

## Rapid-Cycling Populations of Brassicas

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*Rapid-cycling populations of six economically important species in the genus Brassica have unusual potential for resolving many problems in plant biology and for use in education. Rapid-cycling Brassicas can produce up to ten generations of seed per year and serve as models for research in genetics, host-parasite relations, molecular biology, cell biology, plant biochemistry, population biology, and plant breeding. Brassicas are a highly diverse group of crop plants that have great economic value as vegetables and as sources of condiment mustard, edible and industrial oil, animal fodder, and green manure. These plants can also be used in the classroom as convenient, rapidly responding, living plant materials for "hands on" learning at all levels of our educational system.*

Major advances in biology often come when diverse disciplines focus on model organisms. An essential reason why many microbes, *Drosophila*, and mice have continued to be useful as research models is that their genetic information can be readily studied by the developing technologies in molecular biology. These organisms have rapid reproductive cycles and are amenable to laboratory culture in large numbers. Among the higher plants, a short-cycled member of the cabbage family, *Arabidopsis thaliana*, has emerged as an important model for research in molecular genetics (1). Species in the genus *Brassica*, also in the cabbage family, have unusual potential as models for studying plant systems.

### Diversity, Biology, and Production

The brassicas are a diverse group of crop plants with great economic value worldwide [see the "Around the World with Brassicas" slide show]. These plants produce condiment mustard; leafy, stored, processed, and pickled vegetables; seed oils for margarine, salad oil, cooking oil, plastic and industrial uses; animal fodders; and green manures for soil rejuvenation (2).

Although brassicas are known in the United States mainly as highly nutritious vegetables—cabbage, cauliflower, broccoli, collard, kale, mustard greens, and Chinese cabbage—their potential value as oilseed crops and animal fodder is just beginning to be recognized (3). Crucifer oil, known as rapeseed oil, is the fourth most commonly traded vegetable oil in the world. Rapeseed contains 40% oil, which is pressed from the seed, leaving a high-protein seed meal of value for animal feed and nitrogen fertilizer (4). Most Northern European countries produce rapeseed as their main edible oil crop. Salt-tolerant rapeseed is one of the first crops grown on the reclaimed polder land in Holland. China and India each grow rapeseed on over 3 million hectares, and Canada exports rapeseed "Canola," produced on 4 million hectares. An important component of some rapeseed varieties is the 22-carbon unsaturated fatty acid, erucic acid (22:1). Erucic acid is a

component of resins and lubrication oils for jet engines and is used in steel manufacturing. Since it interferes with mammalian metabolism, only plants containing little or no erucic acid are grown for human and animal consumption (5). Brassicas are also grown for animal fodder in regions too cool for maize or during winter months when grass grows slowly. Large acreages of turnips, rutabagas, leafy forms of cabbage, and kales with thickened succulent stems provide winter grazing for sheep and cattle in Northern Europe and New Zealand.

Brassica oil and vegetables are an essential part of the diets of many developing nations. The Chinese consume 0.25 kilogram of crucifer vegetables per capita daily and in Korea consumption is even higher. A close relative of Brassica, radish (genus *Raphanus*), is grown as a vegetable in China, Korea, Japan, and India, where many large root types are dried, brined, pickled, cooked, or fed to animals.

The six major Brassica species of economic importance exist in a natural relationship that was described by the genetic and cytogenetic work of U and Morinaga (6). Three diploid species, *B. nigra* (bb), *B. campestris* (aa) (syn. *B. rapa*), and *B. oleracea* (cc), are the progenitors of the naturally occurring allotetraploid species *B. juncea* (aabb), *B. napus* (aacc), and *B. carinata* (bbcc). Diploid *B. campestris* (aa) therefore has 20 chromosomes and allotetraploid *B. juncea* (aabb) has 36 chromosomes.

Within each of the species there are a range of forms that represent divergent selection during domestication. Within *B. oleracea* are "cole crops" such as cabbage, cauliflower, curly kale, kohlrabi, and Brussels sprouts, and the bizarre tree cabbage or Jersey kale. Tree cabbage, which may be up to 3 meters tall, is grown on the Channel Islands, where the leaves are stripped from the stem as cattle feed in winter and the remaining stalks are cut and dried for manufacture as walking sticks (7). Several oilseed types are found within *B. campestris*, as well as Chinese cabbage, turnip, pak choi, and a host of other forms representing vegetables consumed in various Oriental cultures. Brassica *juncea*, generally known as mustard, also displays a wide divergence of form and is used as a source of oilseed in India and Pakistan, and as a vegetable in western central China. The sharp mustard flavor is imparted by high levels of the mustard oil isothiocyanate in the leaf tissues. The genes controlling mustard oil synthesis are contributed to *B. juncea* (aabb) largely through the genome of *B. nigra* (bb), the black mustard (8). Brassica *napus* varieties are used for oilseed, fodder, and the vegetable rutabaga. Both wild and cultivated forms of *B. carinata* are major sources of leafy greens and cooking oil in Ethiopia.

The wide divergence in form within Brassica species has caused taxonomic confusion (9); considerable variation occurs among the characteristics that differentiate intraspecific taxa. Efforts to clarify the taxonomic status of some subspecific taxa will likely continue for many years. A convenient taxonomy of the major morphotypes within each species makes use of the genomic symbols for each species together with an additional one- or two-letter descriptor representing the intraspecific taxon.

The potential for exchange of useful genetic information between brassicas and the closely related radish was demonstrated in the 1920's by Karpechenko (10). To combine the large root of *Raphanus sativus* (radish) with the head of cabbage, he created the synthetic genus *Raphanobrassica*. As with many such wide crosses in domesticated plants, neither the attributes of radish nor of cabbage were attained. Rather, derived raphanobrassicas are vigorous plants used for sheep and cattle fodder and green manure. *Raphanobrassica* can serve as a bridge for the transfer of useful traits such as cytoplasmic male sterility and disease, as well as nematode resistance from radish into brassicas (11). The relative ease with which diploid and tetraploid species may be intercrossed has permitted the resynthesis of the amphidiploid species, as well as the production of new types with varying numbers of chromosomes. An artificial *B. napus* (aacc), known as "hakuran," has been derived from Chinese cabbage (aa.p) and cabbage (cc.c), and is a new vegetable and fodder crop (12). The transfer of resistance to clubroot and blackleg diseases to susceptible species has also been achieved by interspecies crosses (13). Seed production cycles of different brassicas and radishes can be annual, winter annual, or biennial cycles that require a few days to several months of cool temperatures (<5 degrees C) to induce flowering. Flowering may also be under the control of photoperiod (14). Seed of vegetable and fodder crucifers is produced in regions with mild winters where flower induction takes place. After late summer and fall sowing, flowering occurs in the spring and seed harvest in late summer. Seed of vegetable and fodder crucifers is produced in Australia, China, Europe, India, Japan, Korea, New Zealand, and the United States. The mild Pacific coastal states of Washington, Oregon and California are ideal for crucifer seed production because dry summers minimize seed-borne diseases caused by *Alternaria*, and *Leptosphaeria* fungi, and *Xanthomonas* bacteria (15).

### The Development of Rapid-Cycling Brassicas

In spite of their diversity and importance, relatively little basic genetic information exists for the brassicas and radish, perhaps due in part to the relatively long reproductive cycles of 6 months to a year or more for most horticultural types. In 1970, while growing brassicas from a world collection of over 2000 accessions obtained from the United States Department of Agriculture's National Plant Germplasm System, we noticed that a few plants of each species flowered in a significantly shorter time than others. We could then use these faster flowering types to develop populations that would be tailored strictly for experimental uses under controlled laboratory conditions. By combining the genes of early flowering types from various sources, we might be able to breed for reduced reproductive time. At the same time, if criteria for growing each plant were selected to meet the need for growing large numbers under standardized laboratory or classroom conditions, then the desired model population should result.

To do this, fast flowering plants from each Brassica species and radish were grown at high plant densities in 28 by 55 centimeter plastic multipot trays of 96 pots per tray. We used an "artificial soil" mixture of peat moss and vermiculite (1:1), and watered the plants with balanced nutrient solution. The plants were grown at 24 degrees C and continuously illuminated with very high output fluorescent lamps under 250 micromoles per second per square meter of irradiance (16). Populations were derived by interpollinating diverse

early flowering types within each species. Criteria used in selecting individuals for successive generations were (i) minimum time from sowing to flowering, (ii) rapid seed maturation, (iii) absence of seed dormancy, (iv) small plant size, and (v) high female fertility. Populations of 288 or more were grown at each cycle of reproduction and the 10% of the population that flowered earliest was selected and mass pollinated to produce the next generation (16). When the reduction in the average days to flowering became stabilized and when greater than 50% of the population flowered within a 2- to 3-day period, selection on a population was discontinued. Populations of each species were then increased by mass pollination and designated as rapid-cycling base populations (RCBP's). We characterized the flowering and growth characteristics of each of the RCP's and charted the flowering profile of each species population.

Although the RCBP's were relatively homogeneous with respect to plant morphology and flowering time, they still appeared to contain substantial variation. Preliminary evaluation of the stocks indicated considerable isozyme variation among individuals of each population. This variation will be valuable in the development of gene linkage maps. In addition, when the RCBP's were inoculated with various disease-causing organisms, a wide range of plant-to-plant variation, ranging from resistance to susceptibility, was observed (16). Such observations indicated the RCBP's possess reservoirs of genes useful to plant breeders seeking genetic resistance to pests.

The RCBP's responded strongly to the environment in which they were grown. When we grew them in the standard conditions under which they were developed, the stocks had [certain] phenotypes. When given more space for root and top growth the plants grew many times larger and produced abundant quantities of seed; when restricted to minipots at densities of 2500 plants per square meter *B. campestris* (CrGC-1) produced 25 seeds per plant.

The RCBP's can serve as repositories for genes used in genetic mapping by both conventional and molecular biological methods. They are convenient laboratory plants for studies on plant regeneration, gene transfer, and nuclear-cytoplasmic interactions and are useful as rapidly responding models for gene expression in plant development. These strains are presently serving as model populations for examining aspects of reproduction, adaptation, gene flow, and evolution. Plant breeders and pathologists have found that they also facilitate the recombination of favorable genes for improved crops. Rapid-cycling brassicas also provide convenient, fast growing living plants for "hands on" learning in the classroom.

#### Brassica Research and the Potential for Rapid-Cycling Stocks

A wide range of rapid-cycling brassica stocks is being developed for use in many aspects of basic and applied biology. We are introducing genes for distinctive, simply inherited traits in each species for comprehensive linkage mapping of the chromosomes, and are incorporating all available mutants described in the literature, as well as those sent by brassica researchers (17). We have also developed self-compatible stocks of the three diploid species as starting material for mutation studies, as well as rapid-cycling

tetraploid stocks of *B. oleracea*, *B. campestris*, and *R. sativus*, by colchicine treatment of diploids. These tetraploids are being used to create triploids for the subsequent production of trisomic and other aneuploid chromosome stocks. Among the numerous mutations incorporated into the RCBP's, those of interest to plant biochemists include gibberellin responders, dark green dwarfs, elongated internodes, chlorophyll deficiencies, and anthocyanin suppressors.

The RCBP's can serve as repositories for various classes of genes that have potential economic value. Work is under way to construct gene pools of both major and minor genes for resistance to various crucifer pathogens. Such stocks will be of value to plant breeders seeking sources of resistance. The rapid-cycling stocks of *B. oleracea* and *B. campestris* are also being used in the development of tester stocks containing various known genes (S-alleles) conditioning pollen-stigma incompatibilities. These stocks would provide a readily available supply of pollen of known genotypes for use in evaluating S-alleles of inbreds for F1 hybrid production (18).

Brassicas provide promising systems for combining genetic and biochemical analysis of fatty acid systems. Erucic acid levels are controlled by a relatively simple series of allelic genes (5). Changes in the acetyl coenzyme A carboxylase during embryogenesis and in the acyl carrier protein genes can be monitored to examine developmental regulation of genes controlling fatty acid synthesis in *B. napus* (19). Likewise, genes governing the production of embryo-specific storage proteins of *B. napus* are being used as model systems for examining gene regulation and expression (20). The search for genes triggering and controlling flowering are also under study with photoperiod-responsive, rapid-cycling *B. campestris* and *B. juncea*.

Brassicas are easily grown in cell and protoplast culture. Protoplast fusions and injection have been accomplished in various species, and regeneration of whole plants from cultured cells, protoplasts, and protoplast fusion products has been achieved (21), although success is generally erratic and depends on tissue type, host genotype, and other defined factors. The RCBP's of *B. oleracea* and *B. napus* are highly regenerable. Further reproduction of regenerants by seed will make it possible to extend the selection for regenerative capacity.

The generation of embryos from cell cultures, known as somatic embryogenesis, and the subsequent development of additional embryos from the initial somatic embryos, that is, secondary embryogenesis, occurs with varying frequency in different species (22). Anther-derived haploids are readily produced in most species and these doubled haploids have been used to produce inbred parents for F1 hybrid seeds (23). Regeneration of embryos from pollen has been achieved with all of the polyploid species (*B. juncea*, *B. napus*, and *B. carinata*) and has great potential in brassica breeding and for gene transformation research (24).

Brassicas are well suited for molecular biological studies. Most species are infectable by *Agrobacterium tumefaciens* and *A. rhizogenes* (25). Transformation of *B. napus* with *A. tumefaciens* containing the bacterial neomycin phosphotransferase gene has been

achieved by the cocultivation of the bacterium with tissue explants, and subsequent regeneration from the transformed cells has been accomplished (26). Transformation of *B. campestris* to achieve kanamycin resistance has also been accomplished by direct gene transfer (27). Most brassicas can be infected by cauliflower mosaic virus, a DNA virus with potential as a gene vector (28).

Characterization of various nuclear genes is under way, including genes encoding ribosomal RNA in radish (29), genes for the 12S storage protein in *B. napus*, and genes encoding an S-locus-specific glycoprotein that conditions pollen-stigma incompatibility in *B. oleracea* (20). Brassicas have relatively low amounts of nuclear DNA when compared to many other species. The nucleus of rapid-cycling *B. campestris* CrGC-1 is estimated to contain 0.6 picogram of DNA (30, 31). Several laboratories are mapping the genome of various species by construction of restriction fragment length polymorphisms (RFLP's). As appropriate molecular probes are developed, RFLP maps will become increasingly useful for establishing gene linkage maps and in determining inter- and intraspecific homologies (32).

The close taxonomic relationship of Brassica and Raphanus with *Arabidopsis thaliana* may be of value in plants molecular biology. Both are in the family Cruciferae. Considerable genetic, molecular, and biochemical information exists for *Arabidopsis* and brassicas would be a logical system to investigate the transfer and expression of genes identified in *Arabidopsis*. Cloned DNA from the *B. napus* seed-protein gene shows strong homology with the *Arabidopsis* seed-protein messenger RNA (1). Likewise, *Arabidopsis* gene libraries have been useful in identifying homologies with several brassica genes. Considerable progress has been made in the molecular characterization of the cytoplasmic components of Brassica. The chloroplastic genomes of all brassicas have the typical angiosperm arrangement—a large inverted repeat that divides the genome into small and large single-copy regions (33). A number of chloroplast genes have been mapped in various Brassica species (34). Restriction enzyme mapping of chloroplast DNA's has been used to trace the evolution of several species of brassicas (35). Brassica mitochondrial genomes are of interest because they are several times smaller than most plant mitochondrial genomes (36).

In addition to serving as repositories for nuclear genes, the RCBP's have been particularly useful for introducing the nuclei of the various species into cytoplasms expressing distinct phenotypes such as male sterility and triazine herbicide resistance (16, 37). Because the cytoplasmic traits are transmitted only through the female, the nuclei of a species can be introduced into the cytoplasm of any other species by making crosses between the appropriately selected parents. Normally, after making an interspecific or intergeneric cross, seed set is very low and in some cytoplasmic male steriles, floral morphology and nectary function are also abnormal. However, in RCBP's, normal numbers of seeds produced and normal floral characteristics have been restored. The value of using RCBP's in breeding has been illustrated with Chinese cabbage. We have introduced resistance to clubroot disease from turnip, resistance to viral and mildew infection from Chinese cabbage, and cytoplasmic male sterility from radish into the RCBP's and have determined their combined inheritance patterns. From our

understanding of the combined inheritance for multiple disease resistance and cytoplasmic male sterility, superior Chinese cabbage types were reconstituted within a 2-year period (38).

#### #RCBP's as Educational Models

Perhaps the most exciting use for the rapid-cycling brassicas is their potential utility as living material in the classroom for teachers and students at all levels of education. Most biology courses lack convenient living materials and many use animals predominantly. General and advanced courses in botany, science education, and the applied plant sciences usually lack suitable living plant material that would permit students to explore plant growth development, physiology, reproduction, genetics (39), evolution, and ecology. RCBP's are particularly amenable to classroom settings because they show remarkably rapid development, the plants flower in 14 to 18 days, they are small, and they can reproduce at high densities (up to 2500 plants per square meter) under fluorescent lighting in a classroom. The ease with which plants can be grown and pollinated, together with the wide array of interesting morphological mutations available in the rapid-cycling background, make these plants particularly attractive to teachers and students. The RCBP's have far-reaching potential nationally both in university education and in kindergarten through high school. Teachers at all levels can help students learn more about plant biology through "hands on" exploration with these rapidly responding living plants.

#### The Crucifer Genetics Cooperative

The potential of the rapid-cycling brassicas prompted the formation, in 1982, of the Crucifer Genetics Cooperative (CrGC) as an instrument for dispensing seed of the RCBP's to researchers and educators throughout the world. Members of the CrGC share seed stocks, pollen, gene libraries and technical information. Many specialized stocks that have unique genetic characteristics are being developed and distributed through the CrGC (40). The CrGC also maintains and distributes microbial symbionts of crucifers including parasitic fungi, bacteria and viruses. Information on the CrGC may be obtained from the authors.

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41. We thank S. A. Vicen for assistance with the photographs. Supported by the College of Agricultural and Life Sciences and University Industry Relations Program of the University of Wisconsin-Madison and by the National Kraut Packers Association.
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