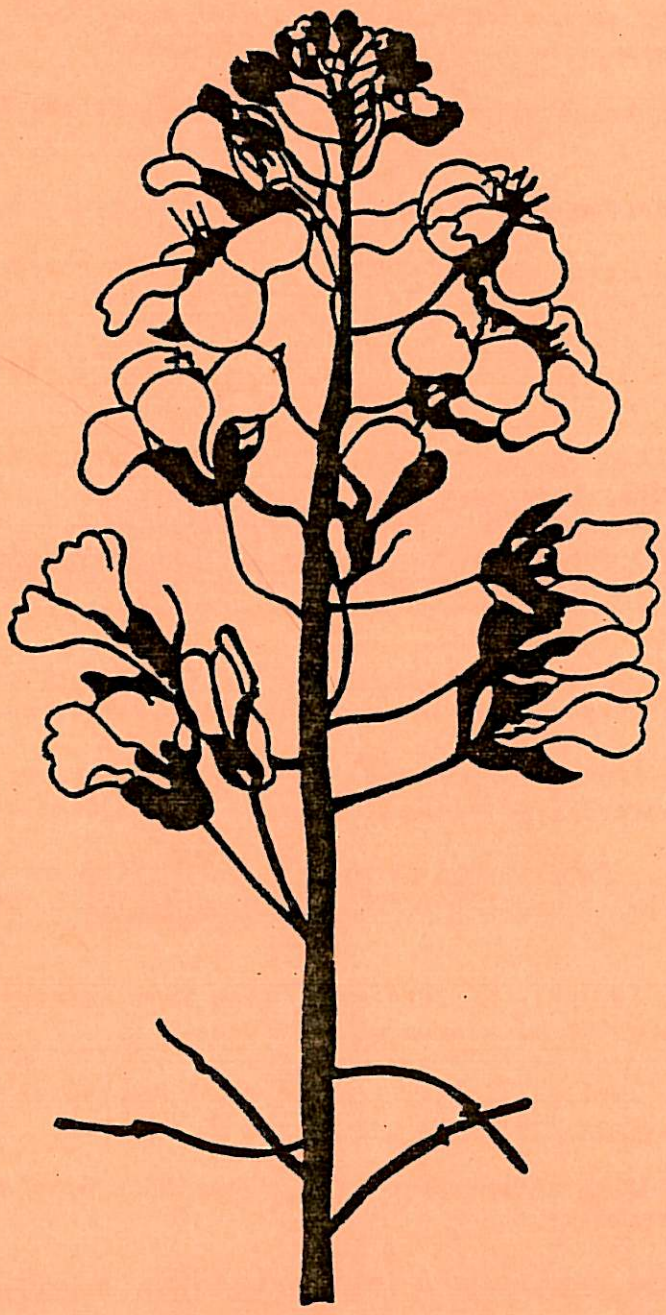
CRUCIFERAE NEWSLETTER No.6



November 1981

EUCARPIA

NOTICE

Mr. Roger Whitehouse has relinquished his joint editorship of the Newsletter and will retire from the Scottish Crop Research Institute, Pentlandfield (formerly the Scottish Plant Breeding Station) on December 31st, 1981.

Roger was one of the original editors, together with Chris North who retired in 1977, he was responsible for steering the development of the Newsletter since its first issue in 1976, and was instrumental in raising funds for publication and by encouraging authors to submit articles.

He is going to live on the Isle of Mull, on the West coast of Scotland, and we wish him a long and happy retirement and pleasant sailing days.

His place as editor has been filled by Ian McNaughton, also of SCRI, Pentlandfield.

A.B.W. & I.H.McN

Editorial

The Editors wish to express their gratitude to those companies who responded to the appeal for donations to support publication of the Newsletter. A list of these firms is appended. So far £562 (after deduction of bank charges) has been received which, with monies from other sources received earlier, brings the balance held in the bank to £1100.

This issue is being distributed to 274 individuals and organisations in 27 different countries and all 5 continents at an estimated cost for printing and mailing of £375. The funds in hand are adequate therefore for at least a further full year.

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REPORT OF INTERNATIONAL CLUBROOT WORKERS MEETING 1981

G.R. Dixon

The Eucarpia Vegetables Section 'Brassica Conference' held at the Agricultural University of Norway provided an opportunity for a meeting of International Clubroot Workers. Papers presented and discussions could be grouped under the following headings:

1. General studies of resistance to Plasmodiophora brassicae in Brassica spp.

Chiang and Crete (Canada) reported resistance in rutabaga to be controlled by small numbers of genes with reasonably high heritability. The latter finding is similar to the results of Hansen (Norway) with clubroot resistant cabbage who also showed "tolerance" to clubroot in B. oleracea material was recessive. A selection of fodder rape (FRM) with field resistance to P. brassicae was reported by Svads and Skaland (Norway) while the use of callus culture to purify P. brassicae isolates was described by Jonsson (Sweden). Evidence that in selecting for P. brassicae resistance there is selection to depress thiocyanate content of B. oleracea was presented by Chiang (Canada).

2. Use of the European Clubroot Differential (ECD) Series and interpretation of results from ECD work

Professor Campbell (USA) provided evidence of the variability present in some ECD differentials and at the same time described the rapidly increasing importance of clubroot in California. Results from a survey of P. brassicae collections from southern Scotland indicated little ability to attack B. campestris differentials (Brokenshire & Lewis, UK). This paper also contained data on the production of monoxenic resting spore suspensions (Fox Roberts & Mohammed, Scotland, UK). Studies of P. brassica populations in Germany (Heyn) and Finland (Linnasalmi & Toivianen) were reported. Use of some ECD hosts to study the composition and nature of P. brassicae collections and factors which complicate the understanding of P. brassicae populations were described by Dixon, Jones & Ingram (UK). Collation, tabulation and analysis of results published in the Clubroot Newsletter were reported by two groups: Toxopeus, Dixon & Mattusch (Netherlands, UK & Federal Republic of Germany) and by Crute, Barnes, Buczacki & Crisp (UK). The latter group concluded that resistance in B. campestris and B. napus was largely differential while that in B. oleracea was non-differential. Tests with ECD hosts plus additional material and use of an hierarchial ranking system indicated the best performance was by Bohmerwaldkol.

3. Chemical and other means of controlling P. brassicae.

Control of P. brassicae by calcium cyanamide and more recently nitrobenzene derivatives in Czechoslovakia was reported by Zvara. Experiments showing the effect of raising soil pH by use of a range of lime forms indicated an increase in cabbage yield but were very environmentally dependent (Balvoll, Norway).

Locations for future meetings are:

International Horticultural Congress, Hamburg 1982
International Congress of Plant Pathology, Melbourne 1983

The Clubroot Group wishes to thank Dr. J. Apeland, Professor A.R. Persson, Dr. N. Skaland and Dr. G. Weisaeth of Agricultural University, Aas, Norway, for their help in arranging these discussions.

FRANCO-BRITISH COLLABORATION

W.H.Macfarlane Smith

International collaboration between French and British forage brassica workers has advanced as a result of a two day meeting held at La Station D'Amelioration des Plantes (INRA), Le Rheu, Rennes, France. The British Group, which was led by Dr. W. H. Macfarlane Smith of the Scottish Crop Research Institute, Pentlandfield, consisted of Dr. J. E. Bradshaw (also SCRI - P), Dr. A. Gosden (Welsh Plant Breeding Station), Mr. J. E. Newton (Grassland Research Institute), Mr. D. Kimber (National Institute of Agricultural Botany) and Mr. M. B. Cooper (Agricultural Development and Advisory Service). The French group, led by Dr. J. Morice, Director of INRA, Le Rheu, consisted of Drs. J. Barloy, M. G. du Crehu, M. R. Giovanni, M. D. Le Floch, Marianne Buson, M. Renard and P. Rousselle.

Initial discussions indicated similar crop interests and breeding objectives in Britain and France, though there is no significant area of swedes (Brassica napus) in the latter. The relative breeding effort in the public and private sectors, and the relationship between them, seemed similar in the two countries, though there is no state marketing equivalent of the National Seed Development Organisation in France. Public/private sector collaborate projects in France are aimed at broadening the genotic base eg. by the production of Brussels sprouts x kale hybrids to provide improved parents. However, there is no possibility of disseminating young breeding material at eg. the F3 stage.

The markets being aimed at consist of 200,000 hectares of forage rape and 150,000 ha of kale in France, and 83,000 ha of swedes and turnips, 33,000 ha of kale and cabbage and 20,000 ha of forage rape in Britain. It is clear that forage brassicas occupy a much more important place in farm rotations in France than in Britain.

While breeding objectives are similar, techniques differ with, for example, the French following a double- or triple-cross kale breeding programme, while the British prefer a population improvement scheme. Similarly the French fodder rape programme has put most of its effort into F1 hybrids while the British programme has concentrated on intervarietal crosses, or crosses between varieties and lines of "artifical napus" produced from the interspecific cross between B.campestris and B.oleracea, followed by pedigree selection.

The agronomy of kale growing is of particular interest in France, as this crop can have an adverse effect on the following maize crop. In addition, there are studies on sowing techniques, timing and level of fertilizer application, soil structure and drainage using lysimeters and including the effect of compaction by grazing annuals, and climate. Techniques such as direct drilling and zero grazing are of proven value, though French farmers are reluctant to change from traditional methods. Conversely in Britain most of the kale and around 40% of the rape crop is sown by direct drilling.

Animal studies are important in both countries with work being carried out on both sheep and cattle. Of particular interest are the anti-nutritional factors , SMCO and the allyl isothiocyanates, with both groups of breeders agreed on the need to reduce the levels of these in kale but as yet uncertain of their importance in rape.

There are proposals for exchange visits between scientists to compare programmes on disease resistance breeding, tissue culture, animal work and infra-red analysis of anti-nutritional factors, and for the evaluation of each other's material. There will also be an exchange of unpublished data to avoid both unnecessary duplication of research and the use of techniques already shown to be unsuccessful. A further meeting to review progress is proposed for 1983 at SCRI - Mylnefield.

REPORT OF THE THIRD ANNUAL MEETING OF BRITISH AGRICULTURAL RESEARCH SERVICE BRASSICA BREEDERS

John Taylor

The third meeting of ARS brassica breeders was held at the Plant Breeding Institute, Cambridge, on 25 and 26 September, 1981. It was attended by 17 specialist brassica research staff from 4 A.R.C. institutes, plus 4 invited representatives from N.S.D.O., N.I.A.B. and F.R.I. Seven members of staff from other P.B.I. departments also attended to discuss topics of more general interest.

Discussions covered such topics as collaborative trials, the incidence and spread of brassica diseases, crop pollution and infestation due to volunteer rape distribution, and the application of infra red techniques in chemical analysis. Practical demonstrations were arranged to illustrate pathological techniques and kale breeding.

To broaden the scope of the meeting talks by specialists in related fields of research were arranged on topics such as genetic engineering, protoclones and tissue culture, and the effects of glucosinolates in the diet of humans and animals. These talks served to provide an insight into some valuable new techniques available to the breeder, drawing attention to the possibilities and limitations of these techniques, and also allowing a useful contact to be made between those engaged in fundamental research and the practical plant breeders.

It was decided that the event might henceforth be held biennially, the next meeting to be arranged in 1983, unless an urgent problem arose in the interval. A meeting of pathology specialists was requested in 1982.

BREEDING RESEARCH ON COLE CROPS AT THE IVT, WAGENINGEN

Q.P. van der Meer

It became obvious that complete elimination of the chilling phenomenon, from the m.s. Bannerot-material, by selection of chilling restoring maintainer lines would be a long way and would result in a rather limited base for the breeding of hybrids. So chilling still looks a severe bottle-neck.
By now the most promising results are those obtained with the (non completely male sterile) material received from Chiang (described by him in recent articles in Euphytica). Further backcrossing of this material (being completely free from chilling) to cauliflower delivered an offspring being attractive to bees and showing a good seed set in the open. Incomplete male sterility seems to be a draw-back of this material. Up to now most plants under glas did show some pollen shedding whereas plants in the open did not. Chiang supposes that older plants give more pollen. We are paying attention to this point and to the influence of environmental factors.
Analysing the back-ground of bolting in Chinese cabbage resulted in the following preliminary conclusions:

- Daylength does hardly influence the bolting process.
- Lower light intensity enlarges the vegetative period.
- Sufficiently low temperatures result in nearly equally long vegetative periods for a vast range of Chinese cabbage and stubble turnip varieties.
- Differences in the duration of the vegetative period are correlated with differences in optimum vernalization temperature and differences in vernalization requirement at supra optimal vernalization temperatures.
- Increasing temperatures shorten the vegetative period, decreasing temperatures do the opposite.

CRUCIFEROUS CROP GENETIC RESOURCES

A.B. Wills

A report on recent and proposed activities of the Eucarpia Crucifer Genetic Conservation Group (CGCG), and the International Board for Plant Genetic Resources (IBPGR) global plan for crucifers was presented at the Eucarpia 'Cruciferae 1981' Conference at Aas, Norway, by Hille Toxopeus and Dick van Sloten. Activities planned on behalf of the CGCG for the near future include the collection of land-races of Brassica species in northern Europe and the sending of a second circular to obtain further information so that accessions held by CGCG members may be better classified.

Over a short period much has been done to establish a basic planning framework for the conservation of cruciferous crops and details of these accomplishments can be found in the reports listed below.

Anon. (1981) Genetic resources of cruciferous crops. AGP/IBPGR/80/100.

Toxopeus, H. and Crisp, P. (1980) Status of genetic resources of cruciferous crops in Europe. AGP/IBPGR/80/70.

Toxopeus, H. and Sloten, D. van (1981) The genetic resources of cruciferous crops - a global plan of action. Proceedings Eucarpia Cruciferae 1981 Conference.

PRODUCTION AND RESEARCH OF RAPESEED IN THE PEOPLES REPUBLIC OF CHINA

Fu Ting Dong

There was about 3,000,000 ha of rapeseed in China this year. Spring rapeseed is about 20% of this total, while the rest is winter rapeseed. Recently, the average yield was about 750 kg/ha.

70% of winter rapeseed growth is in the region of Yangtze River, 80% of varieties grown there are B.napus. Generally, rapeseed and rice or cotton are grown in a two or three cropping rotation system in one year. In the region of the Yangtze River, about 60% of the rapeseed area is in a three cropping system (one rapeseed crop and two rice crops). The rest is in a two cropping system (one rapeseed crop, one rice or one cotton crop). The average yield of two rice (or one rice) crop is about 6,000-10,000 kg/ha. The yield of rapeseed is 750-1,100 kg/ha. The yield of rapeseed in the surrounding areas of Shanghai (the area of rapeseed is about 53,000 ha. there) is the highest in the region of the Yangtze River. In the 1950s, the mean yield was less than 750 kg/ha. there. In the 1960s, it was over 1,150 kg/ha. In the 1970s, it was over 1,800 kg/ha. In the last two years, the yield has reached 2,000 kg/ha.

Spring rapeseed is mainly distributed in the northwest provinces of our country. Most of the varieties grown here are B.campestris and B.chinesis, while the rest are B.napus and B.juncea. The varieties of B.campestris grow well at 3,700-4,200m above sea-level in the western plateau. The density of planting is very close (about 300 - 500 plants/m^2) there. At about 3,000m above sea level there are scarcely any frost-free periods. The varieties are very early in maturing (about 90-100 days). As the sunshine is very strong and there is little disease and insect pest problems, the yield is 1,000 - 2,300 kg/ha.

Long, long ago, our people cultivated rapeseed for vegetable or oil uses. Recently, carbonized seeds of B.juncea and B.rapa from the New Stone Age were unearthed from Ban Po village in Xi An city. They are seeds from 6,000 years ago as identified by C^{14}. However, records from our ancient agricultural books indicate that the period of rapeseed cultivation was over 2,000 years ago.

In about 20 provinces Academy of Agricultural Sciences have departments or groups of rapeseed research. Major research on rapeseed is as follows:-

Firstly, there is breeding of new varieties for improved yield and maturity. Since 1953, 130 varieties and lines of B.napus have been bred in our country. Most of them mature early and are adapted to a two or three crop rotation system. Since 1975, Prof. Dr. Liu Hou Li and I have discovered and studied yellow seeds of B.napus, which originated from the variety Huayu No. 3 coming from the interspecific hybrid (B.napus x B.campestris). At present our emphasis is aimed at (1) the purity of yellow seeds, (2) selection of better agronomic characteristics and (3) research on genetic bases of yellow seedcoat. Certain new strains of low erucic acid were bred by our

scientists in the Institute of Oil Crops.

Secondly, there is cultivation for higher yield of seed, especially the research for higher yield of rapeseed within a three crop rotation (two rice and one rapeseed) in a year. It is possible to obtain a yield of 2,300 - 3,000 kg/ha. of rapeseed in the case of three crop rotation in a large area. The emphasis in managing a normal field is the stage before winter. The number of the green leaves before winter correlates highly with the yield. According to the estimation of regression coefficient, within the extent from 4-12 green leaves per plant, (about 150,000 plants/ha. in the region of the Yangtze River) if the number of thr egreen leaves increases by one, the yield may increase by 225 - 265 kg/ha. During the winter stage, if the number of the green leaves per plant can reach 10-12, the average yield of rapeseed may be over 2,300 kg/ha. in a three crop rotation.

Thirdly, there has been research on the application of trace fertilizer, especially on the application of B(boron). It is quite important for certain kinds of soil lacking B in our country. The yield of rapeseed is severely reduced by the lack of B. It my reduce yield by 30-50%, if it is possible to harvest any at all. But the effect on the yield is much more serious in B.napus than in B.campestris and B.juncea. By applying 5-7 kg/ha. of Boride (Na_2B_4 $10H_2O$), we can avoid such effects.

Fourthly, since 1965, the research on the utilization of hybrid vigour has been started in our country. The main research focuses on the cytoplasmic male sterility (Hunan province and Sichuan province Academy of Agricultural Sciences) and self-incompatibility (my college) of B.napus. Scientists in my college (1972) and Hunan province (1973) discovered natural cytoplasmic male sterility in variety "Bo Li Ma" (from Poland). They are influenced by temperature. When the temperature is high, some of them are fertile (when the temperature is low they are sterile). It is interesting to note that some of them are sterile even when the temperature is high (when the temperature is low they are fertile). Their petals are small. In addition, our scientists discovered some nuclear sterile materials. Their petals are very normal. Since 1972, my colleagues and I have been studying breeding of self-incompatible (SI) lines. In 1975, we bred some SI lines, such as "211", "271", "219" and so on. From 1976 to 1981 we have engaged in regional testing and productive testing for the yielding ability of hybrid rapeseed. (SI x variety or line). But it is necessary to spend much more labour when we reproduce SI lines using selfing of flowerbuds by hand. In order to solve this problem, we began to study breeding of SI maintainers in 1977. Now we are trying to produce and test SI three way hybrids of B.napus.

In addition, research includes work on plasm resource, research on protection from diseases and pests, on physiology, on pollen culture, etc.

In the last years, the planting area of rapeseed has increased very quickly in our country. According to recent estimates, it will be possible to increase the area to 4,000,000 ha. in the future.

SOME RECENT RESEARCH on WILD MEMBERS of the BRASSICEAE

C. Gómez-Campo

Geography. Former Cape Verde Sinapidendron species should be definitely ascribed to the genus Diplotaxis (Rustan & Borgen, 1979)where they show strong affinities to the Sect. Pendulina. They have n=13 chromosome number (Borgen, 1975) and are fully interfertile with D. harra (Sobrino pers. comm.). Madeiran Sinapidendron seems to be an apparently relictic distinct genus. Canarian members were thought to be extinct since the 1930s, but some individuals have been refound by Borgen et al.(1979) who conclude they are members of the n=9 Mediterranean group of Brassica. Whether this is an ancient natural introduction or a recent historic one, remains unknown.

Cariology. Known chromosome numbers in the tribe have increased by a factor of 2, 5 in the last decade (check-list by Gómez-Campo & Hinata, 1980). A polyploid series (4n, 6n, 8n) has been detected in Moricandia by Sobrino (1978); the diploid, if it exists, remains to be found. Other polyploid series has been found in Vella (Gómez-Campo, 1981)where the exaploid level in fact corresponds to the closely related genus Boleum. Also, the basic number n=8 that was apparently missing in Diplotaxis has been found to occur in D. siettiana (Takahata & Hinata, 1978)and D. ibicensis (Gómez-Campo, 1981).

Other aspects. Many characters from the sterile pieces of the flower (sepals, petals and nectaries) have been numerically analyzed by Clemente & Hernández-Bermejo (1980) over a large number of taxa. A multivariate analysis of the genera which are the most closely related to Brassica has been done by Takahata & Hinata (1980).Recent interfertility studies within the Brassica closest allies have been conducted by Harberd (1980). Monographic studies on Hutera (Leadley, 1978)and Brassica (Salmeen, 1979) have been carried out in the University of Reading as Ph. D. theses.

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CRUCIFERAE IN WESTERN ANDALUCIA (SPAIN)

J.E. HERNANDEZ-BERMEJO and M. CLEMENTE-MUÑOZ

As part of the project "Flora of Western Andalucía" patronized by several Botany Departments of three Spanish Universities, the Cátedra de Botánica Agrícola (Escuela Técnica Superior Ingenieros Agrónomos) of Córdoba was asked to study the Cruciferae family. After three years of extensive work, including field prospection in most areas of the four different provinces of Western Andalucía (Cádiz, Córdoba, Huelva and Sevilla), we give a preview here of some of the data to be included in the catalogue and chrological synthesis of this family.

The level of diversification in Cruciferae is especially marked in Southern Spain, and nearby areas of Northern Africa, thus Marocco, Algeria and Andalucía (Spain) are one of the main diversity centers of this family. The floristic richness is seen not only in the number of species but also in the endemicity percentage. We have found in Western Andalucía taxa: a) of the Ibero-North-African area such as: Biscutella baetica Boiss. et Reuter, B. microcarpa DC., B. sempervirens L., Brassica barrelieri (L.) Janka, B. oxyrrhina Cosson, Crambe filiformis Jacq., Diplotaxis catholica (L.) DC., D. siifolia G. Kunze, D. virgata (Cav.) DC., Iberis gibraltarica L., Ionopsidium prolongoi (Boiss.) Batt, Lepidium hirtum (L.) Sm. subsp. calycotrichum (Kunze) Thell., Malcolmia lacera (L.) DC., Sisymbrium crassifolium Cav., b) exclusive of the Iberian Peninsula, such as Draba hispanica Boiss., Iberis crenata Lam., Iberis linifolia Loefl., Moricandia moricandioides (Boiss.) Heywood, Sisymbrium arundanum Boiss. and c) endemics to Andalucía such as Biscutella varieta Boiss. and Reuter, Erysimum rondae Polatschek, Hutera longirostra (Boiss.) Gómez-Campo, Lepidium hirtum (L.) Sm. subsp. petrophilum (Cosson) Thell.

After our research in several herbaria (MAF, MA, SEV, COA, COFC), a bibliographic revision, and numerous field trip to the region, we have found 120 Cruciferae species and about 1000 localities catalogued for western Andalucía.

Besides the compilation of unpublished localities (taken from the herbaria revision), our direct contribution includes new localities, some of which belong to species which have hardly ever been mentioned before such as: Biscutella frutescens Cosson (HERNANDEZ-BERMEJO: Pico Bermejo in Sierra Halconera -Córdoba-, COA: 649), Moricandia moricandioides (HERNANDEZ-BERMEJO, CLEMENTE-MUÑOZ and A. PUJADAS: Rute-Iznájar -Córdoba-, COA: 411; HERNANDEZ-BERMEJO: Coripe-Algodonales -Sevilla-, COA: 328; HERNANDEZ-BERMEJO and CLEMENTE-MUÑOZ: valle del Anzur -Córdoba-, COA: 567; HERNANDEZ-BERMEJO and CLEMENTE-MUÑOZ: Baena -Córdoba-, COA:574), Sisymbrella aspera (L.) Spach subsp. boissieri (Cosson) Heywood (HERNANDEZ-BERMEJO and CLEMENTE-MUÑOZ: Sierra de Cabra -Córdoba- COA: 576 and 592), as well as the mention of new species found for Western Andalucian flora: Brassica repanda (Willd.) DC. subsp. confusa (Emberger and Maire) Heywood (HERNANDEZ-BERMEJO and M.J. PUENTES:Grazalema -Cádiz-, COA: 255), Hutera longirostra (Boiss.) Gómez-Campo (HERNANDEZ-BERMEJO, CLEMENTE-MUÑOZ, COSTA TENORIO and H. SAINZ: valle del Guadiato -Córdoba- MAF:103832; HERNANDEZ-BERMEJO and M. CLEMENTE: Cerro Chimorra -Córdoba- COA 350), and Sisymbrium austriacum subsp. hispanicum (Jacq.) P.W. Ball and Heywood (HERNANDEZ-BERMEJO and CLEMENTE-MUÑOZ: Sierra de Cabra -Córdoba-, COA: 351).

We hope that this study (in preparation), will be of interest to plant breeders and germ-plasm collectors because it deels with the Cruciferae chorology in one of its richest genetic regions.

CRAMBE (CRAMBE ABYSSINICA HOCHST.)

RESULTS FROM SWEDISH CULTIVATION EXPERIMENTS 1976-1979

Anders Bengtsson and Gösta Olsson

During the period 1976-1979 the cultivation value of Crambe was studied at several places in Sweden. In 17 trials,Crambe (variety Meyer) was compared with spring turnip rape (variety Span), and in 15 trials with spring rape (variety Olga). 14 different Crambe varieties were tested in comparison with the variety Meyer. Some trials with different seed rates and harvest times were also carired out.

Species trials. The average yield of 17 trials, Crambe produced 1,435 kg seed per hectare (15 per cent moisture) with an oil content of 38.9 per cent of the dry matter. Thus,the yield of oil was 475 kg per hectare. The crop is high yielding with the seed yield being about the same as that of spring turnip rape, but about 40 per cent lower than spring rape. The yield of seed varied between 520 and 3,200 kg per hectare. Factors causing decreases in yield were frost damage, heavy weed infection, attacks of *Alternaria*, and especially shattering. In all these respects Crambe is more susceptible to damage than spring turnip rape. On the other hand Crambe is less infected by aphids than rape and turnip rape.

Crambe ripened 4 days later than spring turnip rape but 8 days earlier than spring rape. The plant has a very strong resistance to lodging.

The unhulled (normal) seed (it means here the whole pod) has low bulk weight, 25-30 kg per hectolitre. In contrast, hulled seed (seed without pod) has a high bulk weight, about 60 kg per hectolitre. The content of erucic acid varied between 49 and 61 per cent with an average of 57 per cent. The lowest content of erucic acid was in the two northernmost trials where Crambe did not ripen satisfactorily. The contents of crude protein and fibre of the dry matter of the unhulled seed were 23 and 17 per cent respectively. The content of glucosinolates was 5.8 per cent. The hull was 14-16 per cent. The hulled seed had the following contents: oil about 45 per cent of the dry matter, protein about 30 per cent, and crude fibre about 7 per cent.

Variety trials. Only small differences in yield and quality were found between the varieties tested. However, there were distinct differences between the varieties in morphology, earliness, yielding ability, and quality. Nevertheless, the content of erucic acid was almost the same in all the varieties. All the varieties were probably closely related.

Cultivation trials. Mainly, the influence of harvest time was studied in these trials. It was demonstrated that Crambe must be combined early to avoid severe shattering.

MULTIVARIATE STATISTICAL ANALYSIS AS A TOOL FOR GENETICAL AND TAXONOMICAL INVESTIGATIONS IN CRUCIFERAE

Walter Titz

Two years ago I reported the synthesis of an artificial tetraploid in the Arabis hirsuta group as a possible model for the origin of tetraploid A.hirsuta (L.)Scop. s.str. : Arabis sagittata x ciliata, tetraploidized by colchicine (TITZ 1979 a,b). Besides hybridizing the synthetic allotetraploid with true natural A.hirsuta s.str., these both were also compared morphologically in order to examine the correspondence between them. The comparison of 15 morphological characters, evaluated in a 15fold t-test by TITZ (1979b), could be much more effectively performed by means of discriminant analysis: all characters are regarded simultaneously and are weighted in dependence on their ability to discriminate the two different phenotypes. In our case discriminant analysis yielded an extremely clear difference between the artificial and the natural tetraploid (TITZ 1982b): the discriminant scores of artificial Arabis sagittata x ciliata range from -6,5 to -1,5, those of natural Arabis hirsuta s.str. from +1,5 to +6,5. Considerable genetic diversity of the artificial and the natural tetraploid must be the cause for this clear-cut phenotypical difference as well as for the known intersterility between them (TITZ 1979 a,b).

Discriminant (or Canonical) Analysis, Factor and Cluster Analysis proved to be well suited also for solving other taxonomical problems in Cruciferae and other families (e.g. Valerianaceae), especially if there is a great number of quantitative characters to be dealt with (TITZ 1982a and unpublished).

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MULTIPLE REGRESSION AND PARTIAL CORRELATION ANALYSIS OF YIELD CHARACTERS IN OIL SEED RAPE

W. Odenbach and P. Lemansky

The plant material,used in this study,was grown at 4 locations in 1979, including 5 lines,derived from crosses between synthetic rape (no. 129) and zero erucic rape types and the variety "Quinta" (no. 135) as a standard.The following characters were observed: 1.)No. pods/main shoot, 2.)Pod density (= no. pods/shoot length unit),3.)Main shoot yield, 4.)Thousand kernel weight (TKW) and 5.)No. seeds/pod.A multiple regression analysis with four indipendent variables was calculated for each line.Table 1 shows the average response of main shoot yield,when one of the characters was increased by 10%,keeping the others constant.

Table 1: The average response of main shoot yield to a 10%-increase of one yield character

10% increase of	Calculated response of main shoot yield (%)						
	no.59	no.89	no.93	no.109	no.119	no.129	no.135
x_1=no. pods	10,35	13,07	12,65	10,05	10,48	10,35	9,17
x_2=pod density	-0,28	-1.55	-1,55	0,11	-0,41	-0,10	0,44
x_4= TKW	8,68	10,08	8,37	9,45	8,61	9,87	9,27
x_5=no. seeds/pod	8,20	10,73	8,51	8,53	8,66	10,10	8,53

The greatest response gave the increase of no. pods,followed by the increase of TKW and no. seeds/pod.Higher pod densities showed no clear effects to main shoot yield.

The corresponding partial correlation coefficients express the grade of correlation between the 4 characters observed and the main shoot yield. They are given in table 2.Two coefficients of partial correlation analysis with 3 indipendent variables were added.

Table 2:Partial correlation coefficients of main shoot characters

No.	$r_{31.245}$	$r_{34.125}$	$r_{35.124}$	$r_{32.145}$	$r_{32.45}$	$r_{12.45}$
59	0,649	0,421	0,495	-0,024	0,562	0,736
89	0,911	0,874	0,915	-0,254*	0,741	0,818
93	0,904	0,812	0,881	-0,289**	0,804	0,873
109	0,958	0,901	0,915	0,044	0,625	0,634
119	0,878	0,792	0,907	-0,083	0,693	0,759
129	0,946	0,911	0,938	-0,024	0,761	0,781
135	0,927	0,885	0,891	0,208*	0,756	0,745

All coefficients were significant at P=0,1% except $r_{32.145}$

The number of pods is most closely correlated to the main shoot yield. It is followed by the no. seeds/pod and the TKW.From $r_{32.145}$ it seems as if pod density has no influence on main shoot yield. The reason for that is keeping no. pods constant.On the other hand pod density shows a high and significant influence on both number of pods and main shoot yield in $r_{12.45}$ and $r_{32.45}$ .These results demonstrate that pod density is a useful indirect measure of number of pods and by that for high yield of the main shoot.

INHERITANCE OF SELF-COMPATIBILITY AND SELF-INCOMPATIBILITY IN CAULIFLOWER /Brassica oleracea L. var. botrytis L./

Julia Hoser-Krauze

In 1976-80 the investigations were carried out in F_1, F_2, Bc_1 and Bc_2 of crosses between four homozygous self - incompatible lines of Indian Pusa Katki cv. having different S alleles: Sa, Sb, Sc, Sd and self-compatible lines /I_2/ of five summer cultivars such as: Rapid /Poland/, Master /Ohlsen's Enke, Denmark/, Idol, Early Abundance, Super Snowball /Stokes Seed, Canada/.

Sb and Sd alleles showed high activity and dominance in pollen over Sa and Sc alleles. F_1 of crosses between homozygous self-incompatible lines SbSb, SdSd and self-compatible father lines was 100 % self-incompatible. F_2 segregated into self-incompatible plants /less than 1 seed average per pod or lack of pollen tubes in the neck of pistil/ and self-compatible plants /more than 1 seed average per pod/ in 3 : 1 relation. Bc_1 and Bc_2 to self-compatible father lines segregated in ratio 1 : 1.

In the case of recessive in pollen alleles Sa and Sc the situation was quite opposite: F_1 was 100 % self-compatible, F_2 segregated 1 : 3, Bc_1 was 100 % self-compatible.

These results proved that self-compatibility and self-incompatibility were determined by the same locus S. The allele of self-compatibility marked by the author Scm was dominant over weaker in pollen Sa and Sc alleles but recessive in relation to stronger in pollen Sb and Sd alleles.

HAPLOID PRODUCTION FROM ANTHERS AND SECONDARY EMBRYOIDS OF WINTER OILSEED RAPE

C.-S. Loh and D.S. Ingram

METHODS

Six cultivars of Brassica napus ssp. oleifera (winter oilseed rape) were used (Table 1). The seed was sown in the field and in polyethylene tunnels during September 1979 and 1980, and the floral buds were harvested for anther culture in March and April 1980 and 1981. Unopened floral buds of 3-3.5 mm length (pollen either uninucleate or binucleate) were surface sterilised, washed and the anthers isolated and transferred to the following medium in 5 cm plastic petri-dishes: basal medium of Keller et al (1975) + sucrose (10% w/v), Difco agar (0.8% w/v), NAA (0.1 mg l^{-1}), 2,4-D (0.1 mg l^{-1}), glutamic acid (800 mg l^{-1}) and serine (100 mg l^{-1}); pH 5.8 (before autoclaving). Incubation was at 30°C in darkness for 14 days and then at 25°C in darkness until the emergence of embryoids.

Embryoids which emerged from anthers were transferred to medium in which the sucrose level had been reduced to 2% w/v and were incubated in a growth chamber at 25°C with continuous light (1.695 mW cm^{-2}). Subsequently, green plantlets and embryogenic tissues were maintained in Erlenmeyer flasks or 9 cm petri-dishes on Murashige & Skoog basal medium supplemented with sucrose (2% w/v) and Difco agar (0.8%), but without growth substances, and were incubated in the growth chamber at 22°C with a 12 h photoperiod. When plantlets were 5 cm high they were transferred to sterile, peat-based compost in 4 cm pots and maintained in a growth chamber at 20°C with a 16 h photoperiod and high humidity. After 3 to 4 weeks they were transferred to a glasshouse and then to polyethylene tunnels.

RESULTS

Anther culture and establishment of secondary embryogenic tissues: After 3-6 weeks embryoids were successfully obtained from a relatively small percentage of plated anthers of all cultivars (Table 1). Some of the embryoids developed into plants directly, but about 20-60% developed abnormally (hypocotyl swollen and whitish in colour; leafless), and about 3-4 weeks after emergence from the anthers, produced secondary embryoids on the surfaces of their hypocotyls and cotyledons. These secondary embryoids developed through globular, heart and cotyledonary stages. When detached from the parental tissue and transferred to fresh medium, secondary embryoids initiated roots from the radicle end and cotyledons from the proximal end, thus demonstrating their bipolar nature. Normally 40-80% of the plantlets derived from these secondary embryoids developed abnormally and were capable of producing further secondary embryoids on their surfaces; the rest (20-60%) developed into plants directly and did not produce further secondary embryoids. The number of secondary embryoids produced per abnormal plantlet ranged from one to over a hundred, but was usually 40-50 at any one time. Monthly subculture of secondary embryogenic tissue and detached secondary embryoids resulted in the maintenance of the secondary embryogenic potential for well over a year without diminution.

Cytology: The ploidy levels of secondary embryogenic tissues, secondary embryoids and plants regenerated from secondary embryoids were determined cytologically following hydrolysis and staining with 1% natural orcein in 45% acetic acid. The results are as follows.

1) Secondary embryogenic tissues with roots and visible secondary

Table 1

The Production of anther derived embryoids and secondary embryoids of winter oilseed rape

	Cultivar	No. of anthers plated	No. of anthers producing embryoids	Total No. of embryoids	No. of embryoids developing into plants (%)*	No. of embryoids producing secondary embryogenic tissues (%)*	No. of embryoids developing abnormally but without secondary embryoids
1980							
	Brinke	900	4	7	0(0)	0(0)	7
	Jet Neuf	1,647	17	21	15(71)	5(24)	1
	Rafal	240	5	13	7(54)	3(23)	3
	Rapora	400	5	5	1(20)	2(40)	2
1981							
	Jet Neuf	2,000	11	13	2(15)	3(23)	8
	Primor	3,130	83	502	135(27)	197(39)	170
	Quinta	1,206	9	13	2(15)	6(46)	5
	Rafal	1,260	34	101	18(18)	30(30)	53
	Rapora	3,732	4	5	0(0)	3(60)	2

*Figures in brackets are percentages of the total number of embryoids produced.

Table 2. Ploidy levels of secondary embryogenic tissues

Cultivar	No. of tissues examined	No. haploid	No. diploid (or other)
Jet Neuf	8	8	0
Primor	18	18	0
Quinta	3	3	0
Rafal	5	5	0
Rapora	15	15	0
Total	49	49	0

Table 3. Ploidy levels of secondary embryoids produced 1 year after anther culture

Cultivar	No. of parental tissues used	No. of secondary embryoids examined	No. haploid	No. diploid
Jet Neuf	2	3	3	0
Rafal	3	8	8	0
Rapora	8	32	24	8*
Total	13	43	35(81.4%)	8(18.6%)

Table 4. Ploidy levels of plants regenerated from secondary embryoids one year after anther culture

Cultivar	No. of regenerants examined	No. haploid	No. diploid
Jet Neuf	18	14	4
Rafal	5	2	3
Rapora	20	16	4
Total	43	32(74.4%)	11(25.6%)

*All eight embryoids derived from one piece of secondary embryogenic tissue.

embryoids on their surfaces were selected at random and sample root tips fixed and stained. All were haploid (n = 19) (Table 2).

2) Secondary embryoids (newly emerged) were obtained from secondary embryogenic tissues one year after anther culture, and fixed and stained. Most (81.4%) were haploid (n = 19) and the rest (18.6%) were diploid (2n = 38) (Table 3).

3) Regenerants. Sixty-four regenerants from secondary embryoids were planted in polyethylene tunnels on 15 May 1981 and all flowered during July 1981. Another batch of 52 regenerants were planted in the tunnel on 2 July 1981 and 4 flowered in August 1981. None of these regenerants had been vernalized.

Forty-three of the plants were selected at random and sample root-tips excised for cytological examination. Thirty-two (74.4%) of the plants were haploid (n = 19) and 11 (25.6%) were diploid (2n = 38); none were polyploid (Table 4).

DISCUSSION

In oilseed rape the frequencies of naturally occuring haploids are low, ranging from 0.008% to 0.685%. During recent years several attempts have been made to obtain haploid plants through anther culture, but regretably, except for one or two spring cultivars, the frequencies of embryoid production by this means have been low. In order to obtain sufficient haploids for breeding programmes large numbers of anthers therefore have to be plated in a relatively short flowering season. Thus, success in the long-term maintenance of secondary embryogenic tissues following anther culture has important implications in winter oilseed rape breeding. Our pilot results suggest that such secondary embryogenic material can be maintained for very long periods without diminution of the embryogenic potential, that relatively large numbers of regenerants can be obtained in a relatively short period of time from secondary embryoids and that only a relatively small number of anthers have to be plated to initiate secondary embryogenic tissues.

After one year of continuous subculture, 81.4% of secondary embryoids and 74.4% of regenerants from secondary embryoids were haploids, clearly demonstrating thestability of haploidy in the system. All secondary embryogenic tissues examined cytologically were haploid, and we have so far been unable to induce diploid rape tissues to produce secondary embryoids. Thus the presence of secondary embryoids in culture could serve as a useful marker for the presence of haploidy in the system.

By repeated sub-culture of embryoids it is possible to build-up a large number of secondary embryoid producing cultures all derived originally from a single pollen grain. Haploids produced from secondary embryogenic tissues therefore have the advantage of being clonal.

Our future work will be directed towards determining the nature of the factors triggering secondary embryogenesis in haploid tissues and assessing to what extent genetic variation does or does not build up over a period of time in secondary embryogenic systems. If variation does occur, spontaneously or in response to mutagens, we shall screen embryoids and regenerants for novel forms of disease resistance.

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POLLEN VIABILTY AFTER LOW TEMPERATURE STORAGE IN *BRASSICA CAMPESTRIS*, *B. OLERACEA* AND *B. NAPUS*.

Angela Cunningham

In order to make interspecific crosses, it is necessary to manipulate the parent species so that they flower at the same time. When the age and conditions for flower inducton differs in the two species, this can be difficult, or at least inconvenient. If pollen can be collected and stored, even for a few weeks, it may help overcome this problem. Little has been found in the literature on the storage of Brassica pollen. Chiang (1974) observed thelongevity of cabbage pollen (a) stored at room temperature, (b) refrigated at 4 C and (c) stored in the freezer compartment at -15°C. The viability of the pollen was tested by its germination on a particular medium. It was reported that pollen grains lost their viability entirely after 1 day storage in the freezer. When stored at room temperature the germination percentage was about 10% for the first 3 days. When pollen was kept at 4° C the percentage germination up to 50 days storage was recorded, by which time it had reached 4.5%.

This report describes attempts to store pollen by keeping it at sub-zero temperatures. The method used is similar to that used for the storage of potato pollen. (M. De Maine, personal communication).

Materials and Methods

Freshly dehisced anthers of cultivars of *B. campestris*, *B. oleracea* and *B. napus* (see Table 1) were collected and air dried in open petri dishes, at room temperature for 20-24 hours. The pollen was then collected into vials, the lids of which contain silica gel. The vials were all placed in a larger container, which also contained silica gel, and subsequently stored at -20°C to -25°C. In April 1981, plants of the same variety were bud pollinated with pollen which had been stored for periods varying from 21 to 78 weeks. As a control other plants were bud selfed with fresh pollen. The pollinated ovaries were left until they were mature. All the seeds were then harvested and counted and the results used as a measure of the performance of the pollen after storage.

Results and Discussion

It can be seen from Table 1 that the performance of the pollen after a long period of storage varies with the species and the variety.

In *B. campestris* pollen of the cultivar Civasto was inviable after 22 weeks while after a similar period, pollen of the cultivar Ponda showed no loss of viability. For the cultivar Marco it was possible to store pollen for nearly 1 year without loss of viability. In *B. oleracea* pollen of cv. Maris Kestrel can be stored for up to 1½ years without reduction of the number of seeds produced in each silique. In *B. napus* cv. Lair there is some loss of performance after about 1 year storage of pollen. The performance of the pollen may be improved when it is subjected to shorter periods of storage.

Table 1: The performance of Brassica pollen after storage at -20°C to -25°C.

	STORED POLLEN					FRESH POLLEN		
	1	2	3	4	5	3	4	5
B. CAMPESTRIS								
cv. Civasto	22	25	19	0	-	7	68	9.7
cv. Civasto	52	4	3	0	-			
cv. Ponda	21	15	8	83	10.4	6	46	7.6
cv. Marco	50	37	24	203	8.5	17	85	5.0
B. OLERACEA								
cv. Maris Kestrel	26	26	13	143	11.0	30	306	10.2
cv. Maris Kestrel	78	24	13	156	12.0			
cv. Maris Kestrel (4X)	27	20	7	67	9.6	2	2	2.5
B. NAPUS								
cv. Lair	50	30	4	36	9.0	20	354	17.7

Column 1: Storage period (weeks)
2: Number of pollinations
3: Number mature siliquae collected
4: Number seeds obtained
5: Average number seeds/silique

The overall results of stored and fresh pollen suggest that the performance of the pollen in most of these cultivars is not impaired with storage. In some cases the performance of the stored pollen appears to be better than fresh pollen. Chiang (1974) found that fresh pollen yielded lower germination percentage than that of pollen stored at 4°C for 10 days. He suggested this may be due to the higher moisture content of fresh pollen grains. With the cultivars mentioned above, it may be due to the use of pollen from a single plant, which might havedefective pollen; in the bud self controls, whereas pollen collected from several plants is bulked, thus masking any low fertilty individuals, for storage. This may be an explanation of the poor seed set in the tetraploid Maris Kestrel fresh pollen result. To eliminate this possibility a better control would have included fresh pollen bulked from several plants and then bud pollinations carried out.

Further investigation of pollen storage is being undertaken.

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DOUBLING CHROMOSOMES IN INTERSPECIFIC HYBRIDS BY COLCHICINE TREATMENT

Astrid GLAND

More than 100 new Brassica napus forms were resynthesized by crossing B.oleracea and B.campestris. The primary amphihaploids were propagated by cuttings. In order to recover fertility, an efficient colchicine treatment was essential. Three methods were examined based on earlier experiences (DERMEN 1940, BELL 1950, STRAUB 1950, KAUL and ZUTSHI 1971), using the cotton plug, the injection, and the submersion technique for the application of aqueous solutions of 0.05% to 0.3% colchicine, with and without 1.5% dimethyl sulphoxide (DMSO), respectively.

(A) For flowering plants, the cotton plug method was tested. The flowering branches were removed and cuttings were rooted. Onto axillary buds of these, the colchicine soaked cotton plugs were placed. To prevent plugs from drying, the plants were wrapped into plastic bags during the five days of treatment. Desiccated plugs were remoistened by application of the same colchicine solution.

(B) Plants before anthesis were treated by injecting colchicine solutions into the stem with a fine syringe three times at intervals of 24 hours.

(C) Juvenile plants in the 6-8 leaf-stage were removed from their pots; their roots were washed and cut to 4 cm; then, bundles of 10 to 20 such plants were submerged into 0.05% colchicine with roots ahead up to their shoot apex. After this treatment they were carefully washed for 16 hours in current tap water. In one experiment, this procedure was repeated up to six times. In another experiment, the duration of a single treatment was varied from 5 to 25 hours. During the treatment, the vials were kept in a thermostat at 22°C and continuous light. Finally, the plants were replanted into soil.

In evaluating the results (Table 1), one must consider that the treated materials were genetically different amphihaploids in various stages of growth, as they originated from a breeding programme. Nevertheless, one can conclude from the total, that submersion was the most efficient method, if small plants were treated. By this kind of treatment, about 60% of the plants became polyploid and fertile. By dipping the roots into a "Wurzelfix" powder (effective compound: 0.1% α-naphthyle acetic acid)

Table 1: Different methods of colchicine treatment of interspecific hybrids of B.oleracea x B.campestris (= amphihaploid B.napus)

Mode of application and concentration of colchicine	Treated plants	Plants with fertile branches (number)	(%)	($\bar{x}$%)
(A) Cotton plug				
0.1 % Colchicine	14	4	28.6	
0.15% "	16	7	43.6	
0.2 % " + 1.5% DMSO	10	5	50.0	
0.3 % "	9	3	33.3	38.8
(B) Injection				
0.05% Colchicine	5	2	40.0	
0.1 % "	3	2	66.7	
0.15% "	7	1	14.3	
0.2 % "	3	3	100.0	
0.3 % "	5	4	80.0	52.2
0.05% Colchicine	4	2	50.0	
0.1 % "	3	2	66.7	
0.15% " + 1.5% DMSO	4	4	100.0	
0.2 % "	10	2	20.0	
0.3 % "	3	1	33.3	45.8
(C) Submersion				
1 x	38	19	50.0	
2 x	11	9	81.8	
3 x	10	6	60.0	
4 x 8 hrs. in 0.05% Colchicine	8	5	62.5	
5 x + 1.5 % DMSO	11	8	72.7	
6 x	7	2	28.6	
7 x	6	2	33.3	56.0
5 hrs	6	3	50.0	
7.5 "	3	3	100.0	
10 "	5	1	20.0	
12.5 "	6	3	50.0	
15 " in 0.05% Colchicine	7	4	57.1	
17.5 "	8	5	62.5	
20 "	6	3	50.0	
22.5 "	7	6	85.7	
25 "	7	2	28.6	54.5
5 hrs	10	6	60.0	
7.5 "	2	2	100.0	
10 "	9	5	55.6	
12.5 "	5	2	40.0	
15 in 0.05% Colchicine	6	4	66.7	
17.5 " + 1.5 % DMSO	9	6	66.7	
20 "	7	5	71.4	
22.5 "	6	4	66.7	
25 "	5	3	60.0	62.7

before replanting into soil, the number of surviving plants was considerably increased. More than two repetitions of treatment did not increase the percentage of fertile plants (Table 1). No clearcut answer was received on the optimal duration of the treatment. We recommend to treat over night and to use an addition of 1.5% DMSO to the 0.05% colchicine solution.

As compared to earlier reports on the induction of tetraploidy in barley, rye or buckwheat (KAUL and ZUTSHI 1971) and of diploidy in haploid barley plants (SUBRAHMANYAM and KASHA 1973), our methods of colchicine treatment in Brassica reached an almost two times higher effectiveness.

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PLANT REGENERATION FROM PROTOPLASTS OF BRASSICA OLERACEA

H.R. SCHENCK

The limited use of protoplasts in theoretical and applied plant studies is at present mainly caused by their insufficient regeneration capacity. Within the Brassiceae, only in B.napus regeneration of whole plants from protoplasts has been reported (1,2). In B.campestris, B.oleracea, and B.nigra, nothing but callus formation from protoplasts has been achieved (3,4). Recently, our three years' studies in this direction yielded the following first success.

Plant material: Seeds of B.oleracea acephala (fodder kale) were sterilized in NaOCl (5%) for 20 min and placed in petri dishes containing water agar (1%). Seedlings were cultivated in 250 ml Erlenmeyer flasks containing a liquid "S medium" without any hormones (composition will be published in Z.Pflanzenzüchtg.). Flasks were placed in 12 hours day, 3000 lux, 25°C.

Protoplast isolation: From these sterile grown plants, leaves of 2-4 cm length were placed in petri dishes containing 3 ml 0.55 M mannitol, pH 5.7, and cut into 0.5-1.0 mm strips with a scalpell. 1 ml of enzyme solution was added, containing 0.1% pectinase "PATE" (Hoechst AG., Frankfurt, Germany F.R.), 0.5% cellulase "Onozuka R 10" (Kinki Yakult, Nishinomya, Japan) and 0.55 M mannitol, pH 7.0. After 14 hours, the petri dishes were gently shaken for 15 min in a gyratory shaker at room temperature. The released protoplasts were filtered through a 100 µ stainless steel sieve, washed three times by suspending them in 0.55 M mannitol, pH 5.7, and centrifuged at 100 g for 3 min. Finally, the protoplasts were resuspended in the S medium supplemented with 0.2 mg/l 2,4-D, 1.0 mg/l NAA, and 0.5 mg/l 6-BAP, which had been autoclaved prior to use. Subsequent cultivation occurred in petri dishes in the dark.

Protoplast culture: After 2 weeks, they were allowed about 2000 lux. Small calli (0.1-0.2 mm diameter) were transferred to solid (agar) S medium which promoted callus growth. After 4 weeks, the calli (size about 1.5 mm) were cultured in a regeneration "LS medium" (5) with 0.2 mg/l 6-BAP. After another 4 weeks, about 10 shoot buds were visible on one of the calli; but these failed to grow although they were transplanted every 4 weeks to fresh medium. Suddenly, after 12 months in culture, many of these shoot buds developed into shoots which could be transferred to S medium without any hormones. After spontaneous rooting, the plantlets were planted into soil. One part of the shoot producing callus remained in sterile culture as reserve.

This is the first report of plant regeneration from protoplasts of B.oleracea. It is no doubt that one single event is still insufficient for plant breeders and propagators; but it is one step further into the wanted direction.

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THE EFFECT OF SEED GRADING AND SIZE ON FODDER KALE

J.E. Bradshaw

It is common for fodder kale to be sown with a precision drill. The best results are obtained with graded seed. However, in a breeding programme only small quantities of seed are available in the early generations of selection, and seed size can vary between selections. A small experiment was therefore carried out to investigate the effect of seed grading and size on some agriculturally important characteristics of fodder kale.

The cultivar Merlin was chosen and the seed graded as follows: A 2.5 - 2.25 mm, B 2.25 - 2, C 2 - 1.75, D 2.5 - 2, E 2.25 - 1.75, F 2.5 - 1.75. The trial was sown as a six by six latin square on 4 June 1980 with a Webb precision drill set up for grade A seed (i.e. 2.5 mm holes in the wheels). The trial site was the Murrays Farm, Pathhead, Midlothian, Scotland. A fine seed bed was prepared containing 180, 90, and 90 kg/ha respectively of N, P_2O_5, and K_2O fertiliser, and treflan (trifluralin) for weed control. Liquid Dursban (chlorpyrifos) insecticide was applied at sowing, emergence, and three weeks later, to control flea beetles and cabbage root fly. Further weed control was by steerage hoe three weeks after emergence. The five row plots were 6.7 m long with 50 cm between rows and 6.25 cm plant spacing within rows. The trial established and grew well. Plant height was recorded on 17 November 1980. The trial was harvested with a modified Maisprinz forage harvester on 28 November 1980. The fresh weight yield of the centre three rows of each plot was recorded. A representative sample of chopped kale was freeze-dried for dry matter content determination. Milled samples were analysed for organic matter content, digestibility, crude protein content, S-methyl cysteine sulphoxide content, and thiocyanate ion content of the dry matter by the methods described by Dr. M.J. Allison in Cruciferae Newsletter No. 5.

	Rows	Columns	Seed Grade	Mean	C. of V. %
Ht.cm.	NS	XXX	NS	102.2	4.45
F.Wt.t/ha	XX	X	NS	76.42	5.23
DM%	X	X	NS	13.19	1.82
OM%	NS	NS	NS	88.47	0.42
DOMD%	NS	NS	NS	83.04	1.63
CP%	XX	NS	NS	14.92	3.32
SMCO%	XXX	NS	NS	0.7611	4.54
SCN%	NS	NS	NS	0.0142	12.1

Statistical significance: XXX P < 0.1%, XX P 1 - 0.1%, x P 5 - 1%, NS P > 5%.

As no statistically significant differences between seed grades were detected, the present practice of only crudely grading seed (i.e. 2.5 - 1.75 mm.) for use with wheels with 2.5 mm holes is adequate. The trial also revealed that low co-efficients of variation can be achieved with a small trial in which the effects of "environmental" differences in two directions are removed.

COMPETITION EFFECTS BETWEEN ROWS OF NEIGHBOURING PLOTS IN WINTER RAPESEED, (BRASSICA NAPUS L.).

M. LEFORT-BUSON

In micro-plot trials, we often harvest all the plot rows which have been sown ; this would not raise any problem if border rows of neighbouring plots would not compete one with an other. In this paper, the correlation between border rows of two different plots has been estimated, thinking that the occurence of such a correlation could be a fact of competitive effects.

I- METHOD

The correlation has been calculated over two years : 1979 and 1981. The inner and border rows of each plot have been harvested separately, in 4-replication block designs ; in 1979, both the border rows have been harvested together, whereas in 1981 each of them has been harvested alone. The correlation has been estimated in each block between the residuals of the variance analysis for seed yield ; but, for 1979, in order to get a value of the correlation between yield of neighbouring rows and assuming several assumptions, we have corrected the previous estimate as it is shown on figure 1.

II- RESULTS AND DISCUSSION

The results are presented on table 1. The assumption of homogeneity of the estimations in each block has been tested, using the transformed statistics of Fisher $\{q = \sum_{j=1}^{k} (zj - \bar{z})^2/(n-3)^{-1}\}$, and it has been accepted. The average estimation of correlation between residuals of neighbouring rows are significant : -0,546 and -0,486 respectively for 1979 and 1981. So, the values of plots including border rows must be over- or under-estimated whether they stand near low and high yielding plots, the bias of the estimation being all the more important than the plot size is small.

Moreover, for the two years, the order of the varieties for their productivity, when one takes into account only inner rows, is not highly correlated to the order when one takes into account the whole plot ($\hat{\rho}$ =0,780 for 1979, and $\hat{\rho}$ =0,736 for 1981).

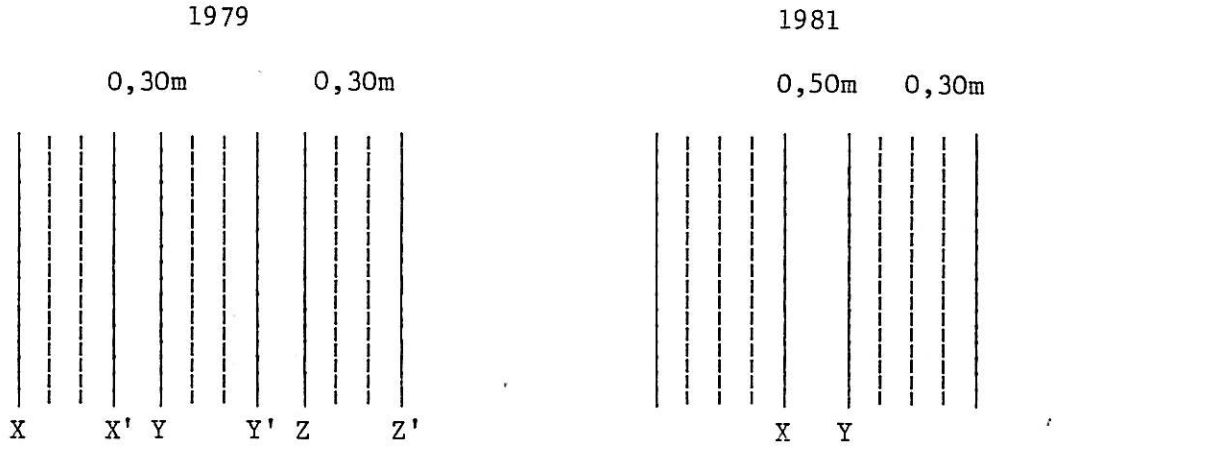
The correlation between the residual of the inner row-yield of one plot and the residual of the border row-yield of the two neighbouring plots has been calculated in 1979 ; it is very low (-0,118) and non significant. So the competition effects must occur essentially on border rows.

The error variance in case of border rows'yield is much more important than the one in case of inner rows' for both years. But, if the whole plot yield is considered, the error variance is about the same as when the only inner rows are considered. So, the consideration of the whole plot does not seem to have any effect on the accuracy of plot value ; (this last result is not statistically established with the results of these two experiments).

CONCLUSION

With rapeseed micro-plot trials, it is recommended to eliminate the border rows of plots in order to well appreciate the plot value, even if these rows do not seem to affect the accuracy of the experiment. In fact, the estimation of plot value is biased if border rows are considered, and this all the more than the number of plot rows is small.

FIGURE 1 : DESIGNS USED FOR THE ESTIMATES OF CORRELATIONS ($\hat{\rho}'1$ and $\hat{\rho}2$) BETWEEN ROWS OF NEIGHBOURING PLOTS.



Harvesting : X + X', Y + Y', Z + Z'

Correlation estimated : $\hat{\rho}1$, between (Y + Y') and (X + X' + Z + Z')

Correlation between neighbouring rows : $\hat{\rho}'1 = \sqrt{2}\,\hat{\rho}1$, between X' and Y or Y' and Z

Harvesting : X, Y

Correlation between neighbouring rows : $\hat{\rho}2$, between X and Y

TABLE 1 : ESTIMATIONS OF THE CORRELATIONS BETWEEN ROWS OF NEIGHBOURING PLOTS FOR THE TRIALS OF 1979 ($\hat{\rho}'1$) and 1981 ($\hat{\rho}2$).

1979

Block	n	$\hat{\rho}'1$	Average Estimate
I	20	-0,662	
II	20	-0,717	-0,546
III	20	-0,071	
IV	20	-0,537	
$-0,748 < \hat{\rho}'1 < -0,202$			

1981

Block	n	$\hat{\rho}2$	Average Estimate
I	15	-0,400	
II	14	-0,639	-0,486
III	15	-0,475	
IV	12	-0,330	
$-0,684 < \hat{\rho}2 < -0,221$			

A CONTRIBUTION TO PLOT SIZE OPTIMIZATION IN MICRO-PLOT TRIALS WITH WINTER RAPESEED (BRASSICA NAPUS L.).

M. LEFORT-BUSON

In rapeseed, for breeding purposes, hybrid seeds are producted by hand till now and are very expensive. So, most of the hybrid trials are done in micro-plots (i.a. 4-row plots, 3m long). Besides, in such trials, it is better to eliminate the border rows as it has been shown in the previous paper ; this means that the character under study are estimated from a very small plot area : the error of measure must then be important. For example, the error variance for plot yield is high very often, and the experiments' accuracy is not sufficient enough to separate the best varieties. So, we wondered wether a small increase in plot size could lead to a decrease in the error variance, thinking that the "technical" errors are all the more important than the harvested quantity is small.

In this paper we have compared the error variances of different trials with respectively 4-row, 5-row and 6-row plots, each 3m long, (i.a. respectively with 2, 3 and 4 harvested rows), for several characters and more particularly seed yield.

I- METHOD

The experiment has been conducted over two years, 1980 and 1981.

In 1980, we have sown a 4-replication block design (with 8 plots of the same variety, "Jet Neuf", in one replication) for each of the following cases : 4, 5 and 6-row plots' trials. Only the plot yield and the 1 000 seed weight have been studied.

In 1981, we have also sown 4-replication block designs (with 6 plots of different varieties in one replication) in cases of 4, 5 and 6-row plots' trials, but the last trial was devastated by the cabbage root fly (Delia brassicae). So, we have compared only the results of 4 and 5 row-plots trials, for the seed yield, the 1 000 seed weight, the average number of seeds per pod and the average seed weight per pod ; both the last character have been evaluated from two 30-pod samples. The error variances, estimated in each design from classical analysis of variance, have been compared two by two using the F-statistic, and assuming that their law was nearly normal.

II- RESULTS

For plot yield the results of all the trials are refering to a constant area. The increase of the harvested rows leads to a significant decrease of the error variance for seed yield in 1980 and 1981, (table 1). Besides from the results of the 1980's trial, the 4-harvested row plots do not seed to increase the accuracy of the experiment, if compared to 3-harvested row plots.

TABLE 1 - COMPARISON OF THE ESTIMATIONS OF THE ERROR VARIANCE OBTAINED WITH DESIGNS OF DIFFERENT PLOT SIZES FOR SEVERAL CHARACTERS

	Number of harvested rows	Estimation of the error variance			
		Seed Yield	1 000 seed weight	Aver. nbr of seeds/pod	Aver. weight of seeds/pod
1980 *	2	10,62	0,0502		
	3	5,24	0,0303		
	4	5,31	0,0190		
1981 **	2	32,50	0,0478	6,90	133
	3	12,35	0,0349	7,63	162

* The error variance has been estimated with 21 degrees of freedom

** The error variance has been estimated with 15 degrees of freedom

For the 1 000 seed weight, there is a little decrease of the error variance when increasing the number of harvested rows from 2 to 3 and from 3 to 4, (see table 1), but the decrease is not significant; the difference between ther error variancesis significant only when comparing 2 and 4-harvested row trials, in 1980.

The value of the error variance is not depending of the plot size for the average number of seeds per pod and the average seed weight per pod, if it is considered the results of the 1981's trial.

- CONCLUSION

As it does not increase too much the number of seeds sown for a plot, it is recommended to have 5-row plot trials rather than 4-row plot ones ; the harvested yield is then increasing for 50 %, because the number of harvested rows is enlarging from 2 to 3. From the above results, it is clear that the accuracy is much better in case of 5-row plots, at least for seed yield.

But, even if it brings some preliminary informations, such an experiment has to be reconducted over several years and localities before we can really conclude to an optimization of the plot size in case of micro-plot rapeseed trials.

THE EFFECT OF PLANT SPACING ON THE SEED YIELD OF WINTER CABBAGE GROWN IN A POLYTHENE TUNNEL

G.J. Faulkner and J.C. Jackson

Pre-basic (breeders') seed of some ten different existing or potential varieties of vegetables bred at the National Vegetable Research Station is regularly produced in polythene tunnels at Wellesbourne (Faulkner and Jackson, 1980). Although capital and maintenance costs and labour inputs are relatively high, the heavy yields of good quality seed more than compensate for the extra costs involved. In order to maximise seed yields, an observation trial was carried out during 1979-80 with the winter cabbage cv. Avon Coronet to determine the effect of plant spacing on seed yield.

From seed drilled in Dutch-light frames in rows 7.5cm apart at the end of May 1979, plants were transplanted into the field at 50 x 50cm about four weeks later, lifted in mid-November and planted into a 8.5 x 24m polythene tunnel at spacings of 90 x 60, 60 x 60, 60 x 45, 45 x 45 and 45 x 30cm. The plots contained 33, 121, 121, 102 and 85 plants respectively and were unreplicated, but each was surrounded by a single row of guard plants. No plant losses occurred during the winter and when the plants re-commenced growth four vertical cuts were made round the perimeter of the heads in mid-February 1980. All loose plant material was removed and shoots emerged in mid-March. Each plant was staked and tied several times as the flowering shoots elongated; flowering commenced in late April and continued until the end of May. Pollination was by blowflies raised from maggots used as a bait for coarse fishing (Smith and Jackson, 1976), and 4.5 l/wk of flies or pupae were placed in the tunnel as soon as flowering commenced, and at weekly intervals until flowering ceased. Routine fortnightly sprays of iprodione (Rovral) were applied as a precaution against fungal attack.

The plants were harvested from mid to late August, and were dried on polythene sheets in situ within the tunnel. Seed was threshed, cleaned and germination tested. Seed yield per plant and seed yield per unit area were calculated (g/m^2).

Plant spacing and seed yield

Plant spacing (cm)	mean yield/plant (g)	yield/m^2 (g)
90 x 60	11.5	21.3
60 x 60	11.6	32.2
60 x 45	10.7	39.6
45 x 45	8.0	39.5
45 x 30	8.4	62.2

Yield increased with plant density from 21.3g/m^2 at the widest spacing to 62.2g/m^2 at the closest spacing (see Table). The mean yield per plant was highest at the two widest spacings. From a spacing of 45 x 30cm about twice the weight of seed per unit area was obtained as that from 60 x 60cm, which has been used in previous seed productions of winter cabbage in polythene tunnels. Seed germination of all treatments was in excess of 90%. Weed growth in the closely-spaced plots was minimal, but regular weeding was necessary in most of the other plots.

These results agree with those of Gray et al, (1980) who found that seed yield in carrot increased with plant density. Further replicated trials are necessary to confirm these preliminary results.

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A NEW METHOD OF FIELD-SCALE SEED PRODUCTION OF SPRING CABBAGE IN THE U.K.

G.J. Faulkner and J.C. Jackson

A cabbage plant will remain in a vegetative state until it is vernalised by a cold stimulus which will initiate flowering. Spring cabbage plants for seed production must reach a stage of maturity to be receptive to the stimulus but should also be sufficiently small and "hardy" to withstand frost damage and low winter temperatures. It is a combination of these two factors (maturity and winter survival) which affects the success or failure of field-scale production of seed.

The most reliable method is the relatively costly '3-year' or 'stump-rooted' method usually used for pre-basic and basic seed production. Seed is sown in a seedbed in mid-July, stecklings are transplanted into the field in early September when they usually make enough growth to overwinter without severe losses. Most plants from a July sowing are insufficiently mature to be receptive to a cold stimulus. In the spring of the second year, the crop is vegetatively mature, bolters are removed, and the mature heads are transversely cut just below the growing point. Side shoots are produced during the summer and autumn and it is in this stage that the plants overwinter for the second time. In the third year the plants flower in May and June, and the seed is harvested during August. The main disadvantages of this method are the length of time the land is occupied and possible plant damage in two winters.

In the 'two-year' method, usually used for commercial seed production, seed is sown under glass in mid-April, seedlings are pricked out into Dutch-light frames, and the stecklings planted out into the field in early June. The plants are relatively large by the end of October (unless the soil is infertile), and although this plant size ensures their acceptance of a cold stimulus, considerable plant losses can occur during winter. Although the two-year crop occupies land for less time, and the plants need to survive only one winter, plant losses can be quite high. In the spring of the second year plants are either cut or more often left to burst, fewer side shoots are produced and the seed yield is often lower than by the 3-year method.

A modified method with the most desirable features from both the 2-year and 3-year systems was investigated during 1979, the objectives being to produce plants with as many side shoots as possible in the first year, for the shoots to overwinter (as in the second year of the 3-year crop) to improve winter survival and increase the seed yield over the 2-year method.

Seed of the NVRS cv. Avon Crest, was sown in boxes in a glasshouse on 22 February 1979, and the seedlings were pricked out to a Dutch-light frame during the second week in March. On 8 May, 6,000 plants were transplanted at a spacing of 83 x 36cm in an isolated site using a two-row planter. The soil, of the Evesham series, was moderately well drained with a high clay content. The soil was deep ploughed but no base fertiliser incorporated as the field was known to be relatively fertile.

The area was arranged in five separate blocks with a tractor-way between each block to facilitate application of insecticides and fungicides against cabbage root fly and Alternaria. Granules of carbofuran (Yaltox) were applied to the base of each plant using a plastic applicator within 48 hours of planting, and Alternaria was controlled by spraying with iprodione (Rovral) as per manufacturers' recommendations. Chlorthal-dimethyl (Dacthal) and propachlor (Ramrod) were applied as per manufacturers' recommendations to control weeds three days after planting. Hoeing was carried out whenever necessary. Hearts were well formed by mid-August and were cut transversely just below the growing point, thus eliminating apical dominance, but leaving enough axillary buds to form shoots. Tripomol 80 (Thiram) powder was applied immediately to the cut surfaces to prevent fungal attack. The plants grew well during a mild autumn and by the end of October 1979 had produced a number of side shoots; a benomyl (Benlate Benomyl fungicide) spray was applied during November to prevent fungal attack. In early January 1980 the plants were lightly covered with straw to prevent frost damage. The plants overwintered as side shoots rather than as hearted cabbage with an average of 6 side shoots per plant; 35% of the plants were killed.

Two beehives were placed in the plot two days after flowering commenced in early May, and although temperatures were relatively low during the flowering period, on some days large numbers of bees visited the flowers. The seed was slow to ripen as relatively low temperatures persistend over the summer period. In July, Alternaria brassicae and A. brassicicola were discovered on the plants, which were immediately sprayed with iprodione (Rovral) and sprayed again 14 days later in order to help control the disease. The

crop ripened unevenly and hand harvesting was carried out from the end of July to the end of August. Harvested material was dried on sheets of polythene inside polythene tunnels; the seed was threshed using a Garvie stationery thresher and cleaned with a mini-Petkus seed cleaner.

Thirty-four kg of seed were obtained from 0.14 ha (a mean of 9 g/plant) with a germination of 88.5%.

Although this method has been tried only once, results were satisfactory. Some seed was lost because of Alternaria which was extremely severe in 1980, and the potential seed yield was further reduced by some side shoots that failed to vernalise. However, shoots that survived the winter grew away quickly in the spring and produced an abundance of flowering shoots. Winter kill was relatively high, but the survival rate was some 5% better than in a 3-year stump-rooted crop started in a nearby field in July of the same year.

From a single multiplication it is not possible to assess the reliability of this method, but it has indicated that spring cabbage plants overwintereed better as shoots than as hearted cabbage and therefore the method merits further trials.

ACKNOWLEDGMENTS

We thank Professor J.K.A. Bleasdale who suggested the new method, the Principal of the Warwickshire College of Agriculture, Moreton Morrell, who made the land available, college staff who co-operated, and Mr John Shepherd who supplied and managed the beehives.

SOME FEATURES OF THE F_2 GENERATION OBTAINED IN THE RESULT OF INTERSPECIFIC CROSSINGS BETWEEN BRASSICA CAMPESTRIS AND BRASSICA OLERACEA

Miroslawa Balicka

Investigated hybrid plants of F_2 generation were obtained from crossings between B. campestris ssp. pekinensis Granaat and B. oleracea var acephala Normal. These plants have the chromosome number of B. napus - 2n = 38. The morphological segregation of the F_2 hybrids shows two types of plants: one similar to the female parent B. campestris Granaat and the second - to the male form B. oleracea Normal.

The plants similar to B. campestris Granaat were of higher frequency. Their growth habit, leaf shape and colour were like these of the female form. But the fertility of these hybrids, expressed in the number of siliques per plant and the number of seeds in silique, is low, amounting on the average 15, 2% siliques and 35, 7% seeds in silique.

The plants resembling the female parent B. oleracea Normal appeared less frequent. Group of these hybrids is similar in their growth habit, leaf shape and colour to the winter forms of rape and has better fertility in comparison to the hybrids similar to the female parent. The mean number of silique per plant in this hybrid group amounts 28, 3% and 56, 5% seeds in 1 silique.

During observations of siliques in the F_2 generation it has appeared that they are longer and their shape is more regular in comparison to the irregular shape of the siliques originating from plants belonging to the F_1 generation. Besides, by siliques in the stage of full maturity, an additional characteristic feature has been noticed - the anthocyanin coating on the peduncle and on the half of the silique, along its surface.

The seeds of selected plants from the F_2 generation have been sown this autumn, for further observations of the next progeny.

INTERSPECIFIC HYBRIDISATION WITHIN THE GENUS *BRASSICA* BY IN VITRO CULTURE

Elzbieta Zwierzykowska

As it was already announced (Cruciferae Newsletter No. 5 p.28) besides conventional methods, in vitro of excised ovaries and embryo culture techniques were recently applied.

During last year, there were obtained 27 plantlets by in vitro culture. After transplanting them to the soil - 13 survived. They originated from the following combinations:

1. *Brassica campestris* cv. "Nagaoka WR 55 Days" x *B. oleracea* var. *sabauda* cv. "Predzvest" - 5 plants,

2. *B. campestris* cv. "Candle" x *B. oleracea* var. *sabauda* cv. "Predzvest" - 3 plants,

3. *B. campestris* cv. "Candle" x *B. oleracea* var. *gemmifera* cv. "Bastion F_1" - 5 plants.

The investigations of pollen grains viability showed complete sterility of plants from the combination 1 and 2. In the third combination only two plants were complete sterile, while the other two showed viability from 10.0 to 20.0%; one plant from this combination had 84.0% of viable pollen grains.

In combination 1 and 2, after colchicine treatment, it has appeared pollen grain fertility and there were obtained seeds of the F_2 generation. The remaining plants were vegetatively propagated aimed at obtaining material for cytological investigations.

Comparative results of interspecific crosses of *B. campestris* and *B. napus* with *B. oleracea* are presented in Table 1.

Table 1. Comparative results of interspecific crosses of *B. campestris* and *B. napus* with *B. oleracea*

Combination		Number of		
		laid out ovaries	isolated ovules	plants obtained
B. campestris x *B. oleracea* var.				
Yellow Sarson	capitata			
	cv. (a) "Head Start"	26	1	0
	(b) "Leo No.80 F_1"	82	0	0
	(c) "Gloria F_1"	25	12	0
B. campestris x *B. oleracea* var.				
ssp. pekinensis	capitata			
cv. "Granaat"	cv. (a) "Market Topper F_1"	163	19	17
	(b) "Tucana F_1"	130	10	6
	(c) "Gloria F_1"	174	12	7
B. campestris x *B. oleracea* var.				
ssp. pekinensis	capitata			
"Chinese cabbage"				
	cv. (a) "Gloria F_1"	166	1	0
	(b) "Leo No.80 F_1"	86	1	0
	(c) "Tucana F_1"	240	34	23
B. campestris x *B. oleracea* var.				
ssp. pekinensis	capitata			
"Kapusta chinska"				
	cv. (a) "Leo No.80 F_1"	50	0	0
B. napus cv. x *B. oleracea* var.				
"Bronowski"	capitata			
	cv. (a) "Gloria F_1"	132	10	4
	(b) "Tucana F_1"	33	1	0
	(c) "Head Start"	119	2	1
B. napus cv. x *B. oleracea* var.				
"Gulliver"	capitata			
	cv. (a) "Gloria F_1"	164	18	7
	(b) "Leo No.80 F_1"	152	20	18

SOME FEATURES OF THE HYBRIDS OBTAINED IN THE RESULT OF INTERSPECIFIC CROSSINGS WITHIN THE GENUS BRASSICA

Waleria Mlyniec

As it was already announced (Cruciferae Newsletter No. 3 p.10, No. 4 p.16, No. 5 p.28) trials aimed at obtaining need seed and leafy genotypes by hybridisation between B. oleracea, B. campestris and B. napus were started in 1976.

Within the variability observed in hybrid progenies there were found forms which can be considered as seed oleiferous types and also very leafy fodder forms.

B.c.ssp. pekinensis in the cross combinations with different winter cultivars of B.c.ssp. oleifera and B.n.ssp. oleifera introduced into hybrids such features as intensity and vigour of vegetative growth and earliness of generative development but, particularly when it was the female parent, their winter hardiness was very low (12 - 35%). Winter hardiness of reciprocal hybrids varied greatly (60 - 100%). Hybrids derived from crosses with B.n. cv. Siberian gave almost 100% winter hardiness with very high green matter production but they were in general slower in vegetative growth and generative development.

The Paper Test Method revealed in F_3 hybrid (B.c.ssp. pekinensis x B.n.ssp oleifera cv. Bronowski) five plants with zero glucosinolates content. Lower than average content was also found in F_3 progeny of hybrid B.c.ssp. pekinensis x B.c.ssp. trilocularis Yellow Sarson. Forms with low or zero glucosinolates content had comparatively high seed production and those derived from hybrid with Yellow Sarson had yellow colour seed coats.

BREEDING OF MAINTAINER AND RESTORER OF SELF-INCOMPATIBLE LINES IN B.NAPUS

Fu Ting Dong

Since 1972, we have been involved in research work on the utilization of the hybrid vigor in B.napus. Our emphasis is aimed at the breeding of self-incompatible (S1) lines.

In order to shorten the breeding procedure, we sow twice a year (in Wuhan, sowing is in September, harvesting is in May; in Chinghai or Yunnan, sowing is in May, harvesting is in September). In 1975, we bred S1 lines "211", "271" (they were from F6 offspring of B.napus x B.campestris) and "219" (from the M5 offspring of Co60 treatment). In the summer of the same year, we began to produce S1 hybrids (S1 x variety or line). From 1976 to 1981, we have utilized the regional test and productive test for the yielding ability of hybrid rapeseed. The advantages of S1 hybrids are early maturity suited for three cropping system and an increase in yield of 10-30% as compared with the control variety. But some combinations of hybrids set seed poorly and gave reduced yields in some places. Because of the peculiarity of cropping systems in many regions in our country, such as the application of sowing nursery and planting field, the sowing rate is only 1.5kg/ha. It may be possible to carry on the selfing of the flowerbuds by hand to reproduce S1 lines. But it is still difficult to do, as it necessitates much more time in labour and higher technical quality.

To solve this problem, in 1977, we advanced the following scheme for the breeding of S1 maintainers and restorers, and began to test crossing (see the material in Chinese rapeseed conference, Wuhan, July 1970).

SI x SI or SC (self-compatible) line
↓ (call SI maintainer)
SI x SC line (call SI restorer)
↓ (maintain SI)
SI three way hybrid (restorer SC)

(1) Maintainer breeding of SI

In order to breed the SI maintainer, a test crossing was conducted. The test crossing hybrids were covered with isolation bags and were self-pollinated and sib-pollinated artificially. We estimated the index of compatibility (IC). The results of this experiment are summarized in Table 1. According to these results it is possible to use some SI or SC as SI maintainers.

Table 1. The effect of different SI maintainers

Combination	Type	Date and Place	Self-Pollination by hand and with isolated bags.				Sib-pollination by hand and with isolated bags.			
			1	2	3	4	1	2	3	4
271x211	SIxSI	1976 Wuhan	22	301	58	0.19±0.18	20	265	78	0.29±0.19
		1979 Chinghai	22	230	7	0.03±0.17	13	114	6	0.05±0.17
		1980 Wuhan	24	238	70	0.20±0.24	22	306	73	0.24±0.29
		1980 Yunnan	12	148	36	0.24±0.23				
211x71-111	SIxSC	1978 Chinghai	10	162	63	0.38±0.30				
271x71-111	SIxSC	1978 Chinghai	10	184	48	0.26±0.30				

Note: 1) 1 — the number of plants
2 — the number of flowers
3 — the number of seeds
4 — the index of compatibility.

2) IC = $\frac{\text{No. of seeds produced}}{\text{No. of flowers treated.}}$

In 1979 and 1980, using as directions of Thompson's paper from the 5th International Rapeseed Conference, we continued series of test crossing:

Table 2. The effect of SI maintainers in different test crossing combinations.

Date and Place	The No. of Combin-ations	Incompatible (IC<1.0)		Less incompatible (IC=1.1-2.0)		Compatible (IC=2.1-12.3)		High Compatible. (IC>12.3)	
		The No. of Combin-ations	%	The No. of Combin-ations	%	The No. of Combin-ations	%	The No. of Combin-ations	%
May, 1980 Wuhan	17	6	35.3	2	11.8	7	41.2	2	11.8
Sept. 1980 Yunnan	227	46	20.1	41	18.1	121	53.3	19	8.4
Total	244	52	21.3	43	17.6	128	52.5	21	8.6

Note: IC of check variety is 12.3

These 224 test crossing combinations may appear in four types (see table 2). We found that 1) the effect of the genes controlling SI may be not single dominant or recessive in our materials, and 2) it would be easier to obtain SI maintainers by test crossing.

(2) Breeding of the restorer in SI three way hybrid:

After test crossing the maintainer, the same principles were utilized to examine the restorers.

In 1980, there were 90 different test combinations crossed. All the SI three way hybrids F_1 progenies were self-pollinated by paper bags and their IC from each combination was estimated.

Table 3. The effect of different restorers in SI three Hybrid F_1

Date and Place	Total No. of Combin-ations	High restorable combinations (IC check variety IC)		Restorable Combinations (IC check variety IC)		Low restorable combinations (IC check variety IC)	
		number	%	number	%	number	%
May, 1980 Wuhan	6	2	33.3	3	50.0	1	16.7
Sept. 1980 Yunnan	84	44	52.4	12	14.3	28	33.3

We could classify the 90 combinations of SI three way hybrids into three classes, ie high restorable, restorable, and low restorable. Most of them set seed very well. The results indicated that it is easier to obtain the restorer of SI three way hybrid.

(3) The effect of seed setting for producing the SI three way hybrids in isolated plot:

In 1980 and 1981, we began to produce SI three way hybrids in isolated plot. The male and female were planted by 1:1 or 1:2 ranges.

Seed setting of female ranges (S1) was normal. For example, when the female was (211 x 271) and male was 75-53, the number of seed settings per pod was 22.71±1.65. When the female was (271 x 71 - 111), and the male was 75-53, the number of seed settings per pod was 17.16±5.37. In general, the seed yield of hybrids in isolated plot was 500-10,000 kg/ha.

Recently, our emphasis is to test better combinations and to study the inheritance of SI three way hybrids.

(This research work is in progress under the direction of Prof. Dr. Liu Hou Li).

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CONTENT OF UNSATURATED FATTY ACIDS IN M_3 AND M_4 GENERATION OF BRASSICA NAPUS

Barbara Barcikowska, Lech Szyld

As it was already announced (Cruciferae Newsletter No. 4 p.16) trials aimed at obtaining better chemical composition by mutagenic treatment of B. napus Janpol and Primor were started in 1976. There was paid special attention on linoleic and linolenic acid content.

Table 1. Linoleic and linolenic acid content in M_3 and M_4 of winter rape (Brassica napus L.) Janpol

No.	No. of plant	Percentage of fatty acids			
		linoleic		linolenic	
		M_3	M_4	M_3	M_4
1.	B_1-2/5 533/1	30,9	23,4	24,7	12,8
2.	" /3		19,3		12,5
3.	" /4		22,2		9,2
4.	" /5		22,9		10,3
5.	" /6		22,6		12,5
6.	B_1-2/28 534/2	27,4	23,3	13,9	15,2
7.	" /3		18,4		11,6
8.	" /4		17,2		7,4
9.	" /5		16,7		9,1
10.	" /6		14,8		9,2
11.	B_1-2/32 535/1	31,7	16,2	13,5	8,6
12.	" /4		17,7		11,6
13.	B_1-5/7 536/1	12,4	14,8	6,7	7,2
14.	" /2		13,5		6,5
15.	" /3		18,2		10,5
16.	" /4		13,5		6,4
17.	" /5		13,9		8,2
18.	" /6		13,4		7,3
19.	B_3-9/48 537/1	28,6	12,5	16,3	9,0
20.	" /2		12,3		9,2
21.	" /3		12,4		8,0
22.	" /4		15,3		11,6
23.	" /5		17,9		12,2
24.	" /6		12,4		8,2
25.	B_3-5/54 538/1	15,2	12,3	6,8	8,6
26.	" /2		14,4		7,0
27.	" /3		14,5		7,4
28.	" /4		14,5		7,4
29.	B_3-9/34 539/1	27,9	18,3	11,2	7,5
30	" /3		19,4		8,8
31.	" /5		17,2		6,0
32.	" /6		16,8		7,8

As it can be seen, there exist no inheritance of linoleic acid content at all. Linolenic acid content, on the contrary, shows certain tendency to be in some degree dependent from the ancestor plant. The results of crossings within the M_4 generation, where the low linolenic form No. 536/1 was the male parent seems to confirm this statement. The linolenic acid content amounted namely in this case 6,8% in the F_2 generation of the cross 533/6 x 536/1, while by crossing in the opposite direction there was 19,5% linolenic acid in the F_2 generation.

SUPEROXIDE DISMUTASE IN BRASSICA SPECIES

M. Coulthart and K. E. Denford

SOD has been identified as an enzyme which catalyzes the reaction $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$ (1). It has attracted considerable attention from both biochemists and geneticists, and has now been shown to be widely distributed among aerobic organisms. In eukaryotes it exists in two distinct forms which are apparently of different evolutionary origin. One active form is mitochondrial and exists as a Mn - containing tetramer; the other is found in the cytosol and is a Cu - and Zn - containing dimer. Evidence for the dimeric structure of the cytosolic form has come from genetic (electrophoretic) and from biochemical (purification) studies. "Hybrid isozymes" in individuals heterozygous for electrophoretically variant forms of SOD have been observed in organisms as phylogenetically diverse as *Helix pomatia* (2), *Drosophila melanogaster* (3), *Salmo gairdneri* (4), *Dipodomys* (5), and man (6). Published reports of electrophoretic hybrid forms of SOD in plants are infrequent, but biochemical studies have indicated that the purified enzymes from *Pisum sativum* (7), *Spinacia oleracea* (8), and *Triticum aestivum* (9) are also dimeric.

In the present isozyme study of *Brassica*, achromatic zones appeared on several types of zymograms in which tetrasolium salts were used as chromogens (dehydrogenases, PGI and PGM). One very slow-migrating zone appeared clearly on GDH gels after overnight staining. In addition to this zone, a faster-migrating form was present which appeared quickly during the staining of IDH gels. Since this faster zone corresponded closely in disc-electrophoretic mobility with that of purified copper-zinc SOD from other plant sources (8 and 9), it was considered most suitable for study in this material. This form showed considerable phenotypic variation within and between taxa, and the results pertaining to this variation are presented. Taxa investigated were of two types Group I representing an established hybrid *B. napus* (Argus) with a Robbelen formula aa cc and its 'parents' *B. campestris* (Rapido II; aa) and *B. oleracea* (Borecole Dwarf Green Curled; cc).

Group II comprised of a synthetic series obtained from Sweden and Germany. Diploid *B. campestris* (Makino; aa), tetraploid *B. campestris* (Chinensis Makino; aa aa), diploid *B. oleracea* Marrowstem Kale; cc) tetraploid *B. oleracea* Marrowstem Kale; cc cc) and Synthetic *B. napus* (a product of both the above tetraploids (aa cc). For ease of discussion, the names of the taxa were abbreviated: the members of Group I are referred to as Rapido II, Borecole and Argus, and those of Group II as Chinensis 2N, Chinensis 4N, Marrowstem 2N, Marrowstem 4N, and Synthetic napus. Isozyme phenotypes are given names based on the abbreviated name of the enzyme plus a superscript. The taxon in which the phenotype occurs is specified by a prefixed abbreviation of the taxon name. For example, SOD phenotype A of Chinensis 2N is referred to as Chin 2-SOD^A. The abbreviated prefixes for the other taxa of Group II are "Chin 4", "Marrow 2", "Marrow 4", and "Syn". Those for the taxa of Group I are "RII", "Bore", and "Arg".

In group II, bands 1 and 2 were consistently present in all

individuals of each taxon; no variations between taxa in the mobilities of these bands were detected. Among the diploids, Chinensis 2N was phenotypically invariant, but certain individuals of Marrowstem 2N showed pairs of variant bands which were both faster and slower than bands 1 and 2. The slower bands (3 and 4) were clearly either present or absent in individuals of this taxon, and two phenotypic classes - Marrow 2-SOD^{A} and Marrow 2-SOD^{B} were distinguished on this basis. However, no such classes could be clearly demarcated for the faster-migrating pair of bands. A continuous range of staining "intensities" of these bands seemed to characterize the zymograms obtained from plants in this group.

With respect to the autotetraploids, Chinensis 4N was invariant, as was its diploid counterpart. Marrowstem 4N showed one variant phenotype in addition to the more common Marrow-4-SOD^{A}. This phenotype, Marrow 4-SOD^{B}, was characterized by the presence of two variant bands of mobilities apparently identical to those of bands 3 and 4 of Marrow 2-SOD^{B}. These bands were widths and intensities such that Marrow 4-SOD^{B} had a markedly asymmetrical appearance, as shown in the diagram.

Synthetic napus displayed three phenotypes - the standard two-banded pattern (Syn-SOD^{A}) and two asymmetric types (Syn-SOD^{B}, Syn-SOD^{C}). In Syn-SOD^{B}, two variant bands are clearly present which correspond in mobility to bands 3 and 4 of Marrow 4-SOD^{B}. However, in Syn-SOD^{C} band 3 is weaker than in Syn-SOD^{B}, and band 4 is very weak indeed.

Turning to Group I, the patterns of phenotypic variation are somewhat similar to those of Group II. Both diploids exhibited bands with higher mobilities than bands 1 and 2. As with Marrowstem 2N, these were of continuously variable intensity relative to bands 1 and 2, and thus were not included in the phenotype classification. When these bands are ingnored Rapido II is internally invariant. Borecole individuals were mostly Bore-SOD^{A}, but two plants were of variant type Bore-SOD^{B}. In this pattern two slower-migrating variant bands were seen, and the three slowest (2, 3, and 4) were of approximately equal intensity.

Argus, the allotetraploid representative of Group I, showed a single phenotype in which two slower bands of the same mobilities as bands 3 and 4 of Bore-SOD^{B} were present. Bands 3 and 4 of Arg-SOD^{A} were clearly fainter than band 1, giving this phenotype an asymmetric appearance also.

The assumption that the active form of soluble SOD in *Brassica* leaves is dimeric appears to be a safe one. On this basis, zone 1 SOD phenotypes in this material may be interpreted as follows:

None of the *B. campestris* taxa showed any variation in zone 1, penotype although this prohibits estimation of structural locus dosage in Chinensis 2N, Chinensis 4N and Rapido II, the two zone 1 isozymes are provisionally considered to be coded by the *Sod-1* locus in all taxa. This locus is electrophoretically homozygous in Chinensis 2N and 4N.

Isozymes 1 and 2 were judged both to be products of the same allele (*Sod-1*^{c}) at the *Sod-1* locus, since they occurred together in every sample and their intensities varied concomitantly. A similar electrophoretic pattern has been reported for human SOD. (10). Since the most common alleles in all the other Group II taxa had the same mobility, these were also named *Sod-1*^{c}.

In interpreting the variant SOD phenotypes seen in Marrowstem 2N, Marrowstem 4N, Synthetic napus, Borecole and Argus, the following Schema was useful. A single-locus heterozygote A/B is assumed to produce subunits *a* and *b* which combine to form catalytically active dimers. If the subunits combine at random three kinds of dimers - *aa*, *ab* and *bb* will result. The proportion of each type of dimer in the mixture is given by the expression,

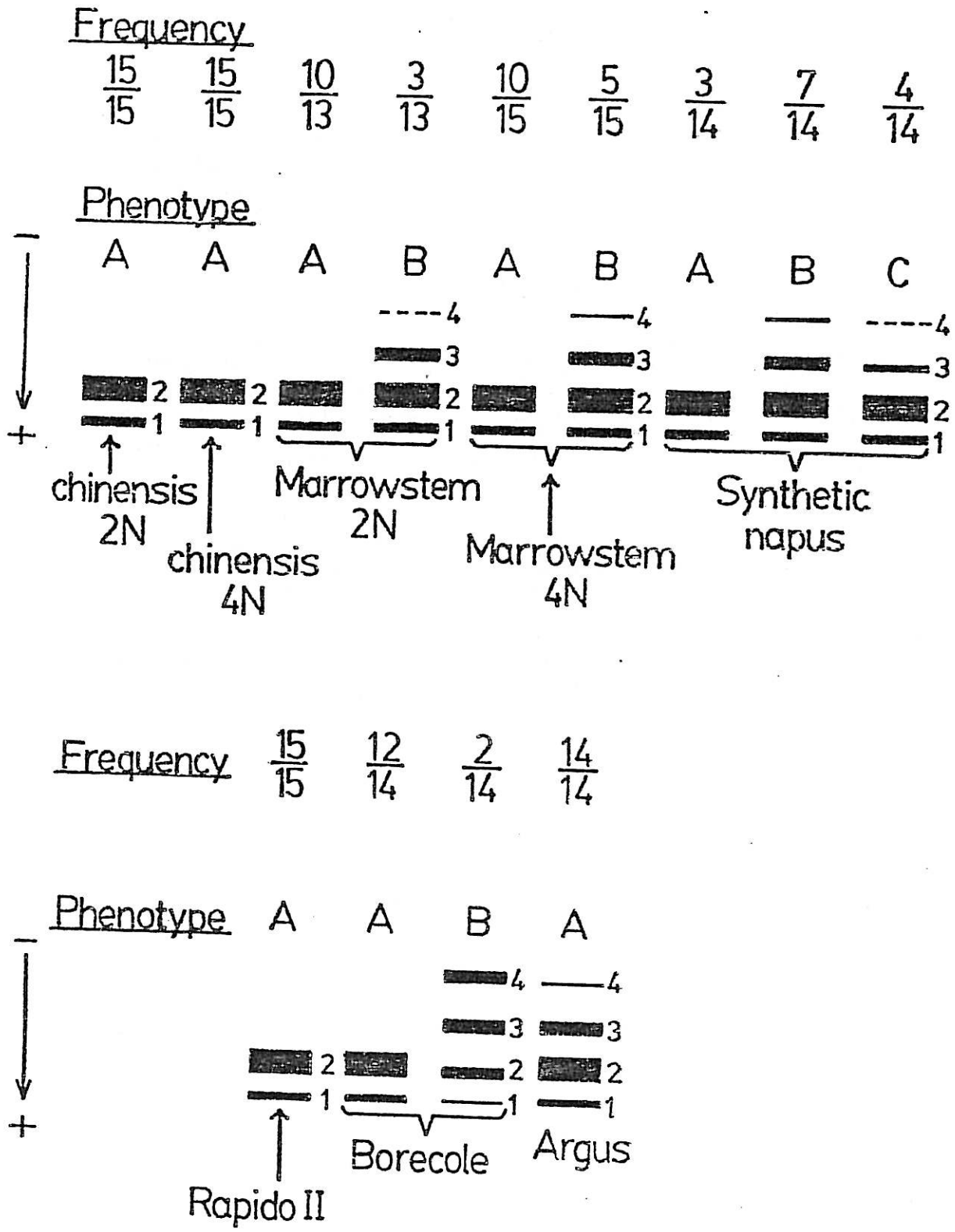
$$(p + q)^2 + p^2 + 2pg + q^2 \qquad (1)$$

where p and q represent the relative quantities of subunits *a* and *b* which are produced. If one assumes further that *a* and *b* are produced in equal amounts and that the dimers *aa*, *ab* and *bb* are equally active catalytically, p = q = 1/2 and the zymogram phenotype of the heterozygote should display 3 bands in the intensity ratio 1:2:1.

The marked asymmetry of Marrow 2-SOD^{B} thus may have a biochemical or a genetic basis. The biochemical explanation suggests that *a* and *b* have different effects on the catalytic efficiency of the dimers into which they are incorporated, so that under the staining conditions employed the order of activities of the dimer types is *aa* > *ab* > *bb*. Thus, although the proportions of dimer types may still by 1:2:1, their unequal activities per molecule produce an asymmetric zymogram profile. The genetic explanation assumes that *a* and *b* have negligibly different effects on activity, but are produced in differing amounts based on gene dosage or expression. This would involve a change in p and q, and an asymmetric ratio of quantities of *aa*, *ab* and *bb* would result.

The SOD data from any one taxon alone did not permit distinction between these two possible explanations. However, comparison of results from all 8 taxa provided a framework for preliminary interpretation. First, Marrowstem 4N as a recently arisen synthetic autotetraploid is almost certainly at least tetrasomic for the chromosome carrying the putative *Sod-1* locus. Therefore, in a simplex heterozygote for a variant *b* subunit which differs from from the *a* subunit only in electrophoretic mobility, p = $\frac{3}{4}$ and q = $\frac{1}{4}$ in expression (1). This gives a ratio of *aa*, *ab* and *bb* subunits of 9:6:1, which approximates the band intensity ratio seen by visual inspection in Marrow 4-SOD^{B}. Thus a genetic explanation of Marrrow 4-SOD^{B} plants as simplex heterozygotes for a slow allele *Sod-1*^{m4} apparently fits the data.

Since if one accepts this genetic explanation for Marrow 4-SOD^{B} *Sod-1* is presumably therefore disomic in Marrowstem 2N, a biochemical explanation seems more likely for Marrow 2-SOD^{B}. The observation of a variant in Borecole which gives the symmetric phenotype Bore-SOD^{B} would also appear to support a hypothesis of disomy for *Sod-1* in the



S.O.D. Zymograms of *Brassica* parental and hybrid profiles.

c genome. Electrophoretic variants of human SOD with diminished activity have also been reported (10). The variant alleles in Marrow-stem 2N and Borecole were provisionally named *Sod-1*^{m2} and *Sod-1*^{b} respectively.

The two variant phenotypes of Synthetic napus are of interest, since they can be explained genetically on the basis of different dosages of *Sod-1* in its *B. campestris* and *B. oleracea* parents. Almost certainly the *Sod-1* locus from each parent is present in at least disomic dosage. In the case of disomy in both parental genomes, one would expect a heterozygote at one of the two homoeologous loci to behave much as a simplex heterozygote in an autotetraploid, presumably giving an asymmetric phenotype resembling Marrow 4-SOD^{B}. One would also expect to observe only one class of asymmetric phenotypes with band intensities weighted towards isozyme 2.

Such is not the case in Synthetic napus; two different asymmetric phenotypes are seen. If one parent (possibly Chinensis 4N) were tetrasomic for the chromosome carrying *Sod-1*, p and q in expression (1) would be $\frac{5}{6}$ and $\frac{1}{6}$ respectively for a *Sod-1*^{c}/*Sod-1*^{m4} heterozygote at the *c* genome *Sod-1* locus, and $\frac{4}{6}$ and $\frac{2}{6}$ respectively for a *Sod-1*^{m4}/*Sod-1*^{m4} homozygote at this locus. The expected band ratios for these two cases are 25:10:1 and 4:4:1, which may be considered to correspond by visual estimate to Syn-SOD^{C} and Syn-SOD^{B} respectively.

The results for Argus may also bear on this hypothesis. The phenotypes of the sample analyzed from this taxon are uniformly asymmetric. All individuals show two bands (isozymes 3 and 4) with mobilities similar to those seen in the *B. oleracea* taxa with substantially lesser intensity. The constant presence of isozymes 3 and 4 suggests homozygosity for an allele similar to *Sod-1*^{b} at the *c* genome *Sod-1* locus in Argus, and the asymmetry is consistent with multisomy of the homoeologous *a* genome locus.

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THE OCCURRENCE AND ROLE OF GLUCOSINOLATES IN BRASSICAS

G.R. Fenwick, R.K. Heaney and E.A. Spinks

Glucosinolates, a group of sulphur-containing glucosides are present in all Brassicas and their enzymic breakdown products contribute significantly to the flavour and aroma of these vegetables but can have undesirable effects in animal feeding stuffs.

a. Rapeseed and Fodder Crops

It has recently been reported [Cruciferae Newsletter, 1980 No.5] that Brussels sprouts buttons and seeds demonstrate a cultivar dependent 'pattern' of glucosinolates and that this property could be used as an aid to cultivar identification. Recent work has attempted to extend this observation to the important area of rapeseed cultivar identification for the purpose of establishing Breeders Rights. Although initial results were encouraging, a more thorough study of the glucosinolate 'patterns' of the first and second or third and fourth true leaves of young rape seedlings has shown that a high level of uniformity exists between different cultivars. This uniformity is demonstrated in the seeds of rape and has also been observed in the seeds of 23 cultivars of cauliflower.

Other Brassica species, in particular forage Brassicas are being investigated in order to establish whether Brussels sprouts are unique in this respect and also to determine whether a correlation exists between the levels of glucosinolates in the seeds and the levels of potential goitrogens in the mature plant. Such a correlation would find application in the selection of improved varieties for breeding.

b. Bitterness of Brassica Vegetables

At the present time approximately a third of the Brussels sprouts grown in the UK are frozen. There has been concern at the presence of undesirable bitterness in certain processed sprouts which leads to reduced consumer acceptability. Recent work has demonstrated the contribution of two major glucosinolate components to this problem. During processing and/or subsequent cooking some enzymatic degradation of glucosinolates would be expected with, in particular the glucosinolate progoitrin yielding the goitrogen, (-)5-vinyloxazolidine-2-thione (goitrin). The latter compound has been shown to be intensely bitter and a good correlation has been obtained for differing cultivars between strength of bitterness and goitrin content. An additional factor is also involved and recent work has shown this to be the intact glucosinolate sinigrin. A method has been developed for the screening of Brussels sprouts for potential bitterness based upon measurement of their individual progoitrin and sinigrin contents. Whilst bitter cultivars have been found to possess high levels of one, or both, of these compounds, cultivars possessing low levels of both are consistently rated 'non-bitter' by sensory evaluation. It is possible

that reported bitterness in certain cultivars of other Brassicas, e.g. rutabaga, is also due to high levels of (potential) goitrin.

c. Effect of cooking

The properties of glucosinolates and their breakdown products described above have led to an examination of the fate of these compounds in Brassica vegetables which have been subjected to the processes of blanching, freezing and cooking. Current work indicates that significant losses of original glucosinolates occur in cooked vegetables due either to decomposition or to leaching into the cooking water. Losses also occur during blanching although at a much lower level. Of the major glucosinolates in Brussels sprouts, the indole glucosinolates glucobrassicin and neoglucobrassicin are the most affected. Such losses, together with the destruction of the glucosinolate degrading enzyme, myrosinase, during cooking/blanching should have a significant effect on the flavour of the product.

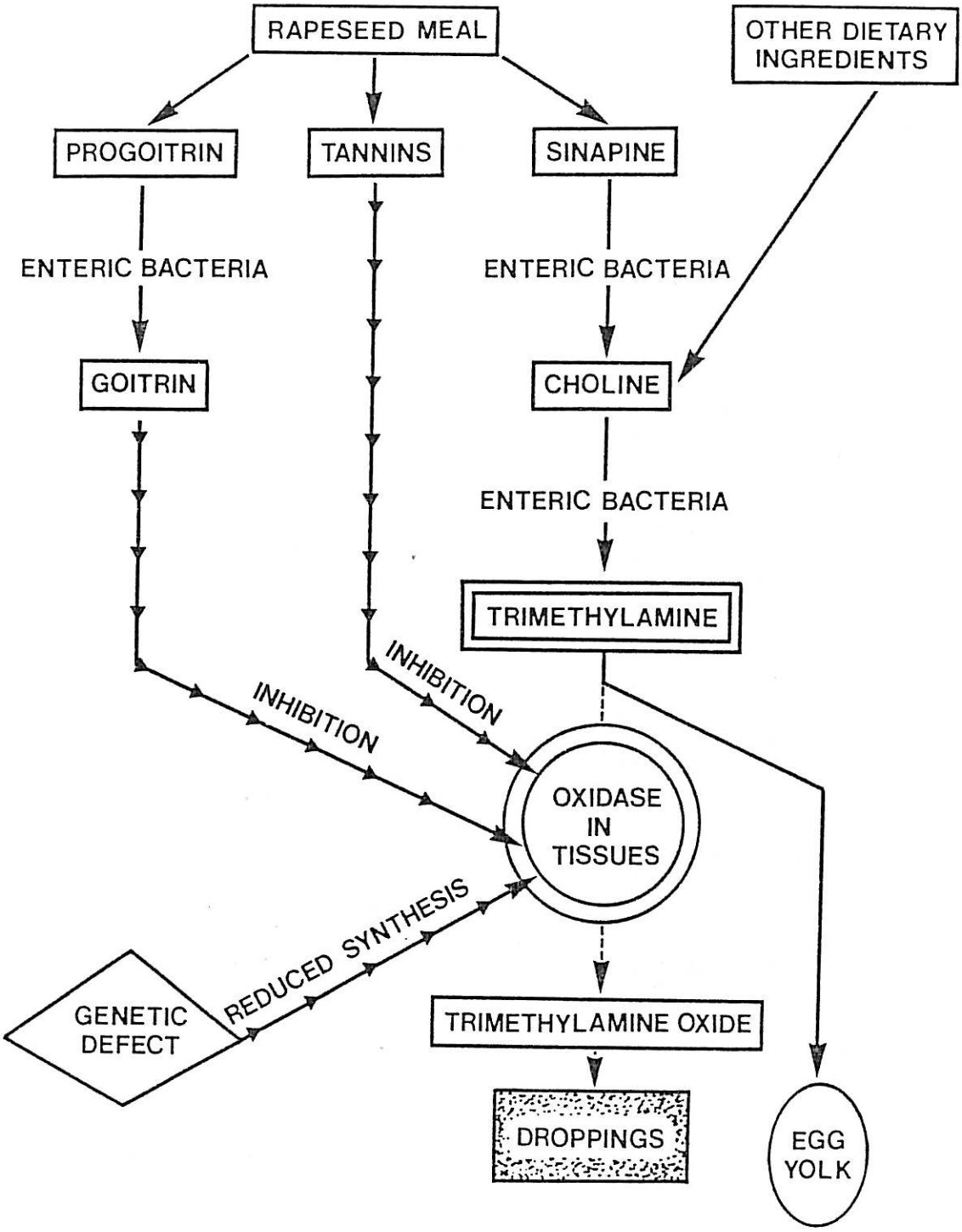
FISHY TAINT IN EGGS CAUSED BY RAPESEED MEAL

G.R. Fenwick, A.W. Pearson and E.J. Butler

Rapeseed, with an annual production of some 11,000,000 tonnes, is now the fifth most important oilseed of commerce and the only one capable of successful cultivation in Northern Europe. In addition to providing edible oil it is a potentially valuable source of protein for farm animals. The protein is contained in the meal which remains after the oil has been removed and when this meal is fed to hens, a "fish" or "crabby" taint appears in the eggs of certain hens, particularly those with brown shells. This problem has severely restricted the use of rapeseed meal as a dietary ingredient for laying hens in many part of the world.

Recent work has shown how the taint is produced and has identified the rapeseed components that are involved. The taint is caused by the presence of trimethylamine in concentrations exceeding 1 ppm. Rapeseed meal contains large amounts of choline in the form of its sinapic acid ester, sinapine, which releases trimethylamine when it is broken down by enteric bacteria. Normally, trimethylamine is oxidised to the odourless, water-soluble N-oxide by a microsomal enzyme in the liver and kidneys and is rapidly excreted in this form. Hens that lay tainted eggs have a genetic defect which greatly reduces their ability to synthesise this enzyme. Furthermore, the rapeseed goitrogen, (-)5-vinyloxazolidine-2-thione which is readily formed by the breakdown of the glucosinolate, progoitrin, has been found to be such a potent inhibitor of this enzyme that whilst "non-tainting" hens are only slightly affected, those possessing the tainting defect have little or no capacity to oxidise trimethylamine when fed a diet containing 10% high glucosinolate rapeseed meal or equivalent amounts of progoitrin or (-)5-vinyloxazolidine-2-thione.

The amount of progoitrin present in low ("zero") glucosinolate meals is sufficient to produce this effect and calculations suggest that it would have to be reduced to less than one fifth in order to prevent the taint. An additional component of rapeseed, *viz.* the soluble tannin-like material has also been shown to inhibit trimethylamine oxidase. Although the removal of sinapine by chemical treatment reduces the tainting potential of the meal it is now clear that (-)5-vinyloxazolidine-2-thione and soluble tannins inhibit trimethylamine oxidation to such an extent that other dietary sources of trimethylamine (*e.g.* lecithins, free choline or trimethylamine oxide itself) are sufficient to cause taint. Thus, without removing these inhibitors, the elimination of sinapine from rapeseed meal does not provide a satisfactory solution to the problem. The most promising way of preventing the taint is to remove the genetic defect from the brown egg breeds by selective breeding and to enable this to be done methods have been developed for detecting it in males and females at an early age.



The production of egg taint by rapeseed meal

DIFFERENTIAL PATHOGENICITY OF PLASMODIOPHORA BRASSICAE

D.R. Jones, D.S. Ingram and G.R. Dixon

INTRODUCTION

Clubroot is probably of greater economic importance than any other disease of Brassicas. It is widespread in almost all temperate countries, and also in some tropical countries. Resting spores of Plasmodiophora brassicae remain viable in the soil for at least 7 years, and probably for much longer, so that crop rotation is seldom a practicable control measure. Some chemical control procedures are available for transplanted crops, but there is little that can be done for drilled crops apart from traditional practices such as liming, which may be of limited value. Because of this there is great interest in breeding for resistance.

Resistance is available in some Brassica species, particularly stubble turnips (Brassica campestris). Some use has been made of this resistance by plant breeders, but it has become apparent that resistance cannot be used rationally until variation in P. brassicae is understood. It has been known for over 50 years that P. brassicae shows differential pathogenicity, and several differential series have been drawn up independently to classify pathotypes. In 1974, the European Clubroot Differential Series (ECD Series) was introduced, to provide an internationally accepted differential series. There are 15 hosts in the series, most taken from earlier differential series. The Brassica campestris hosts, numbers 1 to 4, represent the main resistance genotypes in Dutch Stubble turnips. Host 5 is Chinese cabbage, believed to be universally susceptible. The 5 Brassica napus hosts, numbers 6 to 10, (4 rapes and 1 swede) were identified as a single series at the Welsh Plant Breeding Station, but the Brassica oleraceae hosts, numbers 11 to 15, were derived from several sources and are not a satisfactorily homogeneous set.

In the field P. brassicae is generally though to occur in populations containing a mixture of pathotypes. When the ECD Series was introduced it was recognised that little was known about the composition of such populations, but it was hoped that individual populations could be classified by making a single test on a representative sample of the whole population. In practice, the problem of poor reproducibility of results was soon experienced. We therefore decided to investigate the premise that a single test on a bulk sample of a population of P. brassicae would give a reliable picture of that population.

METHODS AND RESULTS

Three P. brassicae populations, from National Institute of Agricultural Botany regional trial sites, were used. ECD tests were made on these populations in a glasshouse, with 30 plants of each differential host being tested against each population, using a standard inoculation method.

Replicate tests on 2 of the populations did not give consistent results. A total of 14 tests were made with one population, from Trawsgoed in mid-Wales, of which 8 clearly gave one result while the other 6 gave another, different result. This suggested that different samples of the population contained different pathotypes.

The next stage was to prepare spore suspensions from individual clubs and test each against the relevant part of the ECD series. These tests confirmed that different clubs from one host cultivar from one site contained spores of different pathotypes. This means that it is unlikely that different bulk samples of a population would contain the same composition of pathotypes, so it is hardly surprising that inconsistent results were obtained when the ECD series was used to classify P. brassicae populations.

The only reliable way in which the composition of a population can be analysed is by the production of single-spore isolates. Two methods had previously been used for inoculation with single spores, and the method that we preferred was that developed by Tinggal at Exeter University. A dilute spore suspension was spread on agar in a petri-dish, and the plate was scanned under the microscope until a spore was found which was well separated from any other spores. A cutter, mounted on a microscope, was then used to cut out the small piece of agar with the spore on it. This was then placed on the root-hairs of a 5 day old seedling of Chinese cabbage.

Three inoculations were successful in 600 attempts, all from the Trawsgoed population. The 3 isolates all gave different reactions with the ECD series, and 2 of them gave ECD codes different from that of the spore suspension from which they were derived. This provided clear evidence that P. brassicae populations are heterogeneous, and further emphasised that a single ECD test made on a bulk sample of a population is of limited value because it does not show the variation within the population.

We also examined the possibility that there may be interactions between spores of different pathotypes within a population which could restrict or enhance infection by some pathotypes, resulting in a misleading ECD code. This was done by making mixtures containing different proportions of spores of 2 of the single-spore isolates (Isolate No. 1 and No. 2). Each mixture contained the same total number of spores. Two hosts were inoculated, the Chinese cabbage cv. Granaat, which was susceptible to both isolates, and Nevin rape, which was susceptible to isolate No. 1 (Table 1b). In addition, the hosts were inoculated with isolate No. 1 alone, at a range of spore concentrations (Table 1a). These concentrations were chosen so that each had the same number of spores of isolate No. 1 as were present in one of the mixtures.

Table 1 - Results of one experiment on interaction between single-spore isolates.

a. Single-spore isolates Nos. 1 and 2 tested separately

Isolate	Spore concentration (ml^{-1})	% diseased plants*	
		Granaat	Nevin
No. 1	10^6	100	100
No. 1	10^5	100	100
No. 1	10^4	100	100
No. 1	10^3	100	92
No. 1	10^2	100	80
No. 2	10^6	100	0

b. Mixtures of spores of Single-spore isolates Nos. 1 and 2 (Total spore concentration 10^6 ml^{-1} in each mixture).

Proportion of spores of isolate No. 1	Concentration of spores of isolate No. 1 (ml^{-1})	% diseased plants* Granaat	Nevin
10%	10^5	100	85
1%	10^4	100	15
0.1%	10^3	100	1
0.01%	10^2	100	5

*Percentages derived from samples of approximately 90 plants of Nevin and 30 of Granaat.

Taking the results with isolate No. 1 alone, 80% of plants of Nevin were infected by the lowest spore concentration, 100 per ml (Table 1a). When spores of the two isolates were mixed, spores of isolate No. 2 clearly restricted infection by isolate No. 1 (Table 1b). For example, the mixture with 1% of spores of isolate No. 1 infected only 15% of plants of Nevin, even though there were 1000 spores per ml of isolate No. 1, and this concentration was sufficient to infect all plants of Nevin in the absence of isolate No. 2. Three further experiments of this kind were made, and they gave almostidentical results.

How one isolate restricted infection by the other is purely a matter for speculation. The simplest explanation is that there was competition for sites at which the roots could become infected, but it is also possible that the interaction could be due to resistance of Nevin to isolate No. 1 being induced following attempted infection by isolate No. 2.

CONCLUSIONS

To sum up, it is clear that the differential pathogenicity of populations of Plasmodiophora brassicae cannot be classified adequately by making a single test on a bulk sample, however carefully the sample is collected, because there may be many pathotypes in the population. Individual pathotypes can be identified only by producing single-spore isolates. This requires the development of a more reliable and repeatable method for obtaining such isolates. The demonstration that there is intereaction between spores of different pathotypes means that it is not sufficient simply to determine the relative proportions of different pathotypes in a population, because we need to know what proportions of spores of a pathotype must be present to give infection.

A METHOD OF SCREENING FOR RESISTANCE TO CANKER IN OILSEED RAPE SEEDLINGS

Pamela Newman

Introduction A reliable method of screening for resistance to stem canker *(Leptosphaeria maculans)* in seedlings of oilseed rape would be useful to plant breeders, enabling more material to be assessed more rapidly than the field screening procedures used at present.

Method Plants were grown singly in 9 cm diameter pots in the glasshouse and inoculated when the first leaf was fully expanded but the second leaf was still growing (the plants were about three weeks old). The base of the petiole of the first leaf of each plant was wounded with a syringe needle. A 6 mm diameter antibiotic assay disc (Whatman) soaked in pycnospore suspension (1×10^6 spores/ml) was placed onto the wound. The plants were then placed under polythene covers for three days to maintain high humidity. Thirty six plants of each of eleven varieties were arranged in six randomised blocks.

Lesion circumference (C) and length (L) were scored on a 0-6 and 0-8 point scale respectively, and a total score (maximum 14) for each lesion calculated by adding together scores C + L. The scoring system used is shown in the table below.

Score	Lesion circumference (C)	Lesion length (L)
0	no infection	no infection
1	< 25% girdling	< 5 mm
2	25-50% "	5-10 mm
3	50-75% "	10-15 mm
4	75-100% "	15-20 mm
5	stem weak	20-25 mm
6	plant dead	25-30 mm
7	—	> 30 mm
8	—	plant dead

System for scoring stem lesions

Results and Discussion Stem infection was scored 21, 28, 35 and 42 days after inoculation (Fig. 1). The two resistant cultivars, Jet Neuf and Rafal, were significantly different (P=0.01) from the other nine more susceptible cultivars.

Disease ratings produced by the National Institute of Agricultural Botany (NIAB) (0-9 scale, higher being more resistant) give an indication of the field reactions of varieties (Fig. 1). Although this seedling inoculation method distinguished the two very resistant lines from the others, the ranking amongst the susceptible cultivars was not as found in the field. This waspresumably due to the many factors which may affect disease development in the field but which were not reflected in a seedling test. Nevertheless, the method has now been repeated several times, using a number of varieties and breeding lines (some of which are known to be very susceptible), and it has produced results in reasonable agreement with those obtained in the field.

Some evidence of a differential host-parasite interaction has been detected during these experiments. This has already been reported in Australia (Cargeeg and Thurling, 1980). At PBI, the interaction is being further investigated by screening many single-spore isolates on a range of varieties, using this inoculation technique.

Although this method detects resistance derived from Jet Neuf, it is possible that other types of resistance, if they exist, may be missed.

Despite these possible drawbacks, this inoculation method will be useful for initial screening, allowing elimination of very susceptible lines, and the detection of those that are likely to be resistant. Promising lines can then be multiplied and tested in field trials.

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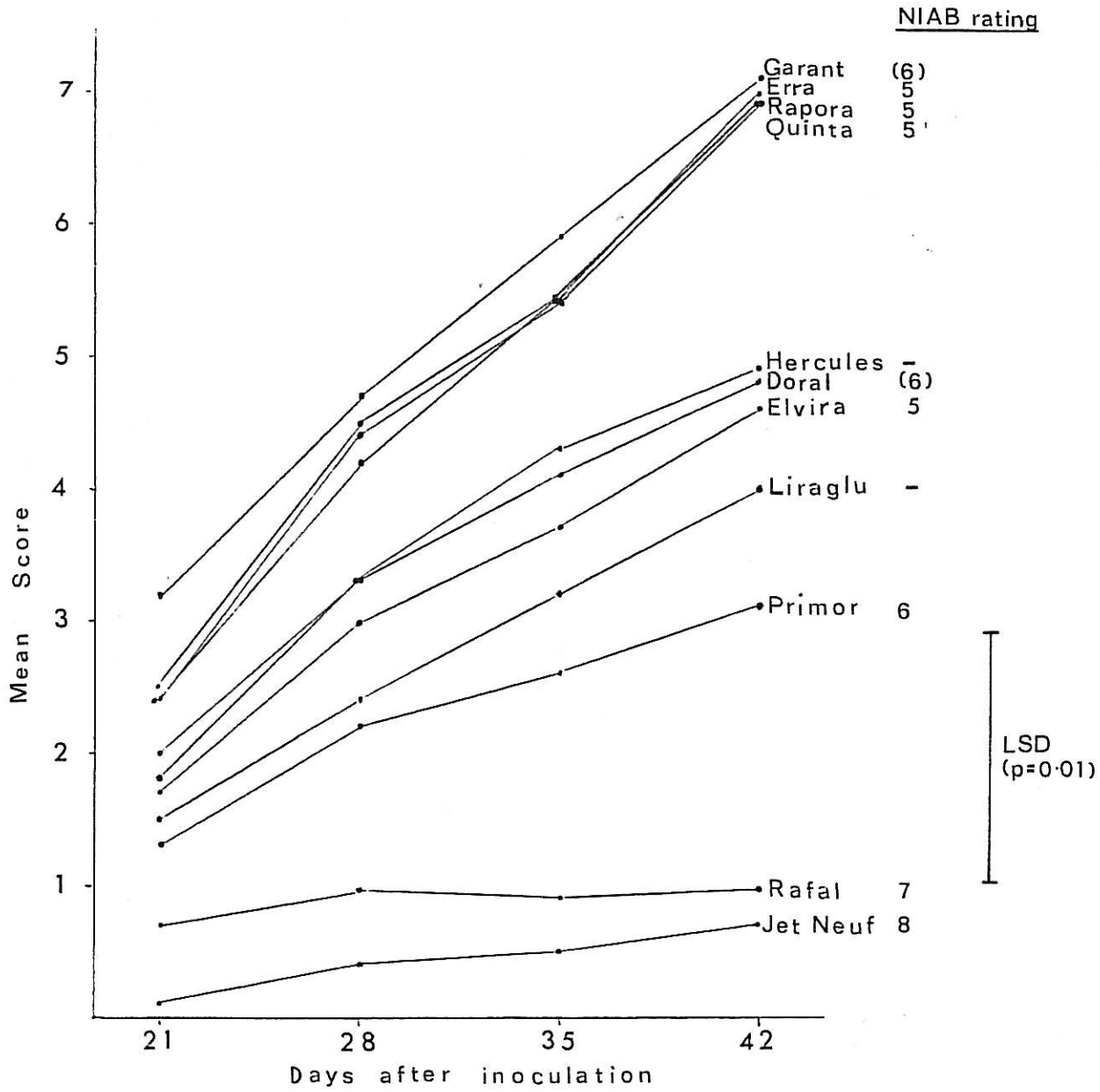

Fig.1 Development of stem canker lesions on eleven oilseed rape cultivars

RESISTANCE TO WHITE RUST IN RADISH (RAPHANUS SATIVUS L.)

A. BONNET

Crucifers white rust, Cystopus candidus (Pers.) de By. = Albugo candida (Pers.) Ktze., is sometimes the cause of serious losses in seed crops of radish. G.S. POUND and P.H. WILLIAMS discovered a resistance to race 1, specific to radish, in 2 varieties "China Rose Winter" and "Round Black Spanish" ; they showed that this resistance is controlled by a single dominant gene, Ac_1, and they developed an artificial inoculation test at the cotyledonary stage.

The material selected at Montfavet Station is very susceptible to white rust and this artificial inoculation test is being used in an attempt to find a resistance. Out of 20 varieties of small radish, 2 varieties have had some resistant plants : these were "Biser", white Bulgarian radish, and "Rubiso", round scarlet radish. "Clovis", fodder radish, "White Long Kenya" and "Purple Long Gournay", with very big root, have also had some resistant plants. However, the 2 varieties "Round Black" and "China Rose Winter" were susceptible but with relatively discrete pustules.

The segregations presented by the progeny of resistant plant "Rubiso 2" and by its cross with a susceptible line (selfing, F_1, F_2, F_3 and BC_1) fully confirm that this white rust resistance is controlled by one dominant gene. Field behaviour, in a very infected environment, gave the same results as artificial inoculation tests.

The homozygous resistant lines have been obtained by selfing resistant plants of the "Rubiso" variety. This resistance is in the process of being introduced into various material.

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CLUBROOT-RESISTANT GENE TRANSFERRED FROM RUTABAGA TO CABBAGE

M.S. Chiang and R. Crête

Third backcross progenies and plants obtained by selfing of B_2 selections derived from interspecific hybridization between rutabaga (Brassica napus) and cabbage (B. oleracea ssp. capitata) were tested for resistance to race 2 (16/02/31) of Plasmodiophora brassicae under greenhouse conditions. The results were presented in Table 1 and Table 2.

Table 1. Disease reaction tests on progenies derived from plant B_1-20

	Disease rating grade				
	Res.	susceptible			
	0	1	2	3 + 4	% Resistant plants
B_2 X cabbage	62	14	5	33	54.39
B_3 X cabbage	223	42	41	170	46.85
B_3 X broccoli	100	26	32	57	46.51
B_3 X cauliflower	41	2	8	33	48.80
B_3 X B_3	70	6	6	20	68.63

Table 2. Chi-square tests on one gene hypothesis

	Number of plants			
	Res.	Susc.	Chi-square	P-Value
B_2 X cabbage	62	52	0.8772	0.25 - 0.50
B_3 X cabbage	223	253	1.8908	0.10 - 0.25
B_3 X broccoli	100	115	1.0465	0.25 - 0.50
B_3 X cauliflower	41	43	0.0476	0.75 - 0.90
B_3 X B_3	70	32	1.7418	0.10 - 0.25

From Tables 1 and 2 it is apparent that only one dominant gene was transferred from rutabaga to cabbage.

All resistant plants formed solid heads with various head size, color and shape.

Contribution no. J.886 of the Research Station, Research Branch, Agriculture Canada, Saint-Jean-sur-Richelieu, Québec J3B 6Z8.

PROGRESS ON BREEDING FOR RESISTANCE TO BLACK ROT (*XANTHOMONAS CAMPESTRIS*) DISEASE IN CAULIFLOWER AND BROCCOLI. Shigemi Honma

Hybridizations made in 1973 between cabbage (Early Fuji) and cauliflower (Self Blanche) have yielded broccoli and cauliflower lines. These cauliflower lines were intercrossed to obtain horticulturally desirable lines. Similar hybridizations were made with the broccoli lines. In order to hasten the obtaining of horticultural types the broccoli lines were backcrossed to the cultivar Spartan Early while the cauliflower lines were backcrossed to the cultivars Self Blanche and Stovepipe. The resulting backcross population did not yield plants with the desired horticultural characteristics and resistance. It appears intercrossing of segregating lines were much more fruitful than backcrossing.

In this study seedlings were inoculated when the first true leaf was fully expanded and were again inoculated 10 days later. Survivors were transplanted in the field and were inoculated when the plants were 6 inches tall and again two weeks later.

PESTS OF RAPESEED IN SOUTHWESTERN SPAIN

Emilio Sabariego and Luis Carlos Alonso

Rapeseed in Spain is presently in a stage of introduction Last season, about 35.000 Ha were grown and most of this crop was sown in the southwestern region of the country. All the rapeseed cultivars grown in this area belong to spring ecotypes of Brassica napus L.

Although rapeseed is a new crop in our country, several - diseases and pests may represent future threat for its growth. This is because the Iberian peninsula is a center of evolu--- tion of several wild relatives of rapeseed, some of which are very common weeds.

In this report, only those insects which have been found feeding either on rapeseed leaves, flowers or pods will be -- listed.

Aphids were common in rapeseed field during the last season, mostly forming clusters on the flower stalks. Other --- insects found were:

Coleoptera: Meligethes aeneus F., Centhorrhynchus napi Gyll., and Ceuthorrhynchus assimilis Payk.

Lepidoptera: Plutella maculipennis Curtis., Pieris rapae L., and Heliothis (Helicoverpa) armigera Hb.

Diptera: Dasyneura brassicae.

Among these pests, Meligethes, Plutella and Dasyneura may be considered the most serious threats. Meligethes causes -- pod abortion because it lais a couple or three eggs in the -- flower buds. Plutella feeds on leaves, flowers and young --- pods. These two pests have been found in almost all the rapeseed fields infested. Dasyneura lais about 50 to 80 eggs --- inside young pods which have been damaged by other insects. -- Seeds from intested pods are mostly destroyed.

Pest damage was not evaluated. Yet, it is significant --- that most of last year rapeseed fields, have never had before this crop.

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2. Brassica campestris (rapa)
3. Brassica napus
4. Brassica other species
5. Other genera
6. Interspecific & inter-generic hybrids

Usage

a. Vegetables and condiments
b. Fodder
c. Green manure
d. Oil and protein

Pests, diseases and weeds

e. Animal
f. Weeds
g. Fungal
h. Bacterial
i. Viral
j. Mineral

Disciplines

k. Agronomy
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m. Physiology
n. Taxonomy and Evolution (including resources and gene banks)
o. Genetics and cytology
p. Breeding
q. Mating systems
r. Nutrition and chemical composition
s. In vitro culture
t. Variety testing (including National Lists and Breeders rights)
u. Seeds, production and performance

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