Chapter 1

Rapid-Cycling Brassicas (RCB's) in Hands-on Teaching of Plant Biology

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Educational Uses of the Rapid-cycling Brassicas

INTRODUCTION

The development of rapid-cycling brassicas (RCB's) as model organisms for research and education is profoundly influencing the quality of science education at all levels by bringing dynamic living materials into the classroom. Most biology courses lack convenient living materials; many use animals predominantly. General and advanced courses in biology, botany, science education and applied plant sciences usually lack suitable living plant material that would permit students to explore plant growth and development, physiology, reproduction, genetics, evolution and ecology. These speedy relatives of mustard are particularly amenable to classroom settings because they show remarkably rapid development (Figure 1), they flower in 13 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 RCB's can be grown and pollinated, together with the wide array of interesting variants available in the rapid-cycling type, make these plants particularly attractive to teachers and students. RCB's have far-reaching educational potential, from kindergarten through college. Teachers at all levels can help students learn more about plant biology through hands-on exploration with these rapidly responding plants.

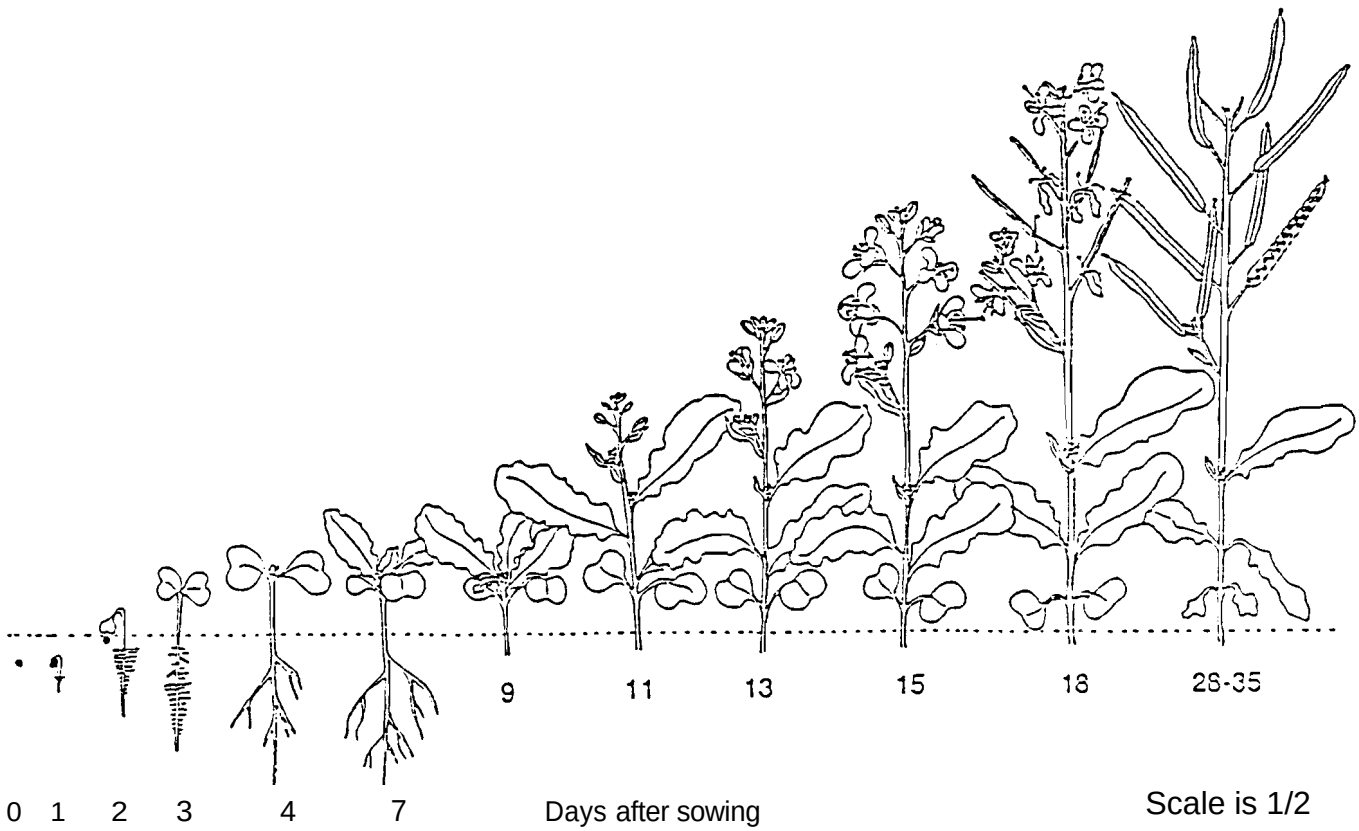

Figure 1. Growth of Rapid-cycling *Brassica rapa* cultivar RCBr showing growth stages at various times from seeding until 28 days.

With support from the Educational Materials Development Program of the National Science Foundation, the Wisconsin Fast Plants Program was initiated to develop a Wisconsin Fast Plant (WFP) kit consisting of 1) specialized genetic seed stocks of rapid-cycling *Brassica rapa* (RCBr) tailored especially for classroom use and 2) self-supporting systems for growing and experimenting with RCB's.

WFP growing systems (Figure 2) are designed to be used in various experiments. The basic growing unit is a 'quad' pot containing four cells, each cell supporting one plant. Seed is sown in a specially tested soil mix to which a slow-release balanced fertilizer is added. A small wick protrudes from the bottom of each cell of the quad, providing a moisture conduit to a water mat lying on a platform over a water containing reservoir. One edge of the water mat extends into the water in the reservoir. The depth of the reservoir is sufficient to provide water to the plants for 3-4 days. Once the seed is sown and watered, only water is added to the reservoir.

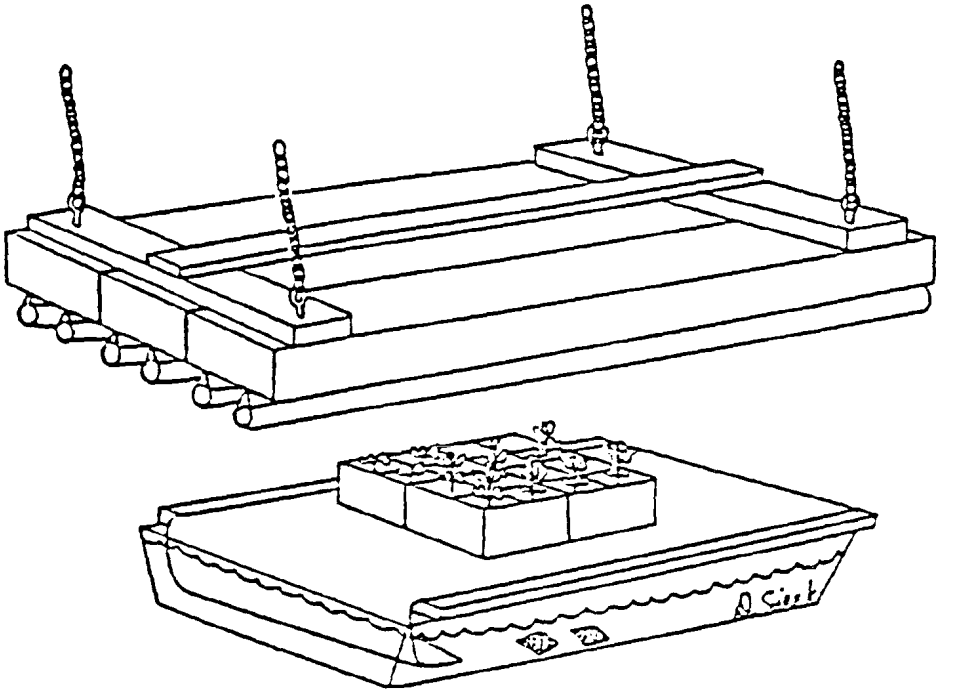

Figure 2. Wisconsin Fast Plants growing system.

Accompanying the seed and growing system is a WFP manual, comprising many exercises which have been developed and tested by teachers participating in the WFP program. These materials address the educational goals of: 1) teaching basic concepts of biology; 2) stimulating inquiry and problem solving; 3) increasing the impact of genetics teaching; and 4) bringing new excitement into the classroom.

The WFP educational materials have been developed to provide teachers and students with the opportunity to investigate a wide range of higher plant biology. Table 1 presents some central topics that can be addressed through the WFP materials.

Table 1. Educational topics that can be addressed using RCB's.

1. Growth and development
   a. Growth; seed germination (plants up in 2 days), leaf formation, stem elongation, flowering (13-16 days), fruit (pod) and seed (embryogenesis) maturation
   b. Growth responses; (plant bends up in 2 hours)
   c. Development/morphology; root, stem, leaf, flower

2. Reproductive biology
   a. Flower development; male and female parts of flower
   b. Pollen and pollination; control of pollination, bee sticks
   c. Fertilization
   d. Embryogenesis

3. Genetics; Mendelian and lion-Mendelian
   a. Mendelian; gene expression, dominance, interaction
   b. Mendelian; gene assortment, independence, linkage, F1, F2 test cross
   c. Non-Mendelian; maternal inheritance
   d. Selection
   e. Evolution

4. Physiology; underlying mechanisms of growth and developmerit
   a. Using numerous physiological mutants: gsowth hormone responders
   b. Photosynthesis; radiant energy utilization
   c. Nutrition; effects of major and minor elements on growth and reproduction
   d. Water relations; excesses and deficiencies
   e. Photoresponses; light intensity, photoperiodand flowering, tropism, etc.

5. Ecology; the plant responding to its environment
   a. Influencesof acid rain on plant growth and development
   b. effects of air pollution;pollution-sensitive mutant stocks
   c. Chemicals in the plant environment;salt injury, herbicide effects
   d. Effects of pests and diseases; disease resistance, microbe-plant interactions

RCB's are suitablefor introducing students to all aspects of growth and developmentfrom germination through to the harvesting of seed. Germinating in less than 12 hours, RCB's emerge in 48 hours, flower buds appear in 7-8 days and flowers begin to open in 12-13 days.

With the initiation of flowering, many aspects of reproductive biology can he learned. Floral morphology and its intimate relationship with the honey bee *(Apis mellifera)* provide an excellent example of coevolutionary interdependence between two organisms. An understanding of the relationships between the bee and the flower can be gained throughthe dissection and close observation of the parts of the flower and the honey bee. Followingthe dissection, students can explore the remarkably efficient pollen collecting ability of the bee by making a beestick from a dead bee and using it as a pollination device for their plants (Figure 3). By investigating pollination and the control of pollen germination, the mechanismsfor ensuring outbreedingof the species can be understood. Following double fertilization, the exploration of endosperm and embryo development through dissections under a microscope can be a challenging and exciting experience in learning.

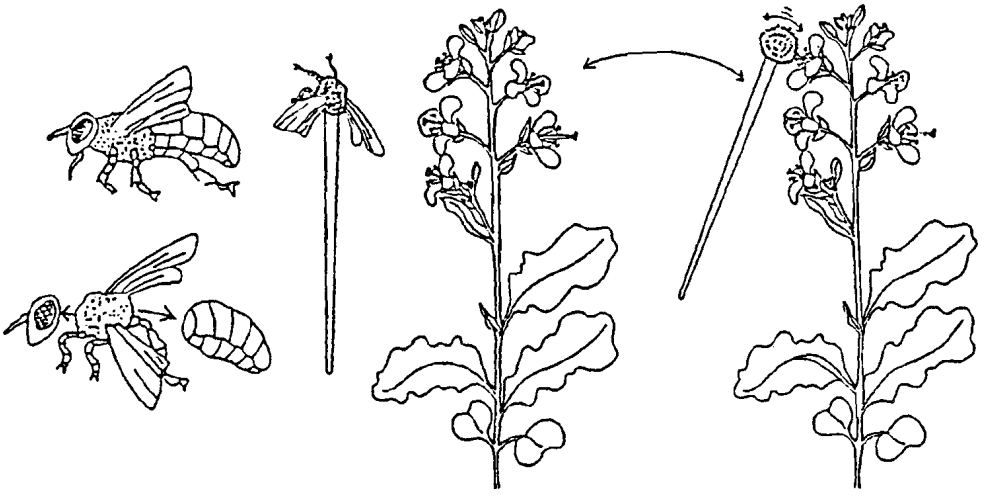

Figure 3. Bees, beesticks, and cross pollination of brassica flowers using a beestick.

An understanding of reproductive biology provides a useful setting in which to present genetics. With many interesting phenotypes and mutants available, Mendelian, cytoplasmic and population genetics can be explored. Ongoing research in the scientific community will soon make available cytogenetic stocks, molecular markers, physiological mutants, cytoplasmic hybrids and, eventually, transformed RCB's. Rapid-cycling *Brassica rapa* can be crossed readily with turnip and Chinese cabbage (see accompanying exercise). The progeny and subsequent F_2 generation of such crosses can provide exciting materials for students interested in evolution, domestication and plant breeding.

Underlying the expression of the phenotype in growth and development is the domain of physiology. The RCB's are well suited for exploring how plants respond to physical and chemical stimuli in their environment. Various physiological mutants are available with which to investigate the influence of light, nutrients and hormones on plant growth and photosynthesis.

Exploring how the RCB's respond to changes in their environment can provide the basis for interesting experiments in ecology. Variation in the acidity of precipitation, the salinity of water and the chemical composition of the soil and atmosphere in which the plants are growing all are excellent avenues for exploratory learning. By growing the RCB's in cages or jars, the effects of various pests on plant growth can be examined. Modifying the chemical, physical and biological environments in which the RCB's grow provides virtually unlimited opportunities for independent investigations and learning by students.

The Need for Good Lighting

The major requirement for successful use of the RCB's is adequate lighting. These plants have been selected to perform best under continuous bright cool-white fluorescent lighting. Adequate lighting can be obtained at 5-10 centimeters distance from banks of six 4-foot cool-white bulbs spaced at approximately 10 centimeters apart. Eight or ten closely spaced cool-white fluorescent bulbs will result in even better plant growth. Providing they have adequate light, the plants grow very well in classrooms and hallways; frequently they do better in these open areas than in the confines of small plant growth cabinets where other environmental parameters such as relative humidity and air velocity are difficult to control.

Taxonomy of Rapid-cycling *Brassica rapa* (RCBr)

Species in the genus *Brassica* belong to the mustard family or Cruciferae (also known as Brassicaceae) so named for the cross-shaped form of its four petals (*crux*= Latin for cross). Higher up the taxonomic ladder (*taxis*= Greek for arrangement or order), the crucifers are part of the Order Papaverales in the subclass Dicotyledonae (flowering plants having two cotyledons and netted leaf venation). All flowering plants are in the class Angiospermae. Angiosperms are in the subdivision Spermatophyta (seed bearing plants) in the division Tracheophyta indicating the presence of vascular tissue (*trachia*= Latin for artery) (Table 2).

The naming of *Brassica* species has been in a state of confusion for more than a hundred years. Because of the great diversity of forms of brassicas, even within a single species, early taxonomists described many of the major forms as separate species. Within *B. rapa*, many forms exist (Figure 4). For instance, wild forms were called *B. campestris* denoting that they were found in fields as weeds (*campestris* =Latin for field). *B. rapa* was the name given to turnip by the Romans and has persisted until now (*rapa*=Latin for root forming). *B. pekinensis* was the heading Chinese cabbage, *B. chinensis*, or pak choi was the large petioled type of oriental brassica.

Table 2. Phylogeny of *Brassica rapa*

KINGDOM—Plantae
—plants have cell walls and chlorophyll
--other kingdoms are: Monera (bacteria), Protista (protozoans), Fungi and Animalia

DIVISION—Tracheophyta
—vascular plants

SUBDIVISION—Spermatophyta
—seed plants

CLASS—Angiosperms
—flowering plants

SUBCLASS—Dicotyledonae (dicots)
—two cotyledons, branching veins in leaves

ORDER—Papaverales
—special anatomy of fruit and embryo
–contains several families

FAMILY – Cruciferae or Brassicaceae (e.g., mustards and cabbages)
—4 petals, 4 sepals, 6 stamens, ovary consists of two carpels
–contains 375 genera and 3200 species

GENUS—*Brassica*
--fruit a silique, embryos conduplicate

SPECIES--*rapa* (formerly *campestris*)
--chromosome number 2n=20
--subspecific groups

SUBSPECIFIC OR CULTIVAR GROUPS--e.g., *chinensis* (pak choi), *pekinensis* (Chinese cabbage), *rapifera* (turnip), *oleifera* (turnip rape)
--cultivar=*culti*vated *var*iety name --domesticated through selection and breeding

B. nipposinica has many small shoots and is found in Japan whereas *B. parachinensis* was an early flowering form with succulent edible leaves, petioles, stems and flower buds. During the first part of this century cytogenetics determined that all of these "species" contained 20 chromosomes and that all could be intercrossed to produce fertile progeny, primary requisites for denoting a single species. The distinctive form-species were therefore designated as subspecies of the common species *B. campestris*. Thus, Chinese cabbage became *B. campestris* ssp. *pekinensis* etc. More recently however, Dutch taxonomists investigating 18th century specimens and records of *Brassica campestris* found that the first authentic description of the 2n=20 chromosome species was actually that for *B. rapa*. *B. rapa* therefore has been adopted as the official name for this species with *B. campestris* considered to be a synonym. Occasionally you may still see the old terms such as *B. pekinensis*, or *B. parachinensis* used for what should now be *B. rapa*.

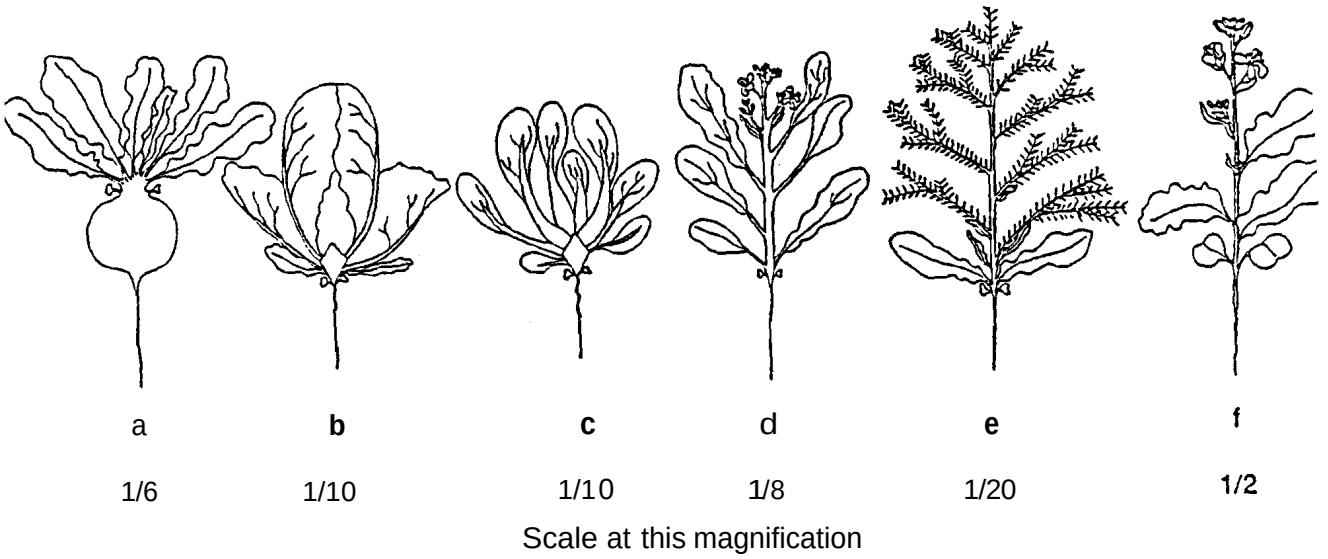

Figure 4. Forms of *Brassica rapa* representing various *cultivar groups*; a. *B. rapa*, turnip group; b. *B. rapa*, Chinese cabbage group; c. *B. rapa*, pak choi group; d. *B. rapa*, saichin group; e. *B. rapa*, turnip rape group; f. *B. rapa*, rapid-cycling.

Scientists are still unsure as to whether to give subspecies names to each of the forms of *B. rapa*. Because the *culti*vated *var*ieties (cultivars) of various forms can be crossed so easily, many intermediate forms are being produced by plant breeders. Rather than designating each form as a subspecies, many scientists are in favor of categorizing cultivars into broad cultivar groups. Thus, cultivars appearing more like Chinese cabbage than any other form are grouped in the *B. rapa* Chinese cabbage group; turnip-like types are in the *B. rapa* turnip group; oil seed types are in *B. rapa* turnip rape group and so forth (see Table 3). Within each cultivar group are many cultivars. A cultivar is given a name by plant breeders and seed companies to differentiate one from other distinctly different cultivars. Cultivar designations can be names, or code numbers. The proper designation for a turnip commonly grown in the United States would be as follows: genus — *Brassica*; species — *rapa*; cultivar group — turnip; cultivar — Purple Top White Globe.

Diversity, Biology and Production of *Brassicas*

Although brassicas are known in the United States mainly as highly nutritious vegetables--e.g., cabbage, cauliflower, broccoli, collards, kale, mustard greens and Chinese cabbage--their potential value as oilseed crops and animal fodder is beginning to be recognized. Crucifer oil, known as rapeseed oil, is the third most commonly traded vegetable oil in the world. Rapeseeds contain 40% oil, which is pressed from the seeds, leaving a high-protein seed meal of value for animal feed and nitrogenous fertilizer. Most Northern European countries produce rapeseed as their main edible oil crop. Salt-tolerant rapeseed is one of the first crops grown on reclaimed polder land in Holland. China and India each grow rapeseed on over **3** million hectares. An important component of some rapeseed varieties is a 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.

Brassicas are also grown for animal fodder in regions too cool to grow maize, or during winter months when grasses grow 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; in Korea consumption is even higher. Radish (genus *Raphanus*), a close relative of *Brassica*, 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 (Figure 5). Three diploid species, *B. nigra* (bb), *B. rapa* (aa), (syn. *B. campestris*) 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. rapa* (aa) has 20 chromosomes and allotetraploid *B. juncea* (aabb) has 36 chromosomes.

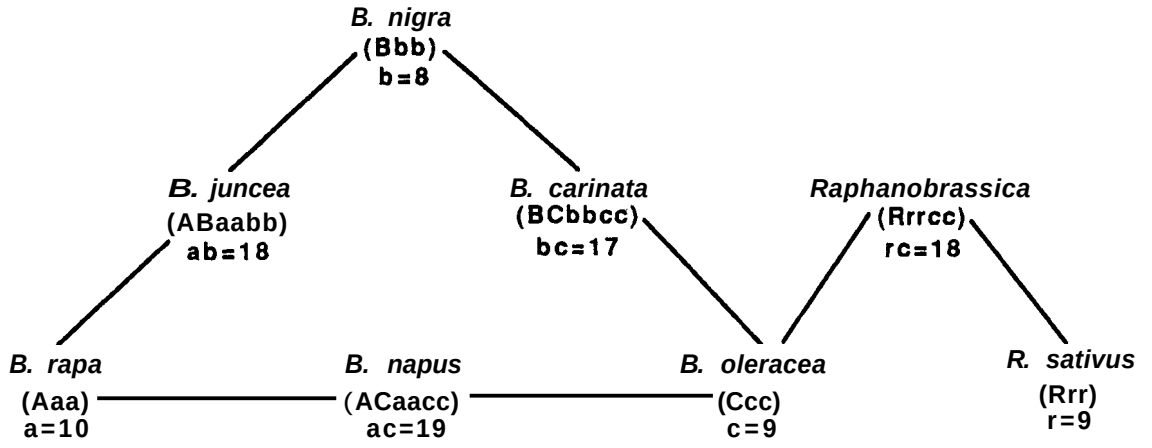

Figure 5. The cytogenetic interrelationships among six *Brassica* species and *Raphanus sativus*. Intergeneric crosses between *R. sativus* and other *Brassica* species are also possible. Cytoplasmic genome is designated by capitals. Nuclear genome is designated by lower case letters, where a = 10 chromosomes; b = 8 chromosomes; c and r = 9 chromosomes.

Within each of the species there is a range of forms that are a result of divergent selection during domestication (Table 3). Within *B. oleracea* are "cole crops" such as cabbage, cauliflower, curly kale, kohlrabi, Brussels sprouts and the bizarre tree cabbage, or Jersey kale. Tree cabbage, which may be up to 3 meters tall, is grown in Portugal and on the Channel Islands, where the leaves are used in a nutritious vegetable soup and as winter cattle feed. The remaining stalks are cut and dried for manufacture of walking sticks. As well as Chinese cabbage, turnip, pak choi and a host of other forms representing vegetables consumed in various Oriental cultures, several oilseed types are found within *B. rapa*. *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, allyl isothiocyanate, in seed and leaf tissue. The genes controlling mustard oil synthesis are contributed to *B. juncea* (aabb) largely through the genome of *B. nigra* (bb), black mustard. *Brassica napus* varieties are used for oilseed, fodder, and as a vegetable, rutabaga. Both wild and cultivated forms of *B. carinata* are major sources of leafy greens and cooking oil in Ethiopia.

Table 3. Names of subspecific taxa of agriculturally important brassicas and radish. considerable taxonomic confusion exists in the literature for *Brassica*. (n) is the haploid complement of chromosomes; a=10, b=8; c and r=9.

Species (genome)	Subspecies or variety	Cultivar group or Common Name
Brassica		
nigra (bb=16)	—	Black mustard
oleracea (cc=18)		
	acephala	Kales
	alboglabra	Chinese kale
	botrytis	Cauliflower, Heading broccoli
	capitata	Cabbage
	costata	Portuguese cabbage
	gemmifera	Brussels sprouts
	gongylodes	Kohlrabi
	italica	Broccoli, Calabrese
	medullosa	Marrow stem kale
	palrnifolia	Tree cabbage, Jersey kale
	ramosa	Thousand-head kale
	sabauda	Savoy cabbage
	sabellica	Collards
	selensia	Borecole
rapa (aa=20) (syn. *campestris*)	chinensis	Pak choi
	narinosa	Taatsai
	nipposinica	Mizuna
	oleifera	Turnip rape, Toria
	parachinensis	Saichin, Choy sum
	pekinensis	Chinese cabbage, Petsai
	perviridis	Tendergreen, Komatsuna
	rapifera	Turnip
	trilocularis	Yellow sarson
	utilis	Brocoletto, Broccoli rab

Table 3 - contd.

Species (n)	Subspecies, variety or group	Common name
carinata (bbcc=34)	—	Ethiopian mustard
juncea (aabb=36)		
	capitata	Head mustard
	crispifolia	Cut leaf mustard
	faciliflora	Broccoli mustard
	lapitata	Large petiole mustard
	multiceps	Multishoot mustard
	oleifera	Indian mustard, Raya
	rapifera	Root mustard
	rugosa	Leaf mustard
	spicea	Mustard
	tsa-tsai	Big stem mustard
napus (aacc=38)	—	Fodder rape
	oleifera	Oil rape
	rapifera	Swede, Rutabaga
Raphanus sativus (rr = 18)	radicola	Radish, dikon
	oleifera	Oil radish
	caudatus	Rat tail radish

The potential for exchange of useful genetic information between brassicas and the closely related radish was demonstrated in the 1920's by the Russian geneticist, Karpechenko. To combine the large root of *Raphanus sarivus* (radish) with the heading form of cabbage, he created the synthetic genus *Raphanobrassica* (Figure 5). As with many such wide crosses in domesticated plants, neither the attributes of radish nor of cabbage were attained. Rather, raphanobrassicas are vigorous plants used for sheep and cattle fodder and green manure. *Raphanobrassica* can serve as a bridge for the transfer from radish into brassicas of useful traits such as cytoplasmic male sterility and disease and nematode resistance (Figure 5).

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 and cabbage, and is a new vegetable and fodder crop. The transfer of resistance to clubroot and blackleg diseases into susceptible species has also been achieved by interspecies crosses.

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° C) to induce flowering. Flowering may also be under the control of photoperiod. 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-coast states of Washington, Oregon and California are ideal for crucifer seed production because dry summers minimize seed-borne diseases caused by the fungi *Alternaria* and *Leptosphaeria,* and by *Xanthomonas* bacteria.

The Development of RCB's

In order to understand the genetic basis for the diversity of forms found in brassicas and to incorporate more efficiently traits of economic importance such as disease and pest resistance, an ideal model plant type or *ideotype* (*ideo*=Greek for idea) was needed to speed research in genetics and plant breeding. Of major importance was rapid flower and seed production, faster than the normal reproductive time of six months to one year for the various crop groups. From a world collection of over 2,000 brassicas obtained from the United States Department of Agriculture's National Plant Germplasm System, a few plants were observed to flower in a significantly shorter time than others. By combining the genes of early flowering types from various sources, plants were bred for reduced reproductive time. These faster flowering individuals could then be used to develop a population that would be tailored to suit the experimental ideotype needed for growing large numbers of plants under standardized laboratory or classroom conditions. To do this, fast flowering plants of various *Brassica* species were grown at 24° C in multipots at plant densities of (880 plants/m2) in a standardized soil mix, irrigated with a balanced liquid nutrient solution (0.5 x Hoagland's solution) and illuminated continuously with bright light from cool-white fluorescent bulbs [250 micro Einsteins per second per square meter (250 mEs-1m-2) of PAR]. Criteria used in selecting individuals for successive generations were: 1) minimum time from sowing to flowering; 2) rapid seed maturation; 3) absence of seed dormancy; 4) small plant size and 5) 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. In each successive generation the plants flowered in less time than the previous one. When the reduction in the average days to flowering became stabilized and when greater than 50% of the population flowered within a 2-3 day period, selection on the populations was discontinued. The resulting model plant of *B. rapa* flowered in an average of 16 days, was 12 cm to the first flower and averaged 78 seeds per plant (Table 4).

Table 4. Phenotypic characterization of rapid-cycling brassica and radish base populations grown at 24° C under continuous high light. Nuclear genome is designated by lower case: a=10 chromosomes; b=8 chromosomes; c and r=9 chromosomes. When grown under lower temperatures and light, development may be delayed. Data are expressed as mean (SD=standard deviation).

Species	Genome & chromosome number	Days to flower	Length (cm) to first flower	Seeds per plant	Days for cycle	Cycles per year
B. rapa	aa=20	16(1)	11.9(3.1)	78(54)	36	10
B. nigra	bb=16	20(2)	27.1(4.9)	69(49)	40	9
B. oleracea	cc=18	30(3)	22.6(5.3)	18(21)	60	6
B. juncea	aabb=36	19(1)	29.6(4.0)	107(46)	39	9
B. napus	aacc=38	25(2)	35.3(7.1)	76(53)	55	6
B. carinata	bbcc=34	26(2)	41.7(6.6)	67(46)	56	6
R. sativus	rr=18	19			48	7

This stock was capable of cycling (seed-to-seed) ten times per year. The seed stocks of the rapid-cycling base population of each species were given code numbers and made available to researchers throughout the world via the Crucifer Genetics Cooperative (CrGC). Many scientists are using the RCB's as model plants for research in genetics, molecular biology, plant breeding, cell biology and physiology. Through the CrGC, new information and new genetic stocks are shared among more than 1000 scientists from 45 countries.

In addition to the development of the rapid-cycling population of *B. rapa* (RCBr), rapid-cycling populations of five other related *Brassica* species, *B. nigra*, *B. oleracea*, *B. juncea*, *B. napus* and *B. carinata* and of radish, *Raphanus sativus* were developed (described in Table 4). More than 100 distinctive genetic traits are being studied in the RCB's. Genetic mapping of the chromosomes is under way using distinctive morphological markers, physiological and disease resistance markers, isozymes and restriction fragment length polymorphisms (RFLP's). Various quantitative traits and cytoplasmically-inherited phenotypes are being incorporated into the rapid-cycling stocks.

Early in their development, stocks of RCBr from the CrGC were sent to Cornell University, the University of California-Davis, the University of Guelph and various other institutions where they were used in plant genetics and plant breeding courses. Today the potential for wider use of the RCB's as model organisms for hands-on learning in the classroom is being realized.

REFERENCES

Williams, P. H. and Hill, C. B. 1986. Rapid-cycling populations of *Brassica*. *Science* 232:1385-1389.

Growing Instructions for Fast Plants

Since the growing conditions for rapid-cycling *Brassica rapa* differ from most traditional classroom plant growing, it is strongly recommended that you grow a cycle of the plants before your students begin experiments. *Continuous bright fluorescent lighting and a constant water supply are critical for the rapid-cycling of these plants.* By explicitly following these guidelines, you and your students will enjoy a successful growing experience.

Read these guidelines completely before you begin planting.

Before Planting

1. Become familiar with the materials in the Fast Plant Kit:
   a. *Brassica rapa* seed—It's small and needs to be handled with care.
   b. quads -- 4-celled planting units in which you will grow one plant to maturity in each cell.
   c. fertilizer pellets—slow-release source of nitrogen (N), phosphorous (P) and potassium (K).
   d. potting mix
   e. wicks-conduct water from water mat to soil in cell of quad.
   f. water mat--conducts water from reservoir to wicks.
   g. pipets—to water cells from above when necessary.
   h. plant labels—see "Records and Terminology" section for labeling suggestions.
   i. dried honeybees—used to make beesticks.
   j. small water mat squares (blue)-contain copper sulfate to prevent algae growth in reservoir.
   k. wooden stakes and plastic support rings—to support the plants if necessary.

2. Lighting:
   Assemble a light bank and the rack to support it. To complete the growth cycle in 40 days, a bank of six or eight, 4-foot cool-white fluorescent bulbs (40 watts/bulb) is necessary (Figure 1). This arrangement can be constructed by fastening lights to a frame. Use a plug adapter and an extension cord. Choose a light-weight light so that your light bank can be moved and transported easily. Suspend the light bank from a wooden rack. This arrangement will permit you to adjust the height of the light bank as the plants increase in height. Plants and quad will need 30-40 cm of space below the bulbs at maturity.

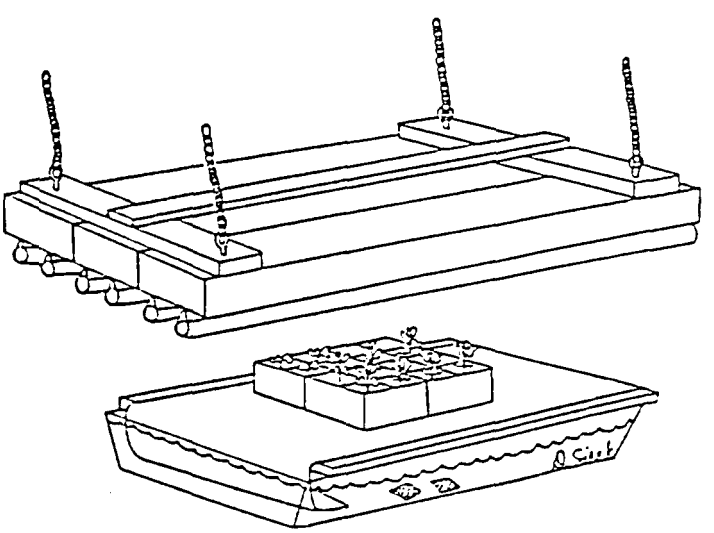

Figure 1. Light bank and watering system.

Keep growing tips of plants about 5-10 cm from the bulbs throughout the life cycle.

The plants will complete their life cycle in 40 days only if they are given 24 hour of light per day and the recommended *light intensity*. If your light source has less than six 40 watt bulbs or you allow more than 5-10 cm from growing tip to bulb, plants will grow tall and spindly. In addition, the time to complete the life cycle will be extended several days.

As an alternative to raising the light bank, set the light bank 40 cm above the table surface and raise the reservoirs initially so that the plants are 5-10 cm from the bulbs. Gradually lower the reservoirs as plant height increases.

If a growth chamber is used, it must be in optimal operating condition.

Planting

1. Plan to begin a Fast Plant cycle by *planting on a Monday or Tuesday*. This schedule will allow you consecutive school days for watering from above for the first three days and align flowering with weekdays.

2. Water
   Prepare the water mat for use. Soak the mat in water, then squeeze water out. Repeat this process two more times. After the final soaking, do not squeeze the water out, but simply lay the mat in the correct position on the platform (Figure 2). Smooth out the mat on the platform, leaving no air pockets under the mat. Fill the reservoir with water.

   This watering system is based on capillary action. Once the water mat is wet, it continues to draw water from the reservoir. Wicks in the bottom of each cell draw water into the potting mix. The reservoir holds enough water for 2-3 days.

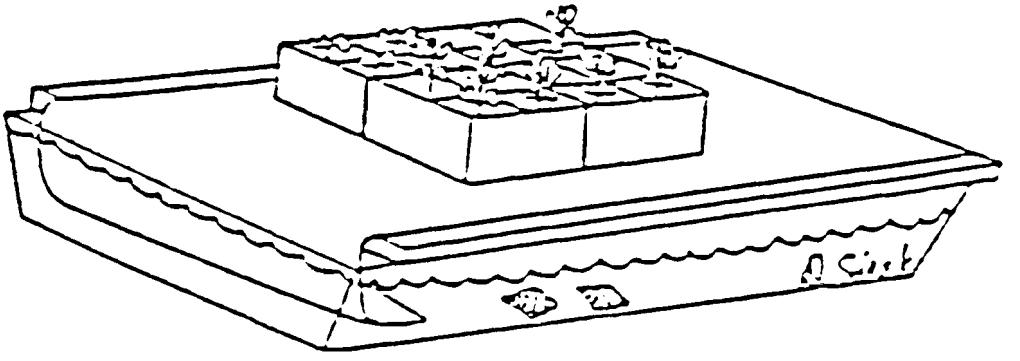

Figure 2. Watering system.

3. *Place copper sulfate squares in the reservoir water.*

4. *Moisten the potting mix* until it is slightly damp.

5. *Drop one wick into each cell* so that the tip extends 1-1.5 cm out the hole in the bottom (Figure 3).

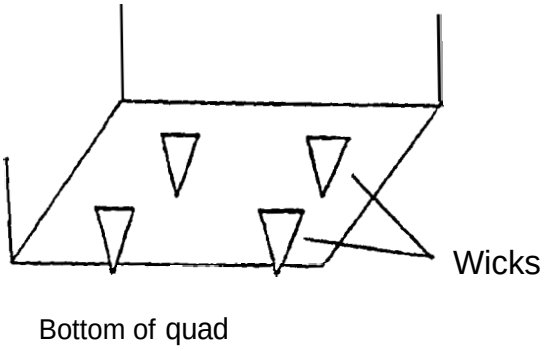

Figure 3. Quad and wicks.

6. *Fill each cell halfway with potting mix* (Figure 4).

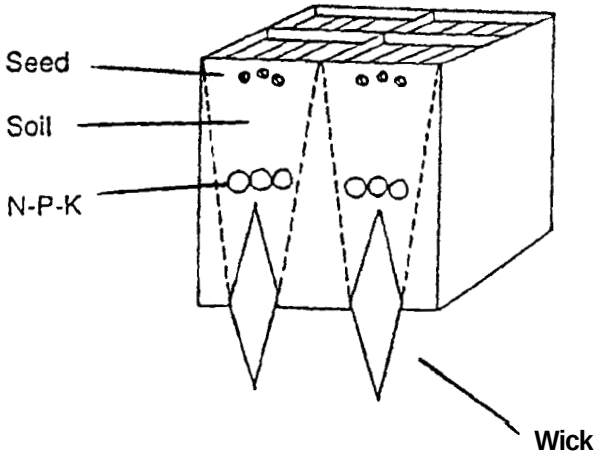

Figure 4. Planting.

7. *Add 3 fertilizer pellets* to each cell.

8. Add more potting mix to *fill each cell at the top. Do not pack soil.* Use your finger to make a 0.5 cm depression on top of each cell.

9. *Drop 3 seeds into the depression in each cell.*

10. *Cover seeds* with just enough potting mix so that the seeds are no longer visible.

11. *Water gently with pipet* until water drips from each wick tip (Figure 5). Place the quad on the water mat. The top of the quad should be 5-10 cm from the bulbs of the light bank.

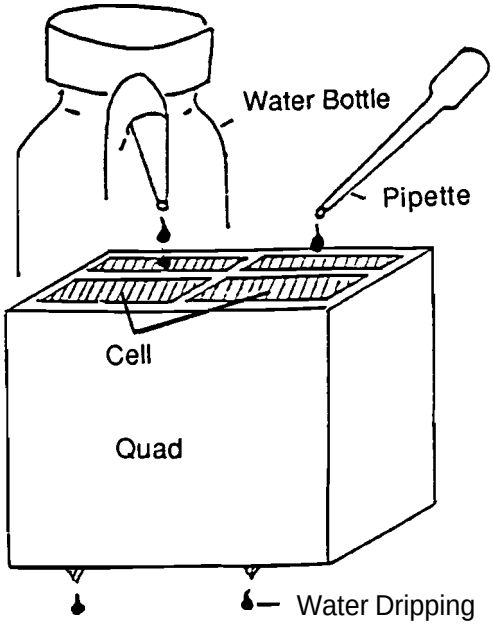

Figure 5. Initial watering.

12. Label each quad by inserting a pot label which has been correctly marked with a waterproof pen (Figure 6). See Records and Terminology section for a method of labeling.

Figure 6. Labeling.

After Planting

1. Be sure to water gently from above with pipets for the first three days to insure adequate moisture for germination.

2. Check the water reservoir *daily* and keep it filled. *Completely* fill the reservoir Friday before leaving for the weekend.

3. Check the water mat and potting mix in each cell daily. Both should be moist at all times. If the mat has dry spots, remove all quads and soak the mat again before returning quads to the mat. If potting mix in a cell appears dry, check the wick. Occasionally a wick dries out because of air pockets in the cell. Add water from above with a pipet until water drips from the wick.

4. Drought
If the worst happens (e.g., *you forgot to fill the reservoir on Friday*) and the plants are wilting (but not yet crispy), you may be able to save your plants. Fill reservoirs with water and float the quads in the water while adding water from above with pipets. Allow the quads to float on the water until plants are turgid again. Re-soak the water mat and return the quads to the mat.

5. Thin Plants
Thin to one plant per cell. Use scissors or tweezers. Transplant extra seedlings to cells without plants (Figure 7).

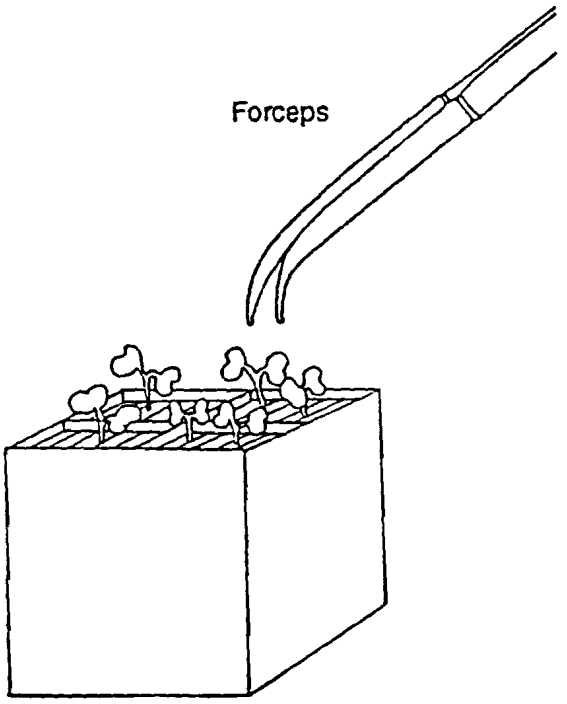

Figure 7. Thinning.

6. As the plants grow, remember to maintain the 5-10 cm spacing between the growing tip and the bulbs.

Although insects are not usually a problem, check plants for insect damage. The most common sources of insects are other plants in the room that are infested or insects such as aphids that attach to clothing outdoors and are then inadvertently brought into the room. Three methods are recommended for insect control:

-First, simply remove the insects from your plants by hand and crush them.
-Second, spray plants with a solution of an insecticidal soap which can be purchased from a local garden store. Be sure to follow instructions on the label.
-The third method will vividly demonstrate the effects of nicotine on insects. Place a large metal waste basket inside a large plastic trash can liner. Place the quads with the infested plants at the bottom of the metal waste basket. Light a cigarette and place it about three inches from the quads and close the plastic liner (Figure 8). After 1-2 hours, remove the quads and check for insects.

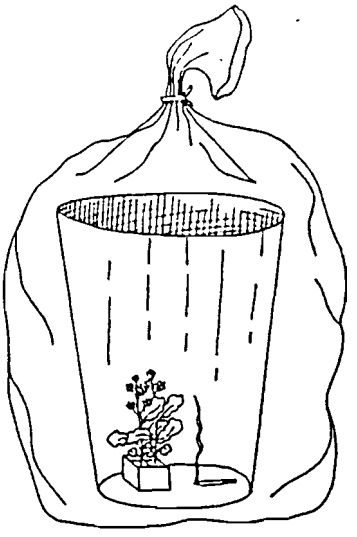

Figure 8. Insect control.

8. As the Fast Plants grow, you may use small wooden stakes and plastic support rings to support the plants (Figure 9). Gently hold plant next to the stake, open the plastic ring and slip ring around both.

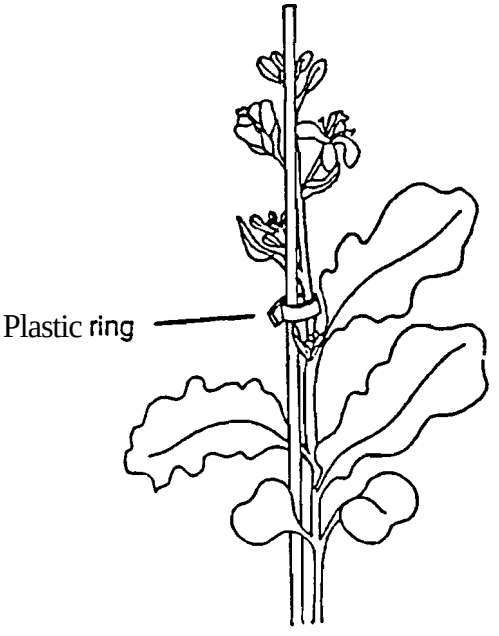

Figure 9. Staking plants.

Pollination

1. *Prepare beesticks* 1-2 days in advance of pollination (Figures 10 and 11). The volatile chemicals in the glue are toxic to the pollen grains and will prevent pollen germination if the bee sticks are used immediately after being constructed. Build and store freshly made beesticks in a different area than where the plants are growing.

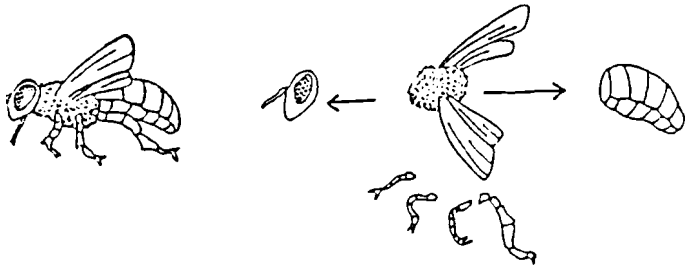

Figure 10. Dissect bees.

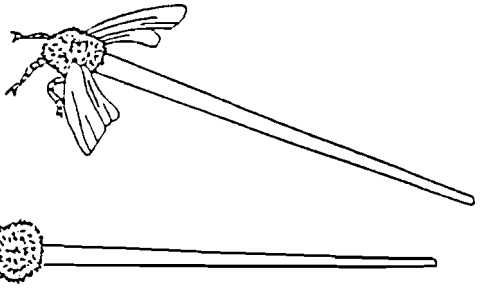

Figure 11. Glue thorax to toothpick

2. *Pollinate* with beesticks by rotating the bee thorax over flowers to pick up and distribute pollen. Transfer pollen back and forth among different plants (Figures 12 and 13). *Brassica rapa* plants must be cross-pollinated, except if you use a bud pollination method (see section "Self-pollinating rapid-cycling *Brassica rapa").*

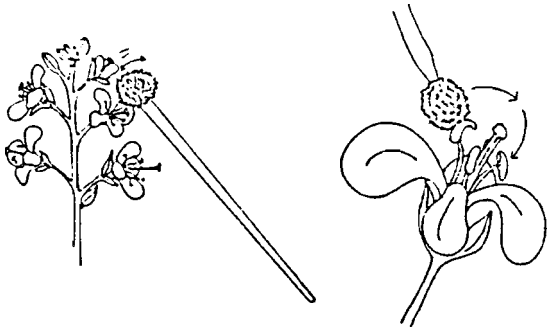

Figure 12. Remove wings and pollinate.

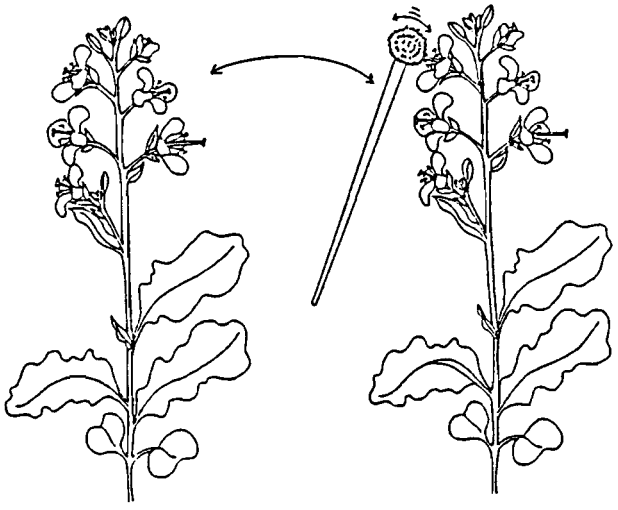

Figure 13. Cross pollinate.

3. Pollinate daily for 2-3 days.

4. On the last day of pollination, *pinch off all other unopened buds* (Figure 14) and mark the date on the pot label.

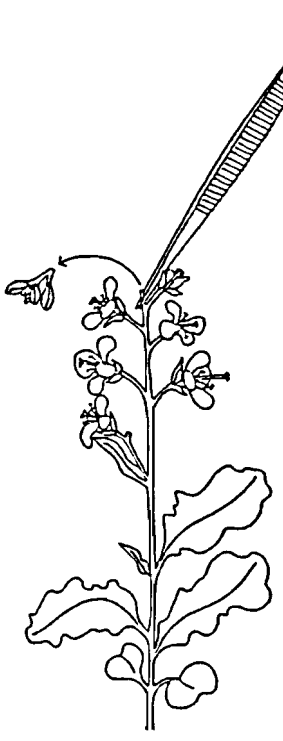

Figure 14. Remove unopened buds.

After Pollination

1. Seed pods and seeds develop. Seed pods will begin to elongate within 3-5 days, and will mature in 20 days (Figure 15).

2. During days 18-36 of the life-cycle, continue to pinch off new flower buds. The plant will then direct its resources to the developing seed pods.

3. Beesticks loaded with pollen may be stored in a glass screw-cap vial with an indicator silica gel desiccant capsule (Figure 16). At 4º C the pollen will remain viable for several months. Periodically check the color of the capsule and replace it if it turns from blue to pink.

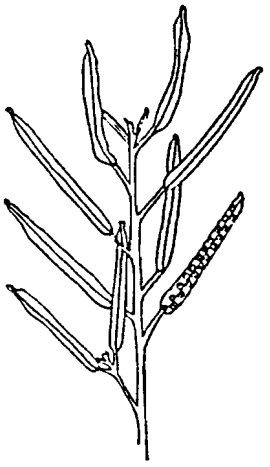

Figure 15. Mature seed pods.

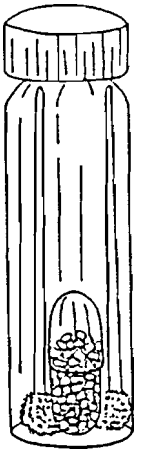

Figure 16. Beestick storage vial.

Seed Harvest

1. Remove plants from water 20 days after the last pollination. *Dry for 5 days.* To cut the drying time to 3 days, cut off seed pods, place in brown paper bags and set the bags on top of the light bank with the lights on.

2. *Harvest seed* by gently rolling dry seed pods between hands over a collecting pan. Place the seed in an envelope like the one you received in the Fast Plant Kit.

3. Seed envelopes should be kept in a cool dry place. Seed stored in your desk retains its viability for about 4-6 months. For longer storage, place seed envelopes in a screw-cap bottle with an indicator silica gel desiccant in a small paper envelope or gelatin capsule. Store the bottle at 4º C. Periodically check the color of the silica gel and replace it if it turns from blue to pink. Indicator silica gel may be reactivated (pink color will return to blue) by drying overnight in an oven at minimum heat (65º C).

Before The Next Cycle

1. Reservoirs, platforms, water mat, quads and wicks should be soaked in a 10% chlorine bleach solution for at least 15 minutes. Then, scrub quads with a brush and rinse all materials thoroughly with water. Let all materials dry completely before reusing.

Records and Terminology

Background

When carrying out any investigation it is important to keep neat, accurate and complete records of what you do. In this way you will be able to analyze and interpret what you have done and communicate it to others. A scientific investigation is only completed when the results of the investigation have been thoughtfully interpreted and clearly communicated. By understanding the specialized terminology for each branch of science and using it accurately, you will be able to communicate effectively your results and ideas. Each part of science has its own specialized set of terms used to describe things, processes and relationships. Many of these terms have been derived from Greek or Latin and often have parts of them that sound familiar to some commonly used English words. In genetics, the core science in biology, symbols are used as a sort of shorthand to designate certain characteristics, processes and relationships among the units being experimented with. This is just the same as is done in chemistry or physics where special symbols are used to designate the chemical elements or various forms of energy. In genetics, the accurate use of terminology is important so that proper analyses and interpretation can be given to the results of an experiment. At the molecular level, the arrangement of nucleotides making up the DNA provides a fundamental understanding of the gene. How the various nucleotide sequences are arranged in the gene and how the genes are expressed in the vast range of phenotypic characteristics within organisms largely is unknown and remains one of the fascinating areas for investigation in biology.

Some of the important terms needed to understand the basic principles of genetics are given below.

Some Important Genetic Terms

allele — one of two or more alternate forms of a *gene* occupying the same locus on a particular chromosome or linkage structure and differing from other alleles at the locus at one or more mutational sites.

dominance — refers to the *expression* of genetically controlled characters (phenotypes) and their corresponding alleles when they are in the *heterozygous* condition.

1. Dominance and recessiveness are not properties of the genes *per se*, but the result of the action of the genetic locus in question within the total reaction system of a particular genotype.
2. Complete dominance and complete recessiveness are the extreme cases between which all transitional degrees of expression are possible.

gene — a particular sequence of nucleotides along a molecule of DNA (or in some viruses, RNA) which represent a functional unit of inheritance.

genotype —

1. The genetic constitution in respect to the alleles at one genetic locus under observation
2. The sum total of the genetic information (genes) contained in chromosomes (linkage groups) of pro- and eukaryotes. The genotype determines not a unique phenotype but a range of phenotypic expression referred to as the individual's *reaction norm.*

heterozygous — in diploid organisms the condition of having different alleles at one or more loci (genes) in homologous chromosome segments, in contrast to *homozygous*, having identical alleles at these loci.

locus — the position of a gene on a genetic map. Allelic genes are situated at identical loci in homologous chromosomes.

phenotype — the observable properties (structural and functional) of an organism, produced by the interaction between the organism's genetic potential (its *genotype*) and the environment in which it finds itself. The term *phenotype* can be applied either to the totality of expressions of the genotype *or* to only a part; i.e., to particular characters or traits. The phenotypic range or expression is referred to as its *reaction norm.*

recessiveness — the *absence of expression* of genetically controlled characters and their corresponding alleles when they are in the heterozygous condition.

wild type — refers to an organism or gene chosen to be the standard for comparing other phenotypes or genotypes.

In genetics, different shorthand schemes have been developed for recording genetic characteristics, processes and relationships of different organisms. This has led to difficulty in communication between scientists. Recently, geneticists have been working to develop more standardized terms for their particular organisms so that they could more easily communicate with other scientists.

Since brassicas are emerging as model organisms, the opportunity exists to adopt genetic terminology for brassicas that will be in conformity with the terminology used by some of the other important model organisms. Thus, in designating symbols to define particular genotypes or phenotypes we suggest using the guidelines of the Crucifer Genetics Cooperative for *Brassica*.

Guidelines for Using Genetic Symbols

In the case of the rapid-cycling *Brassica rapa*, Wisconsin Fast Plants has designated the cultivar name RCBr to the basic fast cycling stock which has been developed for the WFP kits. For most traits the phenotype of RCBr represents the wild type. Various other mutant stocks have been developed in the common genetic background of RCBr. Each of these is accompanied by genotypic or phenotypic symbols that designate the uniqueness of the particular stock.

1. *All* gene symbols should consist of three letters.
2. Underline *genotypes*
3. The genotype designation of wild type is capitalized (e.g. ROS. YGR).
4. The genotype of mutant alleles is in lower case (e.g. ros=rosette; ygr=yellow-green plant).
5. Alleles are designated by a dash followed by a number (e.g. mey-1, mey-2. mey-3). If no allele is specified it is assumed to be allele number 1 (e.g. mey is equivalent to mey-1).
6. Genes with mutant alleles of similar phenotype can (but need not) be given the same three letter designation followed by a different number (e.g. ygr, ygr2, ygr3).
7. Phenotypes are designated by the gene symbol which is not underlined but has the first letter capitalized (e.g. Ygr), a + or - may follow the phenotype symbol to designate expression (+) or absence of expression (-). Degree of expression of a quantitative phenotype may be designated on a 0-9 scale in parentheses following the symbol [e.g. An1 **(7)].**
8. Uniparentally inherited phenotypes are enclosed in parentheses [e.g. (Var)=cytoplasmically inherited variegation].

An example of the name and description of an RCBr mutant stock would be as follows: RCBr, ros/ros; RCBr=rapid-cycling *Brassica rapa* WFP stock; ros/ros=homozygous for the recessive mutant gene, ros, conditioning deficiency in gibberellin and resulting in a rosette plant form with extreme internode compression. The rosette phenotype can be "corrected" by the application of exogenous gibberellin to the plant.

Labeling. Tagging Record Keeping

Accurate labeling and recording of data is vital to an experiment. To insure that you and your students conduct a successful experiment, the following tips are suggested:

1. Obtain planting labels, waterproof marking pens, seed envelopes, and construct data tables *before beginning an experiment.*
2. Number *each* plant in a quad.
3. Label the planting label with:
   - seed type
   - planting date
   - student's initials
   - nature of experiment or treatment to be performed
4. If two or more different genotypes/phenotypesare planted in the same quad, use separate planting labels for each.
5. Seed envelopes should be labeled with:
   - name of grower
   - date of harvest
   - type of seed stock
   - treatments done to plants
   - generation (genetics)
6. In recording a cross between two parents, the female genotype is always written first. In designating the heterozygous condition of a gene, the allele coming from the female is always placed first, separated by a slash (/) followed by the allele from the male, e.g. ros/ROS is heterozygous for rosette. The female parent was homozygous for the rosette allele (ros/ros)and was phenotypically rosette. The male parent was wild type for the rosette allele (ROS/ROS) and, therefore, was normal height. In the case of the rosette, the wild type allele ROS is expressed and, therefore, dominant over the mutant ros allele.
7. Instruct students to return their quads to the same location on the reservoir each day after making observations and recording data.
8. Use data tables provided or construct your own. More recorded information from daily observations is always better than less.
9. Keep a record of class data. You will more than likely want to repeat your experiments year after year and it's handy to look at the data from previous years before you begin again.

Self Pollinating Rapid-cycling *Brassica rapa*

Background

To obtain self-pollination in self-incompatible *Brassica rapa* stocks you must overcome the pollen-stigma incompatibility mechanism. To accomplish this requires manipulation of the flower buds. Observations from growing *Brassica rapa* plants will allow you to predict when the flower buds will open. You must be able to predict when the buds are one to three days from opening. The timing of this procedure is critical. The whorl of developing flower buds is ideal for examining which stages of buds are receptive to "self" pollen. In RCBr each successive swollen bud is approximately 8 hours younger than the next sized one. A new bud will develop approximately every 8 hours. If buds are too young and the stigma too immature, pollen grains will not remain viable long enough to ensure fertilization. If the buds are opening, the incompatibility mechanism will be operational and the pollen tube will be prevented from forming.

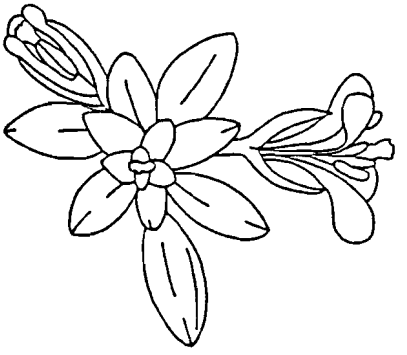

Figure 1. Orientation of buds in apical whorl of RCBr.

Special Techniques/Tips

1. One to three days prior to the buds opening, use forceps to gently pry open the bud (Figure 2). You may want to pull off the sepals and petals. The immature stigma should be exposed (Figure 3).

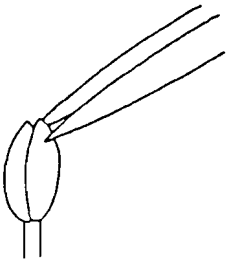

Figure 2. Open bud.

Figure 3. Exposed stigma.

Transfer pollen from a mature anther on the same plant to the immature stigma. The pollen may be transferred by beestick (Figure 4) or by using an excised anther (Figure 5).

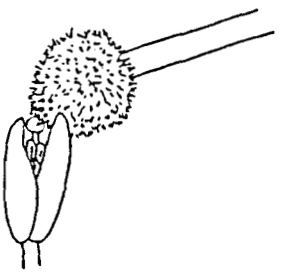

Figure 4. Pollen transfer with beestick.

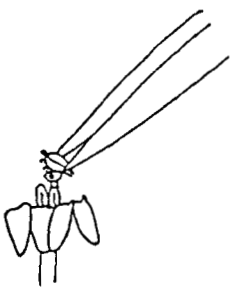

Figure 5. Pollen transfer with excised anther.

3. Examine the pistil for the next 3-5 days (Fig. 6). Elongation and swelling of the pistil indicates that fertilization has taken place.

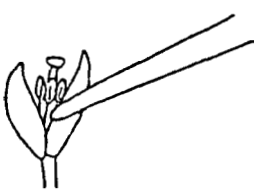

Figure 6. Examine pistil for indications of successful fertilization.

How Can Plants That Look So Different Be the Same?

An Introduction to Plant Breeding

INTRODUCTION

Turnips, Chinese cabbage and Wisconsin Fast Plants (RCBr) look very different. Yet, they actually belong to the same species. This means that they have the same number of chromosomes and they can cross breed and produce fertile offspring. But how can you prove that plants that look so different are really the same species? The following investigations will allow you to explore this question.

TIME REQUIRED

Stage 1 -- 4-6 weeks, requiring no tending
Stage 2 -- 2-3 weeks
Stage 3 -- approximately 20 days

MATERIALS

turnips, Chinese cabbage, Wisconsin Fast Plant (RCBr) seeds and kit supplies
2 liter soda bottles
rooting powder (e.g. "Rootone")
soil mixture (peat moss and vermiculite)

EXPERIMENT I

1. Purchase a turnip with some small buds or shoots at the crown and Chinese cabbage from your local grocery store. With a sharp knife, mm most of the large leaves from the Chinese cabbage leaving about 1 cm of each leaf attached to the core. Use the leaves in your cooking. Trim off leaves until you have a core about 6-10 cm long. Place the turnip and cabbage core in a plastic bag in the refrigerator for 4-6 weeks (Figure 1). This cold treatment, called **vernalization,** simulates over-wintering and the plants will convert from a vegetative to a flowering stage.

Figure 1. Turnip and cabbage core in plastic bag.

2. When small leaves begin to grow from the top of the turnip, make two growing containers from 2-liter soda bottles. (e.g. Fill bottle with hot tap water, replace cap. Hold the bottle firmlyand twist off the opaque bottom. Seal holes on bottom with black electrical tape.) Fill containers with a mixture of equal parts of peat moss and vermiculite (or sand).

3. Remove turnips and Chinese cabbage cores from refrigerator. Cut a thin slice off the bottom of each. Put a little rooting powder on the newly cut surface to help rooting.

4. Place vegetables on growth medium in the containers. Keep the soil moist at all times. Place them in good light, keep cool and partly covered to prevent excess wilting. (e.g. Cover with the top portion of a 2 liter soda bottle which has been cut to be about 8" high (Figure 2).

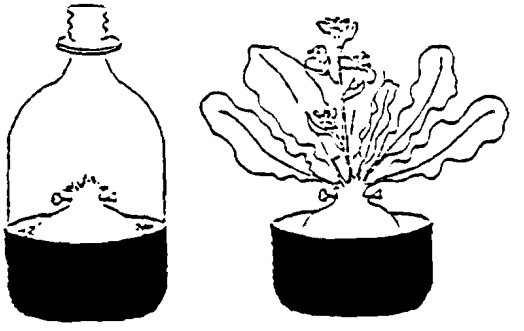

Figure 2. Growing containers.

5. Within 2-3 weeks the plants should begin to produce flowers. At the time the first buds appear, plant at least six quads of Fast Plant (RCBr) seeds. In two weeks all three types of *Brassica rapa* should be flowering.

6. Follow the instructions for pollination found in the Fast Plant "growing instructions." Cross pollinate each of the vegetables to the RCBr as shown in Figure 3. Use separate beesticks for each cross.

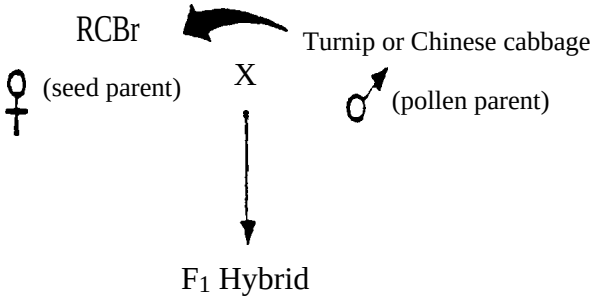

Figure 3. Cross pollination of Turnip or Chinese cabbage to RCBr.

7. After 20 days you should have mature seed pods on you RCBr plants.

Questions

1. Were you able to produce seeds on your RCBr plants?

2. Is this final proof that the different plants are the same species?

EXPERIMENT II

Save the seed you have produced from Experiment I and plant them in soda bottle growing containers in class or in the garden over the summer. These plants constitute the first generation.

1. What is your hypothesis about how these intraspecific (within a species) hybrids will look? (Hint: if the plants get too large, transplant them into a larger container.)

2. If the plants flower do they produce pollen?

3. Can you produce seed by interpollinating two or more hybrid plants?

EXPERIMENT III

If you get seed from the intraspecific hybrid, try sowing this out in your garden (or in class.) What do the plants from this generation look like? IF YOU GET THIS FAR YOU ARE WELL ON YOUR WAY TO BECOMING A PLANT BREEDER and you will understand the riddle of how things that look so different can be the same.