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Full Length Research Paper

Synergistic effects of some plant growth regulators on *in vitro* shoot proliferation of korarima (*Aframomum corrorima* (Braun) Jansen)

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The synergistic effects of some plant growth regulators was investigated upon shoot proliferation and growth of korarima (*Aframomum corrorima* (Braun) Jansen), an important culinary and medicinal plant species native to Ethiopia. Cultures were initiated from axillary bud explants of rhizome using Murashige and Skoog (1962) (MS) medium added with 5% coconut water (CW). The use of 0.5 mg/l thidiazuron (TDZ) in combination with 3 mg/l paclobutrazol (PBZ) gave about 26 shoots/explant (about 12.6-fold than the control) within eight weeks time. Shoot multiplication was also enhanced when TDZ at 0.5 mg/l was simultaneously used with either 2 mg/l imazalil (IMA) or 3 mg/l N^6-benzyladenine (BA) in the culture medium. Subsequent shoot elongation and development of functional roots was attained after one to three monthly-subcultures on a plant growth regulator (PGR)-free basal medium. The protocol developed from the present study could be used for the large-scale multiplication of *A. corrorima* by tissue culture.

Key words: 6-benzyladenine, imazalil, korarima, micropropagation, paclobutrazol, thidiazuron, Zingiberaceae.

INTRODUCTION

Korarima (*Aframomum corrorima*) or the Ethiopian cardamom is a renowned spice and medicinal crop of the family Zingiberaceae, which is native to Ethiopia. The dried fruits are part of the daily dishes of the Ethiopians. They also serve several purposes in the traditional medicine practices (Jansen, 1981). Korarima oil is similar with that of the Indian cardamom (*Elettaria cardamomum*) in its chemical compositions, except for its reduced content of terpinyl acetate (Sebsebe, 1993). Korarima capsules are used as a substitute for the Indian cardamom in the world market. The crop has a relatively wider adaptation and higher productivity (i.e. ca 5.5-fold) than cardamom (Ethiopian Agricultural Research organization, EARO, 2000).

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Abbreviations: BA - N^6-benzyladenine; CW - coconut water, IMA - imazalil; MS - Murashige and Skoog (1962) medium; PBZ - paclobutrazol; TDZ - thidiazuron.

The division of rhizome is the conventional vegetative propagation technique used in korarima. The method shortens the juvenile phase of the stand and enables propagation of true-to-type plants of a desired clone. However, lack of sufficient planting materials to cover large acres of land and sacrifice of potential productive stands are the prominent problems associated with this particular technique. Therefore, it is essential to develop techniques that enable rapid propagation of promising lines. However, no work has so far been conducted to address this issue.

Development of an effective micropropagation protocol requires assessing for the most potent plant growth regulator that could provide high rates of shoot proliferation. In most cases, adenine-type cytokinins are employed to induce shoot multiplication in tissue cultures. This is due to the effects of cytokinin oxidases that cleave unsaturated N^6–isoprenoid side chains. The synthetic cytokinins, such as kinetin and BA, are less susceptible to these degradative enzymes, but they are generally less active than the natural ones (Mok et al., 1987; Hare and van Staden, 1994).

In the past few years, TDZ has emerged as a highly

effective plant growth regulator for use in tissue cultures of different plant species ranging from herbaceous to tree crops (Murthy et al., 1998). However, only few studies have so far been conducted in plants of the family Zingiberaceae involving TDZ (Salvi et al., 2000; Prathanturarug et al., 2003). As reported by Murthy et al. (1998), TDZ is effective both alone and in combination with other growth-regulating substances in inducing high rates of regeneration in tissue and cell culture works. The combined use of TDZ with BA has proved better for shoot multiplication in several plant species (Nielsen et al., 1995; Khalafalla and Hattori, 1999). Since the past decade, some azole derivatives, such as IMA and PBZ, had been found promising to enhance the shoot inducing capacity of different cytokinins, including TDZ. According to Werbrouck and Debergh (1996), TDZ mediated shoot proliferation was improved by the inclusion of IMA in *Spathiphyllum floribundum* culture. Likewise, the combined use of PBZ with TDZ has also been reported to enhance shoot proliferation in banana cultures than the use of the latter alone (Lee, 2001).

Therefore, this study was conducted to investigate the synergistic effects of TDZ when combined with either of BA, IMA or PBZ on the *in vitro* shoot proliferation of korarima.

MATERIALS AND METHODS

Plant material

Rhizomes of korarima (Jimma local) brought from the Jimma Agricultural Research Center (JARC), Ethiopia, were grown in a nursery at Kampangsaen campus, Kasetsart University, Thailand. Axillary buds (3-5 mm) were excised from actively growing rhizomes. Buds collected from the sprouting rhizome were thoroughly washed using laboratory detergent after removal of some outer bud scales. Subsequently, the buds were kept under running tap water for 1.5 h. More scales were then removed, followed by washing with the detergent.

The cleansed buds were rinsed with 70% ethanol for 1 min, followed by a two-step surface sterilization using 20 and 10% Hyter® (ai: 6% sodium hypochlorite, v/v) added with 2 ml/l Tween-80 for 10 and 5 min, respectively. Then, explants were washed four times with sterilized distilled water and were further trimmed to remove dead and chlorine affected tissues. Single explants consisting of a shoot tip with a small portion of the rhizome were cultured on a modified MS medium with 5% CW for culture initiation. Shoot explants were then multiplied on a modified MS medium with 5% coconut water, 3 mg/l BA and 1 mg/l kinetin. They were subsequently sub cultured on a basal MS medium for a month prior to exposure to the different experiments.

Culture conditions

In all cases, 3% (w/v) sucrose was used as a source of carbohydrate and media were gelled with 0.7% (w/v) agar-agar after adjusting the pH to 5.7. All along the experiment, plant growth regulators were added to the media prior to autoclaving and 20 ml of the respective medium was dispensed to each 100 ml baby food jar and covered with plastic cap. The media were autoclaved for 20 min at 121°C (1.06 kg/cm^2). Cultures were incubated in the culture room at a temperature of 25 ± 2°C under cool white fluorescent light of 28 $\mu mol.m^{-2}.s^{-1}$ intensity with 16 h photoperiod.

Shoot proliferation

Three sets of experiments were conducted to evaluate the synergistic effects of TDZ when combined with either of BA, IMA or PBZ on shoot proliferation and growth of korarima. In the first part, four levels of *N*-phenyl=*N*'-(1,2,3-thidiazol-5'yl) urea, DROPP® (thidiazuron, TDZ) (0, 0.25, 0.5 and 0.75 mg/l) and 0, 1.5, 3 and 4.5 mg/l of N^6-benzyleadenine (BA) were evaluated to identify the best combination for shoot proliferation and growth of korarima. In the second study, TDZ at 0, 0.25, 0.5 and 0.75 mg/l was studied in combination with 0, 2, 4 and 8 mg/l Allyl 1-(2,4-dichlorophenyl)-2-imidazol-1-ylethyl ether, Fungaflor® (imazalil, IMA). The third experiment involved the combination of 0, 0.25, 0.5 and 0.75 mg/l TDZ with that of 0, 1.5, 3 and 4.5 mg/l (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pentan-3-ol) [paclobutrazol, PBZ].

Statistical analyses

The design used in all the three experiments was a 4 X 4 factorial in complete randomized design (CRD) with ten replications. Data for shoot number, shoot length as well as dry weight of plantlets were recorded after eight weeks of culture. All experiments were repeated at least three times and data from the last two repetitions were used for analysis. Statistical analyses were done using a PC-SAS program (Version 8e, SAS Institute Inc., Cary, NC, USA). Treatment means showing significant differences were statistically separated using the Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Effects of TDZ in combination with 6-benzyladenine (BA)

Korarima shoot number and dry weight showed a considerable increase with the use of TDZ in the culture medium. But in both parameters, values started to decline after the third level. Shoot length showed a consistent decrease across all levels of TDZ evaluated. Significantly higher shoot number and dry weight were obtained from the treatment involving 3 mg/l BA. However, no statistical difference was observed between 1.5 and 3.0 mg/l BA on dry weight of plantlets. On the other hand, inclusion of BA to the culture medium exerted a negative effect on shoot length (Table 1).

The simultaneous use of TDZ and BA in the culture medium has considerably affected growth and development of korarima. The best medium for shoot proliferation and plantlet growth was the combination of 0.5 mg/l TDZ and 3 mg/l BA (13.89 shoots and 137.48 mg dry weight/explant). However, no significant differences in dry weight of plantlets were observed when 3.0 mg/l BA was combined with either of 0.5 mg/l or 0.75 mg/l TDZ. Of all the levels evaluated, the longest shoot (7.49 cm) was obtained from the PGR-free medium. In general, the sole use of BA in the culture medium seems better for shoot elongation than its combined use with TDZ (Table 2).

Table 1. Mean and probabilities of the independent effects of TDZ and BA on korarima shoot growth after eight weeks of culture.

Treatment	Mean (main effects)		
	Shoot no.	Shoot lg. (cm)	DW (mg)
TDZ (mg l $^{-1}$)			
0	2.77c	4.27a	82.95c
0.25	9.76b	1.52b	99.17b
0.5	11.31a	1.19c	117.59a
0.75	9.89b	0.97d	103.26b
BA (mg l $^{-1}$)			
0	8.38c	2.97a	93.49b
1.5	8.96b	1.89b	111.67a
3.0	10.24a	1.63c	115.39a
4.5	6.18d	1.43d	82.62c
Prob.			
TDZ	***	***	***
BA	***	***	***
TDZ X BA	***	***	***
% CV	**16.26**	**22.77**	**20.59**

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT. Shoot lg. = shoot length (cm), DW = dry weight.

Table 2. Effects of the combined use of TDZ and BA on the *in vitro* growth and development of korarima after eight weeks of culture.

TDZ (mg l $^{-1}$)	BA (mg l $^{-1}$)	Shoot no. (mean ± SE)	Shoot lg. (cm) (mean ± SE)	DW (mg) (mean ± SE)
0	0	1.89k ± 0.08	7.49a ± 0.32	86.66f ± 1.51
0	1.5	2.90j ± 0.16	3.72b ± 0.14	89.05ef ± 2.49
0	3.0	3.40j ± 0.11	3.18c ± 0.09	89.50ef ± 5.07
0	4.5	2.80j ± 0.14	2.99c ± 0.05	66.96g ± 3.00
0.25	0	9.65f ± 0.51	2.16d ± 0.12	96.17def ± 2.59
0.25	1.5	10.55def ± 0.39	1.47e ± 0.03	107.53cd ± 2.59
0.25	3.0	11.44cd ± 0.28	1.29ef ± 0.05	101.61def ± 6.21
0.25	4.5	7.33h ± 0.26	1.08fgh ± 0.08	90.79ef ± 1.94
0.5	0	11.20cde ± 0.25	1.49e ± 0.02	102.71de ± 4.24
0.5	1.5	11.90bc ± 0.30	1.32ef ± 0.04	130.96ab ± 4.54
0.5	3.0	13.89a ± 0.31	1.08fgh ± 0.04	137.48a ± 5.44
0.5	4.5	8.50g ± 0.41	0.86hi ± 0.07	101.19def ± 6.21
0.75	0	10.15f ± 0.29	1.20efg ± 0.03	87.74ef ± 6.47
0.75	1.5	10.50ef ± 0.29	1.04fghi ± 0.05	119.14bc ± 3.47
0.75	3.0	12.70b ± 0.41	0.89ghi ± 0.05	133.81a ± 7.25
0.75	4.5	6.20i ± 0.37	0.74i ± 0.09	72.36g ± 6.15

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT. Shoot lg. = shoot length (cm), DW = dry weight (mg)

The improved shoot proliferation effects observed from the use of TDZ in the present study were in agreement with those of Prathanturarug et al. (2003), on Curcuma longa, and (Tefera and Wannakrairoj, 2004) on Amumum krervanh. Furthermore, our results from the combined use of TDZ and BA are also in accordance with the reports of Khalafalla and Hattori (1999) in faba bean. In substantiating the current findings, Nielsen et al. (1995)

Table 3. Mean and probabilities of the independent effects of TDZ and IMA on korarima shoot growth after eight weeks of culture.

Factor	Mean (main effects)		
	Shoot no.	Shoot lg. (cm)	DW (mg)
TDZ (mg l $^{-1}$)			
0	2.05d	8.19a	89.01C
0.25	13.31c	2.68b	98.40b
0.5	16.76a	1.58c	116.39a
0.75	14.73b	1.12d	94.42C
IMA (mg l $^{-1}$)			
0	9.42c	4.08a	93.58bc
2	14.18a	3.51b	120.28a
4	11.77b	3.07c	96.12b
8	11.18B	3.09c	88.16c
Prob.			
TDZ	***	***	***
IMA	***	***	***
TDZ X IMA	***	ns	***
% CV	20.98	26.55	22.43

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT. Shoot lg. = shoot length (cm), DW = dry weight (mg).

had reported the possible binding of BA and TDZ to a cytokinin-binding protein (CBP), which is a receptor having two different binding sites. Of these two, one site was stated to bind the adenine-type cytokinins, while the other is able to bind the phenylurea-types. Binding of an adenine-type cytokinin to CBP was reported to induce the well-known cytokinin effects, i.e. promotion of cell division and shoot formation, as well as regulation of various developmental events (Mok et al., 2000). The change in cytokinin metabolism due to TDZ results in the induction of cytokinin autonomy by increasing the level of endogenous cytokinins (Victor et al., 1999). Kende and Zeevaart (1997) have also described TDZ as a non-competitive inhibitor of cytokinin oxidase (an enzyme that inactivates free cytokinins), thereby increasing the efficacy of available cytokinins.

Several studies (Murthy et al., 1998; Chand et al., 1999; Fratini and Ruiz, 2002) indicated suppression of shoot elongation due to the inclusion of TDZ in the culture medium. The negative effect of TDZ on shoot length was also aggravated further through addition of BA to the culture medium (Werbrouck and Debergh, 1996). The independent use of BA exerted a relatively milder effect upon shoot length in the absence of TDZ (Rai, 2002). These reports are in complete agreement with our results. The quality of shoots obtained from the experiment involving TDZ and BA was relatively better (Figure 1a) and the shoots resumed normal growth on their subsequent subculture to a PGR-free modified MS medium.

Effects of TDZ in combination with imazalil (IMA)

In this experiment, TDZ significantly improved korarima shoot number and dry weight up to the third level (0.5 mg/l). The use of TDZ in the culture medium greatly reduced shoot length. Significant increase in shoot number and dry weight of korarima were observed in cultures supplemented with 2 mg/l IMA. However, inclusion of IMA in to the culture medium resulted in a slightly shorter shoots (Table 3).

The combined use of these two chemicals has a synergistic effect on the number of shoots and dry weight of plantlets, but not on shoot length. The highest shoot multiplication rate for korarima (11.35-fold than the blank control) was obtained from the culture medium supplemented with 0.5 mg/l TDZ and 2 mg/l IMA, followed by the medium added with 0.75 mg/l TDZ and 2 mg/l IMA. In the absence of TDZ, however, IMA neither affected the number nor the length of korarima shoots (Table 4).

Similar to our findings, addition of IMA to the culture medium resulted in a significant improvement in the efficacy of TDZ in *Spathiphyllum floribundum* (Werbrouck and Debergh, 1996). This enhancing effect of IMA was attributed to its effect upon the general mechanism of cytokinin action, i.e. its effect on changing the metabolism of exogenously applied cytokinins. Imazalil was also reported to have an inhibitory effect on cytokinin degrading enzymes (Werbrouck and Debergh, 1995, 1997).

Furthermore, improvement in shoot proliferation from IMA supplemented medium was recently stated to be due

Table 4. Effects of the combined use of TDZ and IMA on the *in vitro* growth and development of korarima after eight weeks of culture.

TDZ (mg l $^{-1}$)	IMA (mg l $^{-1}$)	Shoot no. (mean ± SE)	Shoot lg. (cm) (mean ± SE)	DW (mg) (mean ± SE)
0	0	1.70 i ± 0.15	8.41 a ± 0.19	90.93 efg ± 2.71
0	2	2.22 i ± 0.10	8.54 a ± 0.30	92.57 efg ± 2.16
0	4	2.10 i ± 0.12	7.59 b ± 0.26	83.82 fg ± 3.30
0	8	2.20 i ± 0.17	8.25 a ± 0.30	89.09 efg ± 3.99
0.25	0	11.50 h ± 0.55	3.52 c ± 0.02	93.83 defg ± 2.65
0.25	2	15.70 de ± 0.66	2.86 d ± 0.34	121.48 b ± 5.91
0.25	4	14.60 ef ± 0.57	2.36 de ± 0.29	93.67 defg ± 4.78
0.25	8	11.22 h ± 0.22	1.90 ef ± 0.24	83.06 fg ± 2.91
0.5	0	13.22 f ± 0.84	2.27 def ± 0.06	102.27 cde ± 1.57
0.5	2	19.30 a ± 1.11	1.72 fg ± 0.19	151.52 a ± 13.87
0.5	4	16.70 cd ± 0.47	1.23 gh ± 0.12	109.35 bcd ± 7.20
0.5	8	17.56 bc ± 0.84	1.14 gh ± 0.12	99.31 cdef ± 2.58
0.75	0	11.89 gh ± 0.48	1.68 fg ± 0.20	87.57 efg ± 1.92
0.75	2	18.78 ab ± 0.56	1.19 gh ± 0.14	111.94 bc ± 3.91
0.75	4	13.89 f ± 0.21	0.89 h ± 0.08	97.80 cdefg ± 1.06
0.75	8	14.40 ef ± 0.54	0.76 h ± 0.02	81.79 g ± 1.74

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT.
Shoot lg. = shoot length (cm), DW = dry weight (mg).

Figure 1. *In vitro* growth of korarima plants (A) Multiple shoots obtained from medium supplemented with 3 mg/l BA and 0.5 mg/l TDZ. (B) Multiple shoots of korarima produced on medium with 2 mg/l IMA and 0.5 mg/l TDZ. (C) Multiple shoots from the treatment involving 3 mg/l PBZ and 0.5 mg/l TDZ. (D) Shoots grown on PGR-free medium (control).

Table 5. Mean and probabilities of the independent effects of TDZ and PBZ on korarima shoot growth after eight weeks of culture.

Factor	Mean (main effects)		
	Shoot no.	Shoot lg. (cm)	DW (mg)
TDZ (mg l $^{-1}$)			
0	2.86c	5.27a	87.04c
0.25	16.48b	1.29b	97.40b
0.5	19.42a	0.95c	112.85a
0.75	16.67b	0.77c	80.04d
PBZ (mg l $^{-1}$)			
0	9.62c	3.18a	91.47b
1.5	13.41b	2.01b	101.02a
3	18.75a	1.60c	102.76b
4.5	13.90b	1.36d	81.29c
Prob.			
TDZ	***	***	***
PBZ	***	***	***
TDZ X PBZ	***	***	***
% CV	22.30	31.53	17.32

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT.
Shoot lg. = shoot length (cm), DW = dry weight (mg)

to inhibition of GA biosynthesis (Werbrouck and Debergh, 1997). However, in all cases, IMA was not observed exerting any cytokinin effect on medium devoid of exogenous cytokinins (Werbrouck and Debergh, 1996), a finding that is in direct agreement with our current results. In the present study, shoots produced on medium added with IMA together with TDZ were dwarf types (Figure 1b), requiring at least two subcultures on a PGR-free medium before attaining normal growth.

Effects of TDZ in combination with paclobutrazol (PBZ)

In this third experiment involving TDZ and PBZ, inclusion of TDZ into the culture medium had a significant positive effect on shoot number and dry weight of korarima. Shoot length was significantly reduced all along the different levels of TDZ used in the experiment. Addition of PBZ to the medium has a considerable effect on all the three parameters evaluated. Paclobutrazol at 3.0 mg/l gave the highest shoot number and dry weight of korarima plantlets. Shoot length was consistently reduced all along the different concentrations of PBZ used in the present study (Table 5).

The combined use of TDZ and PBZ in the culture medium had a significant synergistic effect on the number and length of shoots, as well as dry weight of plantlets. The highest shoot number (25.94 shoots/explant) was obtained when 0.5 mg/l TDZ was combined with 3 mg/l PBZ. The use of thidiazuron at 0.25 and 0.75 mg/l also gave relatively higher number of shoots when combined with 3 mg/l PBZ (22.63 and 22.35 shoots/explant, respectively). In the case of dry weight, the highest values were obtained when 0.5 mg/l TDZ was combined with 3 mg/l and 1.5 mg/l PBZ (133.16 and 125.45 mg/explant, respectively). Unlike those produced from the aforementioned two experiments involving TDZ and BA, or TDZ and IMA, shoots obtained from the PBZ supplemented TDZ medium were very minute in size (Figure 1c). Mean shoot length was reduced to less than half a centimeter when the highest levels of TDZ (0.75 mg/l) and PBZ (4.5 mg/l) were used in the culture medium (Table 6). The tiny shoots obtained from this experiment thus required at least three subcultures to revive and attain normal growth. Similar to the above two experiments, the longest shoots in this experiment were recorded from the PGR-free culture medium (Table 6 and Figure 1d).

In line with this, Lee (2001) has reported enhanced proliferation of banana shoots using TDZ in combination with PBZ, as compared to the use of the former alone. Werbrouck and Debergh (1996) also reported PBZ to have a strong enhancement effects on another cytokinin (BA) in relation to shoot multiplication. In the present study, inclusion of PBZ to the culture medium did not enhance shoot proliferation when TDZ was excluded from the medium. This finding is in direct agreement with the report of Werbrouck and Debergh (1996) that stated lack of any cytokinin effect from the sole use of PBZ in *Spathiphyllum floribundum* culture.

A strong synergistic effect on shoot length was also observed in *S. floribundum* culture when the medium was supplemented with PBZ and another additional cytokinin (BA). As reviewed by Davis et al. (1988), the most obvious plant growth response to triazole treatment was

Table 6. Effects of the combined use of PBZ and TDZ on the *in vitro* growth and development of korarima after eight weeks of culture.

TDZ	PBZ	Shoot no. (mean ± SE)	Shoot lg. (cm) (mean ± SE)	DW (mg) (mean ± SE)
0	0	2.06i ± 0.36	7.79a ± 0.31	86.62de ± 5.82
0	1.5	3.17i ± 0.24	4.79b ± 0.33	91.36cde ± 1.08
0	3	3.44i ± 0.17	4.53b ± 0.14	88.60de ± 2.40
0	4.5	2.76i ± 0.20	3.91c ± 0.16	81.26e ± 4.98
0.25	0	10.94h ± 0.80	2.15d ± 0.08	96.25bcd ± 2.26
0.25	1.5	15.42ef ± 0.78	1.72e ± 0.16	101.68bc ± 7.22
0.25	3	22.63b ± 0.96	0.71g ± 0.10	106.76b ± 3.88
0.25	4.5	16.33de ± 0.66	0.64g ± 0.06	84.08de ± 3.44
0.5	0	13.58fg ± 0.99	1.67e ± 0.18	102.41bc ± 2.70
0.5	1.5	18.33cd ± 1.08	0.84fg ± 0.10	125.45a ± 2.08
0.5	3	25.94a ± 0.63	0.63g ± 0.06	133.16a ± 2.88
0.5	4.5	20.17c ± 0.62	0.62g ± 0.03	90.96bcde ± 2.83
75	0	11.63gh ± 0.80	1.24f ± 0.08	80.84de ± 4.59
75	1.5	16.61de ± 1.00	0.72g ± 0.07	85.56cde ± 5.01
75	3	22.35b ± 0.77	0.66g ± 0.06	84.36ede ± 2.43
75	4.5	15.72e ± 0.50	0.43g ± 0.04	68.87f ± 2.81

Means within a column followed by the same letter are not significantly different at P<0.05 based on DMRT.
Shoot lg. = shoot length (cm), DW = dry weight (mg)

reduced stem elongation and hence reduced height, which is due to a reduction in the internode length. This was also the case in our present study. Being one of the group of chemicals collectively called triazols, all these and similar other effects induced by PBZ could be ascribed to the increased cytokinin levels in the plant system (Fletcher et al., 2000). This group of chemicals is generally known to inhibit GA biosynthesis. Thus, it is such modulation of GA level in the plant that was thought to lead to consequent events, including reduction of plant height (Fletcher et al., 2000; Senaratna et al., 2002).

Conclusion

The combined use of TDZ with either of BA, IMA or PBZ resulted in a considerable synergistic effect on shoot proliferation and growth of korarima. However, considerable differences were observed on the degree of effects exerted by the different combinations evaluated in this study. Relatively speaking, a strong synergism was observed from the treatments involving 0.5 mg/l TDZ and 3 mg/l PBZ in relation to the number of shoots and dry weight of plantlets. Considerable improvements in shoot multiplication were also obtained when 0.5 mg/l TDZ was used together with 2 mg/l IMA. Culture medium supplemented with 3 mg/l BA with this same level of TDZ (0.5 mg/l) gave about 14 shoots/explant that have better growth. In all cases, however, the longest shoots were obtained from the control treatment (PGR-free medium). In the present study, shoots proliferated on BA supplemented media, followed by those obtained from IMA added medium, elongated and rooted at a relative ease. However, the tiny shoots produced on PBZ supplemented medium required repeated subculture and relatively longer time to elongate and produce functional roots for acclimatization.

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