BIBLIOGRAPHY

GALIAN, JAKE L. APRIL 2009. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple. Benguet State University, La Trinidad,Benguet.

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ABSTRACT

This study was conducted at the Horticulture Research and Training Institute, Benguet State University, La Trinidad, Benguet from October 2008 to March 2009. The study aimed to determine the suitable explant, suitable medium and develop techniques in propagating *Alstroemeria in vitro*.

Shoot tip explants grown in MS + .5 ppm NAA and MS + .5 ppm BA + .5 ppm NAA resulted to rhizogenetic cultures. This is the key to *Alstroemeria in vitro* propagation. The easier the decontamination procedure for shoot tip explants makes it a better choice as an explant for *in vitro Alstroemeria* propagation. Rhizome buds are difficult to sterilize.

A more refined system must be established. Auxin-cytokinin concentrations must be considered for further studies. Similarly, position of explant implantation in the growing medium is very important as observed in this study.

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INTRODUCTION

Alstroemeria, a native of tropical North America is also referred to as the "Lily of the Incas" and "Peruvian lily". It is becoming one of the most important cut flower crop worldwide due to its attractive flowers and excellent keeping quality. The genus was named from the Swedish Baron Class *Alstroemeria* by his close friend Caralus Linnaeues. The plant was first described by the French botanist Lous Feuilee (Anon., 2001). This is one of the elegant exotic flower that is introduced in the Cordillera. The exact introduction year of this crop specifically in La Trinidad, Benguet is off the record. There are three main varieties of *Alstroemeria*, the butterfly type which has a shorter stem and peduncles, the orchid type, which has longer stems and peduncles and the aurantica and there are a lot of cultivars, but most of today's types are hybrid.

Alstroemeria belongs to the family *Amaryllidaceae*. Its genus is *Alstroemeria* with about 60 species, and related to the onion, daffodil, agapanthus and nerine. The plant has an underground rhizome where shoot emerges and grow vertically from the ground. *Alstroemeria* is composed of trumpet shaped 1 ½ to 2 inches in diameter, with one or more laterals. Many blooms are grouped atop stems 24 – 86 inches long. The stems of these cut flowers are 2 – 3 feet long and branch into four to six short pedicels, each holding two to four flower buds.

Alstroemeria is not endemic to the Philippines. Other types of *Alstroemeria* loved by enthusiasts include the sweet Laura and Petite Plum, which vary in size and flower color. Colors include yellow, apricot, orange, pink, red, lavender, cream, white, and bi-colors. Perhaps the most fascinating and morphological trait of the *Alstroemeria* and its relatives is the fact that the leaves are resupinate that is they twist from the base so that

what appears to be the upper surface is the lower surface. This is an expensive cut flower but farmers have nowhere to get planting materials. Importation is very expensive.

Due to the nature of the plant that is difficult to propagate vegetative by natural means, *in vitro* technique makes possible mass rapid propagation of *Alstroemeria*. Plant tissue culture is a technique whereby plant parts are grown in artificial environment that can sustain its life. Plant tissue culture has the ability to produce disease free materials.

Presently because of the increasing use of the cut flower and ornamental plant as materials for outdoor home designs, many plants have been tried and cultivated for their beauty and economic value. *Alstroemeria* is one, which interests one's eye and has the potential to be propagated *in vitro* to make available plant material since importation is very costly.

Among the many ornamental plants, *Alstroemeria* is one versatile crop because it is not only used for cut flower arrangements, but also used in landscape gardening or even a houseplant for interior design, because of its beautiful flowers and long vase life.

This study was conducted at the Tissue Culture Laboratory, Horticulture Research and Training Institute, Benguet State University, La Trinidad, Benguet from October 2008 to March 2009. This research work aimed: 1) to determine suitable explants for *in vitro* propagation of *Alstroemeria*; 2) to determine suitable medium for *in vitro* propagation of *Alstroemeria*; and 3) to develop techniques in propagating *Alstroemeria in vitro.*

REVIEW OF LITERATURE

In the development of the country's cut flower industry, *in vitro* culture of flower crops has several advantages besides its usefulness in mass propagation of plants. Tissue culture technique may be employed in the production of disease free and quality mother stock plants. Tissue culture of ornamental crops is an expensive process and its use may not merit its cost (Holdgate, 1977). However, it has been proven to be economically profitable. With further research, tissue culture is of practical value to many ornamental crops.

Hartman and Kester (1959) reported that tissue culture can be started from a variety of plant parts which have cells that are capable of dividing. Moreover, Torres (1989) stated that the primary goals of tissue culture techniques in ornamental plant is the elimination of disease and production of disease-free plant materials and the rapid production of a large number of genetically identical plants. Introduction of a new genotype offers several advantages for a commercial operation.

Moreover, Ammirato (1990) reported that the intent of clonal propagation is to reproduce plants of desirable qualities uniformly and in quantity and that reproduction of tissue culture is faster than by nursery traditional method is generally acknowledged.

Tissue culture is the development of new plants in artificial medium under aseptic condition from very small pieces of plants, such as embryos, stems, shoot tips, root tips, callus, single cell and pollen grains. Haberlandt (1902) has used this in research laboratories throughout the world since it was his first attempt in 1902 to isolate growth in plant tissue culture (Hartman, 1975).

Growth Regulators

Weaver (1972) stated that plant growth regulators play a major role in plant growth and development and that to grow in length, tissue must have received substances. Application of growth regulators on the growing materials accelerates root initiation and development, and increase uniform rooting (Janick, 1972).

Auxin in the media is essential for rooting from shoot tips during the early stage of culture. Cytokinin usually inhibits root formation but induces lateral shoot and leaf growth.

Weaver (1972) reported that the effects of cytokinins are effective for cell division. The relative amount of auxin and cytokinin determine whether shoots or roots develop from callus (Thorpe, 1981). Murashige (1974) reported that a medium high in auxin and low in cytokinin stimulated rooting and reversed balance promoted shoot formation. They explained that to enhance axillary branching in shoot tip explants by high concentrations of cytokinin is presumably due to the suppressive action by cytokinin on apical dominance, a manifestation attributed to auxin. Among various cytokinins used, it is the most commonly employed proliferation culture medium to promote axillary shoot proliferation for different species (Nehra and Karta, 1994).

Vuylsteke (1989) mentioned that plant growth regulators and development of explants *in vitro* depend on their concentrations and ratio in the medium. Often, this determines the pattern of development in culture.

Auxin in the media is essential in the rooting of shoot tip during the early stage of culture. Cytokinin usually inhibits root formation but induces lateral shoot and leaf growth. A negative effect of auxin was observed in the inhibition of plant development in

callus formation. The effect of cytokinins seems to be negligible and generally produces favorable effects only when optimally balanced with auxins (PCARRD, 1975).

Culture Media

One of the most important factors governing the growth and morphogenesis of plant tissue cultures is the composition of the culture medium. The basic nutrient requirements of cultured plant cells are very similar to those of whole plants.

Tissue culture techniques require surgical removal of plant tissues under aseptic conditions; special culture media has been developed for different materials. These involve sugars, inorganic, salts, vitamins, growth regulators and organic substances. Solid media or semi-sold media is made with the addition of agar.

A number of media have been devised for specific tissues and organs. Murashige and Skoog (MS) (1962) medium is widely used to induce organogenesis and regeneration of plant tissue cultures containing the desired salt composition.

Several media formulations are commonly used for the majority of cell and tissue culture work. These media formulations include MS (1962) medium.

Sheuk and Hilderbrandt (SH) and Gamborgs (B-5) medium are all high in macronutrients, while other media formulations contain considerably less of the macronutrients (Torres, 1989).

Smith (1982) stated that the choice of a particular medium depends mainly on the species of the plant, the tissues and organ to be cultured, and concentrations of hormones are the most important considerations in preparation of the medium for monocotyledon and dicotyledonous tissue culture.

The best induction was obtained on MS (1962) medium supplemented with 10 mM Thidiazuron (TDZ) and 0.5 mM Indole butyric acid (IBA) and the shooting medium contained MS medium with 2.2 mM 6 –bezylamino purine (BAP) (Hsueh-Shih Lin, 1998).

Explants

The first step in any successful tissue culture program is the selection of suitable explants source. Almost any plant tissue organ can be used as explants, but the degree of success obtained will depend upon the culture system used, the species being cultured, and the removal of surface contaminants from the explants (Torres, 1989).

Study showed that without understanding of the growth potential within the stock plant is likely that culture lines generated *in vitro* will have quite different growth response and rooting capacity (Marks, 1990). A small piece of plant used to begin culture has been referred to as explant. The smaller the explant, the more effective it is in the elimination of pathogen (Meyer, 1976).

A tissue in micropropagation is called explant. Explants range in size from 1mm to stem pieces 0.2 inch or more (Kyte, 1983). They are usually young tissues consisting of immature cells (Vidalie, 1995) which are composed of differentiated tissues such as fragment of stem, roots, flowers and fruits.

Source of explants should be free from diseases and should be growing vigorously. Meristems, shoot tips, anthers, stems, flowers, leaves, embryos, hypocotyls, seedlings, rhizomes, bulbscales, flower buds, corms or roots can serve as explants or sources of explants (Kyte, 1983).

Alstroemeria can be propagated *in vitro* by variety of techniques. One is through the use of leaves and rhizomes (Hsueh-Shih Lin, 1998).

MATERIALS AND METHODS

Materials

Culture vessels, sterile petri dishes, graduated cylinder, analytical balance, beakers, forceps, scalpels, alcohol lamp, pressure cooker, laminar flow cabinet, laboratory gown, cap and other laboratory equipment were very important for the work.

Studies

For studies 1 and 2, there were 5 treatments, each replicated 3 times with 30 samples. Study 3 on the other hand, consisted of 4 treatments. Studies were laid out using the Complete Randomized Experimental Design (CRD).

The different studies conducted were:

Study 1. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Young Leaf Explants: A Verification

Study 2. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Shoot Tip Explants

Study 3. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Rhizome Buds

Treatments

MS 1962 basal medium enriched with different growth regulators which served as treatments were as follows:

T_1 - MS Basal medium

T_2 - MS + .5 ppm Benzyl-amino purine (BA)

T_3 - MS + .5 ppm Napthalene acetic acid (NAA)

T_4 - MS + 1 ppm BA + .5 ppm NAA

T_5 - MS + .5ppm BA + .5 ppm NAA

Explants

Young leaves, shoot tips and rhizome buds of *Alstroemeria hybrida* cv. Purple were used as explants in the different studies.

Sterilization

Young leaf and shoot tip explants. Young leaves and shoot tips ere isolated from the mother plant and thoroughly washed with detergent and running water. Final washing was done with distilled water. This was followed by sterilization with 5% (v/v) sodium hypochlorite for a period of 10 minutes. Sterile distilled water was used to wash thoroughly the explants. Final sterilization was done with 1% (v/v) sodium hypochlorite for 1 minute, after which explant was thoroughly washed with sterile distilled water. Excess water was removed from the explant making use of sterile tissue paper. Implantation followed.

Rhizome buds. Rhizome buds were isolated from mother plant and thoroughly washed with detergent and running water. Rhizome buds were then soaked with 100% sodium hypochlorite for 20 minutes followed by thorough washing with sterile distilled water. Inside the laminar flow cabinet, sterilization was repeated using 100% sodium hypochlorite for another 20 minutes. Again, rhizome buds were thoroughly washed with sterile distilled water. Excess water were removed with sterile tissue paper. Implantation followed to the respective culture medium.

Maintenance

Cultures were maintained under in a diffused light condition at ambient temperature.

Data Gathered

1. Number of leaves. Numbers of leaves were counted 60 days from implantation (DFI).
2. Number of shoots. Numbers of shoots were counted 60 DFI.
3. Height of shoots (cm). Heights of shoots were taken 60 DFI.
4. Number of tubers. Numbers of tubers were counted 60 DFI.
5. Number of rhizogenetic cultures 60 DFI.
6. Documentation

RESULTS AND DISCUSSION

This section presents the results and observations from the different studies conducted. Figures are also presented.

Figure 1 illustrates the morphological structure of an *Alstroemeria hybrida cv.* Purple. This is presented to help us understand the results.

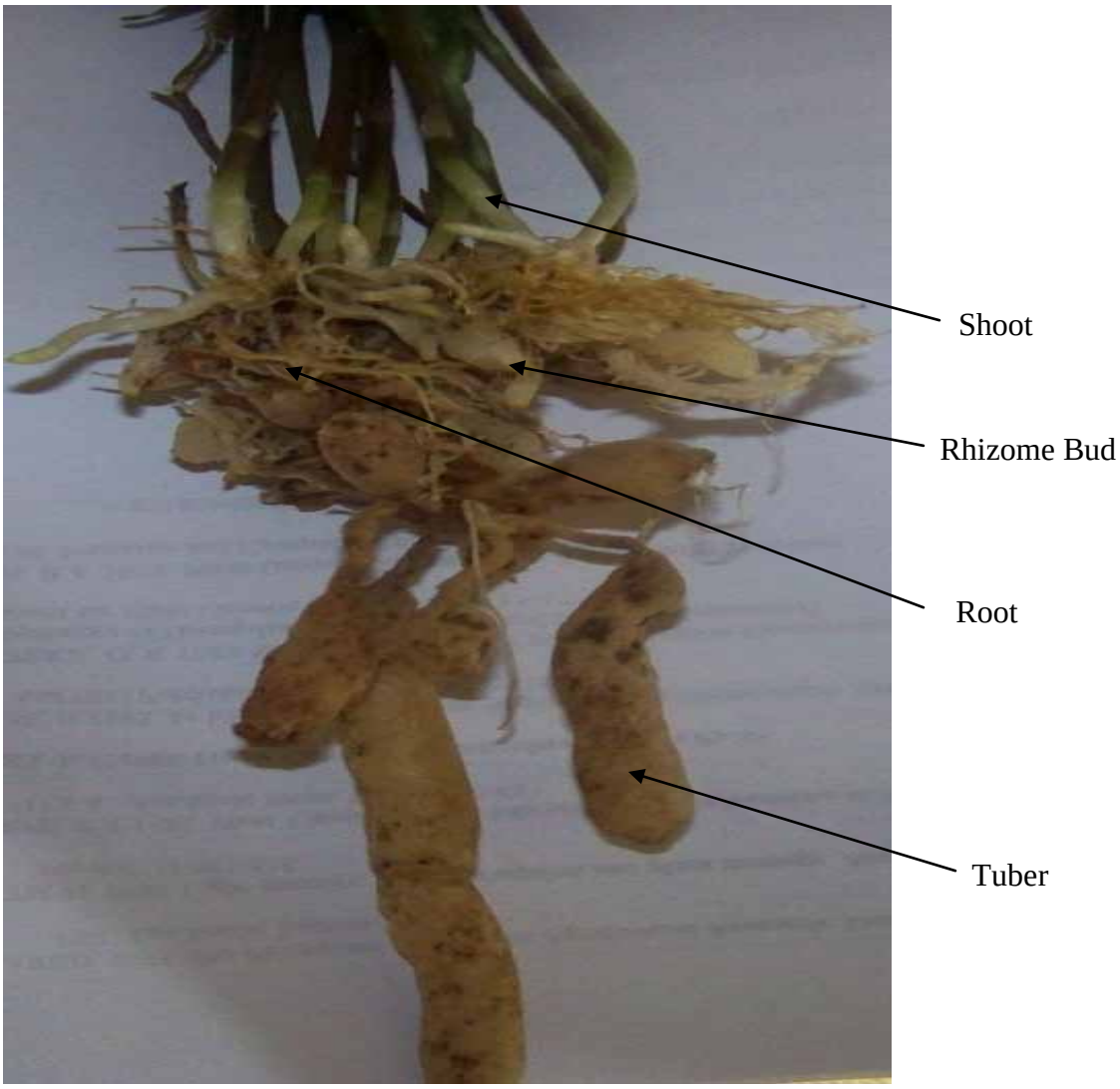

Figure 1 . Morphological structure of an *Alstroemeria hybrida cv.* Purple

Study 1. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Young Leaf Explants: A Verification

Table 1 shows that *in vitro* propagation using *Alstroemeria* young leaf explants is not possible. This confirms the results obtained and reported by Alejandro (2008).

On the contrary, Hsueh-Shi Lin (1998) reported that new shoots were formed from *in vitro* grown *Alstroemeria* leaf explants containing a leaf blade and a stem node. The best induction medium was obtained from MS (1962) medium supplemented with 10 µM thidiazuron (TDZ) and .5µM indole butyric acid (IBA).

The unavailability of TDZ during the conduct of the study prompted the use of BA which is known to be very effective in tissue cultures for shoot formation.

Table 1. Morphogenetic response of young leaf cultures

TREATMENT	MORPHOGENETIC RESPONSE
MS	Swelling no callus formation, no shoot formation, ultimately turned brown
MS + .5 ppm BA	Swelling no callus formation, no shoot formation, ultimately turned brown
MS + .5 ppm NAA	Swelling no callus formation, no shoot formation, ultimately turned brown
MS + 1 ppm BA + .5 ppm NAA	Swelling no callus formation, no shoot formation, ultimately turned brown
MS + .5 ppm BA + .5 ppm NAA	Swelling no callus formation, no shoot formation, ultimately turned brown

Study 2. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Shoot Tip Explants

Study 2 revealed that shoot tip explants are good for *in vitro* propagation of *Alstroemeria*.

Morphogenetic response of shoot tip cultures 60 DFI is summarized and presented in Table 2.

Significant results achieved in this study are the rhizogenetic cultures. These occurred on cultures grown in MS + .5 ppm NAA and MS + .5 ppm BA + .5 ppm NAA (Figures 2 and 3). These could serve as the key to *in vitro* propagation of *Alstroemeria*. It was not significantly different from the other treatments but this is a great result. Further study must be conducted considering the position of the explant implanted. The basal portion of the shoot tip explants should be inserted on the medium. As observed, rhizogenetic cultures resulted when the base of shoot tip explant was implanted unto the medium.

Moreover, BA and NAA concentrations could play significant roles as indicated by the results. As had been reported many times, the relative amount of auxin and cytokinin determine whether shoots or roots develop (Thorpe, 1981).

Table 2. Morphogenetic response of shoot tip cultures 60 DFI*

TREATMENT	NUMBER OF LEAVES	NUMBER OF SHOOTS	HEIGHT OF SHOOTS (cm)	NUMBER OF TUBERS	NUMBER OF RHIZOGENETIC CULTURES	REMARKS
MS	4.63[b]	1[a]	1.84[a]	0[b]	0[a]	
MS+.5 ppm BA	9.40[a]	1[a]	3.60[a]	0[b]	0[a]	
MS+.5 ppm NAA	2.70[b]	1[a]	2.53[a]	1.33[a]	0.13[a]	Key to *Alstroemeria in vitro* propagation
MS + 1 ppm BA + .5 ppm NAA	9.7 0[a]	1[a]	3.80[a]	0[b]	0[a]	
MS +.5 ppm BA + .5 ppm NAA	10.03[a]	1[a]	4.03[a]	0.03[b]	0.03[a]	Key to *Alstroemeria in vitro* propagation

*Means with common letters are not significantly different at 0.05% level DMRT.

T_1 T_2 T_3 T_4 T_5

Figure 2 . Morphogenetic response of shoot tip cultures 60 DFI. T_1 – MS, T_2 – MS +.5 ppm BA, T_3 – MS + .5 ppm NAA, T_4 – MS + 1 ppm BA + .5 ppm NAA, T_5 – MS + .5 ppm BA + .5 ppm NAA

Figure 3. A close up view of the rhizogenetic response of shoot tip explant cultured in MS+ .5 ppm NAA 60 DFI

Study 3. *In Vitro* Propagation of *Alstroemeria hybrida* cv. Purple Using Rhizome Buds

Rhizome buds have been reported as source of explants for *in vitro* propagation of *Alstroemeria*. This has been tried in this study. Table 3 shows the morphogenetic response of rhizome bud cultures 60 DFI.

Rhizome buds grown in MS + 1ppm BA produced the longest shoots, most number of leaves and most number of shoots. Significant differences were observed among treatments. However, longer durations for observations and conduct of the study is important. The very hard disinfestation making use of rhizome buds limited the time span for this study. Many times, contamination as high as 100% occurred. The researcher refused to make use of mercuric chloride as sterilant as had been used and reported by many workers because it is hazardous to health. Moreover, the laboratory used does not have proper sewerage system.

Table 3. Morphogenetic response of rhizome bud cultures 60 DFI*

TREATMENT	NUMBER OF LEAVES	NUMBER OF SHOOTS	HEIGHT OF SHOOTS (cm)
MS	8.16[ab]	2.61[a]	3.83[ab]
MS+.5 ppm BA	4.66[b]	2.33[a]	2.16[b]
MS + 1 ppm BA + .5 ppm NAA	17.33[a]	4.00[a]	5.66[a]
MS + .5 ppm BA + .5 ppm NAA	2.44[b]	3.00[a]	2.08[b]

*Means with common letter are not significantly different at 0.05% DMRT.

T_1 T_2 T_3 T_4

Figure 4. Morphogenetic response of young rhizome buds 60 DFI. T_1– MS, T_2 – MS +.5 ppm BA, T_3 – MS + .5 ppm NAA, T_4 – MS + 1 ppm BA + .5 ppm NAA, T_5 – MS + .5 ppm BA + .5 ppm NAA

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was conducted at the Tissue Culture Laboratory, Horticulture Research and Training Institute, Benguet State University, La Trinidad, Benguet from October 2008 to March 2009. This research undertaking aimed to determine the suitable explant, suitable medium and develop techniques in propagating *Alstroemeria in vitro*.

Results showed that shoot tips and rhizome buds are possible explants for *in vitro* propagation of *Alstroemeria*.

For shoot tip explants, MS + .5 ppm NAA and MS + .5 ppm BA+ .5 ppm NAA resulted to rhizogenetic cultures which is the key for *in vitro* propagation of *Alstroemeria*.

The very hard disinfestations of rhizome buds would make a worker choose the use of shoot tips as explant.

Conclusions

Based on the results obtained, *Alstroemeria* can be propagated *in vitro*. Shoot tips and rhizome buds could served as explants. The easier disinfestation procedure for shoot tip explants makes one decide to choose as explant.

Recommendations

It is highly recommended that further studies on *in vitro* propagation of *Alstroemeria* making use of shoot tip explants should be conducted. A refined system should be established. As found in this study, *in* vitro propagation of *Alstroemeria* is very possible. Optimum cytokinin and auxin concentrations must be studied and

established. Likewise, scaling up propagules *in vitro* is important. Hardening and acclimatization of *in vitro* plantlets must also be considered.

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APPENDICES

Appendix Table 1. Number of leaves produced from shoot tip explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	5.3	5.1	3.5	13.9	4.63
T_2	11.1	8.5	8.6	28.2	9.40
T_3	3.0	2.7	2.4	8.1	2.70
T_4	13.3	6.7	9.2	29.2	9.70
T_5	13.1	7.0	10.0	30.1	10.03

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	4	138.220	34.555	7.309*	.005
Within groups	10	47.2820	4.728		
Total	14	185.280			

*****Significant Coefficient of variation =29.79%

Appendix Table 2. Number of shoots produced from shoot tip explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	1	1	1	3	1
T_2	1	1	1	3	1
T_3	1	1	1	3	1
T_4	1	1	1	3	1
T_5	1	1	1	3	1

No variance within groups – statistics for Table 6, cannot be computed.

Appendix Table 3. Height of shoots (cm) from shoot tip explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	2.35	2.08	1.10	5.53	1.84
T_2	4.88	3.03	2.90	10.81	3.60
T_3	1.65	4.70	1.25	7.60	2.53
T_4	4.91	2.85	3.65	11.41	3.80
T_5	6.00	3.30	2.80	12.10	4.03

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	4	10.498	2.625	1.917^{ns}	.298
Within groups	10	18.524	1.852		
Total	14	29.022			

^{ns}not significant Coefficient of variation=43.02%

Appendix Table 4. Number of tubers produced from shoot tip explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	0	0	0	0	0
T_2	0	0	0	0	0
T_3	2.2	1.8	0	4	1.33
T_4	0	0	0	0	0
T_5	0	0	0.1	0.1	0.03

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	4	4.216	1.054	3.828*	0.39
Within groups	10	2.753	.275		
Total	14	6.969			

*****Significant Coefficient of variation=191.88%

Appendix Table 5. Number of rhizogenetic cultures from shoot tip explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	0	0	0	0	0
T_2	0	0	0	0	0
T_3	0.1	0.3	1	0.40	0.13
T_4	0	0	0	0	0
T_5	0	0	0.1	0.01	0.03

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	4	0.40	0.10	1.875^{ns}	.191
Within groups	10	0.053	.005		
Total	14	.093			

^{ns}not significant Coefficient of variation=2.12%

Appendix Table 6. Number of leaves produced from rhizome bud explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	13.5	6.5	4.50	29.50	8.16
T_2	2.0	10.0	2.00	14.00	4.66
T_4	12.0	10.0	30.00	52.00	17.33
T_5	3.0	3.0	1.33	7.33	2.44

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	3	387.101	129.034	3.111^{ns}	.089
Within groups	8	331.859	41.482		
Total	11	718.960			

^{ns}not significant Coefficient of variation=79.03%

Appendix Table 7. Number of shoots produced from rhizome bud explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	3.5	3.0	1.33	7.85	2.61
T_2	2.0	3.0	2.00	7.00	2.33
T_4	3.0	4.0	5.00	12.00	4.00
T_5	3.5	3.5	2.00	9.00	3.00

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	3	4.787	1.596	1.189^{ns}	.209
Within groups	8	6.749	.844		
Total	11	11.536			

^{ns}not significant Coefficient of variation=30.73%

Appendix Table 8. Height (cm) of shoots produced from rhizome bud explants 60 DFI

TREATMENT	REPLICATION			TOTAL	MEAN
	I	II	II		
T_1	6.50	2.50	2.25	11.5	3.83
T_2	2.00	2.50	2.00	6.50	2.16
T_4	3.00	7.50	6.50	17.00	5.66
T_5	1.75	2.25	2.25	6.25	2.08

ANALYSIS OF VARIANCE

SOURCES OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	COMPUTED F	PROBABILITY
Between groups	3	25.724	8.575	3.095^{ns}	.089
Within groups	8	22.167	2.791		
Total	11	47.891			

^{ns}not significant Coefficient of variation=79.03%