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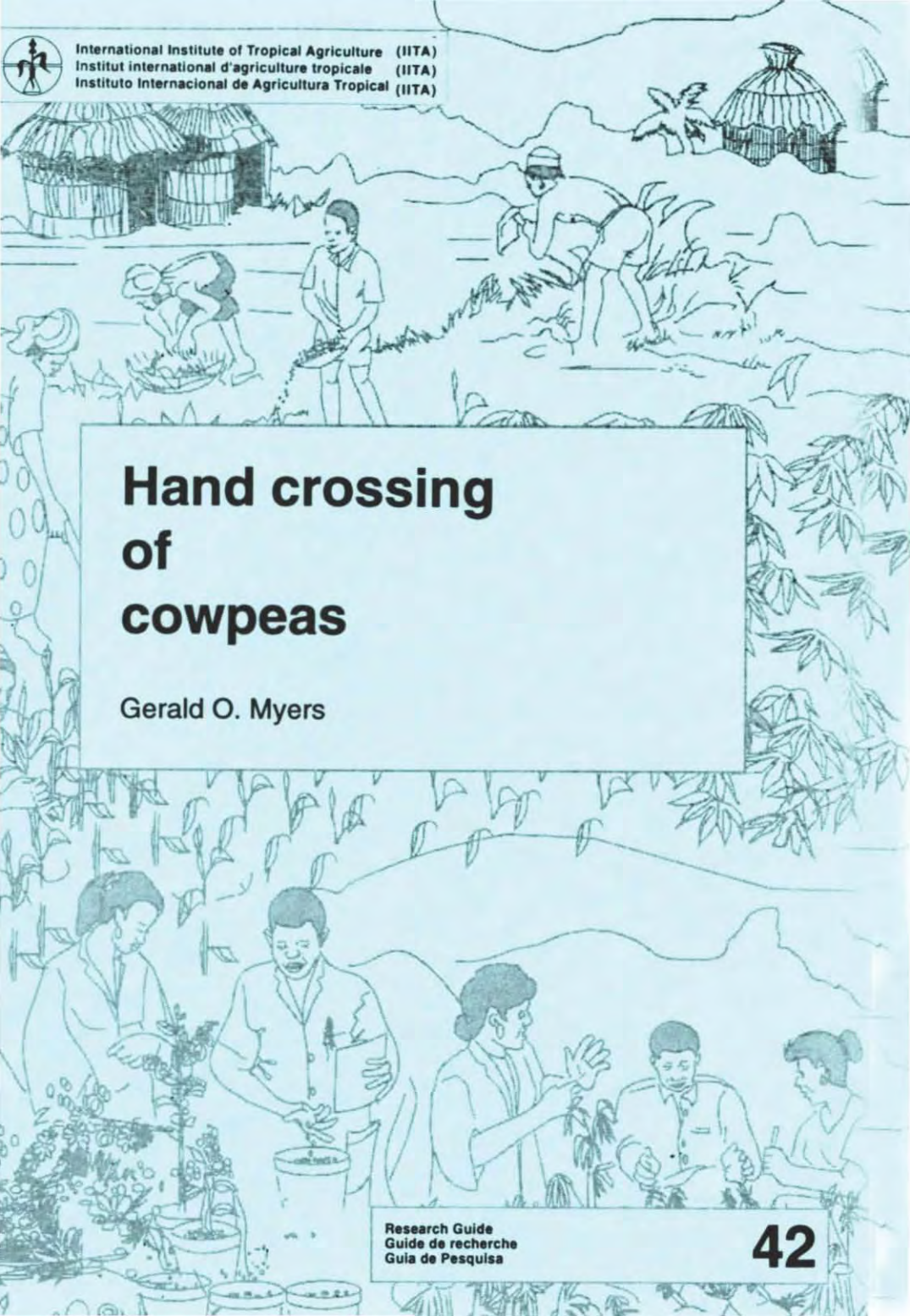

# Hand crossing of cowpeas

Gerald O. Myers

Research Guide
Guide de recherche
Guia de Pesquisa

42

IITA Research Guide 42

# Hand crossing of cowpeas

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**IITA Research Guides**

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# Hand crossing of cowpeas

**Objectives.** This guide is intended to enable you to:

- discuss the principles of cowpeas crossing;
- prepare the environment for crossing;
- emasculate and pollinate;
- maintain plants during post-pollination.

**Study materials**

- Cowpea flowers, pods, and seeds.
- Simple mesh house.
- Cowpea cuttings.
- Materials for emasculation and pollination.

**Practicals**

- Prepare a simple mesh house.
- Prepare cuttings for pollination.
- Emasculate and pollinate.

## Questions

1 What factors limit successful hand emasculation and pollination in grain legumes?
2 Why are cowpeas generally easier to cross than other grain legumes?
3 What are some difficulties in cowpea crossing?
4 How many seeds are produced per developed fruit?
5 What is the advantage of male sterile plants?
6 Why should you preferably carry out crossing in a mesh house or green house?
7 How can you construct a simple mesh house?
8 What problems can day-length sensitivity of *Vigna* germplasm create?
9 How can you delay flowering on a limited scale?
10 How can you start new plants?
11 Why is it desirable to remove the buds that develop in addition to the first bud on a raceme and peduncle?
12 How can you reduce abscission of crossed flowers?
13 When are flowers ready for emasculation?
14 What conditions are most successful for emasculation and pollination?
15 Describe the emasculation process.
16 How and for how long can you store pollen?
17 Describe the pollination process.
18 What portion of the style is receptive?
19 Why should you exclude insects from the plants during and immediately following pollination?
20 When can you check the success of a cross?
21 What rate of success can you expect?
22 When are pods ready for harvest?

# Hand crossing of cowpeas



**Abstract.** In genetic improvement of cowpea, one plant or variety rarely has every desirable characteristic. The recombination of desirable characteristics from different parental sources is achieved by crossing plants and the necessary cross pollinations. This document presents a method for the cross pollination of cowpea developed at IITA.

# 1 Principles of cowpea crossing

Several factors limit successful hand emasculation and pollination in grain legumes:

- flowers are small or have twisted keels and are difficult to manipulate;
- rate of abortion is high, particularly after mechanical manipulation of delicate floral organs;
- receptivity is selective; some genotypes are better seed parents than others;
- insect pollinators often contaminate flowers immediately after crossing and are difficult to exclude under field conditions.

Cowpeas are easier to cross than other grain legumes. Flowers are large and easy to manipulate, the keel is straight, beaked and not twisted. Cowpea flowers have few floral nodes per raceme, and tend to have a lower rate of abortion than many other species. Usually 8-12 seeds are produced per cross. Figures 1 and 2 show an inflorescence and parts of a cowpea flower.

Conventional crossing methods have several disadvantages:

- they are slow;
- insect contamination may occur, especially in the field;
- selective receptivity is a limiting factor;
- a high rate of abscission of manipulated flowers occurs under some conditions.

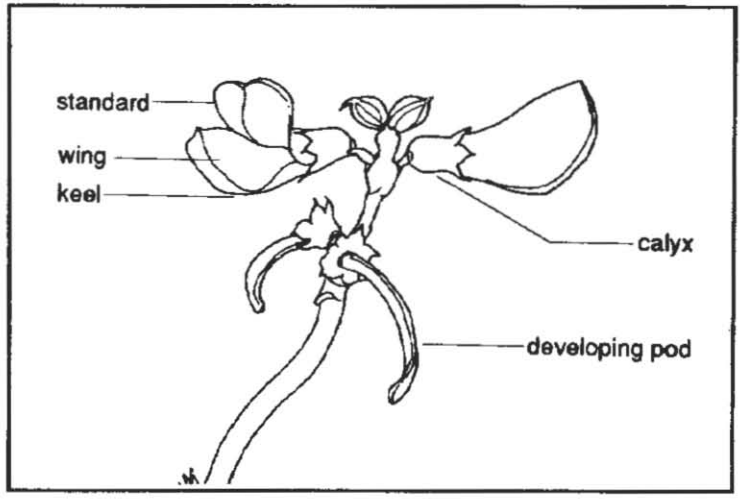
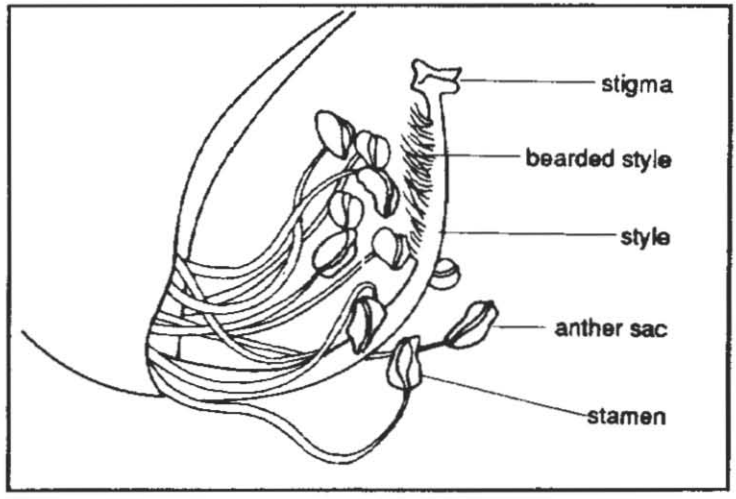



**Figure 1.** Cowpea inflorescence.

**Figure 2.** Parts of a cowpea flower.



Premature flower drops and bud abortion are greatest when the seed plant nears maturation, when the two gametes are incompatible, and when temperatures are high and humidities low.

IITA developed a rapid and effective method of hand emasculating and crossing cowpeas. It consists of removing the upper half of the petals starting with a partial cut opposite the stylar and staminal section. Following pollination with a freshly opened flower, leave the crossed bud uncovered.

The process of emasculation and pollination can be accomplished at the rate of one to two a minute with an average of only 10-20 % success. This means a minimum of 2-10 minutes per cross (5-10 individual flowers crossed) to assure success. However, synchronizing flowering under low temperatures and high humidity increases the success of hand crossing to 50 %.

Normally, at least 8-10 seeds are produced per developed fruit. Attempts on male sterile *($ms_2$ $ms_2$)* plants produce a much higher percentage of success (70-80 % on an average) and they require only pollination – no emasculation.

## 2 Crossing environment

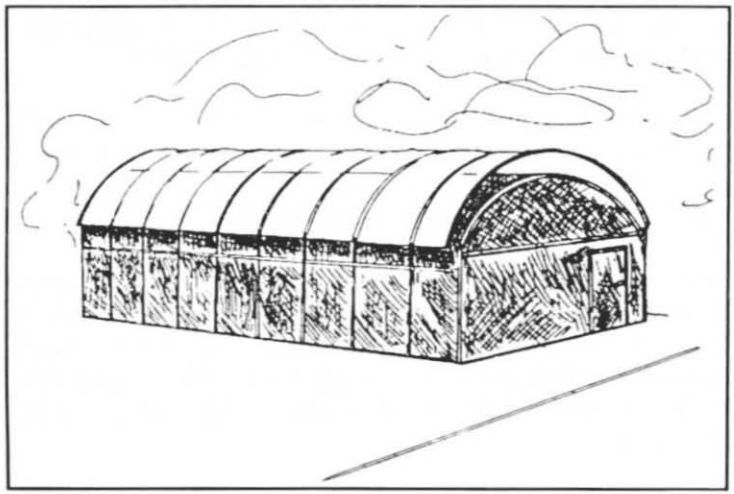
Carrying out the crossing in a mesh house or greenhouse is preferable, to:

- reduce the insect pollen vectors, most of the major pests (especially pod borers), and nearly all important diseases and nematodes;
- permit control of watering, staking, applying nutrients and regulating plant development;
- facilitate moving plants for easier manipulation during crossing operations.

However, potted plants are small and number of pods per plant is low.

In tropical climates, an expensive greenhouse is not essential for crossing purposes. Commonly available wire or nylon mesh over a simple wood frame serves well (Figure 3).

**Figure 3.** Mesh house.



Have the ceiling 2.0-2.5 m high to permit staking of spreading or climbing cowpea types, as slightly reduced light promotes the climbing tendency in many *Vigna* species as well as etiolation. Pitching the roof somewhat helps shed heavy rains, but, even with a flat roof the mesh disperses rain drops and minimizes splashing during heavy downpours.

A considerable proportion of *Vigna* germplasm is day-length sensitive. Inclusion of such types in the crossing program creates problems because of asynchronous flowering. At Ibadan (7° 30' North), planting from late August through early February results in good synchronization of flowering. However, substantial differences between genotypes do occur and staggered plantings about 1 week apart of early parents, particularly when used as females, is usually desirable.

A delay in flowering can be achieved on a limited scale by nipping off the developing flowers and fruits or by pruning the plant more severely. New plants are easily started by placing stem cuttings with a leaf in flats of coarse sand. Cover the flats with plastic to maintain high humidities around the developing plantlet.

Cuttings taken from plants during active fruiting will flower almost immediately on resuming active vegetative growth. Such cuttings are effective as pollen parents, though they are often poor pod setters.

The first developing buds on the plant tend to set pods more easily than later developing buds. Remove other buds on the same raceme and peduncle and leave only one for crossing purposes. This diverts all assimilates in the peduncle into one pod and avoids confusion in labeling.

When making interspecific crosses, a weak solution of silver thiosulfate (AgTS, approximately 4 mm Ag) sprayed on the whole plant till dripping, can reduce the abscission of crossed flowers. The AgTS solution, by interfering with ethylene oxidation, reduces ethylene-induced abscission of buds and flowers.

## 3 Emasculation

In all the flowers of *Vigna* species studied under Ibadan conditions, anthesis took place just before, or simultaneously with, the opening of papilionaceous corolla. Hence, flower buds destined to open the following morning are ready for emasculation. These buds have reached their maximum unopened size and have started to pale slightly from the deep rich green of earlier development.

Emasculation and pollination can be carried out at almost any time of the day. Under Ibadan conditions, emasculation and pollination in the late afternoon were highly successful. Apparently, cool nights provide better conditions for fertilization than the hotter daytimes.

Grasp the bud selected for emasculation firmly but gently in such a way as to avoid any stress at the fragile attachment of the bud and raceme. Cut about two-thirds the width of the unopened bud in the center of the bud starting from its straight edge. (The opposite, curved or hooked edge encloses the style and stamens, which must not be injured).

Use small finely pointed forceps, dissecting scissors, scalpels or even long thumbnails to make the cut (Figure 4). Then, grasp the upper portion of the folded petals by the thumb and index finger and gently tear off the cut segment (Figure 5). This leaves the upper portion of the style, stigma and stamens free and exposed to facilitate removal of the 10 anther sacs with scissors or forceps (Figure 6).

Dip the scissors or forceps in alcohol (75-95 %) between crosses, and do not touch the receptive green tipped stigmatic surface before pollinating. This emasculation procedure requires no longer than 15-25 seconds per flower.

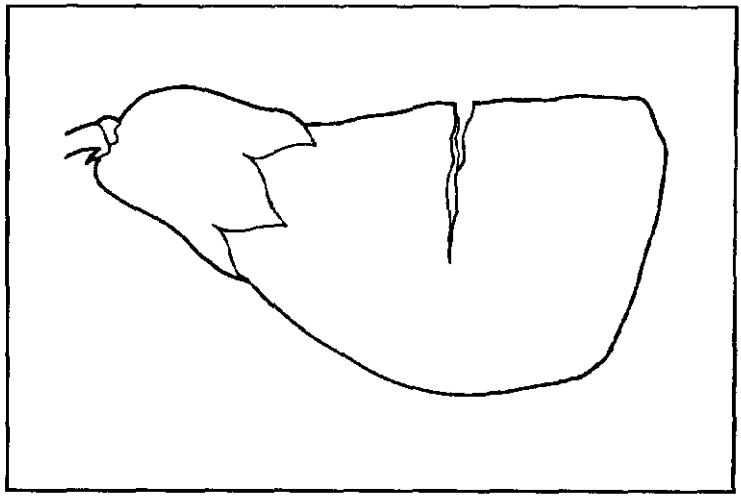
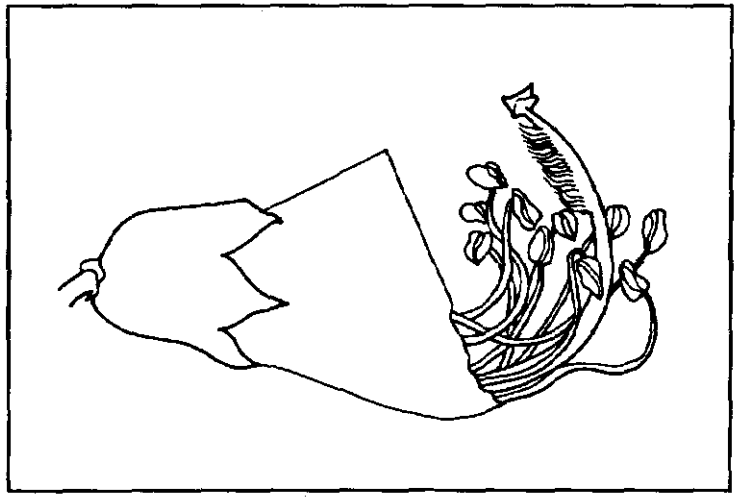


**Figure 4.** Cut about two-thirds the width of the unopened bud.

**Figure 5.** Gently tear off the cut segment.



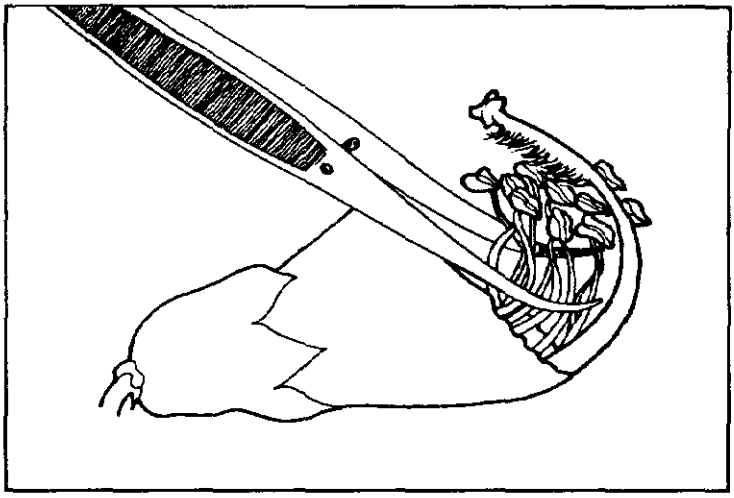
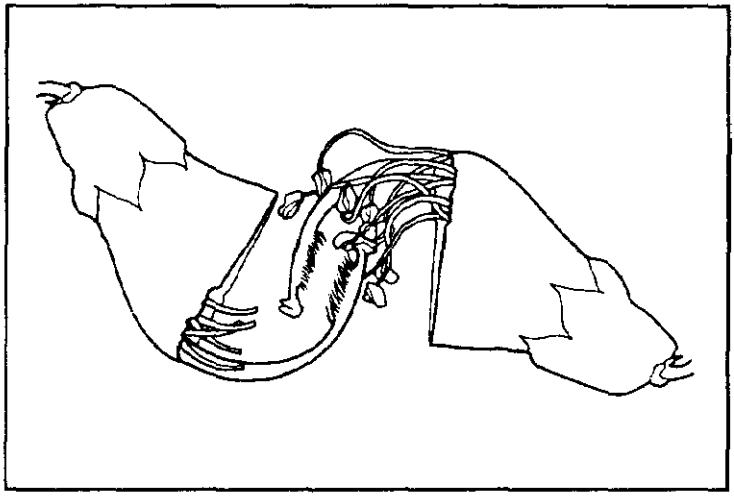


**Figure 6.** Remove all anther sacs.

**Figure 7.** Pollinate the emasculated bud.



## 4 Pollination

Ideally, cross the emasculated flower immediately, or pollinate it the following morning. If crossing is done in the greenhouse, collecting freshly opened male flowers in the morning is no problem and pollen remains viable for 12-15 hours after anthesis. Pollen to be used from several hours to one or two days later can be stored viably in a plastic bag (refrigerated).

Some genotypes are superior pollinators whereas others are better seed parents. Unless special genetic studies are to be made, use the most efficient parental donors. It is usually more convenient, and reduces risks of contamination, to remove the flowers of the male parents and use them to brush pollen on the stigmatic surface.

To expose the anther sacs, remove or slip downwards the innermost petals of mature open flowers. Use the mass of pollen on the hairy-necked style as a brush to deposit pollen grains on and immediately under the green circular disc-shaped stigma (Figure 7).

You may use one flower to pollinate 4 or 5 emasculated buds. Only the obliquely arranged disc-shaped stigma at the tip of the style is receptive (not the hairy portion beneath). Under IITA conditions, anthers usually dehisce before or around sunrise. Pollen grains are somewhat sticky and tend to form clumps that can even be seen with the naked eye or with the aid of slight magnification.

A small tag listing the cross and date is affixed to the raceme or peduncle beneath the pollinated bud. Do not allow hands, instruments and other foreign objects to touch the receptive portion of the stigma and the dehisced anther sacs.

In a well managed mesh house or greenhouse, you may leave the crossed flowers open and uncovered, as risk of contamination is minimal. However, crawling and flying insects must be excluded from the plants during and immediately following pollination. Even ants, which are often attracted to the nectaries, can cause self-pollination. To discourage thrips and other insects likely to carry pollen, apply an insecticide at regular intervals.

## 5 Post-pollination

Unfertilized flowers drop off within 24 hours after anthesis and the unfertilized ovary may remain attached for 48 hours after anthesis. You can make a good check on the success of a cross three days after anthesis.

Moderate temperature and increased humidity appear to increase the percentage of fruit setting in hand emasculated crosses, and results in seasonal variations in the success of hand crossing. At Ibadan, the percentage of successful crosses is higher during the cooler, humid months.

An automatic misting system can be installed over the crossing benches to maintain high humidity and lower temperatures during hot periods.

The rate of setting varies enormously with environmental conditions, genotype and manipulative technique. With the use of the IITA technique, an average 50 % success may be expected with some specific combinations as high as 90 %.

For unexplained reasons, male sterile ($ms_2$ $ms_2$) plants as seed parents seem to produce a high average set (75-95 %) under IITA greenhouse conditions throughout the year, in spite of variation in temperature and humidity. Moreover, male sterile plants appear to accept and hold hand-fertilized fruits more readily than hand crossed fertile plants, producing about as many crossed fruits per plant as unmanipulated fertile plants of similar type.

In crosses between fertile plants it is seldom practical to attempt more than two or three crosses per plant or 30-50 % of the self pollinated fruits normally set by that plant. However, immediate removal of self pollinated

fruits and timely harvest of crossed pods cause a flush of flowers that can be used for crossing – not with the similar frequency of success as the first flush.

Pods are ready for harvest 18-22 days after pollination at Ibadan.

## 6 Bibliography

Ladeinde, T.O.A.; Bliss, F.A. 1977. Identification of the bud stage for pollinating without emasculation in cowpea *(Vigna unguiculata* L. Walp). Nigerian Journal of Science 11: 183-195.

Ebong, U.U. 1972. Optimum time for artificial pollination in cowpea, *Vigna sinensis* Endl. Samaru Agricultural Newsletter 14: 31-35.

## 7 Suggestions for trainers

If you use this Research Guide in training ...

**Generally:**

- Distribute handouts (including this Research Guide) to trainees one or several days before your presentation, or distribute them at the end of the presentation.

- Do not distribute handouts at the beginning of a presentation, otherwise trainees will read instead of listen to you.

- Ask trainees not to take notes, but to pay full attention to the training activity. Assure them that your handouts (and this Research Guide) contain all relevant information.

- Keep your training activities practical. Reduce theory to the minimum that is necessary to understand the practical exercises.

- Use the questions on page 4 (or a selection of questions) for examinations (quizzes, periodical tests, and so on). Allow consultation of handouts and books during examinations.

- Promote interaction of trainees. Allow questions, but do not deviate from the subject.

- Respect the time allotted.

**Specifically:**

- Discuss with trainees experiences and problems of cowpea breeding and crossing (10 minutes).

- Present and discuss the content of this Research Guide, considering the study materials listed on page 3 (45 minutes). You may photocopy the illustrations of the Research Guide on transparencies for projection with an overhead projector.

  Have real samples of different cowpea varieties available for each trainee.

- Conduct the practicals suggested on page 3 in groups (3-4 trainees per group; 2 hours). Keep trainees busy. Make sure that each trainee has the opportunity to practice.

  Have resource persons available for each group. Prevent trainees from scattering around the field.

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