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IN VITRO PLANT FORMATION FROM CALLUS CULTURES OF *JUSTICEA GENDARUSSA*

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Growth hormone requirements of *Justicea gendarussa* explants for callusing and plant regeneration in invitro conditions are being reported for the first time. Nodal explants required 1 mg/l, 2,4-D and leaf explants required NAA (1 mg/l) and BAP (.5 mg/l) for successful callusing. When callus was transferred to 1:3 IAA kinetin supplemented MS medium, organised structures developed from the callus. Root and shoot system was formed. The plantlets were later transferred to the soil where a large percentage grew well.

Justicea gendarussa, a vegetatively propagated plant was brought to Lucknow from South India. Besides its economic significance, it is of special interest to geneticists as it has single B chromosome or accessory chromosome in some cells besides the normal complement of 2n - 30. Propagation of *Justicea* by cuttings is possible only in the rainy season. It may be mentioned that there is no seed setting in this strain, therefore seed propagation is totally absent. Therefore, regeneration of plants from its different explants through tissue culture is an important step towards its propagation throughout the year.

MATERIALS AND METHODS

Explants of *Justicea gendarussa* were inoculated onto MS medium (1962) containing sucrose at 30g/1 at pH 5.7. It was supplemented with different combinations of growth regulators viz. 2,4-D, IAA, Kinetin, NAA and B.A. Various regions of plant were used as explants. These were surface sterilized sequentially with detergent Tween-80, 70% alcohol, 1% mercuric chloride solution and then given a final thorough washing in double distilled water. The inoculated tubes were kept at 25±2°C in the culture room. Observations were made after weekly intervals.

RESULTS AND DISCUSSION

The inoculated stem explants changed from green to brown after a week. Callusing started after one week. Best callus quantitatively, was produced in 1mg/1 2,4-D (Table 1).

Leaf explants showed comparatively earlier initiation of callus which initiated as white postules distributed irregularly on the entire surface. Gradually the number and size of pustules increased, finally coalescing to form a continuous mass. The callus was white and remained so for quite a long time. Leaf explants produced best callus quantitatively in 1 mg/1 NAA and. 5 mg/1 BAP supplemented medium. Later pieces of these calli were transferred to fresh medium having different combinations of IAA and kinetin. After about 15-20 days the first shoots were seen in. 5 mg/1 IAA and 2.0 mg/1 kinetin. Maximum number of shoots appeared in .5 mg/1 IAA and 1.5 mg/1 kinetin supplemented medium (Table 2). After 15 days, well developed shoot system was formed which later gave rise to the roots (Fig.1).

Table 1: A.M.±S.E. values of callus fresh weight (mg) of *Justicea gendarussa* in different combinations of growth regulators.

Growth regulator	Shoot explant	Leaf explant
.5 mg/1 2,4-D	1.03 ± .12	0.67 ± 0.11
1.0 mg/1 2,4-D	1.91 ± .17	0.64 ± 0.08
1.5 mg/1 2,4_d	0.70 ± .09	0.94 ± 0.17
NAA (.5 mg/1) BAP (.2mg/1)	0.99 ± .08	1.74 ± 0.10
NAA (1mg/1) BAP (.5mg/1)	0.82 ± .03	1.84 ± 0.12
NAA (1.5 mg/1) BAP (.75 mg/1)	1.01 ± .11	.79 ± 0.14

These plantlets were transferred to pots initially covered with beakers and kept at the same temperature. About 60% of the transferred plantlets responded well to their changed condition. Initially their growth was slow, but later they grew well in their changed environment (Fig. 2).

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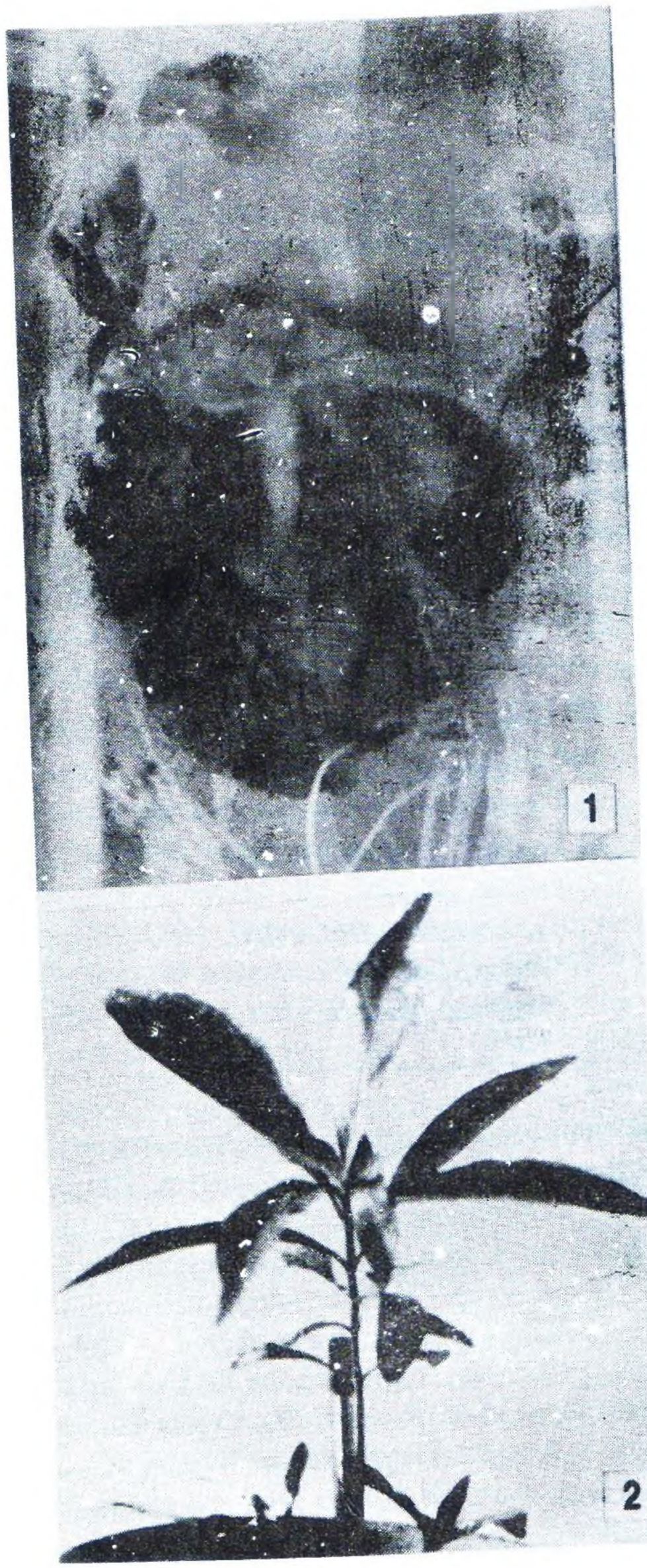

Fig. 1. Formation of shoot and root from callus of *Justicea gendarussa*
Fig. 2. Transplanted *Justicea* plant in 15 cms pot.

It is very important to know the cultural requirements and the responses of various tissues and organs of any plant system. Since different plant systems or even different organs from same plant require different cultural conditions, the various factors effecting the establishment of tissue culture have been worked in various plant species.

Table 2: A.M. ±S.E. values of number of shoots produced in different combinations of IAA and kinetin.

IAA*/ Kinetin*	.05	1.0	1.5	2.0	2.5
.01	C	C	2.1±.3	1.4±.2	-
.05	1.1±.2	1.5±.2	1.1±.1	C	-
.1	C	2.2±.2	1.5±.2	C	-
.5	C	C	4.5±.2	3.4±.3	1.2±.3
1.0	C	C	1.2±.2	2.4±.2	4.0±.3

*mg/l

Callusing of leaf explants of winged bean was done on MS medium with NAA 2 mg/l and BAP 2.5 mg/l. On transfer to regeneration medium, MS with IAA (0.2 mg/l) and BAP (2, 10 mg/l) buds started to appear from the seventh day of culture (Gregory *et al*, 1980).

According to Hedtrich (1979), shoots were formed from leaf tissue during in vitro propagation of *Gerbera* on a modified MS medium supplemented with 1 mg/l benzylaminopurine and 0.1 mg/l GA_3. Meyer and Staden (1988) report multiple shoots from axillary bud cultures of *Gerbera aurantiaca* on MS medium supplemented with 5 µm benzyladenine.

Plants have been regenerated from immature leaflets of *Pisum sativum* by Mroginski and Kartha (1961). In the same plant embryoids were obtained from callus cultures in the presence of picloram 0.6 mg/l in liquid medium. When cultured on solid medium, the callus showed only root formation and no shoot regeneration (Jacobsen and Wilfred, 1984). Regeneration potential of leaf explants of *Glycine max* is enhanced in the presence of 2, 4-5T and BAP in B5 medium (Wright *et al*., 1987).

In vitro organogenesis is found to be directly correlated with the kind and concentration of plant growth regulators. Callusing takes place in almost all growth media but proper growth of this meristematic mass is possible in presence of a specific hormone. The specific requirements of *Justicea gendarussa* explants has become clear through this experiment. The stem explants require 2,4-D 1 mg/l while leaf explants require NAA 1mg/l and BAP. .5 mg/l for callusing Callus from both sources produced plantlets in .5 mg/l IAA and 1.5 mg/l kinetin supplemented MS medium. These plantlets were successfully transferred to the soil.

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