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ASSESSMENT LEVEL FOR COMPLEX ADDITIVES IN THE TISSUE CULTURE MEDIA OF *PHILODENDRON RED EMELARD* PLANTS

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ABSTRACT

Philodendron Red Emerald is a plant that is of great interest due to its indoor and outdoor decorative value this research aims at the assessment of the effect of two complex additives (Malt and Yeast extracts) for the shooting and rooting stage of Philodendron Red Emelard. Shoot clusters of the explants were cultured on MS (Murashige and Skoog), supplement with different concentrations (250mg/l^{-1}, 500mg/l^{-1} and 1000 mg/l^{-1}) of Yeast and Malt extracts and compared with their controls. Results revealed utmost growth and leaf proliferation at concentration 250 mg/$l^{-1}$of Yeast extract. Malt extracts exhibited more profound efficiency in root development. Both extracts showed no apparent effect on the quantity of chlorophyll.

KEYWORDS: Red Emelard, Tissue Culture, Yeast Extraction, Malt Extraction

INTRODUCTION

One of the most prominent names for domestic plants is the *Philodendron Red Emerald*. This plant is common to anyone that is familiar with miscellaneous ornamentals. On the other hand the diverse nature of the plants i.e. the genus being composed of over 250 species is a fact that only knowledgeable nursery growers and interiors capers are aware of **(Dennis *et al,* 2012)**. *Philodendron*s are of prodigious interest due to their indoor and outdoor decorative value. Conventional propagation of *Philodendron* via stem cuttings and seeds is a slow and inconsistent method when paralleled to the demand. As a result micropropagation offers the best solution through the provision of a high quality plants at a desirable pace **(Sreekumar*et al,* 2001).** Plant tissue culturing majorly involves three steps.

The first being isolation of the plant tissue from the typical environment, the second is using aseptic techniques to obtain sterile material that is free of contaminants. And the third is the culture and maintenance of the tissue in vitro in a strictly controlled physical and chemical environment. An additional fourth phase would be that of acclimatization where the whole plants are recovered and transferred to in vivo conditions (**Hall 1999**). The *in vitro* cell and tissue cultures of higher plants are characterized by the use of isolated parts of plants obtained from a full plant body in a suitable nutrient medium aseptically (**Neumann *et al,* 2009**).

Culturing contributes to understanding basic as well as fundamental sciences. The culture can undergo either a callus expansion of differentiation into new plants. Tissue culturing is naturally influenced by the physiological state of the plant meaning that quite often; younger tissues are more responsive to divisions as they possess more actively dividing

cells. Conditions for regeneration diversify according the plant species. The most empirical approach that has been intensively used in studies on *in vitro* organogenesis has demonstrated that success is achieved through the implementation of three factors: choice explant, composition of the media, and of course the control of the physical environment (Molnar *et al*, 2011).

Commercially, self-heading *Philodendron plants* are mostly propagated via tissue culture; "*Philodendron scandensoxycardium"*is normally propagated using (1-1½) inch stem cuttings with a node and an attached leaf. Buds break within (3-5) weeks and rooting occurs within (4-6) weeks (Dennis *et al*, 2012).The regulation of plant growth and biosynthesis of important economic chemical constituents can be achieved through the use of different growth regulating substances. There is a recent trend to use naturally occurring compounds (including amino acids) to achieve such a uniform regulation (Nahed and Abou Dahab, 2006). This trend reported that amino acids, which are organic nitrogenous compounds, act as the building blocks in the synthesis of proteins, which is formed by a process where ribosomes catalyze the polymerizationof amino acids (Davies, 1982).

An outline of cell and tissue cultures of *Philodendron Red Emerald* are presented in this paper while assessing the composition the culture media with regards to natural compounds particularly varying concentrations of Malt and Yeast extracts on the shooting and rooting stages respectively.

MATERIALS & METHODS

This research was carried out in the Biotechnology Laboratory at the Central Laboratory for Data Palm Research and Development (CLDPRD), Agriculture Research Centre, Giza, Egypt.

Explant Materials

Plantlets or proliferated meristems of *Philodendron Red Emerald* produced *in vitro* were used as experimental materials. Explants were isolated at (6-8) weeks; a whole plant culture with the shoot cluster consisting of two shoots with height of 0.5-1 cm without roots.

1-Shooting Stage

Shoot clusters of *Philodendron Red Emerald* explants were cultured on MS (Murashige and Skoog, 1962), with 2 $mg/1^{-1}$ BA, 0.5 $mg/1^{-1}$ of NAA, supplemented with different concentrations of Yeast extract and Malt extract. Data was calculated after three weeks based on the following criteria (Shoot number and Shoot length (cm) and Length of leaves (cm). Each treatment included three replicates each replicate comprised three small jars (200 ml) and each jar containing one cluster of the explant.

Rooting Stage

All pervious shoot clusters obtained from multiplication stage were transferred on MS medium supplemented with, 0.1 $g/1^{-1}$ NAA, 30g/l sucrose, $5gml^{-1}$agar, 3g/l AC and the same different concentrations of Malt and Yeast extraction as shown in Table1.

Table 1: The Difference Concentration of Yeast and Malt Extracts in MS Nutrient Media Formula

Media Composition		Concentrations (Mgl^{-1})		
Malt extract	Control (0.0)	250	500	1000
Yeast extract	Control (0.0)	250	500	1000

Alljars contained one shoot cluster of the explant; the treatment included three replicates each replicate possessed three jars (300 ml). Then the data was calculated after 4 weeks based on the following criteria (Root number, Root length (cm), plant length (cm), pH was adjusted to (5.7-5.8) prior to the addition of agar. The jars were autoclaved at 121°C and 1.2 kg/cm² for 20 min. Cultures were incubated under light for 16 hours and 8 hours in dark conditions at 25± 2 ◦ C.

Statistical Analysis

All data was subjected to statistical analysis according to the procedure reported by (Snedecor and Cochran, 1980) and Fisher's least significant difference (LSD) at 0.05% confidence interval, significance was used to compare between means according to (Steel and Torrie, 1980).

RESULTS

Number of Shoots/Plant

The data recorded in this study Table 2, and Figure 1 were directed towards assessing the effect of the different concentrations of complex additives on the number of shoots per explant. Adding Malt or Yeast extracts to the culture media gaveoptimum results in terms of shoot numbers. The Yeast extract was ideal in the production of the highest number of shoots (3.08shoots/explant).There was no significant difference between the concentrations of complex additives. The interaction between the concentrations and the type of complex additives was significant; the best result was recorded at 1000 mg/l Malt extraction (4.33 shoots /explant).

Table 2: Effect of Malt and Yeast Extraction at Different Concentrations for the Number of Shoots of *Philodendron Red Emelard*

Treatments	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	3.00	2.33	3.33	4.33	3.00
Yeast extract	2.66	4.00	3.00	2.66	3.08
Mean	2.83	3.16	2.66	3.50	

L. S. D at 0.05% A= 0.749 B= 1.05 AB= 1.49

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Number of Leaves/Plant

The addition of the yeast extract to the culture media escalated the number of leaves considerably (11.50 leaves/explant). The highest number of leaves was recorded at a 1000 leaf count at concentration 250 mg/l^{-1}; it bore no significant difference (12.17, 11.50 leaves/explants) when compared to the control, which was recorded at (8.33 leaves/explants). The interaction between the concentrations and the type of complex additives was substantial; the preeminent result was recorded with the Yeast extract at 500mg/l^{-1} (15.67 leaves/explants) as show in the Table 3.

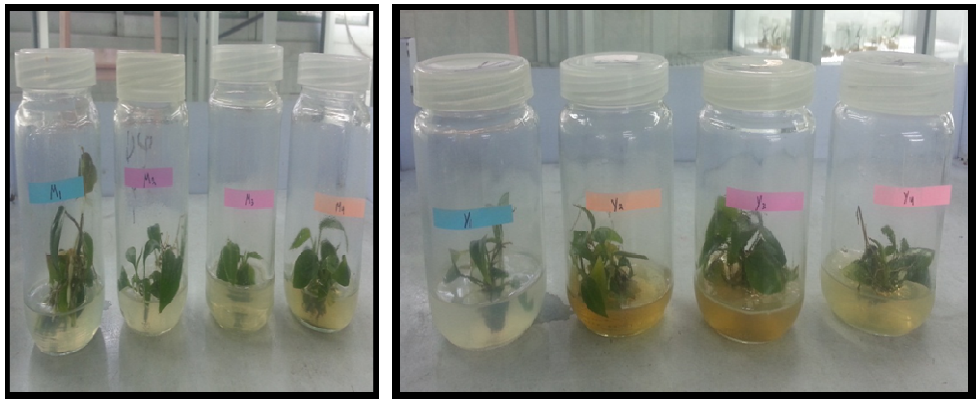

Figure 1: An Image Displaying the Effect of Malt and Yeast Extracts on Number of Shoots

Table 3: The Effect of Malt and Yeast Extraction at Different Concentrations on the Number of Leaves

Treatments	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	9.33	7.33	8.00	13.67	9.58
Yeast extract	7.33	15.67	12.33	10.67	11.50
Mean	8.33	11.50	10.17	12.17	

L. S. D at 0.05% A= 1.82 B= 2.58 AB= 3.65

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Plant Lengths/Plants

The data in **Table 4**revealed that, the response of plant length when cultured with Yeast extract gave the most noteworthy results in terms of plant length (5.29 cm).There is a major difference between the controls, 500 mg/l^{-1}and 1000 mg/l^{-1} for plant length being the highest, while the lowest obtained was with the control (3.08 cm).

The interaction between the concentrations and the type of complex additives was substantial; the unsurpassed result was recorded with Yeast extract at 1000 mgl^{-1} (7.00 cm) as correlated in Table 4.

Table 4: The Effect of Malt and Yeast Extraction at Different Concentrations on Plant Length

Treatments	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	1.50	4.66	4.16	4.00	3.58
Yeast extract	4.66	3.50	6.00	7.00	5.29
Mean	3.08	4.08	5.08	5.50	

L.S.D at 0.05 % A=1.018 B= 1.44 AB= 2.03

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Number of Roots/Plants

The effect of complex additives in the rooting media appeared in Table 5. The finest result for complex additives was obtained with Malt extract (5.67 roots/ explants). Culturing plantlets of *Philodendron* with the control rooting media gave the highest yield (7.05 roots/explants).There is no significant difference between concentrations and complex

additives, culturing plantlets of *Philodendron* on control rooting media gave the most ideal results (11.00 roots /explants).

Table 5: Effect of Malt and Yeast Extraction at Different Concentrations on Root Number

Treatments	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	11.00	3.00	4.67	4.00	5.67
Yeast extract	4.00	2.33	3.00	5.00	3.58
Mean	7.05	2.67	3.83	4.50	

L.S.D at 0.05 % A= 1.92 B=2.71 AB= 3.84

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Root Length/Plants

The effect of complex additives in plant length was apparent in Table 6. There is no compelling difference between Yeast and Malt extracts on rooting length (2.16 and 2.04 cm, respectively). The highest numbers of rooting length was obtained with the control rooting media (3.33 cm). The interaction between the concentrations and the type of complex additives was significant; the best significant value was recorded with the control rooting media.

Table 6: The Effect of Malt and Yeast at Different Concentrations for the Length of the Plant

Treatment	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	3.50	1.83	1.67	1.16	2.04
Yeast extract	3.16	2.33	1.16	2.00	2.16
Mean	3.33	2.08	1.41	1.58	

L.S.D at 0.05 % A= 0.27 B=0.39 AB=0.55

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Chlorophyll A

The effect of Chlorophyll A in *Philodendron* is shown in Table 7.There is no significant difference between complex additives in the quantity of Chlorophyll A. While, the Malt extract gave the highest effect of Chlorophyll A when compared to that of the Yeast extract(0.73mg/g).There is no significant difference between complex additives and concentration type.

Table 7: Effect of Natural Extract on Chlorophyll A in *Philodendron*

Treatment	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	2.75	0.07	0.09	0.04	0.73
Yeast extract	0.12	0.16	1.11	0.16	0.13
Mean	1.43	0.11	0.10	0.10	

L. S. D at 0.05 % A= 1.43 B= 2.02 AB= 2.86

A= Complex Additives.

B= Concentrations.

AB= The interaction between complex additives and concentrations.

Chlorophyll B

There is no suggestive difference between the complex additives; the dissimilarity in concentrations, the interaction between complex additives, and the variance in the concentration of natural extracts was recorded in Table 8.

Carotene

There is no noteworthy distinction between the complex additives; the disparity in concentrations, the interaction between complex additives, and the differences in the concentration of natural extracts were recorded in Table 9.

Table 8: The Effect of Malt and Yeast Extracts on Chlorophyll B in *Philodendron*

Treatment	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	0.02	0.03	0.02	0.04	0.03
Yeast extract	0.02	0.03	0.02	0.02	0.08
Mean	0.01	0.01	0.02	0.02	

L.S.D at 0.05 % N.S

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

Table 9: Effect of Malt and Yeast Extraction at Different Concentration on Carotene

Treatment	Control	250mgl^{-1}	500mgl^{-1}	1000mgl^{-1}	Mean
Malt extract	0.11	0.09	0.07	0.06	0.08
Yeast extract	0.11	0.08	0.07	0.06	0.08
Mean	0.11	0.08	0.06	0.07	

L.S.D at 0.05 % N.S

A= Complex Additives.

B= Concentrations.

AB= the interaction between complex additives and concentrations.

DISCUSSIONS

Previously, attempts at using different extracts for the culturing of explants for *Philodendron Red Emerald.* Extracts have been used predominately as sources of vitamins. Using a basic media supplemented with these extracts has been shown to develop proper yields (Cornet, 2006). Though these extracts i.e. malt and yeast were no longer commonly used. Their efficiency was undisputable for initiation and promotion of growth. The results do not disagree with this statement.

The effect of Yeast extract; Yeast extracts are predominantly used as growth nutrients with multiple cultures such as crown-gall tissue cultures and callus cultures (Jonard, 1960) (Vasil and Hildebrandt, 1966). Currently, yeast extract is ordinarily employed as a biotic elicitor for the induction and enhancement of secondary metabolites production. According to the literature, yeast extract is expended as a supplement for the purpose of promoting plant growth, due to its high amino acid content (George *et al*, 2008). However, several species respond in distinct ways to the presence of yeast extract, e. g. the addition of higher concentrations of yeast extract to the MS medium, inhibits the growth whereas, lower concentrations of yeast extract has demonstrated beneficial responses (Vasil and Hilderbrandt,1966). In this study yeast

was examined to for proliferation of the shoot and leaves in *Philodendron Red Emerald*, a prominent name in the field of decorative plants. The results displayed that yeast extract provided the most optimal results when it came to shoot numbers lengths, and the numeration of leaves. This was particularly evident at the 250 mgl^{-1} concentration interval (Table 2, 3 and 4). Though the means demonstrated no significant difference at the 0.05 confidence level. The interaction symbolized significant results. The findings correlate with the work of Amprayn *et al*, in 2012 and Dawwam *et al*, in 2013 for their assessment of yeast and bacterial promoters on tobacco and potato plants respectively.

The effect of Malt extract; Malt extract, mainly a source of carbohydrates, has exhibited an initiation of embryogenesis in nuclear explants (Rangan *et al*, 1968 and .1984). Mediums that contain malt extract usually give the highest efficiency in terms of shoot lengthening in date palm trees (Zeinab*et al*,2007).Several recent studies showed a role for the extract in the multiplication of *Citrus sinensis* somatic embryos (Das*et al*, 1995), and in other Citrus species (Jumin. 1995). It also aids in the promotion of plantlet formation from somatic embryos derived from the styles of differentCitrus cultivars (De Pasquale *et al*, 1994), and in somatic embryogenesis and plantlet regeneration from pistil thin cell layers of Citruses (Carimi*et al*, 1999). Malt extract also promoted germination of early cotyledonary stage embryos arising from the *invitro* rescue of zygotic embryos of sour orange (Carimi*et al*,1998). With this study, malt extract was the additive under examination for root elongation and number efficiency. The results concluded that yeast produced an impeccable efficiency with root numbers (Table 5). With root lengths the results were not as consistent they showed that the optimal results came with the assessed controls of the experiment (Table 6). There were no significant differences between the means of the assessments at the confidence interval 0.05. The outcomes of the root numbers and elongation are in agreement with the results of Sudipta *et al*, in 2013 for their *in vitro* propagation of croton using natural additives particularly malt extract for their culturing techniques.

CONCLUSIONS

The presence of natural extracts within plant culturing mediums provides significant benefit to the promotion of plants; this was no exception to that of *Philodendron Red Emerald* though the control plants displayed highest efficiency for root formation. The extracts particularly the yeast extract exhibited profound results in terms of shoot lengths, leaf numbering etc. The results also suggest the dire necessity for the implementation of these natural extracts in tissue culturing techniques whether for ornamental plants, date palm, or even citruses.

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