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SHORT COMMUNICATION

EFFECT OF BENZYLADENINE ON PHOTOSYNTHESIS, FLORAL BIOLOGY CHARACTERS AND YIELD IN PIGEONPEA (*CAJANUS CAJAN* L.)

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Genotypes of pigeonpea namely Paras and Manak grown under field conditions were given cytokinin benzyl-adenine (BA; 100 µM) treatment at pre-sowing (seed priming) and flower initiation stage (foliar spray). BA treatments increased the number of branches, flowers and pods in both genotypes. However, seed priming was more effective than the spray treatment. *In vivo* pollen germination was sufficient enough to support fertilisation of ovules in both BA treated and untreated plants. Pollen tubes could not be traced in the lower region of style and ovary due to masking effect of copious hairs. Flowers in which *in vivo* pollen germination was lacking resulted in abscission of flowers. BA spray at flowering stage induced pollen germination in these flowers and thus a decline in percent abscission was evident. Seed treatment with BA resulted in an increase in number of pods in Paras, however, no such increase was observed in Manak. Increase in seed yield was also observed in Paras, the increase being more with seed treatment as compared to foliar spray. However, a decline in test weight of seeds was observed with both the treatments. The relevance of these observations is discussed.

***Key words*: Cytokinin, photosynthesis, pigeonpea, yield**

Cytokinins are implicated in many plant developmental processes and their environmental responses including senescence and abscission of different plant parts, apical dominance, chloroplast development and the regulation of cell division and sink/ source relationship (Nooden *et al*. 1990, Hutchison and Kieber 2002). Cytokinins have been reported to promote plant and leaf growth (Fariduddin *et al*. 2004), number of floral buds and pods (Reese *et al.* 1995, Nagel *et al*. 2001), and yield (Peterson *et al.* 1990). The cytokinin concentration is known to increase after fertilisation in many species which is corroborated with rapid cell division (Lur and Setter 1993). Evidences also implicate cytokinins as promoters in establishing sink potential at early stages of reproductive development (Lur and Setter 1993, Dietrich *et al*. 1995)

Flower abscission is a major physiological constraint which reduces yield significantly in most flowering plants because fruit setting is adversely affected. The phenomenon is very common in grain legumes such as cowpea, soybean, pigeonpea which shed most of their flowers leaving behind only a small proportion of flowers to set fruits (Togun and Tayo 1990). BA is reported to increase pod set and seed yield in legume crops (Barclay and McDavid 1998, Nonokawa *et al*. 2007). BA application to individual flowering racemes or to transpiration stream could prevent pod abortion in well-watered soybeans (Peterson *et al*. 1990, Mosjidis *et al.* 1993, Reese *et al.* 1995, Nagel *et al.* 2001, Cho *et al*. 2002, Nonakawa *et al*. 2007). Furthermore, application of cytokinins stimulated sucrose transport into cultured spikes of wheat resulting in more grain set (Borokovae

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and Prochazka 1992). These lines of evidence indicate that cytokinins can increase sink strength and thus increase the yield potential. Despite such an important role of cytokinins there are only a limited number of studies of cytokinin application in pigeonpea (Barclay and McDavid 1998, Mukherjee and Kumar 2007). Therefore, present investigations were conducted to study the effect of BA (seed priming and foliar spray at flowering stage) on photosynthesis, floral biology characters and yield in two important genotypes of pigeonpea.

Seeds of *Cajanus cajan* L. cv. Paras and Manak were soaked in water (Control and spray at flowering treatment) and 100 µM BA (seed treatment) for 4 h and sowing was done in the field in 24 experimental plots (4 replications for each treatment). Foliar spray of BA (100 µM) was done at the flower initiation stage. Plants sprayed with water served as control. Assimilation rate (µmole m^{-2} sec^{-1}) was measured by using portable Infra Red Gas Analyser (IRGA) between 10.00 and 11.00 AM at the flowering and pod formation stages. Five plants were tagged in each treatment and the number of flowers produced were counted every 5th day until completion. The plant height, number of branches, number of pods was recorded at maturity, test weight of 100 seeds and yield were recorded at final harvest. Stigma receptivity, *in vivo* pollen germination and tube growth was assessed after 48 h of anther dehiscence by aniline blue method using fluorescent microscope (Martin 1959). The data were statistically analysed using Randomized Block Design. Treatment differences were regarded as significant at $p < 0.05$.

No significant changes in plant height was observed with BA treatments in both the genotypes. Cytokinins are known to play an important role in branching of stem in crop plants (Cline 1997). The number of branches increased with BA treatment, the increase being more in Paras than Manak and the higher effect of BA was observed with seed priming as compared to foliar spray.. However, in Manak foliar spray of BA was more effective (Fig. 1). Increased branching with exogenously supplied cytokinins has been reported earlier (Cline *et al*. 2006, Ferguson and Beveridge 2009).

The photosynthetic rate increased with BA treatments, the increase being more in Paras as compared to Manak and BA foliar spray at flower initiation stage was more effective than seed treatment. Increase in photosynthetic rate with cytokinin treatments has been reported earlier (Liu *et al*. 2004). Cytokinins are known to stimulate the expression of photosynthetic enzymes like Rubisco and more generally the development of functional chloroplasts (Chory *et al*. 1994). Liu *et al*. (2004) have also reported that exogenous BA increased assimilation rate in well-watered soybean plants, which leads to a greater availability of photosynthates for pod growth, and may thereby increase pod set.

BA treatments increased quantitative production of flowers and the increase was more pronounced in Paras over Manak and BA applied as seed treatment was more effective than applied as foliar spray. Nooden *et al*. (1990) have demonstrated a correlation between endogenous levels of cytokinins and the level of flower abortion and pod set. Cytokinins are reported to play an important role in regulating flower and pod development in soybeans (Reese *et al*. 1995). Application of exogenous BA has been shown to prevent abortion of flowers and/or pods in legume crops (Peterson *et al*. 1990, Mosjidis *et al*. 1993, Reese *et al*. 1995, Barclay and McDavid 1998, Nonokawa *et al*. 2007).

Cytokinins play an important role in regulating crop reproductive development (Hare *et al*. 1997). The flowers of both the cultivars are yellow sometimes with purple or red streaks. Calyx is represented by five linear teeth. Anthers dehisce a day before anthesis with peak anthesis between 8.00 and 10.00 AM. Pods are flat, beaded, green, purple of mixed green purple, acuminate, pubescent, containing four to five oval to round seeds. According to Reddy (1990) cultivated genotypes of pigeonpea generally possess three to four seeds. The stigma gains receptivity before anthesis. Pods became visible after a week of pollination and attained maximum size by 15 to 20 days. Generally, one to five pods matured in each raceme. BA treatments did not affect time of anther dehiscence and stigma receptivity. Lack of pollen germination on the stigma was associated with abscission of flowers (Fig. 1c). Foliar spray of BA treatment applied at the flowering stage induced pollen germination, thus a decline in per cent abscission of floral buds corroborated with an increase in the number of

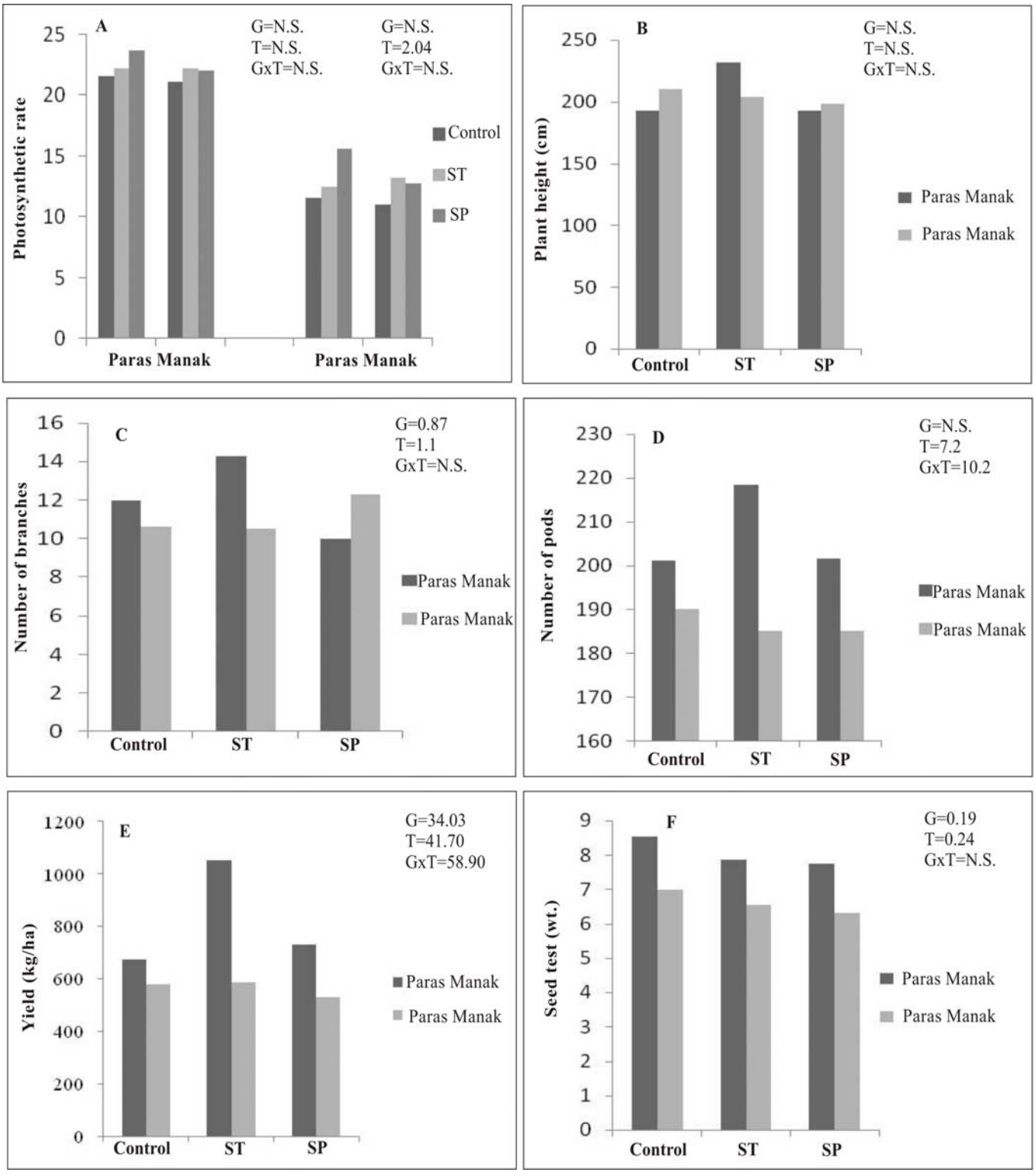

Fig. 1. Effect of BA applied as seed treatment (ST) or foliar spray at flowering (SP) on A-photosynthetic rate (µmol m^{-2} s^{-1}), B- Plant height (cm), C- Number of branches, D- Number of pods, E- Yield (kg/ha), F- Seed test weight (g).

Table 1. Effect of BA applied as seed treatment (ST) or spray at flowering (SF) on per cent increase in the number of flowers as observed every 5th day for 15 days in two pigeonpea genotypes

	Seed treatment		Spray at flowering	
	Paras	Manak	Paras	Manak
1st Day	12.5	10	32	20
5th Day	80	90	70	90
10th Day	416	284	87	90
15th Day	640	200	400	100

pods/plant was evident. Seed treatment with BA failed to show any significant effect. *In vivo* pollen germination in untreated as well as BA treated plants was sufficient enough to support fertilisation of ovules (Fig. 2b). Pollen tubes could not be traced in the lower region of the style and ovary due to masking effect of copious hairs (Fig. 2c). However, Dutta and Deb (1970) reported slow pollen tube growth which took 54 h to reach to the base of ovary.

A significant increase in number of pods was observed in Paras by BA given as seed treatment, however, no such increase was observed in Manak. Similar results were obtained in respect of seed yield.

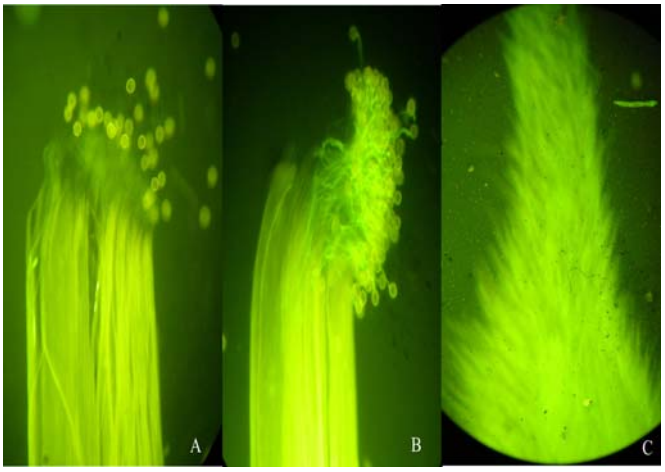

Fig. 2. Flourescent micrograph of pistils of pigeonpea
A. Pistil of abscised floral buds showing ungerminated pollen grains on the stigmatic surface.
B. Pistil showing profuse *in vivo* pollen germination and tube growth.
C. Portion of the style and ovary covered with copious hairs.

Paras registered a higher increase with seed treatment as compared to spray at flowering stage in terms of seed yield. Increased pod and seed yields in Paras in response to the BA treatments supported for the hypothesis that cytokinin availability limits potential seed production in some pigeonpea genotypes. Extensive work done in soybeans, report that cytokinins rescue of soybean flowers and fruits (Reese *et al*. 1995) and suggests that the hormone acts to redirect movement of assimilates into treated tissues, increasing sink strength and subsequent growth rates, and preventing abscission of the developing flowers and pods (Reese *et al*. 1995, Nagel *et al*. 2001). Although no attempt was made to measure flower abortion rates directly in this study, the increase in total number of flowers and pods per plant indicates that total flower set was increased concomitant with a decline in per cent abscission of floral buds by BA applications. A decline in seed test weight was observed in both the genotypes with both the treatments. Increases in pod and seed production in response to cytokinin application may occur at the expense of growth in other parts of the plant, through the recommitment of the available resources from shoot and root development into fruit production. Decreased seed test weight indicates limitation in terms of available resources. Further examination of the effects of cytokinin treatments on plant architecture and dry weight accumulation is needed to fully assess the potential for the redistribution of dry matter into the production of pods and seeds.

REFERENCES

Barclay, G.F. and McDavid, C.R. (1998). Effect of benzylaminopurine on fruit set and seed development in pigeonpea (*Cajanus cajan*). *Sci. Hortic.* **72:** 81-86.

Borokovae, V. and Prochazka, S. (1992). Preanthesis interaction of cytokinins and ABA in the transport of C^{14} sucrose to the ear of winter wheat (*Triticum aestivuum*). *J. Agron. Crop Sci.* **169:** 229-235.

Cho, Y., Suh, S.K., Park, H.K. and Wood, A. (2002). Impact of 2,4-D and BAP upon pod set and seed yield in soybean treated at reproductive stages. *Plant Growth Regul.* **36:** 215–221.

Chory, J., Reinecke, D., Sim, S., Washburn, T. and Brenner,

M. (1994). A role for cytokinins in de-etiolation in *Arabidopsis*. *Det* mutants have an altered response to cytokinins. *Plant Physiol*. **104:** 339–347.

Cline, M. (1997). Concepts and terminology of apical dominance. *Am. J. Bot.* **84:** 1064–1069.

Cline, M.G., Thangavelu, M. and Kim Dong, K. (2006). A possible role of cytokinin in mediating long-distance nitrogen signaling in the promotion of sylleptic branching in hybrid poplar. *J. Plant Physiol.* **163:** 684-688.

Dietrich, J.T., Kaminek, M., Blevins, D.G., Reinbott, T.M. and Morris R.D. (1995). Changes in cytokinin and cytokinin oxidase activity in developing maize kernels and effects of exogenous cytokinin on kernel development. *Plant Physiol. Biochem*. **33:** 327-336.

Dutta, P.C. and Deb, A. (1970). Floral biology of *Cajanus cajan* (L.) Millsp var. bicolor D.C. (Papilionaceae). Bull. *Bot. Soc. Beng.* **24:** 135-145.

Fariduddin, Q., Ahmad, A. and Hayat, S. (2004). Responses of *Vigna radiata* to foliar application of 28-Homobrassinolide and Kinetin. *Biol. Plant.* **48:** 465-468.

Ferguson, B.J. and Beveridge, C.A. (2009). Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. *Plant Physiol.* **149:** 1929-1944.

Hare, P.D., Cress, W.A. and van Staden, J. (1997). The involvement of cytokinins in plant response to environmental stress. *Plant Growth Regul*. **23:** 79-103.

Hutchison, C.E. and Kieber, J.J. (2002). Cytokinin signaling in *Arabidopsis. Plant Cell.* **14:** 547-559.

Liu, F., Andersen, M.N. and Jensen, C.R. (2004). Root signal controls pod growth in drought soybean during the critical, abortion-sensitive phase of pod development. *Field Crops Res*. **85:** 159-166.

Lur, H.S. and Setter, T.L. (1993). Endosperm development of maize defective kernel (dek) mutants. Auxin and cytokinin levels. *Ann. Bot.* **72:** 1-6.

Martin, F.W. (1959). Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol*. **34:** 125-128.

Mosjidis, C.O.H., Peterson, C.M., Truelove, B. and Dute, R.R. (1993). Stimulation of pod and ovule growth of soybean, *Glycine max* (L.) Merr. by 6-benzylaminopurine. *Ann. Bot.* **71:** 193-199.

Mukherjee, D. and Kumar, R. (2007). Kinetin regulates plant growth and biochemical changes during maturation and senescence of leaves, flowers, and pods of *Cajanus cajan* L. Biol. *Plant.* **51:** 80-85.

Nagel, L., Brewster, R., Riedell, W.E. and Reese, R.N. (2001). Cytokinin regulation of flower and pod set in soybeans (*Glycine max* (L.) Merr.). *Ann. Bot.* **88**: 27-31.

Nonokawa, K., Kokubun, M., Nakajima, T., Nakamura, T. and Yoshida, R. (2007). Roles of auxin and cytokinin in soybean pod setting. Plant Prod. Sci. **10:** 199-206.

Nooden, L.D., Santokh, S. and Letham, D.S. (1990). Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. *Plant Physiol*. **93:** 33-39.

Peterson, C.M., Williams J.C. and Kuang A. (1990). Increased pod set of determinate cultivars of soybean, *Glycine Max*, with 6-benzylaminopurine. *Bot. Gaz* **151:** 322–330.

Reddy, L.J. (1990). Pigeonpea :morphology. In: The Pigeonpea (Nene, Y.L., Hall, S.D. and Shiela, V.K. Eds). Wallingford, UK, CAB International pp. 47-86.

Reese, R.N., Dybing, C.D., White, C.A., Page, S.M. and Larson, J.E. (1995). Expression of vegetative storage protein (VSP-Beta) in soybean raceme tissues in response to flower set. *J. Exp. Bot.* **46:** 957-964.

Togun, A.O. and Tayo, T.O. (1990). Flowering and pod and seed development in pigeon pea *(Cajanus cajan). J. Agric. Sci. Cambridge.* **115:** 327-335.