ICARDA Audiovisual Series

Hybridization Techniques in Faba Bean

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ICARDA
المركز الدولي للبحوث الزراعية في المناطق الجافة
International Center for Agricultural Research in the Dry Areas

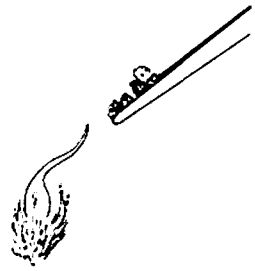
Established in 1977, the International Center for Agricultural Research in the Dry Areas (ICARDA) is one of several centers supported by the Consultative Group on International Agricultural Research -- the CGIAR -- a consortium of over 40 countries, international and regional organizations and private foundations.

ICARDA focuses its research efforts on areas with a dry summer and where precipitation in winter ranges from 200 to 600 mm. It has the world responsibility for research and training on barley, faba bean and lentil, and the regional responsibility -- in West Asia and North Africa -- for bread and durum wheat, chickpea and pasture and forage crops.

Much of ICARDA's research is carried out on a 948-hectare farm at Tel Hadya, Syria -- 30 kilometers south of Aleppo. ICARDA's activities extend throughout West Asia and North Africa. ICARDA has grouped its outreach activities into regional programs -- each with access to several research sites in the national agricultural research systems.

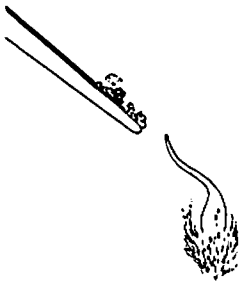
The results of research are transferred through ICARDA's cooperation with national and regional research institutions, with universities and ministries of agriculture. A variety of training programs are offered ranging from general group courses to advanced research opportunities for individuals. These efforts are supported by seminars, publications and by specialized information services.

For additional information on ICARDA's activities, write to ICARDA, P.O. Box 5466, Aleppo, Syria.



INTRODUCTION

Throughout West Asia and North Africa faba bean yields are low and unstable. But yields can be improved through controlled hybridization between known parents. By using hybridization techniques, breeders can develop new varieties by incorporating desirable attributes of one parent into another. These varieties may produce high and stable yields, be tolerant to stress and have high quality seed.



PURPOSE

This slide set is designed to present background information on faba bean hybridization and briefly outlines the crossing technique.

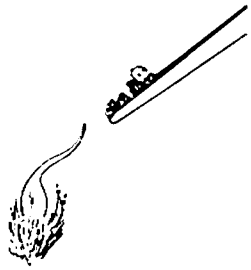
OBJECTIVES

After viewing this module, you should be able to:

1. Recall the scope of hybridization in faba bean breeding, including (a) taxonomic classification, (b) object of hybridization, and (c) steps in hybridization.
2. Describe the crossing block layout for raising parents and making crosses, that is (a) layout of the crossing block, and (b) management practices.
3. Discuss floral characteristics, mainly the flower structure of faba bean.
4. Explain emasculation and pollination and identify appropriate stages in flower development to effect the two processes.
5. Recall eight steps in making artificial crosses which consist of steps in emasculation and steps in pollination.
6. Discuss harvesting and storage of the seed.

USING THE AUDIOVISUAL

This audiovisual training module can be used either as a self-study tool for individual learners or as a presentation to a group. There are several review questions in the audiovisual, if you like, stop the audiotape and discuss these questions. The slide set should be used in conjunction with a field practice session on crossing faba bean. It is not a substitute for individual practice of what is a highly skilled and difficult task. Consequently, the slide set should be used when faba bean flowers are readily available for practice immediately after showing the slide set. The value of the slide set is likely to be completely lost without the integration of theory with practice.



SCRIPT

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This presentation contains basic information on the structure and developmental stages of faba bean and the skills necessary for hybridizing. It will prepare you for hybridization work in faba bean.

3

Hybridization is an important tool for the genetic improvement of crop plants. Acquiring skill in hybridization in faba bean is essential for breeders in improving the faba bean plant.

4

After completing this module, you should be able to:

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5

2. Describe the crossing block layout for raising parents and making crosses, that is (a) layout of the crossing block, and (b) management practices.
3. Discuss floral characteristics, mainly the flower structure of faba bean.

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4. Explain emasculation and pollination and identify appropriate stages in flower development to effect the two processes.

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5. Recall eight steps in making artificial crosses which consist of steps in emasculation and steps in pollination.
6. Discuss harvesting and storage of the seed.

8

Let's begin our discussion with a brief look at faba bean taxonomy. Faba bean is one of 120 species in the genus *Vicia* of the family Leguminosae.

9

Faba bean -- whose scientific name is *Vicia faba* -- has its center of origin in West Asia.

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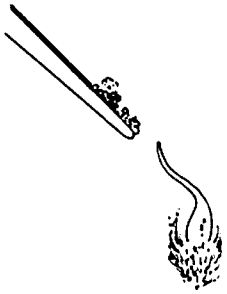
Vicia faba is the most isolated of the *Vicia* species. Breeders have not been successful in making crosses between *Vicia faba* and any other species. This restricts its gene pool to itself.



11 All *Vicia faba* varieties are diploid with 2n = 12. The species has been divided into varieties called minor, equina, and major.

12 The names of the three varieties reflect their seed size. The major variety is large-seeded, the equina is medium-seeded, and the minor is small seeded. Some scientists regard paucijuga -- an Indian-type faba bean with very small seeds -- as the fourth variety.

13 Gene flow between *Vicia faba* and other *Vicia* species is not possible, but crosses between all the botanical varieties of *Vicia faba* are possible.

14 Throughout West Asia and North Africa faba bean yields are low and unstable. But yields can be improved through controlled hybridization between known parents.

15 Crosses between *Vicia faba* varieties -- both naturally and artificially -- have provided breeders with a wide variety of genetically-different parents.

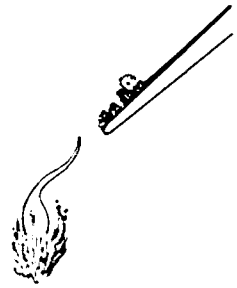
16 Through hybridization, breeders develop new cultivars by incorporating desirable attributes of one parent into another. These cultivars may produce high and stable yields, be tolerant to stress and have high quality seed.

17 Hybridization consists of three major tasks: 1. Making crosses, 2. Managing the hybrid population and 3. Testing and releasing new cultivars.

18 In this module we will focus on the practical skills needed to make successful crosses.

19 Skill in making crosses requires an understanding of the raising of parent material and flower morphology.

20 The parent material can be grown in the field, greenhouse or growth chamber. You will need to adopt appropriate management practices to ensure good flowering and seed set. This requires a layout to facilitate crossing known as a crossing block.



21 The most common layout of the crossing blocks for faba bean is a three-row crossing block.

22 In the three-row crossing block, a male parent row is planted between two female parent rows. The two female rows in this system can be the same or different.

23 For crossing blocks in the field, the plants are seeded at 15 to 30 centimeter spacings in rows 40 to 80 centimeters apart.

24 Proper agronomic practices are necessary for raising healthy parents. This will particularly include an adequate nutrient and water supply and appropriate plant protection measures.

25 The success of a crossing program depends to a great extent on the synchronization of flowering dates of the intended parents. You can achieve this by planting parents at 10-day intervals.

26 The choice of parents is of prime importance in any crossing program.

27 When you select parents consider the complementary desired traits and the possibility of retention of the desired recombinant by the hybrid progeny.

28 Let's pause here and briefly review what we have covered so far. Please answer the following questions.

1. List the three main varieties of faba bean. Name the fourth variety also.

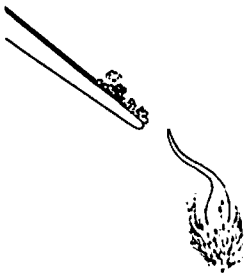
Stop the tape and write your answer and restart thereafter.

29 Some more questions:

2. Name three steps in hybridization.
3. Draw a three-row crossing block showing parental rows and plant spacing.

Stop the tape and write your answers. Restart thereafter.

30 Now compare your answers with the correct answers given in the next two slides.



31 Here are the correct answers:
1. (a) Minor, equina, and major; (b) Paucijuga.
2. The three steps in hybridization are: 1) making crosses, 2) handling hybrid population, and 3) testing and release of promising material.

32 Now for the last question. A three-row crossing block in the field is shown here. If you had problems, repeat the module.

33 Our next topic relates to the floral characteristics of faba bean. Knowledge about the structure of the flower and its development is important in making crosses.

34 The faba bean flower has a typical papilionaceous structure comprising of four main parts -- the calyx, the corolla, the androecium, and the gynoecium.

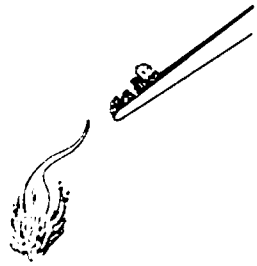
35 The calyx is a five-toothed structure made of five sepals. It forms a tube enclosing the bud.

36 The corolla is irregular in shape and made up of five petals -- the standard, two wings and two lower petals that are united along their outer edge to form a keel. This slide shows the three types of petals and their arrangement.

37 The faba bean flower has 10 stamens. The stamens consist of the filament and anthers and are the main male organ of the flower. The filaments of nine stamens are united in a sheath which encloses the ovary. One stamen is free.

38 The gynoecium -- or the female part of the flower -- has a stigma, a style, and an ovary as the main female organs. The stigma is a swelling at the apex of the filiform tube known as the style. The ovary is the organ containing ovules which develop into seeds after fertilization.

39 Artificial crossing -- whether in the field or greenhouse -- involves two major tasks: emasculation and pollination.



40 Emasculation is the removal of anthers from a flower before they dehisce. It prevents self pollination when preparing a female flower for crossing with other parents.

41 Timing is critical for successful emasculation. If it's done early, the removal of anthers is difficult and the ovary is likely to be damaged. And if it's carried out late, the self pollination would have already taken place.

42 The best indicator for selecting the timing of emasculation is the flower developmental stage. The slide shows the six development stages of a faba bean flower.

43 Anther dehiscence starts about half way through the second stage -- the pointed-bud stage.

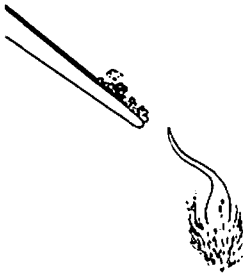
44 Thus the early-to-mid pointed-bud stage is the most appropriate for emasculating a faba bean flower. Emasculation done 12 hours before dehiscence of the anthers is preferred.

45 After you emasculate the flower, pollinate it by transferring male pollen from the desired parent to the emasculated flower. If emasculation is timed correctly, pollination can be carried out immediately.

46 The flower to be used as the male parent should be the one in which the dehiscence has just started. The late pointed-bud stage or freshly-opened flower is normally appropriate for faba bean pollination.

47 With this background information you will be able to make crosses. Before proceeding, let's review what we've covered so far. Draw the sketch of a papilionaceous flower and label the different parts. Stop the tape to draw the diagram. Restart when you have finished.

48 Compare your sketch with the one given in this slide. If you had difficulty, redraw the sketch.



49 You have now covered the preliminaries. Let's move to our main topic on making crosses.

50 To make crosses you will need tools such as a fine forceps or mounted needle, small tags or colored threads, and alcohol.

51 We will do the crossing in steps. Our first step will be to select the proper bud for emasculation.

52 Some useful tips are: 1. Select the inflorescence nearest to the ground. 2. Select the flower on the inflorescence nearest to the stem, 3. Use the first or second bud on the inflorescence, 4. Use a bud in the pointed-bud stage.

53 Step 2: Remove the sepals. Hold the bud between your thumb and forefinger and tear off two to three sepals covering the keel.

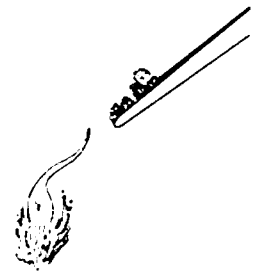
54 Step 3: Expose the staminal column. Insert the points of the forceps by sideways movement through the standard and wing petals. Release the pressure on the forceps gently and fold back the standard and wing petals.

55 Then slit open the keel petals with the help of the forceps along the suture of the two keel petals. Press the calyx at the base gently. You can now see the staminal column.

56 Step 4: Remove the 10 anthers. Be careful not to squeeze out pollen from the anther while removing the filaments. Avoid damaging the style. It's delicate and may break at the bend.

57 Step 5: Rearrange the wing and the standard petals to cover the keel. Then label the emasculated bud.

58 The emasculated flower is now ready for pollination. You can pollinate immediately. However, it's better to cross-pollinate in the afternoon if the bud was emasculated in the morning. A few hours gap is helpful.



59 Step 6: The act of hybridization consists of transferring male pollen to the emasculated bud. So, select an appropriate flower, expose the stamens as before, and collect the sticky whitish-grey pollen at the end of the forceps or mounted needle.

60 Step 7: Smear the pollen on the stigma of the emasculated bud. The emasculated bud already has a slit in the keel petal. Press down the wing and keel petals to expose the stigma. Now, apply ample pollen to the surface of the stigma with forceps.

61 Clean the forceps to be used for pollinating by dipping them into 99 percent ethyl alcohol before changing pollen donor.

62 Step 8: Close the keel petal after transferring the pollen. This step is important to maintain humidity within the flower.

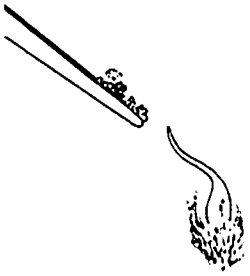
63 Remove all the other buds and flowers from the inflorescence carrying the emasculated. This encourages the development of the crossed bud.

64 After crossing, proper labelling of the crossed bud is essential. Identify crosses either by colored thread or tags. Often a single tagging of an inflorescence is sufficient to identify the emasculated flower.

65 Outcrossing caused by visiting bees causes problems in faba bean hybridization. Control this by using bee-proof cages or bagging the plant or stem before the onset of flowering. Remove the bags after pod initiation.

66 For caging, use a three-millimeter mesh net to keep out pollinating bees. The supporting frame could be galvanized iron pipe or any other material. The cages should be two meters in height and dismantable. The cages are fixed before the onset of flowering.

67 The success of crosses depends on the environment and the skill of the person making the crosses. Under good conditions a success rate of 50 percent in the field and 50 to 70 percent in the green-house is quite possible.



68 A check on correctly hybridized seed is possible only by growing the plant in the F_1 generation. Some of the inherited genetic markers for successful crosses are: 1. Dominant black hilum, 2. Dominant black testa, 3. Flower color.

69 You may now like to review the information. Answer the following questions.

70 1. Define emasculation and pollination.
2. Name flower development stages for emasculation.
Stop the tape and write your answers. Start thereafter.

71 One more question. List the five main steps in emasculating and three steps in pollinating a flower. Stop the tape to write your answer. Start thereafter.

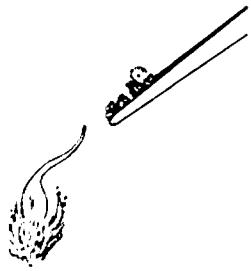
72 Let's look at your answers. 1. Emasculation is the removal of anthers before dehiscence. The transfer of male pollen to the stigma of female part is called pollination. 2. Early-to-mid-pointed-bud stage.

73 3. The five steps in emasculation are: select proper bud, remove sepals, expose staminal column, remove 10 stamens and rearrange petals. The three steps in pollination are: obtain pollen, smear pollen on emasculated bud and rearrange petals. Repeat the module in case of difficulty.

74 Now we proceed to harvesting and storing of the seed. After crossing, the plants are grown with appropriate care. Once pods have developed to a length exceeding three to 3.5 centimeters, they are likely to grow to maturity. Unsuccessful crosses generally abort. The pods of the crossed flowers are harvested and thrashed by hand.

75 Harvested seeds need to be stored properly to keep their viability intact.

76 Breeders hold the key to major improvements in faba bean in the future. And by using hybridization techniques, breeders can develop new faba bean cultivars that are high yielding, early maturing, winter hardy, and resistant to disease.



FABA BEAN GERMPLASM COLLECTIONS

A wide range of genetic diversity already exists within collections of faba bean. Breeders rely heavily on germplasm exploration, collection, evaluation and conservation activities. The major faba bean germplasm collection maintained for breeding is located in Syria. ICARDA is conducting research on barley and distributes germplasm on request.

International Center for Agricultural Research in the Dry Areas (ICARDA)
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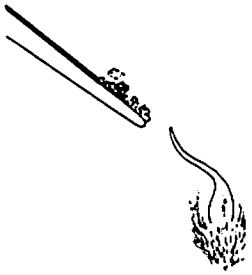
ICARDA maintains a collection of over 4000 faba bean accessions.



GLOSSARY

Androecium: the aggregate of microsporophylls in the flower of a seed plant.
Anther: the pollen-bearing portion of the stamen.
Artificial crossing: transferring the male pollen of one parent to the stigma of the female parent.

Bract: a specialized leaf from whose axil a flower or floral axis arises.

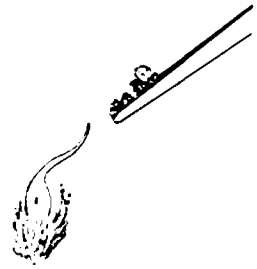
Calyx: the outer verticil of usually green leaves of a flower, the sepals.
Chromosome: one of rod- or thread-like structures, ordinarily definite in number in the cells of a given species, occurring in the nucleus of plant or animal cells, carrying genetics material.
Cleistogamy: pollination and fertilization in an unopened flower bud.
Corolla: the group of petals in a flower.
Cultivar: a variety of plants which are closely related and have similar characteristics to one another.

Dehiscence: splitting open of a fruiting structure or anther.
Diploid: an organism or cell with two sets of chromosomes (2n).
Dominant genes: genes which express themselves at the expense of the other alleles.

Emasculation: the process of removing male pollen from a flower before it falls to the stigma.

F_1, F_2, etc.: symbols used to designate the first filial generation, the second filial generation, etc. after a cross.
Fertilization: the fusion of a male with a female gamete to form the zygote; **self-fertilization** is the fertilization of an egg by a pollen grain from the same plant, also called selfing; **cross fertilization** is the fertilization between gametes produced by separate individuals of different kinds.

Gamete: the mature male or female reproductive cell. The male gamete is the pollen grain and the female gamete is the egg.
Gene: a unit of inheritance located on a chromosome. Genes control the expression of characters either individually or in combination.
Genotype: the genetic constitution of a plant.
Genus: a group of similar species of plants or other organisms.



Glabrous: having a surface without hairs.
Gynoecium: the female part of a flower containing the stigma, style and ovary.

Haploid: a cell or an organism having a single set (genome) of chromosomes in a cell or an individual.
Hermaphrodite: a plant or animal containing both male and female reproductive organs.
Heterozygous: having unlike alleles at one or more corresponding loci (opposite of homozygous).
Homozygous: having like genes at corresponding loci on homologous chromosomes.
Hybrid: the progeny of two homozygous parents, which differ by one or more genes.
Hybridization: the crossing of one plant with another. Crossing between plants of the same species is called **intra-specific hybridization** and crossing between different species is called **inter-specific hybridization.**

Inbreeding: selfing or crossing between closely-related plants for one or more generations.
Internode: the part of the stem between two nodes.
Interrow arrangement of male and female: in a crossing block, alternating a male parent row with a female parent row.

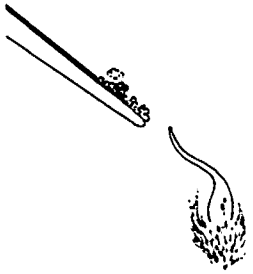
Keel petals: the innermost petals of a flower surrounding the androecium and gynoecium.

Mass selection: a system of breeding in which seed from individuals selected on the basis of phenotype is composited and used in the next generation.
Micropyles: a small opening in the seed cover through which pollen penetrates to the embryo sac.

Node: the enlarged portion of the stem from which the leaves arise and where the buds originate.

Out-breeding: crossing between two plants.
Ovary: the enlarged basal portion of the pistil containing the ovule. The structure of the ovary becomes the fruit at maturity.

Papilionaceous: a flower that is hermaphroditic, has a tubular and five-toothed calyx, and ten filaments bearing anthers.

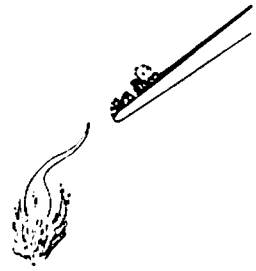


Pistil: the seed-bearing organ in the flower composed of the ovary, the style and the stigma.
Plant density: number of plants per unit area.
Pollen: the male gamete of a plant which is produced in the anthers.
Pollen germination: initiation of growth of the pollen on the stigma.
Pollen shedding: distribution of pollen.
Pollen penetration: penetration of pollen into the stigma.
Pollination: the transfer of pollen from the anther to the stigma; **self-pollination** is the transfer of pollen from an anther to the stigma of the same flower or another flower on the same plant, or within a cultivar, **cross-pollination** is the transfer of pollen from an anther on one plant to a stigma in a flower on a different plant.
Protogynous: when the stigma is receptive before the pollen is shed.
Pure line: a strain in which all members have descended by self-fertilization from a single homozygous individual. A pure line is genetically pure (homozygous).

Recombination: the combining of characters in an offspring in a different combination from that in the parents.

Staggered planting: successive sowing of seeds.
Stamens: the pollen-bearing organ of a flower which consists of an anther and a filament.
Staminal column: the structure bearing the pollen.
Standard petals: the uppermost petal.
Stigma: the tip of the female style to which pollen adhere during pollination.
Style: the slender portion of the pistil between the ovary and the stigma.

Zygote: the cell resulting from the fusion of the gametes.



FURTHER READING

Bond, D.A., D.A. Lawes, and M.H. Poulsen.
1980. "Broadbean (Faba Bean)." In *Hybridization of Crop Plants*, edited by W.R. Fehr and H.H. Hadley, pp. 203-213. Madison, WI, USA: American Society of Agronomy and Crop Science Society of America.

NOTES

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