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Effect of IBA on root development and associated biochemical changes in single- and double-node stem-cuttings of spine gourd

Jitendra Kumar Tiwari[1]*, Gajala Ameen[1], Vivek Kumar Sandilya[1], Ashish Kumar[2] and AK Singh[3]

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Abstract

The present study was carried out to induce rooting in single- and double-node cuttings of *Momordica dioica* Roxb. variety Indira Kankoda-2 (RMDSG-3) under controlled conditions and to investigate associated biochemical changes during root development. The cuttings were treated with six different concentrations of IBA (0, 1100, 1200, 1300, 1400, 1500 mg/l) at four soaking durations (5, 10, 15 and 20 second). Among five concentrations of IBA tested, 1500 mg/l IBA + 5 seconds soaking duration gave the highest percentage of rooting, number of roots, and root length in both single and double node stem cuttings. As a result, IBA 1500 mg/l was used further in this investigation for biochemical studies. The adventitious rooting was obtained in three distinct phases *i.e.*, induction (0–12 days), initiation (12–14 days) and expression (14–18 days). IAA-oxidase activity of cuttings treated with IBA increased slightly as compared to control, this activity was found to decrease during induction and initiation phases and increase during the expression phase. The peroxidase activity of cuttings treated IBA-1500 mg/l increased up to initiation phase and decreased during the expression phase. Polyphenoloxidase enzyme activity/mg protein/min increased in both treated (0.9-1.75) and control (0.5-0.8) cuttings during induction and initiation phase but declined slowly during the expression phase. Total phenolic content was measured in mg/gm fresh weight, and it was found to be higher (6.55 mg/g fresh weight) in IBA- 1500 mg/l treated cuttings, particularly in initiation and expression phases, and it was also positively correlated with peroxidase activity. Phenolic compounds may be used as a rooting enhancer in spine gourd, and they can play a key role in inducing adventitious rooting. IBA is found efficient in spine gourd rooting, and by utilizing this protocol *i.e.*, double node cuttings treated with 1500 ppm IBA for 5 seconds duration, spine gourd can be commercially propagating.

Keywords: IBA, Stem cutting, Spine gourd, *Momordica dioica*, Rooting, Vegetative propagation

Introduction

Spine gourd (*Momordica dioica* Roxb.) belongs to family *Cucurbitaceae*. It is widely cultivated in India, Sri Lanka, Myanmar, China, Nepal, Pakistan and Bangladesh. In India, it is distributed in all the states except north-east region (Bharathi et al. 2011), but mostly found in Orissa, Bihar, Uttar Pradesh and West Bengal. *M. dioica* grows very well in hot and humid areas and almost behave as day-neutral plant (Bharathi et al. 2013). The nature of this vegetable crop is perennial, rhizomatous and climbs up to 3-10 m height with tapering root. Immature tender green fruits are used as vegetable and other parts such as young leaves, tuberous roots and flowers are also consumed. This vegetable has high demand in market but still remains underutilized and underexploited due to its dioecious nature and vegetative mode of propagation (Bharathi et al. 2007). Conventional commercial propagation of spine gourd largely depends on tuberous roots followed by seeds (Nabi et al. 2002). Tubers are not multiplying and only one tuber will get in one generation from a single plant. Further, due to dioecious nature of spine gourd, male plant has heterozygous (XY) and female has homozygous (XX) sex type. So, after fertilization in a single fruit both male and female seeds are present in 1:1 ratio (Ameen 2020). Due to these major constrains in propagation of spine gourd, asexual propagation through stem cutting and *in vitro* propagation will show their significance. *In vitro* propagation increases the cost of planting material as well as time for field planting. Whereas, plants developed from stem cutting, on the other hand are inexpensive and can be planted in the field within few days.

[1]Department of Genetics and Plant Breeding, RMD College of Agriculture and Research Station, IGKV, Ambikapur- 497 001, Chhattisgarh
[2]Department of Biotechnology, Sant Gahira Guru University Sarguja, Ambikapur- 497 001, Chhattisgarh
[3]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi- 221 005, UP
*Corresponding author, Email: tiwarijk5@gmail.com

Stem cuttings in spine gourd is limited due to unavailability of standard propagation method; as a result, it has not been commercially exploited. Normally, rooting in stem cuttings is difficult without treatment of any growth regulator. The successful establishment from stem cuttings depends upon many factors, such as stem portion, stem diameter, seasonal and age variation, moisture level, growing media, temperature and nutrient status (Kristiansen et al. 2005). Plant growth regulatory hormones particularly 'auxins' play a vital role in adventitious root formation in plants (Leakey et al. 2004). Adventitious rooting is a complex developmental process which is increased by initiation of the root primordium and growth *via* cell division (Fogaça et al. 2005). IBA has been known to be involved in the process of adventitious root formation (Haissig 1974; Wiesmann et al. 1988) and the interdependent physiological stages of the rooting process are associated with changes in endogenous auxin concentrations (Gaspar et al. 1997). In addition, several studies have emphasized that polyamines and auxins played major role during the induction of rooting (Davis et al. 1988; Nag et al. 2001). The formation of adventitious roots involves the process of rediûerentiation in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia (Aeschbacher et al. 1994). Among these changes, the process of ligniûcation in the cell wall, catalyzed by a particular peroxidase, may occur during rooting (Sato et al. 1993). The effect of leaf area had its own significant role in rooting of cuttings, as it is directly correlated with relative water content, stomatal conductance and photosynthetic rates (Newton et al. 1992). Number of roots increases with the increase in IBA concentration and this response has been enhanced by increased leaf area (Kamaluddin and Ali 1996). It is well recognised that adventitious root formation can be stimulated by the application of exogenous auxins but, its concentration, the effects of leaf area and the accompanying biochemical changes on rooting of spine gourd cuttings have not been extensively studied so far. Thus, the main aim of the present study is to establish the rooting efficiency of *M. dioica* from single and double node cuttings under controlled conditions and to investigate the associated biochemical changes that occur during the adventitious root development process.

Materials and Methods

Vines of Spine gourd variety Indira Kankoda-2 (RMDSG-3) were used as planting materials in this study. This variety is high yielding with a yield potential of 40 q/ha (Annual report Potential crops, 2018). Vines were taken to the shed net in a box filled with water from experimental field at Raj Mohini Devi College of Agriculture and Research Station, Ambikapur, Chhattisgarh, India. Each vine was then divided into different smaller segments i.e., one node with a single leaf (SNC) and two nodes with double leaves (DNC) in each section. Vines were collected in the mid-June to September and adjusted to 10-12 and 15-25 cm long leafy cuttings in the case of single leaf and double leaf cuttings with apical meristem, respectively. After initial cleaning, the cuttings were placed in a glass beaker containing 1.5 L double distilled water to clean field soil and retain cutting turgor pressure until they were not planted in soil after treatment. On the cutting node, the fungicide Bavestine (2 g/L) was used as a disinfectant. The cuttings were treated with different concentrations of IBA (0, 1100, 1200, 1300, 1400, and 1500 ppm) in combination with dipping for four time durations (5, 10, 15 and 20 second).

Pre-treated stem cuttings were immediately inserted or placed into the potting trays containing a substrate mixture of FYM: Soil: Sand (2:1:1). During the day time, the temperatures in the net house varied dramatically (up to 25-35°C and between 15-20°C during the night). To maintain a relative humidity of 90-95 %, water was sprinkled manually in form of mist for 05 seconds after every 03 hrs, except during the night time (6 pm-6 am). For biochemical investigation Double node pre-treated with or without 1500 ppm IBA for 5 sec. were used for biochemical studies because this combination of pre-treatment of IBA showed higher percentage of rooting (97.34%) as well as higher number of roots per cutting within 20 days of transfer to potting medium. Biochemical estimation consisted of 50 cuttings with double node pre-treated with or without 1500 ppm IBA for 5 sec. and subsequently potted in the rooting medium and replicated thrice. The growth conditions were same as used for rooting experiment. Root samples were collected from 10 to 20 days after pre-treatment. The basal part (about 0.5 of the rooting zone) of the cuttings was taken, after 10, 12, 14, 16, 18 and 20 days of transferring to potting tray, for biochemical analysis. Extraction for enzyme assay were done using one gram fresh cuttings, it was grind in mortar and pestle with 10 ml of phosphate buffer pH 6.8 (0.1 M) and divided into two equal 5 ml portions. The homogenate was centrifuged at 20000xg for 20 minutes, after centrifugation the pellet was discarded and clear supernatant was taken for different enzyme assay *viz*., peroxidise, IAA oxidase, polyphenol oxidase and total phenol content as per the procedure below:

Peroxidase assay: 05 ml of the peroxide activity assay mixture consisted of 125 µM of phosphate buffer, pH

6.8, 50µM of pyrogallol, 50 µM H_2O_2, and 1 mL of the 20 times diluted enzyme extract. This was incubated for 5 minutes at 25°C after which the reaction was stopped by adding 0.5 mL of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbency at 420 nm (Kar and Mishra 1976).

IAA oxidase assay: The reaction mixture was prepared by mixing the 0.2 ml enzyme extracts, 0.78 m L of 50 mM potassium-phosphate buffer (pH 6.0), 0.01 mL of 5mM $MnCl_2$, 0.01 mM 2,4-dichlorophenol and 0.02 ml of 2.5 g/l IAA. Assays were conducted at 25±0.5°C for 30 minutes. The Salkowski reagent (2.0 mL) was then added and destruction of IAA was determined by measuring the absorbance at 535 nm after 30 minutes (Rout 2006). One unit of IAA oxidase activity is equivalent to a ÄA535 of 1.0 for mg of protein in 30 minutes reaction (Rout 2006).

Total phenol content: Total Phenol was extracted and estimated by Foline-Phenol reagent (Bray and Thorpe 1954) and expressed as mg/g fresh weight.

Polyphenol oxidase assay: Pyrogallol was used as the substrate for the polyphenol oxidase enzyme assay (Kar and Mishra 1976). The activity was determined in terms of enzyme activity per mg protein/min.

Five characters namely, sprouting %, no. of roots per cutting, root length (cm), no. of leaves and shoot length (cm) was recorded at five days interval up to 15 days after planting of the single and double node stem cuttings. All experimental treatments were replicated three times with 100 cuttings per plot in a completely randomized block design (CRBD). The data collected were analyzed using SPSS software version 20.0 to perform univariate analysis including means, standard error (SE), range and coefficient of variation (CV). Duncan's multiple range test (DMRT) have been applied to detect the differences between treatment means. T-test was used to compare means of treatment at same temperature with and without scarification. Data related to sprouting frequency (%) were first angular transformed.

Results and Discussion

Stem rooting characteristics were significantly influenced by different IBA treatments, soaking duration of cuttings and number of node per cuttings as per ANOVA (data not shown). Cuttings that were not treated with IBA, showed their response in terms of root development, 18 days after potting (Figure 1). General statistics for single and double node cuttings in response to different IBA treatments presented in Table 1, which showed the effect of pre-treatment with different IBA concentrations on root development and other characters. Number of roots per cutting induced by various treatments ranged from 9.35-21.65 (SNC) and 10-26.67 (DNC). The length of the longest root did not

(A) Double node cutting (B) Single node cutting (C) Cutting without any treatment

Figure 1: Single and double node cuttings treated with 1500 ppm IBA after 20 days of potting; (A) Double node cutting; (B) Single node cutting and (C) Cutting without any treatment

differ much in both single (5.85-13.77) and double node (7.44-14.52) cuttings. The length of shoot ranged from 11.61-15.74 (SNC) and 12.40-16.40 (DNC). Number of leaves per cutting ranged from 1.33-3.33 (SNC) and 1.66- 4.34 (DNC) and did not show much difference. Leaves are essential for rooting and growth of leafy stem cuttings. Leaves are a source of carbohydrates, mineral nutrients, hormones i.e. auxins (Reuveni and Raviv 1981). Leaves, via photosynthesis and transpiration activate movement of solutes, and water as well as of hormones (e.g. auxins and cytokins), within the cutting and influence the temperature regulation of the cutting. Similar results were obtained by Ahmad et al. 1992. Pointed gourd (*Trichosanthes dioica* Roxb.) shows increased rooting by using IBA @ 100 mgL^{-1} (Pandey and Ram 2000).

Root initiation was observed after 12 days of potting in most of the nodal cuttings treated with IBA. The nodal cuttings pretreated with IBA showed a higher response on double node than single node cuttings (Table 1). The double node cuttings treated with 1500 ppm IBA + 5 seconds dip time showed the highest percentage of rooting (97.34 %) and the highest roots length (14.52 cm) followed by 1400 ppm + 5 second dip time (93.00%) and IBA 1500 ppm + 10 second dip time (91.00%) (Table 2). Among the different concentrations of IBA used for pre-treatment of single node cuttings, the highest percentage (92.33%) of rooting was obtained in cuttings treated with 1400 ppm IBA + 5 second dip time followed by IBA 1500 ppm + 5 second dip time (91.33%) and IBA 1400 ppm + 10 second dip time (88.00%). However, the double node cuttings pretreated with 1500 ppm IBA + 5 second dip time showed the highest percentage of rooting (97.34%) as well as higher number of roots per cutting within 20 days of transfer to potting medium. As a result, this concentration was used further for biochemical studies during rooting process.

The adventitious root formation is significantly influenced by IBA dosage, soaking duration of cuttings and number of nodes. Similar observations have been reported by different workers i.e., rooting ability of cuttings varied among clones and node positions in spine gourd different plant species (Vishnu et al. 2018, Ahmad et al. 1992 and Dick and Magingo 1998). When different doses of IBA were compared, it was observed that the higher dose of IBA caused a significant increase in rooting ability. High IBA concentrations along with shorter soaking times showed positive association among rooting traits. OuYang et al. (2015**)** found 150 mg kg^{-1} IBA with soaking for 0.5 h and 200 mg kg^{-1} IBA with soaking for 2 h were the optimum rooting combinations and suggested positive relationships among rooting traits, cutting length, and cutting diameter. The ability of auxins to promote adventitious root development in spine gourd stem cuttings is well known (Ahmad et al. 1992). Applying different IBA treatments has signiûcantly enhanced rooting and other rooting traits than the control. Applying IBA may have an indirect inûuence by enhancing the speed of translocation and movement of carbohydrates to the base of cuttings and consequently stimulate rooting (Davies et al. 1990; Aminah et al. 1995), so the rooting rate may be enhanced by the IBA treatment (Aminah et al. 1995). This may also be related to total phenolic content and peroxidase activity, which are higher in IBA-treated cuttings, particularly during the initiation and expression phases (Moncousin and Gaspar 1983; Rout 2006).

The biochemical observations were made from 10 to 20 days at every 2 days intervals during the rooting process. Generally, the adventitious rooting occurred in three distinct phases *i.e.*, induction, initiation and expression, as proposed for poplar species (Hausman et al. 1997). The results showed that the IAA oxidase activity of the IBA-treated cuttings decreased (95-75 one unit of IAA-oxidase activity is equivalent to a $ÄA_{535}$ of 1.0 for 1 mg of protein in 30 min.) during induction (0-12 days) and initiation phases (12-14 days) and increased (80-100) during the expression phase (14–18 days). IBA-treated cuttings have higher peroxidase activity than control cuttings (Fig. 2A). Peroxidase activity increased significantly in IBA-treated cuttings up to the expression phase, while it remained stable or increased slightly in control cuttings during the initiation and expression phases (Fig. 2A). It has been suggested that the reduction in adventitious rooting that occurs by reduced auxin signaling can be partially rescued by treatment with hydrogen peroxide (Li et al. 2009). The

Table 1: General statistics for single and double node stem cuttings in response to different IBA treatments.

General Statistics	Single node cutting					Double node cutting				
	SP	NRC	RL	NL	SL	SP	NRC	RL	NL	SL
Range	39.0-92.33	9.35-21.67	5.85-13.77	1.33-3.33	11.61-15.74	44.34-97.34	10-26.67	7.44-14.52	1.66-4.34	12.4-16.40
Mean	62.13	16.13	9.34	2.62	13.32	74.01	18.34	11.01	3.26	14.40
± SD	18.03	4.73	2.72	0.73	1.25	18.65	5.21	2.85	0.74	1.31
CV (%)	5.59	18.06	8.85	26.59	6.93	5.21	18.32	7.93	24.76	6.04

SP = Sprouting %; NRC = No. of roots per cutting; RL = Root length (cm); NL = No. of leaves and SL = Shoot length (cm).

increase in peroxidase activity observed in IBA treated cuttings during induction and initiation phase might serve as a good marker for rooting ability in cuttings (Li et al. 2009). Plant peroxidases are known to be involved in auxin metabolism as well as lignification processes in the cell wall in the presence of phenol, and the present results showed that peroxidase activity increased later than IAA-oxidase activity. It indicated that peroxidase activity is more involved in cell wall synthesis at the later phase and played an obligatory step in root formation (Sato et al. 1993). The IAA-oxidase activity was higher (103-120) in control cuttings as compared to IBA-treated one (75-100) (Fig. 2B). These findings are consistent with the results on root induction from Camellia cuttings (Rout 2006). The low IAA-oxidase activity during the induction period in IBA-treated cuttings appears to be responsible for better development of adventitious roots with IBA, possibly serving as the source of free auxin (Rout 2006; Shao et al. 2017). According to Hartmann et al. (1993**)**, there are two phases to adventitious root formation (i) an auxin-sensitive process and (ii) an auxin-insensitive phase. At

Table 2: Pretreatment effect of different concentrations of IBA on rooting from single and double nodal cuttings of *Momordica dioica* var. Indira Kankoda-2 after 20 days of transferring to potting medium.

IBA (ppm)	duration (Second)	Sprouting (%)	Number of roots per cutting	Root length (cm)	Number of leaves	Shoot length (cm)
Single node stem cutting						
Control	0	17.31	4.21	2.44	1.21	10.25
1100	5	39.00z	10.33op	6.40ij	1.33cd	11.61ef
	10	39.67z	12.67lm	7.33hi	3.00 b	12.18de
	15	39.33z	13.33kl	6.51ij	3.00 b	12.96de
	20	44.33y	9.33pq	7.67hi	2.67 bc	13.04cd
1200	5	53.67w	10.33op	5.85jk	1.67cd	12.92de
	10	45.33y	12.33lm	7.04 hi	1.67c d	12.65de
	15	51.00wx	13.33kl	7.81hi	1.67cd	12.88de
	20	41.33yz	11.33mn	7.54hi	3.00 b	12.59de
1300	5	86.33fg	16.67ij	8.55gh	2.33 bc	11.92ef
	10	76.00m	17.67hi	11.52 cd	3.00 b	12.15de
	15	60.00s	18.67gh	11.73 cd	2.67bc	14.26 ab
	20	46.67 e	15.00jk	10.29ef	3.00a b	15.10 a
1400	5	92.33bc	17.67hi	11.92 cd	3.33 ab	15.17 a
	10	88.00ef	20.67bc	11.62 cd	2.67b c	13.18 bc
	15	76.33mn	19.00fg	12.40 bc	2.67b c	13.29 bc
	20	59.33st	20.67bc	13.60 ab	2.33bc	12.74 d
1500	5	91.33 b	21.67bc	12.23 bc	2.67b c	13.47 bc
	10	73.33o	20.33bc	13.77 ab	3.00ab	15.74 a
	15	70.33p	21.33bc	13.03 ab	3.33a b	14.77 b
	20	68.67q	20.33bc	11.97 cd	3.33 ab	13.82 bc
Double node stem cutting						
IBA (ppm)	duration (Second)	Sprouting (%)	Number of roots per cutting	Root length (cm)	Number of leaves	Shoot length (cm)
Control	0	19.11	6.52	5.34	1.22	11.15
1100	5	46.00 e	11.34mn	8.40gh	1.66 d	13.92cd
	10	49.67 e	14.00de	7.44hi	2.34 bc	14.75 ab
	15	44.34 e	14.34de	7.67hi	3.34 ab	13.14cd
	20	51.34 e	10.00 e	8.85gh	2.00 bc	13.23cd
1200	5	58.34tu	17.67hi	7.65hi	3.00 ab	12.40de
	10	58.00tu	17.00hi	7.99hi	3.00a b	12.77de
	15	57.00uv	10.00 e	8.43gh	3.67 ab	13.71cd
	20	67.67qr	17.67hi	8.52gh	3.34 ab	12.62de
1300	5	89.67cd	18.60gh	12.15cb	3.34 ab	15.91ab
	10	85.67gh	15.67cd	9.91fg	3.34 ab	14.65bc
	15	82.00j	22.00cd	10.38ef	3.67 ab	16.09 a
	20	80.00kl	16.67cd	13.36 ab	2.67b c	14.92bc
1400	5	93.00b	26.00 a	13.76 ab	3.67 ab	15.33ab
	10	88.00ef	22.67cd	14.50 a	4.00 a	15.62ab
	15	86.67fg	20.00ef	13.48 ab	2.67 bc	14.72bc
	20	81.67jk	21.00de	12.62bc	3.00 ab	14.60bc
1500	5	97.34 a	24.67ab	14.52 a	4.34 a	14.70bc
	10	91.00cd	23.67bc	13.41 ab	4.34 a	13.94cd
	15	89.00e	21.00de	13.96 ab	4.00 a	15.62ab
	20	84.00hi	26.67 a	13.32ab	3.67 ab	15.44ab

the expression phase, the IAA-oxidase activity was high which might be related to low IAA content (Nag et al. 2001). Auxin-induced changes in peroxidase and IAA-oxidase have also been reported during the rooting process (Liu et al. 1996**;** Rout et al. 2006; Yan et al. 2014).

Similarly, total phenol content was found highest in IBA-treated cuttings, particularly during the initiation and expression phases (Fig. 2C). It might be possible that phenolics play key role in the induction of adventitious rooting. Since, phenolic compounds helps to protect against reactive oxygen species (Jaleel et al. 2009), it is not surprising that they also increase in response to wounding. Phenol is also involved in different steps of adventitious root formation (Rout et al. 2006**)**. Phenolics cause changes in IAA-oxidase activities and appear to be involved in rooting as cofactors (Hess 1962; Haissig, 1974). De Klerk et al. (1999) investigated a wide variety of polyphenols and found that all are promoting adventitious rooting. Phenolic compounds act as antioxidants, thereby protecting IAA from oxidation and plant tissue from oxidative stress due to wounding (Berthon et al. 1993; Rout 2006). Polyphenoloxidase catalyzes the oxidation of polyphenols and the hydroxylation of monophenols and lignification of plant cells during the rooting process (Khorsheduzzaman et al. 2010) and higher amount of polyphenoloxidase and lignin contents improved rooting and resistance to biotic stress (Beffa et al. 1990). During the induction and activation phases, polyphenoloxidase activity increased in both IBA-treated and control cuttings, but it gradually decreased during the expression phase (Fig. 2D).

IBA concentration and the duration of its application are crucial in adventitious root formation and associated changes in nodal cuttings of spine gourd. The double node cuttings treated with 1500 ppm IBA + 5 seconds dip time showed the highest percentage of rooting (97.34 %) and the highest roots length (14.52 cm) followed by 1400 ppm + 5 second dip time (93.00%) and IBA 1500 ppm + 10 second dip time (91.00%) has significant effect on rooting in spine gourd. Hence, it could be an efficient and cost-effective method for ensuring satisfactory genetically uniform population for plantation. This phenomenon could be linked to physiological factors. Among the treatments studied, the ability of IBA along with different concentration to improve rooting is of practical importance for spine gourd growers.

Among all the biochemical parameters evaluated, the most distinct between IBA-treated and control cuttings were IAA-oxidase (which includes a subgroup of

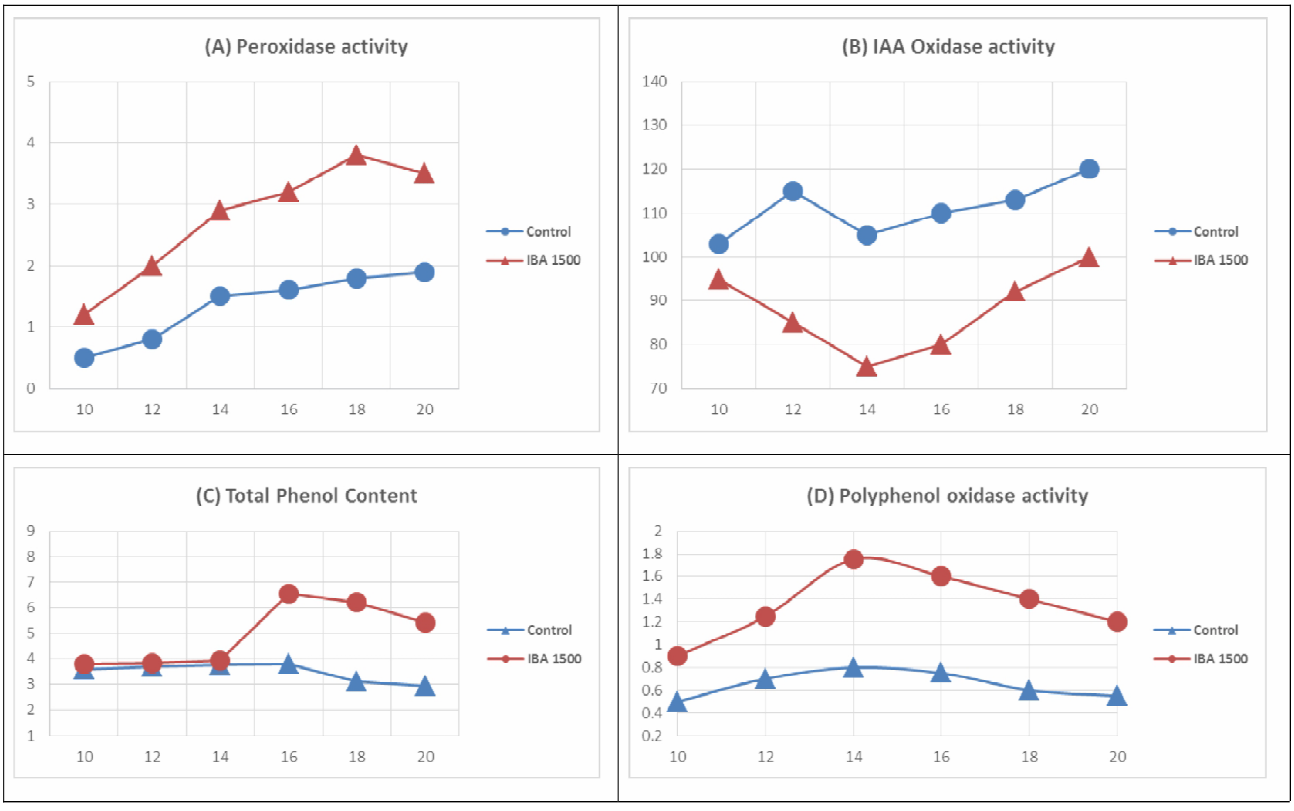

Figure 2: Biochemical changes during different time period of root development from nodal cuttings of spine gourd pre treated with or without IBA (1500 ppm)

peroxidases, and whose activity was lower in rooting cuttings during the induction phase) and total phenolic content (higher in rooting cuttings during the initiation phase). Peroxidase activity was found higher in IBA-treated cuttings up to the expression phase, while polyphenol oxidase activity was higher up to the initiation phase and then declined. Higher activities of peroxidase, polyphenol oxidase, and lower IAA-oxidase activity in the cuttings might be the critical factors to improve rooting, and to prevent membrane damage. Phenolic compounds, which act as both inhibitors and stimulators of peroxidase and polyphenol oxidase activities, can affect the emergence of roots from nodal cuttings of *Momordica dioica* var. Indira Kankoda-2. The present finding could be used for commercial propagation of spine gourd.

Lkkjka'k

वर्तमान अध्ययन ककोड़ा (*मोमोर्डिका डायोका* रॉक्सब.) के किस्म इंदिरा कंकोडा–2 (आर.एम.डी.एस.जी.–3) को एकल और दो गांठ कलम में जड़ आने को प्रेरित करने के लिए नियंत्रित परिस्थितियों में और जड़ विकास के दौरान संबद्ध जैव–रासायनिक परिवर्तनों की जांच करने के लिए उपयोग किया गया। कलम को चार भिंगोने की अवधि (5 सेकेंड, 10 सेकेंड, 15 सेकेंड और 20 सेकेंड) में आई.बी.ए. (0, 1100, 1200, 1300, 1400 तथा 1500 मिली. ग्राम प्रति लीटर) के छः अलग–अलग सांद्रता के साथ किया गया था। आई.बी.ए. परीक्षण की पांच सांद्रता में से 1500 मिग्रा. प्रति लीटर आई.बी.ए. + 5 सेकेंड भिगनों की अवधि ने एकल और दो गांठ कलम में जड़ विकास, जड़ों की संख्या और जड़ की लंबाई का उच्चतम प्रतिशत स्पष्ट किया। परिणामस्वरूप, जैव–रासायनिक अध्ययनों के लिए इस परीक्षण में आगे आई.बी.ए. 1500 पी.पी.एम का उपयोग जैव–रासानिक अध्ययनों के लिए भी किया गया। अपस्थानिक जड़ों का विकास तीन अलग–अलग चरणों जैसे–विप्रेरण (0–12 दिनों), प्रारंभन (12–14 दिनों) एवं अंग प्रदर्शन हुआ। आई.बी.एस. से शोधित कर्तनों में इण्डो एसीटिक एसीड–आक्सीडेंज प्रक्रिया नियंत्रक की तुलना में कम तौर पर पायी गयी जो विप्रेरण एवं प्रारंभन के दौरान कम पायी गयी एवं अंग प्रदर्शन के दौरान बढ़ता हुआ पाया गया। आई.बी.ए.–1500 मिग्रा. प्रति लीटर से शोधित कर्तनों में पेराक्सीडेज प्रक्रिया प्रारम्भिक दशा में अधिक एवं अंग प्रदर्शन दशा में कम पाया गया। प्रेरणा और प्रारम्भ चरण के दौरान उपचारित (0.9–7.75) और नियंत्रण (0.5–0.8) कटिंग दोनों पॉलीफेनोल आक्सिडेज एंजाइम गतिविधि प्रति मिग्रा. प्रोटीन प्रति मिनट में वृद्धि पायी गयी, लेकिन प्रदर्शन चरण के दौरान धीरे–धीरे गिरावट देखी गयी। कुल फेनोलिक सामग्री को मिग्रा. प्रति ग्राम ताजा वजन में मापा गया और आई.बी.ए.–1500 मिग्रा. प्रति लीटर उपचारित कर्तन में अधिक (6.55 मिग्रा. प्रति ग्राम ताजा वजन) पाया गया जो विशेष रूप से प्रारम्भ और प्रदर्शन चरणों में और पेरोक्साइड गतिविधि के साथ सकारात्मक रूप में सहसंबद्ध था। फेनोलिक यौगिकों का उपयोग ककोड़ा में जड़ बढ़ाने के रूप में किया जा सकता है और आकस्मिक जड़ निकलने की प्रक्रिया में प्रेरक के रूप में महत्वपूर्ण भूमिका निभा सकते हैं। आई.बी.ए. ककोड़ा के जड़ को प्रेरित करने में सक्षम पाया गया और इस नवाचार का उपयोग (5 सेकेंड की अवधि के लिए 1500 पी.पी.एम. आई.बी.ए. के साथ शोधित) दो गांठ कलम, ककोड़ा को व्यावसायिक रूप से प्रवर्धित किया जा सकता है।

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