SYNTHESIS OF NEW GENOTYPES WITHIN THE GENUS BRASSICA BY IN VITRO CULTURE

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It has appeared, as well during the course of own experiments as in the opinion of other scientists /Inomata 1977/, that crossings of diploid species Brassica campestris /2n=20/ and B. oleracea /2n=18/, aiming at obtaining favourable oleiferous and fodder forms of artificial B. napus /2n=38/ with help of traditional methods, has little effectiveness. The applying of in vitro techniques in the interspecific crossings, evidently increases the amount of successful crosses. These techniques, as it has been stated in many experiments allow to omit a range of barriers during the course of interspecific crossings and enable to shorten considerably the time of obtaining favourable hybrids.

MATERIALS AND METHODS

Biochemical characters, and especially the erucic acid and glucosinolates content, were the main criterion in selection of plant material for inteded crosses. As maternal forms, the following varieties of B. campestris were used :

1. ssp.oleifera - c.v. : Candle and Torpe,
2. sp.pekinensis - c.v. : Granaat, Nagaoka WR 55 Days, Chinese cabbage
3. ssp.trilocularis -c.v. : Yellow sarson.

The Canadian turnip variety Candle, besides low glucosinolates, has also lower erucic acid content and yellow seed coat. Varieties belonging to the subspecies pekinensis have in general also lower glucosinolates content. Because of the usefulness from the agronomical point of view of the yellow seed coat, the yellow seeded variety Yellow sarson was also included to the crossings.

After checking up glucosinolates content by gas chromatography method, varieties with lower glucosinolates content have been selected, from the possessed plant material of B. oleracea, and following forms have been crossed:

1. var. capitata - c.v. Gloria, Darkrii, Taurus, Chogo, Leo No. 80, Tucana, Market Topper, Head Start,
2. var. sabauda - c.v. Predzvest,
3. var. gemmifera- c.v. Bastion.

The flower buds of B.campestris have been emasculated and isolated in isolators of thin aluminium foil. After two days, emasculated flowers have been pollinated with fresh pollen of B.oleracea. Pollinated ovaries have been gathered in different time-limits from the date of pollination /4-10 days/ and sterilized /70% C_2H_5OH, 0,1% $HgCl_2$ solution, H_2O dest. sterile/. The ovaries have been layed out on agar medium in sterile conditions and cultured in a culture room during about 30-40 days in temperature 22°C and 12 hrs illumination.

Then in sterile conditions, embryos isolated from the ovaries have been transferred to the agar medium. The tubes with transplanted embryos have been placed at first in the darkness. During the course of their development, the embryos have been transferred to the culture room in conditions identical to these applied for the cultured ovaries. Plants, which have developed 3 to 4 leaves and proper root system, have been placed in pots filled with soil for further cultivation in the greenhouse.

The somatic chromosome number was determined in the root tips stained with Schiffs dye and squashed in acetocarmin. The viability of pollen grains was established according to the number of dyed grains in Bellings mixture.

RESULTS

During the course of initial investigations, different methods of sterilizing of plant material and different mediums have been tested. The best results have been obtained on the medium of Murashige and Skoog /1962/ with modified organic substances, containing 5% saccharose, whereas the isolated embryos were transplanted on medium with 1-2% saccharose. It has been stated, that too high sugar concentration and overabundance of protein substances as well, on the medium on which the embryos have been cultured, cause the disturbances in the root system development. Similar observations have been done by Harberd/1969/, who stated, that too rich medium causes the drying up of radicle and after transferring of such embryo on a poor medium the root system does not develop.

From the observations dealing with the dependence between the number of embryos isolated and the time of isolation of pollinated ovaries after pollination it may be concluded, that seed setting in ovaries growing in vitro is higher when the ovaries are transferred to the medium in later term from the date of pollination. On this account more favourable seems to be the in-vitro method of isolated embryos cultivation proposed by Gland /1980/, who isolated embryos direct from the pods, after 28 days from the date of pollination, it means at the moment when the embryos according to Håkansson/1956/should reach the full maturity.

During the embryos isolation, different kinds of their degeneration have been observed. Very often in welldeveloped seeds, filled with endosperm, there have been found embryos in the early developmental stage of "Torpedo" or "Walking-stick". Embryos in the stage of full maturity have been isolated from the drying up, devoided of endosperm seeds. It happened very often, that the embryos during the stage of full maturity were in far advanced decay, brown, or there remained, instead of them, the rest of a slimy tissue. Similar observations have been noticed also through other authors: Harberd /1969/, Gland /1980/.

The embryos, after transfer in a noninjuried state on the medium, developed regularly, forming the shoot and roots. Besides normally developing embryos, different deformations have been noticed. One of them was the excessive development of the cotyledons, which formed thick formations in fantastic shapes - mostly such seedlings stopped in their further development. Another example of disturbances were embryos, by which the root system developed normally, but instead of cotyledons, callus tissue aroused, from which, after the change of medium and the addition of growth substances normally growing seedlings regenerated. There were also plants with welldeveloped roots and normal cotyledons, which - during further development had disturbances in the growth of leaves appearing in strong leave blades deformations. These seedlings, after forming some deformated leaves, died. In spite of strong deformations, some of the injured seedlings were able to develop and formed normal plants. Similar remarks are to be found in the publication of Gland /1980/, who is of the opinion, that the injuries of the embryos during their isolation have a stimulating effect on their development.

The quantitative results of plant material by in vitro cultures of hybrid embryos are reduced, in a great extent, by the difficulty of keeping alive the seedlings, after transferring them to the soil. Great amount of plants dies on account of fungal infection or drys up because of low air humidity in the growth chambers.

In general there have been cultured on mediums 2188 ovaries, from which 252 embryos have been isolated in different developmental states. 74 of them gave rise to the plantlets. But unfortunately only 19 plants survived after transferring to the soil. At present we're possessing hybrid plants from the following crossing combinations:

1. B. campestris ssp.oleifera Candle x B.oleracea var.sabauda Predzvest
   2 plants completely sterile,
   1 plant partly fertile, pollen viability 10%, 2n=19.
   Till now, the doubling of chromosomes was not successful. All these three plants have been crossed with their maternal form. The progeny obtained from these crosses will be evaluated during this vegetation period.

2. B.campestris ssp.oelifera Candle x B.oleracea var.gemmifera Bastion
   3 plants completely sterile,
   1 plant partly fertile, pollen viability 10%,

1 plant, whose pollen grains viability amounted 84%. As the results of selfpollination of this plant, seeds of F_2 progeny have been obtained. This progeny will be evaluated during current vegetation period. These seeds have been partially sown in the autumn 1982 in the experimental field aiming at checking their winterhardiness. They overwintered in 73%.

3. B.campestris ssp.pekinensis Nagaoka WR 55 Days x B.oleracea var.sabauda Predzvest
   4 plants with markedly lower fertility amounting from 8-10%, 1 plant completely sterile, whose somatic chromosome number 2n=19. After effective colchicine treatment and doubling of chromosomes to 2n=38, artificial B.napus was obtained. At present the socalled C_1 and C_2 progeny of this hybrid has been sown in the field conditions, to check the winterhardiness of these plants. C_1 progeny overwintered in 74%, while the plants of C_2 - in 85%.

4. B.campestris ssp.pekinensis Granaat x B.oleracea var.capitata Market Topper
   2 plants completely sterile. They have been crossed with yellow seeded forms of B.campestris. Seeds were obtained from one crossings combination only. The obtained hybrids will be evaluated during this vegetation period,
   3 plants with markedly lowered pollen grains viability, inside of the limits 10-15%. Thanks to the self-fertility of these plants a small amount of F_2 progeny seeds was gained. These hybrids will be evaluated during this vegetation period.

5. B. campestris ssp.pekinensis Chinese cabbage x B.oleracea var.capitata Market Topper
   1 plant, pollen viability 15%, number of chromosomes 2n=19.
   6 seeds have been gathered from the F_2 progeny, they will be evaluated during this period of vegetation.

Conclusions

1. The essential problem in obtaining new artificial forms of B.napus on the basis of crosses between the diploid species B.campestris and B.oleracea is the lack of favourable oleiferous forms of rape, which is rather difficult.

2. The use of in vitro technique in the work dealing with new rape genotypes synthesis is undoubtedly necessary in the cases when obtaining positive results in crossings with the help of traditional method is difficult. This happens especially, when the material for crosses must be selected according to the agronomical and biochemical values and not according to the mutual crossability.

3. On the basis of observations, dealing with morphological characters of the plant material obtained, it is not yet possible to draw clear conclusions, according to the utilitarian value for oleiferous or fodder purposes. This will be possible during the course of obtaining further generations and higher amounts of plants in each combination.

Reference :

1. Gland A. 1980. Glucosinolatgehalt und Muster in den Samen resynthetisierter Rapsformen. Dissertation Univ. Göttingen.

2. Hakansson A., 1956. Seed development of Brassica oleracea and B.rapa after certain reciprocal pollinations. Hereditas 42:373-396.

3. Harberd D.J., 1969. A simple effective embryo culture technique for Brassica. Euphytica 18:425-429.

4. Inomata N., 1977. Production of interspecific hybrids between Brassica campestris and Brassica oleracea by culture in vitro of excised ovaries.
I. Effects of yeast extract and casein hydrolizate on the development of excised ovaries.
Jap. J. Breed. vol. 27, NO; 4.

5. Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tissue cultures.
Physiol. Plant. 15: 473-479.