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THIDIAZURON INDUCED MULTIPLE SHOOT IN PIGEONPEA (*Cajanus cajan*)

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ABSTRACT

Thidiazuron (TDZ) a substituted phenylurea has shown various cytokinin like activity and is primarily used as a cotton defoliant has proved to induce shoot proliferation in pigeonpea (*Cajanus cajan* L). Cotyledonary nodes were more responsive in producing multiple shoots. Maximum number of shoots were induced at concentration 1mg/L TDZ but higher concentration of TDZ reduces shoot proliferation. Roots were induced in these shoots in MS (Murashige & Skoog's) medium supplemented with 0.2mg/L IBA.

INTRODUCTION

Among pulses, pigeonpea (*Cajanus cajan* (L) Mill sp) alone shares around 21% of the total area under pulse production in India. More than 90 percent of the world's area and production of pigeon pea is in India. It plays a significant role in farming system where it contributes towards the soil nitrogen thereby reducing the need for nitrogenous fertilizers through symbiotic fixation of atmospheric nitrogen. One of the major problems limiting pigeonpea cultivation is pod borer (Helicoverpa armigera) a lepidopteran insect, which causes extensive damage to the crop (Lateef and Reed, 1983). Due to this pest infestation the production goes down by more than 20% (Verma and Verma, 1991). Genetic engineering approaches to introduce genes coding for insecticidal proteins into pigeonpea may prove useful in obtaining pest-resistant genotypes (Kumar *et al.*, 1996). Limited progress in the development of plant regeneration system has seriously impeded the application of gene transfer technology to the cultivated grain legumes. Although regeneration by organogenesis or somatic embryogenesis has been reported in pigeonpea, the procedures generally yield unsatisfactory frequency of regeneration and often involve extensive manipulation of culture conditions to obtain regenerants.

A successful and efficient system for multiple shoot induction of pigeonpea is being reported here, which can be utilized in the genetic transformation experiments. Thidiazuron, a substituted phenyl-urea (N-phenyl-N-1,2,3-thiadiazol-5yl urea) has been shown in various cytokinin bioassays to exhibit strong cytokinin like activity similar to that of N6 substituted adenine derivatives.

MATERIAL AND METHODS

Multiple shoots were induced from the cotyledonary nodes cultured on Murashige & Skoog's (MS - Murashige and Skoog, 1962), medium supplemented with thidiazuron. Surface sterilized seeds of UPAS 120, AF 239 and TRA 99-1 were germinated on MS medium containing 3% sucrose and B5 vitamins (Gamborg et al., 1968) along with different concentrations of thidiazuron (0.5mg/L, 0.75mg/L, 1mg/l, 2mg/l, 2,5mg/L and 3mg/L). Multiple shoots were formed at the cotyledonary nodes after 7-8 of inoculation. Small shoots, from14-15 days old seedlings, were separated from the cotyledonary nodes and cultured on hormone free MS medium while the cotyledons were re-inoculated in the same media for further induction

of new shoots. Rooting was induced in the elongated shoots on different strength of MS medium (MS, 1/2MS, 1/4MS) and MS supplemented with 0.2mg/L IBA (Indole butyric acid).

RESULTS AND DISCUSSION

Among the combinations tried, MS along with TDZ 1mg/L proved to be the best for all the three genotypes. Seeds of all the three genotypes responded to thidiazuron. The number of shoots produced per cotyledon after 15 days of inoculation varied from 18 - 25. More than 30 shoots were obtained per cotyledon after two subcultures (Table I). At higher concentration of TDZ, the number of shoot formation was reduced. 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. Singh et al. (2002) reported multiple shoot formation in chickpea using thidiazuron. Genotypic differences in callusing and organogenesis were observed by Singh et al., (1994) in 8 genotypes of pigeonpea. Benzyladenine (BA), either 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). Thidiazuron induces callus formation from leaf explants and subsequent plant regeneration. In our experiment, the average number of shoots increase with the increase of TDZ up to a certain level i.e 1mg/l. Above this concentration there was a fall in the number of shoots obtained. These results suggest that a thidiazuron upto a certain concentration enhances multiple shootings beyond that shoot formation was greatly reduced.

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

Varieties	Different media composition					
	MS + 0.5mg/L	MS + 0.75mg/L	MS + 1.0mg/L	MS + 2.0mg/L	MS + 2.5mg/L	MS + 3.0mg/L
UPAS 120	4-5	7-9	19-22	12-15	3-4	3-4
AF 239	7-8	6-9	21-23	14-17	5-6	4-6
TRA 99-1	4-8	2-4	18-25	10-14	8-10	8-10

The above study presents a reproducible protocol for multiple shoot regeneration from cotyledonary nodes thus paving the way for genetic manipulation in pigeonpea.

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