

GROWTH AND DEVELOPMENT OF PROTOCORM LIKE BODIES HYBRID DENDROBIUM ORCHIDS ON MS MEDIUM WITH CYTOKININ AND AUXIN COMBINATION

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ABSTRACT

The aim of this research was to find out the proper growth regulators for orchid *Dendrobium* hybrid growth crossed Anching Lubay with Bandung White. The experimental was carried out at Tissue Culture Laboratory of Seed Technology, Faculty Agriculture, Padjadjaran University from middly of August 2006 until middly of December 2006. Completely Randomized Design with eighteenth combination treatments and replicated twice was used in this experiment. The treatments were combination NAA a1,3,5 mg L⁻¹ with BAP 2,4,6 mg L⁻¹ and Thidiazuron 0.02, 0.04, 0.06 mg L⁻¹. Monitoring has been done in a month. Result of experiment showed that number of plb, number of shoots, number of leaves, number of roots, height of shoots, length of leaf and length of root were affected by combination growth regulators. The best result for number of plb was achieved by combination NAA 3 mg L⁻¹ with thidiazuron at 0.06 mg L⁻¹. Combination NAA 3 mg L⁻¹ with thidiazuron 0.04 mg L⁻¹ result the best number of shoots and number of leaves. The best number of root and length of root was achieved by combination NAA 3 mg L⁻¹ and BAP 2 mg L⁻¹. Combination NAA 1 mg L⁻¹ with BAP 4 mg L⁻¹ gave the best length of leaf and height of shoots. The conclusion, combination NAA with TDZ give the best result for number of plb, number of shoots and number of leaves, combination NAA with BAP gave the best result for the number of root and length of root.

Keywords: PLB, BAP, TDZ, NAA, MS

INTRODUCTION

Orchids are one of the unique plants groups, highly esthetics, it has become one of important export commodities. Among the commercially important orchids, *Dendrobium* accounts for about 80% of the total of micropopagated tropical orchids. *Dendrobium* plants are used for the production of orchid flowers and in traditional Chinese medicine.

Dendrobium is micropopagated in tissue culture by "protocorm –like bodies (PLBs)", but the growth is very slowly. They are generally propagated through PLB . Methods for rapid multiplication of orchids are essential to the commercial demand or conserve the orchid hybrid or the wild orchid population in forest. To stimulate more efficient micropopagated of PLB ,has been directed to used plant growth regulators such as; N- benzylaminopurine, alpha-naphthaleneacetic acid dan thidiazuron (TDZ), (Nge,K.L. , Nitar We, Suwalee, C, dan Willem F.S.,2006). TDZ (N-phenyl-N 1,2,3-thidiazol-5 phenylurea) has been used extensive in tissue culture. It exhibits strong cytokinin-like activity and promotes proliferation of axillary shoots as well as stimulating adventitious organ regeneration of woody plant. In orchids tissue culture, several effects of TDZ have been reported, such as increased protocorm, proliferation rate, induced protocorm- like bodies, enhanced adventitious bud initiation. A concentration of 0.05 – 0.1 uM thidiazuron was more active than 4 – 10 uM BA (Pierik, 1987). The synthetic auxin (NAA, IBA and 2,4-D) generally cause the formation of roots whereas cytokinins (BA and TDZ) are often used to stimulate growth and development. In higher concentration (1 – 10 mg/l),they can induce adventitious shoot formation. Plant tissue culture is particular suitable to compare the effect of TDZ and BA on plants that growth and multiply slowly, like orchids.

In this study presented, the growth and development of PLB have been studied under the controlled conditions of tissue culture in the presence of cytokinin (TDZ and BA) and NAA have been compared in various concentration.

MATERIALS AND METHODS

Plant materials

PLBs of three months old from germination hybrid *Dendrobium* (D. Bandung White x D. Anching Lubay) were used as the source of explants.

Culture medium and conditions

The MS medium were used in these experiments and supplemented with nutrition medium, sugar, vitamin and growth regulator. PLBs (0.03 – 0.04 g fresh weight) were planted in agar medium. The culture were maintained at 27 C and provided photoperiods of 16 h per day using cool white fluorescent tube.

Experiment methods

The experiment was used CRD with 18 combination in two replicates on treatments ; TDZ (0.02; 0.04; 0.06 mg/l) + NAA (1.00; 3.00; 5.00 mg/l) and BAP (2.00; 4.00; 6.00 mg/l) + NAA (1.00; 3.00; 5.00 mg/l).

Treatments combination were:

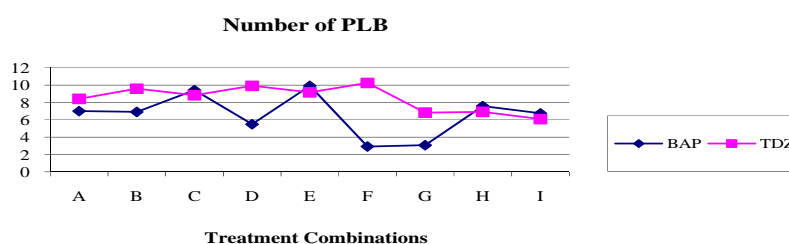
A = NAA 1 mg/L + BAP 2 mg/L / TDZ 0.02 mg/L	F = NAA 3 mg/L + BAP 6 mg/L / TDZ 0.06 mg/L
B = NAA 1 mg/L + BAP 4 mg/L / TDZ 0.04 mg/L	G = NAA 5 mg/L + BAP 2 mg/L / TDZ 0.02 mg/L
C = NAA 1 mg/L + BAP 6 mg/L / TDZ 0.06 mg/L	H = NAA 5 mg/L + BAP 4 mg/L / TDZ 0.04 mg/L
D = NAA 3 mg/L + BAP 2 mg/L / TDZ 0.02 mg/L	I = NAA 5 mg/L + BAP 6 mg/L / TDZ 0.06 mg/L
E = NAA 3 mg/L + BAP 4 mg/L / TDZ 0.04 mg/L	

Data collection and analysis

Visual observations were made everyday and data observations every month until four month. Various variable such as; total PLBs, shoots, leaf, root, length of leaf, length of root, maintained on agar medium were counted after 16 weeks of cultivation. All data were analysed using anova and then F test 5% and Scott Knott test 5%.

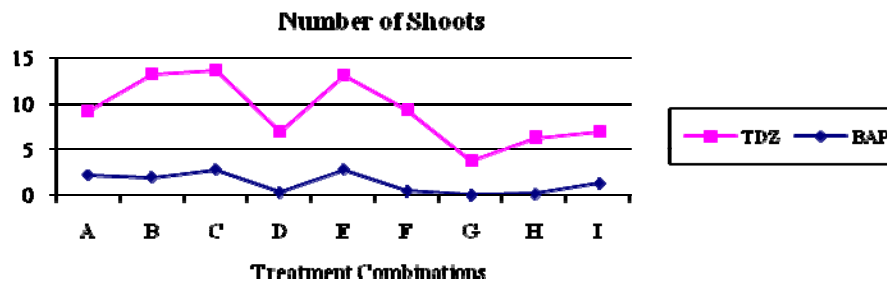
RESULTS AND DISCUSSION

The effect of TDZ+NAA with BAP+NAA on average number of shoots per explant



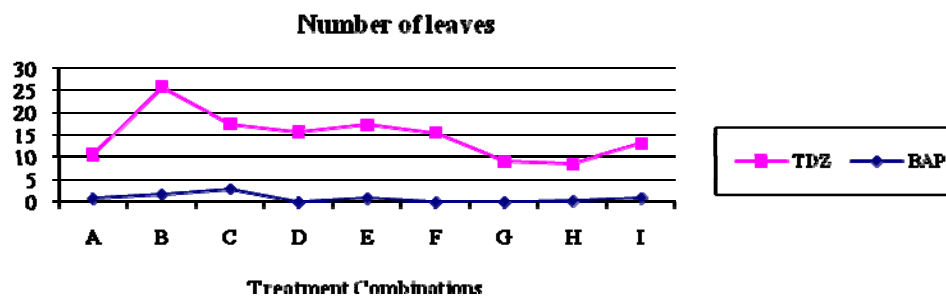
Based on Scott-Knott test 5% level show that generally TDZ result ni more PLBs than BAP. It is in line with Ernst R experiment (1994), that the effect of TDZ on orchid tissue culture increase protocorm proliferation dan induce *plb*. Sankhla (1994) also explained that TDZ has level biological activity compared adenine. The highest total of PLBs was achieved on combination of 0.06 ppm TDZ + 3 ppm NAA. Level concentration of NAA at 1-3 ppm gave more total of PLBs than at 5 ppm, it's because NAA has high activity to stimulate the form of root and inhibit the PIB multiplication. Bretagne et.all declare that efficiently of NAA various depend on kind and concentration of sitokinin.

The effect of TDZ+NAA with BAP+NAA on average number of shoots per explant



The combination using TDZ result in total average of shoot higher compared to the combination using BAP. These show that the activity of TDZ greater than BAP. The TDZ is known as inhibitor of sitokinin oxidase more effective than the others (Pierick, 1987). Chen and Piluek (1995) experimental show that TDZ was more effective in stimulation of shoot and also to regenerate adventitious shoot of *Epipremnum* sp. Surprisingly, the combination of 0.02 ppm TDZ and 2 ppm BAP in various of NAA tend result in total of shoot less than others. It is caused by the concentration of sitokinin was not enough to form shoot.

Average number of leaves per explant

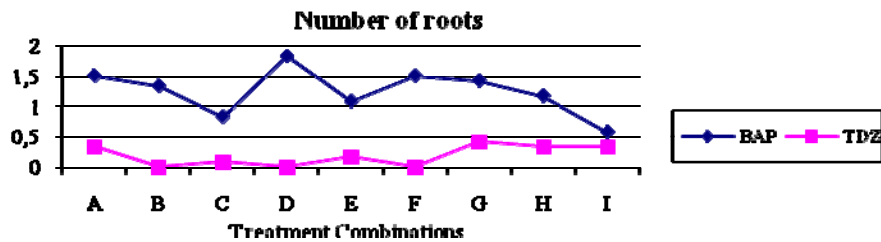


Scott-Knott test of level 5% shows that the combination of growth regulators using TDZ produced the average number of leaves per explant higher BAP. This relates to the ability of TDZ in forming the axillary buds and adventitious shoots. Number of shoots directly proportional to the number of leaves formed, bringing the total number of leaves also become more numerous. Results of research on aspen plants using BAP and TDZ at various concentrations can increase the formation of buds, also found that the effects of TDZ in the development of shoots greater than BAP which induces 10 leaves that increase as the increased shoots.

Average Number of roots per explants

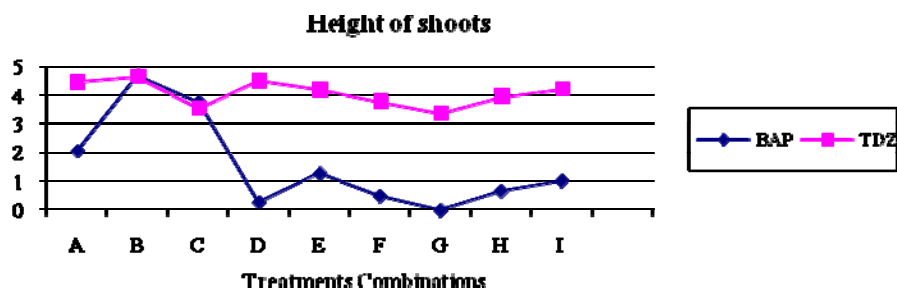
Further analysis of Scott-Knott 5% level of treatment using TDZ. There are two opinions that can describe this condition. From the research results Khalafalla and Hattori (2000) states that, like ethylene are considered as a barrier in the formation of roots, the ability of TDZ to stimulate ethylene biosynthesis is caused by difficulty in inducing the formation of roots than shoots. This is in line with the statement Chang and Chang (2000) stating that TDZ inhibits root initiation. Different things stated that ethylene stimulates root formation of hypocotyls cut Norway spruce plants. Other studies that support is that ethylene stimulates root formation Hazelnut cotyledon plants. Another possibility that might happen in this case that endogenous ethylene produced by TDZ can actually stimulate root formation proved it on a combination of 0.02 ppm TDZ + 5 ppm NAA which produce roots that tend to be higher than the other TDZ treatment. Root formation can occur due to the accumulation of

ethylene, but at a subsequent increase in TDZ concentration was decreased root formation, this may be due to TDZ can increase endogenous auxin, that all types of auxin can stimulate the synthesis of ethylene, also NAA stimulated the synthesis of ethylene in plant tissues provided NAA treatment. The formation of roots on tomato plants is inhibited when the endogenous ethylene level increases or decreases, it explains that the ethylene promote root only in a narrow concentration interval. In the treatment using TDZ gave the formation of a better number of roots on the use of NAA 5 ppm, it is clear that the NAA will work effectively if administered at higher concentrations when combined with TDZ at low concentrations due to accumulation of ethylene is produced at intervals no spur formation roots. In many species, TDZ did not inhibit the formation of roots when the shoots appear.



The treatment using BAP tends to produce more number of roots but at high concentrations in combination with NAA 5 ppm actually reduce the amount of root formation, it explains that the BAP is suitable for the formation of roots at a concentration of 2-4 ppm, whereas at higher concentrations would inhibit root formation and further spur the formation of the shoot. BAP especially at high concentrations usually inhibit the formation of roots and suppress growth, besides reducing the influence of auxin in promoting root formation (George and Sherrington, 1984). George (1991) also stated that BAP expected can induce endogenous auxin hormone in leek explant. This case is similar to that TDZ also can induce endogenous auxin, which in turn it will synthesize ethylene then collaborate to enhance root formation, because the interval use of ethylene is very narrow on the excessive or less condition able to inhibit root formation.

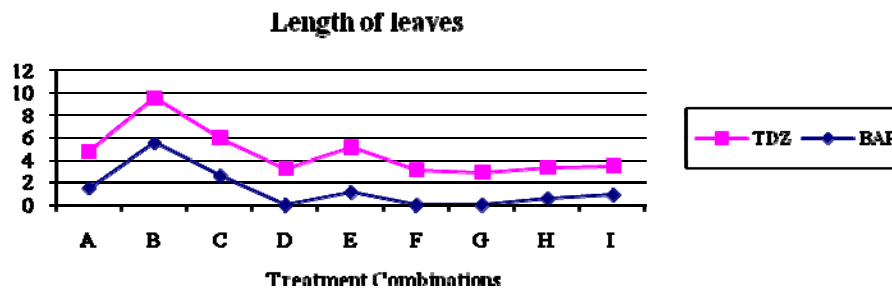
Average Height of shoots Per explants



Further analysis of Scott-Knott 5% level showed that the all of TDZ combination produced higher shoots, while in treatment using BAP only occur in combination 2-6 ppm BAP with 1 ppm NAA. This indicates that TDZ is more effective in shoot elongation of hybrid orchids because it has greater effectiveness than BAP, it is because all of explant in each treatment using TDZ grow and form shoots, while in treatments using BAP just form PLB, so that in statistics calculating the value was small. According to Chein and Chang (2000) states that TDZ at concentrations (0.1 to 1 mg / L) inhibited shoot elongation. The same case was happen in this experiment that formed short shoots, and experienced vitrification and has an irregular shape, so that although this treatment using TDZ produced a lot of shoots but the shoots did not grow tall and experienced irregularity shape. the

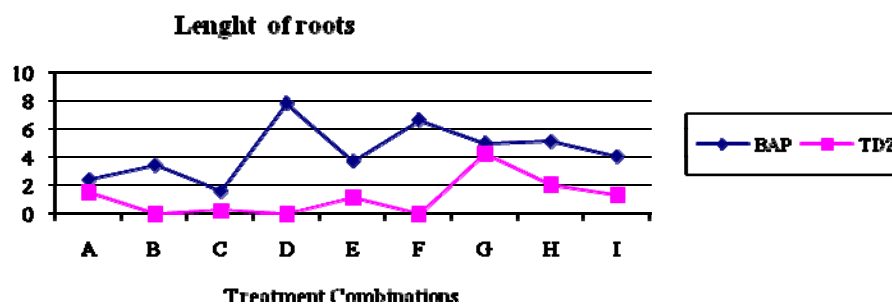
increase of TDZ concentration on medium containing NAA inhibits budding, buds become more succulent and enlargement growth becomes obstructed. The organic components of TDZ at high concentrations can increase ethylene production inhibited shoot growth in eight weeks.

Average length of leaves per explant



Further analysis of Scott-Knott 5% level showed that the combination using 4 ppm BAP + NAA 1 ppm produces the highest average length of leaves compared to other treatment combinations. While in general the combination using TDZ produced high average length of leaves than using BAP. This is because of such treatment using BAP at 16 week has not been formed a lot of shoots and still in forming PLB, thus formed a little small leaves even on a combination of 2 ppm BAP + NAA 3 ppm, 6 ppm BAP + NAA 3 ppm and 2 ppm BAP + NAA 5 ppm not yet formed at the age of 16 strands of leaf MSI. All treatment using TDZ forms a lot of leaves, thus result in high average length of leaves, due to the number of axillary shoots and adventitious buds are formed even though most of the leaves are small and lack of chlorophyll in the beginning of its growth, this is one of the symptom vitrisence shown in the use of TDZ. Vitrisence symptom is characterized by short or stunted plants, growth tends toward the addition diameter of trunk, and less chlorophyll. Some cases also show the stems and leaves look like out of water, translucent and fragile. Vitrification can occur due to failure or constraints on the process of cell wall formation (parenchymal tissue) and the constraints on the process of lignin formation.

Average Length of Root per Explant



Analysis of Scott-Knott 5% level showed that the combination using BAP in general, produce a longer root length compared with all treatments using TDZ. Treatment with a combination of 2 ppm BAP and 3 ppm NAA produced the highest average root length. While the combination of TDZ 0.02 ppm + NAA 5 ppm produced the highest average root length compared with all treatments that use combinations of TDZ, this was due to NAA at high concentration can stimulate the formation of ethylene, as well as TDZ which enhances the formation of endogenous auxin spurred the formation of ethylene. Ethylene is known can make a change to the plant roots, other studies have proved the existence of cooperation between auxin and ethylene in the swelling and root formation

by auxin applied after ethylene play a role in the tissue. regularly reciprocal mechanism of high auxin concentration, causing the formation of ethylene. But the presence of ethylene causing low auxin concentration in the tissue, further argued that ethylene inhibits basipetally and laterally auxin transport. Thus inhibited the forming, tend thickening and irregularly swollen roots. The ethylene inhibit stem elongation, increasing enlargement sideways, causing leaves aging and encourage the formation of roots on stems, especially in wild tomato. Furthermore, he said if the elongation of stem and root obstructed become more thick it caused by more triggered horizontal enlargement.

CONCLUSIONS

Conclusion

Based on the research and discussion, it can be concluded as follows:

1. The combination of NAA and BAP and NAA and TDZ on MS medium significantly affect the growth of hybrid Dendrobium PLB on the character of the average number of PLB, the average number of shoots, average number of leaves, the average number of roots, shoots high average, average leaf length and average root length.
2. The combination of NAA and TDZ gave better results for the number of PLB, the number of shoots, leaf number, shoot height and leaf length, whereas the combination of NAA and BAP gave better results for the number of roots and root length.

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