Artificial Hybridization in Groundnut

Information Bulletin no. 29

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Nigam, S.N., Vasudeva Rao, M. J., and Gibbons, R. W. 1990. **Artificial Hybridization in Groundnut.** Information Bulletin no. 29. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Artificial hybridization is an integral part of groundnut breeding. In groundnut, the success rate in artificial hybridization depends largely on a proper understanding of the flower structure and its biology, adoption of an appropriate hybridization procedure, adequately trained personnel, and careful environmental control during and after the pollination stage. This bulletin describes the procedures of artificial hybridization in groundnut followed at ICRISAT Center. It also presents suggestions on how to organize a successful hybridization program in groundnut. This bulletin is intended to be used as a practical guide to plan and carry out artificial hybridization in groundnut, both in the field and in the greenhouse.

Résumé

Nigam, S.N., Vasudeva Rao, M.J. et Gibbons, R.W. 1990. **L'hybridation artificielle de l'arachide.** Bulletin d'information n° 29. Patancheru, A.P. 502 324, Inde : International Crops Research Institute for the Semi-Arid Tropics.

L'hybridation artificielle fait partie intégrante de la sélection de l'arachide. Pour cette culture, le taux de réussite de cette méthode est étroitement lié d'une part à une bonne connaissance de la structure des fleurs et de leur biologie et à l'adoption d'un procédé approprié d'hybridation, et d'autre part au niveau de formation du personnel ainsi qu'à la régulation précise des conditions du milieu pendant et après le stade de pollinisation.

Ce bulletin présente les divers procédés de l'hybridation artificielle adoptés au Centre ICRISAT, et offre des propositions pour l'organisation d'un programme efficace d'hybridation de l'arachide. Le bulletin a pour objectif de servir de guide pratique pour la planification et l'exécution de l'hybridation artificielle de l'arachide que ce soit en milieu réel ou en serre.

Resumen

Nigam, S.N., Vasudeva Rao, M.J., y Gibbons, R.W. 1990. **La hibridación artificial del maní.** Boletín de Informacion nu 29. Patancheru, A.P.502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

La hibridación artificial forma parte integral de la cría del maní. En este caso, la tasa del éxito del dicho método depende mayormente de un conocimiento preciso de la estructura de la flor y su biología, y la adopción de un procedimiento apropiado de hibridación, y por otra parte del nivel de formación del personal asi como la regulación cuidadosa de las condiciones ambientales durante y después de la etapa de polinización. Este boletín presenta los diversos procedimientos de la hibridación artificial del maní adotados por el centro ICRISAT. También ofrece sugerencias para la organización de un programa eficaz de hibridación del maní. El boletín se proyecta para servir de guía práctica en la planificación y la ejecución de la hibridación artificial del maní, sea en el campo o en el invernáculo.

Cover: *Pollen mass being transferred artificially on to the stigma of an emasculated groundnut flower.*

Artificial Hybridization in Groundnut

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Preface

Of late, some scientists have been arguing that groundnut genetic improvement through introduction and selection is much too limited and that progress in future could come only through artificial hybridization where genes from diverse gene pools are brought together in a single cultivar. The Groundnut Breeding Unit of the Legumes Program at ICRISAT Center has spared no efforts over the past 15 years to artificially hybridize groundnut cultivars of diverse origins and supply the segregating populations to groundnut scientists in semi-arid and other parts of the world. In the process, they have accumulated a wealth of information on the mechanics of organizing the hybridization activities in groundnut, especially those of large-scale field hybridization operations.

We hope that this information bulletin, which consolidates ICRISAT's past experiences, will assist groundnut breeders all over the world and hasten the process of human-mediated gene transfer among diverse varieties of groundnut.

L.D. Swindale
Director General

Introduction

Artificial hybridization between parental lines to bring together a desirable combination of genes is an integral component of any crop improvement program. In groundnut *(Arachis hypogaea* L.), Norden (1973) indicated that having exhausted the possibilities for improvement by introduction and selection, further progress can be achieved only through artificial hybridization.

The cultivated groundnut, a native of South America, is regarded as a self-pollinated leguminous crop (normally with less than 1% cross-pollination), but cross-pollination up to a maximum of 10% has been reported (Knauft et al. 1987), resulting in natural hybridization. This outcrossing is related to the level of bee activity prevalent on a genotype in a season and at a specific location. Bees of the genera *Apis, Bombus, Steganomous, Megachile, Nomia, Pithitis, and Lasioglossum* act as cross-pollination agents (Hammons 1963, Gulp et al. 1968, ICRISAT 1978).

The genus *Arachis* belongs to the family Fabaceae, subfamily Papilionaceae. It is placed with its related genera, *Stylosanthes, Chapmannia, Arthrocarpum, and Pachecoa* in the subtribe Stylosanthinae of the tribe Aescynomenae (Rudd 1981). The genus *Arachis* is morphologically well defined and is clearly delimited from its closest related genera by the development of a 'peg' and geocarpy.

The genus *Arachis* has more than 70 wild species existing in nature, of which only *A. hypogaea* is commonly cultivated (Ramanatha Rao 1988). *A. hypogaea* is a segmental amphidiploid (2n = 4x = 40) with a basic chromosome number (x) of 10, but it behaves cytologically like a diploid.

Cultivated groundnuts are classified as follows (Krapovickas 1968):

Arachis hypogaea L. (Linnaeus 1753)

subspecies *hypogaea* Krapovickas and Rigoni (1960)

variety *hypogaea* (the Virginia group. Gregory et al. 1951)

variety *hirsuta* Kohler (1898)

subspecies *fastigiata* Waldron (1919)

variety *fastigiata* (the Valencia group, Gregory et al. 1951)

variety *vulgaris* Harz (1885) (the Spanish group, Gregory et al. 1951)

The Virginia group consists of plants that have spreading (runner), spreading bunch, or upright (erect bunch) growth habits and an alternate branching pattern; they lack flowers on the main stem leaf axils, possess fresh seed dormancy, flower longer, and mature later than those of the Valencia or Spanish groups. Both the Valencia and Spanish groups consist of plants that are mostly upright in growth habit, have sequential branching patterns, and have flowers on the main stem leaf axils, and possess no or very limited fresh seed dormancy. In the Valencia group, secondary branches are absent or occur on distal nodes, while in the Spanish group, they are occasionally found irregularly located on primary branches. The first branch on the cotyledonary lateral is always vegetative in subspecies *hypogaea,* while in *subspecies fastigiaia,* it is reproductive (Gibbons et al. 1972). However, with extensive inter- and intra-subspecific hybridizations being carried out in breeding programs, true breeding intermediate forms have been established due to selection, thus blurring the differences between the groups.

Flower structure and floral biology

Inflorescence

An inflorescence in groundnut is either represented by a solitary flower (simple inflorescence) or by a raceme containing two to five flowers (compound inflorescence) in the axils of the cataphylls. Flower number per inflorescence

varies with the cultivar (Umen 1933). A flowering branch never occurs at the same node as a vegetative branch, although they may appear to do so because of the shortness of the internode below the first cataphyll of a branch (Norden 1980).

In variety *hypogaea,* the inflorescences are simple and expand slightly in length during maturation. In variety *vulgaris,* the inflorescences are compound and expand moderately (Fig. 1, left). In variety *fastigiata,* the inflorescences are simple, but may elongate to form a conspicuous long branch that may occasionally terminate in leaves (Fig. 1, right). The length of the inflorescence is also dependent on the cultivar and may exceed 10 cm in some varieties (Ramanatha Rao 1988).

Flower structure

Groundnut flowers are typically papilionaceous and zygomorphic with a reduced pedicel (Fig. 2). What appears to be a pedicel is actually an elongated hypanthium. The calyx has five lobes—in two groups of one and four. The single sepal is juxtaposed to the keel while the other four are fused except at their tips. The standard petal has a range of colors from yellow to orange to dark orange or garnet, and in rare cases, it is white or creamy white. It has a central area called the standard crescent which has darker lines (or markings) radiating from the base to the periphery of the standard in most cultivars. The wings are generally yellow and wrap around the keel. The keel is pale yellow, and closely wraps

Figure 1. A compound (left) and a simple (right) inflorescence in groundnut.

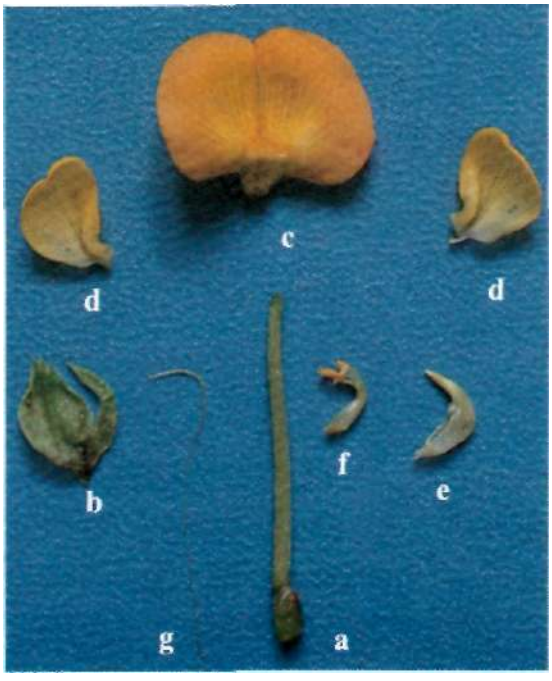


Figure 2. A dissected groundnut flower showing:* (*a*) *hypanthium,* (*b*) *single and fused sepals,* (*c*) *standard,* (*d*) *wings,* (e) *keel,* (*f*) *stamens, and* (*g*) *style and stigma.

around the stamens and the upper parts of the style and stigma. There are 10 monadelphous stamens (Fig. 3) of which two are staminodes (sterile) represented only by filaments (Smith 1950). The remaining eight are dimorphic. Of these, four are with globose, dorsifixed and monothecous anthers, alternating with four having adnate, introrse, oblong anthers, three of which are bithecous and one, opposite the standard, monothecous. The basal two-thirds length of the filaments are fused. The filaments of the globose anthers are initially shorter than those of the oblong ones, but they elongate and become equal to or longer than them a few hours after pollination (Gregory et al. 1973). The ovary is situated at the base of the hypanthium; it is superior, about 1.5 mm long, and normally has two to four ovules, occasionally five, and rarely six(Ramanatha Rao 1988). The style is long and filiform with two bends; the first bend is close to the upper end of the hypanthium, and the second one occurs along with the bend in the keel petal. The style has upward-slanting hairs on its distal portion. The stigma is club-shaped and usually on level with, or slightly above, the anthers.

Figure 3. The three groups of stamens:* (*a*) *two sterile staminodes,* (*b*)*four stamens with globose, dorsifixed, and monothecous anthers, and* (*c*) *four stamens with adnate, introrse, oblong anthers.

Floral biology

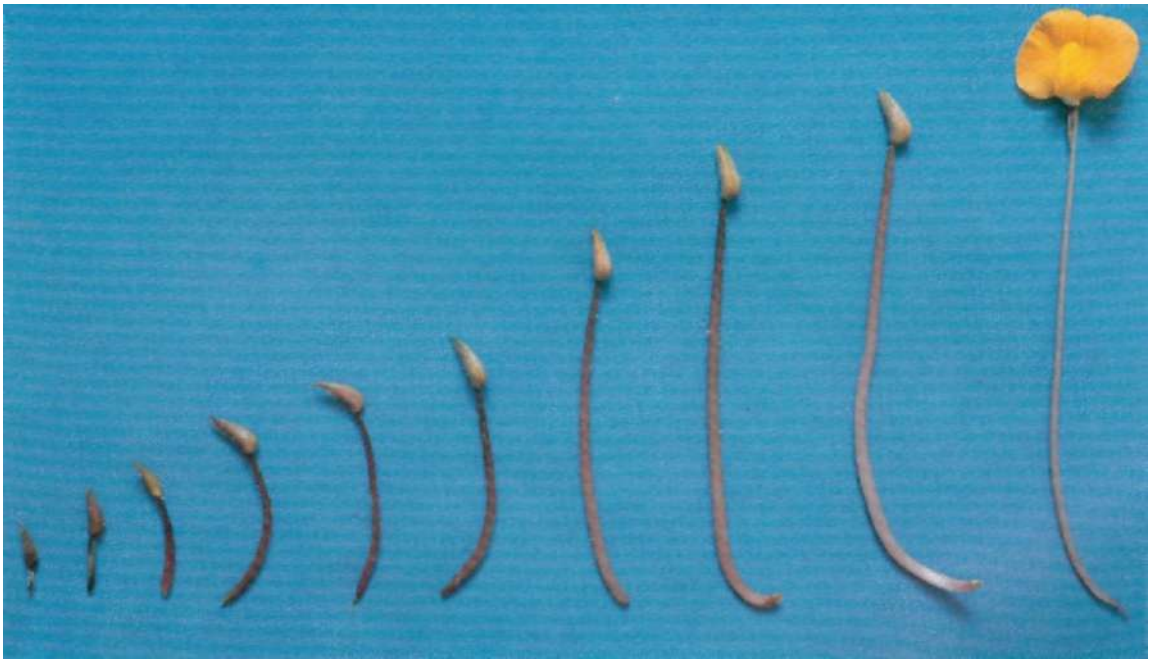
Flowering begins 17-35 days after seedling emergence, depending on the cultivar and environmental conditions. Flowers open in centripetal order. Flower size differs with the cultivar and environment. Low temperatures generally delay flowering.

The flowering pattern varies among and within botanical varieties. Generally the valencias flower before the other types and have a short time of flowering. The Spanish types also flower early, but the first flowering peak may be broader than that in the valencias and some varieties have multiple peaks. The Virginia types take more time than the other two types to start flowering and have multiple flowering peaks. However, cultivars within a subspecies vary in their flowering patterns (Seshadri 1962, Muhammad and Dorairaj 1969).

One bud per inflorescence usually reaches anthesis on a given day, but occasionally two or more buds per inflorescence may open on the same day. The bud is 6-10 mm long 24 h before anthesis and, during the day, the hypanthium elongates slowly (Fig. 4) and the bud attains a length of 10-20 mm. During the night, elongation of the hypanthium is faster. The flower reaches a maximum length of 50-70 mm at the time of anthesis (Smith 1950). Flower opening is normally at sunrise, but may be delayed by low temperatures. Anthers may dehisce 7-8 h before flowers open in some varieties whereas in others they may not do so even at flower opening (Bolhuis et al. 1965). Stigma becomes receptive about 24 h before anthesis and its receptivity persists for about 12 h after anthesis (Hassan and Srivastava 1966). However, Sastri and Moss (1982) reported that the stigma was not receptive before or after the day on which anthesis occurred.

Pollen grains are smooth, oval, and sticky. Fertilization occurs about 6 h after pollination. In general, self-pollination is the rule. However,

Figure 4. Elongation of hypanthium and development of buds in groundnut.



in situations where the stigma and anthers are exerted from the keel and the stigma is still receptive, the flower is more vulnerable to cross-pollination by bees. Extra-floral parts start withering about 6-12 h after flowers open but withering may be delayed or hastened by the environmental conditions, high temperature and low humidity in particular, during the postflowering phase.

Fertilization of the egg activates the growth and elongation of the intercalary meristem which is located at the base of the ovary. As a result, a stalk-like structure or 'peg' becomes visible within 4-6 days after fertilization under normal environmental conditions. A peg, otherwise known as carpophore is, at this stage, a small pinkish, purplish, or greenish growth (depending upon the cultivar). When temperatures are low, appearance of the peg may be delayed by up to 14 days. The peg bears the ovary with the fertilized ovules at its tip. It has a positively geotropic growth behavior and can reach a length of up to 15 cm. Once the peg enters the soil and penetrates to a depth of 2-4 cm, the tip becomes diageotropic and the ovary develops into a pod.

Hybridization in groundnut

For convenience of operation, hybridization in groundnut is normally carried out on plants grown in pots or boxes. These pots are placed on raised benches or ta-bles inside a greenhouse or outside in the open. Use of a greenhouse permits better control of environmental conditions, but limits the number of crosses that can be made. At ICRISAT Center, where large numbers of crosses are made each year, hybridization is routinely carried out both in the field and in the greenhouse.

Choice of parents

The choice of parents is the first and most important step in hybridization and depends on the breeding objectives. Parents are chosen for various reasons—as locally adapted cultivars, gene donors for resistances, or for specific morphological/physiological traits. A breeder's knowledge of the parental lines and their behavior in segregating generations helps in making a suitable choice of the parents and the direction of the cross. The hybridization plan should clearly state the objectives and the number of hybrid seeds desired to be obtained from each cross.

Hybridization techniques

For hybridization in the field, rainy season is the best season as atmospheric humidity is high. In greenhouses or growth chambers where it is possible to control light, humidity, and temperature, hybridization can be carried out at any time of the year. Hybridization should be restricted to the early phase of flowering because of higher success rates in the production of mature pods from early-formed flowers (Muhammad and Dorairaj 1969, Bear and Bailey 1973, Ramanatha Rao 1988). The skill of the operator and environmental conditions (high humidity in particular) influence the success rate.

Before beginning the hybridization, it is very important to ensure that all the plants to be used are true-to-type. Plants that are vigorous and healthy are selected for hybridization. At the onset of flowering, for 1-2 days, all the flowers on the female parents should be removed to help stimulate profuse flowering.

CONVENTIONAL TECHNIQUE

The conventional technique for hybridization in groundnut was described by Norden (1973). The procedure followed at 1CRISAT Center is similar except for the modifications described by Nigam et al. (1980). Equipment required for hybridization includes forceps with fine points, colored nylon threads, petri dishes, and alcohol for rinsing forceps between pollinations (Fig. 5).

Figure 5. Equipment used in artificial hybridization in groundnut.

Emasculation

Emasculation (removal of anthers from buds flowers before their dehiscence to avoid self-pollination) (Fig. 6) is carried out between 1330-1630 at ICRISAT Center (18° N, 78° E, 545 m above sea level). By this time of day, the hypanthium is sufficiently elongated and the bud is big enough to be handled easily during emasculation, and the anthers are not dehisced. At other locations, buds may not be ready for emasculation until later in the evening depending on the prevailing environmental conditions. Once a well-developed bud is selected, all other buds at that node (axil of the leaf) are removed with forceps (Fig. 6a). Removal of these buds ensures that only one flower is allowed to set a peg at each node and this facilitates the identification of hybrid pods. The leaf is pulled down gently to expose these buds. Care is taken to avoid injury to the selected bud. The bud is held gently between the thumb and index finger of the left hand (in case of a right-handed person). Using forceps held in the right hand, the single sepal opposite the standard petal is pulled down (Fig. 6b). The fused sepal is also folded down and held back (Fig. 6c). The standard is then gently and carefully opened with forceps (Fig. 6d) and is held back by the thumb and index finger (Fig. 6e). The wing petals are pulled down locking them with the standard (Fig. 6f). The keel is pulled outwards by its ridge with forceps to expose the anthers (Fig. 6g). All the anthers are removed with the filaments from their bases (Fig. 6h). If the filaments are not removed, they may be confused with the style during the pollination process. This leaves only the stigma and style, which are now well exposed (Fig. 6i), The standard, wing, and keel petals usually return to their normal positions after emasculation to cover the style and stigma. If not, they should be carefully folded back to cover the style and stigma to prevent desiccation of the style (Fig. 6j). Any damage to the style and stigma during emasculation makes the bud unfit for pollination. The internode just above the emasculated bud is then marked with a date-coded colored nylon thread (Fig. 6k). A thread of a different color is used every day to help identify the buds for pollination the next day.

Stages in the emasculation of a bud

Figure 6a. Selecting the right-sized bud and removing other buds at the node (node cleaning).

Figure 6b. The right way of holding the bud and removing the single sepal.

Figure 6c. Folding down and holding back the fused sepal.

Continued

Figure 6d. Opening the standard petal.

Figure 6e. Holding back the standard petal with thumb and index finger.

Figure 6f. Pulling down the wings and locking them with standard.

Figure 6g. Pulling back the keel petal to expose the anthers.

Continued

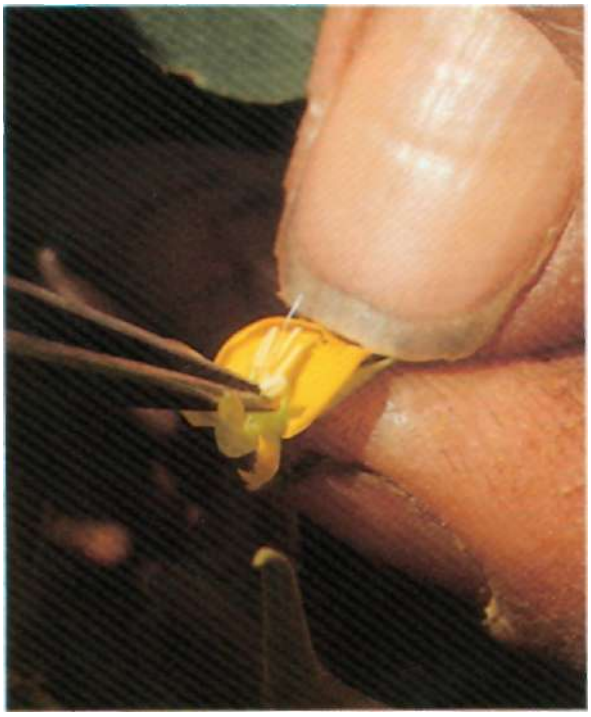


Figure 6h. Removing the anthers.

Figure 6i. Fmasculated flower showing only style and stigma.

Figure 6j. Folding back the standard, wing, and keel petals to their normal positions after emasculation.

Figure 6k. Tying a date-coded color thread on the stem for the identification of the emasculated bud for pollination.

Stages in the pollination of an emasculated flower

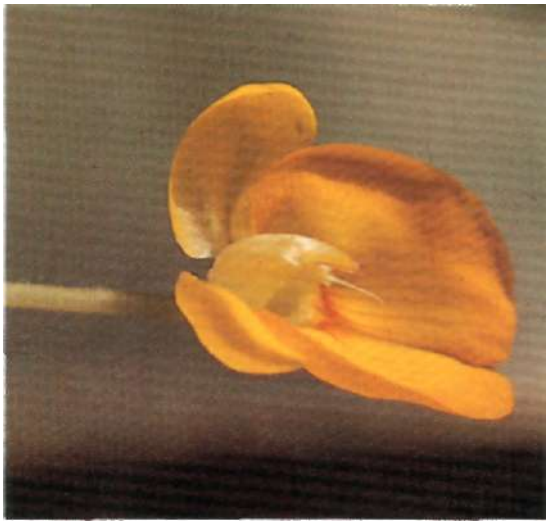


Figure 7a. Emasculated flower ready for pollination.

Figure 7 b. A healthy flower from a male parent plant with its calyx, standard, and wings removed and pollen mass squeezed out, ready to be used for pollination.

Figure 7c. Depositing the pollen on the stigma.

Figure 7d. A pollinated female flower with pollen sticking to the stigma.

Though cross-pollination is reported to occur in groundnut, there is generally no need to protect the emasculated bud from unwanted pollen if insects are properly controlled.

It may take about 40-60 days from fertilization to pod maturity (Norden 1980, Sastri and Moss 1982). Therefore, emasculations should be stopped at least 60 days before the normal harvest time to permit full development and maturity of hybrid pods from the last-pollinated flowers.

Pollination

Pollination (transference of pollen grains from the anthers of one flower to the stigma of another flower) is carried out the day after emasculation. Normally buds start opening at sunrise. However, sometimes, bud opening may be delayed due to an overcast sky or low night temperatures. Pollination soon after buds open is best to achieve a high success rate. During this period, atmospheric humidity, stigma receptivity, and pollen viability are high. If pollination is delayed, the success rate goes down because the stigma receptivity is reduced. At ICRISAT Center, pollinations are carried out between 0600 and 0800.

Before pollination is effected, the emasculated flower should be checked for the condition of the style (Fig. 7a). If the style is fresh and of normal length, the flower should be pollinated. If the style has withered or turned brown due to injury during emasculation, the flower should be rejected. When an immature bud has been emasculated, the style coils, and grows longer than its normal length by the next day. Such flowers should not be used.

For pollination, a healthy flower from a preidentified male parent plant is removed by breaking the hypanthium. The calyx, standard, and wing petals are detached for ease in operation. The keel petal is gently pressed between the thumb and index finger to squeeze the sticky pollen mass out from the anthers (Fig. 7b). The sticky lump of pollen is deposited on the tip of the stigma of the emasculated flower (Fig. 7c). It is possible to pollinate up to 15 female flowers with 1 male flower, depending on the environmental conditions at the time of pollination. The pollinated flower should not be disturbed for some time after pollination to avoid dislodging the pollen from the stigma (Fig. 7d). The forceps and fingers of the operator should be rinsed with alcohol when changing from one male parent to another to avoid contamination with unwanted pollen.

All flowers except those that are artificially pollinated, should be removed every day soon after pollination from the base of the hypanthium. This helps in prolonging the duration of flowering of the female parent plant. This flower-removal operation should be carried on for at least 2 weeks after completing the last pollination of the season. This reduces competition for the development of hybrid pods.

Checking for success

If the pollination is successful, a peg will be seen emerging from the axil of the leaf just below the colored thread 4-6 days after fertilization (Fig. 8a). Monitoring of the developing hybrid peg (Fig. 8b-d)—peg checking—should be routinely carried out without disturbing the plant to avoid damage to older pegs. While carrying out peg checking, if new buds/flowers are found, they should be removed. These buds/flowers appear if all immature buds are not removed from the node at the time of emasculation. If a peg is not observed even up to 2-3 weeks after pollination, the pollination is considered unsuccessful. If success is low, more buds can be emasculated and pollinated to obtain the desired number of hybrid seeds. If the pollinated flower is from a higher node and if it is difficult for the peg to reach the ground, then, iron/zinc U-pins can be used to bend the branch and hold it near the ground, facilitating the entry of the peg into soil (Fig. 9). Once the branches are pinned into the soil, they should not be disturbed. Alternatively, small pots could be placed below the nodes to allow entry of pegs into the soil (fig. 10). These pots must be watered regularly.

Monitoring the development of a hybrid peg—stages of development

Figure 8a. A peg-initial seen 4-6 days after fertilization.

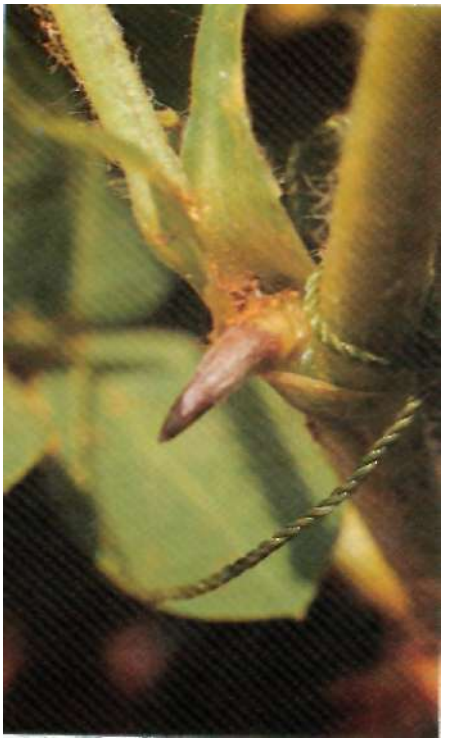


Figure 8b-d. Stages before the peg enters the soil.