A COMPARATIVE STUDY OF THE N, P AND AMINO ACID CONTENT OF COLCHICINE TREATED POLYPLOID PAPRIKA PLANT

by

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The cultivation of paprika can at present be successful in Hungarv only by planting seedlings owing to the short vegetation period. Cultivation on a large scale would be greatly promoted by sowing the seeds instead of planting seedlings. Since according to certain authors, the polyploid forms endure cool weather much better than the diploids (HERTZSCH, 5) we have tried to solve the problem of field sowing by using polyploid plants (BARANOV, 1). We wanted to produce the polyploid plants by colchicine treatment. It is known that the samples so bred are usually misshapen, poorly developed and the new tetraploid type is shown only in the following generations (GYÖRFFI, 3, 4; PÁLFI, et al. 8; SEDLMAYER, 10). The process of re-balancing the inner conditions, disturbed by the increase of genoms, goes on for several generations before the vitality of the plant is restored since with the doubling of the genoms several genetic factors may also be doubled, and so it can be assumed that the vitamin „C" content may become greatly increased in the tetraploid paprika. On the basis of such considereations other useful qualities such as the colour, vitamins, flavour and volatile substances may be improved (MÜNTZING, 7).

For a systematic study it seemed necessary to carry out continuous observation of the morphological and physiological or biochemical differences appearing as the result of the colchicine treatment and their eventual relations.

Materials and Methods

The expperimental plant was capsaicin-free spice paprika (*Capsicum annuum* L. var. *longa*, forma *Szegediensis*). The plants sprouted in Petri dishes and then planted into growing vessels with rich soil, were treated when growing cone appeared between the cotyledons. During treatment the plants were kept at 28° C with a 90% relative humidity under artificial light, then they were planted in the open field. In order to ensure identical and perfect nutrition, the

plants were watered with a full nutrient solution from the time when they had 10 to 12 leaves till flowering.

For the treatement 0,2% of colchicine was used dropped every 3 hours for 48 hours on the growing top between the two cotyledons. In order to control the genoms, after the development of the fourth foliage leaf, tissue samples were taken from the growing tops (stem-meristen). The preparations optained by the carmine acetic acid method were examined with the help of an oil immersion phase contrast apparatus (SÁRKÁNY—SZALAI, 9).

Fully developed leaves were used for analysis and they were picked every 5 days. First their fresh weight was determined, then after drying at 65° C their air-dry weight. After grinding to a fine powder the NO_3—N was demonstrated by means of phenol disulpho acid, the inorganic phosphorus by means of molybdic sulphuric acid reagent, and all the nitrogen and the total amount of phosphorus after destruction with sulphuric acid — salicylic acid — perchloric acid by means of Nessler' reagent and molybdic sulphuric acid.

In the ascending paper chromatography method for the demonstration of free amino acids we made use of repeated runnings. This we modified by bringing up also amino acids of known quality and quantity besides the extracts because identification of the different amino acids on the basis of the Rf value is no longer possible owing to the repeated runnings. The amino acid standards were set up according to the expected amino acid compounds after long experimentation with extracts from several species of plants belonging to different families. Of course, at the beginning of the analyses in the case of a plant with an unknown amino acid compound isatin identification was also necessary for the setting up of the standard, while the quantitative analysis needed the eluation of the spots developed with 0,5% of ninhydrin dissolved in aceton and fixed with copper salt and the photometric examination of the coloured alcoholic solution. To prepare leaf grist (ground leaf) of 50 mg dry weight is 3 or 4 times rubbed away with 100 mg of quartz sand and 10 ml of 50% etanol is gradually added or washed through in a centrifuge tube. This is centrifuged at 5000 rpm for 15 minutes, then the liquid part is carefully poured into a porcelain bowl and dried at 65° C. Then the free amino acids are dissolved with 1 ml of 50% watery isopropyl alcohol and from this a quantity of 0,05—0,2 ml is put on Whatman No. 1 paper. The solvent used in the 3 or 4 times repeated running is a mixture of butanol: acetic acid : water (2 : 1 : 1). The complex spots of the amids were dissolved and, after hydrolysis with hydrochloric acid, run again.

Results

About 80% of the colchicine-treated plants became polyploid, 10% remained diploid, and another 10% perished. We found that the polyploid plants developed more slowly than the diploids but by the time of examination they reached about the same degree of development as the latter concerning the number and size of the leaves. Morphological differences: The polyploid plants mostly developed shoots with short stems and thick pulpous, darkgreen leaves. The leaf stems are longer and thicker, the leaves and the leaf stems are asymmetrically constructed. In the polyplois plants the number of the lobes of the petals is often greater, the number of the pollen sacks is also nearly double. The

number of the stomata in the epidermis of the leaves is greater in the diploid plants, but the stomata are smaller than in the polyploids.

The change in the arrangement of the chromosomes is shown in Fig. 1. Laying the chromosomes in one plane is rarely made successfully. The chromo-

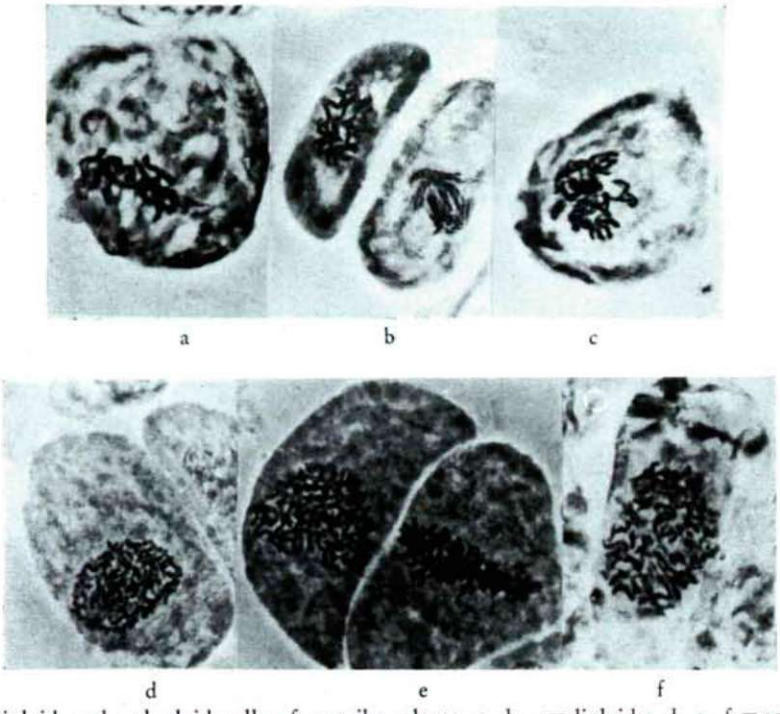

Fig. 1. Diploid and polyploid cells of paprika plants. a, b, c=diploids; d, e, f,=polyploids. Magnification: × 2700

somes of the polyploid cells are generally smaller and thinner. The tissues are, here too, chymeric in structure, but mixoploidia is not unknown in nature either (BRUNKENER, 2; LIPAEVA, 6).

As shown in Fig. 2 the average dry weight of the diploid leaf is always greater then that of the polyploid leaf suggesting that the productivity of the polyploid individuals decreases in the year of the treatment. Also in regard to the nitrate-nitrogen and total nitrogen content of the leaves conditions were more favourable in the case of diploid individuals (Fig. 2). We found more inorganic phosphorus in the polyploid in three cases out of four analyses, yet the amount of organic phosphorus and total amount of phosphorus was greater in all four analyses. This seems to indicate a less intensive synthesis of organic phosphorus int the polyploid plant. The free amino acid content of the polyploid plants was in all cases greater than in the diploid. In this respect no amino acid or amid is an exception. In the leaves of the paprika plant glutamine acid, aspartic acid, γ — amino butyric acid, alanine, leucine, valine, lysine, and cistine can be demonstrated to be present in larger quantities. In this order we plotted curves of the quantities of the first four amino acids (Fig. 3.). The

greater total N and inorganic P content of the leaves of diploid plants and the greater amino acid content of polyploid plants seems to indicate that the development of the polyploid form is accompanied by disturbance of the protein synthesis. This is proved also by the smaller dry matter content of poly-

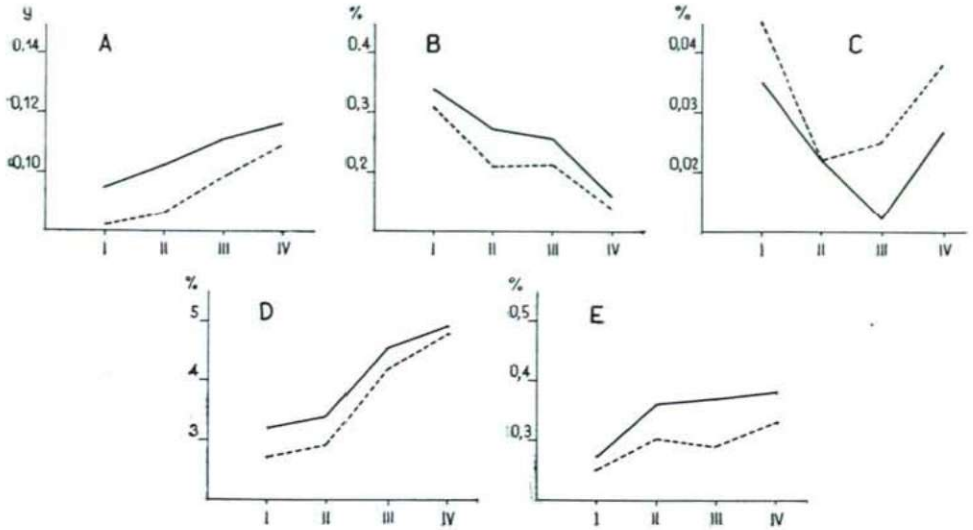

Fig. 2. Average of dry weight of the one leaves (A), nitrate-N (B), inorg.-P (C), total-N (D), and total-P (E) contents of the leaves in the dry weight percentage. ——— = diploids; — — — — = polyploids plants. I, II, III = vegetative development, IV. = flowering

ploid paprika. This polyploid form on he other hand may give better developed materials for heterosis crossfertilization than the diploid. We found that spontaneous crossfertilization was succesful between the polyploid form and such distantly related kinds as do not otherwise crossfertilize with the kinds common in our country. We will report on the results of similar investigations of the already developed polyploid forms (C_3 and C_4) in the future.

Summary

We have carried out polyploidisation with colchicine to breed paprika that can be sown into the field without seedling planting and which can endure the cool, unfavourable spring weather of our country. Microscopis examination revealed mixoploidia but the majority of the cells (80%) were tetraploid. In order to elucidate the physiological changes we examined semples taken on four occasions to compare the nitrogen phosphorus and amino acid quantity in the polyploid and in the original diploid paprika. The results of the examinations were as follows:

1. In all each of the four examinations the nitrate and total nitrogen content of the leaves was higher in the diploid plants than in the tetraploid.

2. The organic and total phosphorus content of the diploid plants exceeds the values of the polyploid plants, although the amount of inorganic phosphorus was greater in the latter. This indicates that owing to the distru-

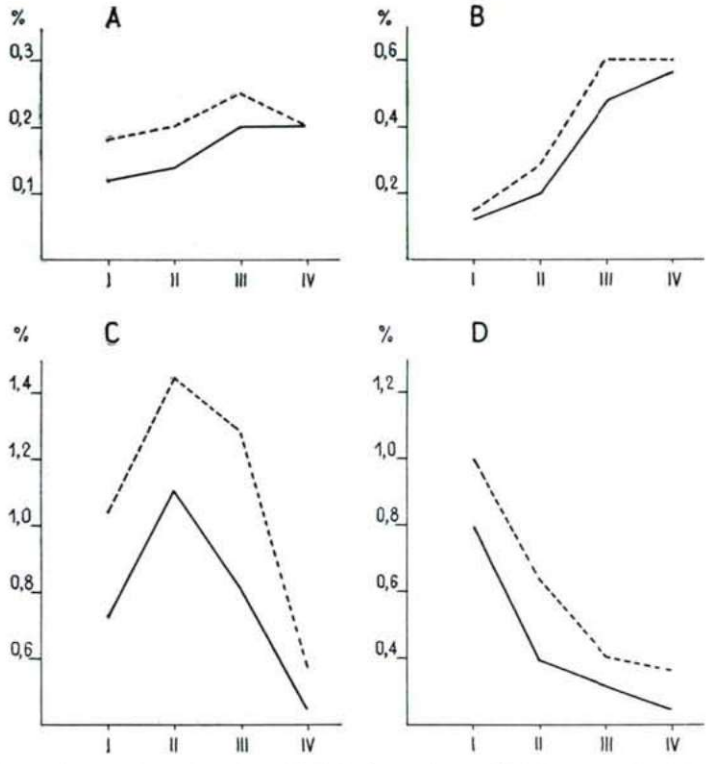

Fig. 3. Alanine (A), γ-amino butyric acid (B) glutamine acid (C), aspartic acid (D) content of diploid (————) and polyploids (— — — —) paprika leaves in dry weight percentage I, II, III = vegetativ development,, IV. = flowering

bance caused by the treatment the synthesis of the organic phosphates containing great energy is slower in the polyploid paprika.

3. The amount of free amino acid of the leaves of the polyploid plants was greater in the case of each amino acid than in the leaves of the diploid plants and this fact is a sign of disturbed protein synthesis. This assumption is further corroborated also by the lesser dry matter content of polyploid paprika plants.

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