Effect of Auxins on *in vitro* Rooting and Effect of Different Growing Media on the Growth of Two Hybrid Pineapples

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Abstract : The effect of different type and concentrations of auxin on *in vitro* rooting and the effect of different growing media on the growth of two hybrid pineapples (Smooth cayenne and Queen) were conducted. The effect of auxin on *in vitro* rooting were studied by using two types of auxin, NAA and IBA, at the concentrations of 1, 2 and 4 mg/l in MS medium. Results showed that after 12 week cultivation, the MS supplemented with NAA at 2 mg/l gave the highest number of roots, 33.4 roots per shoot, in hybrid no. 4 and MS supplemented with auxin at 2 mg/l gave the highest number of roots, 24.8 roots per shoot, in hybrid no. 18 ($p<0.05$). Seven formulas of growing media were used to examine the effect of different growing media on pineapple growth after in vitro root induction. Analysis revealed that four formulas of growing media - sand:coconut compost:charcoal husk at the ratio of 1:1:1, sand:charcoal husk at the ratio of 1:1, soil:sand:charcoal husk at the ratio of 1:1:1 and soil:sand:charcoal husk at the ratio of 1:1:1 - significantly produced the highest growth for both hybrid pineapples ($p<0.05$).

Keywords : pineapple, rooting, auxin, growing media.

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1. Introduction

Pineapple (*Ananas comosus* Merr.) is one of the most important economic plants in Thailand. The fruit is utilized in the processing and canning industries. Nutritionally, freshly harvested pineapple is an inexpensive source of vitamins A, B, and C, calcium, phosphorus, and iron. Vegetative propagation of pineapple from suckers and crowns is often restricted in some cultivars by the limited availability of propagules (Popluechai, *et al*. 2007). Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well defined tissue culture media under aseptic conditions. Micropropagation of plants has largely been through proliferation of axillary shoots derived from shoot or meristem tips followed by rooting of elongated shoots generally on a low salt medium with auxin, although rooting can often occur on hormone free or charcoal containing medium. Auxin, known to be involved in cell enlargement, was long thought to be the controlling factor in the rooting process (Ali, *et al* 2009). Growing medium is not only as a growing place but also as a source of nutrient for plant growth. Growing media constituents are the basic components of substrates, which are generally formulated on a percentage volume basis. Such materials include soil, coconut compost, sand and charcoal husk and others. Growing media constituents can usually be sensually detected in the mix (Schmilewski, 2008). The objective of this work was to define an efficient protocol for the micropropagation of hybrid pineapple, that were bred in Research Institute of Agricultural Technology, Rajamangala University of Technology Lanna, by the manipulation of type and concentrations of auxin for root induction in cultures. Furthermore, the experiment of the appropriate growing media for tissue transplanting from the cultures was also determined.

2. Materials and Methods

2.1 Study of types and concentration of auxin on pineapple growth

The research was conducted in Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang. Two hybrids pineapple shoots, no. 4 and no. 18, regenerated from the axillary buds of crossing between 2 varieties of pineapples (Smooth cayenne and Queen), were used in this research. The studied of auxin on root induction of six treatments were arranged in a Completely Randomized Design with 10 replications. The MS medium (Murishige and Skoog, 1962) with each type of auxins, NAA (naphthaleneacetic acid) and IBA (indole butyric acid), at the concentration 1, 2 and 4 mg/l were used. Cultures were incubated at 25^{O}C with photoperiod of 16 hours/day at 3000 lux light intensity by cool white fluorescent light. The rooting ratio were determined by the number of roots, root length, shoot height, number of leaf and length of leaf and were recorded 12 weeks after cultured. The data were analyzed at 5% significance level using analysis of variance (ANOVA).

2.2 Study of growing media on pineapple growth

Seven different growing media were used as source for growth of seedling of pineapple from auxin study experiment including, sand:coconut compost:charcoal husk 1:1:1, soil:sand:charcoal husk 1:1:1, sand:charcoal husk 1:1, soil:charcoal husk 1:1, soil and sand 1:1,

soil:coconut compost 1:1 and soil. Experiment were carried out in Completely Randomized Design with 10 replications and data were collected weekly for 12 weeks. Five different physiological parameters including number of shoots per plant , number of leaves per plant, plant height, leaf length and leaf width were used for this study.

3. Results and Discussion

Rooting of adventitious *in vitro* shoots is normally induced on auxin-containing media. These hormones differently affect *in vitro* rooting of various species differently. In this study, combinations of NAA and IBA elicited root formation. The root formation was observed 14-21 days after culturing on rooting medium. Both concentration and type of auxins used markedly influenced percentage of root formation (number of rooted shoots) on both hybrid pineapples. The results showed that the medium containing different concentrations of auxin significantly affected rooting of the hybrid pineapples ($p<0.05$). Supplementation IBA in the medium with a concentration of 4 mg/l produced the maximum rooting of 21.3 and 20.7 roots for the hybrid no.4 and no.18, respectively whereas supplementation NAA in the medium with concentrations of 2.0 and 4.0 mg/l yielded maximum rooting of 33.4 and 24.8 roots for the hybrid no.4 and no.18, respectively (Table 1 and Figure 1). The hybrid no. 4 gave the highest root of 33.4 roots per explant in the medium containing 2 mg/l of NAA after 12-week cultivation. In contrast, significant difference was not observed in the hybrid no. 18 grown in the medium containing different concentrations of NAA ($p>0.05$). Measurement of pineapple root length in both 2 hybrids indicated that IBA in the medium gave longer root length than NAA. However, NAA supplementation in the medium exhibited better characteristics of new shoot proliferation, shoot length and leaf length than IBA supplementation in the medium.

In *Citrus reticulata* Blanco and *C. limon* Burm., rooting was found to favor a medium containing both NAA and IBA (Singh *et al*., 1994). In this present study, though the rooting response varied with type and concentration of auxin, the results revealed supplementation 2 and 4 mg/l of NAA into the medium was appropriate for root induction. Similar observation was also reported by Hamad *et al*. (2013). The researchers studied the in vitro induction and proliferation of adventitious roots in pineapple by using different concentrations of IBA and NAA. They described that the MS solid medium enriched with 1.0 mg/L NAA was the best choice for rooting resulting in the tallest plantlets, highest number of roots per shoot and intermediate rooting percentage. However, Akbar *et al*. (1992) reported that IBA was found to be the best treatment for root induction. This might be caused by the cultivar of pineapple.

It is well established that growth medium has a large effect on plants. Seven different media used in this study had significant impact on some selected parameters of vegetative growth of pineapples. In general, mixture of sand:coconut compost:charcoal husk (1:1:1), sand:charcoal husk (1:1), soil:sand:charcoal husk (1:1:1) and soil:sand:charcoal husk (1:1:1) gave higher values of growth parameter such as number of leaves per plant, plant height, leaf length and leaf width in comparison to other media ($p<0.05$). However, the medium prepared from the mixture of soil:sand

(1:1) gave the highest shoot per explant in both 2 hybrid pineapples (Table 2 and Figure 2). Considering the physical characteristics, aeration and water holding capacity are probably the most important factors while chemical characteristics, e.g. nutritional status and salinity level have a crucial role on plant development (Dewayne *et al*., 2003). Mixture of sand:coconut compost:charcoal husk, sand:charcoal husk, soil:sand:charcoal husk and soil:sand:charcoal husk were apparently appropriate for physical characteristics and high nutritional levels. Therefore, these mixtures gave the highest values in most of the growth parameters measured in this study.

4. Conclusion

This study investigated the efficient protocol for the micropropagation of 2 hybrid pineapples, that were bred in Research Institute of Agricultural Technology, Rajamangala University of Technology Lanna, for *in vitro* root induction and in cultures and the appropriate growing media for tissue transplanting from the cultures. There was 100% root induction of shoots after transferring onto MS medium with every concentration of NAA and IBA. The MS medium with 2 and 4 mg/l NAA gave the best result of the quality and quantity of shoots and roots of both hybrids. The results also revealed that three growing media, sand:coconut compost:charcoal husk 1:1:1, soil:sand:charcoal husk 1:1:1 and sand:charcoal husk 1:1 gave the highest growth of both hybrid in every parameters.

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Table 1 Effect of different hormone types and concentration on rooting of 2 hybrid pineapples after 12 weeks in culture.

Type of auxin	Conc. (mg/l)	No. of roots per explant		Root length (cm)		No. of new shoots		Shoot length		No. of leaf per explant		Leaf length (cm)	
		No.4	No.18	No.4	No.18	No.4	No.18	No.4	No.18	No.4	No.18	No.4	No.18
NAA	1	24.0^{bc}	20.2^{abc}	3.7^{b}	3.1^{bc}	1.9^{b}	2.8^{a}	7.9^{b}	6.8^{b}	16.8^{bc}	23.9^{a}	8.3^{b}	5.7^{c}
	2	33.4^{a}	23.0^{ab}	4.3^{b}	5.0^{a}	3.5^{a}	1.4^{a}	9.5^{a}	9.1^{a}	27.5^{a}	16.2^{a}	9.3^{a}	8.1^{a}
	4	28.8^{ab}	24.8^{a}	3.1^{b}	2.4^{c}	2.3^{ab}	2.5^{a}	9.6^{a}	8.9^{a}	19.1^{bc}	18.6^{a}	9.7^{a}	8.4^{a}
IBA	1	12.5^{d}	15.8^{c}	6.5^{a}	3.6^{abc}	1.3^{b}	1.9^{a}	6.2^{c}	7.2^{a}	12.2^{c}	19.3^{a}	7.1^{c}	6.7^{bc}
	2	20.6^{c}	18.1^{bc}	5.7^{a}	4.5^{ab}	1.4^{b}	1.5^{a}	6.6^{c}	8.1^{ab}	15.4^{bc}	15.7^{a}	7.2^{c}	7.4^{ab}
	4	21.3^{c}	20.7^{abc}	6.1^{a}	5.2^{a}	2.5^{ab}	1.5^{a}	8.0^{b}	8.7^{a}	20.9^{ab}	16.1^{a}	7.4^{c}	7.9^{a}

Means in a column followed by different letters are significantly different at *P<0.05*.

Table 2 Effect of various growth media on vegetative plant characteristics of 2 hybrid pineapples after 12 weeks**.**

Growing media	No. of shoots per explant		Shoot height (cm)		No. of leaves per explant		Leaf length (cm)		Leaf width (cm)	
	No.4	No.18	No.4	No.18	No.4	No.18	No.4	No.18	No.4	No.18
sand:coconut - compost:charcoal husk 1:1:1	1.0^{b}	1.0^{b}	15.8^{a}	15.9^{a}	24.5^{a}	26.3^{ab}	15.1^{a}	15.2^{a}	1.9^{a}	2.1^{a}
soil:sand:charcoal husk 1:1:1	1.0^{b}	1.0^{b}	16.1^{a}	16.6^{a}	24.6^{a}	26.3^{ab}	15.0^{a}	15.5^{a}	1.9^{a}	2.0^{a}
sand:charcoal husk 1:1	1.0^{b}	1.0^{b}	15.8^{a}	16.9^{a}	25.6^{a}	28.5^{a}	15.2^{a}	16.0^{a}	1.9^{a}	1.9^{a}
soil:charcoal husk 1:1	3.6^{a}	2.3^{a}	12.6^{b}	11.3^{d}	26.6^{a}	21.1^{bc}	11.4^{b}	10.9^{c}	1.1^{b}	1.4^{c}
soil:sand 1:1	3.9^{a}	3.1^{a}	13.6^{b}	14.5^{b}	27.0^{a}	26.8^{a}	12.3^{b}	13.5^{b}	1.1^{b}	1.6^{b}
soil:coconut compost 1:1	2.8^{a}	2.7^{a}	12.9^{b}	12.9^{c}	22.2^{a}	24.2^{abc}	11.7^{b}	11.7^{c}	1.2^{b}	1.3^{c}
soil	3.2^{a}	2.3^{a}	12.3^{b}	12.7^{c}	23.3^{a}	20.0^{c}	11.5^{b}	11.3^{c}	1.2^{b}	1.1^{d}

Means in a column followed by different letters are significantly different at *P<0.05*

Figure 1 Effects of varying concentrations of IBA and NAA on the root induction of 2 hybrid pineapples after 12 weeks.

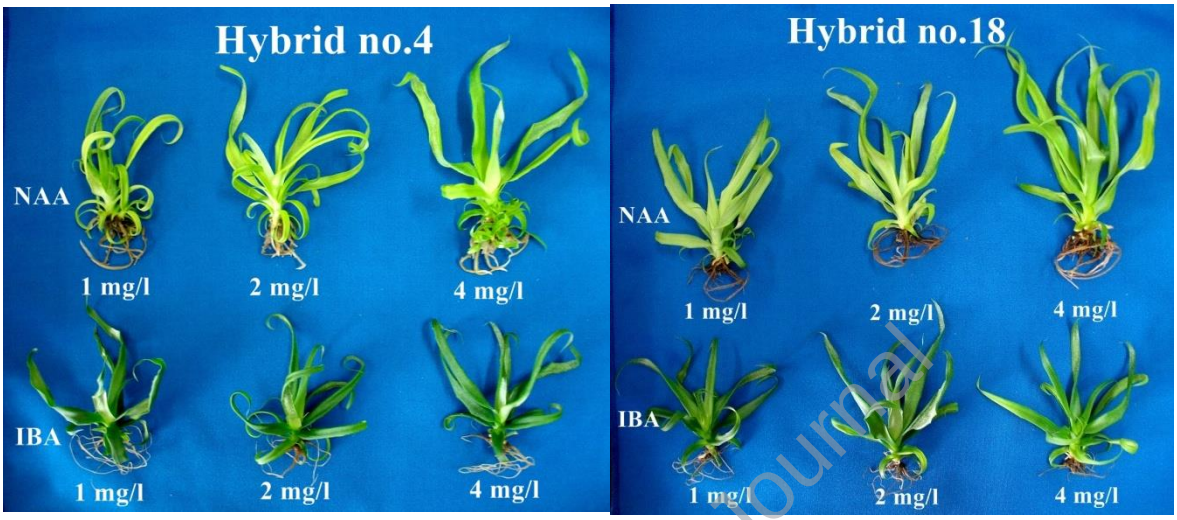

Figure 2 Effects of various growth media on vegetative plant characteristics of 2 hybrid pineapples after 12 weeks.