Hardening of *Alpinia galanga* (L.) Willd.

Tint Khine Aye[1]

Abstract

The soil medium was prepared by mixing 2 parts of humus: 1 part of sand: 1 part of burnt rice husks. Then the soil mix was sterilized in an autoclave for 12 hrs to diminish the soil born disease. For liquid medium, the medium was supplemented with different concentration of MS solution. The survival rate from both media was 100%. Therefore, the survived plantlets were acclimatized in the prepared soil mix for the next step of hardening. In this step, the micropropagated plantlets were propagated together with rhizomatic plantlets. These were arranged in two factors factorial of CRD. Factor A was the planting materials while factor B was the chemical treatments. The results of factor A showed that the micropropagated plantlet had the highest number in plantlets, leaves, plant height, leaf length and root diameter. In factor B, PBZ treated plantlets had optimum number of plantlet. Atonik treated plants had maximum in plant height, leaf length and roots diameter. However, fertistart treated plants had more root and higher root length. Interaction of factor A and factor B revealed that the chemical treated micropropagated plants had maximum plant growth. Both stages of acclimatization, the plantlets covered with the plastic bags had more survival rate.

Key words: hardening, micropropagated, sterilized, acclimatization, survival rate

Introduction

Alpinia glanga (L.) Wild. (Padegawgyi) is belonging to the family *Zingiberaceae*. It is rhizomatous perennial herbs. Plant tissue culture refers to various methods are presently employed for shoot multiplication, shoot growth, rooting and root growth. Rooted shoots from multiplication cultures were transferred to individual containers having an appropriate potting mix and maintained in a greenhouse to allow gradual acclimatization (Anderson, 1980). Hydroponic is a method of growing plants using mineral nutrient solutions, in water, without soil (kyte and Kleyn, 1996). The growth rate on a hydroponic plant is 30-50 percent faster than a soil plant, grown under the same conditions. The yield of the plants is also greater. The nutrients in a hydroponic system are mixed system hydroponic plants also have fewer problems with bug infestation, funguses and disease. In general, plant grow hydroponically are healthier happier plant (Driver *et al* 1986). Most of the principles that apply to soil fertilizers also apply to hydroponic fertilizers, or nutrient solutions (Hoagland and Arnon, 1938). Most plants can grow hydroponically within a pH range of 5.8 to 6.8, 6.3 is considered optimal (website – 3).

[1]Head of Professor, Dr. Department of Botnay, Yeanchaung Degree Collage

MATERIALS AND METHODS

1. Hardening Process of micropropagated *Alpina galanga* (L.) Willd plantlets in soil and hydroponic systems

1.1 Location of the study and plant material

Hardening process was carried out in VFDRDA, Hlegu Township, Yangon. The micropropagated plantlets from previous experiment were used as the planting materials for hardening.

1.2 Media preparation

Soil medium was prepared by mixing 2 parts of humus: 1part of sand :1 part of burnt rice husks . Then the soil mix was sterilized in an autoclave for 12 hrs to diminish the soil born disease.

- After sterilizing the soil mixture was put into the plastic bag (7.5 cm x 12.5cm) for hardening of Alpinia galangal plantlets.

Experimental Layout and Treatments

- In both systems, there were 4 treatments and had 3 replicates (Fig. 4. A).

The treatments for both systems were:

T1= Control
T2= Ms (1/ 4 does)
T3= Ms (1 / 2 does)
T4= Ms (Full does)

- The survived plantlets from both systems were transplanted into the soil medium containing the prepared soil medium. In there, 4 treatments with 5 replicates each were arranged in two factorial of CRD. Factor A was the planting materials and factor B was the treatments (Fig.4.B).

The treatments were:

T1= Control
T2= PBZ (1g L-1)
T3= Atonik (1 ml L-1)
T4= Fertistart (10ml L-1)

1.3 Process of hardening

- The healthy uniform and aproximately the same height and weight plantlets of *Alpinia galanga* were selected for hardening processes.
- The selected cultures were maintained in a room with the ambient temperature for one week.
- Then these plantles were taken out from medium and washed with water to remove agar especially from the roots. And then the plantlets were sterilized in 2g l-1 Homifungicide solution for 10 minutes to prevent contamination.
- After sterilization, the plantlets were allowed to rinsed in the solution of 5cc L-1 Fertistart + 1cc L-1 of Atonik for 10 minutes to absorb required minerals and compounds for their growth.
- After absorbing, the rooted plantlets were grown in the plastic cup of 5 cm in diameter. After growing, the plantlets were covered with transparent plastic bags and without plastic bags.
- After absorbing, the rooted plantlets were grown in conical flasks containing the prepared MS medium. After growing, some of the plantlets were covered with transparent plastic bags and some had without plastic bags.
- After covering the plastic bag, all treatments were incubated in incubation room at the temperature of (28+2˚C) for one week.

- ❖ The survived plantlets were again hardened in the polybag (7.5 cm x 12.5 cm). In this growing, the micropagated plantlets were grown together with rhizomatic plantlets.

1.2.5 Data collection and Statistical Analysis

Three weeks after hardening in both media, the survival plantlets were recorded. The survival rate was calculated using the following formula:

$$\text{Survival rate (\%) plantlets} = \frac{\text{Total number survived exolants}}{\text{Total number cultured explants}} \times 100$$

In the second step of hardening, the following data were collected.

(a) Survival rate (%)
(b) the number of survived plants
(c) plant height
(d) the number of leaves
(e) leaf length
(f) leaf width
(g) number of root
(h) root length and
(i) root diameter

The collected data were statistically analyzed using IRRISTAT software

RESULTS AND DISCUSSION

Factor A	Number of plantlet	Plant Height (cm)
Tissue	3.96	14.52
Rhizome	3.30	13.93
F-Test	**	**
5% LSD	0.121	0.532
cv %	5.2	5.8

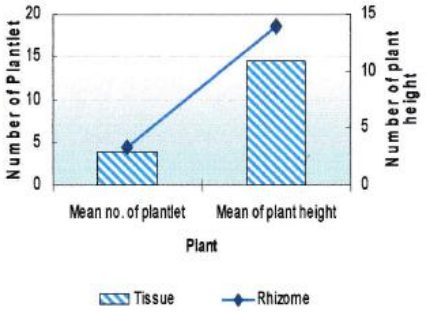

Table.1 Number of plantlet and plant height of micropropagated and rhizomatic plants

Fig.1 Number of plantlet and plant height of micropropagated and rhizomatic plants

Table.2 Number of plantlet and plant height from respective treatment

Factor B (Treatment)	Number of Plantlets	Plant Height(cm)
Control	3.47	12.75
PBZ	4.60	11.25
Atonik	3.70	17.05
Fertistart	2.75	15.83
F-test	**	**
5 % LSD	0.171	0.754
cv %	5.2	5.8

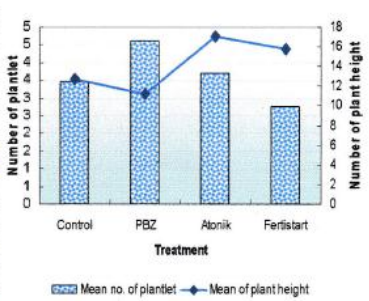

Fig.2 Number of plantlet and plant height from respective treatment

Table.3 Number of plantlet and plant height from interaction of factor A and B

Factor A	Factor B (Treatment)	Number of Plantlets	Plant Height (cm)
Tissue	Control	3.14	14.3
	PBZ	5.20	11.00
	Atonik	4.30	18.10
	Fertistart	3.20	14.66
Rhizome	Control	3.80	11.20
	PBZ	4.00	11.50
	Atonik	3.10	16.00
	Fertistart	2.30	17.00
F-test		**	**
5% LSD		0.242	1.065
CV %		5.2	5.8

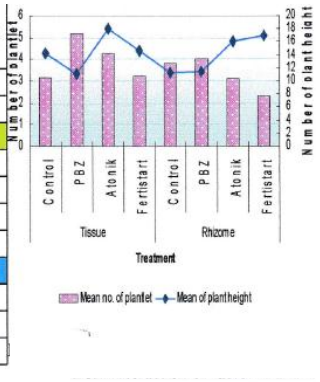

Fig.3 Number of plantlets and plant height from interaction of factor A and B

Table.4 Leaf length, leaf width and number of leaves of micropropagated and rhizomatic plants

Factor A	Leaf length (cm)	Leaf width (cm)	Number of leaves
Tissue	9.003	1.633	6.88
Rhizome	8.66	2.848	3.29
F-test	**	**	**
5% LSD	0.255	0.69	0.95
cv %	4.5	4.8	2.9

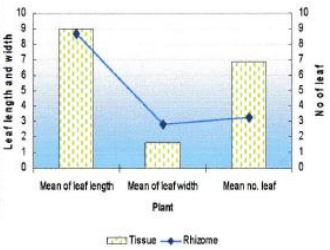

Fig.4 Leaf length, leaf width and number of leaves of micropropagated and rhizomatic plants

Table.5 Leaf length, leaf width and number of leaves from respective treatment

Factor B (Treatment)	Leaf length (cm)	Leaf width (cm)	Number of Leaves
Control	8.5	2.56	5.787
PBZ	8.655	2.01	3.575
Atonik	9.54	2.2	5.58
Fertistart	8.625	2.19	5.415
F-test	**	**	**
5 % LSD	0.362	0.973	0.135
cv %	4.5	4.8	2.9

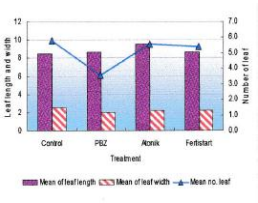

Fig.5 Leaf length, leaf width and number of leaves from respective treatment

Table.6 Leaf length and width, number of leaves from interaction of factor A and B

Factor A	Factor B (Treatment)	Leaf length (cm)	Leaf width (cm)	Number of Leaves
Tissue	Control	9.8	2.07	8.324
	PBZ	7.71	1.18	4.40
	Atonik	9.75	1.90	7.50
	Fertistart	8.75	1.38	7.33
Rhizome	Control	7.20	3.05	3.25
	PBZ	9.60	2.84	2.75
	Atonik	9.33	2.50	3.66
	Fertistart	8.50	3.00	3.50
F-test		**	**	**
5% LSD		0.511	0.137	0.191
CV %		4.5	4.8	2.9

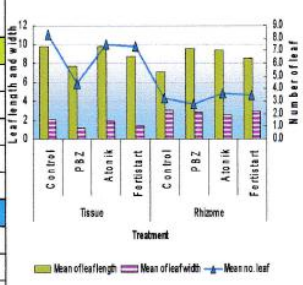

Fig.6 Leaf length, leaf width and number of leaves from interaction of factor A and B

Table.7 Root length, root diameter and number of root of micropropagated and rhizomatic plants

Factor A	Root length (cm)	Root diameter (cm)	Number of root
Tissue	8.313	0.30	2.713
Rhizome	10.21	0.25	3.34
F-test	**	**	**
5% LSD	0.293	0.360	1.08
cv %	4.9	20.3	5.5

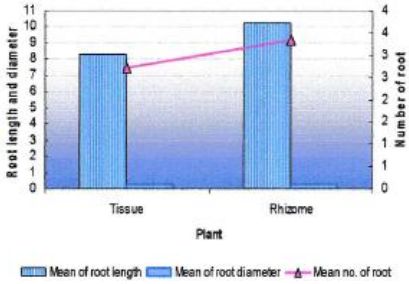

Fig.7 Root length, root diameter and number of root of micropropagated and rhizomatic plants

Table.8 Root length and diameter from respective treatment

Factor B - Treatment	Root length (cm)	Root width (cm)	Number of root
Control	10.0	0.25	2.65
PBZ	8.04	0.25	2.60
Atonik	9.0	0.35	2.96
Fertistart	10.0	0.25	3.90
F-test	**	**	**
5% LSD	0.416	0.509	0.153
cv %	4.9	20.3	5.5

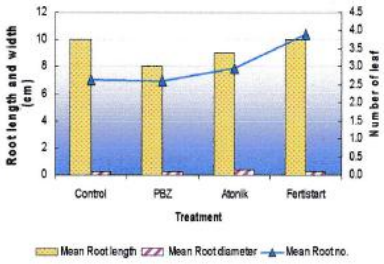

Fig.8 Root length and diameter from respective treatment

Table.9 Root length and diameter, number of root from interaction of factor A and B

Factor	Treatment	Root length (cm)	Root Diameter (cm)	Number of root
Tissue	Control	9.00	0.30	3.30
	PBZ	6.75	0.20	2.00
	Atonik	10.00	0.40	2.25
	Fertistart	7.50	0.30	3.30
Rhizome	Control	11.00	0.20	2.00
	PBZ	9.33	0.30	3.20
	Atonik	8.00	0.30	3.66
	Fertistart	12.50	0.20	4.55
F-test		**	**	**
5% LSD		0.587	0.720	0.216
cv %		4.9	20.3	5.5

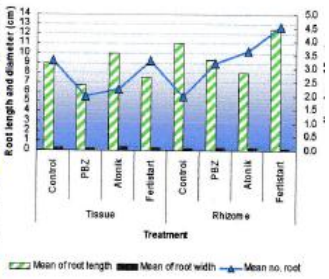

Fig.9 Root length and diameter, number of root from interaction of factor A and B

Table.10. Number of survived plants of various ratio of MS treatments with plastic bag cover

Treatment	1st week	2nd week	3rd week	4th week
T1 (Control)	1	1	0	0
T2(1/4 MS)	1	1	0.33	0
T3 (1/2 MS)	1	1	1	1
T4 (MS)	1	1	0.66	0
F-test	ns	ns	**	*
5% LSD	1	1	0.11	1
cv %	13.46	11.35	14.26	15.16

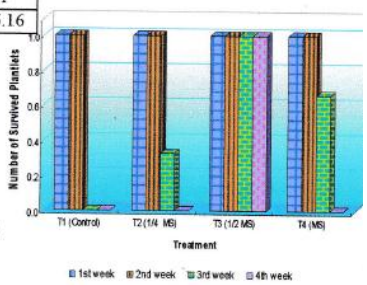

Fig.10 Number of survived plants of various ratio of MS treatments with plastic bag cover

Table.11 Number of survived plantlets of various ratio of MS treatments without plastic bag cover

Treatment	1st week	2nd week	3rd week	4th week
T1 (Control)	0.66	0.66	0	0
T2(1/4 MS)	1	0.66	0.33	0
T3 (1/2 MS)	1	1	0.66	0.66
T4 (MS)	1	0.33	0.66	0
F-test	ns	ns	**	*
5% LSD	0.17	0.23	0.15	0.26
cv %	10.48	9.41	12.15	14.22

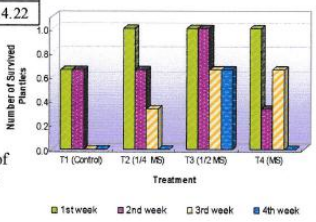

Fig.21. Number of survived plantlets of various ratio of MS treatments without plastic bag cover

Fig.11 Number of survived plantlets of various ratio of MS treatments without plastic bagcover

Fig.12 Hardened micropropagated and rhizomatic *Alpinia galangal* (L). Willd

DISCUSSION AND CONCLUSION

The results of the experiment showed that in factor A, micropropagated plantlet had the highest number in plantlets, leaves, plant height, leaf length and root diameter. In factor B, PBZ treated had optimum number of plantlet. Atonik treated plants had maximum in plant height, leaf length and root diameter. However fertistart treated plants and more root and higher root length. Interaction of factor A and factor B revealed that the chemical treated micropropagated plants had maximum plant growth.

Most species grown *in vitro* require an acclimatization process in order to ensure that sufficient number of plants survive and grow vigorously when transferred to soil. This article reviews current the developing methods for the acclimatization of micropropagated plantlets (Hazarika, 2003).

In 1996, John Woodward published his water culture experiments with spearmint. He found that plants in less pure in less pure water sources grew better than plants in distilled water. Borthakur *et al.* (1999) found that in *Alpinia galanga* proliferated shoot of 4-6 weeks old with developed root systems were transferred to liquid nutrient solution containing half strength of MS medium. Plantlets were hardened for 6-8 weeks and then transferred to potting soil composed of a 1:1 mix of autoclaved sand and soil, and were kept at 23 ± 2C with 16-h photoperiod. 80% of the transferred plantlets survived in the potted soil and did not show any morphological abnormalities.

PBZ inhibited leaf development and induced the formation of bud clusters. Regenerated plants resumed normal growth after transplanting. Effect of PBZ *in vitro* relates to their ability to reduce wilting, improve desiccation resistance, and increase post transplant survival in number of plant species. (Website-1)

Paclobutrazol (PBZ), a triazole growth retardank know to improve tolerance of various species to stress. PBZ significantly reduced shoot growth *in vitro* but increased/improved the quality and coloration. The percentage of water loss from detached leaves of *in vitro* plantlets was significantly improved by PBZ, IBA, and the combination. Incorporation of PBZ *in vitro* better enables *prunus serotina* plantlets to withstand the stresses associated with acclimatization (Westie-2)

When PBZ application was studied on website (1), it was revealed that the effect of PBZ increased post transplant survival in a number of plant species. In the study of Fertistart, according to the instruction, Fertistat solution promoted the root formation and multiplication. In this study, it was found that rhizome and Fertistat is the best for root formation and multiplication. All of the above reason, tissue plantlet and PBZ concentration should be selected for plantlet multiplication and rhizome plantlets and Fetistart should be selected for root formation.

The hydroponic system proposed, allowed high levels of acclimatization and survival of the *in Vitro* plants when these are transferred to pots, probably due to the gradual controlled decrease of the relative humidity. Because the acclimatization of plants is not so clear, it would be interesting to evaluate the biochemical, physiological and structural changes in the tissues of the leaves during this process (Biotechnologia Aplicada 2003)

Acknowledgments

The author would like to greatly acknowledge Dr. Soe Win, Principal, Yenanchaung Degree Collage, for his kind permission and supports for this study. I am also thankful to U Khin Maung Sein, Pro-Rector (Retd), Taungoo University, for his valuable comments, suggestions, and supports and guidance for this study. Lastly, but not the least ,I am indebted to Dr. Thanda Aye, Professor, Department of Botany, Yangon University, for her valuable advices, suggestions, guidance and comments for this study.

References

Anderson, W.C 1980. Mass propagation by tissue culture principles and practice U.S. Dept of Agri. Sci and Education Administration

Borthaku M., Hazarika, J. and Singh, R.S. 1999. A protocol for micropropagation of *Alpinia galanga*. Plant cell Tissue Organ Culture, 55:231-233.

Driver *et al* 1986 Hortiscience 19:507-509. Method for Acclimatizating and propagating plant tissue culture shoots. Plant Reserch laboratories, modesfo, calif.

Hazarika 2003. Acclimatization of tissue-cultured plants. Current science, vol 85. No 12, Department of Horticulture, Assam Agricultural University, jorhat 785013, India.

Hogland, D.R. and D.I Arnon. 1938. The water culture mentod for growing plants without soil. California Agr. Expt. Sta. cric. 347.

Kyte, L.and J.Kleyn. 1996. Plants from test tubes chapter 5, Media Ingredients p:61-71.

Wesite

1. http;//www.pubhort.org/members/showdocument?series=hr&volume=24&article = 3 & bestand = 2& layout = pubhort & action = showdocument
2. file:///E:share –the-server/school.2/prunus%20tc.htm.
3. http:///www.free encyclopedia.june 2009