ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.31067-31070, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

TDZ INDUCED *IN VITRO* PLANT REGENERATION OF *MOMORDICA BALSAMINA* AND *MOMORDICA CHARANTIA*, IMPORTANT MEDICINAL CUCURBITS

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ARTICLE INFO

Article History:
Received 27th February, 2016
Received in revised form
04th March, 2016
Accepted 21st April, 2016
Published online 20th May, 2016

Key words:

Thidiazuran,
Multiple shoots,
Regeneration,
Hardening.

ABSTRACT

The comparative study was conducted to analyze the effect of TDZ on Caullogenesis of M. *balsamina* and M. *charantia* a closely related important medicinal cucurbits. The highest number of (10.9 ± 0.05) multiple shoots were observed on MS medium fortified with TDZ+ CSH (2.0 + 200 mg/L) from cotyledonary explant of M. *balsamina* However cotyledonary explants were produced maximum number of shoots (9.00± 0.23) on MS medium augmented with TDZ + CSH (2.0 + 200 mg/L) in M. *charantia*. Best rooting response obtained from regenerated shoots on MS medium supplemented with IBA (3.0 mg/L) in both plants. The plantlets raised in vitro were transferred in to green house and successfully acclimatized to natural condition with 75 % survival rate. This study is used for the analysis of effect of various concentration of TDZ on both plants and it is tool for genetics and transformation applications.

Citation: Peer Mahammad Shaik, Raju Samraju, Ravi Chithakari and Mustafa Mohammad, 2016. "TDZ induced *in vitro* plant regeneration of *Momordica balsamina* and *Momordica charantia*, important medicinal cucurbits", *International Journal of Current Research*, 8, (05), 31067-31070.

INTRODUCTION

Cucurbitaceous plants are playing an important role in nutritional, medicinal and economical affairs of man. Bitter gourd (*Momordica charantia*) and Balsam apple (*Momordica balsamina*) which belongs to the family Cucurbitaceae, is an important medicinal and nutritional vegetable crop (Heiser, 1979). Bitter gourd is a tropical and subtropical annual twine, widely grown in Asia, Africa, and the Caribbean for its edible fruit, Balsam apple is an annual to perennial vine native to tropical regions of Africa. Bitter gourd fruits are a good source of carbohydrates, proteins, vitamins and minerals. It has a highest nutrient value among the cucurbits (Miniraj *et al.,*1993; Desai and Musmade 1998). Moreover, the crude protein content of bitter gourd fruit is higher than of tomato and cucumber (Xiang *et al.,* 2000). Balsam apple fruits are a good source of carbohydrates, proteins, vitamins and minerals. The leaves are important source of nutrients having 17 amino acids (Hassan *et al.,*2006) and adequate mineral composition like potassium, magnesium, phosphorous, calcium, sodium, zinc, manganese and iron and high protein and fat with low fiber content (Michael and Anthony, 2007; Hasan *et al.,*2006). Bitter gourd has been used in traditional medicine of India, China, Africa and plant parts extracts possess antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic properties while having the ability to lower blood sugar (Wellihinda *et al.,*1986). Balsam apple have various medicinal properties such as anti HIV property (Bot *et al*., 2007), antiplasmodial activity (Benoit-Vical *et al.,* 2004), Shegellocidal, anti- diarrhoeal, antiseptic, antibacterial, antiviral, anti-inflammatory and anti microbial activity (Tommasi *et al*., 1995, Karumi *et al.,* 2003, Thakur *et al.,* 2009, Jigam *et al.,* 2004). Pierik (1987) stated that cytokinins are often used to stimulate growth and development, Kn and BAP being in common use. Agarwal and Kamal (2004) observed shoot differentiation in *Momordica charantia* when alone BAP was used. Tang *et al.,* (2011) investigated the effects of Thidiazuran (TDZ), silver nitrate (AgNO3) and triacontanol on adventitious buds induction from stems of balsam pear (*Momordica charantia*) and found that TDZ was necessary for bud development. (Thakur *et al.,* 2011) reported that high frequency *in vitro* shoots were produced form nodal explants on MS medium supplemented with BAP (1.0 mg/L) in *M. Balsamina.* In cucurbits, the *in vitro* plantlet regeneration offers an excellent model system for biotechnological studies. However, *in vitro* plantlet regeneration depends on genotype, explants and medium their interaction and other environmental factors. Therefore, protocols for *in vitro* plant regeneration have to be developed for each and every species, variety and cultivars. Due to high medicinal and nutritional value, low seed viability of these two plants, there was a need to develop

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specific concentrations of phyto hormones for rapid production of in vitro regenerated plant lets.

MATERIALS AND METHODS

M. balsamina and *M. charantia* seeds were collected and sterilised with 0.1% $HgCl_2$ solution for 5 minutes, after that the seeds were rinsed with double distilled water for four to five times to remove the traces of mercuric chloride under aseptic condition. The surface sterilised seeds were placed on MS basal medium for germination. After germinating the cotyledons were taken and cultured on Murashige and Skoog (MS) medium fortified with various concentrations of Thidiazuran (TDZ), 6- Benzylaminopurine, (BAP) and Kinetin (Kn) + Casein Hydrolysate (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 +200 mg/L) for organogenesis studies. The clumps of shoots obtained from explants were taken and sub cultured on MS medium supplemented with GA_3 for shoot elongation. The regenerated shoots derived from explants were taken and transferred in to MS medium fortified with IBA (3.0 mg/L) for rooting. The regenerated plantlets were transferred to green house.

RESULTS AND DISCUSSION

TDZ + Casein hydrolysate

Statistically highest number of shoots, (10.9 ± 0.05 and 9.00± 0.23) and shoot length (0.45 ± 0.32 and 0.67 ± 0.12 cm) was observed in cotyledon explants cultured on MS medium supplemented with TDZ (2.0 mg/L) + Casein hydrolysate (200 mg/L) in *M. balsamina* and *M. charantia,* respectively.

BAP + Casein hydrolysate

Statistically highest number of shoots, (7.00 ± 0.25 and 7.87 ± 0.45) and shoot length (1.26 ± 0.20 and 0.96 ± 0.12 cm) was observed in cotyledon explants cultured on MS medium supplemented with BAP (2.0 mg/L) + Casein hydrolysate (200 mg/L) in *M. balsamina* and *M. charantia,* respectively.

Kn + Casein hydrolysate

Statistically highest number of shoots, (3.55 ± 0.10, and 2. 98 ± 0.45) and shoot length (1.45 ± 0.05 and 2.23 ± 0.26 cm) was observed in cotyledon explants cultured on MS medium supplemented with Kn (2.0 mg/L) + Casein hydrolysate (200 mg/L) in *M. balsamina* and *M. charantia,* respectively.

***In vitro* shoot bud elongation**

Clumps of small shoots and shoot buds (non-elongating rosette) obtained from MS medium supplemented with TDZ + L- glutamic acid or Casein hydrolysate treatment elongated to 2.0 cm in length when cultured on MS medium supplemented with GA_3 (1.0 mg/L), after 3 weeks of culture.

***In vitro* rooting**

In vitro shoots (2.0 cm long) were separated from shoot bud clumps when transferred to MS medium supplemented with IBA (3.0 mg/L) developed roots. Highest percent cultures (90) developed roots on MS medium supplemented with IBA (3.0 mg/L). A total of 9.81 and 8.5 shoots produced roots (Plantlets, R_0) in cotyledon explants in *M. balsamina* and *M. charantia,* respectively.

Plantlet acclimatization and field transfer

About 75 % of the plantlets (R_0) were successfully transferred to field after acclimatization. A total of 7 and 6 plantlets (R_0) survived in field conditions and produced flowers and fruits. In this study we observed that, among three cytokinins tested, TDZ induced maximum number (10.9 ± 0.05 and 9.00± 0.23) of shoots in cotyledon explants of *M. Balsamina and M. charantia*, respectively. Tang *et al*., (2011) investigated the effects of TDZ, silver nitrate ($AgNO_3$) and triacontanol on adventitious buds induction from stems of balsam pear (*Momordica charantia*) and found that TDZ was necessary for bud development and the higher concentration of it could induce adventitious buds efficiently.

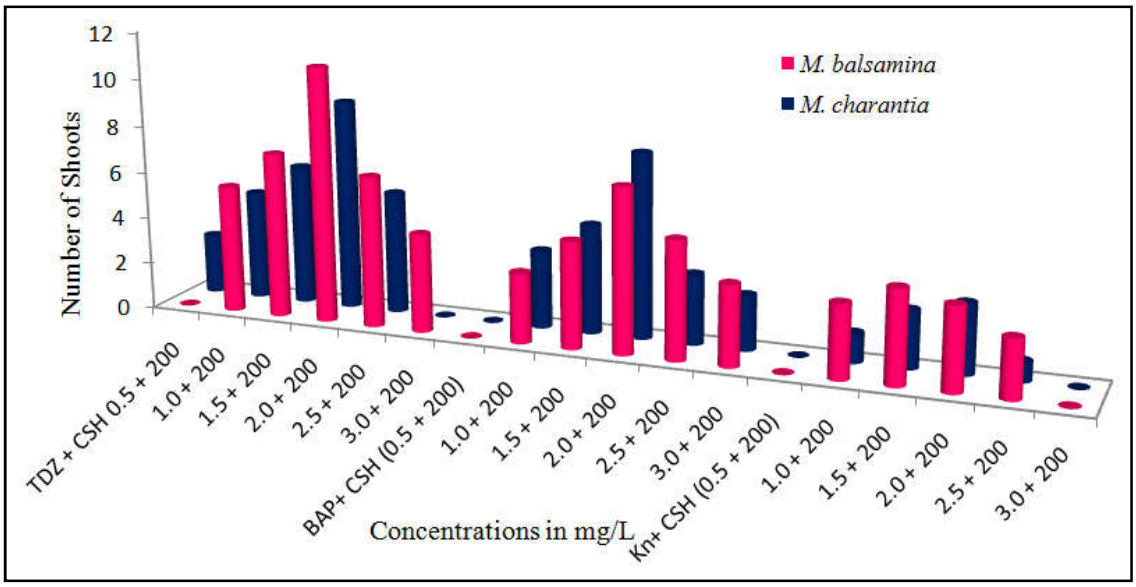

Figure 1. ***In vitro*** **shoot bud induction via organogenesis from cotyledon explants cultured on MS medium supplemented with TDZ, BAP and Kn (0.5 – 3.0 mg/L), combination with Casein hydrolysate (200 mg/L) in** ***M. balsamina*** **and** ***M. charantia*** **L., after 3 weeks of culture**

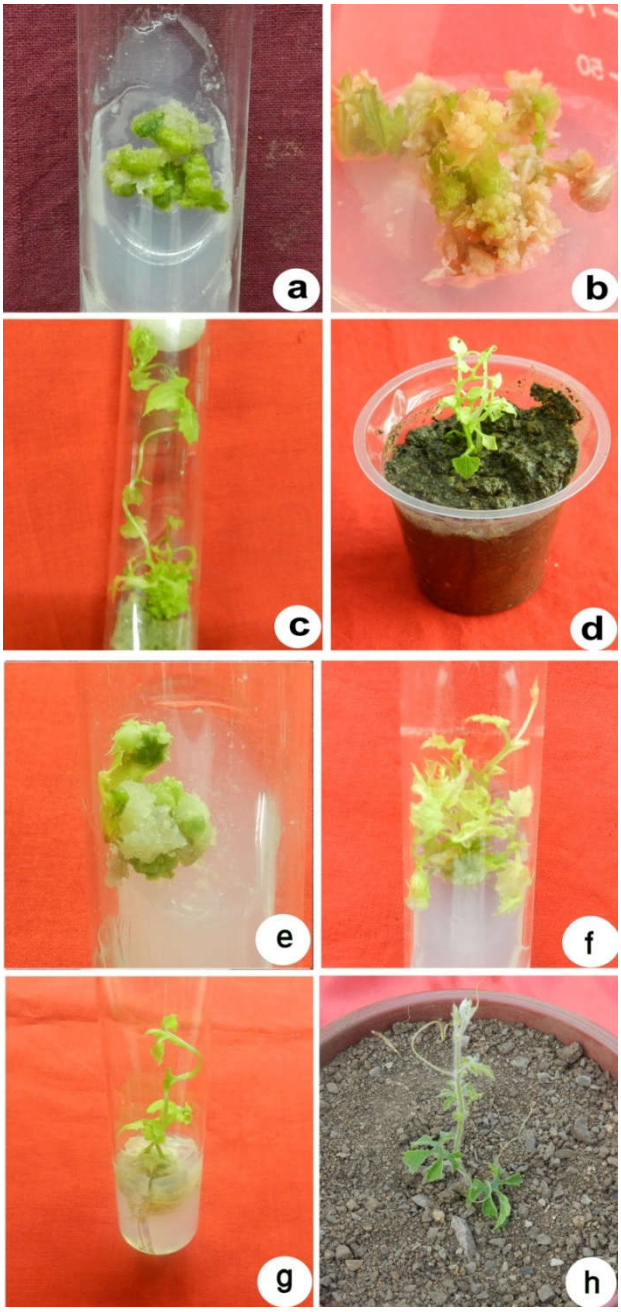

Fig. 2. I*n vitro* shoot bud induction via organogenesis from cotyledon explants cultured on MS medium supplemented with TDZ, BAP and Kn (0.5 – 3.0 mg/L), combination with Casein hydrolysate (200 mg/L) in *M. balsamina* and *M. charantia.* a & e) Cotyledon explant inoculated on MS + TDZ (2.0 mg/L) + Casein hydrolysate (200 mg/L), in *M. balsamina* and *M. charantia*, after 1 weeks of culture. b & f) Adventitious shoot bud induction in cotyledon explants on MS + TDZ (2.0 mg/L) + Casein hydrolysate (200 mg/L) in *M. balsamina* and *M. charantia*, after 2 weeks of culture. c & g) Shoot bud elongation on MS+ GA_3 (1.0 mg/L) medium in *M. balsamina* and *M. charantia* after 2 weeks of culture. d & h) Hardening of the plant into research field

Savitha *et al*. (2010) reported that MS medium with 2, 4-D (2.5 mg/L) and TDZ (0.5 mg/L) produced high frequency shoot regeneration from leaf derived callus of *Citrullus colosynthes*. The effectiveness of TDZ over other cytokinins has also been reported in other cucurbits such as *Cucurbita pepo* (Pal *et al.,* 2007), and *Melothria maderaspatana* (Baskaran *et al.,* 2009). In this study, MS medium containing TDZ (2.0 mg/L) and various concentrations of casein hydrolysate significantly increased adventitious shoots from cotyledon explants of *M. Balsamina and M. charantia*. In this study we observed that, TDZ is more effective in shoot regeneration as compared to BAP. In our study the maximum number (7.00 ± 0.25 and 7.87 ± 0.45) of shoots produced from in cotyledon explants of *M. Balsamina and M. charantia*, respectively. Our results also supported by Sultana and Bari (2003), Hoque *et al.* (1995). Sultana and Miah (2003) observed best response towards shoot regeneration obtained from the nodal segments of *Momordica charantia* on MS supplemented with BA (2.0 mg/L) and NAA (0.2 mg/L).In this study we observed that low number (3.55 ± 0.10 and 2. 98 ± 0.45) of shoots induced from in cotyledon explants of *M. Balsamina and M. charantia*, respectively. A clumps of micro shoots obtained from MS medium containing TDZ (2.0 mg/L) + CSH (200 mg/L) from cotyledons explants of both plants. These shoots were transferred to MS medium fortified with GA_3 (1.0 mg/L) for shoot elongation. Huda *et al* (2006) observed the growth of Meristem on semisolid MS medium supplemented with Kn (0.05 mg/L) + GA3 (0.1 mg/L). While root formation the roots was achieved from, when regenerated shoots were transferred to MS medium both full strength supplemented with IBA (3.0 m/L) concentrations in both plants.

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