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Impact of Benzyladenine, Brassinolide and IBA on the Proliferation of Shoots and Initiation of Roots in *Stevia rebaudiana* Bertoni

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Abstract: Lab trial was laid out at University of Anbar to assess the effect of benzyl adenine on stevia initiation and proliferation via single node which possessed one leaf primordia on MS and to increase percentage of node response to produce the shoots at initiation after that the increase of shoots number and length at proliferation. To induce root, 0, 1, 2 and 3 mg IB L^{-1} and 0, 1, 2, and 3 µg L^{-1} brassinolide were applied. Murashige and Skoog solidified with 2mg BA gave highest shoots (11.38) with shortest length of 2.94 cm. The combination of IB and brassinolide with concentration of 2 mg IB L^{-1} X 2m BL L^{-1} produces highest number of root (10.04 root explant^{-1}) with longest root (4.02 cm) 4 weeks after culture. The application of Indole butyric acid and brassinolide was efficient in solidified media culture for initiation and proliferation of Stevia from node.

Keywords: Benzyladenine, Brassinolide, Indole butyric acid, Stevia

Stevia rebaudiana Berton was originated from tropical and subtropical region and is categorized as short day plants that suitable to temperate temperature in winter where it was originated. *Stevia* possessed the ability synthesize a big group of chemicals namely, steviol. This glycoside has sweet taste and is used as natural sweeteners (Kaplant and Tuyout 2019) and is used as carbohydrates, proteins, filers and antioxidants (Al-Amrani et al 2018). *Stevia* is propagated sexually via seed or vegetatively using stem cutting. However the propagation by seeds is accompanied by many problems which reduced germination. Individuals that produced from seeds possessed quality and quantity traits different from their parents due to high genetic variation in seed. The current focus is on tissue culture technique because this technique produces uniform individuals in short time. The study was aimed to propagate stevia in *vitro*, and test combination between different concentrations of indole butyric acid and brassinolide fortified with banzyladenine on root initiation.

MATERIAL AND METHODS

Lab trial was conducted out at University of Anbar to propagate stevia in *vitro*. Explants were cut from stevia plants grown in greenhouse 2m long then washed under tap water with drops of cleaner to remove the suspended impurities and dust and were sterilized (Namdari et al 2015). The explants were soaked with 70% ethanol for few second then put into Sodium hypochlorite ($NaClO^{-}$) solution 2.5% with continuous agitation for 4 min and washed with distilled water many times. In Vitro culture media of Murashige and Skoog (MS) (1962) was fortified with 30 g sucrose then different concentrations of benzyl adenine 0, 0.5, 1 and 2 mg L^{-1}, was added. Thereafter, pH was adjusted upto 5.7 using sodium hydroxide and hydro chloric acid (1N). Final volume of prepared media was completed to 10^3 cm^3 with distilled water. Agar was applied with 7g L^{-1}. Consequently, medium was heated on magnetic hotplate stirrer up to boiling. The medium was poured in gars. The poured jars were left till be Cooled. Thereafter jars were sterilized via Autoclave at 121° c and pressure of 1.04 kg cm^{-2} for 15 min.

Shoot initiation and multiplication : Explant were cut at long of 0.5 cm using surgical blade and were cultured on MS solidified with different concentrations of benzyl adenine of 0, 0.5, 1 and 2 mg L^{-1}. This process was applied in layer flowing air table and were incubated in growth chamber on 25±2° C, 16h illumination and 8 h darkness. After 4 weeks, the cultured explants were sub cultured on the same MS to proliferate. Date was recorded on shoots, fresh and dry weight.

Root induction: Shoots produced from proliferation were transferred into rooting MS supplemental by different concretions of IBA Indole butyric acid viz., 0, 1, 2 and 3 mg L^{-1} and brassinolide of 0, 1, 2, and 3 µg L^{-1} such as to shoots be to rooted. After 4 weeks. Data were recorded on number root and their lengths.

Statistical analysis: All trials were done using CRD Design categorized means were analyzed using GENSTAT12.

RESULTS AND DISCUSSION

Proliferation and propagation: Number of fresh shoots and

dry weight increased with increased the concentration of benzyl adenine into MS (Table 1). Benzyl adenine with 2 mg L^{-1} gave highest number of shoots (11.38 shoots per explant), fresh weight (0.848 mg explant) and dry weight (0.047 mg $shoot^{-1}$), respectively. The control possessed the lowest averages of these parameters high concentrations of benzyl adenine caused reduction of soot length where the decrease was occurred with increase of applied benzyl adenine into MS. So , control (without benzyl adenine) produced longest shoot of 5.97 cm then minimized at 0.5 mg L^{-1} of 4.27 cm reached to lowest of 2.94 cm at 2 mg L^{-1} of benzyl adenine. Aziz and Al- Taweel (2019) also observed highest number of shoot when MS solidified with 2.5 mg benzyl adenine per liter. Furthermore, decrease in shoot length with increase concentration of benzyl adenine.

Root formation: The supplementary MS with indole butyric acid or Brassinolide led to succeed the root formation on stevia shoots. The brassinolide resulted in higher number of roots (6.52 root $shoot^{-1}$), as compared on indole butyric acid (3.46 root $shoot^{-1}$). MS fortified with 2mg L^{-1} from each growth regulator caused highest response of root formation on shoots (8.48 root $shoot^{-1}$). The application of 1 mg L^{-1} lowered the root number to 1.71 root $shoot^{-1}$.

The addition of brassinolide into MS produced longest root (3.27cm) as compared on indole butyric acid possessed lowest roots (1.70 cm). The root length is increased with increase of applied concentrations into MS (Table 2). The application of 2mg resulted in maximum root (3.51 cm) and 1 mg IBA or 1 µg BL decreased root length (1.27 cm). The reduction of root number and length could be due to high concentrations of auxins when applied into MS led to produce ethylene thereby would inhibit the growth of root (Ladaniy 2008). Hu et al (2016) observed that low concentrations of brassinolide (0.1 and 0.01 mg L^{-1}) caused root elongation and lateral root development in potato. The highest concentrations (1-100 µg L^{-1}) caused inhibition the root number and elongation. The brassinolide (BL), as a new type of plant hormone play significant roles in plant tissue culture and able to promote cellular division, increase of regeneration and growing the adventitious buds or shoots (Sasaki 2002) and forming the tissue of embryo (Pullman et al 2003). Lu et al (2003) indicated that BL increased the fresh weight and shoot regeneration rate of calli at concentration of 0.04 mg L^{-1} and stimulated the shoots growth that had regenerated at maximum concentration of 0.05 mg L^{-1} in *S.*

Fig. 1. Effect of benzyl adenine on stevia explant proliferation

Table 1. Effect of BA concentrations on shoot initiation of Stevia *In vitro*

BA(mg L^{-1})	Shoot (shoot $explant^{-1}$)	Shoot length (cm)	Fresh weight (mg $shoot^{-1}$)	Dry weight (mg $shoot^{-1}$)
0	6.90	5.97	0.335	0.026
0.5	8.53	4.57	0.411	0.030
1	11.17	3.07	0.669	0.045
2	11.38	2.94	0.848	0.047
L.S.D 5%	2.12	0.67	0.135	0.004

Table 2. Effect of brassinolide and indole butyric on stevia root number and length after 4 weeks of culture

Conc.	Root number			Root length		
	BL (µg L^{-1})	IBA (mg L^{-1})	Average	BL (µg L^{-1})	IBA (mg L^{-1})	Average
1	3.22	0.24	1.71	2.45	0.10	1.27
2	10.04	6.92	8.48	4.02	3.00	3.51
3	6.30	3.27	3.27	3.35	2.00	2.67
Average	6.52	3.46		3.27	1.70	
LSD 0.05	0.84		1.03	1.06		1.30

Fig. 2. Root growth and development under brassinolide and indole butyric acid

patens. The calli were formed in end of shoot at the zone of root initiation when explants were culture on MS solidified with 1 mg L^{-1} indole butyric acid (Fig. 2), whereas the application of 2 µg L^{-1} brassinolide resulted in maximizing root number per shoot. The brassinolide was the most efficient and effective in forming and developing root growth especially lateral roots. .

CONCLUSION

BA and BL could directly differentiate stevia explant into shoots. Conclusively, the study pointed that BA and BL could possess a focal role i.e. this hormone could regulate the elongation of stem, and that it would conserve the membrane stability over abiotic stresses, thereby confers thermal tolerance via increase the activity of enzymes and metals.

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