



SHORT RESEARCH ARTICLE

Adhesive bud method: A novel crossing technique in sesame (*Sesamum indicum* L.)

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Abstract

Sesame (*Sesamum indicum* L.) is an important oil seed crop of tropical and subtropical countries. Sesame is an autogamous indeterminate crop. Crossing programme plays a major role in creating variability. The main objective of the present work is the development of Adhesive Bud Method, a novel crossing technique in sesame. Here, emasculation and pollination were carried out parallelly during 3.00 PM to 6.00 PM. Foreign pollen contamination is completely restricted using this procedure. Pollination is affected whenever the pollen dehisces and stigma attains receptivity. Five straight cross combinations are attempted using Adhesive Bud Method, crossing technique in sesame. It resulted in 52–68 percentage of capsule development with effective hybridization of the desirable parents. This novel Adhesive Bud Method, crossing technique, helps the sesame breeders adapt an easy, effective and efficient crossing programme

Keywords: Sesame, crossing, emasculation, pollination, Adhesive Bud Method

Sesame (*Sesamum indicum* L.), popularly known as til, benni seed is queen of oil seeds and belongs to the family Pedaliaceae. Sesame is diploid (chromosome no. $2n=26$), indeterminate, autogamous crop and reported to have outcrossing of 1–42% (Kumar and Lenin 2000, Anonymous 2018). The cross-pollination is effected due to honey bees (Andrade et al. 2014). Sesame flowers develop solitarily in the leaf axil and are arranged alternately on the stem (Bedigian et al. 2010). Sesame flowers are bisexual and zygomorphic with two green bracts. The corolla tube of sesame flowers exteriorly white in colour and have a light pink wash on the interior corolla. Ovary is superior, bicarpellary, forked stigmas. Anthers are epipetalous with four stamens with two long and two short stamens, attached on the inner side of the corolla tube, dorsifixed and positioned higher over the stigma (Andrade et al. 2014). Flowers generally open from the base to the apex in acropetal manner. Flower anthesis generally occurs in the morning between 6–9 AM. The stigma is receptive for 48 hours and pollen is viable for nearly 24 hours (Bedigian 2010; Ramya et al. 2019). Variability in breeding material is of paramount relevance for developing desirable high-yielding cultivars. Hybridization serves a crucial function in generating variation. Numerous sesame cultivars, such as, YLM 66, GT-4, Swetha til, RT 351, etc., are developed using the hybridization approach, which involves a crossing programme (Parameshwarappa et al. 2017; Kumhar et al. 2013).

The main objective of the study was to develop easy, effective, efficient and feasible crossing technique for hybridisation programme in sesame adaptable for sesame breeders. The adoption of efficient crossing techniques has a significant influence in the generation of variation. In general, sesame breeders adopt the soda straw method (https://agritech.tnau.ac.in/crop_improvement) as an age-old method for crossing and hybridization programmes. In this old method, the female flower buds are emasculated between 3:00 PM and 6 PM on day one, and a soda straw is inserted on the emasculated stigma to prevent external pollen from adhering. The following day (day 2), between 7 AM and 9 AM, fully opened male flowers are gathered and

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pollen is dusted onto the stigma that has been emasculated. In the soda straw approach, the inserted straws may fall and introduce unintended or undesired pollen. The process of emasculation and pollination is extremely arduous. The selfing techniques has been earlier reported in sesame by Ramya et al. (2019).

In light of the foregoing, a revolutionary easy-to-use technique for crossing sesame called the Adhesive Bud Method was developed at Agricultural Research Station, Yellamanchili (Acharya N.G. Ranga Agricultural University ANGRAU), Visakhapatnam, Andhra Pradesh, India. During Rabi-summer 2019 and 2020, the experiment was performed at the Agricultural Research Station, Yellamanchili (Acharya N.G. Ranga Agricultural University). The Agricultural Research station, Yellamanchili, is located in the Viskahapatnam district of Andhra Pradesh, India, and focuses primarily on sesame. The hybridization programme in sesame is an ongoing, variable-creating initiative. Seven parents, namely, Madhavi, VZM-10, YLM-66, SKL-19, VZM-5, YLM-17, and EC 370360 of sesame (*Sesamum indicum* L.) brown seed varieties were used to attempt five straight crosses (Table 1). Once flowering has initiated, the Adhesive Bud Method crossing technique was utilized to attempt the crossing programme.

The detailed step-by-step technique of the Adhesive Bud Method is given below:

Selection of Parents

Desirable healthy male and female parents are selected and tagged. Once flowering commences crossing programme is initiated.

Collection of male corolla tubes

The corolla tubes carrying the epipetalous anthers of the flower buds to be opened on the next day are collected at 3 PM to 6 PM from the male parents in a petri plate. (Fig.1).

Emasculation of female flower buds

On the female parent, select the flower buds to be opened on the next day. Emasculate the flower buds by removing the corolla tubes at 3.00 PM to 6.00 PM Emasculation is done by removing the corolla tube (As the anthers are epipetalous, removal of the corolla tube results in emasculation). (Fig. 2. and Fig. 3.).

Insertion of male corolla tube

Immediately after emasculating the female flower buds,

the male corolla tubes collected are inserted over the emasculated stigma of the female flower buds carefully at 3 PM to 6 PM The male corolla tubes are inserted with adhesive (fevicol). A thin layer of the adhesive (fevicol) is applied in the inner side, at the basal part of the corolla tube (Fig. 4.) with a thin stick. After applying the adhesive, the male corolla tube is carefully inserted over the stigma of the emasculated flower of the female parent and adhesion of the male corolla tube is done to the base of the female emasculated stigma (Fig. 5.). Now, it looks like a natural flower bud. Care should be taken that the applied adhesive on the male corolla tube should not stick to the stigma during insertion.

Tagging

The crossed female flower bud is tagged with a woollen thread tied at the base of the flower and the leaf axil. The woollen thread should be tied to the stem connecting the leaf axil and flower bud. (Fig. 6.).

Development of the capsule

The inserted male corolla tube acts as a cover to the stigma. The epipetalous anthers on the inserted male corolla tube physiologically dehisce naturally next day morning, and the stigma becomes receptive and the pollination is effectively executed. The inserted male corolla tube dries off but the epipetalous anthers on the male corolla tube remain fresh. (Fig. 10.). The inserted male corolla tube dries off (Fig. 7.) naturally and consequently, the capsules development occurs. (Fig. 8.).

The male corolla tube found intact and unable to open serves as a cover for the female stigma. When the female stigma becomes receptive, the anthers on the epipetalous corolla dehisce physiologically (Fig. 10) and become exposed, allowing for natural pollination. The targeted male pollen is pollinated effectively and without contamination on the female stigma. Unique to the Adhesive Bud Method is the simultaneous emasculation and pollination procedure. For every emasculated female flower, a single male corolla tube is required. The implanted male corolla tube naturally dries out over time. However, pollination occurs efficiently. The growth of the capsule begins four to five days after crossing. The capsule is well developed (Figs. 8 and Fig. 9.) with the seeds. After the seeds have matured, the capsules are promptly harvested. The seeds are extracted from the capsules, dried, and preserved (Fig. 9). The crossed seeds (F0)

S.No	Cross	No.of crosses attempted	No.of crosses effected or capsules developed	Percentage of mature capsules developed
1	Madhavi X VZM-10	22	13	52
2	YLM-66 X SKL-19	28	21	68
3	YLM-66 X VZM-10	29	20	57
4	YLM-66 X VZM-5	37	27	58



Fig.1. Male flower bud collected for emasculation (day1)



Fig. 2. Female flower bud selected for emasculation (day1)



Fig. 3. Emasculation of the female flower bud selected for emasculation (day1)



Fig. 4. Application of glue to inner side of the basal side of the corolla tube of Male flower bud (day1)



Fig. 5. Insertion of the male flower bud corolla tube on the emasculated flower stigma (day1)



Fig. 6. Coloured woollen thread is tied to the pedicel of the female flower (day1)



Fig. 7. Intact Flower bud status on day 3



Fig. 8. Developed capsule with dried corolla tube at tip



Fig. 9. Seeds developed in the crossed capsule



Fig. 10. Proof of pollen dehisced from the male inserted corolla tube (day2 morning 9.00 am)

Step wise procedure of AdhesiveBud Method crossing technique in Sesame (*Sesamum indicum* L.) developed at Agricultural Research Station, Yellamanchili

are sown and the plants are germinated. The experiment was undertaken using seven parents and five straight crossings of *Sesamum indicum* L. (Table 1) displays the pooled data for the number of pollinated flowers and the number of ripe capsules formed.

The percentage of mature capsules developed ranged from 52 to 68. The seeds (F0) extracted from the capsules grew well and vigorously. This demonstrates the efficiency of the novel Adhesive Bud Method for crossing in sesame.

Advantages of the adhesive bud method crossing technique

- It is easy, and effective crossing technique for the breeder.
- The emasculation and crossing procedure done at once at 3 PM to 6 PM.
- The laborious process of emasculation on day1 and pollination on day 2 as in soda straw method is not required.
- The cross-pollination, physiologically, takes place efficiently.
- Contamination of the cross pollination of foreign pollen is restricted as the corolla tube of the male flower itself acts as natural cover.
- No need of paper covers or soda straws to restrict the foreign pollen.
- The targeted cross is attempted even without one percent contamination.

The sesame breeders could adapt this Adhesive Bud Method crossing technique and carry out the crossing programme in sesame with much ease and efficiency.

The importance of the present new methodology "Adhesive Bud Method" is the development of a successful and simple technique of the desired, targeted cross. The process is simple since emasculation and pollination are performed simultaneously from 3 PM to 6 PM, and capsules are developed without contamination of foreign pollen. As a result of this experiment, sesame breeders are encouraged

to adopt the Adhesive Bud Method sesame crossing technique and implement a successful crossing programme with ease and efficiency.

Authors' contribution

Conceptualization of research (ABMS); Designing of the experiments (ABMS); Contribution of experimental materials (ABMS); Execution of field/lab experiments and data collection (ABMS, HB, TLT); Analysis of data and interpretation (ABMS, HB, TLT); Preparation of manuscript (ABMS, HB, TLT).

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