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GENETIC RESOURCES

The Crucifer Genetics Cooperative

Paul H. Williams

Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706

The Crucifer Genetics Cooperative (CrGC) was established in November 1982 in the Department of Plant Pathology at the University of Wisconsin, Madison for the purpose of acquiring, maintaining, and distributing seed stocks and pollen of various crucifers and cultures of crucifer-specific symbionts. Emphasis is on genetic, chromosomal and cytoplasmic variants useful for expediting a wide range of research in breeding, genetics, cytogenetics, genetic engineering, molecular biology, plant physiology, entomology, phytopathology, ecology and other biological studies. Included among the crucifer symbionts are parasites, pathogens and pests. Current emphasis is on genera within the tribe *Brassicaceae*. Stocks are stored and maintained in Madison with duplicate seed samples to be stored in the USDA National Seed Storage Laboratory at Fort Collins, Colorado, and symbionts and pollen to be stored at the American Type Culture Collection, Rockville, Maryland.

Seed, pollen and symbiont cultures are received from those who have unique genetic stocks and who would like to share them with others interested in crucifer genetics. Annual summaries of CrGC activities are submitted through the *Eucarpia Cruciferae Newsletter* (Wills and McNaughton, 1984). More detailed reports are sent to subscribing members of the CrGC and are also available upon request. Reports contain descriptions of the stocks held in the CrGC, accounts of accessioning and distribution of stocks, gene lists, allozymic variants, linkage groups, cytoplasmic variants and cytogenetic stocks such as trisomics and nullisomics. Technical information on plant maintenance, seed production and on the establishment of crucifer-symbiont interactions are also available.

Anyone having genetic material (qualitative, quantitative or cytoplasmic) and information that they would like to share with others may send seed and write to the CrGC. Arrangements can also be made for shipment of pollen carrying useful genes. Stocks bearing unique genes or phenotypes are normally backcrossed for several generations to the rapid cycling base population stocks of the appropriate species while selecting for the trait of interest. In the case of highly adapted stocks containing unique quantitative phenotypes (e.g., collections of wild populations or advanced breeding lines and cultivars), the CrGC requests that the donor take the responsibility of supplying sufficient seed for long-term storage and distribution; normally 20-100 grams of each stock is sufficient. At the time of assignment of a CrGC accession stock number to a line, the donor is provided with copies of descriptor, lineage and accession information for the purpose of verifying the accuracy of the CrGC records.

The CrGC will also receive genetic material on a confidential, proprietary or prepublication basis, and will honor the donor's wishes not to make the particular trait generally available through the CrGC until permission for release is given in writing. Normally with proprietary stocks, the CrGC begins introducing the traits into the rapid cycling background, then notifies the donor when the stock is sufficiently well developed for release.

Persons providing genes and stocks to the CrGC are recognized as the source of the traits. In the case of where the supplier of a trait is not the originator of the trait, the supplier is asked to provide as much information as possible on the lineage and origins of the trait. A record of stock origins, stock development (lineage), maintenance and distribution is kept by the CrGC. This information will become increasingly valuable to users of the stocks.

Through the CrGC, a range of stocks of diverse genotype and phenotype is being developed. Thirty-eight stocks are currently available for distribution, and over 100 more stocks will be added during the coming year. The CrGC lists over 250 members, in 22 countries, with professional interests in: genetics, plant breeding, molecular-, cell-, population- and developmental biology, ecology, biochemistry, pathology and teaching.

Crucifer Genetics Cooperative Resource Book

The development of the CrGC *Resource Book* provides a mechanism for supplying information among members of the CrGC. The Resource Book consists of sections representing various categories of information relating to crucifers. Information documents (IDs) are coded as to the subject and originator and are dated to indicate the most current version of the document. Members are identified with a code comprising the first three letters of their last name followed by the first three letters of their first name (e.g., Paul H. Williams

= WILPAU). Codes are referenced to a complete membership listing. Members of the CrGC are encouraged to submit IDs that become part of the resource book. IDs normally contain summarized descriptive information or techniques that are generally useful in research or teaching. IDs could also contain lists and descriptions of seed stocks, cell clones, gene libraries, etc., available from the CrGC member's laboratory or organization. The information presented in an ID should be sufficiently complete so as to be useable either on its own, or with reference to other existing CrGC IDs in the Resource Book, or with reference to the published literature. IDs received from CrGC members are codified, copied and sent to the CrGC membership. Originators of IDs may suggest new file categories for documents which would appropriately represent new areas of information in the Resource Book. The system is constructed to be open ended and to accommodate new categories and subcategories.

The initial CrGC Resource Book issued in May 1985, contains 55 IDs in 12 subject areas totaling 150 pages (CrGC, 1985).

Operation of the Crucifer Genetics Cooperative

In order to partially defray the costs of operating the CrGC, individual members are encouraged to pay a subscription fee of $25.00 covering a three year period. Membership subscriptions should be made to the Crucifer Genetics Cooperative, Department of Plant Pathology, University of Wisconsin. Upon payment of the fee, subscribing members receive the CrGC Resource Book complete with all current information documents including current seed stock lists. Subscribing members also receive a mailing list of the membership which includes a detailed listing of member's professional interests. All members, subscribing and non-subscribing, are entitled to prorated amounts of all available seed stocks of the CrGC without charge. Non-subscribing members may also obtain individual information documents without a charge.

In addition to support from subscribing members, the CrGC is seeking assistance from individual companies and research organizations in the form of sustaining memberships to support its mission and activities. Companies and research organizations are encouraged to subscribe to the CrGC as sustaining members for $250.00 for each three year period. Sustaining members are recognized individually for their contribution to the CrGC.

At present the CrGC receives no support from public agencies other than the University of Wisconsin-Madison.

Biology, Taxonomy, Production and Uses of Brassica

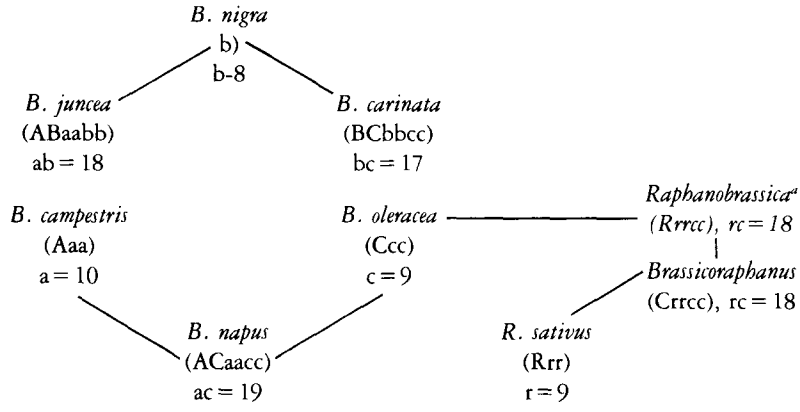
Cultivated brassicas are represented by six interrelated species, three of which are diploids, *B. nigra*, bb (n = 8), *B. oleracea*, cc (n = 9) and *B. campestris*, aa (n = 10) and three of which are the amphidiploid derivatives of the diploid

species, *B. carinata*, bbcc (n = 17), *B. juncea*, aabb (n = 18) and *B. napus*, aacc (n = 19).

Many of the *Brassica* species consist of numerous subspecies or varieties representing a diverse range of morphotypes and utilization, from oils and condiments (Harapiak, 1975) to vegetables (Vaughan et al., 1976) and animal fodders (McFarlane-Smith and Hodgkin, 1984). Brassica oil (rapeseed oil) ranks fifth in world commerce as a major edible and industrial oil, kales, rapes, turnips and swedes are important sheep and cattle fodder in climates too cool for maize or soybeans; whereas the cole crops (Nieuwhof, 1969) and oriental brassica greens are a primary dietary vitamin source for over half of the world's population (Talekar and Griggs, 1981).

The cytogenetic interrelationships of the six *Brassica* sp. were first described by Morinaga (1934) and U (1935) and since then, numerous studies have been made on the interspecific transfer of genes among various species of *Brassica* (Yarnell, 1956). More recently, intergenetic relationships between various brassicas and radish, *Raphanus sativus*, rr (n = 9) have demonstrated the transfer of potentially useful characters such as disease resistance and high dry matter content, and have resulted in the development of the new crop genus *Raphanobrassica* (McNaughton and Ross, 1978).

Figure 1. The cytogenetic interrelationships among six *Brassica* species and with *Raphanus sativus*.



[a]Intergeneric crosses between *R. sativus* and the other *Brassica* species are also possible.

The three diploid species of *Brassica* are insect pollinated and strongly outbreeding with self incompatibility controlled by a multiple allelic series of genes at the *S*-locus under sporophytically controlled expression (Wallace, 1979). Occasionally genetic self compatibility can be found, and is predomi-

nant in cauliflower and sarson (yellow mustard). Selfing of incompatible plants can be accomplished by bud pollination; the placing of "self" pollen on the immature stigmas, or by breaking incompatibility with CO_2 treatment. Selfing in the diploid species is normally accompanied by inbreeding depression. Amphidiploid species are predominantly self pollinating (75% in oil seed rape) though *S* alleles do exist in some populations (Tsunoda et al., 1980).

The reproductive forms of different brassicas range from biennial and winter annuals which may require from a few days to several months of cool temperatures (less than 5°C) to induce flowering, to annual and ephemeral types which flower without vernalization. Seed production of most brassicas occurs in regions with cool (mild) winter climates where vernalization of the biennial forms takes place. Following late summer and fall sowings, flowering occurs in the spring with seed harvest in late summer (Shinohara, 1984).

Many vegetable brassicas and radishes are produced as F1 hybrids using *S* allele incompatibility (Hinata and Nishio, 1980). Self- and sib-incompatible inbreds having different *S* alleles are interplanted in F1 hybrid seed production. Depending on the degree of *S* allele control in the particular breeding program, one-, two-, three- and four-way cross hybrids can be made. Male sterility controlled by recessive *ms* genes may be used to produce F1 hybrids (Shiga, 1980). Fertiles, occurring at approximately the 50% level in the stocks, are rogued from inbreds which are maintained through backcrossing.

Cytoplasmically controlled male sterility (CMS) is also used in F1 hybrid production (Shiga, 1980). This form of hybrid production has greatest utility when nuclear genes capable of restoration of the CMS are available. Fertility restoration is essential for crops whose seed is the commerical product. In the case of cytoplasmic-genic systems of pollen control, selection for bee attraction (nectary function) is very important to insure F1 hybrid production.

Attributes of Brassicas for Research

Seed production cycles of most crucifers are annual or biennial with the result that improvement through breeding has been slow and fundamental biological and genetic information generally lacking. Despite these impediments, the brassicas and radish are recognized by increasing numbers of plant biologists and molecular biologists as useful species for their research. Among the attributes of interest are the following:

Rapid Cycling Crucifer Stocks

The recent development at the University of Wisconsin-Madison of rapid cycling populations of each of the important crucifer species has circumvented the problems of long sexual generation times. The Wisconsin stocks have seed-to-seed generation cycles ranging between 35 and 60 days, are selected

TABLE 1. *Rapid cycling base populations of* Brassica *sp. and* Raphanus *in the Crucifer Genetics Cooperative (CrGC)*

CrGC #	Species	Days to flower	Days for cycle	Cycles per year
1	*B. campestris*	16	36	10
2	*B. nigra*	18	38	9
3	*B. oleracea*	29	59	6
4	*B. juncea*	20	40	9
5	*B. carinata*	28	58	6
6	*B. napus*	26	56	6
7	*R. sativus*	19	49	7

for homogeneous flowering, are adapted to high density plant spacing (833 plants per meter square) and are highly amenable to laboratory cultivation and *in vitro* propagation (CrGC, 1985).

Genetics

Genetic markers are being collected by the CrGC and incorporated into the rapid cycling base populations of each species. In *B. campestris* 61 marker genes including those described by Cours (1967), James and Williams (1980), and Stringam (1969, 1970, 1971, 1973, 1976, 1977, 1978 and 1980) are represented. Among these are genes for male sterility and incompatibility, pollen markers, various host-pathogen interaction phenotypes, gibberellin responders, dwarfs, chlorophyll deficiencies, etc. An additional 31 distinctive phenotypes are under genetic analysis. Marker stocks of *B. nigra* are limited to the 13 genotypes described by Delwiche and Williams (1981); however, work is underway to generate more marker traits via chemical mutagenesis and also to develop self compatible rapid cycling stocks. The *B. oleracea* collection comprises 22 genotypes many of which have been described by Sampson (1958, 1966, 1967a, 1967b, 1970, 1978a, 1978b). Eight other distinctive phenotypes are under study. In *B. oleracea,* a number of *S* alleles (Ockendon, 1982) conditioning self incompatibility are being introduced into the common background of the RC,BP. Stocks of markers in *B. napus* consist of seven genotypes including a dominant dwarf, *Du;* glossys *Gl* and *gl* and tendril, *T* and *t* (all obtained from K. F. Thompson), the dominant male sterile *Msj* (Mathias, 1985) and the dominant white *W* from a *Raphanus* alien addition line (2n = 40) (Sernyk and Stefansson, 1982). Phenotypes in *B. napus* include five flower colors from Heyn (1977), and a dwarf, a yellow green plant, white flowers and shatter resistance from G. Seguin-Swartz. In *Raphanus,* eight genes include those described by Humaydan and Williams (1976), and Williams and Pound (1963, 1967). Disease resistance phenotypes described by Humay-

dan et al. (1976) and Peterson and Pound (1960) are also in the collection.

In addition to stocks with distinctive morphological markers, the collection also contains a number of stocks conditioning carefully described interaction phenotypes to numerous crucifer pathogens. In the case of disease reaction stocks, isolates of the particular pathogens and carefully prescribed disease screening protocols are available for characterization of the host-pathogen interaction phenotypes (CrGC, 1985).

A number of cytoplasmic phenotypes are available, including cytoplasmic male steriles, CMS, derived by Ogura (1968) from *Raphanus*, by Pearson (1972) from *B. nigra*, by Hinata and Konno (1979) from *Diplotaxis muralis*, by Shiga (1980) from *B. napus* (known as 'polima' type) from *B. juncea* by Anand (personal communication), and by Chiang and Crete, (1985) from *B. napus*. Other cytoplasm with unique phenotypes include chloroplastic mosaics producing distinctive chimeral variegation, and a triazine resistant cytoplasm (Souza-Machado et al., 1978).

Interspecific and intergeneric crosses have permitted the substitution of the nuclei of the rapid cycling base populations of various species into a number of the distinctive cytoplasms given above. Nuclear substitution is a continuing part of the stock development research of the CrGC.

A putative transposable element system in *Raphanus* is expressed at a locus conditioning yellow green plant color. Mutability occurs as the highly variable appearance of normal green flecks and sectors (Williams, unpublished). Expression and transmissibility of the green flecking is similar to some of the transposable element systems reported in maize. Using rapid cycling marker stocks, experiments are under way to determine the genetic control of the instability, and the potential mobility and transmissibility of the trait from *Raphanus* into *Brassica* via *Raphanobrassica* bridging.

Cell Biology

Generally *Brassica* spp. are highly amenable to cell and protoplast culture (Chuong et al., 1985; Glimelius, 1984; Newell et al., 1984; Robertson et al., 1984, 1985; Jourdan and Earle, 1985). Protoplast fusions and injections have been accomplished (Hoffmann and Adachi, 1981; Pelletier et al., 1983; Jourdan and Earle, 1985; Schenck and Robbelin, 1982). Though regeneration procedures exist for several species, it is still erratic, dependent on tissue type, host genotype and other unknown factors (Keller and Armstrong, 1983; Lazzeri and Dunwell, 1984a, 1984b). Somatic embryogenesis and secondary embryogenesis occur with varying frequency in different species (Bhattacharya and Sen, 1980, Loh and Ingram, 1983; Loh et al., 1983). Androgenic haploids are readily produced in most species, and sometimes at high frequency (Keller and Armstrong, 1981; Leelavathi et al., 1984; Lichter, 1981; Ock-

enden, 1984). Microspore culture has great potential, but is presently limited to *B. napus* and *B. carinata* (Dunwell et al., 1983, 1985). As Thurling and Choy (1984) illustrate for microspore culture, success in generating whole plants from various cell types is still highly genotype dependent. The use of the CrGC rapid cycling stocks in cell biology has the advantage of shortening the time for which genes favoring regeneration can be accumulated. The CrGC base populations are being used for this purpose in a number of laboratories.

Molecular Biology

Brassicas are particularly suitable for molecular biological approaches. Most species are infectible by *Agrobacterium tumefaciens* and *A. rhizogenes* (De Cleene and De Ley, 1981; Tanaka et al., 1985). Experiments comparable to co-cultivation reported for solanaceous species (Hain et al., 1985) have also been achieved with *B. napus* (Moloney and Hera, 1984; Moloney, personal communication). Potrykus (1985) mentions a manuscript by Paszkowski et al., (in preparation) reporting the transformation of protoplasts of *Brassica (syn. B. campestris)* to kanamycin resistance via direct gene transfer. Most brassicas are infectible by cauliflower mosiac virus (CMV), one of a few DNA plant viruses with potential as a gene vector (Hohn et al., 1985). Characterization of nuclear genes is underway in a number of laboratories (Delseny, 1983). The cloning of genes is underway (Crouch et al., 1983; Simon et al., 1985) and gene libraries are being developed and are available for some species. Brassicas have relatively low amounts of nuclear DNA. Michael Bennett (personal communication) estimated the nucleus of the rapid cycling *B. campestris* stock (CrGC-1) to contain 0.6 pg DNA at the C level.

Considerable progress has been made in the molecular characterization of the cytoplasmic components of *Brassica* (Chetrit et al., 1984, 1985; Link, 1984). The chloroplastic genomes of all *Brassica* species have the typical angiosperm arrangement consisting of a large inverted repeat dividing the genome into small and large single copy regions. About 20 genes have been mapped (J. Palmer, personal communication). Palmer et al. (1983b) have traced the evolution of a number of *Brassica* species based on restriction enzyme fragment mapping of chloroplast DNAs. *Brassica* mitochondrial genomes are several times smaller than most plant mitochondrial genomes (Palmer and Shields, 1985). All fertile and CMS lines of *B. campestris* and *B. napus* contain a linear 11.3 kb mitochondrial DNA plasmid (Palmer et al., 1983a) which is inherited nonmaternally (Palmer, personal communication).

An important consideration in the molecular biology of *Brassica* is the fact that *Arabidopsis* for which considerable genetic (Koorneef et al., 1983) molecular (Leutwiler et al., 1984), and biochemical (Browse et al., 1985) information exists, is also a member of the *Cruciferae*. The recent report by North

(1985) cites Meyerowitz's work on the *in situ* hybridization of the cloned *Arabidopsis* seed protein gene with seed protein mRNA. Cloned DNA from the *Brassica* seed protein gene also shows strong homology with the *Arabidopsis* seed protein message (M. Crouch, personal communication).

Biochemistry and Developmental Biology

Among the various problems in which biochemical and physiological research may be of value in improving crucifers, oil content and quality (Röbbelen and Thies, 1980a) glucosinolate content (Röbbelen and Thies, 1980b) and seed meal protein have received attention. Though there exists considerable analytical information of the glucosinolates of both vegetative parts (Sang et al., 1984) and seeds (Gland et al., 1981) of crucifers, little definitive biochemical or genetic information exists on the complex glucosinolate biosynthesis.

The most promising system for genetics and biochemical analysis appears to be fatty acid synthesis. The genetics of the fatty acid, erucic (22:10) is relatively simple, and stocks with alleles controlling various levels of erucic acid exist. The changes in activity in the acetyl Co-A carboxylase during embryogenesis (Turnham and Northcote, 1983) and acyl carrier protein genes appear to be useful systems for examining the developmental regulation of genes governing fatty acid synthesis. Likewise, the genes governing the production of embryo specific storage proteins (Crouch and Sussex, 1981; Crouch, 1982) appear to be excellent candidates for examining gene regulation and expression using biochemical and molecular biological methods (Crouch et al., 1983; Simon et al., 1985).

Among the CrGC stocks are a number of mutants that have potential for biochemical studies or studies in developmental biology. Some of these are: 1) *ro/ro* = rosette, an extreme dwarf which is a strong gibberellin responder; 2) *dw/dw* = dwarf, a dark green dwarf plant type which resembles abscisic acid deficient mutants in other species; 3) *eh/eh* = elongating hypocotyl, a pale green mutant which shows extreme hypocotyl and nodal elongation in the light, and resembles some phytochrome deficient mutants; 4) *y/y* = yellow embryo, where the developing embryo remains completely free of green pigmentation throughout embryogenesis; 5) numerous other chlorophyll deficient mutants (Stringam, 1969, 1973) and anthocyanin mutants (Sampson, 1967b, Stringam, 1971).

Genes of Economic Importance

In addition to the genes described above, there are other genes which impact more directly with the objectives of plant breeders. Important among these are the *S* alleles conditioning self-incompatibility (Redfern, 1982; Roberts et

al., 1980). The *S* locus is amenable to both genetic (Wallace, 1979) and to molecular analysis. The glycoprotein products of *S* alleles have also been examined biochemically (Hinata and Nishio, 1980; Okazaki and Hinata, 1984). The CrGC is undertaking the incorporation of various *S* alleles in the common backgrounds of the rapid cycling base populations.

Another group of important genes are those conditioning the interaction phenotype between crucifers and various pathogens. The specificity with which various pathogens interact with particular host genotypes makes some host-pathogen systems attractive for basic studies on gene action. The CrGC maintains a collection of numerous isolates and pathotypes of the following pathogens: Turnip mosaic virus (Green and Deng, 1984), *Xanthomonas campestris* pv. *campestris* (Alvarez et al., 1985; Daniels et al., 1984), *Albugo candida* (Pound and Williams, 1963; Fan et al., 1983), *Peronospora parasitica* (Kluczewski and Lucas, 1983), *Leptosphaeria maculans* (Newman, 1984; Roy, 1984) and *Fusarium oxysporum* f. sp. *conglutinans* (Ramerez-Villupadua et al., 1985).

A major portion of the research of the author, P. H. Williams, and his collaborators is in the development of methodologies that permit the identification of, and genetic analysis of host-pathogen interaction phenotypes ranging from high resistance to very high susceptibility in seedling populations of the various *Brassica* species and *Raphanus*. Highly reproducible host-pathogen interaction phenotypes are being introduced into the rapid cycling stocks for distribution and use by plant breeders and plant biologists interested in research on host-pathogen, specificity, evolution, ecology and epidemiology.

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