ANDROGENESIS IN WINTER RAPESEED /BRASSICA NAPUS L./

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To gain haploid and then dihaploid plants through androgenesis is a difficult task. This technique, however, may be very advantageous for plant breeding. Dihaploids obtained in this way are fully homozygous plants, which allows to shorten the breeding cycle by 6-10 years. Also, segregation ratios for traits conditioned by a few genes are more profitable on the haploid level.

Works on androgenesis in B. napus were carried out by : Thomas, Wenzel /3/, Keller, Armstrong /2/, Stringam /4/, Hansson /5/, Loh, Ingram /6/.
They obtained a very differentiated embryoids yield, from 1 embryo per 7000 anthers to 2 embryoids per 1 anther. Despite the same media and incubation conditions, the repeatability of results is very variable. In our Institute attempts to obtain haploid plants have been made since three years, however, only in 1981 a larger set of embryoid was obtained.

METHOD

The method applied in 1981 may be divided into the following stages :

- choice of plants - inflorescences were collected from plants of F_1 generation, healthy, with undamaged rosette and a root after vernalization. Buds 3,0-3,5 mm large were taken for preparation.
- surface sterilization - buds were sterilized by method elaborated in our Institute. In this method "Pertlenon" preparation which contains urea hydroperoxide is used; atomic oxygen appearing in the solution is an aseptic factor.

Solution composition:

1 tablet of Pertlenon,
20 ml H_2O

2 drops of detergent solution.
Buds were sterilized for 15 minutes and then rinsed in sterile water. Then anthers were prepared,

- anther preparation - attention was paid to complete separation of anther thread from the anther. Leaving that tissue causes diploid rhisogenesis,
- medium - agar medium B_5 according to Gamborg /1/ was applied. This medium was supplemented with :

  saccharose to 10%,
  0,1 mg/l 2, 4 D,
  0,1 mg/l NAA,
  800 mg/l L-glutamine,
  100 mg/l L-serine,

- anther incubation was conducted at 28°C in darkness, and after two weeks, temperature was changed to 25°C,
- embryoid incubation - when the embryoids appeared, they were transferred into B_5 medium with normal saccharose concentration /2%/, and additional lightning with glow-lamps "Flora" for 12 per 24 hours was applied.

RESULTS

Totally, in 1981, 3109 anthers were laid out on agar medium. The obtained results are shown in table 1.

Thermical shock was applied to first samples of inflorescences by placing them for 16 hours at 35°C. In this experiment not any embryoid was obtained per 387 anthers. In the remaining experiment, samples of inflorescences for anther preparation were taken directly from the refrigerator. Embryoids were obtained in all these experiments however with a very differentiated yield. Further studies are needed to reveal which factors cause that differentiation.

In the next year /1982/ 41 embryoids of winter rape were obtained from one thousand of inoculated anthers. Similarly to the work of Loh and Ingram /6/ a vigorous development of secondary embryoids was observed. These embyoids were passaged for one to following mediums:

1. B_5 10% $C_{12}H_{22}O_{11}$ + 0,1 mg/l 2,4D + 0,1mg/l NAA
2. B_5 2% $C_{12}H_{22}O_{11}$
3. MS 3% $C_{12}H_{22}O_{11}$ + 2mg/l Glicyne + 1mg/l BAP
4. B_5 2% $C_{12}H_{22}O_{11}$
5. B_5 2% $C_{12}H_{22}O_{11}$ + 1mg/l BAP+0,1mg/l NAA+0,1 mg/l 2,4D
6. B_5 2% $C_{12}H_{22}O_{11}$
7. MS 3% $C_{12}H_{22}O_{11}$ + 2mg/l Glicyne + 1mg/l BAP
8. B 2 $C_{12}H_{22}O_{11}$

Beginning from the third medium, a part of embryoids started to develop into plantlets. One part of embryoids did not develop into plantlets at all. Using different combinations of medium 250 plants belonging to 34 clones were obtained.

CONCLUSION

Obtained results indicate the possibility of using androgenesis in rape breeding. This problem, however, still needs futher elaboration.

REFERENCES

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3. Wenzel G., Hoffmann F., Thomas E., 1977, Z. Pflanzenzüchtg. 78, 149-155.
4. Wilfred A., Keller W.A., Gary R. Stringam, 1978, Proceedings of the 4th International Congress of Plant Tissue and Cell Culture, Calgary, Alberta, Canada, August 20-25, 1978, 113-122.
5. Badil Hansson, 1978, Sveriges Utsädesförenings Tidskrift, 3, 141-148.
6. C.S. Loh and Ingram, Cruciferae Newsletter No. 6, Eucarpia 14-17.

Table-1 : Results of anther incubation in 1981.

Start of anther culture	additional thermical shock	No. of anthers planted on medium	No. of obtained embryoids	No. of anthers giving embryoids
21 April 1981	+	54	–	–
22 April 1981		333	–	–
23 April 1981	–	339	1	1
24 April 1981		451	1	1
25 April 1981		606	14	9
27 April 1981		407	42	10
30 April 1981		234	182	6
5 May 1981		298	3	3