

## Synergistic effect of kinetin and benzyl adenine improves the regeneration of cotyledon explants of bottle gourd (*Lagenaria siceraria*) on ethylene production

Shyamali, S.\* and Kazumi, H.

Laboratory of Plant Genetics and Breeding, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601 Japan. \*Corresponding author: Shyamali Saha (\*Corresponding author: shyamali2000@gmail.com)

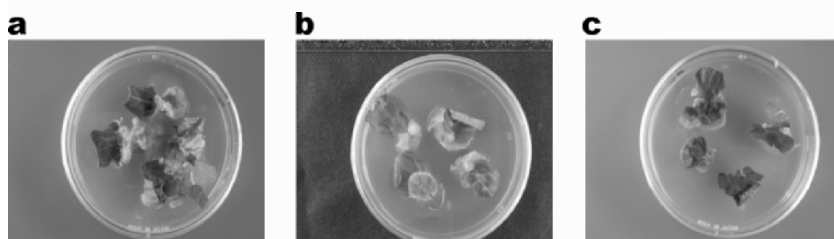
In this study, we show the capacity of de novo shoot organogenesis from cotyledon explants of bottle gourd in vitro in relation to ethylene, ethylene inhibitor, phytohormones and growth supplements. The physiological relevance of the hormonal interaction with respect to regulation of ethylene initiation and effect on regeneration is discussed. In this communication, we report the stimulatory effect of N6-benzyladenine (BA) and kinetin on adventitious shoot organogenesis leading to high frequency plant regeneration from cotyledon explants of bottle gourd on ethylene production. In recent years, there has been increasing evidence that the occurrence of morphogenesis in cultured plant cells may be associated with ethylene. Cytokinins are also known to promote ethylene production several-fold in many plants partially through the increase in ACC synthase activity (Abeles *et al.*, 1992). BA, a synthetic cytokinin, synergistically enhances ethylene production in the presence of IAA in mungbean hypocotyls (Yoshii and Imaseki, 1982).

**Table 1.** Effect of different plant growth regulators on shoot regeneration.

BA	kinetin	Bud proliferation	Regeneration %	Shoot/explants	Shoot length (cm)
2	0	3.5	25.33bc	3.80a	2.85c
3	0	3.5	20.00cd	3.07b	2.34d
0	1	2.0	18.67d	2.66bc	3.70a
0	2	2.0	24.00bcd	2.80bc	3.36b
2	1	3.8	80.60a	4.06a	1.18e
2	2	3.8	33.33b	1.01d	0.70f

Decoated seeds were sterilized by soaking in 70% ethanol for 1 min followed by 45 min in 20% sodium hypochlorite (1% a.i.) containing

0.2% Tween-20, rinsed 4 times and then sterilized seeds were placed on germination medium containing Murashige and Skoog (MS) basal salts and vitamins with 2% (w/v) sucrose. The pH of the medium was adjusted to 5.8 before the addition of the 0.8% agar (INA AGAR BA-30). After five days proximal parts of the cotyledons were isolated from seedlings and cultured on basal medium supplemented with different levels of BA and kinetin alone or in combination. Data on the bud proliferation state, percentage of regeneration, number of shoots per explants and shoot length were statistically tested by analysis of variance (ANOVA). Ethylene was quantified by comparison with an ethylene standard.



**Fig. 1.** (Color figure in the Annex, p.457) (a) Bud state showed a higher brownish expression on MS medium containing 2 mg/l BA. (b) No brownish tissue was observed in 2 mg/l kinetin and (c) Bud showed a moderate brownish tissue on MS medium containing 2 mg/l BA with 1 mg/l kinetin combination.

Bud proliferation, number of shoots per explant, shoot regeneration and shoot elongation varied depending upon the kind and concentration of cytokinins, which are shown in Table 1. Combined cytokinins (BA and kinetin) produced more bud (3.8) than when cytokinins were used individually. Kinetin was less effective as compared to BA in terms of bud proliferation as well as shoot bud induction but positively influenced shoot elongation ( $>3.36$  cm). Better shoot bud differentiation on combined BA and kinetin has been reported in *Feronia limonia* L. hypocotyle explants (Vyas *et al.*, 2005), cotyledonary explants (Hossain *et al.*, 1994) and *Vigna radiata* L. (Gulati and Jaiwal, 1990). (Fig. 1). BA showed a more brownish phenotypic expression ( Fig. 1a) than the BA and kinetin combination (Fig. 1c). No browning of tissue was observed when cotyledon explants were cultured on the kinetin containing medium (Fig. 1b). A positive relation was found between the tissue browning and ethylene production. A shoot generation capacity of explants and stimulation of ethylene biosynthesis may vary depending on the growth regulator used (Kumar *et al.*, 1987). From these data it can be concluded that the combination of kinetin and BA in the growth medium induces less

ethylene emissions than BA and decreased brownish content, suggesting cell differentiation and ensured higher regeneration.

## References

- Abeles FB, Morgan PW and Salveit ME (1992) Ethylene in Plant Biology. 2nd Edn. Academic Press, San Diego, Canada.
- Gulati A and Jaiwal PK (1990) Culture conditions beffecting plant regeneration from cotyledons of *Vigna radiata*(L.) Wilczek. *Plant Cell Tissue and Organ Cult* 23:1–7.
- Hossain M, Biswas BK, Karim MR, Rahman S, Islam R and Joarder OI (1994) In vitro organogenesis of elephant apple (*Feronia limonia* ). *Plant Cell Tissue Organ Cult* 39:265–268.
- Kumar PP, Reid DM and Thorpe TA (1987) The role of ethylene and carbon di oxide in differentiation of shoot buds in excised cotyledons of *Pinnus radiata* in vitro. *Physiol. plant* 69:244–252.
- Vyas S, Joshi N, Tak K and Purohit SD (2005) In vitro adventitious shoot bud differentiation and plantlet regeneration in *Feronia limonia* L.(Swingle). *In Vitro Cell. Dev. Biol. plant* 41:296–302.
- Yoshii H and Imaseki H (1982) Regulation of auxin- induced ethylene biosynthesis.Repression of inductive formation of 1-aminocyclopropane-1-carboxylate synthase by ethylene. *Plant Cell Physiol* 23:639–649.