Indian J. Hill Farmg. 14 (1) : 144 — 145 2001

BENZYL ADENINE INDUCED MULTIPE SHOOT INDUCTION FROM THE COTYLEDONARY NODE OF PIGEONPEA

Alpana Das, A. Pattanayak, H. S. Gupta* and B. K. Sarma
Division of Plant Breeding, ICAR Complex for NEH Region,
Umiam, Meghalaya

Pulses are the second most important crop in India occupying an area of 22-23 million hectares producing 10 –12 million tones of grain to the food basket. Among the pulses, pigeon pea (*Cajanus cajan* (L) Millsp.) occupies an important place in Indian house hold. High protein content and the presence of fats with a low degree of saturation have contributed significantly to the increasing popularity of pigeonpea. However, pod borer *(Helicoverpa armigera)* attack causes severe loss of this crop throughout the world in general and India in particular. There is no known source of resistance against this pest in pigeonpea. Introduction of insect resistant gene through genetic transformation has long been considered as an alternative strategy. But, *in vitro* plant regeneration is a limiting factor in the transformation of pigeonpea.

A successful and efficient system for multiple shoot induction of pigeonpea is being reported here, which can be utilized in the genetic transformation experiments. Multiple shoots were induced from the cotyledonary nodes cultured on Murashige & Skoog's (MS – Murashige and Skoog, 1962), medium supplemented with benzyl adenine (BA) with or without indole – 3 butyric acid (IBA). Surface sterilized seeds of UPAS 120, AF 239 and Tripura local were germinated on MS medium containing 3% sucrose and different combinations of benzyl adenine (1mg/l, 2mg/ l and 5 mg/l) and Indole 3 butyric acid (0.1 mg/l). Small shoots, from 1-12 days old seedlings, were separated from the cotyledonary nodes and cultured on hormone free MS medium while the cotyledons were reinoculated in the same media for further induction of new shoots.

Among the combinations tried, BA 2mg/l along with IBA 0.1 mg/l proved to be the best for all the three genotypes. Seeds of all the three genotypes responded to the combination of BA along with IBA. The number of shoots produced per cotyledon after 12 days of inoculation varied from 12 – 17. More than 25 shoots were obtained per cotyledon after two subcultures (Table I). Geetha *et al.* (1998) reported high frequency of multiple shoot formation from seedling explants of pigeonpea in MS medium containing either benzyladenine (BA) or kinetin. Callusing from epicotyl explants of pigeonpea in medium containing BA or kinetin has also been reported (Thatikunta and Baldev, 1994). Sreenivasu *et al.* (1997) reported plant regeneration via somatic embryogenesis in pigeonpea. Genotypic differences in callusing and organogenesis was observed by Singh *et al.*, (1994) in 8 genotypes of pigeonpea. Benzyladenine (BA), either

* *Present address : VPKAS, Almora, Uttaranchal*

alone or in combination with IAA has also been reported to induce callus from leaf explants of pigeonpea. However, shoot regeneration was observed when both were used (Yadav and Chand, 1998). In our experiment, the average number of shoots increase with the increase of BAP up to a certain level i.e. 2mg/l BAP. Above this concentration there was a drastic fall in the number of shoots obtained. IBA at a concentration of 0.1 mg/l showed an enhancing effect on multiple shoot induction when used in combination with BAP.

These results suggest that a balanced combination of auxin and cytokinin is required for shoot regeneration in pigeonpea and that the required concentration of cytokinin is higher than that of auxin.

Table 1. Influence of different media composition and varieties on average number of shoots / cotyledonary nodes after initial culture.

	Different media composition					
Varieties	BAP 1 mg/1	BAP 1mg/l + IBA 0.1 mg/l	BAP 2 mg/1	BAP 2mg/l + IBA 0.1 mg/l	BAP 5 mg/l	BAP 5 mg/l + IBA 0.1 mg/l
UPAS 120	4 – 5	7-9	11-14	12-15	3-4	3-4
AF 239	7 – 8	6 – 9	6 – 10	14 – 17	5 – 6	4 – 6
Tripura Local	4 – 8	2 – 4	10 – 12	10 – 14	8 – 10	8 – 10

REFERENCES

Geetha, N., Venkatachalam, P., Prakash, V. and Sita, G.L. (1998). *Current Sci.* 75: 10, 1036 – 1041

Mohan, M.L. and Krishnamurthy, K.V. (1998). *Plant Cell Rep.* 17: 9, 705-710

Murashige, T. and Skoog, F (1962). Physiol. Plant 15: 473-497

Sreenivasu, K., Malik, S.K., Kumar, P.A. and Sharma, R.P. (1997). *Plant Cell Rep.* 17: 4 292-297.

Singh , A. K., Sahi, V.K. and Kumar, H. (1994). *Int. Chickpea and Pigeonpea Newslett.* 1: 43-44

Yadav, Vichita. Laxmi, Chand., Yadav, V. and Chand, L. (1998). *Int. Chickpea and Pigeonpea Newslett* 5 : 48-50.

Thatikunta, R. and Baldev, B. (1994), Ind. Journal of Plant Physiology. 37 : 1,53-55