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Research Article

Studies on efficiency of artificial hybridization in groundnut (*Arachis hypogea* L.)

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Abstract
Groundnut being an important oilseed crop worldwide poses many challenges in its improvement. One such factor is hybridization. The crop produces self-pollinated papilionaceous flowers for the crossing of which hand emasculation and dusting are the best methods employed. In the present study, 10 cultivars of groundnut varying in agronomic traits were crossed in L × T fashion of 5 × 5 set to obtain 25 crosses to study the efficiency of hybridization. The F_1s of the crosses were raised and their truthfulness was identified and confirmed by the varying traits between male and female parent and also at the molecular level. Hybridization success rate recorded a range of 25.4% to 42.3%.

Keywords
Papilionaceous flower, emasculation and dusting, hybridization, L × T crosses

INTRODUCTION

Groundnut is a very potential oilseed crop which provides huge opportunities to plant breeders for its improvement. The increased use of groundnut oil around the world and use of groundnut for various purposes like soap making, and manufacturing cosmetics and lubricants, roasted or sweetened confectionery, residual oilcake as cattle feed and fertilizers, use of shell as fuel are the standard proof of the crop's growing demand.In addition to the exploitation of available germplasm for cultivar development, three main approaches have been applied in crop improvement: 1) artificial hybridization is commonly known as crossing, 2) mutagenesis by chemicals or radiation, and 3) genetic transformation.

The latter two methods are not widely used in peanut breeding programs owing to economic feasibility and their difficulty in handling the populations with identifiable traits. Artificial hybridization is the most preferred method which has given rise to most of the released peanut cultivars by the Groundnut research institutions around the world. Furthermore, artificial hybridization has been employed in the effort to identify genetic components conferring phenotypic traits of interest. The establishment of gene-trait associations for mapping populations segregating for traits of interest and molecular marker development is all based on the crossing program in the crop.

Groundnut is a highly self-pollinated crop belonging to the *Fabaceae* family. The flower has one large standard petal, two lateral wing petal, and a keel petal that encloses the staminal tube. At the distal end of the staminal tube, ten anthers surround a club shaped stigma (Smith, 1950). Anther dehiscence and self-pollination occur during floral expansion shortly after sunrise. Since groundnut artificial hybridization is low yielding and time consuming, reported costing 10 minutes per flower (Hammons, 1964), maximizing the success rate of artificial hybridization is desirable for groundnut breeding programs. The present study is taken up to estimate the efficiency of artificial hybridization of groundnut by hand emasculation and pollination.

MATERIALS AND METHODS

The parental lines that varied in agronomic traits and maturity durations viz., CO 7, ICGV 07222, VRI 6, VRI 8 and GPBD 4 were selected as a female parent while VRI

3, Chico, Gangapuri, ICGV 91114 and ICGV 93468 were selected as the male parent in the study conducted during *Rabi,* 2018 at the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore. The male and female parent was sown in adjacent rows that could enable to hybridize the parents in L × T fashion of 5×5 set.

The crossing methodology: In Groundnut flowering has been reported to occur as early as between 0600 to 0800 h in India (Daniel and Thulasiada, 1976). Dehiscence of the anthers has been reported to occur early, prior to flower opening which enables self-pollination to take place within the closed petals (Culp *et al.,* 1968). The technique of hand emasculation and pollination was carried out as described by Norden and Rodriguez, 1971.

The flower buds that are ready to open in the next day are selected and emasculated in the evening after 4 pm. They are identified by either the length of the calyx tube or different colored threads used every day to tie around the pedicel of the emasculated flower. During emasculation, the bud is held between the thumb and the index finger of the left hand and with the help of a razor blade in the right hand, a cut is made below the tip so as to cut the standard and a portion of the wing petals. Then the calyx and corolla are gently pulled by holding at the tip of the flower bud. By doing this, the sepals and the petals except the keel would be removed and with the help of the fine forceps the bundle of stamens are liberated from the keel and the anthers are nipped off.

The emasculated flower bud is covered with a piece of the straw tube closed on one side by bending by slowly inserting the calyx tube into it. This ensures perfect protection to the stigma from any natural cross-pollination. The next day morning pollen grains are collected early in the morning between 7 am and 11 am from mature yellow anthers of the selected male parent flower. The flower is held between the thumb and the middle finger after the standard and wing petals are removed. The flower with keel protruding is taken to the stigma of the emasculated flower. The straw tube is taken off, from the stigma of the emasculated flower. A gentle push on the keel of the selected male flower by the finger forces lumps of pollen grains to cover the entire stigmatic surface. Pollination between 7 and 8 am was found to give more success. If the stigma is found dry, smearing of pollen with 2 per cent sucrose solution is done again. Five to seven days after pollination successful crosses will produce gynophores (pegs) with the dried flowers at their tips. The number of flowers hybridized in each cross is taken record of daily.

The process of crossing was carried out for a period of 14 days or till the pollen parent ceases to flower. After the period of crossing, steps are taken to remove the flowers from the female parent to avoid mixing up of the selfed flowers in forming the pegs. The crossed seeds are harvested after the maturity duration of the female parent and the number of pods formed was recorded. The seeds were examined for the change in characteristic colors of the seed coat if any. The harvested crossed seeds were sown and raised in the next season for the study of F_1 characters.

Identification of True hybrids: 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. Moreover, exact identification is done when the F_1s are raised and examined at the field level.

Table 1. The per cent success rate of artificial hybridization in groundnut

Crosses	Total no. of Flowers hybridized	No. of Pods yielded	% Pod set	% Seed set	No. of true F_1s identified	% of successful hybridization	Polymorphic marker used to identify true F_1
CO 7 X VRI3	239	53	22.2	71.7	13	34.2	GM1076
ICGV07222 X VRI3	238	62	25.9	45.8	12	42.3	GM1076
VRI 6 X VRI3	230	57	24.8	48.3	10	36.3	GM1076
VRI 8 X VRI3	217	35	16.0	89.9	9	28.6	GM1076
GPBD 4 X VRI3	219	35	15.7	73.3	14	32.5	GM1076
CO 7 X CHICO	234	35	14.7	63.2	12	33.1	GM2265
ICGV07222 X CHICO	208	29	14.0	75.5	15	29.4	GM2265
VRI 6 X CHICO	227	49	21.4	87.3	12	27.9	GM2265
VRI 8 X CHICO	216	51	23.5	70.9	12	33.2	GM2265
GPBD 4 X CHICO	228	32	13.9	86.2	10	34.6	GM2265
CO 7 X GANGAPURI	247	58	23.3	60.6	11	31.3	TC4H07
ICGV07222 X GANGAPURI	241	58	24.1	78.4	12	26.4	TC4H07
VRI 6 X GANGAPURI	237	42	17.8	80.1	11	32.7	TC4H07
VRI 8 X GANGAPURI	206	14	6.7	83.2	13	27.9	TC4H07
GPBD 4 X GANGAPURI	211	51	24.1	65.0	12	36.2	TC4H07
CO 7 X ICGV91114	256	28	11.0	54.6	14	33.2	GM2407
ICGV07222 X ICGV91114	290	42	14.5	83.7	15	35.4	GM2407
VRI 6 X ICGV91114	262	32	11.9	79.8	12	31.3	GM2407
VRI 8 X ICGV91114	291	32	10.8	77.6	14	37.2	GM2407
GPBD 4 X ICGV91114	253	31	12.1	63.5	12	37.5	GM2407
CO 7 X ICGV93468	215	45	20.9	80.6	12	33.1	GM1311
ICGV07222 X ICGV93468	234	31	12.9	89.5	11	35.3	GM1311
VRI 6 X ICGV93468	258	44	16.8	89.0	13	33.2	GM1311
VRI 8 X ICGV93468	249	37	14.6	67.5	16	34.1	GM1311
GPBD 4 X ICGV93468	271	51	18.8	85.6	12	27.5	GM1311

1. In the F_1 crop cultivated the male parent characters are looked upon for their identification. In the present study, the female and male parents differed in duration of maturity. Therefore the plants that showed first flowering along with its respective male parent and before its female parent were identified as true F_1s and tagged.
2. The seed coat colour was also used to identify true F_1s. A mixture of both parental seed coat colour was observed in the F_1 seeds (**Fig.1**).
3. Molecular identification: The DNA from the tagged F_1 plants was extracted by the CTAB method. SSR markers referred by Sujay *et al.* (2012), were amplified in PCR to study the polymorphism among the parents. The markers identified as polymorphic among the parents were used to identify the F_1s in which both the parental bands observed due to the co-dominant nature of the marker.

The polymorphic SSR markers which could be used to identify the F_1 of the crosses under study are presented in **Table.1.** Thus, the truthfulness of the tagged F_1s was confirmed at the molecular level using SSR marker.

With the data of the number of flowers hybridized, the number of pods formed and the number of healthy seeds obtained the percentage of pod and seed set were calculated. The total number of seeds from the sound mature pods was counted and the number of true F_1s identified was recorded to obtain the percentage of success of crossing. The calculation of successful crossing percentage for each of the crosses was done by the formula:

$$\frac{\text{Number of True hybrids identified}}{\text{Number of seeds sown}} \times 100$$

RESULTS AND DISCUSSION

The number of flowers hybridized in each cross, the number of pods yielded from the crosses, percentage of pod and seed set, the number of true F_1s identified in each cross and their respective hybridization success rate are furnished in **Table1**.Success rates of artificial hybridization can be affected by multiple factors such as humidity, temperature, crossing schedule, peanut genotype, operators and integrity of emasculated flowers, pollen pistil compatibility etc. In the present study withering

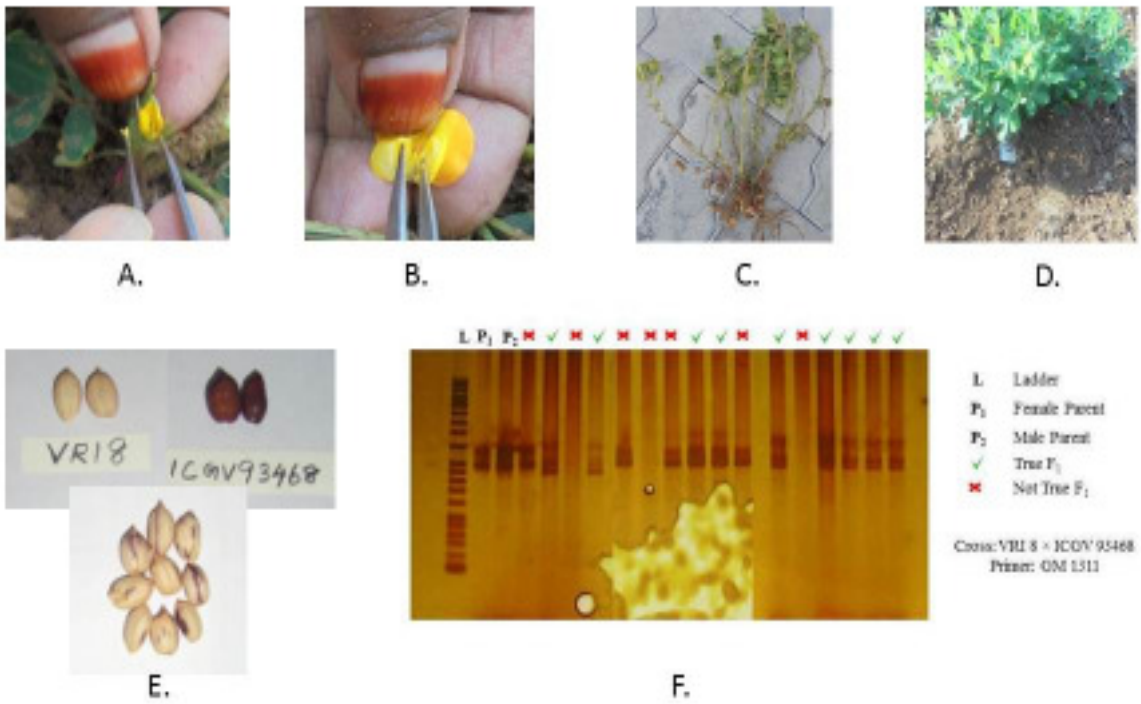

Fig.1 A. Emasculation, B. Dusting of pollen, C. Crossed pods, D. Tagged F_1s in field, E. F_1pod colour along with parents,
F. Screening of F_1s in cross : VRI 8 ×ICGV 93468 with GM 1311

of crossed flowers and ill filled pods formed the major criterion for the reduction in the success rate of hybridization. Also, selfed seeds were developed along the F_1s which were discarded after identification of hybrids. The true hybrid yield ranged from 25.4% to 42.3% in the crosses VRI 8 X ICGV93468 and ICGV07222 X VRI3 respectively.

The success of artificial cross-pollination in groundnut varies from 38 to 70 per cent depending upon the efficiency of the operator as reported by Halim and Ahmad(1980) and Nigam *et al. (*1981). In earlier studies, 70 to 90% of hand pollinations were reported to achieve fertilization (Norden and Rodriguez, 1971) and 26% to 89% of pollinations have resulted in viable hybrids (Banks, 1976). The fact that each successful cross-pollination yields a few seeds, there is always emerging modifications in the technique of artificial hybridization to achieve a high success rate. The heterogeneity of crosses from parental lines was assessed conventionally performed by visual selection of dominant phenotypic traits transferred from male parents. When there is no apparent visual marker there is a chance of that seeds from self-pollination could be mixed with hybrids. This increased cost and difficulty of subsequent generation advancement and selection. With the implementation of genetic markers, homozygous parental lines for the traits of interest could be selected prior to the crossing and F_1 hybridity can be checked at the earliest stage of seed germination (Favero *et al.*, 2006; Chu *et al.*, 2011).

Thus, from the above study conducted the hybridization of groundnut flowers was successful to a level of 42.3%. The usefulness of molecular markers in the detection of the true hybrid is also well understood.

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