# Practical Manual on Hybridization Systems in Temperate Root Vegetable Crops





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# Practical Manual on Hybridization Systems in Temperate Root Vegetable Crops

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# Hybridization Techniques-Introduction

Genetic variation is essential for making selections. When the variation is absent or is of low magnitude it is created artificially. Valuable characters may be found scattered in different varieties, species or genera. Through hybridization, desirable characteristics of two or more parental lines may be combined into a single genotype. Hybridization may result in transgressive segregation which may surpass the parents involved in a cross.

#### **Choice of parents:**

In hybridization, the choice of parents is of utmost importance. The breeder must be clear about the objectives and plan of hybridization. Generally one parent is an improved and predominant variety of the area where on the other parent(s) should complement the first parent in respect of the character(s) which it lacks. Greater success is achieved if both the parents are vigorous and healthy. Weak, old and sick plants should be avoided. The plants should be well spaced and, if possible, grown in green house or glasshouse in which greater environmental control can be expressed with regard to factors such as humidity, temperature and light.

#### **Techniques of hybridization:**

To produce hybrid seed, the knowledge of floral biology is important. It is necessary to study the compatibility of the parents and the physiology of flowering. The knowledge of the number of chromosomes and their homology is also essential. In nature, plants may be self or cross fertilized. The plants may bear hermaphrodite or unisexual flowers. The latter may be monoecious or dioecious. The dioecious plants are compulsorily cross pollinated. The technique of hybridization consists of processes like emasculation and pollination, ensuring the union of male and female gametes of the known parents. For this purpose, the time of shedding of pollen, the viability of pollen, the emergence and receptivity of the stigma are to be studied.

#### **Emasculation:**

Emasculation is the removal of stamens manually from the female parent before the bursting of anthers and the shedding of pollens. The emasculated flowers are protected against agencies such as wind, water and insects. When the stigma is receptive, the pollen from the desired male parent is dusted on it. The stigma is protected till it loses its receptivity.

# **Anthesis and Pollination:**

The phenomenon of flower opening and pollination is termed "anthesis" *i.e.* when the stamens mature, the anther-sacs burst open and shed the pollen and when the pistil is mature, the stigma is receptive (it appears fresh and coated with a sugary liquid. The dusting of stigma with pollen grains takes place at different stages of flower opening and even at closed bud stage, depending upon the mechanism in the plant and the relative timings of various processes.

Before any crossing programme is undertaken, a thorough knowledge of the floral parts and anthesis in the crops concerned is essential to enable the breeder to emasculate and cross-pollinate the flowers at the proper time. The actual time at which the various process takes place in a crop varies with environmental conditions such as temperature and humidity.

After studying the floral parts, it is necessary to study the time of the day when the following phenomenon take place so the proper time for emasculation and pollination is determined:

- a) The time when the accessory floral parts begin to fall apart or open out, the total time taken to complete this cross, the duration for which flower remain open, the time when they begin to close and whether the process is repeated in the same flower on succeeding days etc.
- b) If the flowers open and close repeatedly, the proportion of flowers self-pollinated at different periods. The time when stamens begin to emerge or when the anthersacs burst open; whether these place before, during or after the accessory parts have opened out; whether the stigma is receptive when the pollen grains are shed and whether there is mechanical obstruction between the two sexes to prevent selfpollination
- c) The time when stigma protrudes, whether at that time the pollen grains are mature or not; the study of the agents which aid or prevent self-pollination or cross pollination, such as the visits by bees and butterflies or other insects to the flowers
- d) The period for which the pollen grains are viable and the stigma remains receptive
- e) The effects of climatic and other environmental factors on the timing of various processes
- f) Varietal differences, if any.





Fig-1. Instruments used during emasculation and pollination

# **Instruments required**

- 1. Magnifying glass
- 2. Forceps
- 3. Scissors
- 4. Needle
- 5. Brush
- 6. Pencil
- 7. Tag
- 8. U-Clip
- 9. Paper bag
- 10. Measuring tape
- 11. Alcohol

# Labelling

After emasculation, the following information should be written on the tag with a lead pencil:

- 1. Name of the parent
- 2. Date of Emasculation
- 3. Date of Pollination
- 4. Name of the male parent
- 5. Name of the technician
- 6. Symbol of parents either male ( $\eth$ ), female ( $\bigcirc$ ), crossed (×) or selfed ()

# Assignment:

• Collect the flowers of vegetable crops. Differentiate them into self and cross pollinating behaviour depending upon their floral morphology

# **Practical Exercise:**

# **Hybridization Techniques**

- 1. Emasculation means
- 2. What is the procedure to write name on the tag?
- 3. Pollen will be present in-----
- 4. Symbol for male, female, crossing and selfing respectively?
- 5. Root vegetables are .....

# Breeder's kit and its components - uses.

A breeder require the following tools for controlled selfing, artificial pollination and for field observation.

S.No.	Items	Purpose
1.	Magnifying lens	To observe small flowers, stigmatic surface, dehiscence of anthers etc.
2.	Forceps	Fine forceps are required for emasculation
3.	Scissors	Required to remove unwanted buds, awns, etc.,
4.	Needles	Required to open small buds and separating the floral parts.
5.	Brushes	Camel hair brushes of size 3 or 4 for collection of pollen and transfer to stigma.
6.	Bags	Parchment paper bag, khaki cloth bags, muslin cloth bag, and paper bags of different sizes for different crops.
7.	Alcohol or Methylated spirit	A small vial of alcohol or methylated spirit is required to sterilize forceps, scissors, needles, brushes etc.,
8.	Tags	Paper, cardboard or aluminium tags are required for labeling the units in the field. In the case of paper or cardboard tags, they have to be dipped in wax after labeling and tags are tied in bamboo stakes.
9.	Meter scale	Required for plant measurement in the field.
10.	Field note books	Field note books are required to note down daily observation in the field, regarding germination, flowering, morphological description, initial and final stand, wet weight of grains, haulms etc.

# **Practical Exercise**

1. Draw the different components of Breeder's kit.

# Selfing and crossing techniques in temperate root crops

Selfing and crossing are the essential procedures in crop improvement process. The exact procedures used to ensure self or cross-pollination of specific plants will depend on the floral structure and normal manner of pollination. Generally effecting cross-pollination in a strictly self-pollinating species is more difficult than *vice-versa* because for instance preventing self-pollination occurring inside the unopened flowers is cumbersome.

# Selfing

In the selfing of cross-pollinated species, it is essential that the flower are bagged or otherwise protected to prevent natural cross-pollination. Selfing and crossing are essential in crop breeding. It is important that the breeder, master these techniques in order to manipulate the pollination according to his needs. The exact procedure that he may use to ensure self or cross pollination of specific plants will depend on the particular species with which he is working. The structure of the flowers in the species determine manner of pollination. For these reasons, the breeder should acquaint himself with the flowering habit of the crop.

In the case of Solanaceous and Leguminous crop, the plant is permitted to have self pollination and the seeds are harvested. It is necessary to know the mode of pollination. If the extent of natural cross pollination is more, then the flowers should be protected by bagging. This will prevent the foreign pollen to reach the stigma. Seed set is frequently reduced in the inflorescence enclosed in bags because of excessive temperature and humidity inside the bags. In certain legumes which are almost insect pollinated, the plants may be caged to prevent the insect pollination. In carrot, a paper bag is placed over the umbel to collect pollen and the **umbel** is bagged to protect from foreign pollen. The pollen collected from the **covered umbel** is transferred to another umbel.

# Emasculation

Removal of stamens or anthers or killing the pollen of a flower without the female reproductive organ is known as 'emasculation'. In bisexual flowers, emasculation is essential to prevent of self-pollination. In monoecious plants, male flowers are removed. In species with large flowers e.g. (Cucurbitaceous and Solanaceous crop) hand emasculation is accurate and it is adequate.

# **Practical Exercise**

- 1. Effecting self-pollination in a cross pollinated crop (or) effecting cross pollination in a self-pollinated crop which one is easier why?
- 2. Note down the type and size of bags used in crossing blocks of carrot, radish, turnip and beetroot and offer your remarks.

# Breeding Systems in Temperate Root Vegetable Crops

#### Introduction

In India, main temperate root crops are radish, carrot, turnips and beetroot.. Radish (*Raphanus sativus*) and Turnip (*Brassica rapa*) belong to family Cruciferae, carrot (*Daucus carota* L.,) belongs to Apiaceae (Umbelliferae), and beetroot (*Beta vulgaris* L.,) to family Chenopodiaceae. All the crops are cool season and are highly cross pollinated. First three (radish, turnip, carrot) are entomophilous and beet root are anemophilous. In these crops except beet root, two types i) Asiatic/Tropical ii) European/temperate cultivars are found. Beet root cultivars belong to European type only.

#### **Devices promoting self and cross pollination**

#### **1. Self-pollination:**

The essential pre-requisites for self-pollination are hermaphrodite (bisexual) and homogamous flower (female and male parts maturing at the same time). The following devices promote self-pollination in flowers.

#### A) Cleistogamy:

The flowers do not open before anther dehiscence. Therefore self-pollination is the rule eg. Lettuce

#### **B) Chasmogamy:**

The receptive stigma is pollinated before or just as the flower opens eg. Peas and Beans

#### C) Protruding pistil:

The stigma may elongate through the staminal column at the time of anther dehiscence just after flower opening and this results in self-pollination in tomato since the elongating stigma gets the pollens from the laterals of anthers

#### 2. Cross pollination:

Cross pollination in a unisexual flower is the rule. The plants bearing unisexual flowers, may be monoecious (the flowers of both sexes are present on the different parts of the same plant) eg. Cucurbits or may be dioecious (flowers of different sexes are present on different plants) eg. Asparagus, spinach and pointed gourd

Male sterility is also present in some crops eg. onion, cole crops, carrot etc. Even in hermaphrodite flowers cross pollination may occur because of the following mechanisms.

#### A) Dichogamy:

When male and female parts mature at different times. This is of two types

#### i) Protandry:

When anthers mature earlier than the stigma eg. Carrot, onion, leek, garden beet and parsnip

## ii) Protogyny:

When stigma matures earlier to anther dehiscence eg. Cole crops

#### **B) Heterostyly:**

The styles and filaments are of different lengths eg. Tomato, brinjal, Brassica sp.

## C) Herkogamy:

Some physical barriers between the anthers and the stigma may prevent self-pollination *eg.* Protruding stigma in tomato and capsicum

## D) Self incompatibility:

The pollen grains are viable but fail to fertilize the ovule of the same flower as in kale, cabbage, knol-khol, radish and turnip

#### E) Male Sterility

It is an inability of the plant to produce viable pollen with active and compatible stigmatic surfaces. eg. Carrot, onion

# Methods of determination of mode of reproduction

- Flower characteristics
  - If chasmogamy, cleistogamy, self compatible then it is self pollinated eg. Solanaceous and leguminous crop
  - If monoecious, dioecious, protandry, protogyny and self incompatibility then it is cross pollinated crop eg. Cucurbits, carrot, radish, cruciferous crop
  - Characteristic feature of cross pollinated crop: attractive flower colour, larger size of flower, stigmatic surfaces are wider, attractive honey dew, inconspicuous size of flower, easy dispersal nature of pollen grains, sticky nature of pollens, small size of pollen grains
- Growing of plant under cage/bag
  - If there is seed setting then it is self pollinated crop e.g. Solanaceous crop
  - If there is no seed set then it is cross pollinated crop e.g. onion and carrot
- Effect of selfing on vigour of plant
  - Inbreeding depression and loss in vigour of the plant then it is outcrossing crop
  - Eg. Carrot and onion
  - No inbreeding depression and no loss in vigour then it is inbred crop eg. brinjal

Сгор	Mode of pollination	Breeding system	Pollination vector
Carrot	Cross	Protandry & Male sterility	Honeybees
Radish	Cross	Protogyny & Sporophytic SI	Bumble bees & Honeybees
Turnip	Cross	Protogyny & Sporophytic SI	Bumble bees & Honeybees
Beetroot	Cross	Protandry	Wind

#### Table 1: Breeding system of root crops which are used for hybrid seed production

# **Practical Exercise:**

- 1. Protandry means-----
- 2. Self incompatibility is defined as ------
- 3. Male sterility occur in -----
- 4. Inbreeding depression occurs in -----pollinated crop
- 5. Cleistogamy leads to -----pollination

# Handling of $\mathbf{F}_1$ and $\mathbf{F}_2$ generations

#### Introduction:

#### Planting of a crossing block and raising of F<sub>1</sub> and F<sub>2</sub> generations:

#### A. Crossing block:

A vegetable breeder working for the improvement of any crop has many projects in hand at one time such as breeding for high yield, disease resistance, earliness and quality. There may be a set of varieties/lines carefully chosen under each project and have to be crossed inter-se. It is advisable to grow all these parents together in a crossing block either in the glass house/greenhouse or in the field.

#### **B.** Raising of F<sub>1</sub>:

A large number of  $F_1$  seeds of different crosses are produced by the breeder every year. It is necessary to plant the  $F_2$  seeds of each cross along with the parents. If the parents of  $F_1$  hybrid are homozygous cultivar in self pollinated crop (SPC) and true breeding inbred line in cross pollinated crops (CPC), then all the  $F_1$  individuals of the same cross are genetically similar and therefore, should be uniform for all the characters. In other words, the  $F_1$  is heterozygous but homogeneous. The  $F_1$  plants should not resemble female parent is respect of all the characters, but should exhibit some characters of the pollen parent as well.

#### C. Raising of F<sub>2</sub>:

Seeds harvested from the true  $F_1$  plants are the  $F_2$  seeds. Seeds produced by the different  $F_1$  plants are bulked and sown in the next year. The  $F_2$  population is the first segregating generation in which maximum variability is generated upon the genetic differences between the parents used.

#### Procedure

#### The crossing block:

If all the cultivars that are to be crossed do not flower at the same time, then three to four sequential sowings of all these lines after every 7-10 days are made so as to get the synchronized flowering of very early and very late cultivars. However, most of the vegetables have prolonged duration of flowering where this problem is automatically contained. Since the emasculation and pollination involve the physical handling of plants, sufficient alleys or paths are provided between the pairs of rows so as to prevent the smothering of plants.

#### The raising of F<sub>1</sub>:

The  $F_1$  seeds of all crosses are space planted. The parents are also grown along with the  $F_1$ s. This procedure allows the identification of any selfed plants. Plants should be exercised as  $P_1$ - $F_1$ - $P_2$ .

#### The raising of F<sub>2</sub>:

The method of planting the  $F_2$  depends upon the procedure of handling the segregating generations and the following generations. In the pedigree method,  $F_2$  seeds are grown in widely spaced rows with an appropriate distance from plant to plant, so that each and every  $F_2$  plant can be examined and selected or rejected. The parents of the cross the  $F_1$  as well as the standard check upon which the improvement is sought are also planted along with the  $F_2$  to aid in the selection of desirable crosses as well as the desirable plants of a particular cross. When the bulk method is followed, the  $F_2$  is planted just like a commercial crop and no selection is practiced. Planting may be exercised as  $P_1$ - $F_1$ - $F_2$ - $P_2$ -Check.

#### **Observations**

These are made on the following parameters:

- a. The number of cultivars in a crossing block
- b. The date of sowing
- c. The crosses made (Pedigree: The number of  $F_1$  seeds)
- d. Notes on important characters of the F<sub>1</sub>s
- e. Sketch plan of F<sub>1</sub>s and parents
  - i. The number of cultivars in the crossing block
  - ii. Date of sowing
  - iii. Crossing made
  - iv. Notes on important characters of F<sub>1</sub>s
- f. Sketch plan of the F, population under pedigree method
  - i. No of  $F_{2}s$
  - ii. Date of sowing

# Botany, Floral Biology, Selfing and Crossing-Emasculation and Pollination Techniques, Pollination Control Mechanisms, Hybrid Seed Production in Carrot

#### Introduction:

Carrot is a dicotyledonous herbaceous crop belonging to Apiaceae (Umbelliferae) with 2n=2X=18. The wild form is annual but the cultivated crop is biennial. Like radish, the carrot also has two groups-Asiatic and European (temperate) types. The Asiatic type produce roots (conical) and seeds freely in plains whereas European types produce good roots (blunt) but fail to produce seeds in plains. In Asiatic type roots are red with more anthocyanins whereas root is orange and rich in carotene in temperate types.

#### **Systematics:**

Crop: Carrot Family: Apiaceae (Umbelliferae) Genus: Daucus Species: carota L. Chromosome number: 2n = 2X= 18 Origin: Afghanistan Related Species: D. carota L. var. sativa DC (purple), D. carota L. var. dentatus D. carpillifolius L.

# **Part A: Botanical features**

Habit: Carrot is annual in habit as vegetable crop and biennial as seed crop

Root: It is an enlarged tap root consisting of phloem (cortex) and xylem (core)

**Stem:** Stem is reduced and discoid in young plants. The stem elongates and produces rough hipid branches during the second year

Leaf: Cauline and small, radical in young plants, usually exstipulate

**Inflorescence:** It bear compound umbel. It is the king/primary umbel that flowers first. The umbels terminating anther branches of the main stem are known as secondary umbel and third and fourth order umbels develop in succession. The interval of flowering between the umbels of different order is usually 8-12 days. The flowers are perfect and anthesis in a single umbel is completed in 7-9 days. The peripheral umbellate flowers open first followed by the inner umbellates. The umbels which terminate the branches are known as secondary umbels.

Calyx: 5 sepals, gamosepalous

Corolla: 5 petals

Androecium: 5 free, altermipetalous, filaments equal in length

Gynoecium: Bicarpellary, syncarpous, inferior, bilocular, plancentation marginal, parietal

Fruit: Fruit is schizocarp separating at maturity into two segments producing one seed, hairy

Seed: Seeds are flat, ribbed, spiny and vary greatly in size. 1000 seed weight is 0.8g

# Part B: Emasculation and Pollination

**Mode of pollination:** Principally a cross pollinated crop-entomophilous, pollinated by honeybees

Anthesis: Flower opens in the morning hours

Anther dehiscence: Shortly after anthesis

Pollen viability: Pollen become viable 3-4days before stigma becomes receptive

**Stigma receptivity:** Stigma becomes receptive 5 days after anthesis and it remains so for 8 days. Better fruit set is on 6-11 days after anthesis

**Flowering stage:** Flowering starts as early as in March-April and special attention should be given to irrigation and other cultural practices required for successful seed production.

**Emasculation and pollination:** Umbel having maximum buds at the time of emasculation are selected and opened flowers along with young buds are removed. Maximum number of buds are emasculated for pollination. The bagged and emasculated umbel is pollinated by enclosing an umbel from the male umbel on emasculated umbel



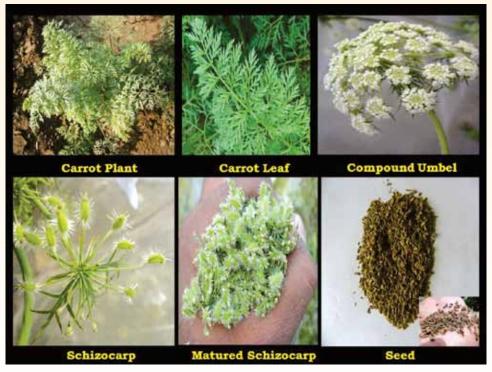


Fig-2. Botany of carrot

#### Stage of emasculation and pollination:

Sufficient buds at peak stage of flowering are emasculated and the remaining ones are removed. Then umbel of pollen parent is cut off and its stalk placed in a glass bottle filled with water. The pollen parent umbel is enclosed in the same bag along with the emasculated umbel of the female parent. Daily for a few days in the morning, the male umbel is gently rubbed over the female to ensure cross pollination. In the presence of some dominant marker gene for distinguishing hybrids in the seedling stage, emasculation of flowers is not necessary.

#### **Natural Pollination:**

Special attention is required to prevent practices that will negatively influence bee activity as bees are very important for pollination for a good seed setting. Pollination is best performed by using honey bees or flies for pollen transfer during the period of flowering. Natural populations of bees and other insects will sometimes be adequate. As an alternative, pollen movement is possible by hand or brush but seed set will often be low. Within 4 to 6 weeks after pollination the developing seed turns brown.

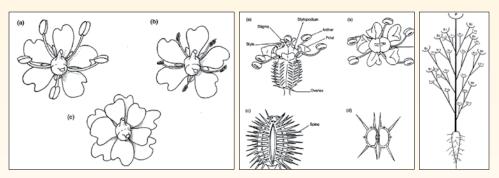


Fig-3. a. Carrot fertile, b. carrot sterile flower, c. petaloid type flower d. Carrot seed structure and umbel development

#### **Maintenance of Inbred Lines:**

For effective individual plant pollinations for maintenance of inbred lines, the roots should be planted at  $60 \times 50$  cm spacing and cages of 50-80 cm long wire cylinders, 40-60 cm diameter, covered with muslin cloth and open at each end are used. These are placed over umbels to be pollinated and tied tightly with a wire closure around seed stalks at the bottom, just below the umbels. Similarly, top is also tied tightly and flies are added through a tube inserted in the top or side. Self-pollination and sib mating for maintenance of inbred lines and varieties, respectively, can also be made in an insect free area by rubbing umbels of parental stocks together or moving pollen with brushes or by hand. These can be maintained by controlled pollination under mesh screen cages of sizes 1 m × 2 m to 8 m × 30 m depending on plant numbers with the help of honeybees or flies added to distribute pollen. Besides, inbred lines and varieties can also be maintained by planting in isolation.

#### **Three Way Cross:**

Carrot hybrids are usually threeway crosses i.e.  $(A \times B) \times C$ , since the hybrid vigour in a single-cross  $F_1$  female seed parent usually results in much greater seed production than that of an inbred male-sterile parent. Single cross hybrids (A × B) are on average more uniform than three-way crosses and they do not require an extra year to produce  $F_1$  seed

	Single- cross	Three-way hybrid	Backcross hybrid
Year 1	A x B	AxB	АхВ
Year 2		(A x B) x C	(A × B) × B
Year 3			$(A \times B^2) \times C$

Fig-4. Scheme for production of hybrid carrots. A. male-sterile inbred; B and C, male-fertile inbreds.

parent stock. Thus, if seed productivity of single-crosses is adequate, they are used.

#### **Techniques of hybrid seed production:**

The two basic steps involved in the hybridization for production of hybrid seeds are emasculation and pollination. Hand emasculation and hand pollination of individual flowers is most expensive method and may not be economically worthwhile in carrot where floral parents are so arranged that manipulation of flowers for emasculation is difficult. Therefore, any method which is convenient and economical in two basic procedures is worthwhile for adoption. The three commonly employed mechanisms for overcoming emasculation and hand pollination are sex expression, self-incompatibility and male sterility. In many crops the genetic emasculation has become feasible by using male sterility and hence hybrid seeds are produced from the male sterile plants in the female parents with the help of natural pollination as in onion and carrot.

#### **Production of F**<sub>1</sub> hybrid seed:

Hybrid cultivars of carrot have the advantage of relatively uniform roots and have been produced by using of two methods. The first system is with cytoplasmic male sterility (CMS) in which the pollen does not develop beyond the microspore stage, sometimes referred to as the 'brown anther form'. The other type is the petaloid form in which the five anthers are transformed into petaloid structures during their early development and do not produce any pollen. The final morphology of the petaloid anthers varies from petal-like to filamentous. Most commercial  $F_1$  carrot hybrids are produced from the petaloid CMS. Generally the ratio of female-to-pollinator rows is from 2:1 to 4:1. Production of parental lines and the hybrid seed by either system should be carried out in accordance with the instructions of the maintenance breeder.

Hybrid seed yields can be relatively low, about 500 kg/ha, after taking into account the exclusion of the seed from pollinator rows. The lower yield is frequently attributed to less insect activity because of smaller petals on the male sterile flowers of the seed-producing lines. Seed producers frequently use honeybee colonies to supplement the natural level of pollinating insect activity when producing hybrid carrot seed.



Fig-5. Crossing techniques in carrot root

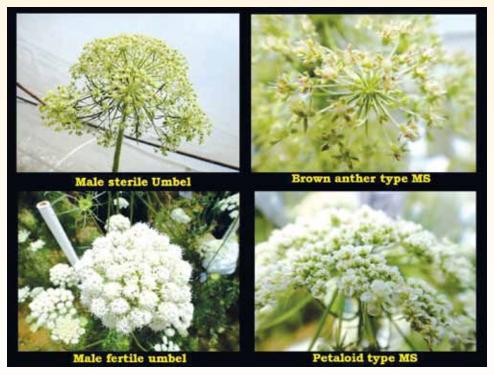


Fig-6. Structure of fertile and male sterility in carrot



Fig-6A. Pollination isolation cage for (a) single plants and (b) a large number of plants for carrots



Fig-6B. Characteristics of male sterile lines and stage of seed maturity

# Male sterility:

It is an inability of the plant to produce viable pollen. There are two types of male sterility in carrot:

- 1. The brown anther type, in which the anthers are shriveled and brown without any viable pollen
- 2. The petaloid type in which anthers are modified into petaloid structures

# **Exploitation of cyto-genic male sterility:**

The sterility factor is determined by the interaction of genes and cytoplasm but none of them singly can control sterility. This type of male sterility is reported in carrot and been extensively exploited for hybrid seed production. Brown anther type is conditioned by dominant and recessive genes in (S) cytoplasm while petaloid type is conditioned by three genes in (S) cytoplasm. In carrot, the sterility is of unstable in nature. It gets altered with the changed in climate which may be due to presence of modifying genes. The male sterile line is maintained by crossing it with a male fertile sister line. In this case, the progeny of male sterile plants is not necessarily male sterile but can be male fertile which certain stocks are used as pollinators. Such male plants are reported carry gene having power to restore pollen producing ability in the genotypes possessing the male sterility cytoplasm.

## **Characteristics of male sterile lines:**

- 1. The male sterile plants are less vigorous than male fertile plants
- 2. Male sterile lines produce off-white to green colour flowers, while fertile produce white flowers
- 3. Anthesis in male sterile plants starts later than fertile plants
- 4. Male sterile flowers are less visited by bees, since nectar produced in them is often poor quality
- 5. Male sterile lines produce indistinct carrot like or no aroma in the flowers

# Problems in exploitation of C-GMS:

Since the system is sensitive to environmental factors, change in climate may lead to restoration of fertility, thus contaminating hybrid with self pollinated seeds.

# Hybrid Seed Production System:

The hybrid seed production using male sterility needs location of male fertile plants (maintainer) which in successive generations will yield all male sterile progenies. In the heterosis breeding programme, male sterile line (A), the male fertile line (B) and the pollinator lines are used. The male sterile and pollinator lines are inbred for several generations for attaining uniformity. Usually 4 rows of male sterile seed parent are planted alternatively to 2 rows of pollinator line in an isolated field for the production of  $F_1$  hybrid seeds. The seeds are harvested from male sterile line and the pollinator plants are removed before collecting the seeds from male sterile female parent.

The seed production is taken in the temperate region of country for European types and in the plains for Asiatic types. European types require high chilling of 4-7°C for a period of about 2 months. The mild summer and low rainfall of hills or higher altitudes especially at flowering and seed setting is beneficial. Root to seed method is preferably used for seed which becomes ready for harvesting in July and August. Threshing is done by rubbing the umbel with hand. The seed produced by first few umbel order is vigorous and should be utilized.

# **Practical Exercises:**

- 1. Carrot is scientifically known as -----a. Daucus carota L. b. Daucus capilliformis c. D. maritima
- 2. Carrot belongs to the family-----a. Umbelliferae b. Apiaceae c. Both d. None
- 3. Breeding system of carrot-----a. Protandry b. Protogyny c. Distyly d. Herkogamy

- 4. Flower colour in carrot is-----a. White b. Pink c. Yellow d. Lilac Purple
- 5. Chromosome number of carrot-----a. 16 b. 18. c. 20 d. 22
- 6. Type of inflorescence: Umbel/Racemose/Cymose/Perianth
- 7. A) Type of pollination: Cross/Self; B) Mode of pollination:
- 8. Anthesis time:
- 9. Type of Fruit: Siliqua/Simple/Pepo/Scizhocarp
- 10. Which umbel is most suitable and effective for hybridization?  $1^{\circ}/2^{\circ}/3^{\circ}$

# Introduction, Botany, Floral Biology, Selfing and Crossing-Emasculation and Pollination Techniques, Pollination Control Mechanisms, Hybrid Seed Production in Radish

#### Introduction:

Radish (*Raphanus sativus* L.) a member of the family Brassicaceae, is a cool season, fast maturing, easy to grow annual or biennial herbaceous plant that is grown for its roots. Radish is mainly grown for its thickened fleshy root used mainly as *salad*. Its young leaves are also consumed after cooking. Although radish is grown and consumed worldwide, it contributes little to the nutrition.

#### **Systematics:**

Family: Cruciferae/Brassicaceae Genus: *Raphanus* Species: *sativus* L. Chromosome number: 2n = 2X= 18 Origin: Asia and Europe Related Species: *R. raphanistrum* L. *R. landra R. maritimus* 

# **Part A: Botanical features**

Habit: Annual and biennial herb depending on types
Root: Uniform taproot, swollen, cylindrical or conical shape
Stem: Simple or branched
Leaf: Radical and Cauline, simple, alternate
Inflorescence: Terminal raceme
Flowers: White, rose or lilac in colour with purple veins in bractless racemes
Calyx: 4 sepals, polysepalous

Corolla: 4 petals, polypetalous. Regular, each petal differentiate into claw

Androecium: 6 stamens in two whorls, 2 outer stamens are short while 4 interior stamens are long

Gynoecium: 2 carpels, syncarpous, overy superior

Fruit: Siliqua

Seed: Exalbuminous, embryo curved, attached to septum. 1000 seed weight is 10g.

## Part B: Emasculation and Pollination

Mode of pollination: Cross pollination is carried out by honey bees and bumble bees

Anthesis: Flowers open in the morning 9.00am to 10am

Anther dehiscence: It coincides with the anthesis

**Pollen viability:** Maximum on the day of anthesis. But when stored at 3°C, the pollen remains viable for 60days

**Stigma receptivity:** Stigma becomes receptive at the time of anthesis and continues for four days

**Emasculation and pollination:** Young buds of suitable size are selected one or two days prior to anthesis. Emasculation is done on 3-4 flowers in a single branch. For pollination, racemose of opened flowers is picked up from the male parent and dusted on the stigma of the emasculated flowers

#### **Pollination Control Mechanisms:**

Radish is a cross pollinated species due to presence of self-incompatibility. It is entomophilous root vegetable where pollination is greatly influenced by insect activity, especially honey bees. Pollination is normally limited by environmental conditions, especially temperature, during the flowering season. Wild honeybees mainly pollinate radish and wild flower flies. During rainy winter and low temperature insect pollination is rare. With the discovery of male sterility in radish, it became possible to have a control over pollination. Male sterility in radish was controlled by the genetic interaction between cytoplasmic and nuclear genes. There are two pairs of *ms* genes in the nucleus, the genotype of the male sterile plants being  $Sms_1ms_1ms_2ms_2$  and the maintenance line  $Nms_1ms_1ms_2ms_2$ . Selfing can be accomplished by bud pollination.

#### Hybrid seed production:

Both self-incompatible line and male sterile lines are being used for producing hybrid seed, commercially. Single crossing, three way crossing and double crossing are three methods of hybrid seed production in radish. However, double-crossing method is the most effective means of hybrid seed production in radish. The field for hybrid seed production should be 500-1000 m apart or even more from other radish fields to avoid cross pollination. The proportion of the two parental lines in mixed planting is influenced by the level of self-incompatibility, vigour in growth of seed parents, plant

posture and quality and quantity of pollen. The seed parents are planted at a ratio of 1:1 where  $F_1$  hybridization is between two inbreds with the same level of traits. Maximum rate in mixed planting is 1:3. The ratio of female to male parent rows is usually 1:1.

# Maintenance of Inbred/Pure Lines:

Due to existence of self-incompatibility in radish, selfing can be accomplished by bud pollination. The flower buds should be pollinated 2 days prior to their opening by their own pollen from previously bagged flowers of the same plant when the plant has about 30 percent flowering than just at the commencement of flowering. At this stage self-incompatibility is not active. Usually about 8 to 10 flower buds were pollinated in each inflorescence branch to ensure better seed set and the unopened young flower buds at the terminal end are removed. An alternative to conventional bud pollination method for overcoming self-incompatibility in radish by the application of carbon dioxide at relatively high concentration (3-5%). Normally CO<sub>2</sub> at this concentration is applied for 2 hours in the evening of every other day and this treatment is continued for 3-4 weeks. By this method, self-fertilized seeds can be obtained in pollination at flowering and sufficient original seeds of inbreds/pure lines as parents of F<sub>1</sub> hybrids maintained.

# **Practical Exercises:**

- 1. Radish is scientifically known as....a. Raphanus sativus b. R. maritima c. R ficifoliformis
- 2. Radish belongs to the family----a. Cruciferae b. Brassicaceae c. Both of these d. None
- 3. Bud pollination means-----
- 4. Bud pollination is done because of -----a. Self or b. Cross-----incompatibility
- 5. Flower colour of radish....a. White b. Lilac Purple c. Both of these d. None
- 6. Type of inflorescence: Umbel/Racemose/Cymose/Perianth
- 7. Type of pollination: Cross/Self/Often-cross B) Mode of pollination:
- 8. Anthesis time:
- 9. Type of Fruit: Siliqua/Simple/Pepo/Schizocarp/Hesperidium
- 10. At which stage we need to do emasculations and pollination?

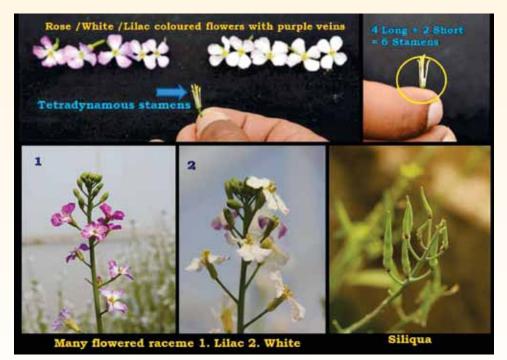


Fig -7. Botany of radish



Fig-8. Bud pollination scheme at ICAR-CITH, Srinagar



1. Selection of terminal inflorescence



3. Removal of all opened flower from inflorescence



2. Selection of flower from female line



4. Removal of younger buds and selection of optimum buds



6. Selection of bud for emasculation



7. Circular incision on the bud



Fig-9. Crossing techniques in radish root crop



# Introduction, Botany, Floral Biology, Selfing and Crossing-Emasculation and Pollination Techniques, Pollination Control Mechanism, Hybrid Seed Production in Turnip

#### Introduction:

Turnip (*Brassica rapa* L.; 2n = 2 x = 20), a member of family Brassicaceae, is a cool season, frost hardy crop mainly grown for its fleshy roots. Turnip is used in roasts, stews, soups, casseroles, and as a boiled or cooked vegetable or sliced in salads.

## **Systematics**

Family: Cruciferae
Genus: Brassica
Species: campestris L. var. rapifera, Metz.
Chromosome number: 2n = 2X= 20
Origin: Central and Southern Europe possibly the Mediterrenean region
Related Species:
B. campestris L. ssp. chinensis Jusl.,
B. campestris L. ssp. pekinensis (Lour.) Rupr.,
B. napus L.
B. campestris

# **Part A: Botanical features**

Habit: Botanically a biennial herb, grown as annual root crop

Root: Swollen, tuberous tap root-rapiferous

Stem: Flattened, conical at the apex of the root

**Leaf:** Pinnate or simple leaves, basal leaves light green, petiolate, pinnatid with large terminal lobe, hairy without wax. Leaf oblong to oval.

**Inflorescence:** Racemose. It is typical raceme on the main stem. The flowers are attached with short pedicel.

Calyx: 5 parted and erect sepals

Corolla: 5 petals, persistent, pale yellow coloured and clawed.

Androecium: 5 stamens, the 2 outer stamens are curved outwards at base and are shorter than the inner ones

**Gynoecium:** Ovary superior, three celled with large lobed disc at at the base. Styles-3, distinct or united. Stigma capitates.

Fruit: Siliqua with converse values, tipped by usually seedless beak.

Seed: Round, black or reddish brown containing oil. 1000 seed weight is 4g

# Part B: Emasculation and Pollination

**Mode of pollination:** Turnip is a cross-pollinated crop-mainly entomophilous. Honey bees, bumble bees and blow flies are the usual pollinators

Anthesis: Flowers open during morning hours i.e. 8.00AM to 10.30AM

Anther dehiscence: It coincides with the anthesis

Pollen viability: Maximum on the day of anthesis.

Stigma receptivity: 2-3days before the anthesis

**Emasculation and pollination:** Young buds of suitable size are selected one or two days prior to anthesis. Emasculation is done on 3-4 flowers in a single branch. For pollination, racemose of opened flowers is picked up from the male parent and dusted on the stigma of the emasculated flowers

# **Pollination Control Mechanisms:**

There are two pollination control mechanisms in turnip, viz., sporophytic selfincompatibility system and cytoplasmic male sterility (CMS). Effective selfincompatibility avoid the need for emasculation and crosses can be made by enclosing flower heads from two compatible plants in a cellophane or muslin bag with blowflies as pollinators.

# **Topping:**

In turnip, 'topped' to encourage the development and growth of secondary inflorescences from the main flowering shoots. This 'topping' also reduces the overall height of the crop and the possibility of lodging at a later stage. The removal of the growing points also reduces the seed crop's range of maturity period. The top 10 cm of the terminal shoots are removed when the flowering shoots are between 30 and 40 cm high.

# **Hybrid Seed Production:**

A distance of 1000 m to avoid out crossing must separate the fields for  $F_1$  hybrid seed production. The ratio of female to male parent is usually 1:1 while utilizing cytoplasmic male sterility as well as seed parents planting in case of self-incompatibility.

#### Maintenance of Inbred/Pure Lines:

Self-incompatibility does pose a problem in producing selfed seed of inbreds/pure lines, but this can be overcome if style is pollinated after removing the stigma. Besides, salt (NaCl) solution and carbon dioxide could also be used to overcome self-incompatibility that will facilitate self-seed production, thus maintenance of inbred/ pure lines.

#### **Double cross:**

A combination of self-incompatible (SI) and self-compatible sister lines to produce a double-cross hybrid was suggested as the most practicable method. In a comparison of CMS and modified SI systems, the main differences are that two self-sterile lines need to be maintained with SI but only one for CMS, as opposed to the whole  $F_1$  crop being sown and harvested together with SI, whereas pollinator rows have to be sown separately and then cut out with CMS. For hybrid seed production of turnip the ratio of female-to-male parent is usually 1:1.

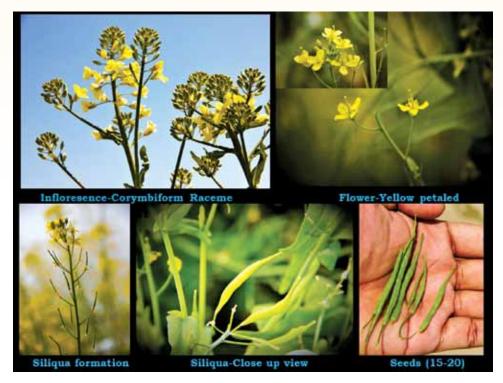


Fig-10. Botany of turnip

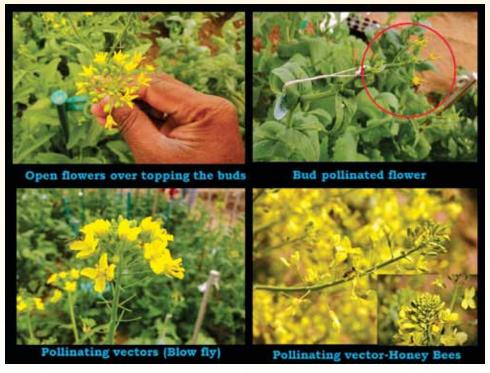
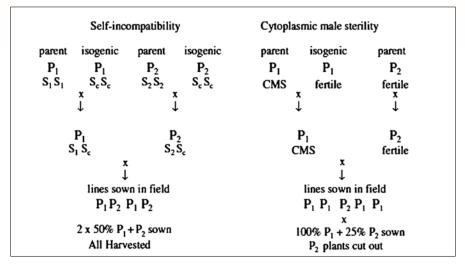


Fig-11. Flowering and pollination in turnip



**Fig-12.** Simplified diagram to show the main differences between hybrids produced by a modified double cross using self-incompatibility and a F1 hybrid using cytoplasmic male sterility, from parental lines P1 and P2. Two self-sterile lines (S1S1 and S2S2; both dominant to ScSc fertile, self-compatible lines) have to be produced by CO2-enhanced pollination with self-incompatibility but only one sterile line with CMS; however, a greater area of crop has to be sown for the final seed production with CMS and the pollinator line cut out, whereas the whole crop is simply sown out and harvested with self-incompatibility. As only a vegetative crop is required, a restorer line is not needed with CMS

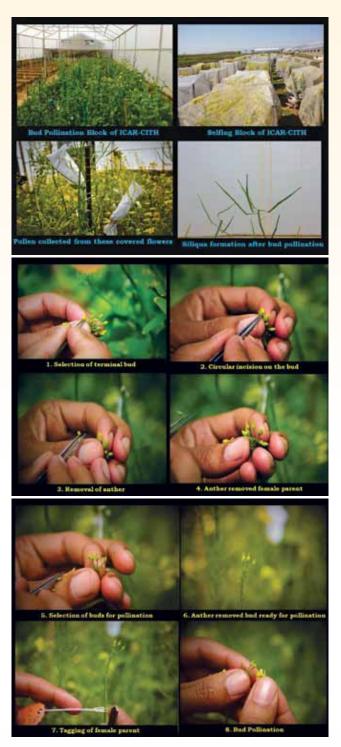


Fig-13. Crossing techniques in turnip root crop

#### **Practical Exercises:**

- 1. Turnip is scientifically known as-----a. *Brassica rapa* b. *Brassica acephala* c. *Brassica botrytis*
- 2. Turnip belongs to the family-----a. Cruciferae b. Brassicacae c. Both of these d. None
- 3. Flower colour of turnip-----a. Yellow b. White c. Pink d. Green
- 4. Type of self-incompatibility-----a. Sporophytic SI b. Gametophytic SI b. Gametophytic SI
- 5. Chromosome number of turnip-----a. 16 b. 18. c. 20 d. 22
- 6. Type of inflorescence: Umbel/Racemose/Cymose/Perianth
- Type of pollination: Cross/Self/Often-cross; Mode of pollination: Water/Air/ Insects
- 8. Anthesis time:
- 9. Type of fruit:
- 10. At which stage we need to do emasculation?

# **Chapter-9**

# Introduction, Botany, Floral biology, Selfing and Crossing Techniques, Pollination Control Mechanisms, Hybrid Seed Production in Beet root

#### Introduction:

The Beetroot (*Beta vulgaris* L.), a member of the family Chenopodiaceae, is grown for a fleshy root, a marketable product composed of hypocotyl and crown. The roots of beet are eaten boiled either as a cooked vegetable or uncooked as a salad after adding oil and vinegar. Betanins/Betalins, obtained from the roots, are used industrially as red food colorants, e.g., to improve the color of tomato paste strains with strikingly colored, large leaves are used as ornamentals.

# **Systematics**

Family: Chenopodiaceae
Genus: Beta
Species: vulgaris L.
Chromosome number: 2n = 2X= 18
Origin: Mediterrenean Region
Related Species:
B. vulgaris ssp. maritima L.

# **Part A: Botanical features**

Habit: Botanically a biennial herb, grown as annual root crop

**Root:** The tap root is tuberous. The lateral roots arise on the swollen tap root in two opposite vertical rows

Stem: The stem is very much reduced and the leaves arise in the basal rosettes

**Leaf:** Leaves are succulent, spiral and are much variable in size, shape and colour. They are ovate to oblong-ovate, entire or wavy, surface smooth or crinkly, base cordate. The colour is dark red to light green.

**Inflorescence:** Inflorescence is a terminal open panicle with spicate branches, bearing small sessile flowers either singly or in clusters of 2 to 7, in linear bracts.

Flowers: Flowers are bisexual, nearly epigynous, actinomorphous, pentamerous

Perianth: Perianth five lobed, lobes concave and incurved

Androecium: 5 stamens, opposite perianth lobes and slightly united to them. Anthers dithecous

**Gynoecium:** Ovary embedded in the fleshy unilocular receptacles and three carpelled, syncarpous with a single ovule arising just above the base. Style short. Stigmas short and awl shaped

**Fruit:** Corky enoclosed in hard woody perianth. Fruits often formed in aggregates (seed ball) by the fusion of a few flowers and called 'multiple beet seed' or multi germ. If the fruit is formed from a single ovary, it is termed as 'single germ beet seed' or monogerm

Seed: Small, kidney shaped and brown in colour. 1000 seed weight is 17g

# **Part B: Emasculation and Pollination**

Mode of pollination: Cross pollinated crop-anemophilous

Anthesis: 7.00am to 5.00pm

Anther dehiscence: 8.00am to 6.30pm

Pollen viability: Maximum on the day of anthesis.

Stigma receptivity: 6 hours before to 8 hours after anthesis

**Emasculation and pollination:** Since the flower is hermaphrodite, emasculation is necessary for crossing. Select the matured buds on a spike and remove the anthers with forceps. Pollination may be carried out simultaneously by dusting the male spike on the emasculated buds, or the male spike may be bagged along with.



Fig-14. Bolted beet in greenhouse condition

#### **Flowering and pollination:**

The beetroot is a quantitatively long-day biennial with a cold requirement for flower initiation. The minimum and maximum temperatures for vernalization in beetroot were  $0^{\circ}$  and  $15^{\circ}$  C, respectively, with the fastest flowering response occurring at  $12^{\circ}$ C. The inflorescence emerges from the growing point relatively early in the March. 'Topping' has to be done in the flowering shoot when it is approximately 40-50 cm high. It increases seed yield by reducing the duration of flowering and concentrates the seed maturity period, which in turn reduces seed losses from shattering. Beetroot flowers are predominantly wind-pollinated, although there is also some insect pollination by *Diptera* species. The gene '*ffs*' which conditions fasciation of the flower stalk that primary characteristics are a flattened flower stem with petioles coalesced into a twisted, ribbon like appearance. Fasciation due to '*ffs*' can be used in seed production, as fascinated plants tend to exhibit heavy seed set with seed maturity occurring in a synchronous fashion.

## **Pollination Control Mechanisms:**

Beetroot is predominantly wind pollinated. Its pollens are dust like and produced abundantly. For isolation of plant for breeding work pollen proof conditions are required. Since for hybridization, emasculation process in cumbersome, therefore mass pollination of selected root was used for development of improved cultivars of beetroot. The transfer of CMS system from sugar beet has now facilitated this process.

#### **Pollen Isolation:**

Beetroot is normally allogamous and self-sterile. Pollen granules are spherical with a diameter varying around 16–20µm. Control of pollination is necessary during breeding and the reproduction of basic and commercial seed. Isolation systems include (i) paper or cloth bags for one or more branches of the seed stalk (ii) cloth or plastic coverings for one or two plants (iii) glass and metal structures for up to about ten plants and (iv) space isolation for more numerous groups and for commercial seed crops. Using bags or isolators of small dimensions, the isolation can be completely controlled, but often the yield and quality of the seed are lower due to higher temperature and humidity inside the enclosure.

#### **Hybridization Techniques:**

Beetroot is wind pollinated and is a very prolific pollen producer. Breeding work requires some form of pollen-proof conditions for isolation of plants. The near-impossibility of emasculating the flowers meant that until the introduction of CMS from sugar-beet, cultivars were produced by mass pollination of selected roots. The introduction of CMS into beetroot has meant that  $F_1$  hybrid cultivars can be produced. For hybridization, if dominant marker is available it can be used to avoid emasculation of female parent and the selfed plants can be rogued either at seedling stage or at root

stage. If the pollen parent does not possess any marker genes it would be necessary to emasculate the flowers before crossing since these are hermaphrodite. During pollination care should be taken to avoid contamination from foreign pollen by wind. The bags covering the plants of the male and female parents should not be opened on a windy day and pollination must be done when the air is still, preferably in a glasshouse or plastic cage

#### **Hybrid Seed Production:**

Production of beet seed for commercial purposes is usually accomplished over the course of two seasons in a climate with mild winters (8°C) and cool dry summers (24° C). Sowing seed parent and pollen parent in 1:1 ratio can produce the commercial  $F_1$  hybrid seed in fields isolated from other compatible crops or varieties. Isolation of at least 1000 m is required, since beetroot is an anemophilous. Zoning scheme can also be employed to confine seed production of different hybrids in separate geographical areas. Hybrid seed production in beetroot is feasible only if monogerm character and cytoplasmic male sterility (CMS) system is available.

#### Maintenance of Inbred/Pure Lines

Selfing is necessary for maintenance of inbreds/pure lines for this purpose, the entire plant or a few flowering branches are enclosed in a thick muslin cloth bag or preferably a Kraft-paper bag so that pollen does not get blown away by wind. The bags must be shaken once or twice daily to ensure better seed-set and must not be opened on windy days.

#### Three way cross

Methods for the synthesis of hybrid varieties are becoming quite similar among the few seed companies currently active. In Fig. 15 is represented the method for the synthesis of three-way hybrids employing a monogerm CMS  $F_1$  crossed with a multigerm 2x pollinator. As previously mentioned, the CMS inbred line is usually crossed with a different O-type. The selection of the best combination CMS ×

O-type is made testing their general combining ability (GCA). The traits to consider in the  $F_1$  progeny are also seed production, a high degree of male sterility, monogermy, the traits of the seed stalk, etc. The selected CMS F1 is crossed with different pollinators each in an isolated field. The seed of test crosses is harvested from the CMS and is accurately tested for the germination traits. The year later, test crosses are drilled in multi-year field trials organized in localities where the future variety should be cultivated. The crosses with superior yield and quality performances are mixed in different ways and go on with testing for at least 3 years. According to the results, the seed of the new variety is reproduced in large amounts for registration procedures and commercialization.

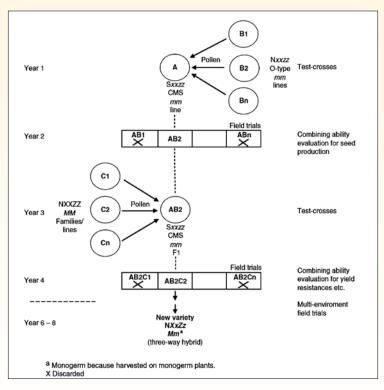


Fig-15. Synthesis of three-way hybrid variety including tests of combining ability for O-types and pollinators

#### **Practical Exercise:**

- 1. Beet-root is scientifically known -----a. *Beta vulgaris* var. *vulgaris* b. *B. vulgaris* var. *maritima*
- 2. Beet-root belongs to the family-----a. Chenopodiaceae b. Umbelliferae c. Convolvulacae
- 3. Hybridization is done by shaking of male and female parents a. inside muslin bag or b. outside muslin bag
- 4. Chromosome number of beet-root is-----a. 16 b. 18. c. 20 d. 22
- Red-violet Colour of beet-root is due to the presence of ---a. β-cyanin b. β-xanthin c. β-carotene
- 6. Type of inflorescence: Umbel/Racemose/Cymose/Perianth
- 7. Type of pollination: Cross/Self/Often-cross
- 8. Mode of pollination: Water/Air/Insects
- 9. Anthesis time:
- 10. Type of fruit:

# Chapter-10

# Seed Production in Temperate Root Vegetable Crops

#### Introduction:

Radish (*Raphanus sativus*), turnip (*Brassica rapa*) and carrot (*Daucus carota*) have varieties of both European and Asiatic types whereas all cultivars of beet (*Beta vulgaris*) are European or temperate. Hence their seeds can only be produced in the hills of India. Like temperate carrot, beet requires exposure to low temperatures for initiation of flower stalks. Being cross-pollinated due to one or the other reasons like self-incompatibility (radish, turnip and beet) and protandry (carrot), these root crops behave similarly as for as their methods of seed production are concerned.

#### European carrots, turnip and radishes:

Roots are raised by planting seeds in the month of September to December in temperate conditions and true-to-type roots are pulled out in late winter. The roots are selected on the basis of root shape, colour and other varietal characteristics. The leaves of the roots are chopped off and the stecklings are stored in 2 to 2½ months in trenches for vernalization in case of heavy snow conditions. In the month of February-March, the selected roots are replanted in the field. Flower shoots start emerging in April. Seed bearing umbels and shoots are harvested periodically from May to June. After curing and threshing, seeds are extracted and dried for subsequent processing and storage.

## Methods of seed production:

Both seed-to-seed and root-to-seed methods are employed for seed production in root crops. The former method is not followed unless the seed is of the highest quality. Replanting method (root-to-seed) is preferred for producing nucleus and breeder seeds.

#### A) Root-to-seed method:

When the roots are fully mature, the crop is harvested and true-to-type roots are selected. The selection and roguing are done on the basis of foliage characters and root characters both external and internal (colour, shape, size, flesh colour, core size, pungency, indistinct rings etc.). Undesirable roots are discarded. After selection of true-to-type roots, their tops and tips are cut and transplanted in a well prepared field. The stecklings may be given one-third top (shoot) cut and one-fourth to one-half root cut to obtain higher yield of better quality seeds. In case of turnip and beet, after selection the tap root and tops of the roots are trimmed taking care not to injure the crown and planted in a well prepared field.

#### Advantages

- i. Opportunity to discard all the undesirable roots
- ii. Smaller land requirement for root production
- iii. Production of high quality seed

#### Disadvantage

Requirement of extra man power at the time of selection and replanting

#### **B) Seed-to-seed method**

The seed-to-seed method differs from root-to-seed in that the roots are allowed to remain and produce seeds in the same location where they were initially raised from seed. This method is not common in the seed trade. For success in this method the only requirement is the sowing of seed of the highest quality to produce the roots to be left *in situ*.

## **Advantages**

- i. No extra expense on land, labour, replanting and storage of roots
- ii. Seed yields are generally high
- iii. Delayed sowing can be done

#### **Disadvantages**

- i. Lack of proper roguing and selection of true to type roots
- ii. Uneven distribution of plants for seed
- iii. Quality of seed produced is not of the highest order

#### Nucleus and breeder seed production of varieties:

The root-to-seed (replanting) method is used for nucleus and breeder seed production of root vegetable crops. If there are no isolation problems, the breeder seed can be produced in the open fields conditions otherwise on the safest side it is better to cover the crop of radish, turnip and carrot with isolation chamber of nylon or wire netting and introduce the honeybees at the flowering. In beet and crossable species the pollen is dispersed by the air and is very fine to pass through the mesh of nylon or wire netting, so these chambers should be lined with a fine muslin cloth to prevent the entry of foreign pollen coming from the contaminating plants of garden beet itself, sugar beet, swiss chard and spinach beet. In beet, beehive is not required in the isolation chambers since it is a wind pollinated crop. Often hand shaking of plants inside the chamber is useful practice for better seed setting.

#### Seed Source:

Nucleus seed plot forms the base population for nucleus seed production. Breeder seed is produced from the pooled nucleus seed.

#### **Isolation and pollination:**

All the root vegetables are cross pollinated owing to one or the other genetic mechanisms. Radish, carrot and beet do not cross with cruciferous crops belonging to genus *Brassica*. Turnips do not cross naturally with any other member of the sp. *Brassica oleracea* but cross easily with Chinese cabbage (*B. pekinensis* and *B. chinensis*) and *B. juncea*. Insects are the main agents to carry out pollination in these crops. Beet (*Beta vulgaris*) is pollinated by the agency of wind however, insects also visit the crop at flowering which may be a source of contamination. Garden beet crosses easily with sugar beet, spinach beet, swiss chard etc. Keeping in view the pollination behavior of these crops an isolation distance of 3000 m should be maintained for undergoing breeder seed production. If there are any chances of out-crossing it is better to cover the crop with isolation cages. Nucleus seed production should be carried out in the isolation chambers to ensure controlled pollination and genetic purity. A honey bee colony is introduced inside the chamber to effect pollination in case of carrot, radish and turnip whereas beet being wind pollinated, hand shaking of plants inside the chamber is practiced.

## **Roguing and Selection:**

Roguing and selection are the most important operations in seed production and must be carried out at specific stage of crop growth to remover off types and undesirable plants to maintain the purity of the variety. While selecting true to type roots, besides foliage characters, the various traits of roots are of utmost importance and examined thoroughly. A minimum of 3 inspections and rouging are necessary to maintain the purity of a variety.

- i) **Pre-uprooting stage**: Inspection at this stage can be made any time from root development stage but before pulling the roots. Rouging based on foliage characters is made as the roots cannot be examined at this stage. The amount of vegetative growth, size, shape, colour, and distinction of leaf-lamina are observed carefully. Diseased, unhealthy, weak or very vigorous plants should be discarded.
- ii) Uprooting and replanting stage: At this stage size, shape, colour, shoulder and typical shape of the roots (stumpy, semi-stumpy, tapering or globe) are examined critically. All the cracked, diseased hairy, deformed roots are discarded. Extremely large or small roots are not desirable. The internal characters viz., pithiness, colour of flesh, core colour and size and presence/absence of blackish tissues in the core of the roots can be observed when stecklings are prepared for replanting.
- iii) Bolting and pre-flowering stage: Consideration is given to remove very early and late bolters. Plants showing poor growth due to disease infection should be rouged out. This inspection should be done before flowering to avoid contamination with undesirable plants. Volunteer plants growing here and there need to be removed before their flowering.

## **Practical Exercise**

- 1. Isolation distance for carrot and radish------
- 2. Steckling preparation is done during the month of ------
- 3. Selfing means-----
- 4. Seed harvesting month of carrot and radish is------
- 5. Roguing stages in carrot and radish are-----

# **Radish seed production cycle**



Fig-16. Radish Seed Production Cycle



Fig-17. Carrot Seed Production Cycle

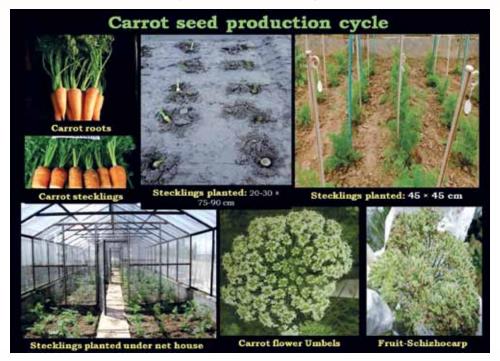


Fig-18. Seed Production cycle in turnip

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# N O T E S











