Short Communication

J Indian bot Soc Vol 75 (1996) 129-130

IN VITRO RESPONSE OF CULTURED TISSUE OF *HYOSCYAMUS MUTICUS* L. TO ANTIBIOTIC KANAMYCIN

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(Accepted December 1995)

Dose response experiments with antibiotic kanamycin is pre-requisite step in the development of any new transformation system. With an aim to develop *Agrobacterium* madiated transformation system in *Hyoscyamus muticus* L. which is potential source of pharmaceutical important tropane alkaloids, *in vitro* response of cultured tissue of *H. muticus* to Kanamycin has been worked out. Leaf segments and shoots were subjected to increasing dose of Kanamycin (5-100 mg/l) to find out lowest concentration required to inhibit callus growth and shoot development. Callus initiation frequency, growth and shoot proliferation were inhibited by 50 mg/l of Kanamycin which suggest that tolerance level 25 mg/l and 10 mg/l should be included in selection medium for callus and shoots transformation in the present case.

Key Words : *Hyoscyamus muticus*, Callus, Kanamycin, antibiotic, transformation.

Sensitivity affect the recovery of transformed plants and varies widely among tissues and species, kanamycin sensitivity is a pre-requisite during intitial stage of developing any new plant transformation system. (Colby and Meredith 1990). With an aim to develop *Agrobacterium* mediated transformation system in *Hyoscyamus muticus* L. (Family : Solanaceae), which is a potential source of tropane alkaloids hyoscyamine and scopolamine, growth responses of leaf disc explants and split shoots to kanamycin has been determined and reported in this communication.

In vitro shoot cultures initiated from apical meristems as described earlier (Ahuja *et al.* 1992) maintained on MS (Murashige and Skoog, 1962) medium containing Kn, 5 X 10^{-6}M were utilised to harvest fully expanded leaves and split shoots for present experimentation. Leaves harvested from shoot cultures 3 weeks after subculture were punched in disc (5 mm) aseptically. Discs were immersed in sterile distilled water and blot dry on sterilized Whatman filter paper before they were transferred to callus initiation medium (MS + BAP 5 X 10^{-6} M + NAA 5 x 10^{-6} M) with 0-100 mg/L kanamycin dispensed in petridishes 9.0 cm dia. Ten replicates Petridishes were used for each treatment. Shoot splits about 3.0 cm were excised aseptically and cultured on MS medium with Kn (5 x 10^{-6}M) with 0-100 mgL^{-1} kanamycin.

All cultures provided with 16 hr. light/8 hr. dark incubation. Kanamycin sulphate (Alembic, India) at 0, 5, 10, 25, 50 and 100 mgL^{-1} was dissolved in double glass distilled water and filter sterlized (0.22 µm), added aseptically in medium after autoclaving.

The response of leaf-disc explants and split shoots to varying concentration of kanamycin is summarised in Table-I. Callus induction from leaf-disc explants was not affected below 25 mg/l. Increasing dose of kanamycin beyond 50 mg/l greatly reduced the percentage of leaf disc forming callus. Light greenish friable callus proliferated from cut edge of leaf discs. Kanamycin treated leaf disc became completely white beyond 25 mg/l concentration, degree of bleaching of leaf-disc increased with increasing dose of antibiotic (fig. 1. a). Axenic shoot regeneration from transferred split shoot was inhibited by 25 mg/l which continued upto higher concentration of antibiotic used. Rate of shoot multiplication decreased with increased bleaching with increasing dose of kanamycin (fig. 1. b).

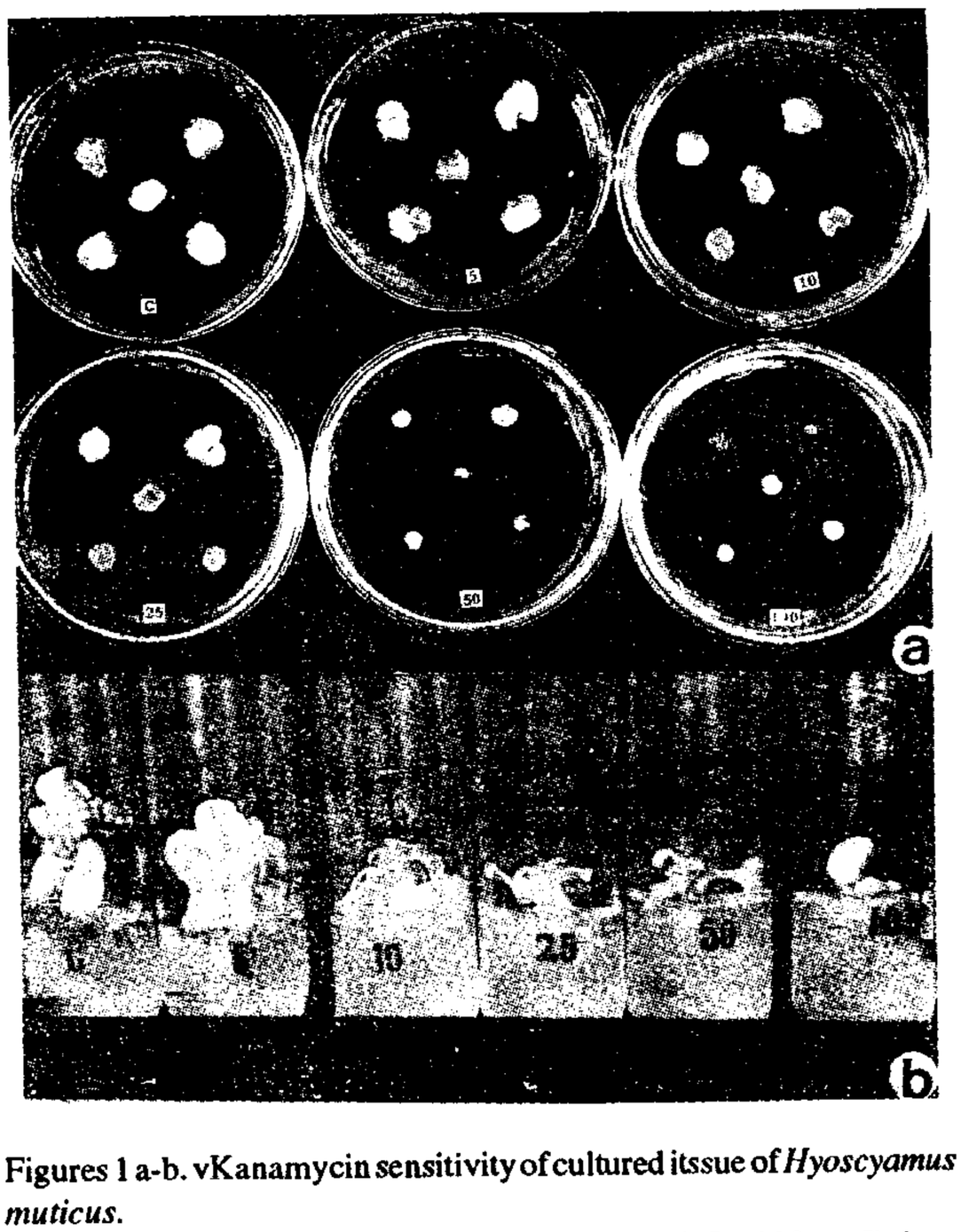
It is evident from present results that callus growth, adventitious shoot proliferation and their development were very sensitive to high dose of kanamycin used. The split shoot transferred to kanamycin were more sensitive than callus proliferation from leaf-discs. Growth in the presence of normally inhibitory concentration is taken as indi-

Received August 1995

Table 1. Response of leaf segments and shoots of *Hyoscyamus muticus* L. to different level of antibiotic kanamycin

BM + Kanamycin conc. (mg/l)	Leaf Disc.*			Split Shoots**		
	Leaf Segments Callusing			Number of Shoot/ Culture	Average Length (cm)	Appearance of Shoot
	Explant cult./Resp	% callusing	Appearance			
0	5/5	100	Friable green callus with good growth	5	3.0	Healthy green.
5	5/5	100	Friable green callus with good growth	5	2.75	Healthy green.
10	5/4	80	Friable light green callus moderate growth with moderate growth.	4	1.75	Slight bleaching.
25	5/3	60	Friable light green callus moderate growth with moderate growth.	3	1.0	Slight bleaching.
50	5/1	20	No callus complete decoloured explants.	2	1.0	Week, Complete bleaching.
100	5/0	0	No callus complete decoloured explants.	1	0.75	Week, complete bleaching.

BM - Basal Medium (Murashige & SKoog 1962) + * BAP (5 x 10^{-6} M) + NAA (5 x 10^{-6} M), ** Kn (5 x 10^{-6}M) Incubation : 25±2°C, 16 hr light/8 hr dark. * Values are mean of 10 replicates. ** Values are mean of 5 replicates.



Figures 1 a-b. vKanamycin sensitivity of cultured itssue of *Hyoscyamus muticus*.
Fig. 1a. leaf-disc response to varying (0, 5, 10, 25, 50, 100 mg/l) dose of kanamycin.
Fig. 1b. Split shoot response to varying (0, 5, 10, 25, 50, 100 mg/l) concentration of kanamycin.

cator of NPT-II enzyme activity which represent the lowest concentration appropriate for the selection of kanamycin resistant tissue in *Agrobacterium* co-cultivation experiments (Colby and Meredith, 1990). Thus for the development of *Agrobacterium* transformation system in *H. muticus* using leaf disc, at least 25 mg/l and 10 mg/l of kanamycin addition would be necessary to select out transformed callus and shoot regenerants, respectively. It is reported that level of kanamycin tolerance and sensitivity varies from plant to plant as well as different explants of the same species.

One of us (RKS) is thankful to the Director, Regional Research Laboratory, Jammu for providing Research Fellowship.

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