EFFECTS OF THIDIAZURON AND BENZYLADENINEONFORMATION OF SHOOTSANDEMBRYOGENIC NODULESIN BANANA(Musaspp.)TISSUE CULTURE

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EFFECTS OF THIDIAZURON AND BENZYLADENINE ON FORMATION OF SHOOTS AND EMBRYOGENIC NODULESIN BANANA (*Musa* spp.) TISSUE CULTURE

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ABSTRACT

A Study was conducted to investigate the effect of thydiazuron (TDZ) and benzyladenine (BA) on shoot and embryogenic nodule formation in banana tissue culture. The study consisted of two experiments. The first experiment was conducted to study response of banana cv Kepok Kuning to thidiazurona (TDZ) and benzyladenine (BA) in vitro. Shoot tips were used as explants and cultured on MS medium containing MS salts, 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glyciene, 30 g/l sucrose, combinations of TDZ (0.005, 0.01, 0.05, and 0.1 mg/l) and BA (0 and 2 mg/l) as treatments. The medium was solidified with 8 g/l agar. Some cultures formed embryogenic nodules, and these structures were then cultured on MS medium supplemented with 0.1 mg/l TDZ or 0.1 mg/l TDZ + 2 mg/l BA as treatments. The second experiment aimed to investigate response of banana (cv. Raja Bulu) embryogenic nodules to TDZ and BA as previously described in the first experiment. The medium was as previously described for the first experiment. Results of the first experiment revealed that BA did not significantly affected shoot and shoot bud. The highest number of shoots was attained at 0.05 mg/l TDZ (2.6 shoots per explant). Treatment 0.1 mg/l TDZ and 0.1 mg/l TDZ + 2 mg/l BA resulted in formation of embryogenic nodules. Results of the second experiment showed that increased in TDZ concentrations did not significantly affect embryogenic nodule proliferation. In contrast, addition of 2 mg/l BA significantly decreased number of embryogenic nodules, from 12.5 to 6.2 embryogenic nodules per explant.For propagation purpose, cv. Kepok Kuning required 0.005 mg/l TDZ for shoot and shoot bud multiplication and 0.1 mg/l TDZ for embryogenic nodule proliferation and cv. Raja Bulu required 0.005 mg/l TDZ for embryogenic nodule proliferation.

Key Words: Benzyladenine, Kepok Kuning, Raja Bulu, shoot tips, thidiazuron,

INTRODUCTION

Banana (*Musa* spp.),which belongs to the family of Musaceae, is one of the most important sources of food for human beings after wheat, rice, and corn (Remakanthan et al., 2014). Indonesia has been recorded as the sixth largest producers of banana fruit, producing 6.19 million MT, with India being the largest, with production of 26.5 million MT, followed by (in million MT), China 10.6, The Philippine 9.2, Ecuador 7.0, and Brazil 6.9 (Indian Horticulture Database, 2014). Indonesian production of banana has been mostly for domestic market, which is due to relatively low quality of products and their unreliable continuity. To supply the global market, this problem should be solved, among other things, by improving crop production practices and genetic quality of plants.

Most of banana production practices in Indonesia use suckers as planting materials, which are not reliable because they are in variable quality and difficult to supply in large number and in uniform size. One way to cope with the problem is by applying plant tissue culture technology because it could be used to produceuniform planting materials in a large number in a relatively short time.In addition, plant tissue culture could be used to support plant breeding through somaclonal variation, mutation, and genetic engineering. This technology is especially essential for commercial banana plants, since they are seedless and vegetatively propagated.

Plant tissue culture through axillary branching has been widely used to clonally propagate banana plants.Clonal propagation of banana using axillary branching are usually conducted by culturing shoot tips from rhizome on media containing cytokinins to induce shoot multiplication, subculture each shoot on media containing auxins to promote rooting;and acclimatize the plantlets (Yusnita et al., 2015; Yusnita, 2015). At the base of proliferating shoots sometimes appear scalps, a structure containing clumps of meristematictissue showing an embryogenic characteristic. Under favorable condition scalps could grow into multiple shoots.This research aimed to investigate effects of thidiazuron and benzyladenin that have strong cytokinin activity on shoot multiplications and scalp formation in tissue culture of banana cv Kepok Kuning and Raja Bulu.

MATERIALS AND METHODS

Experiment 1: Response of shoot tips of banana cv Kepok Kuning to concentrations of thidiazuron and benzyladenine

In this experiment, shoot tips of sword suckers as explants were cultured aseptically on a precondition medium(P) for 2 weeks and subcultured onto shoot-inducing media (SI) with several subcultures. After for weeks in culture, the shoot tips were longitudinally sliced four times down to meristem tips to suppress apical dominance. After 10 weeks in culture, shoots and embrygenic nodules (scalps) originated from the base of the explants were observed. The shoots were induced to form roots and the plantlets were acclimatized. The embryogenic nodules were subcultured for proliferationin embryogenic-nodul-proliferating media (NP).

Plant materials were derived from banana plant cv. Kepok Kuning from Lampung Province. Sword suckers with the rhizome of approximately 10-15 cm in diameter were used as source of explants. The pseudostems were separated from the rhizome in such a way that the apical shoots were still intact with the pseudostems. The pesudostems were cut off and the sheaths were peeled off, leaving shoot tip of 15 cm in length, which were then rinsed under running tap water and soaked for 30 minutes in a solution containing 2 g/l fungicide mankozeb and 150 mg/l ascorbic acid, and rinsed again under running tap water. Sheath peeling were done again and the shoots were cut to the size of 5 cm in length and rinsed under running tap water. The sterilization was continued by shaking the shoots in 2.13% NaOCl solution added with 5 drops of Tween-20. Sheath peeling and cutting was done again until the shoots were 1.5 x 1.5 x 1 cm (W x L X H) in size. The shoots were soaked for 15 minutes in ascorbic acid solution (150 mg/l), sterilized for 10 minutes in 0.8% NaOCl solution under vacumed condition in a dessicator and then rinsed three times with sterile distilled water.The procedure was repeated one more and the shoots as explants were ready for culture initiation. Initially the explants were aseptically cultured on P medium and then transfered to SI medium.

The P medium was composed of MS (Murashige and Skoog, 1962) salts, 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glyciene, 30 g/l sucrose,2 mg/l BA, 0.005 mg/l TDZ; 50 mg/l citric acid, and 150 mg/l ascorbic acid. The SI medium was the same as the P medium except that the added plant growth regulatorswere combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (BA) (0 and 2 mg/l).The NP medium was the same as the P medium except that the supplemented plant growth regulator were 0.1 mg/l TDZ.The pH of medium was adjusted to 5.8 by adding HCl 1 N or KOH 1 N. The medium was added with 8 g of agar as solidifying agent,boiled and then dispensed into 250-ml culture bottles, 25 ml/bottle. The

bottles containing the medium were capped with transparent plastic sheets and autoclaved at a pressure of 1.2 kg/cm^2 and a temperature of 121°C for 10 minutes.

Shoot tips as explants were initially cultured on P medium for 2 weeks and then subcultured to SI medium.The SI mediumwassupplemented with combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (0 and 2 mg/l). The treatments were arranged in a completely randomized design with 5 replications, 5 culture bottles per replication, 1 explant per bottle. All cultures were incubated in a culture room at 25 ± 2°C, under continous light of fluorescent lamps of approximately 1000 lux, and After 10 weeks in culture, data on shoots were recorded and embryogenic nodules were separated and cultured on NP medium containing 0.1 mg/l TDZ.Data were subjected to analysis of variance and difference between two data due to treatments was subjected to a least-siginificant difference (LSD) analysis.

Experiment 2: Response of embryogenc nodules cv. Raja Bulu to concentrations of thidiazuron and benzyladenine

Embryogenic nodules were initiated from proliferating axillary shoots of banana cv. Raja Bulu reported by Yusnita *et al* (2015) by culturing the shoots on media MS media containing 0.05 mg/L of TDZ for 8 weeks (Fig.). The embryogenic nodules were treated as previously described using SI medium supplemented with combinations of TDZ (0.005, 0.01, 0.05 and 0.1 mg/l) and benzyladenine (0 and 2 mg/l). Media preparation and culture condition was as previously described. After 8 weeks in culture the proliferating embryogenic nodules were observed and recorded.

RESULTS AND DISCUSSION

Results of Experiment 1 showed that different concentrations of TDZ had significant effects on number of shoots and propagules (shoots and shoot buds), while BA had no significant effects. Both TDZ and BA did not significantly affect number of shoot buds. Interaction of both factors did not significantly influence all of the variables. Increase in TDZ concentrations from 0.9 - 0.1 mg/l resulted in an increase in numberof shoots and propagules (Figure 1). Shoots were defined as those having length 0.5 cm or more, while shoot buds are those having length less than 0.5 cm. Highest number of shoots was attained at treatment of 0.05 mg/l TDZand propagules at 0.1 mg/l TDZ (Figure 1). Based on LSD analysis, number of shoots and number propagules at 0.05 mg/l TDZ and 0.1 mg/l TDZ were not significantly

different, so 0.05 mg/l TDZ was considered the best treatment for maximum shoot proliferation.

Figure 2 showed that treatment 0.1 mg/l TDZ and 0.1 mg/l TDZ + 2 mg/l BA resulted in formation of embryogenic nodules (scalp) in addition to shoots. The nodules from 0.1-mg/l-TDZ treatment were then subcultured onto the same medium for proliferation. Subcultures were conducted every 4 weeks. Subculture started with embryogenic nodule clumps of 0.5 cm, each then proliferated. After 12 weeks, the culture produced 42 clumps containing 433 embryogenic nodules (Figure 3)

Experiment 2 was conducted to confirm whether embryogenic nodules of cultivar Raja Bulucould also proliferate as cv. Kepok Kuning did when they were cultured on media containing TDZ or TDZ + BA. Results of the experiment revealed that all treatment led to proliferation of embryogenic nodules. Range ofTDZ concentrations (0.005 – 0.1 mg/l) resulted in non-significantly different number of nodules, while additions of 2 mg/l BA significantly suppress nodule proliferation (Figure 4 and 5). Therefore, MS medium supplemented with 0.005 mg/l TDZ was optimum for embryogenic nodule proliferation.

The data showed that shoot multiplication response varied with TDZ concentrations. In general the higher the TDZ concentrations the more shoots or the propagules were formed. Even though there was no control treatment i.e. without growth regulator, based on experience and many reports, no shoot multiplication occured in shoot tip culture of banana in media devoid of growth reguators. Therefore, it could be concluded that in this experiment TDZ could induce shoot multiplicationof banana cv. Kepok Kuning, indicating that TDZ showed a cytokinin activity in banana tissue culture. TDZ has beenalso reported to have cytokinin activity in other plants , that was to induce shoot de novo or shoot multiplication in other plants, for example in tissue culture of *Viola odorata* (Mokhtari et al., 2015), *Aloe vera* L. (Lavakumaran and Seran, 2014), *Vitex trifolia* L. (Ahmed and Anis , 2014), *Crocus sativus* L. (Sharifi et al., 2010), *Curcuma vamana* L. (Beyoy et al., 2012), *Saussurea involucrata*Kar. et Kir. (Guo et al., 2012), *Curculigo latifolia* L. (Babaei et al., 2014), *Populus ciliata* Wall.(Aggarwal et al., 2012), and *Salix tetrasperma* Roxb. (Khan and Anis, 2012).

The mode of action of cytokinin activity of TDZ, which was initially produced as a defoliant, has not been conclusively elucitated. However, a group of researchers showed that shoot differentiation response to TDZ had been correlated with an increase in a compound having cytokinin activity such as N^6-(Δ^6-isopentenyl)adenine (iP)(Casanova et al., 2004).

This increase might be due to inhibition of cytokinin oxidase by TDZ, so that more iP was available to tissue. Casanova et al. (2004) did not exclude the posibility that TDZ had its own biological activity based on other reports that receptor proteins for both adenine-type and phenylurea cytokinins had been discovered.

In Experiment 1, TDZ not only induced shoot multiplication but also the formation of scalps, structures containing meristematic nodules. Scalps were considered to be embryogenic bacause they could be turned into embryogenic callus and then turned into somatic embryos (Sadik et al., 2015). In our experiment, the scalps were induced in media containing TDZ, and were then proliferating when cultured on media containing the same regulator (Figure 3).

That TDZ induced the formation of scalp, which was embryogenic in nature, indicates that TDZ was correlated with auxin activity since auxins has been known to induce somatic embryogenesis. Guo et al (2011) reviewed biochemical and biophysical responses of plant cell to TDZ. They made a list of 58 families of plants that has been reported to respond to TDZ. While most of the families on the list showed shoot formation response, 13 families showed somatic embryogenesis response (Guo et al., 2011), meaning that this was related to endogenous auxin metabolism. Using leaves of *Echinacea purpurea* L. cultured in vitro as a model system, it was demonstrated that TDZ-induced regeneration was correlated with level of auxins (indole-3-acetic acid) in the regenerant tissue. Number of regenerant and auxin level increased by exposure to TDZ. Number of TDZ-induced regenerant was decreased by exposure to auxin transport inhibitor, 2,3,5-triindobenzoic acid (TIBA). Number of TDZ-induced regenerant was also decreased by exposure to auxin action inhibitor, p-chlorophenoxyisobutyric acid (PCIB). Those data suggest that TDZ did not act directly to regulate regeneration, but its action was mediated by endogenous auxin.

The optimal concentration of TDZ for propagation of banana cv. Kepok Kuning was 0.05 mg/l TDZ. Number of propagules should be a variable of consideration because number of shoot buds is important since they could be turned into shoots. In addition, lower concentrations poses less genetic abberation, a requirement for clonal propagation. For embryogenic nodule proliferation, 0.1 mg/l TDZ was found to be optimum for cv. Kepok Kuning and 0.005 mg/l TDZ for cv. Raja Bulu.

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REFERENCES

Aggarwal, G., Sharma,C. and Srivasta,D.K. 2012. Thidiazuron: a potent cytokinin for efficient plant regeneration in Himalayan poplar (*Populus ciliata* Wall.) using leaf explants. Ann. For. Res. 55(2):179-188.

Ahmed, M.R. andAnis, M. 2014. In vitro regeneration and the antioxidant enzymatic system on acclimatization of Vitex trifolia L. Agroforest Syst 88:437-447.

Babaei, N., Abdullah,N.A.P., Saleh, G. and Abdullah, T.L. 2014. An efficient in vitro plantlet regeneration from shoot tip cultures of *Curculigo latifolia*, a medicinal plant. The Scientific World Journal 2014: 1-9.

Bejoy, M., Dan,M.,.Anis,N.P., Nair, A.R.G., Radhika, B.J. and Manesh,K. 2012. Micropropagation of an Indian Ginger (*Curcuma vamana* Sabu and Mangaly): A wild relative of turmeric. Biotechnology 11(6):333-338.

Casanova E., Valdes,A.E., Fernandes, B., Moysset, L., and Trillas, M.I. 2004. Level and immunolocalization of endogenous cytokinins ini thidiazuron-induced shoot organogenesis in carnation. J.Plant Physiology 161:95-104.

Guo, B., Stilles,A.R. and.Liu, C.Z. 2012. Thidiazuron enhances shoot organogenesis from leaf explants of *Saussurea involucrata* Kar.et Kir. In Vitro Cell.Dev.Biol.48:609-612.

Guo, B.,.Abbasi,B.H., Zeb,A., Xu, L.L. and Wei,Y.H. 2011. Thidiazuron: a multi-dimensional plant growth regulator. African J.Biotech.10(45):8984-9000.

Indian Horticulture Database. 2014. Indian Hortikulture Database-2013. New Delhi. India.

Khan, M.I. and Anis,M. 2012. Modulation of in vitro morphogenesis in nodal segments of *Salix tetrasperma* Roxb. through the use of TDZ, different media types and culture regimes. Agroforest Syst 86:95-103.

Lavakumaran, L. and Seran,T.H. 2014. Effects of 6-benzyl-aminopurine and thidiazuron on in vitro shoot organogenesis of Aloe vera (L.) Burn.f. Chilean J.Agric.Res. 74(4):497-501.

Mochtari, A., Otroshy,M. and Barekat, T. 2015. Plant regeneration through callus induction on medicinal herb *Viola odorata*: role of plant growth regulators and explants.

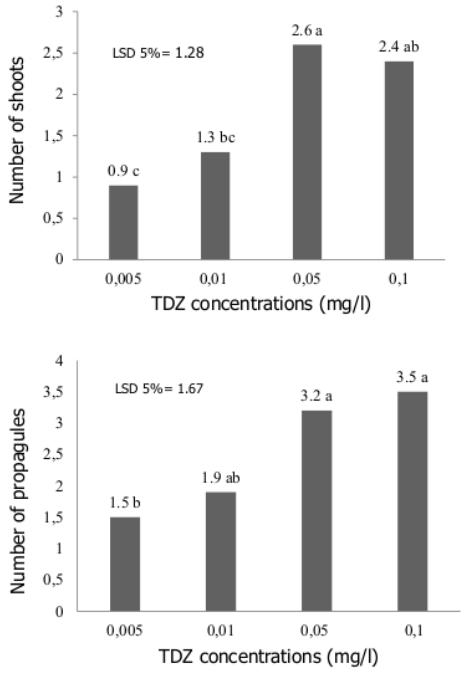
Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Remakanthan, A., Menon, T.G.,and Soniya, E.V. 2014. Somatic embryogenesis in banana (Musa acuminata AAA cv. Grand Naine): effect of explant and culture conditions. In Vitro Cell.Dev.Biol.—Plant 50:127–136.

Sadik, K.,.Arinaitwe,G., Rubaihayo,P.R. and Mukasa, S.B.. 2015. TDZ and 4-CPPU induce embryogenic response on scalps of recalcitrant east african highland banana. J.Agric.Sci.7(8):215-224.

Sharifi, G., Ebrahimzadeh, H., Ghareyazie, B., and Karimi, M. 2010. Globular embryo-like structures and highly efficient thidiazuron-induced multiple shoot formation in saffron (*Crocus sativus* L.). In Vitro Cell.Dev.Biol. 46:274-280.

Yusnita, Danial,E. and Hapsoro, D. 2015. In vitro shoot regeneration of Indonesian banana (Musa spp.) cv. Ambon Kuning and Raja Bulu, plantlet acclimatization and field performance. Agrivita 37(1): 51-58.



Yusnita. 2015. Kultur Jaringan Tanaman Pisang. Aura Publishing. 104p.

Figure 1. Effects of thidiazuron on number of shoots (above) and propagules (below) in tissue culture of banana cv. Kepok Kuning after 10 weeks in culture.

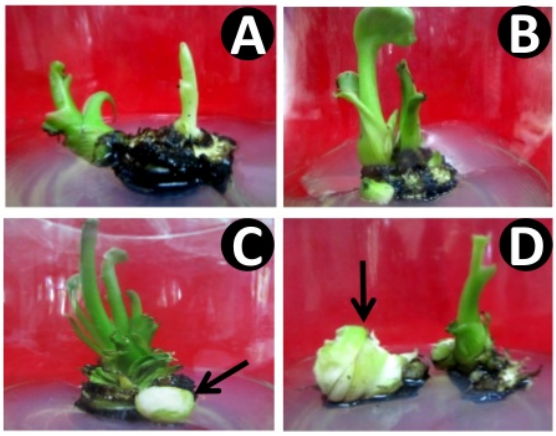


Figure 2. Shoot and scalp (embryogenic nodule) formation (indicated by arrow)in response to thidiazuron (TDZ) and benzyladenine (BA) in tissue culture of banana cv. KepokKuning after 10 weeks in culture. The media was MS containing (A) 0,05 mg/l TDZ. (B)0,05 mg/l TDZ + 2 mg/l BA. (C)0,1 mg/l TDZ. (D) 0,1 mg/l TDZ + 2 mg/l BA.

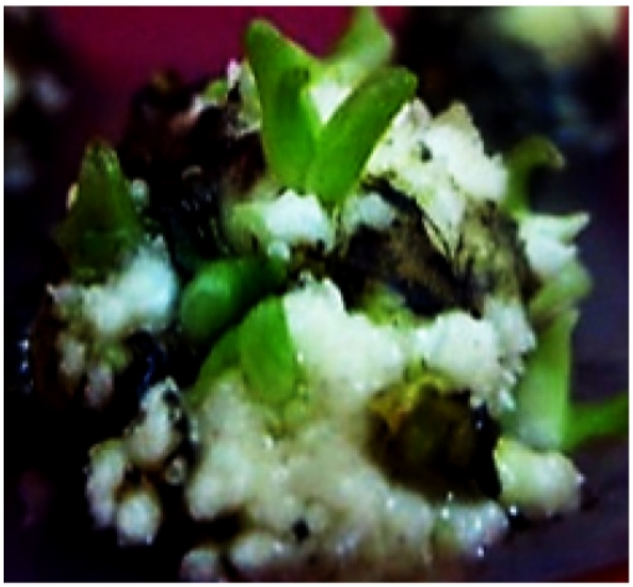


Figure 3. Shoots and proliferating embryogenic nodules in tissue culture of banana cv. Kepok Kuning after 3 subcultures. Subculture was done every 4 weeks. The embryogenicnodules looks white in color. The shoots, that was developed from nodules, appear green in color

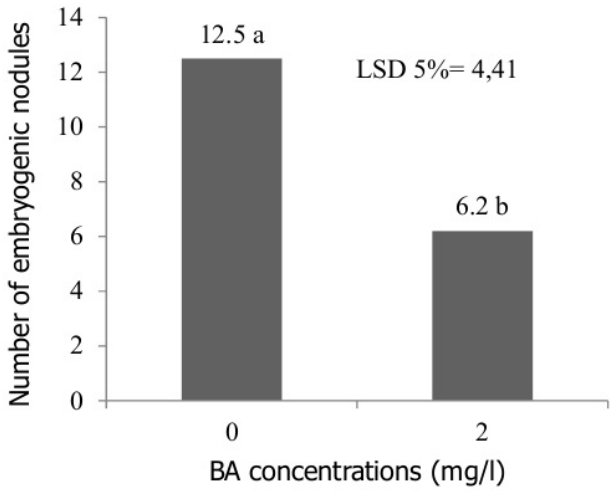


Figure 4. Effects of benzyladenine (BA) on number of embryogenic nodules in tissue culture of banana cv. Raja Bulu.

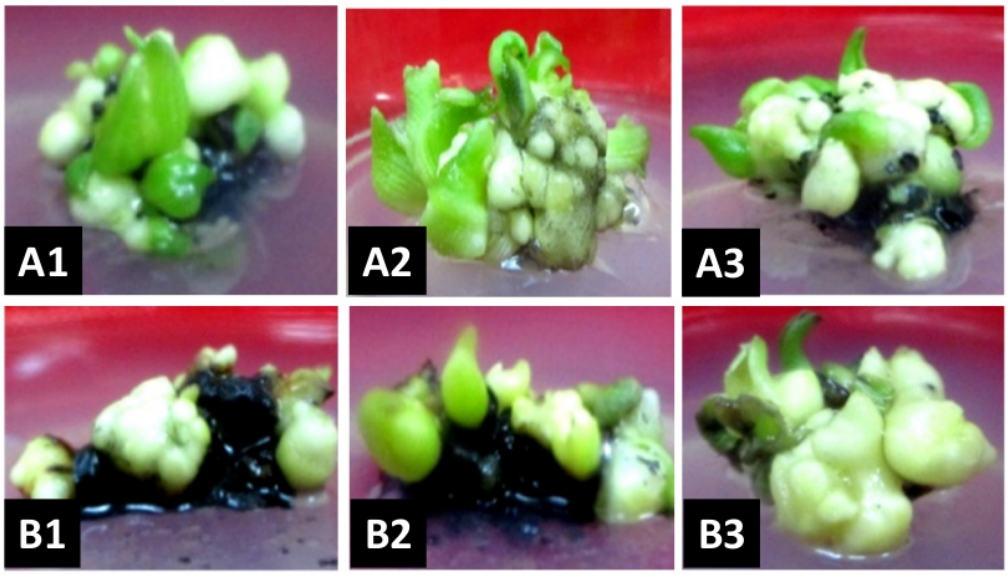


Figure 5. Response of embryogenic nodules to proliferation media containing thidiazuron (TDZ) and benzyladenine (BA) after 8 weeks in culture.Proliferating embryogenic nodules on media containing TDZ (Row A) and TDZ + BA (Row B).(A1) 0,005 mg/l TDZ, (B1) 0,005 mg/l TDZ + 2 mg/l BA, (A2) 0,01 mg/l TDZ, (B2) 0,01 mg/l TDZ + 2 mg/l BA, (A3) 0,05 mg/l TDZ, (B3) 0,05 mg/l TDZ + 2 mg/l BA.

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19 Rosaria Perrini, Vittorio Alba, Claudia Ruta, Irene Morone-Fortunato, Antonio Blanco, Cinzia Montemurro. "An evaluation of a new approach to the regeneration of Helichrysum italicum (Roth) G. Don, and the molecular characterization of the variation among sets of differently derived regenerants", Cellular and Molecular Biology Letters, 2009 — 9 words — < 1%
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Internet

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