ICARDA Audiovisual Series

Hybridization Techniques in Chickpea

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

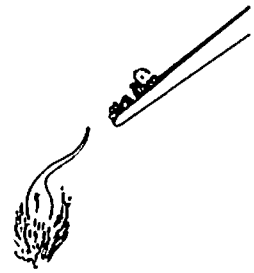
Established in 1977, the International Center for Agricultural Research in the Dry Areas (ICARDA) is one of several centers supported by the Consultative Group on International Agricultural Research -- the CGIAR -- a consortium of over 40 countries, international and regional organizations and private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

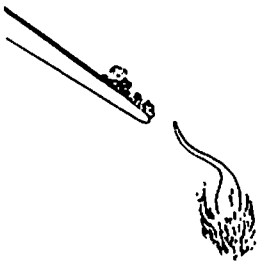
For additional information on ICARDA's activities, write to ICARDA, P.O. Box 5466, Aleppo, Syria.



INTRODUCTION

Chickpea is the second most important pulse crop in the world. It is an important source of protein and plays a significant role in the farming systems of many countries. Chickpea has been cultivated for at least 7,000 years, and man, consciously or unconsciously, has selected improved types as he has done with other crops.

No recorded history is available to indicate when, where, or how chickpea breeding efforts began. Now, breeders are increasingly using hybridization techniques to create the genetic variability they need to select improved cultivars.



PURPOSE

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

OBJECTIVES

After viewing this presentation, you should be able to:

1. Define hybridization and list three steps involved in hybridization.
2. Diagram and describe the four main parts of a papilionaceous flower including the calyx, corolla, androecium, and gynoecium.
3. List and relate the six main stages in the development of a chickpea flower such as, closed-bud, hooded-bud, half-open flower, fully-open flower, and fading-flower.
4. Identify the two major tasks in making crosses and describe the procedure for raising parental material.
5. Discuss the two main steps in artificial crossing: emasculation and pollination.

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 chickpea. 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 chickpea 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

1 (ICARDA logo -- no text)

2 This module contains basic information on the structure and developmental stages of chickpea flowers and describes the steps in making crosses. It will prepare you for hybridization work in chickpea.

3 Breeders are increasingly using hybridization techniques to create the genetic variability from which they can select improved cultivars. Knowledge and skill in hybridization techniques are important to everyone involved in crop improvement work.

4 After completing this module you should be able to:

1. Define hybridization and list three steps involved in hybridization.

5 2. Diagram and describe the four main parts of a papilionaceous flower including the calyx, corolla, androecium and gynoecium.

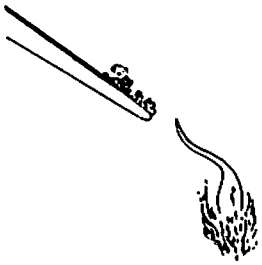
6 3. List and relate the six main stages in the development of a chickpea flower such as, closed-bud, hooded-bud, half-open flower, fully-open flower, and fading-flower.

7 4. Identify the two major tasks in making crosses and describe the procedure for raising parental material.

8 5. Discuss the two main steps in artificial crossing: emasculation and pollination.

9 Chickpea belongs to the genus *Cicer* -- a genus with 43 species of which 42 are wild and one -- the chickpea -- is cultivated. Crosses are possible between the cultivated species and with only two of the 42 wild species.

10 Chickpea has the specific name *Cicer arietinum*. The word 'arietinum' is derived from the Latin word 'aries' which means a ram's head. The shape of the chickpea seed resembles a ram's head.



11 Chickpea is divided into the kabuli and desi groups. The kabuli chickpea has large beige-colored seeds shaped like a ram's head. The seeds of desi chickpea are angular, small and multi-colored.

12 Chickpea is the most important food legume in West Asia and North Africa and occupies an area of 1.7 million hectares. However, yields are low when compared to the potential of the crop.

13 Breeders in the region are striving to improve chickpea productivity by using a number of breeding methods.

14 The simplest and easiest-to-adopt method would be mass and pure line selection within germplasm accessions or landraces.

15 In mass selection, a group of similarly-appearing superior plants are harvested and the seed is composited to grow the next generation.

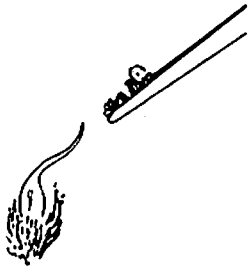
16 The pure line selection is similar to mass selection but it focuses on selecting from a homogeneous group of plants descending from a single homozygous individual.

17 Although, mass and pure line selection methods are easy, they offer only limited potential for increasing yields. To make greater advances, it's usually necessary to introduce new desired genes into existing landraces or cultivars.

18 This is especially true for chickpea which is almost 100 percent self-fertilized. So there's little possibility of new gene recombination in nature.

19 Accordingly, we have to look for some method other than mass and pure line selections -- and this method should have gene recombination capability. The breeding technique most widely used to generate additional genetic variability is hybridization.

20 Hybridization is the crossing of two or more genetically-different parents from the same, or different, species. The progeny of the cross will have genes from both parents.



21 There are three major steps in hybridization: 1. Making crosses, 2. Handling hybrid population, and 3. Testing and releasing the new cultivars.

22 By using hybridization techniques, breeders have successfully increased and stabilized crop yield, and improved quality.

23 You may also use hybridization to improve chickpea. So let's learn in detail about it. But before we go further let's review what you have learned so far.

24 Please answer the following questions:
1. How many species does genus *Cicer* have?
2. Are crosses possible between cultivated and wild species?
Stop the tape to write your answers. Restart thereafter.

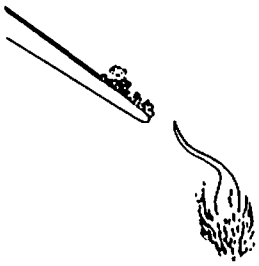
25 Some more questions:
3. Why can't mass and pure line selection methods lead to dramatic increase in yield?
4. Name three major steps in the hybridization process.
Stop the tape again to write your answers, restart thereafter.

26 Now compare your answers. The answers are:
Question 1: 43.
Question 2: Yes, with two only.
Question 3: Because new gene recombination is not possible with these methods.

27 Question 4: The three steps in hybridization are: 1. Making crosses, 2. Handling hybrid population, and 3. Testing and release of new cultivars.
Repeat the module if you had difficulty in answering.

28 The first step in hybridization is making the crosses. We'll discuss this in detail.

29 Before we discuss making crosses, let's review the flower structure of the chickpea.



30 The four main parts of the chickpea flower are: the calyx, the corolla, the androecium and the gynoecium.

31 While making crosses you will mainly be concerned with the androecium and gynoecium but to reach these two parts you have to work through the calyx and corolla as well.

32 Let's examine the four main parts individually. The calyx forms a tube and encloses the bud. It has five pointed lobes or sepals at the open end.

33 The corolla consists of five petals: one standard, two wing and two keel. The standard petal -- the largest and uppermost -- is broad and clawed. The keel petals are the innermost enclosing the androecium and gynoecium. Wing petals flank keel petals.

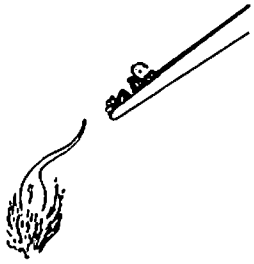
34 The androecium consists of 10 stamens; each stamen consists of a anther and a filament. The anthers store male pollen. Of the 10 stamens, nine are usually joined in a staminal column surrounding the pistil and one is free.

35 The gynoecium has three major parts: the stigma, the style and the ovary. The stigma is located at the end of the style. The ovary contains the ovules which develop into seed after fertilization.

36 Knowing the main parts of a flower -- particularly the androecium and gynoecium -- is essential for understanding how the flower produces seeds.

37 Now let's learn three key words: emasculation, pollination, and fertilization. We'll come across these words often. They're important for understanding how the seed is formed in the flower.

38 Emasculation is the removal of anthers before dehiscence. Pollination is the transfer of pollen from the male parent to the stigma of the female parent. The union of the male and female gametes in the ovule is known as fertilization.



39 If pollen falls on the stigma of the same flower the process is called self-pollination. But if pollen produced in the flower of one cultivar is transferred to the stigma of another cultivar, the process is called cross-pollination. Chickpea is self-pollinated.

40 In order to pick the right time for making the cross, you should understand the stages in development of a chickpea flower.

41 Scientists recognize six distinct stages in flower development for chickpea: 1. Closed-bud, 2. Pointed-bud, 3. Hooded-bud, 4. Half-open flower, 5. Fully-open flower, and 6. Fading flower.

42 The first three development stages are shown here. During the first two stages, the stigma is not receptive to pollen. At the hooded-bud stage, the stigma of a chickpea flower becomes receptive to pollen, but the pollen is not yet fully developed in the anther.

43 The last three stages of flower development are shown in this slide. By the time a chickpea flower attains the half-open stage, the filaments have elongated and the anthers are mature. Pollination generally takes place during this stage. Fertilization occurs in the fifth stage.

44 This means that to make artificial crosses we should be ready for emasculation by the third stage of flower development. The fourth stage is the most suitable for collecting pollen to apply on the emasculated bud. However, pollen can also be collected in the fifth stage.

45 Before we discuss crossing techniques, let's review what you have learned under objectives two and three. This will give you feedback and also prepare you better for making crosses.

46 Please answer these two questions:
1. Draw a chickpea flower and identify the important parts, and
2. Name six stages in flower development. Stop the tape to write your answers; restart when you have finished.

47 Compare your drawing with this diagram of a chickpea flower.

48 This slide lists the six stages in flower development. If you had difficulty in answering the two questions, you can replay the relevant portion of the module.

49 Let's proceed to the central topic of the module: crossing techniques. The process of making crosses involves two tasks: 1. Raising parents and 2. Crossing the parents.

50 With respect to raising parents, we must keep in mind that the goal of 'crossing' is new gene recombination. Therefore, the most critical factor in the process of making crosses is the choice of parents.

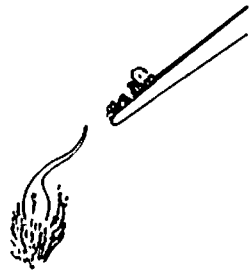
51 When choosing parents for hybridization you should consider these four factors: 1. Eco-geographical diversity, 2. Presumed complementary characteristics, 3. Biotic and abiotic stress resistance and 4. Seed characteristics.

52 After you choose the parent materials, grow them in the field or greenhouse. You must follow proper cultural practices to obtain good flowering and seed set.

53 For all crossing block nurseries in the field, a minimum spacing of 45 centimeters between rows and 10 centimeters between plants within rows is recommended.

54 Further, plant a male parent row with a female parent row, thereby making a two-row crossing block with one row vacant. Label the two rows for one cross. This system provides the opportunity of using either parent as male or female.

55 In most crossing-block layouts, provision is made for planting parents on different dates in close proximity. This helps in synchronizing the flowering of parents. You can also achieve synchronization by removing the flower from early parents.



56 Growing of parents at different dates also extends the period of crossing. In chickpea, two planting dates are adequate.

57 The proper control of diseases and insects is essential in the crossing-block growing parents. Control these by using appropriate insecticide and fungicide.

58 You can also raise the chickpea parents for crossing in a controlled environment. You can obtain satisfactory flowering of most cultivars in the greenhouse within a temperature range of 17 to 25 degrees Celsius. The use of extended light hastens flowering.

59 We have finished our lesson on raising parent material for crossing; now we will discuss the techniques of artificial crossing.

60 Artificial crossing, whether in the field or greenhouse, involves two major practical tasks: emasculation and pollination.

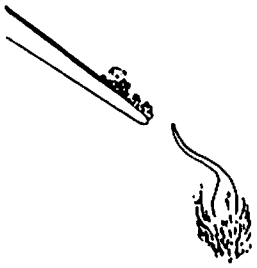
61 The easiest method for learning the techniques of crossing is by task analysis. We will break the technique into a series of tasks and each task into a series of steps. Let's begin with the task of emasculation.

62 Step 1: Collect the tools needed for crossing such as fine forceps or mounted needle, alcohol for sterilizing the forceps, and tags or threads. You may also want to use a hand lens, scissors, and small matting.

63 Step 2: Select a large flower bud in the hooded-bud flower stage for emasculation. As you'll recall, we discussed this point earlier.

64 Step 3: Hold the bud tightly at its base, using the thumb and first finger of your left hand, and cut off the front sepals.

65 Step 4: Slit the bud open along the dorsal surface using the fine forceps or a mounted needle.



66 Step 5: Press the calyx at the base of the slit to open the flower and expose the anthers. You can also achieve this by gently pushing the keel petal downward with forceps or the needle.

67 Step 6: Remove the anthers using the forceps or needle. The process of emasculation is now complete. Tie a colored thread around the pedicel below the emasculated flower for identification.

68 Now get ready for pollination. Immediate pollination is effective. But it can also be done successfully within four to 24 hours. Pollination is the transfer of pollen from the anther to the stigma.

69 In step 1 for pollination, select a half-open flower for pollen collection. Pollen at this stage is mature. It's yellow and sticky.

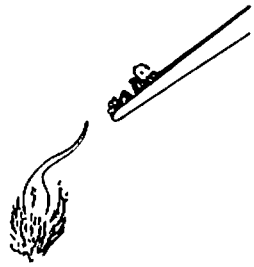
70 Step 2: Remove or fold back the petals of the selected flower so that the androecium is clearly visible. Now grasp the filaments with forceps, and pull them out gently or collect the pollen at the tip of the needle or forceps.

71 Step 3: Apply the collected pollen liberally on the stigma of the emasculated flower. Be careful not to damage the stigma, style or pedicel.

72 Here are some tips for successful crossing: 1. Begin crossing when pods appear on the plant; 2. Avoid using the last flower towards the tips of a branch for crossing;

73 3. Handle the flower gently. It's advisable to pull the branch bearing the flower nearer to you rather than to stretch the flower; and 4. Remove all non-crossed pods and flowers from the plant.

74 You have completed the major steps in pollination. If your crossing was successful, the pedicel will remain fresh and you will see chickpea pods forming within five to six days.



75 Your post-crossing activity will now comprise of: 1. Caring for the pod until it reaches maturity, 2. Harvesting, 3. Threshing, 4. Packaging the seed with proper cross name or number, and 5. Storing.

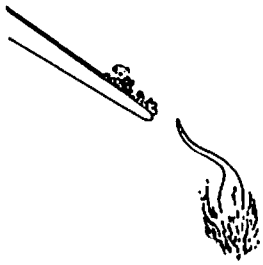
76 The success rate of crossing depends on the environment and the skill of the crosser. You can measure the success with a genetic marker in the F_1 seeds. In chickpea, dominance of the purple flowers over white is a good genetic marker. (The F_1 plants of crosses between tall and dwarf, large pod size and small pod size, and large leaf size and small leaf size are intermediate in expression of these characters.)

77 Let's review what we've covered.
1. Define emasculation and pollination, and
2. Name the flower development stages appropriate for emasculation and pollination.

Stop the tape to write your answers. Restart thereafter.

78 The answers are:
1. Emasculation is the removal of anthers before they dehisce on stigma. Pollination is the transfer of male pollen to the stigma.
2. Hooded-bud stage and half-open flower stage.

79 In order to meet the rising food demands, we must strive to develop chickpea cultivars that are more productive and stable. Your skill in hybridization techniques may be one step in this direction. Good luck.



CHICKPEA GERMPLASM COLLECTIONS

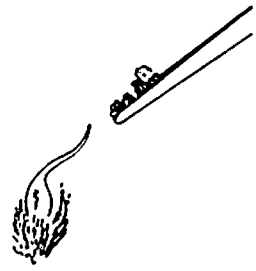
A wide range of genetic diversity already exists within collections of chickpea. Breeders rely heavily on germplasm exploration, collection, evaluation and conservation activities. The major chickpea germplasm collections maintained for breeding are located in Syria and India. Two international crop research institutes, ICRISAT in India and ICARDA in Syria, are conducting research on chickpea and distribute germplasm on request.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
Patancheru P.O.
Andhra Pradesh 502 324, India

> ICRISAT has assembled over 15,000 germplasm accessions of both desi and kabuli type chickpeas.

International Center for Agricultural Research in the Dry Areas (ICARDA)
P.O. Box 5466, Aleppo, Syria

> ICARDA maintains a collection of over 8,200 kabuli type 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.

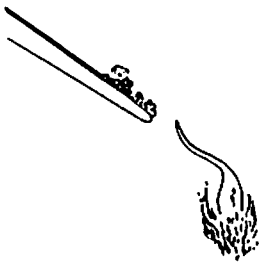
Calyx: the outer verticil of usually green leaves of a flower, the sepals.
Chromosome: one of rod- or thread-like structures, ordinarily definite in number in the cells of a given species, occurring in the nucleus of plant or animal cells, carrying genetics material.
Cleistogamy: pollination and fertilization 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,*** crossing between different species is called ***inter-specific hybridization***, and crossing between different genus is called ***inter-generic 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.

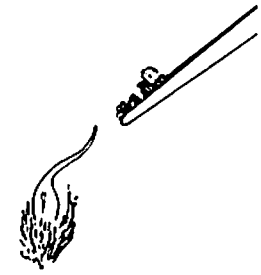
Keel petals: the innermost petals of a flower surrounding the androecium and gynoecium.

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.

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.

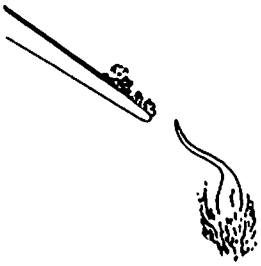


Plant density: number of plants per unit area.
Pollen: the male gamete of a plant which is produced in the anthers.
Pollen germination: initiation of growth of the pollen on the stigma.
Pollen shedding: dispersal of pollen from the anther.
Pollen penetration: penetration of pollen into the stigma.
Pollination: 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

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