form. These plants were accreted.

**Table 5.** Morphological characteristics of fruits of twin plants, representing the maternal phenotype, obtained from $F_1$ seeds

| Cultivars and hybrids | Plant symbols | Fruit | | | | | |
|---|---|---|---|---|---|---|---|
| | | number | mean weight (g) | lenght (cm) | width (cm) | wall thickness (mm) | shape coefficient |
| HPS | | 7 | 30 | 13.6 | 2.3 | 2.5 | 6.0 |
| Poznańska Słodka | | 5 | 49 | 11.0 | 4.9 | 3.0 | 2.2 |
| Sarga chilli | | 7 | 30 | 6.8 | 4.2 | 2.7 | 1.6 |
| Cuneo | | 4 | 41 | 4.4 | 4.0 | 4.5 | 1.1 |
| Paradisoni | | 3 | 26 | 3.2 | 4.5 | 5.4 | 0.7 |
| HPS × Cuneo | 19b | 7 | 29 | 14.6 | 2.0 | 2.5 | 7.4 |
| | 27a | 2 | 24 | 13.8 | 1.9 | 2.5 | 7.1 |
| | 29a | 5 | 25 | 12.2 | 2.6 | 2.2 | 4.7 |
| | control[1] | 11 | 56 | 9.4 | 4.4 | 3.3 | 2.1 |
| HPS × Paradisoni | 3a | 9 | 31 | 15.0 | 2.5 | 2.6 | 6.3 |
| | 5a [2] | 7 | 48 | 7.7 | 4.5 | 4.1 | 1.7 |
| | 5b | 3 | 24 | 13.1 | 2.4 | 2.1 | 5.7 |
| | 12a | 15 | 21 | 11.4 | 2.2 | 2.0 | 5.2 |
| | control[1] | 8 | 37 | 6.5 | 4.3 | 3.5 | 1.5 |
| Poznańska Słodka × Paradisoni | 1a [2] | 7 | 56 | 5.2 | 5.2 | 4.1 | 1.0 |
| | 1b | 7 | 45 | 9.2 | 4.6 | 3.0 | 2.0 |
| | 5a | 7 | 53 | 11.3 | 4.9 | 3.3 | 2.3 |
| | 5b | 6 | 53 | 11.2 | 4.8 | 3.5 | 2.4 |
| | control[1] | 4 | 42 | 7.9 | 4.8 | 3.5 | 1.7 |
| Sarga chilli × Paradisoni | 10a | 8 | 31 | 9.9 | 4.7 | 3.0 | 2.1 |
| | 10b | 3 | 25 | 9.5 | 4.5 | 2.4 | 2.1 |
| | 15a | 3 | 12 | 4.7 | 2.9 | 1.6 | 1.6 |
| | 15b | 4 | 19 | 5.9 | 3.4 | 2.2 | 1.8 |
| | 21a [2] | 4 | 36 | 4.6 | 5.0 | 4.0 | 0.9 |
| | 21b | 2 | 29 | 7.0 | 4.4 | 3.0 | 1.6 |
| | 22a [2] | 3 | 40 | 5.4 | 5.2 | 3.9 | 1.1 |
| | 22b | 1 | 28 | 7.1 | 4.5 | 2.3 | 1.6 |
| | control[1] | 5 | 58 | 5.7 | 5.7 | 4.5 | 1.0 |

[1] the mean for $F_1$ plants from monoembryonic seeds
[2] a twin partner representing hybrid phenotype

Two plants of the (Poznańska Słodka ×Paradisoni) $F_1$ hybrid, representing the phenotype of the maternal form constituted a twin pair. The third one designated with the symbol 1a, was from another pair, where a twin plant was a hybrid form. These plants were not accreted.

Four plants of the last hybrid, (Sarga chilli ×Paradisoni) $F_1$, made two pairs originating from two seeds. The remaining ones designated 21b and 22b, was obtained from the pairs, where twins showed character of $F_1$ hybrid. These plants were not accreted.

The fruits of all twin plants were red.

**Table 6.** Morphological characteristic of fruits of the twin plants obtained from $F_2$ seeds

| Hybrids | Plant symbols | Fruit | | | |
|---|---|---|---|---|---|
| | | length (cm) | width (cm) | wall thickness (mm) | shape coefficient |
| HPS × Cuneo | 2a | 5.4 | 3.6 | 2.0 | 1.5 |
| | 2b | 5.4 | 3.6 | 2.0 | 1.5 |
| | 3a | 5.1 | 2.9 | 2.1 | 1.8 |
| | 3b | 4.1 | 2.4 | 2.0 | 1.7 |
| | 4a | 7.6 | 3.9 | 2.4 | 1.9 |
| | 4b | 6.2 | 3.7 | 2.2 | 1.7 |
| | 5a | 7.3 | 2.4 | 1.7 | 3.1 |
| | 5b | 9.1 | 3.1 | 1.8 | 3.0 |
| | 6a | 9.4 | 4.9 | 3.7 | 1.9 |
| | 6b | 10.0 | 5.2 | 3.2 | 1.9 |
| | 7a | 6.6 | 2.9 | 2.1 | 2.2 |
| | 7b | 6.2 | 2.9 | 1.4 | 2.1 |
| | 10a | 7.7 | 4.4 | 2.5 | 1.8 |
| | 10b | 6.4 | 3.6 | 2.2 | 1.8 |
| HPS × Paradisoni | 1a | 16.2 | 3.1 | 2.4 | 5.2 |
| | 1b | 12.3 | 2.4 | 2.2 | 5.0 |
| | 2a | 4.7 | 4.3 | 3.5 | 1.1 |
| | 2b | 6.6 | 5.0 | 4.4 | 1..3 |
| | 3a | 4.5 | 5.2 | 5.8 | 0.09 |
| | 3b | 3.9 | 3.9 | 4.1 | 1.1 |
| | 4a | 6.2 | 5.5 | 4.0 | 1.1 |
| | 4b | 3.7 | 3.4 | 3.4 | 1.8 |
| | 6a | 3.4 | 4.1 | 4.5 | 0.0 |
| | 6b | 4.4 | 4.6 | 4.0 | 1.3 |
| | 8a | 6.7 | 5.0 | 3.9 | 1.5 |
| | 8b | 6.5 | 4.3 | 3.1 | 1.4 |
| | 9a | 6.2 | 4.5 | 3.8 | 1.[illegible] |
| | 9b | 5.9 | 3.9 | 3.5 | 1 |
| Poznańska Słodka × Cuneo | 4a | 6.3 | 4.2 | 2.5 | 1.5 |
| | 4b | 4.7 | 3.6 | 2.1 | 1.3 |
| | 5a | 6.4 | 4.8 | 3.1 | 1.3 |
| | 5b | 9.2 | 6.4 | 3.5 | 1.4 |
| | 6a | 8.9 | 5.5 | 3.1 | 1.6 |
| | 6b | 7.3 | 5.6 | 3.1 | 1.3 |
| | 7a | 5.3 | 4.0 | 2.3 | 1.3 |
| | 7b | 6.2 | 5.0 | 3.6 | 1.2 |
| | 8a | 8.0 | 5.1 | 3.3 | 1.6 |
| | 8b | 6.4 | 4.0 | 2.8 | 1.6 |
| | 9a | 6.6 | 5.9 | 3.5 | 1.1 |
| | 9b | 8.0 | 6.4 | 3.7 | 1.2 |
| | 10a | 7.9 | 5.7 | 3.7 | 1.4 |
| | 10b | 8.5 | 6.0 | 4.5 | 1.4 |
| | 11a | 7.5 | 4.4 | 3.0 | 1.7 |
| | 11b | 5.5 | 3.2 | 2.7 | 1.7 |
| | 12a | 10.0 | 4.3 | 2.6 | 2.3 |
| | 12b | 9.2 | 4.1 | 3.4 | 2.3 |
| Poznańska Słodka × Paradisoni | 1a | 10.5 | 4.6 | 3.1 | 2.3 |
| | 1b | 9.0 | 4.3 | 2.3 | 2.1 |
| Sarga chilli × Cuneo | 1a | 4.1 | 2.7 | 1.5 | 1.6 |
| | 1b | 5.2 | 3.3 | 1.7 | 1.6 |
| | 3a | 7.9 | 5.1 | 2.4 | 1.5 |
| | 3b | 6.3 | 4.5 | 2.5 | 1.4 |
| | 5a | 7.1 | 5.5 | 2.9 | 1.3 |
| | 5b | 5.0 | 3.9 | 2.5 | 1.3 |
| Sarga chilli × Paradisoni | 4a | 7.0 | 5.4 | 3.0 | 1.3 |
| | 4b | 5.6 | 4.8 | 3.2 | 1.2 |

## Twin plants in the progeny from twoembryonic seeds

In the phenotype analysis of plants, using morphological characters of fruits, attention was paid to the generation $F_2$, presented by the obtained twin plants. The phenotypes were also compared within twin pairs (Table 6).

The progeny of $F_2$ generation of twin plants of all the hybrids showed significant morphological differentiation. Some of them in which the paternal form was the cultivar Cuneo, had yellow and red fruits, characteristic of the parental forms. In the progenies of the remaining hybrids the presence of numerous recombinants was also observed.

Comparing twin plants within each pair, one can see their morphological similarity. Significant differentiation between individual pairs was accompanied by phenotypic similarity of twin plants from single seeds.

## Haploids from plants treated with growth regulators

An analysis was limited to three twin pairs of plants, where one of the partners was a haploid and the second one a diploid. This statement was made after some observations of meiosis in immature anthers of these plants. Indirect methods of estimating the ploidy level were used. However, they did not guarantee a proper classification of twin plants. Determination of the chromosome number at the first meiotic anaphase permitted to exclude haploid forms among plants, which they were initially referred to.

88 twin plants were brought to physiological maturity. This constituted 76% of their total number (Table 3). Three of them were haploids. All of them originated from twoembryonic seeds of the cultivar Sarga chilli. Two were obtained from the seeds of plants treated with 2, 4-dichloricnaphtoxyacetic acid, one of the plants hormonised by alpha-naphtalenacetic acid. The fruits of haploid plants were small and deformed (Table 7).

Two of the mentioned haploids designated as 21b and 25b, set only one fruit each. The first of them had eight seed including four with embryos. Four seeds

Table 7. Morphological characteristics of the fruits from $n$-$2n$ twin plants

| Plant origin | Plant symbols | | Fruit | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | number weight | mean weight (g) | lenght (cm) | width (cm) | wall thickness (mm) | shape coefficient |
| Sarga chilli — castration and 2,4 D hormonisation | 21a | $2n$ | 2 | 6 | 3.0 | 2.0 | 1.5 | 1.5 |
| | 21b | $n$ | 1 | 6 | 3.0 | 2.0 | 1.5 | 1.5 |
| Sarga chilli — 2,4D hormonisation | 25a | $2n$ | 8 | 27 | 8.1 | 3.9 | 2.0 | 2.1 |
| | 25b | $n$ | 1 | 14 | 4.5 | 3.0 | 1.5 | 1.5 |
| Sarga chilli — NAA hormonisation | 32a | $2n$ | 11 | 33 | 7.3 | 4.3 | 2.0 | 1.7 |
| | 32b | $n$ | 2 | 5 | 2.8 | 1.5 | 0.9 | 1.9 |
| Sarga chilli[1] | | $2n$ | 8 | 31 | 7.4 | 4.2 | 2.1 | 1.8 |

[1] control — the mean of 12 diploid plants obtained from monoembryonic seeds.

in the second fruit had embryos. Plants obtained from the seeds were diploid and presented the phenotype of the cultivar Sarga chilli.

Fruits of the third haploid, designated 32b, had numerous seeds, devoid of embryos.

## DISCUSSION

As mentioned in the introduction, a practical use of polyembryony in breeding depends on its frequency and origin of additional embryos. The undertaken studies are an attempt to determine the source of polyembryony under natural conditions and to induce it by growth regulators.

It should be recognized that the frequency of polyembryony determined as mean for cultivars and hybrids was lower than that found by Christensen and Bamford (1943) and Novak and Betlach (1969) for other cultivars of *Capsicum annuum* L., and by Morgan and Rappleye (1950) for *Capsicum frutescens.*

Significant is a comparison of hybrids and cultivars used for their production. Like in the studies of the above authors, marked differences were observed between the parental cultivars. Differentiation of the frequency was characteristic of material representing the effect of their crossing. It was, however, smaller, though in extreme cases it reached nearly 100 per cent. A similar range of variability was found in $F_2$ generation, which was the progeny of plants obtained from twoembryonic seeds. The values characterizing individual hybrids were similar to those observed in $F_1$. This fact suggests no influence of the seeds origin, i.e. the frequency of this phenomenon did not depend on whether the observed material was the progeny of plants from monoembryonic seeds, as it was in the $F_1$ generation, or whether it consisted of the progeny obtained from twin plants like $F_2$ generation. This suggestion seems to be proper only with regard to whole heterozygous material. Making an analysis of the results concerning the progenies of individual twin plant, it was found that there exists a very significant differentiation. Limiting our considerations to those twin plants whose seeds were found to have at least two two embryos it was detected that their portion increased. It was characteristic that they were the most among hybrids showing the highest frequency of polyembryony. The last one, therefore, may be treated as the effect of exceptional inclinations towards production of twoembryonic seeds in hardly several plants. It is more convincing, that since a small number of $F_1$ twin plants gave seeds among which some polyembryonic forms were observed. It is known at the same time that seeds of these plants constituted less than one half of the total number of germinating seeds.

This interpretation of the results permits to infer that forms with especially large inclinations towards the production of twoembryonic seeds may be selected. This conclusion is substantiated by the fact that similar results were obtained in some other hybrid material (Nowaczyk, Kryger, 1986). It was determined then that the heritability of this feature was high and amounted to 80%. Its value for the presented herein hybrids was 65%. Differences undoubtedly resulting from the application of different research methods in consequence of which material prepared in a different way was used. Neverthless, the heritability of polyembryony should be recognized to be large, and in a similar way the possibility of selection in this respect may be determined.

Therefore, crossing and selection of hybrids with regard to the tendency to polyembryony may permit the obtaining of forms with especially large abilities to produce seeds with many embryos. Practical results of these works on pepper are known (Selivanov, Tyrnov 1975).

Recognizing the above as one of possibilities permitting to increase the portion of twoembryonic seeds, the search for other possibilities was based on the use of growth substances applied for hormonisation of plants. A methodolical character of these studies was preserved by using some established cultivars instead of hybrids.

Like in the cultivars and hybrids discussed previously distinct differences in the frequency of the phenomenon were observed in the material from the control plants of different cultivars, which were not subjected to the effect of growth regulators. This confirms, therefore, the thesis about genetic control of the tendency to give twoembryonic seeds at a definite frequency. An increase of polyembryony frequency under the influence of growth regulators in three genetically different cultivars permits to suggest that similar results may be expected in other cultivars as well.

There would be only a problem of choosing the right growth regulator. The obtained results permit to solve this problem. A considerable growth of the phenomenon under the influence of 2,4-dichloricphenoxyacetic acid, and a similar reaction with beta-naphtoxyacetic acid indicate an especial suitability of just these growth substances, whereas the lack of plant response to 3-indolylbutric acid and alpha-naphtaleneacetic acid indicates that the last growth regulators should be excluded from such experiments at last at the concentration used during these investigations.

The lack of data concerning the induction of polyembryony by growth regulators inclined us to use results of one of the papers dealing with other species characterized by a specific biology of embryo development. They are formed in *Eranthis hiemalis* L. only for the period of some months after attaining maturity by the seeds, when they are alredy in the soil. Treating the seeds with 2,4 D soon after their falling from the plants caused that the number of twin embryos increased several tenfold (Haccius 1955).

The hybrids material and the parental forms were not found to have any haploid twin plants whose particular suitability was underlined before. The possibility of using diploid twins in breeding depends on their origin. Phenotype differentiation within several twin pairs of the $F_1$ generation, one of the partners of which was of matroclinal origin, whereas another developed in a sexual way, which was determined on the basis of the inheritance of some fruit characters in the $F_1$ hybrids (Nowaczyk 1982), seems to give some hopes. It is impossible, however, to determine accurately the origin of twin forms representing maternal phenotype. Exceptionally attractive from the practical point of view would be an attempt to elucidate the tendency towards reduced apogamy, explaining the diploid number of chromosomes by their spontaneous doubling before embryogenesis. This was confirmed by conclusions of Morgan and Rappleye (1954), on the basis of the results of investigations on the twin plants of intercultivar hybrids of *Capsicum frutescens* L. They simultaneously found that adventitious embryony and any other form of apomixis do not appear in the development of many diploid embryos of this species. A close relationship with *Capsicum annuum* L. make possible to use these conclusions. At this place it is worthwhile to quote a fragment of Kruse's work (1960), concerning beetroot. The author confirmed that in many cases monoploid twin plants developed seeds, which was the effect of a spontaneous chromosome doubling without colchicine-treatment. The homozygous character of diploid twins of matroclinal origin did not give the possibility of attestation the presented considerations. Not excluding adventitious embryony as a source of these diploids, it should be confirmed that the formation of numerous embryos in *Capsicum annuum* L. is the effect of splitting and apomixis polyembryony. There is, however, no place for independent development of twin embryos resulting from the egg cell fertilization and from fusion of the male gametes with the other haploid element of the embryo sac. This conclusion was based on the phenotypical similarity of the $F_2$ twin plants within pairs. The source of twin embryos in this case was only splitting polyembryony. Trying to explain that state of things, it cannot be excluded that castration of maternal plant flowers when preparing $F_1$ seeds could, being undoubtedly a stressogenic factor, induce adventitious embryony or apogamy, which were classified as possibilities leading to apomixis polyembryony.

An attempt to explain the presence of twin embryos in the $F_1$, of which each one represented the maternal phenotype, would be a mere speculation. It should be assumed that with the method used for the preparation of hybrid material, no self-pollination could occur. Accepting even such a possibility, the probability of the presence of twoembryonic seeds among several, which could be formed as a result of self-pollination, would be small, taking into account the frequency of this phenomenon.

Haploid forms were obtained exclusively in the material originating from plants treated with growth regulators. In the presence of a limited number of haploids no attempt was made to determine their frequency or to compare the obtained results with those of the other authors (Morgan, Rappleye 1954, Novak, Betlach 1969). It should be underlined once more that haploid forms were obtained only in the material originating from plants with hormonisation, whereas in other cultivars used in the mentioned papers they occurred at various frequency in the material collected from plants not exposed to the action of growth regulators. It is seems interesting, why in seeds constituting the material of these investigations and obtained from nonhormonized plants, no haploids were observed. The frequency of twoembryonic seeds in the plants of some hybrids was similar to that observed in both species of *Capsicum*. The presence of haploids among seedlings dying during germination cannot be excluded, since a lower vitality of these forms, particulary in competition with diploids in twin pairs is known. The survival of seedlings and twin plants was similar or larger than in other cultivars investigated by Christensen and Bamford (1943) and Novak and Betlach (1969). Therefore the possibilities of obtaining haploids were similar. Thus the lack of haploids permits to suggest the existence of genetic control of the tendency towards the development of apomictic haploid embryos in the cultivars representing different genotypes. At the same time, it seems doubtful to generalize conclusions concerning correlation between a high frequency of twoembryonic seeds and the presence of haploids under natural conditions, without the use of growth regulators or other physical or chemical factors. The reliability of the above is confirmed by results of earlier investigations (Nowaczyk 1979, Nowaczyk, Kryger 1986), where other hybrids of the cultivars presented herein were used as the studying material. A high frequency of polyembryony in some of them was not accompanied by the presence of haploid forms.

A stimulating effect of growth regulators due to a reduced apomixis should be recognized as doubtless. The number of the obtained haploids does not permit to determine clearly the effectiveness of the applied substances. However, limitation of our considerations to 2,4-dichloricphenoxyacetic acid and alpha-naphtaleneacetic acid is justified. Their application made possible the obtaining of twoembryonic seeds of the $n$-$2n$ type. Taking into consideration the action of the first of them increasing the frequency of polyembryony and its inducing effect on the occurrence of haploid forms, this growth substance should be recognized as especially suitable. It also plays an exceptional role among growth regulators used in the media of in vitro anther cultures. Many authors (Harn et al. 1975a, 1975b, Sibi et al. 1975, Dumas de Vaulx et al. 1981) show that 2,4-dichloricphenoxyacetic acid makes possible the formation of haploid callus tissue or induction of androgenesis.

Since haploids were observed in the established cultivar, an analysis of their origin was impossible. According to Morgan and Rappleye (1954), haploid forms in twin pairs occur as a result of embryogenesis from elements of the embryo sac with a reduced number of chromosomes. The most probable is their formation from synergids. Dumas de Vaulx (1985) report that they were not degenerated in 1 per cent of observed embryo sac after fertilization of the egg cell. Treatment of plants with $N_2O$ may increase their embryogenetic potency (Dumas de Vaulx Pochard 1974). Similar effects were obtained using pollen grains affected by X-rays (Campos 1955) for cross pollination.

However, such classification of haploid origin from the seeds of plants treated with growth regulators raises doubts, since a specific effect of 2,4-dichloricphenoxyacetic acid towards the induction of androgenesis is well known. Such origin of haploids induced by growth regulators after pollination cannot be excluded. Besides, it is known that the formation of androgenetic haploid embryos in natural conditions without growth substances (Campos, Morgan 1958) is possible.

Haploid plants appeared to be sterile to a high degree. In two of them only several normally-developed seeds were obtained. As it appeared, they had diploid embryos representing the genotype of the parental cultivar. It should be suggested that they were occurred as a result of selfpollination.

A similar situation was also observed in haploids of other cultivars (Pochard, Dumas de Vaulx 1979) setting few seeds after selfpollination. Studies of Thombre and Mehetre (1979) shows that chromosomes of some pollen mother cells of haploids may form six bivalents and, in consequence, normal pollen grains.

Setting seeds as a result of cross-pollination with pollen of diploid plants growing in the nearest neighbourhood of haploids cannot be excluded. Also, controlled pollination of haploids by pollen from diploid plants enabled obtaining normally developed seeds (Morgan 1975).

Summing up the results, it may be optimistically inferred that polyembryony can be used in breeding. Selection of forms with especially large tendencies to produce seeds with two or more embryos, a further increase in the portion of these seeds by treating plants with growth regulators at a simultaneous action in the direction of the induction of haploids in vivo create possibilities for working out the technology of a new breeding method. The haploids obtained from cultivar, which under natural conditions gives only diploid twin plants, seem to support this conclusion.

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## NATURALNA I INDUKOWANA POLIEMBRIONIA U PAPRYKI (*CAPSICUM ANNUUM* L.)

### Streszczenie

W badaniach nad naturalną poliembrionią u papryki wykorzystano odmiany oraz uzyskane w wyniku krzyżowania tych odmian mieszańce $F_1$ i $F_2$. Niektóre z obserwowanych w $F_2$ potomstw roślin bliźniaczych charakteryzowały się dużym udziałem nasion dwuzarodkowych.

Zasadniczym źródłem dodatkowych zarodków była poliembrionia rozszczepieniowa. Obserwowano także zarodki diploidalne o pochodzeniu matroklinalnym, towarzyszące w parach bliźniaczych zarodkom płciowym. Zaklasyfikowano to jako poliembrionię apomiktyczną. Niemożliwe było ustalenie, czy rozwinęły się one w wyniku embrionii przybyszowej, czy też były efektem embriogenezy z elementów woreczka zalążkowego o zredukowanej liczbie chromosomów, z poprzedzającą ją spontaniczną diploidyzacją. Wykluczono możliwości tworzenia się zarodków bliźniaczych w wyniku zapłodnienia komórki jajowej i innego elementu woreczka zalążkowego.

W badaniach nad poliembrionią indukowaną stosowano w czasie kwitnienia roślin regulatory wzrostu (kwas beta-naftoksyoctowy (BNOA) oraz 2,4-dwuchlorofenoksyoctowy (2,4-D)), które spowodowały kilkakrotny wzrost zjawiska poliembrionii.

Formy haploidalne w parach bliźniaczych $n$-$2n$ uzyskano tylko w materiale z roślin traktowanych regulatorami wzrostu. Szczególnie przydatny dla zwiększenia częstotliwości poliembrionii, jak też indukowania embriogenezy haploidalnej okazał się kwas, 24-dwuchlorofenoksyoctowy.

Stwierdzono genetyczne uwarunkowanie zjawiska poliembrionii. Selekcja form o skłonnościach do wydawania nasion dwuzarodkowych oraz zwiększanie częstotliwości zjawiska poliembrionii działaniem regulatorami wzrostu przy jednoczesnym indukowaniu haploidów in vivo, otwierają możliwości wykorzystania tego zjawiska w hodowli.

## НАТУРАЛЬНАЯ И ИНДУЦИРОВАННАЯ ПОЛИЭМБРИОНИЯ У СТРУЧКОВОГО ПЕРЦА (*CAPSICUM ANNUUM* L.)

### Резюме

В исследованиях натуральной и индуцированной полиэмбрионии у стручкового перца были использованы его сорта и полученные в результате скрещивания этих сортов гибриды $F_1$ и $F_2$. Некоторые из наблюдаемых в $F_2$ потомоств растений-близнецов характеризовались большой долей двузародышевых семян.

Основным источником дополнительных зародышей была расщепляющаяся полиэмбриония. Наблюдались также диплоидные зародыши матроклинного происхождения, сопровождающие половые зародыщи в парах близнецов. Классифицируется это как апомиктическая полиэмбриония. Нельзя было установить: развились ли они в результате адвентициальной эмбрионии или были результатом эмбриогенеза из элементов зародышевого мешка с сокращённым числом хромосом с предществующей ей спонтанической диплоидизацией. Была исключена возможность образования близнячих зародыщей в результате оплодотворения яйцеклетки и другого элемента зародышевого мешка.

В исследованиях индуцированной полиэмбрионии во время цветения растений использовались регуляторы роста (бета-нафтоксиуксусная кислота — BNOA и 2,4-дихлорфенолукснусая кислота — 2,4-D), которые вызвали взрост явления полиэмбрионии в несколько раз.

Гаплоидные формы в близнячих парах с $n$-$2n$ были получены только в материале растений, к которым были применены регуляторы роста. Особенно способствовала увеличению частоты полиэмбрионии, а также индуцированию гаплоидной эмбриогенезы 2,4-дихлорфенолуксусная кислота.

Установлено, что явление полиэмбрионии является генетически обусловленным. Селекция форм со склонностью к образованию двузародышевых семян, а также увеличение частоты полиэмбрионии в результате действия регуляторов роста при одновременном индуцировании гаплоидов in vivo открывают возможности использования этого явления в разведении растений.