Evaluation of Pollination Control Methods for Pigeonpea (*Cajanus cajan* (L.) Millsp.) Germplasm Regeneration

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Maintaining the genetic integrity of germplasm accessions during regeneration is of paramount importance in *ex situ* conservation of plant genetic resources. In pigeonpea (*Cajanus cajan* (L.) Millsp.) where outcrossing by insects ranges from 3 to 26% (Reddy et al. 2004), regeneration is costly in terms of time and resources (Remanandan et al. 1988). The problems are compounded when several hundred germplasm accessions need to be regenerated in a season. Nestor and Ramanatha Rao (1998), analyzing the information on seed germplasm regeneration, noted much conjecture and uncertainty over regeneration procedures employed by genebanks. Therefore, the development of optimal procedures for regeneration, to preclude contamination of pollination, is vital to maintain genetic integrity of pigeonpea accessions. The RS Paroda Genebank at ICRISAT conserves 13,632 accessions of pigeonpea from 74 countries, including landraces, breeding lines, cultivars and wild relatives. Bagging individual plants/branches of pigeonpea with muslin cloth bags to control outcrossing was used for the past several years while regenerating the germplasm accessions at ICRISAT and elsewhere. The disadvantages of this method include mainly the high cost of muslin bags, time and labor required for bagging and its removal, and difficulty in bagging all plants when the number of accessions to be regenerated is high, particularly when these accessions belong to the same maturity group. In addition, inadequate plant protection and high humidity and temperature within the bag result in high flower drop and low seed yield.

In view of the above limitations of the bagging method, a new method of growing accessions under net cages was developed at ICRISAT, Patancheru. In the present study, the two methods were compared for cost benefits and the performance of the crop for important agronomic traits, including seed yield.

To evaluate the two pollination control methods, six accessions of pigeonpea germplasm (ICP 28, ICP 6907, ICP 7057, ICP 8863, ICP 8865 and ICP 11289) belonging to different maturity groups and flowering patterns were sown during the rainy season 2003/04. The experiment was conducted at ICRISAT research farm, Patancheru, India, laid out in split plot design with method of pollination control as main plot and genotype as subplot with two replications. To reduce the vegetative growth and facilitate easy bagging of plants and avoid damage to the net under cage method, the crop was sown late, during the 1st week of August in both years in Alfisols (Remanandan et al. 1988). Each accession was grown on a nine-meter long ridge, spaced 75 cm apart. Plant to plant spacing was 25 cm, accommodating about 72 plants in 36 hills per accession. Crop was fertilized with 20 kg N and 40 kg P_2O_5 ha^{-1} as basal dose. The experiment was provided with life-saving irrigations and protected from pests and diseases adequately before bagging in the bagging method and throughout the crop growth period under cages.

In the bagging method, two plants of the same hill were covered with a muslin cloth bag of size 100×75 cm, after bud initiation but prior to flowering in any accession and the bag was closed tightly at the base of the plants to prevent the entry of insects. About 36 bags were used to cover 72 plants of an accession (Fig. 1). As a precautionary measure against insects, plants were sprayed with appropriate insecticide just before bagging.

The other method of pollination control used cages made of prefabricated iron frames of 3 m $\times$ 3 m size and polypropylene net. Iron frames were fabricated such that they can be conveniently erected and dismantled. The iron frames can be used for 15 seasons or more and the polypropylene net can be used for 5-6 seasons. After bud initiation but prior to flowering in any accession, frames were fixed in the field and several such frames joined together to cover about 0.5 ha accommodating 550 accessions. These frames were covered with eight polypropylene net pieces measuring 25 $\times$ 25 m each stitched together. The cages were sealed all around with soil at the ground level to prevent the entry of pollinating agents and other insects as shown in Figure 2. Adequate plant protection measures were taken inside the cage.

At maturity, dry pods from all plants of an accession were harvested, bulk threshed and processed for conservation. Costs common to both methods of regeneration were not included in estimating the costs of individual pollination control methods.

To study the agronomic performance of accessions grown under two pollination control methods, observations on 10 important agronomic traits (days to 50% flowering, plant height, number of primary and secondary branches, days to 75% maturity, seeds per pod, 100-seed weight, seed yield $plant^{-1}$, harvest index (%) and plot seed yield (kg ha^{-1}) were recorded in accordance with the 'Descriptors for Pigeonpea' (IBPGR and ICRISAT

1993). Data were analyzed using GENSTAT 6.1. The cost of pollination control per accession in perpetuity with a regeneration frequency of 15 years was estimated using the following formula of Koo et al. (2002).

The in-perpetuity cost of an operation that is performed every n^{th} year from zero with a cost of X is given by

$$C_0^n = X + \frac{X}{(1+r)^n} + \frac{X}{(1+r)^{2n}} + \ldots = X\left[1 + \frac{X}{(1+r)^n} + \frac{X}{(1+r)^{2n}} + \ldots\right] = \frac{X}{(1-a)^n}$$

Where, C= Cost of pollination control per accession in perpetuity, n = frequency of regeneration, a = 1/1+r, r = rate of interest and X= cost of one cycle of regeneration per accession.

The cost estimates revealed that pollination control using cages was 3 times less expensive than the bagging method. The estimated cost saving per accession was US$ 7.83. With a 15-year regeneration interval, the cost of pollination control per accession would be US$ 26.33 for the bagging method and US$ 8.72 for the cage method when real rate of interest is 4%. The estimated net saving in perpetuity over the entire collection of 13,632 accessions by switching to cage method would be US$ 2,40,176 (Table 1). The net savings will increase with the increase in number of accessions in the genebank. The difference in initial investment on purchase of bags (US$ 11,250) and cages (US$ 12,435) for 550 accessions is not much (US$ 1185). In addition, we need to purchase bags every alternate year.

Analysis of variance over ten agronomic characters showed significant differences ($p < 0.0001$) between the methods for plant height, number of primary branches, days to 75% maturity, 100-seed weight and highly significant differences for seed yield. All accessions except ICP 28, a short-duration and short-height accession with determinate flowering pattern, performed well under cages and yielded significantly high yields. Optimum seed yield in accessions like ICP 28 can be achieved by growing them as separate groups. This grouping will reduce the problem of shade due to tall, spreading, indeterminate and late-maturing accessions grown in adjacent rows. Grouping also facilitates adequate plant protection.

Relatively higher temperature and humidity inside the muslin cloth bag resulted in increased flower drop and reduced seed yield. It is also more likely that the microclimate within the bag may facilitate the growth of seedborne fungi, thus affecting the seed quality. Krishnasamy (1990) reported that growing eggplant crop in net cages results in the exclusion of insects that damage the crop. In addition, in the bagging method, covering all branches of two plants with a muslin cloth bag may not be possible and the seed from open pollinated branches cannot be used for conservation. It is clear from the results of the present study that we can regenerate large number of accessions at a time safely and cost-effectively under cages, even when many accessions to be regenerated belong to same maturity group. Increased seed yield under cage method minimizes the regeneration frequency of

Table 1. Cost (US$) of pollination control methods in pigeonpea.

Items	Bagging	Cages
Cost of pollination control materials per year(muslin cloth bags, iron frames and net)	5625	1656
Labor (for bagging and bag removal, construction and dismantle of cages)	803	436
Plant protection	14	41
Total cost for 550 accessions	6442	2133
Cost for one accession	11.71	3.88
Cost for one accession in perpetuity	26.33	8.72
Cost for 13,632 accessions in perpetuity	359 193.86	119 018.18

Figure 1. Field view of pigeonpea germplasm accessions covered with muslin cloth bags to prevent outcrossing.

Figure 2. Pigeonpea germplasm accessions grown under pollination control cages to prevent outcrossing.

accessions, thereby reducing the maintenance costs of total collection in perpetuity.

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