Direct regeneration of *stevia rebuadiana* Bertoni through node culture

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Introduction

Stevia rebaudiana Bertoni, belonging to the family *Asteraceae*. The leaves of stevia are the source of the diterpene glycosides, vis. Stevioside and rebaudioside (Ramesh et al, 2006, Lemus-mondaca et al,2012). The seeds of stevia show a very low and poor germination percentage. Therefore, there are basically two options for multiplication: tissue culture and steam cutting (puri et al, 2011). Different explants of *stevia rebaudiana* Bertoni can regenerate shoots when cultured on murashige and skoog (MS) medium supplemented with different combination growth regulators (Ramesh et al, 2006).

Materials & methods

shoot segments obtained from in vitro regenerated plantlets of stevia were immersed in detergent solution and washed thoroughly under running tap water, then disinfected in 70% ethanol for 3 min and rinsing several times with sterile water, after the sterilization treatment, 1 to 2 cm sized nodal segments were excised aseptically and were implanted vertically onto culture glass containing MS medium fortified with 1-naphthaleneacetic acid (NAA:0,0.5, 1.5, 2.5), indole-3-acetic acid (IAA:0, 0.1, 0.3, 0.5, 1.3, 1.5, 2.5, 4.5, 5.5), Indole-3-butyric acid (IBA:0, 1, 1.1, 1.3), 6-benzylaminopurine (BAP: 0, 0.5, 1, 1.1, 1.5, 2.5, 8.5) and kinetin (KIN:0, 0.5, 9.5).

Result and Discussion

Nodal segments of stevia were used as explants testing the effects of different concentration cytokinin & auxine on the induction of direct regeneration of stevia. After four weeks, shoots and roots formed from nodes on semi-solid MS medium supplemented with BAP (1.3mg/l) and IAA (1.1mg/l). However, NAA+BAP (1.5+2.5mg/l) had positive response on direct shoot & root induction. In addition, increase the level of BAP in combination of IAA+BAP highly retarded the frequency of rooting and also using of KIN+NAA or KIN+BAP can repress rooting from explants. A maximum of in vitro cultured explants (78%) showed multiple shoot formation with an average of 3.5 shoots per explants on MS medium supplemented with BAP. The initiation of shooting could not be achieved on medium with NAA+IAA+BAP (1+0.1+1.5) and the maximum height of shoots (16.2, 14.3, 13.5 cm) were obtained in the media containing (0.5, 1.1and 2.5) mg/l BAP in combination with 1.5mg/l NAA, 1.3 IAA and 1.3IBA after culture initiation. The increase of NAA concentration higher than 1.5 mg/l suppressed the rate of shoot bud regeneration and slow growth of the rooting. Unlike our findings, Ibrahim et al (2008) reported that the highest heights of shoots were obtained in MS supplemented 2 mg/l BA, but these shoots were very thin and not suitable for multiplication. MS medium with high level of kinetin (9.5mg/l) did not promote root induction, however, root were observed in media supplemented with IBA (1.1mg/l) or BAP (1and 2.5) in combination with IAA (0.3 and 4.5 mg/l). Optimal rooting and growth of macro shoots were observed in medium containing BAP (1.1mg/l) in combination with IAA (1.3mg/l). The maximum percentage of rooting (93%) was obtained in the medium containing (1.3+1.1 mg/l) IAA+BAP. This protocol provides an efficient and repeatable way to regenerate stevia through direct regeneration. In conclusion, a successful production of shoot & root regeneration from nodal explants were dependent on the nutrient medium. This study might provide new opportunities for propagation of an important medicinal plant, *stevia rebuadiana* Bertoni.

Keywords: stevia- Stevioside- explants- medium- BAP.

References

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