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Short Communication

Stigma receptivity in pigeonpea [*Cajanus cajan* (L.) Millspaugh]

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Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a partially out-crossed species and on average, the insect-aided natural out-crossing varies around 25-30% [1]. The pigeonpea breeders have used crop improvement procedures relevant to both self as well as cross pollination mechanisms. Since, hybridization is an integral part of genetic enhancement programmes, an adequate knowledge of pollination behaviour helps in deciding mating schemes. In pigeonpea, where hybrid breeding technology has been developed [2] , the duration of stigma receptivity of female parent plays an important role in enhancing the quantities in insect-aided seed production of hybrids and their parents. Therefore, a study was undertaken at ICRISAT to understand the stigma receptivity pattern in pigeonpea.

Seeds of a cytoplasmic-nuclear male-sterile line ICPA 2039 and its maintainer [3] were sown during 2006 rainy season in field inside an insect-proof nylon cage. To study the stigma receptivity hand pollinations were carried out on male-sterile plants using pollen of the maintainer line at different stages of flower bud growth of female parent. These materials and methods not only excluded the need of emasculation for controlled pollinations but also avoided chances of accidental self- or cross-pollination.

To identify appropriate bud size for maximizing pod set after hand pollinations, five young buds with petals just emerging (Fig. 1, inset) were selected randomly on five male-sterile plants and photographs were taken at 24 h intervals starting 0830 hrs on December 15, 2006. The day when the flowers opened was designated as 'Day 0'. It took four days for the selected young buds to open and another four days to drop from the pedicle base. The initially selected bud stage was designated as 'Day -4' and the subsequent stages after each 24 h were designated as 'Day -3', 'Day -2', and 'Day -1'. Similarly, the stages after flower opening were designated as 'Day +1', 'Day +2', 'Day +3', and 'Day +4' (Fig 1). To develop a visual bud selection index for hybridization, the lengths of corolla and calyx were measured on each day and the ratios of corolla to calyx were estimated. These indices ranged from 0.14 ± 0.06 (Day -4) to 3.0 ± 1.34 (Day +4) and at 'Day 0' stage this ratio was 2.6 ± 1.16.

Ten male-sterile plants of ICPA 2039 were selected randomly and one plant was assigned for pollinating one stage of bud. In male-fertile plants of the maintainer line the pollen dehiscence started a day before flower opening i.e. at Day -1 stage and the dehisced pollen grain remained intact on the anther lobes up to Day +1. During this period the pollen grains exhibited >95% viability when examined under microscope using 2% aceto-carmine solution. Therefore, for pollinations fully developed but unopened flower buds were harvested from the maintainer line and 100 pollinations were done at each stage (from Day -4 to Day +4). To minimize the possible effect of micro-environment on fertilization only 10 pollinations were done daily on each bud stage. The targeted pollinations were completed between December 15 – 24. Each pollinated bud was tagged with a thread for identification and pod set was recorded three weeks after completing the pollinations. During the experimental period the mean minimum and maximum temperatures ranged between 8.6 and 13.2^{o}c and 25.8 and 29.4^{o}c, respectively with maximum relative humidity ranging between 92 – 98%. The pod set after hand pollinations was considered as indicator of stigma receptivity.

On average it took nine days for unfertilized young floral buds to complete their life cycle and abscise. Only 2% pod set was recorded when the pollinations were made 48 h before (Day-2) flower opening and prior to this no pod set was observed. On the subsequent day (Day-1), the pod set improved rapidly and it was highest (98%) when pollinations were made on the day of flower opening (Day 0) and it remained in the high regime for another two days. Subsequently, the pod set declined with time and there was no pod set 96 h after Day 0. The variation observed in pod setting at different stages could be attributed to the inherent developmental changes in stigma and embryo sac of the female flowers. The high pod set on the day of flower opening suggests that perhaps most of the egg cells developed on that day. The process of fertilization may also be influenced by the moisture availability at stigmatic surface, ability of pollen germination and pollen tube growth. These aspects, however, were not studied in the present experiment. The large variation observed in grain setting in silky oat [4] was attributed to the changes in stigma and embryo sac structures while in buffelgrass [5] it was due to the protogynous nature of the flower. In pigeonpea such studies are needed to understand the role of developmental changes in stigma and embryo sac in pod set. The decline in the pod set after flower opening could be due to withering of the embryo sac and/or desiccation of stigmatic surface.

The results of the present experiment revealed that in pigeonpea the receptivity of stigma started 48 h before flower opening and continued to be receptive 72 h there after, but within this period a considerable variation for pod set was observed on different days. Prasad *et al.* [6] reported that at Ranchi, Bihar the stigma receptivity in pigeonpea started 68 h before flower opening and it continued up to 20 h after flower opening. The differences observed in the present study and that of Prasad *et al.* [6] could be attributed to the differences in the methodology and/or the genotypes used in the studies, and environmental conditions. From the present study it is concluded that for optimizing pod set in Patancheru environment the pollinations should be initiated a day before flower opening and be continued for three days. To select the appropriate floral buds for pollination the corolla to calyx index should be between 1.8 ± 0.80 to 3.0 ± 1.34. This index, however, should be considered with caution as in the present study the error components were high due to small sample size.

The present studies showed that at Patancheru the stigma of ICPA 2039 remained receptive for a total of about 120 h. Since honey bees (*Apis spp.*) visit

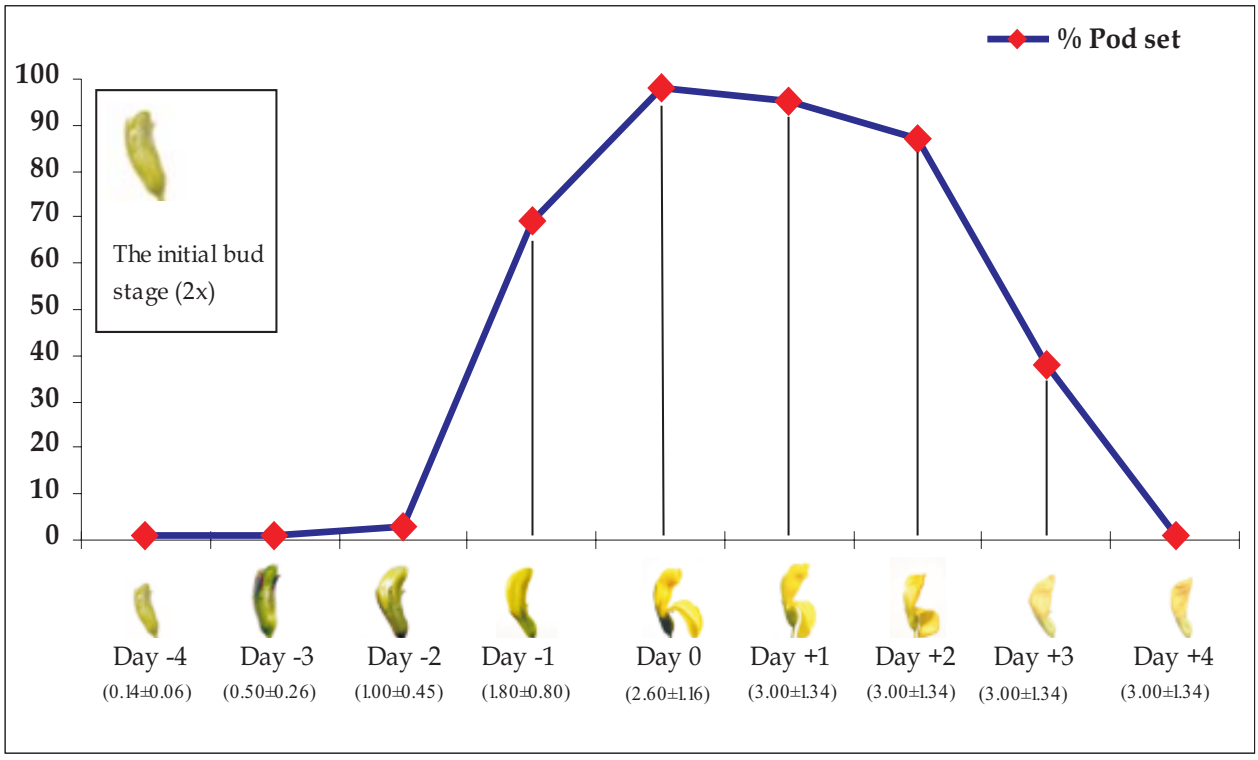

Fig. 1. Percent pod set on the male-sterile plants of ICPA 2039 at Patancheru, 2006 rainy season

pigeonpea flowers after they open and from this time the stigma remains receptive for 72 h. This period coincides with high activity of pollinating insects, which are responsible for cross pollination in this crop. The high yields recorded in the large-scale hybrid (male-sterile × male-fertile line) pigeonpea seed production studies under natural conditions [2,7] confirm this hypothesis. The information generated from this study can also be used to optimize the pod set when crosses are made between two male-fertile lines where emasculation of female flowers is essential. Since pollen dehiscence starts a day before flower open and maximum pod set is observed on the day of flower opening it may be recommenced that for optimizing the pod set emasculations be done at Day -2 stage and pollinations could be made either on Day -1 or Day 0 stages, provided humidity is not a constraint.

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