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In vitro Propagation of *Ziziphus nummularia*

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Abstract An *in vitro* method for mass multiplication of *ziziphus nummularia*, a highly drought resistant shrub of the Indian Desert, has been developed. Maximum and the best induction of multiple shoots (15-20) was in the explants from shoot tip, closely followed by that of cotyledonary node (10-15), nodal region (8-10) and hypocotyl (6-8) on Murashige and Skoog's (MS) medium containing 2.5 mgL^{-1} kinetin (kn). These *in vitro* raised shoots were excised and cultured on same but fresh medium for further multiplication. In addition original explants were also repeatedly subcultured on the fresh medium to obtain new shoots. Rooting in these isolated shoots from both the original explant and induced shoots could be induced by placing them for 12h on filter paper bridge in White's liquid medium containing 8.0 mgL^{-1}. Indole Butyric acid (IBA). Thereafter, the shoots were transferred to agar-gelled hormone free White's medium. Regenerated plants were hardened and transferred to the pots.

Key words *In vitro* propagation, Plantlets, Shoot tip explant, *Ziziphus nummularia*

Ziziphus nummularia [Burm. f. Wt and Arn. family Rhamnaceae] is drought and heat resistant plant of arid and semi arid part of India with manyfold uses in horticulture and forestry (Mann & Saxena 1981). Much efforts have gone into its improvement and its proven success as root stock for budding improved cultivars of *Ziziphus manuritiana* is well known. Since it is a cross pollinated plant, *in vitro* propagation assums importance for mass and clonal multiplication. (Dunstan & Thorpe 1986, Cheliak & Rogers, 1990, Hammat 1992). Results of the *in vitro* propagation of *Ziziphus nummularia* in establishment of cultures from various juvenile explants, proliferation of multiple shoots from axillary and nodal zones, and induction of roots from *in vitro* produced shoots and plantlet formation are, therefore described in this communication.

Materials and Methods

Seeds of *Ziziphus nummularia* were collected from campus of CAZRI, Jodhpur. Seeds were separated out from the dried friuts, they were washed with tap water with a few drops of a detergent Tween-80, and then surface sterilized with 70% alcohol (1 min) followed by mercuric chloride (0.2%, 6 min) solution and then rinsed several (5-6) times with sterilized distilled water. Thereafter, these were germinated. Seedlings were raised aseptically on hormone-free Murashige and Skoog medium (Murashige & Skoog 1962). Different parts of 8-10 days old seedlings were used as explants.

These explants viz., hypocotyl (10 mm), shoot tip (4-5 mm), cotyledonary node (6-8 mm) and nodal segment (10 mm). (Fig 1) were cultured on MS, (Murashige & Skoog, 1962), B_5 (Gamborg *et al.* 1968), White (White 1963), WP (Lloyd & MC Cown 1980) and Heller's (Heller 1953) media with various concentrations of cytokinins (Kinetin or BAP 0.5 to 10.0 mg L^{-1}) and auxin (NAA or IAA 0.1 or 0.5 mg L^{-1}) for shoot induction. The growth regulators were used either individyally or in combinations. The cultures were incubated at 28 ± 2°C and 60% relative humidity and 35-43 μEM^{-2} s^{-1} photon flux density for 12 h day^{-1} photoperiods for 4 weeks.

Full, three fourth, half, and one fourth strength of MS, White, Heller and full and half strength of B_5 basal agar gelled media were used with IBA, NAA and IAA (0.1-5.0 mg L^{-1}) for root induction from regenerated shoots. In addition to agar media, shoots were also kept in White's liquid medium (on a filter paper bridge) containing 8.0-10.0 mg L^{-1} of

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Table 1 *Effect of auxins and cytokinins on direct regeneration from juvenile explants of Z. nummularia*

MS Medium mg L^{-1}	Number of Shoots explants^{-1}			
	Type of Explant			
	Hypocotyl	Cotyledonarynode	Shoot tip	Nodal segment
MS – HF	–	–	–	–
NAA 0.1 + BAP 1.0	1.2±0.7	1.4±0.5	2.3±1.2	1.3±0.9
NAA 0.1 + BAP 2.5	2.2±1.1	2.1±0.7	3.2±1.4	2.8±1.1
NAA 0.1 + BAP 5.0	2.1±0.9	2.4±1.2	3.6±1.5	2.9±1.0
NAA 0.1 + BAP10.0	2.2±0.8	1.2±0.8	3.9±1.7	2.6±1.3
IAA 0.1 + BAP 1.0	1.4±0.6	1.6±0.7	2.8±1.0	1.9±0.6
IAA 0.1 + BAP 2.5	2.4±0.9	2.8±1.1	3.6±1.3	2.8±1.2
IAA 0.1 + BAP 5.0	2.7±0.8	2.1±1.0	4.8±2.1	2.9±1.6
IAA 0.1 + BAP10.0	2.6±1.1	2.9±1.7	4.1±1.8	2.2±1.0
NAA 0.1 + Kn 1.0	1.6±0.7	2.8±1.6	2.8±1.2	2.7±1.0
NAA 0.1 + Kn 2.5	2.8±1.2	2.9±1.4	3.9±1.9	3.1±1.8
NAA 0.1 + Kn 5.0	3.2±1.6	3.1±1.9	4.2±2.3	3.2±1.9
NAA 0.1 + Kn 10.0	4.2±1.9	3.6±1.8	5.3±2.0	4.1±2.6
IAA 0.1 + Kn 2.5	2.3±0.8	2.2±0.9	3.2±1.8	2.5±1.2
IAA 0.1 + Kn 5.0	3.1±1.4	2.5±0.8	2.8±1.6	2.2±0.9

IBA and NAA for 0,6, 12 and 24 h and then transferred to hormone free White's semi solid medium. After initiation of roots these were kept at elevated temperature of 35°C under 100 μ Ems^{-1} photon flux density for hardening. Rooted plantlets were washed throughly with water and 0.05% Bavistin (1 min.) and transferred to pots containing sand : vermiculite (3:1).

Results and Discussion

Shoot formation was observed on almost all the treatments of plant growth regulators incorporated in MS medium containing various auxins and cytokinins. (Table 1) It was observed that presence of an auxin caused callusing in all the explants except shoot tip. Maximum number (15-20) of shoots were induced in shoot tip explants closely followed by cotyledonary node (10-15) nodal region (8-10) and hypocotyl (6-8) on MS medium containing Kinetin 2.5 mg L^{-1} (Mathur 1992).

On B_5, White's, WP and Heller media only few treatments containing growth regulators responded positively and induced three to five shoots per explant. BAP was found to be less effective than kinetin for shoot proliferation (Table 2). Regenerated shoots of shoot tip explant could be

Table 2 *Effect of cytokinins on direct regeneration from juvenile explants of Z. nummularia*

MS medium mg L^{-1}	Type of Explant		Shoot tip	Nodal segment
	Hypocotyl	Cotyledonarynode		
Kn 1.0	1.8±1.13	2.8±1.16	3.2±2.15	2.5±1.82
Kn 2.5	7.3±1.64	12.2±0.68	17.2±1.84	9.4±1.49
BAP 1.0	2.9±1.00	3.1±1.23	3.6±2.82	3.6±0.78
BAP 2.5	3.8±1.25	5.6±1.72	6.6±1.85	4.4±1.44
2 ip 1.0	2.7±0.92	2.7±1.45	4.2±2.26	2.8±1.25
2 ip 2.5	4.2±1.38	5.0±1.36	7.3±1.73	5.1±1.58

Kn = Kinetin

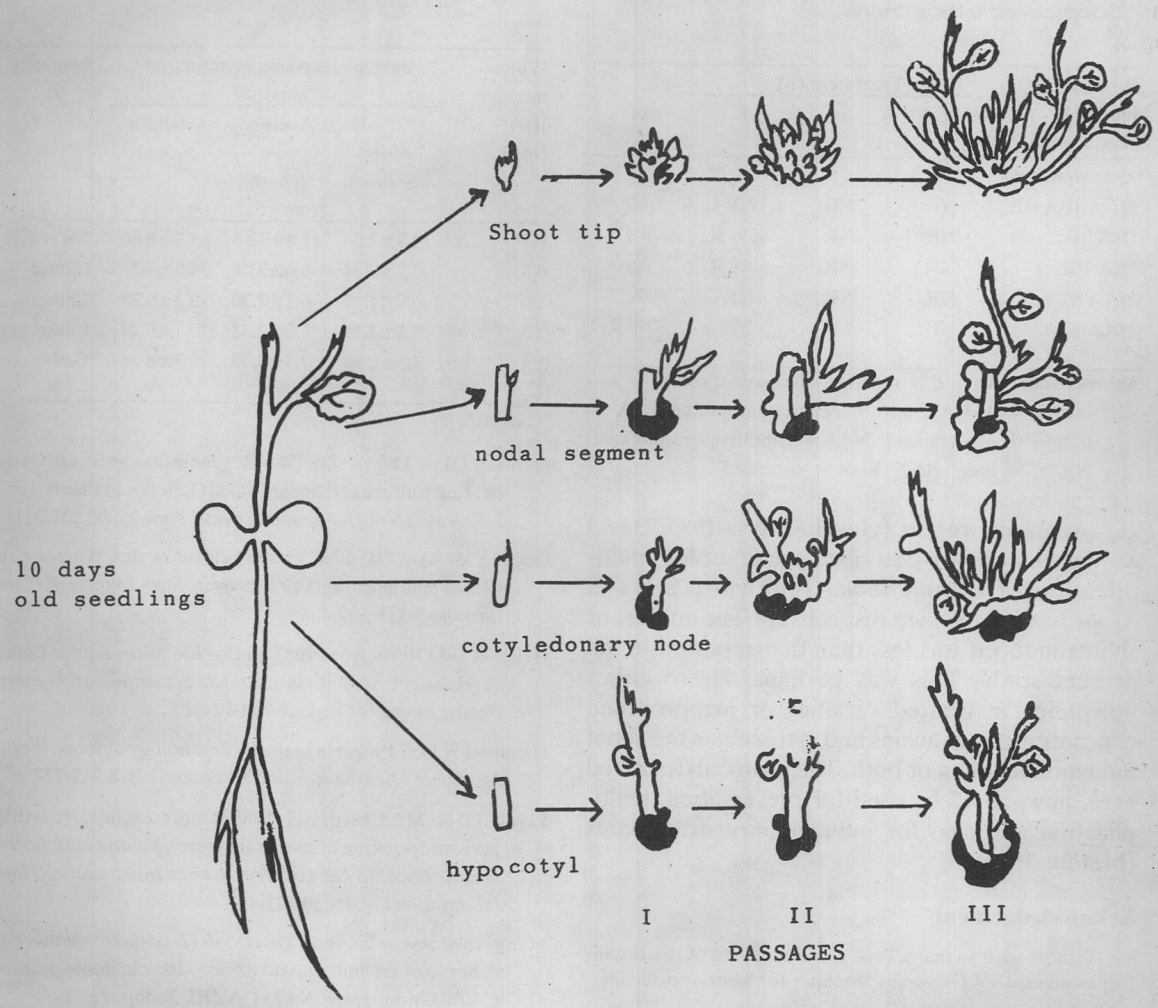

Fig 1 Regeneration from juvenile explants of *Z. nummularia* on MS medium containing Kinetin (2.5 mg L^{-1}).

further multiplied on the same medium; each segment produced (8-10) new shoots within four weeks.

During second passage on the same medium, number of shoots per explants did not increase markedly in all the explants except shoot tip. In remaining explants, callus grew fast, and only shoot elongation was observed (Fig 1). Shoot buds from shoot tip explants proliferated rapidly in subsequent passages without producing intervening callus.

Shoots differentiated *in vitro* rooted best when pretreated with 8.0 mg L^{-1} of IBA in White's liquid medium for 12 h in the dark, followed by transfer to semi-solid hormone-free White's medium at 28 ± 2°C (Table 3 and 4). About 80- 90% of *Z. nummularia* shoots rooted by this method. Plantlets could be easily transplanted in the pots. After hardening in the culture room, the potted plants could be transferred to a net house. More than 70% of the field transferred plants survived.

Table 3 *Pretreatment of shoots on white liquid medium containing different concentrations of IBA and NAA. only significant results are presented.*

White liquid media + Auxin (mg L^{-1})	Treatment (h)			
	O	6	12	24
Z. nummularia				
NAA/IBA 0.0	NR	NR	NR	NR
IBA 8.0	NR	NR	80% R	RS
IBA 10.0	NR	NR	50% R	RS
NAA 8.0	NR	NR	NR	40% R
NAA 10.0	NR	NR	NR	30% R

Abbreviation : Kn = 6 – furfurylaminopurine; indole – 3– butyric acid; BAP = 6 – benzylaminopurine; IAA = indol – 3 – acetic acid; NAA = naphthaleneacetic acid. NR = No roots, RS = Root senescenced

Table 4 *Effect of IBA on pretreated shoots of Z. nummularia (Average of 12 replicates at four weeks growth in diffuse light)*

White media + IBA (mg L^{-1})	Juvenile explants regenerated shoots			Remarks
	No. of roots per shoot	Average root length (mm±SD)	% shoot rooted	
HF	12.8±3.06	11.4±3.51	83.3±6.86	No callus
0.5	7.2±3.34	8.6±3.46	74.5±7.28	Callus
1.0	6.9±1.97	6.1±2.20	70.3±6.33	Callus
2.0	5.2±3.29	7.0±2.32	30.1±6.44	Callus
2.5	4.5±2.84	7.4±3.99	79.3±6.44	Callus

An earlier report (Goyal & Arya 1985, David & Vasaikar 1980) described methods for multiplication of *Z. mauritiana* (cultivars, *'seb'* and *'Gola'*) and *Z. xylopyra* respectively. The number of shoots induced was less than that reported in the present study. This was perhaps due to either polyploidy in grafted varieties or inappropriate concentrations of auxins and cytokinin in the shoot induction medium or both. The protocol described here, now, could be used for preservation, multiplication and also for inducing virus-free plants (Mathur 1992).

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