A novel, low-cost and throughput selfing technique in sesame

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ABSTRACT

Sesame (*Sesamum indicum* L.) is an oilseed crop, highly valued for its quality edible oil and direct confectionery uses. Though sesame is a self-pollinated crop, the opened flowers attract several groups of bees which promote cross pollination. Genetically pure seed obtained by ensuring self-pollination is an essential requirement for fixing the traits in genotypes, and for employing them in molecular and genomic studies. As of now, bagging technique is followed for selfing and this is not only tedious but also detrimental to the developing flowers. In order to overcome the limitations of the traditional selfing technique, a novel selfing technique has been developed using liquid glue, which is low cost, less labour intensive and high throughput method. This technique has no seasonal limitations and is applicable across any region where sesame is grown.

Keywords: Bag, Bees, Glue, Insects, Sesame, Self-pollination

Sesame (*Sesamum indicum* L.) is an Indian origin oilseed crop grown in tropical and sub-tropical regions which belongs to genus Sesamum and family Pedaliaceae. Sesame is predominantly an autogamous crop, nevertheless reported outcrossing rates are quite high ranging from 1 to 42 per cent (Pathirana, 1994; Baydar and Gurel, 1999; Anonymous, 2018) due to insect visits to the flowers (Andrade *et al*., 2014; Kumar and Lenin 2000). This wide range of cross pollination is reported to be due to local bee population as well as availability of different genotypes within the vicinity.

According to Bedigian (2010), the flower buds develop at each leaf axil within 25-40 days after sowing depending on the genotype. It takes 10-12 days from the time of visibility of the bud (size 1mm) at the tip of the apical meristem to opening of the bud (10-12 mm) to bell shaped flower (Fig.1). Flowers are generally solitary or in small fascicles in upper leaf axils, bisexual, zygomorphic, 5-merous, with 2 bracts at the base, each bract with an axillary gland; calyx with oblong lobes 4-7 mm × 1-1.5 mm, slightly fused at base, apex acute, long-hairy. The corolla is fused and lobed, white, rose-pink, lavender, or mauve, in many cultivars with dark purple interior markings. The lowest lobe is often as much as 1 cm longer than the others, and often shaded purple which is commonly referred as flower lip. The ovary is superior, oblong-quadrangular, approximately 5 mm × 2 mm, and grayish hairy. Style is 1 cm long, with 2-lobed or 3 lobed stigma covered by 4 stamens, filament is fused near the base of corolla tube and included, the upper 2 stamens are shorter than the lower 2 stamens.

Flowers open in acropetal sequence, from the base of the stem toward the apex. Flower anthesis takes place early in the morning between 6 and 9 AM, and the pollen remains viable for >24 hours while the stigma remains receptive for up to 48 hours (Bedigian 2010). Sesame is an indeterminate plant with flowering usually starting from 30-35 days after sowing in the main stem and continues until harvest. It has been observed and that in commercially cultivated varieties such as TKG-22, PHULE TIL-1, RT-346, RT-127, GT-2 etc. flowering ceases at 70-85 days after sowing. This observation is supported by the reports of earlier workers (Kumhar *et al.,* 2013; Parameshwarappa 2017). During flowering, each branch will have 2-4 opened flowers every day until bud production stops. Sesame flowers are visited by a wide group of honey bees and other insects for nectar and pollen and during this process they cause cross pollination. Longer duration of stigmatic receptivity coupled with longer pollen viability increases the window of time for cross pollination. Although honey bee pollination is encouraged during commercial cultivation of sesame, it is a disadvantage in breeding programmes where directed crossing or selfing is intended. Homozygous and homogeneous lines are required for carrying out several molecular, biochemical and genomic studies and under such conditions unintended cross pollination poses a problem. Therefore, it is essential to ensure proper selfing of plants under several situations and to meet this requirement, the development of a fool proof, easy and high throughput selfing method in sesame is advantageous. Even while maintaining the germplasm accessions through self pollination, it becomes essential to ensure that cross pollination has not occurred and genetic identity of lines is maintained. Covering the flower buds from top of the branches with butter paper is commonly followed by sesame breeders. When the branches from selected plants are covered using butter paper bag and the base of the cover is tied to the branch using a thread, the indeterminate growth of the braches poses difficulty in maintaining the butter paper bags on the branches. The flower bud as well as immature capsule drop is observed inside the bag along with fungal mycelium growth and only one or two capsules with normal seeds could be harvested. Due to these disadvantages of covering the branches, a method which can cover only individual flowers so that

honey bee cannot enter inside the flower is essential. In Tamil Nadu Agriculture University, Coimbatore, smearing clay solution on tip of the mature flower bud is being practiced. We understood that this method, basically does not allow the corolla to open up on bud maturation, hence, honey bee cannot enter the flower. On perusal of this idea of smearing the clay, we communicated with the senior workers of sesame in TNAU, Coimbatore and we were informed that the clay used by them was the natural clay available particularly in their area and they also mentioned that the clay may be washed away due to rain thus allowing the corolla to open (personal communication from Dr. V. Muralidharan, former Head, Oilseeds TNAU, Coimbatore and former Director ICAR-DGR, Junagadh). Therefore, we searched for the material which is easily available, handy to use and wash proof with the capacity to hold the corolla tight without opening. We initially thought of adhesive strips (was difficult to place it on the flower buds), fevicol (costly and damages the bud tissue in 2-3 hours of application), starch solution prepared using starch powder (attracts ants and flakes out from the bud when it dries up). We identified liquid glue which is easily available, thick paste like starch but does not flake out and does not attract insects, less concentrated and odor free when compared to fevicol and liquid based which facilitates for easy application unlike adhesive tapes. This article describes, a new technique developed to ensure self-pollination using liquid glue.

In this study undertaken with popular cultivated variety SHWETA TIL, mature flower buds (Fig. 1) which were ready to open the next morning were selected and tagged using a piece of thread. Initially, liquid glue was directly used to apply on the mature flower bud so that glue holds the corolla tight without opening, but it was observed that the thick concentration was too heavy on the soft tissues of mature flower buds (tip of the bud turned brown on next day). Therefore solution was prepared using water and commercially available liquid glue in 1:1, 2:1 and 3:1 raito and these solutions were used for application on 10 flower buds. The corolla of the buds were closed upon maturity in all three solution, but there was some damage (browning of the tip) in 1:1 ratio. The buds on which 2:1 and 3:1 solution was applied resulted in normal healthy flower with closed corolla in all 10 buds. Therefore, 2:1 dilution was considered in all subsequent experiments. The mature flower buds were dipped to third of its length in the glue solution of 2:1 ratio carried in a small container (say like a cap of a bottle as shown in Fig 2 or any tiny equivalent container). The tip of the flower bud was dunked into the glue solution and it was ensured that tip of the flower bud where it opens into flower was closed with the glue solution. When the tip of the bud was covered with glue solution, the corolla gets glued up and does not open up. The following morning, it was observed that the corolla was intact due to the glue and did not open. However, the bud had grown normally and matured into flower with normal anthesis and ensured self-pollination (Fig.3). Normal seed setting and seed filling was observed in all the flower buds treated with the glue solution and there was no difference between these capsules and the capsules formed from flowers that were not treated (Fig.4). This experiment was taken up during Summer-2018. The success of capsule set on treated flower buds encouraged us to treat 20 flower bud in each of the released varieties *viz.,* E-8, GT-2, GT-4, HT-1, MT-75, PHULE TIL, RT-351, RAJESHWARI, HIMA, SWETHA TIL, TSS-6, VRI-3 and germplasm accessions *viz.,* IC-132201, IC-500472, IC-96160, IC-204618, IC-204613, IC-96227, IC-204300 and IC-204444. The selfed seeds from these capsules were collected and used for crossing during late *kharif*-2018. The new technique described above was used to self 4608 flower buds of 768 F_2 plants from the cross IS-49-1A x RT-346 during late *kharif*-2018 (August sown) crop. The selfing spanned from 10 September 2018 to 23 September 2018 using 2 women labour for this particular activity. There was normal capsule and seed setting in these treated buds which were used to raise F_3 generation. During this selfing activity there were rains on 12th September (12.4 mm) and 22nd September (4.87 mm), however, it was observed that the glue treated flowers were closed even after the rains. With this observation we were convinced that the rain does not wash away the glue on the flower bud. The three popular selfing techniques practiced by breeders to ensure selfing and the merits and demerits of each of these techniques are presented in Table 1.

Lower seed weight per capsule has been reported when selfing is ensured by covering the capsules using butter paper covers or tulle covers or nets (Das and Jha 2019). Light interception is restricted, which in turn reduces photosynthetic rate in top leaves and stem resulting in low seed weight per capsule. Green capsules in sesame are also known to be photosynthetically active and expected to contribute significantly for increased carbon fixation and seed weight (Weiss, 1983).

The simple liquid glue is available in stationery stores and the same could be diluted with water and used to dip the bud. In our experiments we found that the glue does not get easily washed out due to rain or dew. Dipping a bud in the glue solution takes just a second and so large number (up to 1000) of flower buds could be treated in less than 2 hours. This technique is less labour intensive, with no special skill requirement and is high throughput. The present method of glue application overcomes the disadvantages in other selfing techniques followed by the breeders. However, tagging of selfed buds is time consuming, which can be avoided by selfing all the available buds every day up to one week of peak flowering or as per the breeder's choice.

This technique of glue based self-pollination in sesame is currently being practiced in ICAR-IIOR, Hyderabad since summer 2018 and it is working satisfactorily, to maintain purity in germplasm as well as in advancing the generations through selfing. Based on these observations, the present method is being advocated as a simple and effective method for selfing flower buds in sesame. However, identification of selfed flower bud needs labelling of the buds and the presently followed method of tying a thread around is quite time consuming. Therefore there is a need to look for alternative ways of labeling the flower buds selfed using glue.

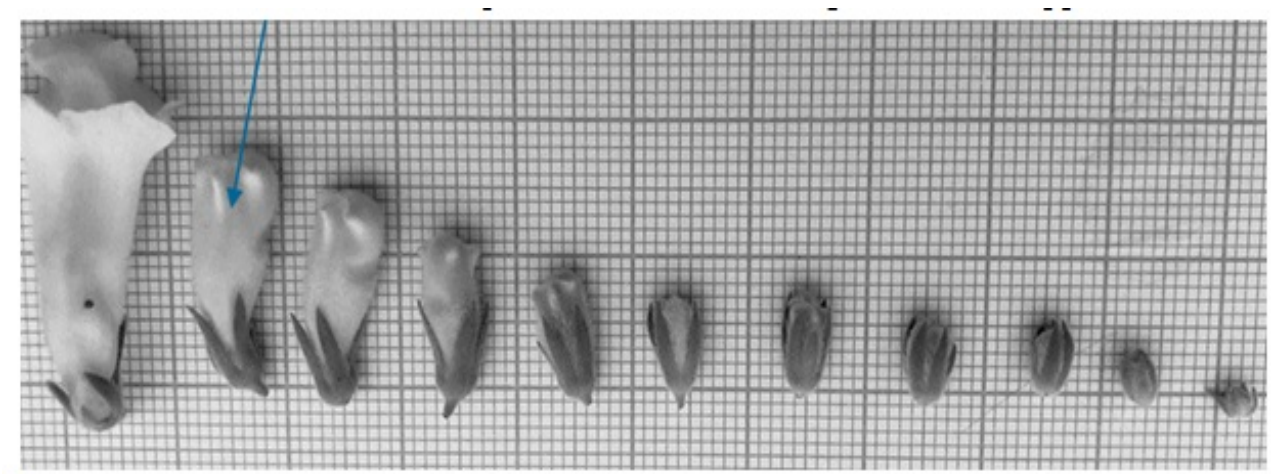

Fig. 1. Development of sesame flower bud. Mature flower bud (with arrow mark is ready to open in the next morning) is selected for treating with glue

Fig. 2. Dipping of mature flower bud (which is ready to open in the next morning) in glue solution using a small container (cap of water bottle) to carry the glue

Fig. 3 (a). Treated (with glue) flower buds matured normally but did not open for insect pollination; (b). Treated flowers dropped off after fertilization without the corolla opening; (c). Untreated flowers opened completely and then dropped off after fertilization

Table 1 Selfing techniques practiced and their merits and demerits

Selfing technique	Merits	Demerits
Covering of buds at top of branches with butter paper bags or tulle covers to protect the flowers from insect visit to ensure self-pollination.	1. Ensures selfing 2. Widely adoptable 3. Less laborious and not highly skilled.	1. Very difficult to cover individual flower bud due to fragile pedicel. Therefore, bags are placed on top of the branches which arrests further production of the buds. 2. Very few buds can be selfed, therefore less amount of selfed seeds produced 3. Humidity and temperature in the bag increases leading to dropping of the capsule before maturity as well as favours growth of saprophytic and opportunistic fungi 4. Usually leads to reduced seed set and seed weight
Coving the plots by nylon net	1. Easy and less laborious 2. Large quantity of self-seed obtained	1. Does not ensure 100% self-pollination as there are chances of insects getting inside the net resulting in cross pollination 2. Difficult for inter-cultural operations and increased humidity, reduced seed set and seed weight
Applying paste of clay on the tip of the buds and once the clay dries it prevents flower opening as it forms a tight cap preventing the corolla from opening developed by TNAU, Coimbatore (agritech.tnau.ac.in/crop_improvement/crop_improv_emasculation_oilseeds.html)	1. Individual flowers can be selfed 2. Ensured selfing 3. Good seed set 4. Selfed seeds can be obtained early by selfing early buds	1. Smearing clay with fingers on individual bud is laborious 2. Requires skill for preparing right clay consistency as well as for application. 3. Clay may wash out due to rain and dew. (Refer agritech.tnau.ac.in/crop_improvement/crop_improv_emasculation_oilseeds.html) 4. Ensuring uniformity in clay quality is difficult and varies with location and time. 5. Maintaining consistency though out the process is difficult

Fig. 4. Normal capsule maturity and seed filling from glue treated flower buds (ensured self-pollination) when compared with seeds from untreated flower buds (open pollination)

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