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SHORT RESEARCH ARTICLE

An easy method of artificial hybridization in two arid legumes, guar (*Cyamopsis tetragonoloba* Taub.) and moth bean [*Vigna aconitifolia* (Jack.) Marechal]

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Abstract

Moth bean and guar are important rain-fed crops of the western arid region of India. The genetic improvement mostly realized through germplasm evaluation and selection has successfully increased the yield potential and stability. However, continued improvement has been hampered due to the inefficient or non-availability of crossing techniques for recombination breeding. We attempted direct pollination without emasculation and found it very successful compared to earlier techniques used in both crops. The article provides a detailed method for the selection of buds and pollination.

Keywords: Cluster bean, Guar, Hybridization, Inheritance, Moth bean

Both moth bean (*Vigna aconitifolia* Jack.) and guar (*Cyamopsis tetragonoloba* Taub.) are an integral part of subsistence farming in the western arid region of India. Their capacity to tolerate high temperatures and prolonged drought spells adapt to this region's growth. Both the crops serve the purpose of food and fodder, and roots improve soil health by adding atmospheric nitrogen to the soils. Guar has industrial importance for its high-quality gum (galactomannan) accumulated in endosperm during seed development (Sharma et al. 2021b; Kumar et al. 2017). Moth bean seed is an important ingredient of "Bikaneri Namkeen"; a big food industry, in addition to routine household use as pulse, sprouts, and breakfast items (Sharma 1 et al. 2020).

Most of the produce (>80%) of these two crops is contributed by the Rajasthan state of India since these crops provide the required nutrition and income in a climate that supports limited crop species to grow. The area and production of guar fluctuated from 2.5 to 5.0 mha and 1.0 to 2.9 mt, respectively, while that of moth bean from 1.0 to 1.6 m ha and 0.04 mt to 0.8 mt, respectively in the last 10 years in Rajasthan. The cultivation of these two crops depends largely on climatic conditions, especially rainfall during the crop growing period. Their productivity remains very low (usually below 400 kg/ha for guar and less than 300 kg/ha for moth bean) despite the availability of advanced varieties.

Understandingly, poor climatic conditions and management contribute to poor productivity of the crops. Nevertheless, the genetic improvement for increased yield and stable performance of genotypes under challenging growing conditions remains the most important strategy to make crop more remunerative. Germplasm collection activities in guar were started in 1961 by the Division of Plant Introduction, Indian Agricultural Research Institute (Now National Bureau of Plant Genetic Resources-NBPGR), New Delhi. The genetic improvement activities were started by various state universities in both the crops before the commencement of the All India Coordinated Research Project on Arid Legumes in 1995. The first variety by selection in guar was released in 1964 (Type 1) and moth bean in 1967 (Type 1). Since then, about 40 varieties in guar and 14 varieties in moth bean have been released at the

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national level.

Moth bean has a chromosome complement of 2n = 22 that is common to most *Vigna* spp. with one known exception. The karyotype formula, as per Bhatnagar et al. (1974), is 1-long (2.7-3.5μm) sub-median centromere + 5 medium (1.96-2.6 μm) sub-median centromere + 1 medium median centromere and four small (<1.95μm) median centromere. Rangaswamy and Krishnaswamy (1933) observed 7 and 14 as the guar's haploid and diploid chromosome numbers. Frahm-Leliveld (1966), Srivastava and Naithani (1964), and Bir and Sidhu (1967) confirmed these numbers. Germplasm evaluation contributed most of the early released varieties in guar. Artificial hybridization was delayed, and Agaita Guara 111 was the first released variety in 1978. Later breeding efforts included selection, mutation, and hybridization methods with the dominance of hybridization program in varietal contribution. However, mutation breeding was followed in moth bean germplasm selections without any systematic hybridization program for genetic improvement. Only limited inheritance studies are available in guar (Mahla et al. 2020; Sharma 2 et al. 2021a; Chaudhary and Lodhi 1981; Ray and Stafford 1985), while such studies are absolutely lacking in moth bean.

A strong hybridization program is basic to recombination breeding allowing novel combinations of desired traits for the improvement of yield. Systematically developed segregating populations are also important for genetic and molecular studies of traits to identify genes, biochemical pathways, and precision breeding. The small flower size and sensitivity to manipulation have been restrictive to a strong recombination breeding support in these two species. Alike in most other leguminous crops, moth bean and guar have strict self-pollination imposed by cleistogamy. The efforts for emasculation and pollination are thwarted due to very small flower size (5-7 mm in guar and even smaller 4 to 4.5 mm in moth bean) and high sensitivity to manipulation and receptivity of stigma. In both the species though anthesis may continue throughout the day starting from early morning (before 5 am), the maximum is around sunrise 7:0 to 9:0 am. A high proportion of pod settings during artificial hybridization is a prerequisite for successful employment in legume genetic improvement programs. Considering the above limitations, we are proposing an efficient artificial crossing protocol in guar and moth bean.

Guar racemes borne in the axils of leaves contain about 10- 30 or more flowers but convert 4 to 8 into pods. Newly emerging raceme with mature lower buds is preferred for artificial crossing. We tried emasculation of buds that were just turning yellow or light green from immature green buds. Evening and early morning (5-6 am) emasculated buds were pollinated after sunrise (7-9 am) while simultaneous emasculation and pollination was also tried after sunrise. These efforts could produce <1% hybrid pods (Table 1). Emasculation proved to be highly inefficient, tiresome, and time-consuming. Alike in legumes, the ten stamens are arranged in two groups where filaments of 9 are fused to make a tube around style, leaving one stamen free (diadelphous). All the stamens, style and stigma are tightly enclosed by a keel that is further surrounded by wing and standard petals. This whole complex is a very small and closed system for damage-free and effortless emasculation.

To increase the frequency of pod setting, we tried pollination without emasculation, where buds were critically selected for optimal maturity to have a receptive stigma but have not yet shed pollen. Such buds opened easily with an outward push of forceps on standard petals, followed by tilting out the wings and pushing stigma out of the keel while holding them between left-hand thumb and forefinger. Open flowers, older and younger buds were removed from the inflorescence. Buds that had anthesis during maneuvering also produced hybrid seeds on immediate pollination. Exposing the stigma head with a gentle push of forceps on petals in comparatively mature buds is enough for pollination. Pollination is carried out by extracting the diadelphous stamens and gynoecium complex from a mature bud of the donor plant and rubbing dehisced anthers on the stigma head after confirmation of effective anthesis (Fig. 1). This method can be perfected to have a 10 to 30% pod setting from cross-pollinated buds. Three years of use of this technique produced about 28% success in pod setting, and 60% of the seeds obtained were hybrid as identified by the appearance of a dominant male

Table 1. Relative efficiency of pollination without emasculation for hybridization in Guar

Method	Period of experiment	Crossing attempts	Total pods harvested	Per cent pod setting	Total seeds	Hybrid plants	Per cent hybrid plants
Evening emasculation and morning pollination at 7:30 to 9:30 am	2014-2020	1600	12	0.8	43.0	34.0	79.1
Early morning emasculation (5-6 am) and pollination at 7:30 to 9:30 am	2016-2020	1200	15	1.3	35.0	23.0	65.7
Emasculation and pollination simultaneously at 7:30 to 9:30 am	2016-2020	1100	13	1.2	41.0	25.0	61.0
Pollination without emasculation at 7:30 to 9:30 am	2018-2020	200	56	28	125	75	60

Table 2. Relative efficiency of pollination without emasculation for hybridization in moth bean

Method	Period of experiment	Crossing attempts	Pods harvested	Per cent pod setting	Total seeds	Hybrid plants	Per cent hybrid plants
Evening emasculation and pollination at 7:30 to 9:30 am	2016-2020	600	0	0	0	0	0
Early morning emasculation (5-6 am) and pollination at 7:30 to 9:30 am	2016-2020	600	0	0	0	0	0
Emasculation and pollination simultaneously at 7:30 to 9:30 am	2016-2020	600	0	0	0	0	0
Pollination without emasculation at 7:30 to 9:30 am	2018-2020	100	8	8	42	23	54.8

Fig. 1. Exposed stigma of guar from mature yellow bud showing method of pollination

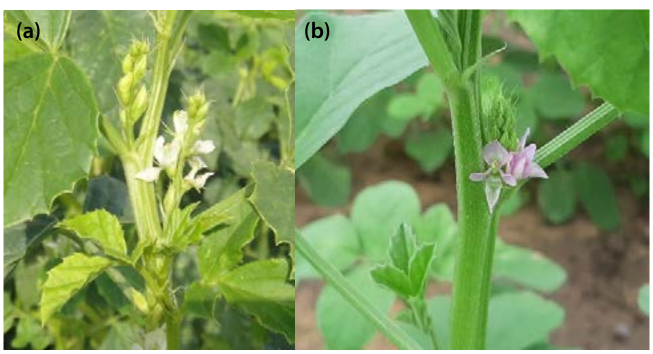

Fig. 2. Guar plants with inflorescence having contrasting traits: (a) hairy plant surface with pink flower and (b) glabrous plant with white flowers

trait in F_1 and segregation in F_2 (Table 1). Identification of F_1 hybrids is important with crossing techniques that evade emasculation. Parents harboring contrasting but simply inherited morphological traits are thus valuable for making crosses. Guar offered a large number of contrasting traits like; smooth and hairy surface, violet and white flower (Fig. 2a and Fig. 2b), growth habit (single stem regular bearing, single stem alternate bearing, branched, bushy, and basal branching), and inflorescence size (small and long). The appearance of a male trait in F_1 indicates to successful hybrid plant. Seed of individual hybrid plants was harvested separately and used to raise the F_2 population.

Fig. 3. Exposed stigma of moth bean from bud

Moth bean flower is smaller than guar and borne on smaller (2-5cm) or long (10-15 cm) delicate peduncles making handling tedious. Sessile flowers are delicately attached to the peduncle and detach with little pressure; for effective pollination, stigma was pushed out of the keel by inserting forceps between standard petals moving down through wing petals and entering the keel by tilting the forceps. The upward swapping of forceps unfolded the feathery stigma and exposed it out of the bud for easy pollination (Fig. 3). The feathery stigma was rubbed by dehisced anthers gently for achieving pollination. Moth bean offered a limited number of contrasting traits to identify hybrids. Shallow and deep leaf lobes (Fig 4), peduncle length (short thick v/s long thin), and plant stature (small branches and spreading types) are a few important traits. This method produced about 2-5% hybrid seeds successfully. We achieved about 8% success in the hybrid pod setting (Table 2). There is a possibility of introducing a limited number of contrasting morphological traits in diverse material to make crosses among desired parents. However, the use of molecular markers would extend the scope of crossing among any pair of diverse parents. Aroraand Jeena (2000), found pollination without

Fig. 4. Detecting deep lobed hybrid plants from maternal shallow lobed moth bean plants

emasculation to improve hybrid seed set by around 14% (8 to 22 %) in chickpea. The proposed technique would enable inheritance studies and systematic recombination breeding in the arid legumes, guar, and moth bean.

Authors' contribution

Conceptualization of research (HRM, RS); Designing of the experiments (HRM, RS); Contribution of experimanetal material (HRM, RS); Execution of field/lab experiments and data collection (HRM, RS); Analysis of data and interpretation (HRM, RS); Preparation of a manuscript (HRM, RS).

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