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## 4. PERSONAL AUTHORS (100)

Kachic, K. O.; Rawal, K.; Franckowiak, J.D.

## 5. CORPORATE AUTHORS (101)

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## 11. ABSTRACT (950)

## 12. DESCRIPTORS (920)

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Grain legumes

Plant breeding

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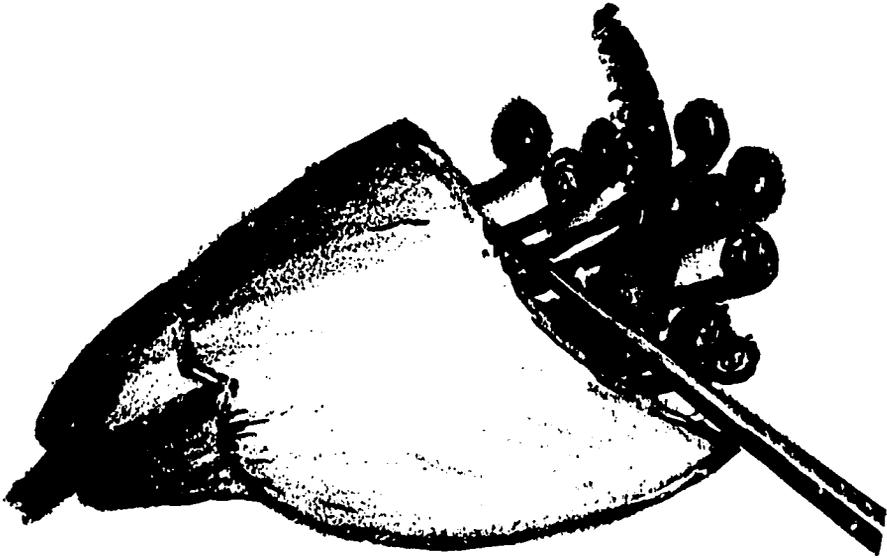
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# A RAPID METHOD OF HAND CROSSING COWPEAS

by  
K. O. Rachie,  
K. Rawal and  
J. D. Franckowiak



**Technical Bulletin No 2**

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**K. O. Richie, K. Rawal and J. D. Franckowiak were plant breeder and program leader, botanist and visiting plant breeder (8/73 - 12/73) respectively in IITA's Grain Legume Improvement Program.**

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# **A Rapid Method of Hand Crossing Cowpeas**

K. O. Rachie, K. Rawal and J. D. Franckowiak

INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE  
IBADAN, NIGERIA

## A Rapid Method of Hand Crossing Cowpeas

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

1. Flowers are small or have twisted keels and are difficult to manipulate.
2. A high rate of abortion normally occurs, particularly after mechanical manipulation of delicate floral organs.
3. There is selective receptivity; some genotypes make better seed parents than others.
4. Insect pollinators often contaminate flowers immediately after crossing and are difficult to exclude under field conditions.
5. Seeds produced per cross are few, ranging from one or two in soybeans to eight or ten in beans.

Cowpeas are generally easier to cross than other grain legumes. Cowpea flowers are large and easy to manipulate, the keel is straight, beaked and not twisted. There are only a few floral nodes per raceme, which tend to have a lower rate of abortion than many other species, and 8–12 seeds are usually produced per cross. Nevertheless, conventional crossing methods are slow, insect contamination does occur, especially in the field, selective receptivity is a limiting factor and a high rate of abscission of manipulated flowers occurs under some conditions. Premature flower drop 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.

A rapid and effective method of hand emasculating and crossing cowpeas was developed at IITA. This consists of removing the upper half of the petals starting with a partial cut opposite the styler and staminal section. Following pollination with a freshly opened flower the crossed bud remains uncovered. The process of emasculatation 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 to 10 minutes per cross (5–10 individual flowers crossed) to assure success. However, synchronizing flowering under low temperatures and high humidity conditions

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.

## THE TECHNIQUE

When possible actual crossing is done in a mesh house or greenhouse. This effectively reduces the insect pollen vectors, most of the major pests (especially pod borers) and nearly all the important diseases and nematodes. It also permits control of watering, staking, applying nutrients and regulating plant development, and facilitates moving plants for easier manipulation during crossing operations. However, potted plants are small and number of pods per plant is low.

*Mesh house.* In tropical climates an expensive greenhouse is not essential for crossing purposes. Commonly available wire mesh over a simple wood frame serves well. However, it is desirable to have a 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. Peaking the roof somewhat helps shed heavy rains but even with a flat roof the mesh disperses rain drops and minimizes splashing during heavy downpours.

*Synchronizing flowering.* 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' N Lat.) planting late August through early February results in good syn-

chronization of flowering although substantial differences between genotypes do occur and staggered plantings 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 more severe pruning of the plant. New plants are easily started by putting 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 but they are often poor pod setters. They are however, effective as pollen parents.

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

*Emasculation:* In all the flowers of *Vigna* species studied under Ibadan conditions anthesis took place just prior to, or simultaneous with, the opening of papilionaceous corolla. Hence, flower buds destined to open the following morning are ready for emasculation (Plate 1). 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 done at almost any time of the day. Under Ibadan conditions emasculation and pollination



**A flower bud ready for emasculation.**

done in the late afternoon were highly successful. Apparently, cool nights provide better conditions for fertilization than the hotter daytimes.

The bud selected for emasculation is grasped firmly but gently in such a way as to avoid any stress at the fragile attachment of the bud and raceme. A cut about two-thirds the width of the unopened bud is made 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.) Small, finely pointed forceps, dissecting scissors, scalpels or even long thumbnails can be used to make the cut (Plate 2). The upper portion of the folded petals is then grasped



**Make a cut 2/3 the width of the bud.**

by the thumb and index finger and lifted outward tearing the upper portion of the petals free (Plate 3).

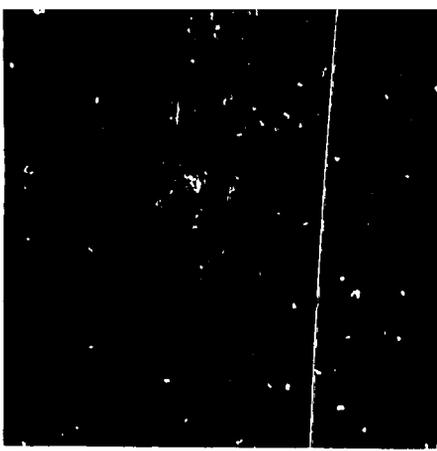


**Gently tear off the cut segment.**



**Remove all anther sacs.**

This leaves the upper portion of the style, stigma and stamens free and exposed to facilitate removal of the 10 anther sacs with a scissors or forceps (Plate 4). The scissors or forceps should be dipped in alcohol (75-95%) between crosses and the receptive green tipped stigmatic surface should not be touched prior to pollinating. This emasculation procedure should require, not longer than 15-25 seconds per flower (Plate 5).



Remove other buds on the raceme.

*Pollination.* The emasculated flower is pollinated the following morning if emasculated in the evening. If crossing is done in the greenhouse, collecting freshly opened male flowers 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 viably stored in a plastic bag (refrigerated).

Some genotypes are superior pollinators whereas others are better seed parents. Unless special genetic studies are to be made, time and efforts in crossing are better spent utilizing 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 the innermost petals of mature open flowers are removed or slipped downwards and the mass of pollen on the hairy-necked style can be used as a brush to deposit pollen grains on and immediately under the green circular disc-shaped stigma (Plate 6).

Another technique for pollination involves removal of the mass of pollen grains from the hairy-necked style with a thumb nail. For effective transfer the thumb nail is applied gently to the stigma of the receptor. With this technique, however, extreme

care is essential to prevent damage to the stigma and to avoid contamination.

One flower can be used 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.



Pollinate the emasculated bud.

A small tag listing the cross and date is affixed to the raceme or peduncle beneath the pollinated bud. Hands, instruments and other foreign objects should not touch the receptive portion of the stigma and the dehisced anther sacs. The crossed flowers are left open and uncovered as risk of contamination is minimal in a well managed mesh house or greenhouse. However, crawling and flying insects must be excluded from the plants during and immediately following pollination. Even ants, which are often attracted to the nec-

taries, can cause selfing. To reduce thrips and other insects likely to carry pollen an insecticide can be applied at regular intervals.

*Post-pollination.* Unfertilized flowers drop off within 24 hours after anthesis whereas the unfertilized ovary may remain attached for 48 hours after anthesis. Therefore, a good check on the success of a cross can be made three days after anthesis. Moderate temperature and increased humidity appear to increase the percentage of fruit setting in hand emasculated crosses. This reflects in a seasonal effect because 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 humidities and lower temperatures during hot periods.

The rate of setting varies enormously with environmental conditions, genotype and manipulative technique. With the use of this technique an average 50% success may be expected

with some specific combinations as high as 90%. For unexplained reasons male sterile (*ms2 ms2*) plants as seed parents seem to produce a high average set (75–95%) under IITA greenhouse conditions throughout the year and in spite of variation in temperature and humidity. Moreover, the 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 to 50% of the selfed fruits normally set by that plant. However, immediate removal of selfed fruits and timely harvest of crossed pods causes a flush of flowers that can be used for cross-although not with the similar frequency of success as the first flush. Pods are ready to harvest 18 to 22 days after pollination at Ibadan.

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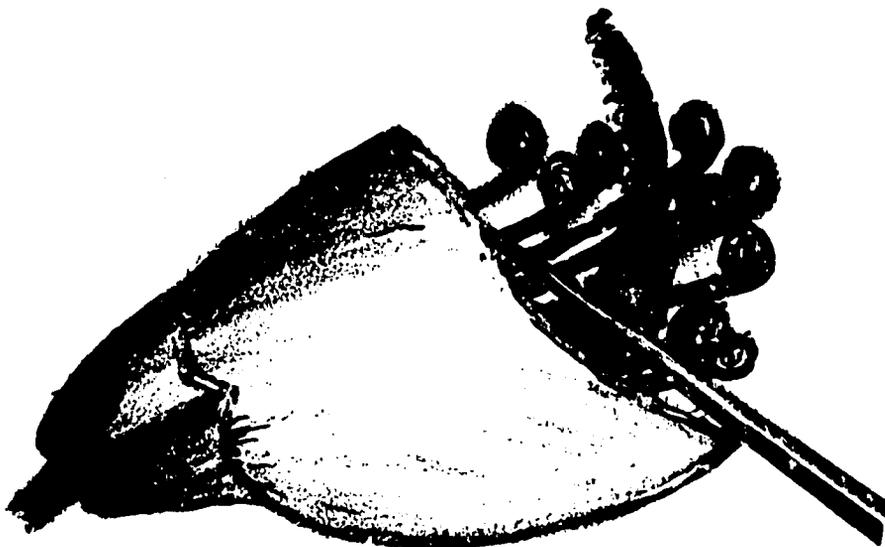
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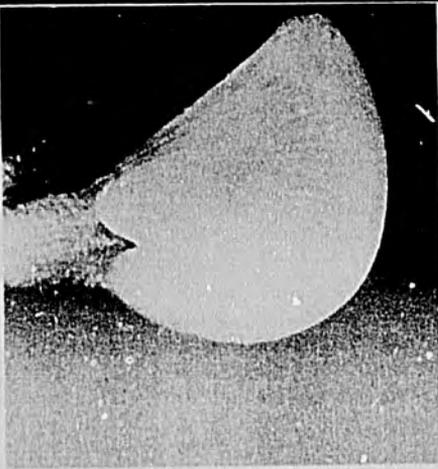
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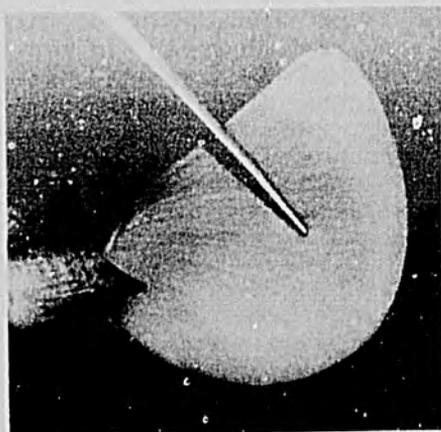
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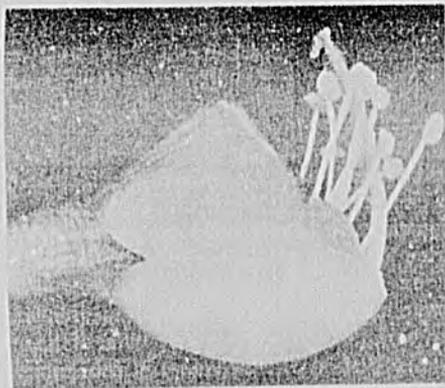
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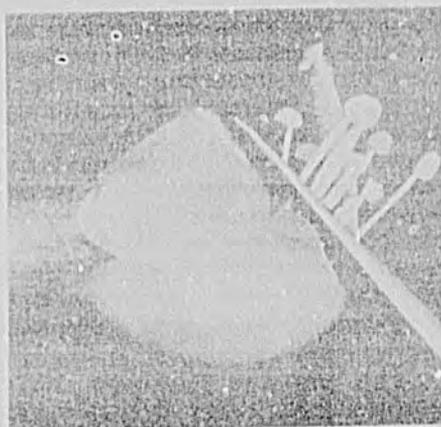


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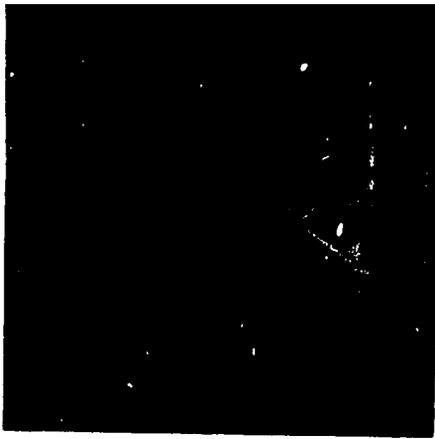


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