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Full Length Research Paper

A study on stigma receptivity of cytoplasmic-nuclear male-sterile lines of pigeonpea, *Cajanus cajan* (L.) Millsp.

R. H. Luo[1], V. A. Dalvi[1], Y. R. Li[1] and K. B. Saxena[2]

[1]Crop Genetic Improvement and Biotechnology Laboratory, Guangxi Academy of Agricultural Sciences, Nanning, P.R. China 530007.
[2]International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, A.P. India.

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Stigma receptivity in pigeonpea [*Cajanus cajan* (L.) Millsp.] was studied using 2 male-sterile lines (ICPA 2039 and ICPA 2043) under field conditions. An experiment was conducted to observe the stigma receptive period at Nanning (22°N 108°E) in Southern China. The study revealed that the stigma was receptive 48 h before flower opening and continued up 4 days after flower opening. The peak stigma receptivity was on the day of flower opening with 84 and 86% pod set after hand pollination in the male-sterile lines ICPA 2039 and ICPA 2043, respectively. This floral stage is characterized by the corolla to calyx length ratio of 2.75 ± 0.075 (ICPA 2039) and 2.60 ± 0.283 (ICPA 2043). The long time span of stigma receptivity in pigeonpea encourages insect-aided natural out-crossing. This information will help breeders to carry out hybridization activity with high success rates. Also the long receptivity may facilitate more seed yield in isolated seed production blocks.

Key words: CMS lines, pigeonpea, stigma receptivity.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a commercially important legume crop. In recent past, hybrid breeding is considered as an effective tool in this crop (Stakstad, 2007). In crop plants like pigeonpea where male-sterility system is recently developed, it is necessary to study the environmental effect on stigma receptivity duration. Stigma receptivity is an important factor to have higher success rate of natural out-crossing. The literature reports some studies on this aspect but very few with male-sterile lines. With the development of the cytoplasmic-nuclear male-sterile (CMS) lines, it is necessary to study this aspect in detail to enhance the utilization of CMS technology.

For successful commercialization of any hybrid, easy seed production method is a pre-requisite, which is dependant on insect behavior in the particular location, stability of male-sterile line, and duration of stigma receptivity (Saxena et al., 2006). This paper emphasizes the research need of stigma receptivity in China. Already some research has been done in India on pigeonpea (Prasad et al., 1977) and other crops such as silk oat (Kalingnire et al., 2000) and bullelgrass (Shafer et al., 2000). This research paper confirms the previous findings of stigma receptivity in pigeonpea but at different environmental conditions. This will help in standardization of the stigma receptivity time at different locations.

*Corresponding author: E-mail: vijay_dalvi79@rediff.com. Tel.: +86 -13407704550.

MATERIALS AND METHODS

For the present study the seeds of cytoplasmic-nuclear male-sterile (CMS) lines ICPA 2039 and ICPA 2043 (along with its maintainers) were sown in isolation during 2008 rainy season (Crop duration from June - January) in field at Guangxi Academy of Agricultural Sciences, Nanning (22°49′ N), China. 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 buds. As the female parent is male-sterile, there was no need of emasculation for controlled pollinations and this avoided chances of accidental self- or cross-pollination due to isolation.

To identify appropriate bud size for maximizing pod set after hand

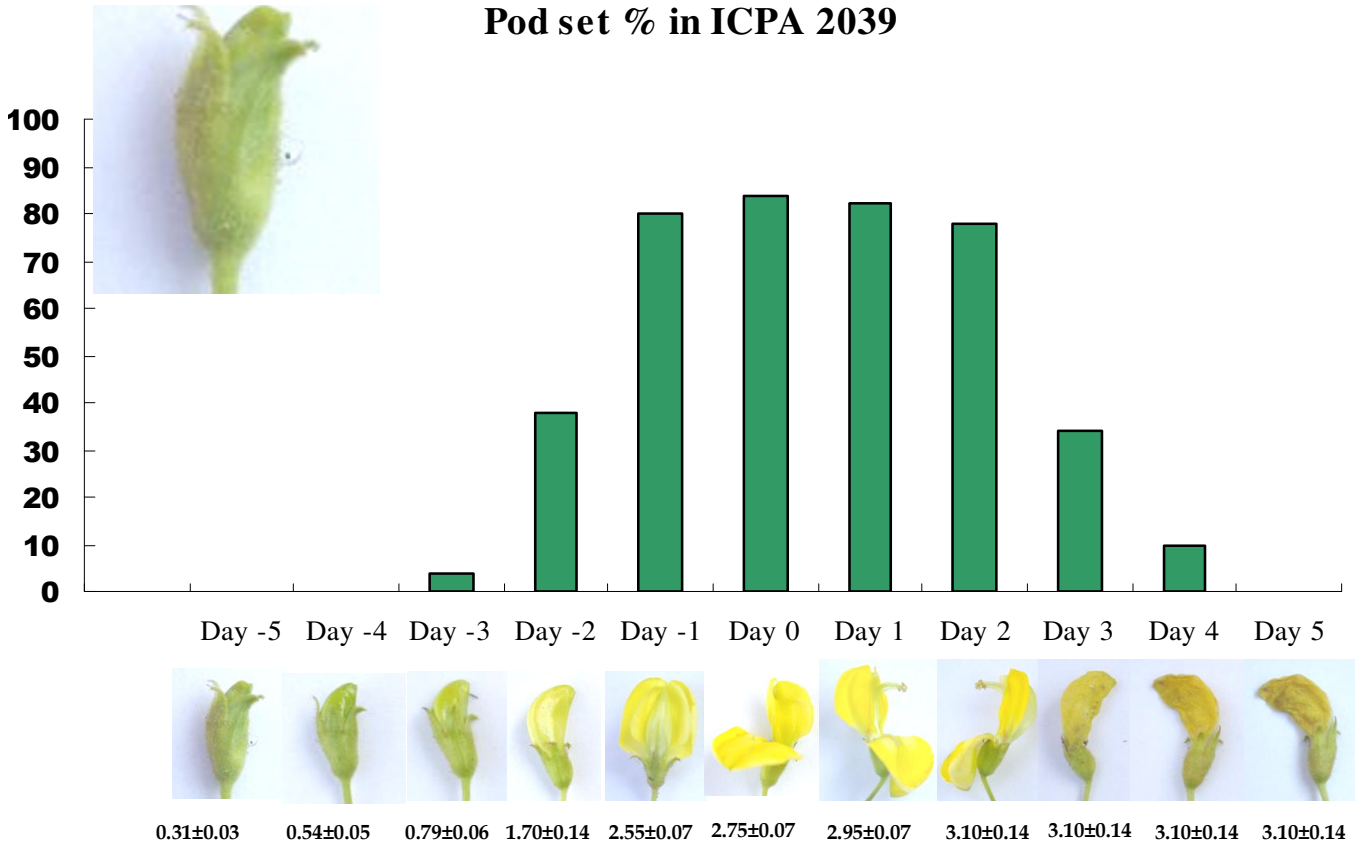

Figure 1. Pod setting (%) in male-sterile line ICPA 2039 at Nanning, China.

pollinations, 5 young buds with petals just emerging (Figure 1, inset) were selected randomly on 5 male-sterile plants and photographs were taken at 24 h intervals starting 0930 h. The procedure used by Dalvi and Saxena (unpublished) was used for this study. The day when the flowers opened was designated as 'Day 0' and it took 5 days for the selected young buds to open and another 5 days to drop from the pedicel base. The initially selected bud stage was designated as 'Day 5' and the subsequent stages after each 24 h were designated as 'Day -4', 'Day -3', 'Day -2', and 'Day -1'. Similarly, the stages after flower opening were designated as 'Day +1', 'Day +2', 'Day +3', 'Day+4' and 'Day +5' (Figure 1). To develop a visual bud selection index for hybridization, the lengths of corolla and calyx were measured on each day and their ratios were estimated. In case of ICPA 2039 these indices ranged from 0.31 ± 0.034 (Day -5) to 3.10 ± 0.141 (Day +5) and at 'Day 0' stage this ratio was 2.75 ± 0.075. Similar indices were recorded in case of ICPA 2043 male-sterile line. The Day-5, Day 0 and Day+5 indices were 0.27 ± 0.025, 2.60 ± 0.283 and 2.90 ± 0.141, respectively.

11 male-sterile plants each of ICPA 2039 and ICPA 2043 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 that is, 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 respective maintainer lines and 50 pollinations were done at each stage (from Day -5 to Day +5). To minimize the possible effect of micro-environment on fertilization only 10 pollinations were done on each bud stage every day. The targeted pollinations were done during 21st - 25th December 2008. Each pollinated bud was tagged with a thread for identification and pod set was recorded 3 weeks after completing the pollinations. The pod set after hand pollinations was considered as indicator of stigma receptivity.

RESULTS

The present experiment revealed that it needs on average of about 11 days for a tiny bud to complete its life as flower. In this study we have examined 2 cytoplasmic-nuclear male-sterile lines viz. ICPA 2039 and ICPA 2043. Only 4% pod set was recorded in ICPA 2039 when the pollinations were made 72 h before anthesis and prior to this no pod set was observed indicating stigma was not receptive at that time. On the subsequent day, the pod set improved rapidly and it was highest (84%) when pollinations were made on the day of flower opening (Day 0) and it remained in the high regime for another 2 days with 82 and 78% pod set (Figure 1). Subsequently, the pod set declined with time and there was no pod set 96 h after flower opening (Day 0). In case of ICPA 2043 the pod set was considerable (14%) 72 h before flower opening and the maximum pod set was 86% on the day of flower opening (Figure 2). The pod set declined further

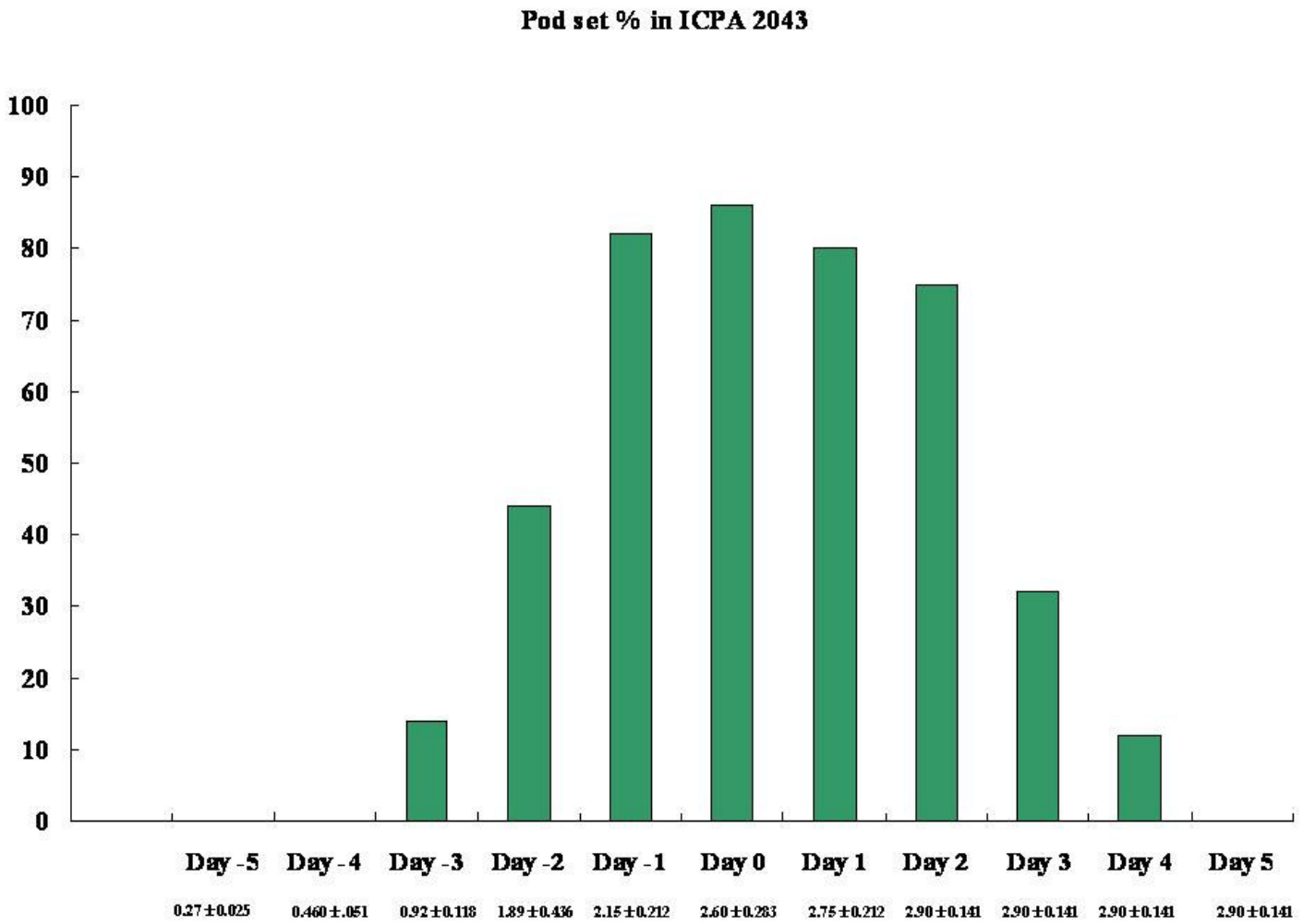

Figure 2. Pod setting (%) in male-sterile line ICPA 2043 at Nanning, China.

and no pod set was observed on Day +5. This experiment showed that in ICPA 2039 the stigma was receptive for 6 days. Such longer stigma receptive period in male-sterile lines will help for better pod set by hand pollination. Similarly, in ICPA 2043 the stigma was receptive for 6 days. There is need to study the effect of environmental factors such as temperature and humidity, whether these parameters have any effect on the duration of stigma receptivity. The mean temperature during the study period was 13°C with a mean relative humidity of 72%.

DISCUSSION

The previous studies predicted that 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 large variation observed in grain setting in silky oat (Kalinganire et al., 2000) was attributed to the changes in stigma and embryo sac structures while in buffelgrass (Shafer et al., 2000) 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 rate is attributable to the age of the flower.

The results of the present experiment showed that in pigeonpea the receptivity of stigma started 48 - 72 h before flower opening and continued to be receptive 96 h there after, but within this period a considerable variation for pod set was observed on different days. Dalvi and Saxena (unpublished) also observed a similar trend for stigma receptivity duration at Patancheru, India. The previous studies reported 68 h stigma receptivity before flower opening and 20 h after flower opening at Bihar, India by Prasad et al. (1977). The differences observed in various studies could be attributed to the differences in the methodology and/or the genotypes used in the studies and environmental conditions. From the study conducted at Patancheru, India it is concluded that for

maximizing pod set 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 calyx to corolla index should be between 1.8 ± 0.80 to 3.0 ± 1.34. Our results are in accordance with this study.

The present study showed that at Nanning the stigma of ICPA 2039 remained receptive for a total of about 192 h (including the short period at Day -3). In case of ICPA 2043 the stigma receptivity duration was the same. Since honey bees (*Apis spp*.) visit pigeonpea flowers after they open and from this time the stigma remains receptive for 120 h and 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 (Saxena, 2006; IIPR, 2007) confirm this hypothesis. The information generated from this study can also be used to maximize the pod set when crosses are made between 2 male-fertile lines where emasculation of female flower is essential. Since pollen dehiscence starts a day before flower opening and maximum pod set is observed on the day of flower opening it may be recommenced that for maximizing 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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