REVIEW OF SESSION G
GENETICS AND METHODS - MICROSPORE CULTURE

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INTRODUCTION

Remarkable progress has been made throughout the last fifteen years in the field of cell and tissue culture techniques. As a consequence, techniques like embryo and microspore culture are often considered as routine methods for rapeseed breeding. Nevertheless some problems still need further consideration. A general conclusion from microspore culture studies reported in this session is that genotype, physiological state of the donor plant, bud size and microspore stage, culture medium and incubation temperature are crucial to the production of haploid embryoids in *Brassica*. For example, genotypically different responses need to be solved in order to allow a broader application of isolated microspore culture techniques in practical breeding programmes for *Brassica napus, B. campestris* (*B. rapa*), *B. oleracea*, and *B. juncea*.

CONTENT OF POSTERS

Thirteen posters were included in the session on 'Genetics and Methods - Microspore Culture' and they could be divided in five groups:

- Culture conditions, *in vitro* development and applications of DH lines
  (Ferrie and Keller, 1995, Gland-Zwerger, 1995, Jang *et al.*, 1995, Minami, 1995, Ohkawa *et al.*, 1995, Sodhi *et al.*, 1995, Wakui *et al.*, 1995)
- Inheritance and *in vitro* selection for quality traits
  (Möllers and Albrecht, 1995, Naleczyńska and Cegielska, 1995, Stringam and Thiagarajah, 1995)
- Nitrogen efficiency
  (Gerath and Balko, 1995)
- Microspore cultures of interspecific hybrids
  (Zhou and Scarth, 1995)
- Field evaluation
  (Dewan *et al.*, 1995)

Culture conditions, *in vitro* development and applications of DH lines

The most effective way to produce haploids has been through isolated microspore culture. The effects of genotype, bud size, microspore stage, culture conditions and different media on embryoid production and plantlet regeneration were investigated (Ferrie and Keller, 1995, Gland-Zwerger, 1995, Jang *et al.*, 1995). Five different media, four *B. napus*, four *B. campestris* and three *B. oleracea* genotypes were included in an experiment to improve the yield of embryogenesis from isolated microspore culture (Gland-Zwerger, 1995). In most cases, an incubation treatment at 35 °C only for 18 h followed by 10 days at 30 °C increased the number and quality of embryoids per bud considerably.

The number of embryoids per bud ranged from 1 to 33 for *B. oleracea*, from 1 to 89 for *B. campestris* and from 9 to 465 for *B. napus*, depending on genotype, medium, and temperature treatment.

The relationship of bud and anther size to the precise stage of microspore development at the beginning of culture and the difference between donor plant genotypes had been investigated by Jang *et al.* (1995). They used three winter and three spring cultivars of *B. napus* for their experiments. The petri dishes were treated at 32.5 °C for 3 days and then maintained at 25 °C. Embryoid yields and frequency of normal developed plantlet production showed remarkable contrast according to the six genotypes. The highest frequency of embryogenesis was obtained from cultures of bud sizes from 2.8 to 3.2 mm with mainly late uninucleate stage of microspores. The efficiency of spring types was much better than in winter types. The embryo yield of the spring types differed obviously, according to the genotype.

Ferrie and Keller (1995) used 23 *B. campestris* cultivars for their investigations. Sufficient numbers of embryoids were obtained from all genotypes except the cultivar Horizon which did not respond. The embryoid yield per bud ranged from 0.02 to 20.06. The observed genotypic differences in the study are similar to the results of other laboratories. Selection studies within the line DSC-3 resulted in the identification of a highly responding line. This line produced from 20 to 160 embryoids per bud depending on the treatment. Highly embryogenic lines were used for the mutagenesis studies. Mutagen has been applied to the microspores and embryoids have been recovered. Desiccation studies have been conducted using mirospore-derived embryoids. A system for microspore transformation is being developed.

Nitrogen assimilation during androgenetic embryogenesis was investigated in *B. napus* (Ohkawa *et al.*, 1995). Nitrate salts were not used while cultured microspores developed to heart-shaped embryoids. Once androgenetic embryogenesis was initiated, microspores developed to torpedo-shaped stage in a medium with ammonium salt and glutamate. Glutamine was an essential component for initiating androgenetic embryogenesis as well as for normal embryo development in *B. napus*.

Minami (1995) established an *in vitro* selection methodology for obtaining nitrate-reductase mutants of *B. napus* cv. Lisandra. Screening is carried out for $KClO_3$ resistance of the embryoids which derived from isolated microspores mutagenized with gamma rays.

The successful induction of haploid plants from cms juncea and cms Tokumasu in rapeseed was reported by Sodhi *et al.* (1995). Haploid plants have been obtained from these cms lines despite of low concentration of potential microspores. Microspore development is inhibited at premeiotic stage in cms juncea and at postmeiotic stage in cms Tokumasu. The bud size was approximately 2 mm. Total DNA of 26 cms juncea and nine cms Tokumasu regenerants was digested with Hind III and Eco R1. Hybridization with the mitochondrial specific gene probes cox III and atp 9, respectively, showed the banding pattern of the corresponding cms cytoplasms. Experiments have been initiated to fuse haploid protoplasts from cms juncea and cms Tokumasu. An attempt will be made to search for male fertile diploid (2n) plants, because fusion of two different cytoplasmic male sterile lines has been reported recently to restore male fertility in *Nicotiana tabacum*. RFLP analysis will be conducted on mitochondrial DNA from male fertile rapeseed plants and compared to parental male sterile cms lines. Sodhi *et al.* (1995) expect to ascertain the mitochondrial DNA fragment associated with male sterility in *B. napus*.

To examine LEA (late embryogenesis abundant) gene expression in *B. napus* and *B. campestris* on microspore-derived embryoids, Wakui *et al.* (1995) isolated total RNA from the embryoids which were treated with ABA (desiccation tolerant) for one week and without ABA (desiccation sensitive). Northern analysis was carried out using *B. napus* LEA 76 clone as a probe. The accumulation of LEA mRNA is detected in desiccation

tolerant embryoids, but not in desiccation sensitive ones. LEA mRNA expressed in the microspore-derived embryoids of *B. napus* as well as those of *B. campestris*. The DNA sequences of cDNA of LEA genes isolated from embryoids were determined. The predicted amino acid sequence of a clone of *B. napus* had 95 % of homology with LEA 76 clone, and that of *B. campestris* had 70 % with this clone. The induction of desiccation tolerance of microspore-derived embryoids will be of great importance for the production of artifical seeds.

Inheritance and *in vitro* selection for quality traits

Alkenyl glucosinolate segregation patterns were studied in F_2, BC_1, and F_1-derived doubled haploid populations arising from a cross between a high glucosinolate East Indian cultivar, RLM 514, and a low glucosinolate *B. juncea* breeding line from the University of Alberta breeding programme (Stringam and Thiagarajah, 1995). A wide array of total glucosinolate levels were observed consisting of various combinations of propenyl, butenyl, and pentenyl glucosinolates. The glucosinolate content of the DH lines was skewed towards higher total alkenyl glucosinolate levels. In some DH lines, individual glucosinolate levels for propenyl or pentenyl, surpassed the levels found in the high parent. Segregation ratios observed in the DH lines for total alkenyl glucosinolates fitted both the 1:31 and 1:255 ratios, indicating that 5 to 8 recessive allels control the complete absence of alkenyl glucosinolates in *B. juncea*.

The availability of new qualities of rapeseed oil with, e. g., a very high oleic acid content, a very high erucic acid content or a low linolenic acid content is increasing rapidly due to the tools of mutation breeding, interspecific protoplast fusion, and molecular gene transfer (Möllers and Albrecht, 1995). From the segregating microspore population derived from F_1-plants arising from the cross between DH lines of 'Duplo' and 'Janetzki' a total of 134 regenerants were obtained. The genotypes were divided into three classes based on the sum of eicosenoic acid (C20:1) + erucic acid (C22:1). The results demonstrated that 60 - 70 % of the regenerants may be discarded (89 of 134) at an early *in vitro* stage because of the low content of C22:1 + C20:1 and only the remaining regenerants with the highest erucic acid content *in vitro* have to be transferred to the greenhouse in order to obtain the 10 genotypes with the highest erucic acid content in the seeds. The production of DH lines in combination with an early selection for the desired fatty acid composition represents an efficient tool for the selection of specific genotypes from a reduced population size.

Microspore-derived embryoids of *B. napus* as a potential system for rapid screening for fatty acid composition have been studied by Naleczyńska and Cegielska (1995). They recommend to use the early *in vitro* selection for detecting desirable genotypes.

Nitrogen efficiency

DH lines are a useful basic material for the development of *B. napus* genotypes with improved N-efficiency (Gerath and Balko, 1995). The N-efficiency of DH lines was influenced significantly by the genotype of the donor plant and single DH lines from one donor plant differed significantly in their N-efficiency. The authors suggested that the test for N-efficiency should be carried out under a low medium and high N-supply to assign DH lines to N-reaction types and to make it possible to calculate indices of N-efficiency.

Microspore culture of interspecific hybrids

Zhou and Scarth (1995) studied the feasibility of applying microspore culture in the

stabilization of interspecific hybrids from reciprocal crosses between *B. napus* x *B. campestris* and between *B. napus* x *B. juncea*. Among the 57 microspore-derived plants from *B. napus* x *B. campestris* hybrid and its reciprocal, 25 had chromosome numbers from n=10 to n=19 and 32 had chromosome numbers greater than n=19.

The six regenerants from the *B. napus* x *B. juncea* hybrid had chromosome numbers ranging from 21 to 42, only two plants had 27 chromosomes. The study confirmed that the gametes produced from interspecific hybrids have embryogenic capacity under *in vitro* culture conditions.

Field evaluation

Dewan *et al.* (1995) present data on seed yield of microspore-derived DH lines of *B. campestris* and their parent populations. In 1993, 43 DH lines from three populations and in 1994, 131 DH lines from five populations were tested. The average seed yield per plant of DH lines was 31 % and 25 % of parent populations in 1993 and 1994, respectively. The lower average seed yield of DH lines compared to the parent populations was due to the expression of deleterious recessive allels in the homozygous state. The best DH line yielded 80 % of its parent population. The ranges for seed yield for the DH lines and the parent populations were significantly different in both years. The higher yielding DH lines had growth characteristics similar to their parent populations and suffered significantly less from inbreeding depression.

CONCLUSIONS

Androgenetic embryogenesis is principally inducible in *Brassica* species with sufficient frequencies, however, it still strongly depends on the genotype. Genotype screening, identification of an embryogenic line, and an efficient microspore culture system are necessary for the application of haploid technology. Microspores, haploid embryoids, and doubled haploid plants can used in mutagenic treatment and transformation studies as well as in plant breeding programmes.

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