# PHYSIOLOGICAL ASPECTS OF EX VITRO ESTABLISHMENT OF TISSUE CULTURED ORCHID (*Dendrobium* sp. var. SONIA 17) PLANTLETS

By

**SAMASYA K. S.**

**THESIS**

*Submitted in partial fulfilment of the requirement for the degree*

Master of Science in Agriculture

*Faculty of Agriculture*
*Kerala Agricultural University*

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI
THIRUVANANTHAPURAM

**2000**

# DECLARATION

I hereby declare that this thesis entitled "Physiological aspects of ex vitro establishment of tissue cultured orchid (Dendrobium sp. var. Sonia 17) plantlets " is a bonafide record of the research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society

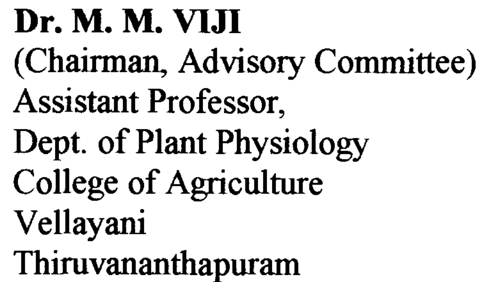
Vellayani.

8.05.2000

SAMASYA K. S.

# CERTIFICATE

Certified that this thesis entitled "Physiological aspects of *ex vitro* establishment of tissue cultured orchid (*Dendrobium sp.* var. Sonia - 17) plantlets" is a record of research work done independently by Mrs. Samasya K. S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



Vellayani
8 -05-2000

**Dr. M. M. VIJI**
(Chairman, Advisory Committee)
Assistant Professor,
Dept. of Plant Physiology
College of Agriculture
Vellayani
Thiruvananthapuram

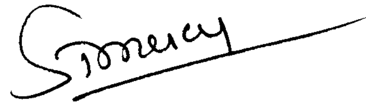
# APPROVED BY

## CHAIRMAN

Dr. M. M. VIJI

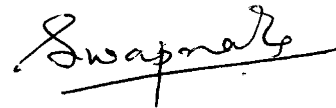
## MEMBERS

1. Dr. S. T. MERCY



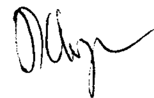
2. Dr. D. S. RADHADEVI

3. Smt. SWAPNA ALEX



## EXTERNAL EXAMINER

Dr. P. S. SRINIVASAN



# ACKNOWLEDGEMENT

*I wish to extend my profound gratitude and indebtedness to* **Dr. M. M. Viji**, *Assistant Professor and Chairman of Advisory Committee, for her inspiring guidance, valuable suggestions and constant encouragement rendered to me throughout the course of my study. I owe my flow of thanks to her for her helpful criticism ever willing help, friendly approach and affection which have greatly facilitated the production of this thesis.*

*Grateful and Sincere thanks are due to* **Dr. S. T. Mercy,** *Professor and Head of the Department of Plant Physiology for her expert advice, critical comments and constructive persual of the manuscript.*

*I am particularly grateful to* **Dr. D. S. Radha Devi**, *Associate Professor, Department of Plant Breeding and Genetics for her valuable guidance and whole hearted approach throughout the investigation.*

*I wish to express my sincere and profound gratitude and obligations to* **Smt. Swapna Alex** *for her timely help and valuable advices during the course of this investigation.*

*I express my profound gratitude **to Dr. K.RajMohan**, Associate Professor, Department of Horticulture for his whole hearted technical help, inspiring guidance, helpful criticism and valuable suggestions.*

*Grateful acknowledgement to **Dr. D. Chandramony**, Associate Professor, Department of Plant Breeding and Genetics for her expert advice, kind help and whole hearted co-operation towards me. I place on record of my gratitude to **Dr. P. Sivaprasad**, Associate Professor, Department of Plant Pathology., for all the help he had offered to me in taking photographs. **Dr. P. Saraswathi**, Professor and Head, Department of Agricultural Statistics for her expert advice in planning of the experiment.*

*I am particularly thankful to Dr. R.R. Nair, Dr.R. Vikraman Nair, Director of Research., N.A.R.P., Dr. S.Mohan Kumar, Prof and Head, Dept. of Agronomy, C.T.C.R.I. and Dr. K. Ravi, Senior scientist, for their sincere help which enables me in taking observations at C.T.C.R.I.*

*I also express my sincere thanks to Rekha, Lekha Rani teacher, Sreelatha teacher, Satheeshna Kumari and Uma Maheswary for their constant help during the course of my research work.*

*Kerala Agricultural University for granting me the Junior Research Fellowship.*

*My dear friends especially Lekshmi, Rakhee, Pournami, Rani, Romi, Radhika, Suja, Manoj, Ullas, and Binduji who were always with me during the preparation of the thesis and whose voluntary and selfless help I cannot express in words. My juniors Beena and Sreeja who helped me a lot during the entire period of investigation.*

*I wish to express my sincere thanks to Dr. M. Thankaraj, Mrs.M.R. Kalarani and all the friends at T.N.A.U. who have helped me a lot in the collection of literature.*

*Words fail to express my deep sense of gratitude and indebtedness to my dear husband for his constant encouragement, countless help and care without which I could not have completed this venture.*

*From deep with in my heart I express my sincere and profound gratitude to my father, mother, sister, father in law, mother in law and relatives for their constant encouragement and moral support.*

*Above all I bow before the Almighty for all the blessings showered upon me.*

***SAMASYA K. S.***

# CONTENTS


| | | Page No. |
|---|---|---|
| **INTRODUCTION** | .... | **1-2** |
| **REVIEW OF LITERATURE** | .... | **3-21** |
| **MATERIALS AND METHODS** | .... | **22-35** |
| **RESULTS** | .... | **36-94** |
| **DISCUSSION** | .... | **95-133** |
| **SUMMARY** | .... | **134-140** |
| **REFERENCES** | .... | **i - xix** |
| **APPENDIX** | .... | |
| **ABSTRACT** | .... | |

## LIST OF TABLES


| Sl. No. | Title | Page No. |
|---|---|---|
| 1a. | Effect of sucrose in the rooting medium on CGR of orchid plantlets at 45 days after rooting | 37 |
| 1b. | Effect of triazole treatment at planting out on CGR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 37 |
| 1c. | Effect of light and humidity treatment in the hardening chamber on CGR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 38 |
| 2a. | Effect of sucrose in the rooting medium on NAR of orchid plantlets at 45 days after rooting | 40 |
| 2b. | Effect of triazole treatment at planting out on NAR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 40 |
| 2c. | Effect of light and humidity treatment in the hardening chamber on NAR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 41 |
| 3a. | Effect of sucrose in the rooting medium on RGR of orchid plantlets at 45 days after rooting | 43 |
| 3b. | Effect of triazole treatment at planting out on RGR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 43 |
| 3c. | Effect of light and humidity treatment in the hardening chamber on RGR of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days | 44 |

interval

| | | |
|---|---|---|
| 4a. | Effect of sucrose in the rooting medium on photosynthetic rate of orchid plantlets at 45 days after rooting | 45 |
| 4b. | Effect of triazole treatment at planting out on photosynthetic rate of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 45 |
| 4c. | Effect of light and humidity treatment in the hardening chamber on photosynthetic rate of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 46 |
| 5a. | Effect of sucrose in the rooting medium on transpiration rate of orchid plantlets at 45 days after rooting | 48 |
| 5b. | Effect of triazole treatment at planting out on transpiration rate of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 48 |
| 5c. | Effect of light and humidity treatment in the hardening chamber on transpiration rate of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 49 |
| 6a. | Effect of sucrose in the rooting medium on plant height of orchid plantlets at 45 days after rooting | 51 |
| 6b. | Effect of triazole treatment at planting out on plant height of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 51 |
| 6c. | Effect of light and humidity treatment in the hardening chamber on plant height of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 52 |

orchid plantlets at 45 days after rooting

10b. Effect of triazole treatment at planting out on total chlorophyll of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 62

10c. Effect of light and humidity treatment in the hardening chamber on total chlorophyll of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 63

11a. Effect of sucrose in the rooting medium on chlorophyll a of orchid plantlets at 45 days after rooting 64

11b. Effect of triazole treatment at planting out on chlorophyll a of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 64

11c. Effect of light and humidity treatment in the hardening chamber on chlorophyll a of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 65

12a. Effect of sucrose in the rooting medium on chlorophyll b of orchid plantlets at 45 days after rooting 67

12b. Effect of triazole treatment at planting out on chlorophyll b of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 67

12c. Effect of light and humidity treatment in the hardening chamber on chlorophyll b of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 68

13a. Effect of sucrose in the rooting medium on carotenoids of orchid plantlets at 45 days after rooting 70

7a. Effect of sucrose in the rooting medium on number of leaves per shoot of orchid plantlets at 45 days after rooting 54

7b. Effect of triazole treatment at planting out on number of leaves per shoot of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 54

7c. Effect of light and humidity treatment in the hardening chamber on number of leaves per shoot of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 55

8a. Effect of sucrose in the rooting medium on number of roots of orchid plantlets at 45 days after rooting 56

8b. Effect of triazole treatment at planting out on number of roots of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 56

8c. Effect of light and humidity treatment in the hardening chamber on number of roots of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 57

9a. Effect of sucrose in the rooting medium on survival percent of orchid plantlets at 45 days after rooting 59

9b. Effect of triazole treatment at planting out on survival percent of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 59

9c. Effect of light and humidity treatment in the hardening chamber on survival percent of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 60

10a. Effect of sucrose in the rooting medium on total chlorophyll of 62

orchid plantlets at 45 days after rooting

10b. Effect of triazole treatment at planting out on total chlorophyll of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 62

10c. Effect of light and humidity treatment in the hardening chamber on total chlorophyll of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 63

11a. Effect of sucrose in the rooting medium on chlorophyll a of orchid plantlets at 45 days after rooting 64

11b. Effect of triazole treatment at planting out on chlorophyll a of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 64

11c. Effect of light and humidity treatment in the hardening chamber on chlorophyll a of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 65

12a. Effect of sucrose in the rooting medium on chlorophyll b of orchid plantlets at 45 days after rooting 67

12b. Effect of triazole treatment at planting out on chlorophyll b of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 67

12c. Effect of light and humidity treatment in the hardening chamber on chlorophyll b of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval 68

13a. Effect of sucrose in the rooting medium on carotenoids of orchid plantlets at 45 days after rooting 70

| | | |
|---|---|---|
| 13b. | Effect of triazole treatment at planting out on carotenoids of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 70 |
| 13c. | Effect of light and humidity treatment in the hardening chamber on carotenoids of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 71 |
| 14a. | Effect of sucrose in the rooting medium on protein content of orchid plantlets at 45 days after rooting | 73 |
| 14b. | Effect of triazole treatment at planting out on protein content of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 73 |
| 14c. | Effect of light and humidity treatment in the hardening chamber on protein content of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 74 |
| 15a. | Effect of sucrose in the rooting medium on carbohydrate content of orchid plantlets at 45 days after rooting | 76 |
| 15b. | Effect of triazole treatment at planting out on carbohydrate content of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 76 |
| 15c. | Effect of light and humidity treatment in the hardening chamber on carbohydrate content of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 77 |
| 16a. | Effect of sucrose in the rooting medium on leaf area of orchid plantlets at 45 days after rooting | 78 |
| 16b. | Effect of triazole treatment at planting out on leaf area of rooted (at | 78 |

$S_2$ sucrose level) orchid plantlets at 15 days interval

| | | |
|---|---|---|
| 16c. | Effect of light and humidity treatment in the hardening chamber on leaf area of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 79 |
| 17a. | Effect of sucrose in the rooting medium on root length of orchid plantlets at 45 days after rooting | 81 |
| 17b. | Effect of triazole treatment at planting out on root length of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 81 |
| 17c. | Effect of light and humidity treatment in the hardening chamber on root length of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 82 |
| 18a. | Effect of sucrose in the rooting medium on root shoot ratio of orchid plantlets at 45 days after rooting | 84 |
| 18b. | Effect of triazole treatment at planting out on root shoot ratio of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 84 |
| 18c. | Effect of light and humidity treatment in the hardening chamber on root shoot ratio of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 85 |
| 19a. | Effect of sucrose in the rooting medium on total fresh weight of orchid plantlets at 45 days after rooting | 86 |
| 19b. | Effect of triazole treatment at planting out on total fresh weight of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 86 |

| | | |
|---|---|---|
| 19c. | Effect of light and humidity treatment in the hardening chamber on total fresh weight of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 87 |
| 20a. | Effect of sucrose in the rooting medium on total dry weight of orchid plantlets at 45 days after rooting | 89 |
| 20b. | Effect of triazole treatment at planting out on total dry weight of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 89 |
| 20c. | Effect of light and humidity treatment in the hardening chamber on total dry weight of rooted (at $S_2$ sucrose level) orchid plantlets at 15 days interval | 90 |
| 21. | Stomatal size of various leaf types of plantlets | 92 |
| 22. | Stomatal count of various leaf types of plantlets | 92 |
| 23. | Cuticle thickness of various leaf types of plantlets | 92 |
| 24. | Number of mesophyll layers of various leaf types of plantlets | 94 |

# LIST OF PLATES


| Sl. No. | Title | Between Pages |
|---|---|---|
| 1. | Meristem cultured plantlets before rooting. | 22-23 |
| 2 a. | Coconut husk balls, the potting media used. | 24-25 |
| b. | Sonia-17 plantlets in the coconut husk balls at 45 days after planting out | " |
| 3. | Hardening chamber for maintaining light and humidity | 26-27 |
| 4. | Effect of triazole treatments on number of leaves produced at 30 days after planting out | 54-55 |
| a. | Maximum number of leaves produced by plantlets under the treatment $T_1$ (5 mg/l) | |
| b. | Number of leaves produced by plantlets under the treatment $T_2$ (10 mg/l) | |
| c. | Minimum number of leaves produced by plantlets under the treatment $T_3$ (Control) | |
| 5. | Effect of different sucrose concentrations in the rooting media on number of roots produced | 56-57 |
| a. | Maximum number of roots produced by plantlets under the treatment $S_2$ (40 g/l) | |
| b. | Number of roots produced by plantlets under the treatment $S_3$ (60 g/l) | |
| c. | Minimum number of roots produced by plantlets under the treatment $S_1$ (20 g/l) | |
| 6 a. | Abaxial surface of field grown leafs showing more number of stomata per unit area | 90-91 |

# LIST OF FIGURES


| Fig. No. | Title | Page No. |
|---|---|---|
| 1. | Effect of sucrose (S) and triazole(T) on growth parameters | 98 |
| 2. | Effect of light and humidity on growth parameters | 100 |
| 3. | Effect of sucrose (S) and triazole (T) on relative growth rate (RGR) | 102 |
| 4. | Effect of light and humidity on relative growth rate (RGR) | 103 |
| 5. | Effect of sucrose, triazole, light and humidity on photosynthetic rate | 107 |
| 6. | Effect of sucrose, triazole, light and humidity on transpiration rate | 110 |
| 7. | Effect of sucrose, triazole, light and humidity on survival percent | 118 |
| 8. | Effect of sucrose on photosynthetic pigments content. | 120 |
| 9. | Effect of triazole on photosynthetic pigments content. | 121 |
| 10. | Effect of light and humidity on photosynthetic pigments content. | 123 |
| 11. | Number of mesophyll layers of various types of plantlets | 130 |

b. Abaxial surface of *in vitro* leaf showing less number of stomata per unit area

7 a. Cross section of field grown leaf showing distinct layers of cuticle above the epidermis. 92-93

b. Cross section of *in vitro* leaf showing the absence of cuticle layer above the epidermis.

8 a. Abaxial surface of field grown leaf showing elliptical closed stomata with small aperture 132-133

b. Abaxial surface of *in vitro* leaf showing fully opened circular stomata with large stomatal aperture

# ABBREVIATIONS USED IN THIS THESIS

| | | |
|---|---|---|
| **@** | - | At the rate of |
| **°C** | - | Degree Celsius |
| **cm** | - | Centimetre |
| **CGR** | - | Crop growth rate |
| **Fig.** | - | Figure |
| **g** | - | Gram |
| **Kg.** | - | Kilo gram |
| **m** | - | Metre |
| **mm** | - | Millimetre |
| **mg** | - | Milligram |
| **µm** | - | Micrometre |
| **NAR** | - | Net assimilation rate |
| **%** | - | Percent |
| **RGR** | - | Relative growth rate |
| **s** | - | Second |

# INTRODUCTION

# 1. INTRODUCTION

Orchids with flowers of exquisite beauty and variety of patterns belong to the family orchidaceae, which comprises of about 800 genera and more than 35,000 species. Orchids are known for their bewitchingly beautiful flowers with long lasting shelf life, which fetch a very high price in the international market. They occupy prime position among all the flowering plants.

Kerala has been earmarked by the government of India as a zone for intensification of orchid cultivation and was given special emphasis in the developmental programmes during the eighth plan period itself. *Dendrobium* hybrids are the most popular among the orchids commercially grown in Kerala. In India export earnings from floriculture has increased from 2.67 per cent to 17.00 per cent (Raghava and Dadlani, 1997).

The conventional methods of vegetative propagation is laborious and time consuming. So commercialization of orchid cultivation necessitates the mass production on a scale sufficient to meet the steady demand from internal and export markets which in turn urge more production of tissue cultured planting material of these varieties. Several minor factors are found to influence the per cent success with special reference to shoot or root formation as well as the performance of plantlets *in vitro* and *ex vitro* conditions.

The success of micro propagation depends on the field establishment of *in vitro* derived plantlets. Since within the *in vitro* system, the plantlets are hetrotrophic, they are characterised by some physiological, morphological and anatomical peculiarities. During *ex vitro* establishment the plantlets have to switch over to autotrophic nutrition, involving normal photosynthetic activity. Normally the tissue cultured plantlets of orchids show high rate of mortality in *ex vitro* establishment. This is mainly because the *ex vitro* plantlets will not be able to with stand the sudden shock of environmental changes and improper functioning of stomata. (Brainerd and Fuchigami, 1981; Wardle *et al.*, 1983).

Studies regarding the physiological changes that occur during *ex vitro* establishment of orchids and how they influence the plant growth and survival in the new environment are very much limited. Hence investigations into the physiological, morphological, biochemical, anatomical and biometric changes taking place during *in vitro* propagule multiplication and *ex vitro* establishment of tissue cultured plantlets of orchids will be helpful in finding out measures to overcome the field mortality rate and to improve propagation efficiency.

The commercial significance of the present study becomes evident in this context. Considering the above aspects, research focused on the understanding of physiological aspects of *ex vitro* establishment of orchid plantlets has been initiated.

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

Orchids, one of the major cut flowers of Kerala belong to the family orchidaceae. Among orchids *Dendrobium* is a sympodial orchid variety of great demand in Kerala. Conventional methods of propagation of orchids are slow and time consuming. So in recent years propagation of orchids by tissue culture is gaining popularity. The propagation of orchid clones through meristem culture was the first commerically successful venture in tissue culture. Eventhough considerable progress has been made in the *in vitro* propagation of orchid, the *ex vitro* establishment of plantlets remains critical. Detailed study of the physiological aspects of *ex vitro* establishment of orchid plantlets have not been attempted so far. Understanding the physiological aspects of *ex vitro* establishment of tissue cultured plantlets will be helpful to evolve measures to overcome the field mortality and improve propagation efficiency of orchids.

This review encompasses the research on the various aspects of *in vitro* culture and *ex vitro* establishment of plantlets, mainly physiological, morphological, biochemical, anatomical and biometric characteristics of micro propagated plantlets.

## 2.1 *In vitro* rooting

Root production is of extreme importance for the survival and growth of plants and orchids are not an exception. Compared to the roots found in field condition, the *in vitro* roots of cauliflower plantlets were non functional because they had poor vascular connection with the shoot, resulting in restricted water transfer from roots to shoot (Grout and Aston, 1977). Debergh and Maene (1981) reported that *in vitro* roots of ornamental plants died after transplanting to green house and delayed the plant growth. In micropropagated apple, Welander (1985) observed that rooting ability was increased by dark treatment of the culture during root initiation. Root induction in darkness could increase the number of roots formed in apple and peach ( Zimmerman, 1984; Hammerschlag, *et. al*. 1987). Madhuri and Vasundhara (1990) reported that auxins were not necessary for root formation in *Dendrobium* and rooting was observed to be rapid when treated with 1.0 percent sucrose. Honmode and Sehgal (1991) opined that good rooting could be obtained from nodal sections of *Dendrobium transpersensis* in MS medium containing 5.0µm of IBA. Increased rooting can be obtained in terresrial orchids by increasing the sucrose concentration to 40.0g/l and by addition of 0.05 per cent activated charcoal to the rooting medium (Collins and Dixon, 1992).

## 2.2 Effect of sucrose on *in vitro* rooting

Sucrose concentration significantly increased the fresh weight of protocorm like bodies of *Cymbidium* orchids (Honjo *et. al.* 1988). Wainright and Scrace (1989) studied the influence of sucrose on *in vitro* rooting of *Ficus lyrata* and obtained maximum fresh weight and dry weight of the cultures conditioned with 2.0 or 4.0 percent sucrose. Better *ex vitro* establishment was also observed for these plantlets than that of control. Bertrand *et. al.* (1992) suggested that the cultures of coffee with sucrose in the rooting medium exhibited better growth, rooting and survival rate than that of control. According to Ault (1994), rooting percentage and root length were reduced when the sucrose concentration in the rooting medium of *Eriostemon myoporides* was reduced from 50.0 to 25.0 g/l. Sudeep (1994) obtained maximum number of shoots from axillary buds of *Dendrobium* and high rooting percentage, when the medium was supplemented with 3.0 percent sucrose along with BA 5.0 mg/l.

### 2.2.1 Effect of sucrose on physiological and biochemical characters

Fevereiro *et. al.* (1986) observed that there was an increase in inter cellular protein production of *Silybum marianum* with an increase in the concentration of sucrose from 30.0 to 40.0 g/l. Hess *et. al.* (1986) suggested

that generally there is an increase in fresh weight and dry weight of the micro propagules with increased concentration of sucrose due to enhanced cell division. According to Langford and Wainwright (1987) an increase in sucrose level in the nutrient medium of rose plantlets resulted in a decrease in the rate of photosynthesis which is due to impaired regeneration of RuBP carboxylase. They also reported that the starch grain formation in the chloroplast also depend on sucrose level. Kubota and Kozai (1991), studied the effect of initial amount of sugar in the medium on *in vitro* growth of *Cymbidium* orchids and reported that the fresh weight and dry weight of plantlets were greater at higher glucose concentration. De Rick and Van (1992), found that sucrose supplemented in stage three improved acclimatization of micropropagated rose plantlets and exhibited increased growth and transpiration rate at higher relative humidity. The stomata of such plants were possibly more closed and also with higher pigment and carbohydrate content. Rahman *et. al.* (1992), reported that in rose plantlets sucrose at 40.0 g/l concentration gave significantly more number of roots, higher rooting frequency, maximum root length and shoot length during *in vitro* period as compared to 20.0 g/l and 60.0 g/l concentration. When these plantlets were kept in the humidity chamber for one week and then transferred to the field, 70 percent plantlets had survived. Yue *et. al.* (1993), reported that photosynthetic rate of *in vitro* cultured plantlets of asparagus were as high as those of seedlings grown in green house and the rate of transpiration was higher. They also observed that at the early stage of *in vitro* rooting, the net photosynthetic rate of leaves of tissue culture

plantlets was about 50 percent that of the leaves of green house grown plants. But later on the leaves expanded and the net photosynthetic rate increased as high as that of leaves of green house grown plants. In kiwi fruit sucrose resulted in an increase in the number of chloroplast, but negatively affected the size of mesophyll cells by its addition to the substrate medium (Kortessa and Artemios 1997). Sunitibala *et. al.* (1998) studied the effect of sucrose on growth and chlorophyll synthesis of teak shoots and observed that growth, fresh weight of shoot, chlorophyll a, chlorophyll b and total chlorophyll content of leaves were stimulated with an optimum sucrose level of 30.0 g/l. It was found that higher concentration inhibited the shoot growth.

## 2.3 Acclimatization and planting out

Acclimatization is the process to make an organism to adapt to environmental changes (Brainerd and Fuchigmi, 1981). The plantlets produced *in vitro* cannot be transferred straight away to *ex vitro* environment due to dessication that may result after the transfer (Corner and Thomas, 1982). The micro propagants have poorly developed cuticle, stomatal aperture and less photosynthetic ability. So they fail to withstand direct exposure to environment. Acclimatization or hardening helps in cutting down their mortality rate during field transfer by correcting the morpho-physiological aberrations (Vij, and Promila,1989).

During hardening the plantlets of orchids are generally kept either in community pots or in individual pots of 5.0 cm diameter. The bottom half of the pots are first filled with broken pieces of charcoal and above this a mixture of smaller broken bricks (Sharma and Chowhan, 1995). They also reported that the potting mixture comprising brick, charcoal, tree-fern, bark pieces, leaf mould and dry sphagnum in 1:1:1:1:1:2 ratio with green sphagnum on top supported the maximum survival of *Dendrobium*.

Transplanting of the shoots or plantlets raised *in vitro* typically requires prolonged regulation of both temperature and relative humidity to allow acclimatization of the plantlets to *ex vitro* condition thus minimizing the mortality percent (Grout and Crisp, 1977). The major problems commonly encountered at transplanting are inability of plantlets to regulate transpiration and insufficient photosynthetic capability to achieve a positive carbon balance and this was reported in micro propagated cauliflower (Grout and Aston, 1978). A period of humidity acclimatization was suggested for the newly transferred tissue cultured plantlets to make them adapted to the outside environment (Hu and Wang, 1983). Sharma and Tandon (1990) observed 65 percent survival of *Dendrobium* when transferred to a potting medium containing charcoal fragments, brick pieces and coconut fibres. Kumar (1991) observed that seedlings of *Dendrobium* grown *in vitro* showed best survival percentage in charcoal followed by fern roots and rubber husk when maintained at a

temperature of 29°C and 35°C with relative humidity between 70 and 90 percent and illumination between 1000 to 1500 lux at pot level. Lakshmi Devi (1992) reported 70 percent survival of *Dendrobium* when transplanted in a potting medium containing brick and charcoal in equal proportions. Keeping the plantlets in the open under shade with intermittent water spray was found to be best for hardening of *Dendrobium* (Sudeep, 1994).

The acclimatization of micro propagated orchid seedlings from *in vitro* to *in vivo* environment involves their transfer to humidity chamber before they are subjected to harsher conditions. Subsequently plantlets in the community pots can be transferred to green house. Sharma and Chauhan (1995) reported that acclimatization of *in vitro* raised seedlings of *Dendrobium* is essential for their successful transfer to community pots. The cultures under reduced relative humidity may develop normal wax formation and hence may not need a long period of acclimatization for survival (Byoung *et. al.* 1995).

## 2.4 Effect of triazole in tissue culture

Steffens and Wang (1985), observed the retarding growth of young apple plant at lower concentration (0.25 mg/l) of triademefon - a triazole derivative. Davis *et. al.* (1986) reported that triazole treatment while transplanting to green house causes better survival of ornamental plants. Triazole exhibited varying degrees of both plant growth regulating and

fungicidal activity (Fletcher *et. al.* 1986). Williamson *et. al.* (1986) reported that triazole treated peach plants have increased root to shoot ratio compared to untreated plants. They also observed that triazole reduced shoot length, stem weight and leaf area and increased leaf thickness. Bausher and Yelenosky (1987) observed an increase in root diameter and root number in triazole treated citrus plants. The increased root diameter is due to increased size of cortex parenchyma cells. Triazole promoted adventitious root formation in cuttings (Davis and Sankhla, 1988). In grape wine triazole treatment as well as reduced relative humidity, strongly improved the resistance of plantlets to wilting after transplanting (Novello and Roberts, 1992). Eliasson and Beyl (1993), observed that paclobutrazole, a triazole growth retardant was useful in hardening of cherry plantlets when incorporated into the *in vitro* rooting medium. In this case the roots appeared shorter, thicker, numerous and rooting percent was higher whereas shoot growth was reduced. Rong *et. al.* (1995) observed that the treatment of rice seedlings with multi-effect triazole resulted in increase of plant height, dry matter per plant, root number, protein content, chlorophyll content and the photosynthetic rate. Yin-Liquing *et. al.* (1996) found that root growth and differentiation of *Gerbera jamesoni* could be improved when rooting medium was supplemented with a triazole derivative - amino benzo triazole. At 0.5 mg/l concentration it gave 100 percent rooting and 98.9 percent survival of the transplanted plantlets.

### 2.4.1 Effect of triazole on physiological and biochemical characters

According to Bachenauer *et. al.* (1984) higher amount of chlorophyll was found in primary leaves of barley seedlings treated with paclobutrazole. Triazole treatment reduces plant bio-mass and leaf area of soya bean (Sankhla *et. al.* 1986 ). Triazoles have only little direct effect on net photosynthetic rates on leaf area basis (Andersen and Aldrich, 1987). Marini (1987) noted that triazole treated peach plants had delayed onset of leaf senescence and thereby prolonged the period of photosynthetic activity. It was observed that paclobutrazole significantly increased the rooting percentage treated black cherry plants and produced thicker roots. The rate of water loss from their leaves was also reduced which in turn enabled black cherry plantlets to withstand the stresses associated with acclimatization (Eliasson and Beyl, 1993).

Kane and Smiley (1983) reported that the triazole derivative triadimefon increased the total non structural carbohydrate content in mature leaves of blue grass. Steffens and Wang (1985) found that triazole reduced shoot respiratory activity in apple. Wang *et. al.* (1995) noted that triazole could alter plant nutrition thus influence root growth and morphology of wheat seedlings. They also observed that uniconazole - a triazole derivative treated wheat seedlings showed increased photosynthetic efficiency and chlorophyll content thereby increasing the yield to 6 to 14 percent. Triazole treated plants of *Cucumis*

*sativus* appeared dark green than control and this is due to increased chlorophyll content (Fletcher *et. al.* 1986). Triazole reduced chlorophyll catabolism which in turn resulted in an increase of chlorophyll content. Sairam *et. al.*(1995) found that the triazole derivative triadimefon treated plantlets of wheat exhibited a significant increase in chlorophyll content (24 %), protein content (56%) and leaf area per plant. Sheela and Alexander (1995) reported that seed treatment of rice with triazole, $NaH_2PO_4$ and KCl resulted in an increase of chlorophyll and proline content. In *Cucumber* Thomas and Singh (1995) observed that triazole enhanced the accumulation of chlorophyll and carotenoid due to enhanced cytokinin levels in the treated cotyledons. Saha and Guptha (1998) reported that triazole when applied as soil drench improved photosynthetic activity, growth and yield in mung bean.

## 2.5 Effect of humidity on *ex vitro* establishment

Humidity and light are two important physical factors which influence the *ex vitro* establishment of plantlets (Wardle *et. al.* 1983 ; Desjardins *et. al.* 1987). Donnelly *et. al.* (1985) found that the tissue cultured raspberry plantlets lack effective stomatal closure mechanism which resulted in excessive water loss leading to increased field mortality at lower relative humidity. In cauliflower and chrysanthemum, Short *et. al.* (1987) reported that plantlets cultured at 80 percent relative humidity has increased wax deposition on their leaves and they had got better *ex vitro* establishment because of reduced water

loss. Mathur *et. al.* (1988) observed that when the relative humidity of 60 to 70 percent was maintained in the growth chamber 100 percent establishment of *Valeriana wallichi* resulted.

Several methods have been used for gradual adaptation of *in vitro* plantlets to *ex vitro* condition. The regenerants should preferably be transferred first to green house and then to the field. The humidity is controlled in apple during acclimatization by covering the plantlets with transparent plastic sheets with holes in them and giving intermittent misting (Brainerd and Fuchuigami, 1981). Poole and Conover (1983) found that in order to provide humidity for *in vitro* cultured *Diffenbachia* plantlets, intermittent misting was better than growing them under tents. Rajmohan (1985) reported 55 to 60 percent survival of *in vitro* produced jack plantlets when the humidity is maintained at 90 to 100 percent using plastic sheets. Nobuoka *et. al.* (1996) opined that transpiration rate of tomato increased with decrease in relative humidity and increased light intensity. Shimizu *et. al.* (1997) found that low relative humidity decreased the leaf area and relative growth rate (RGR) of wheat and increased the transpiration rate.

*al.* 1990). Salisberry and Ross (1992) reported that light is an important factor for leaf expansion.

### 2.6.2 Effect on physiological characters

Donnelly and Vidaver (1984), found that in red raspberry, the pigment contents (chlorophyll a, chlorophyll b and carotenoids on fresh weight basis) were significantly higher in the newly produced leaves of *in vitro* cultured plantlets than the leaves formed *in vitro*. Das and Mukherji (1995) reported that chlorophyll and carotenoid contents of light and dark grown seedlings of *Vigna radiata* increased sharply at 40°C and 50°C, but there was a decline in the carotenoid level at 60°C. Shaheen *et. al.* (1995) observed a reduction in fresh weight, dry weight and total chlorophyll content at higher shade level. Fukuoka *et. al.* (1996) conducted a study on the effect of shading in cabbage seedlings on their physiological processes and rooting ability after transplanting. They observed that the rate of photosynthesis and root respiration declined in shaded plants. Galyuon *et. al.* (1996) observed a reduction in total leaf area, dry matter per plant, NAR and RGR of cocoa under full sun light, but leaf thickness, specific leaf weight and stomatal density was increased. However RGR remained unaffected at different irradiance levels. Leonardi (1996) studied the effect of shading on green house grown pepper and observed that shading reduced chlorophyll content, photosynthetic rate and transpiration rate. Swapna (1996), reported that with decreasing intensity of

shade there is a decrease in chlorophyll content of *Philodendron* 'Wendlandi' when the effect of shade alone is considered. Park *et. al.* (1997) studied the effect of light acclimatization on photosynthetic activity of foliage plants and observed that plants under 80 percent shade showed high photosynthetic rate.

In the case of cauliflower and strawberry (Grout and Aston, 1978; Grout and Millam 1988), the leaves formed *in vitro* failed to develop photosynthetic activity during *ex vitro* condition and finally degenerated. The leaves formed *ex vitro* were photosynthetically comparable to that of field grown normal plants. George and Sherrington (1984), reported that *in vitro* grown plantlets have low photosynthetic rate and require an external carbon source. Clarkson (1986), reported that uptake of nitrate by *Dendrobium* plantlets was slow and it could be related to photosynthesis and growth of plantlets in the culture. Mary *et. al.* (1986) studied the anatomical and physiological characters of micro cultured seedlings and green house grown Asian white birch plants and observed that the photosynthetic ability of the leaves formed *in vitro* was only one third of that of the leaves of green house grown plants. Photosynthetic rate of *in vitro* plantlets were low due to structural difference in the leaves, feedback inhibition from sugar in the medium, lack of $CO_2$ in the vessels and restricted development of components of the photosynthetic apparatus, which in turn affects the plant quality (Dube and Vidaver, 1987). Wainwright and Scrace (1989) observed that sucrose level should be maintained at the level of 30.0 g/l or even more prior to

acclimatization of *Ficus lyrata* to maximise plant quality. It is because acclimatization environment may not allow occurrence of appreciable amount of photosynthesis. Arne *et. al.* (1995) reported that the survival of birch plants during acclimatization to green house and field conditions depend on transpiration and carbohydrate supply from the leaves.

## 2.7 Biometric characteristics

Yoshida (1972) found that 70 percent shade was optimum for anthurium plantlets resulting in an increase of photosynthetic rate with increase in leaf area index. Lalithabhai (1981) reported that the plant height of anthurium was increased with increasing shade. Lim *et. al.* (1992) reported that in *Dendrobium* the fresh weight as well as relative growth rate of *in vitro* plantlets increased with the presence of sugar in the rooting medium. Pre conditioning of *Ficus* plantlets with 2 to 4 percent sucrose recorded maximum shoot length, fresh weight and dry weight of the above (Wainright and Scrace, 1989). Midmore and Prange (1992), reported a reduction in the growth of potato plants at higher temperature which was due to the reduction in leaf area and NAR.

## 2.8 Anatomical characteristics of tissue cultured plantlets

Epicuticular wax has been found to be either reduced or absent in the *in vitro* formed leaves of cabbage and carnation, which leads to higher rate of water loss (Grout, 1975; Sutter and Langhani, 1979). The density of wax deposition was found to be increasing during acclimatization of aseptically cultured plantlets (Wardle *et. al.* 1983). Dhawan and Bhojwani (1987) observed that during the shoot multiplication stage, leaves of *Leucaena leucocephala* had only amorphous wax and structural wax appeared during *in vitro* rooting and increased in quantity during *ex vitro* establishment.

Donnelly *et. al.*(1985) in silvan blackberry and Donnelly and Skelton (1989) in rose examined the hydathode anatomy. They found that the apex and marginal serration of the leaves of *in vitro* and green house grown rose plants had terminal hydathode regions, composed of scattered, primary, adaxial group of sunken water pores. Water pores and stomata of *in vitro* leaves of rose plants were open with large apertures while those of the green house grown plants were closed with small apertures.

Wetzstein and Sommer, (1983) studied the surface morphology of *in vitro* cultured *Liquidambar styraciflua* leaves and field grown leaves. They observed that the *in vitro* leaves had superficial circular stomata and irregular epidermal cells. In the acclimatized leaves, the stomata are ellipsoid and

depressed but the epidermal cells were irregular. In the field grown leaves, the epidermal cells were well defined and iso-diametric in shape. The shape of stomata was found to be similar as in the above case. Fabbri *et. al.* (1986) reported that persistent leaves of tissue cultured strawberry plantlets formed during acclimatization exhibits anatomical characters intermediate between that of *in vitro* formed leaves and the leaves of green house grown plants. Mary *et. al.*(1986) observed that the reduced stature of micro propagated shoots of Asian white birch plants were due to reduced cell division. Reuther (1988) observed in *Zier pflanzenbau* that the roots formed *in vitro* had no root hairs.

Brainerd and Fuchigami (1981) observed that stomata of tissue cultured apple plantlets regained their normal responsiveness to water stress, only after several days of exposure to low relative humidity. They also reported that apple root stock lost 50 percent of the total leaf water content at the *in vitro* condition. This rate was three times faster than that of the excised green house grown leaves. Marin *et. al.* (1988) reported that the nonfunctional state of the stomata in the persistent leaves of cherry were reversible to certain extent. The shape of stomata in persistent leaves changed from round to normal elliptical during acclimatization.

Grout and Aston (1978), reported that the mesophyll cells and palisade cells were found to be limited in the *in vitro* grown leaves of cauliflower than that of the field grown leaves. Fabbri *et. al.* (1986) observed that the *in vitro*

leaves of micro propagated strawberry plantlets lack well differentiated palisade parenchyma and spongy parenchyma whereas they are having large inter cellular space resulting in occurrence of vacuoles in the cells. Chloroplast was flattened and irregularly arranged in this case. Donnelly and Vidaver (1984) reported in red raspberry that palisade cells to epidermal cells were lesser in the *in vitro* plantlets than the field grown plants. They also found that transplanting shock in red raspberry had been attributed to desiccation and wilting which is due to poor epicuticular and cuticular wax formation, reduced stomatal control and lower stomatal distribution as compared to green house plants. Donnelly *et. al.* (1985) observed that the roots of *in vitro* cultured red raspberry plantlets had less periderm than the hardened plants, whereas the roots of the field grown plants had multi layered periderm. Sutter (1988), studied stomatal and cuticular water loss during acclimatization in apple, cherry and sweet gum plantlets. In the acclimatized plantlets, stomatal conductance of persistent leaves decreased to about half of that in the *in vitro* leaves while cuticular conductance remained the same. He concluded that increased stomatal closure reduced the conductance.

Blanke and Belcher (1989), showed that small humidity gradient between the inter cellular leaf space and the saturated atmosphere was responsible for the poor development of morphological stature of leaves and lack of stomatal functioning in tissue cultured apple, which in turn resulted in high mortality of plantlets after transfer to the *ex vitro* conditions. The

capability of *in vitro* stomata to adapt to the new environmental conditions by modifying guard cells during acclimatization enlighten the role of stomata in the death of micro propagated *Prunus cerasus* plants after the transfer to external environment (Marin *et. al.* 1988). Isiah and Rao (1992), studied the anatomy of *Dendrobium* and reported that the leaves were hypo stomatal with para cyclic stomata, they possessed large and compound marginal vascular bundles. The epidermal cells were polygonal covered by a thick cuticle which was comparatively larger on the adaxial surface than the abaxial surface. The stomata were paracytic and with prominently pointed outer edges. They were present only on the abaxial leaf surface. The mesophyll comprises of polygonal to isodiametric cells and are differentiated into palisade cells and spongy parenchyma.

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

The present investigation on "Physiological aspects of *ex vitro* establishment of tissue cultured orchid (*Dendrobium* hybrid, Sonia - 17) plantlets" was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani. during the year 1997 to 1999. A brief account of materials used and methodologies followed in this study are presented below. The experiment consisted of *in vitro* trials and *ex vitro* trials.

## 3.1 Materials Utilized :

The orchid cultivar chosen for the experiment was *Dendrobium* hybrid viz. Sonia - 17, a commercially important variety of Kerala. Previously meristem cultured plantlets at four leaf stage before rooting (Plate 1) comprised the experimental material. These plantlets were produced through shoot meristem culture based on the following protocol. Explants ie. shoot apices were excised properly and surface sterilised. After that they were inoculated in Vacin and Went (1949) medium with NAA 1.5 mg/l and BA 1.0 mg/l. Half MS medium (Murashige and Skoog, 1962) was used for shoot proliferation. Plantlets regenerated after 3-4 subculture were used for the study.

**Plate 1. Meristem cultured plantlets before rooting.**

## 3.2 Methods :

### 3.2.1 Design of the experiment :

The experiment was laid out in CRD with ten replications with a single plant per treatment.

### 3.2.2 Rooting *in vitro*

Trials on the *in vitro* rooting of *Dendrobium* hybrid, Sonia 17 were conducted in half MS medium containing IBA 10 ml/l, 2,4 - D 2.5 ml/l, 100 mg/l coconut water 150 ml/l and agar 6g/l. Individual shoots measuring 2 - 3 cm length excised from the shoot proliferating cultures were used as the explants.

#### 3.2.2.1 Sucrose

Studies were conducted to find out the effect of sucrose on the *in vitro* rooting.

Different levels of sucrose tried were given below.

$S_1$ - 20.0 g/l (Control)

$S_2$ - 40.0 g/l

$S_3$ - 60.0 g/l

Observations were recorded on number of days taken for root initiation, roots per shoot, length of roots and survival percent at 45 days after rooting. The best medium identified on these aspects was used for further study.

#### 3.2.2.2 Intensity of light during incubation

The cultures were kept under dark for a period of thirty days for rapid root initiation and then kept at a constant light intensity of 3000 lux for a period of two weeks. The light intensity was provided *in vitro* by fluorescent tubes and lux meter was used to measure the light intensity.

### 3.2.3 *Ex vitro* establishment

The culture vessels were opened and plantlets were taken out using sterilized forceps. The agar adhering to the roots were completely removed by thorough washing. For this first the plantlets were kept under the flow of running tap water and then kept in a beaker of distilled water, so that agar was completely removed. During all these process, care was taken for not damaging the roots. The plantlets were subjected to fungicide treatment in 0.2

**Plate 2. a. Coconut husk balls, the potting media used.**

**b. Sonia-17 plantlets in the coconut husk balls at 45 days after planting out**

per cent indofil (Dithane M-45) by dipping in it for a period of twenty minutes. These plants were then planted directly into coconut husk balls. The coconut fibres were autoclaved for forty five minutes before being used for planting. The fibres were then subjected to a dip on 0.2 per cent bavistin and water was squeezed off to remove excess fungicide, leaving it just moist. The coconut fibre ball was made by tying fibres at the centre(Plate 2). Rooted plantlet was placed on the top of the ball and the roots were spread on it.

#### 3.2.3.1 Triazole

Before placing the rooted plantlets on to the husk ball, they were subjected to a dip of thirty minutes in different concentrations of triazole. The different concentrations of triazole tried were given below.

$T_1$ - 5ppm

$T_2$ - 10ppm

$T_3$ - 0(Control)

Observations were recorded on different physiological, morphological, biochemical and biometric characters at fifteen days interval and the best level identified was used for further study.

### 3.2.3.2 Effect of light intensity and humidity on *ex vitro* establishment

Studies were conducted on the effect of light intensity and humidity on *ex vitro* establishment. The plantlets were grown in four different levels of light intensity. They were as follows.

$L_1$ - 25 per cent

$L_2$ - 50 per cent

$L_3$ - 75 per cent

$L_4$ - 100 per cent (Control)

The three different levels of light intensity were provided by using different shade nets and the control plants were maintained at normal light conditions.

The different levels of humidity treatments were also given simultaneously along with light treatment.

The details of treatment were given below.

$H_1$ - below 70 per cent (Control)

$H_2$ - 70 - 90 per cent

For maintaining two levels of humidity two chambers with polythene sheet were constructed within the shade nets(Plate 3). By adjusting misting the

**Plate 3. Hardening chamber for maintaining light and humidity**

required humidity was maintained. In the 90 per cent humidity chamber the plots were placed in a tray with water so as to maintain the high level of humidity. By using hygrometer humidity was monitored throughout the day. Observations were recorded at fifteen days interval on physiological, morphological, biochemical and biometric characters. The data on meteorological parameters of the tropical climate prevailed viz. minimum and maximum temperature, relative humidity, evaporation and rainfall collected during the *ex vitro* establishment of the orchid plantlets is furnished in appendix I.

#### 3.2.3.3 After care of plantlets

After 15 days of planting out the plants were irrigated twice a week with a ten times distilled solution of *in vitro* rooting medium that was used. Indofil 0.2 percent was sprayed twice weekly during the first few weeks of planting out and later at weekly intervals.

### 3.3 Observations

Observations on physiological, morphological, biochemical, anatomical and biometric characters were taken at 15 day intervals.

### 3.3.1 Physiological characters

#### 3.3.1.1 Crop growth rate (C. G. R)

The CGR was worked out by using the formula of Watson (1971) and expressed in mg $cm^2$ day $^{-1}$

$$CGR = \frac{W_2 - W_1}{p(t_2 - t_1)}$$

Where $W_1$, $W_2$ - Whole plant dry weight at $t_1$ and $t_2$ respectively.

$t_1$ , $t_2$ - Time in days.

p - Ground area on which $W_1$ and $W_2$ was estimated.

#### 3.3.1.2 Net assimilation rate (N.A.R)

The method proposed by Gregory (1917) and modified by Williams (1946) was employed for calculating the NAR on leaf dry weight basis and the values were expressed in mg $cm^{-2}$ $day^{-1}$

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e L_2 - \log_e L_1}{L_2 - L_1}$$

Where $W_1$ and $W_2$ - Dry weights of whole plant at $t_1$ and $t_2$ respectively

$L_1$ and $L_2$ - Dry weight of leaf at $t_1$ and $t_2$ respectively

$t_1$ and $t_2$ - Time in days

#### 3.3.1.3 Relative growth rate (RGR)

The RGR was determined by utilizing the following formula (Williams, 1946) and expressed in mg $g^{-1}$ $day^{-1}$

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

$W_1$, $W_2$ - Plant dry weight at $t_1$ and $t_2$ respectively

$t_1$, $t_2$ - Time in days

#### 3.3.1.4 Photosynthetic rate

It was measured directly by using LCA-4 (Leaf Chamber Analyser or portable $CO_2$ analyser) manufactured by Analytical Development Co. Ltd., U.K. The values were expressed in $\mu$ mol $m^{-2}s^{-1}$

#### 3.3.1.5 Transpiration rate

Transpiration rate was measured directly by using the portable $CO_2$ analyser or leaf chamber analyzer or LCA-4, manufactured by Analytical Development Co. U.K. The values were expressed in $\mu$ mol $m^{-2}s^{-1}$

### 3.3.2 Morphological characters

#### 3.3.2.1 Height of the plant :

This was measured from the collar region to the tip and the mean length was expressed in millimetre. The observations were made at 15 days interval.

#### 3.3.2.2 Number of leaves :

The total number of fully opened leaves developed per plantlet was counted and the mean value was expressed.

#### 3.3.2.3 Number of roots :

Total number of roots per plantlet was counted and the mean value was recorded at 15 days interval.

#### 3.3.2.4 Survival percentage

In order to measure the per cent mortality the survival per cent of the cultures and the hardened plant were noted at 15 days intervals.

### 3.3.3 Biochemical characters

#### 3.3.3.1 Chlorophyll content (Chlorophyll a, Chlorophyll b, total Chlorophyll and carotenoids) of leaves

Photosynthetic pigments namely chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were estimated by the following method described by Arnon (1949).

**Procedure**

A representative sample of 100 mg of leaf tissue was weighed and ground with 10 ml of 80 per cent acetone using a pestle and mortar. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatent was collected and was made up to 10 ml with 80 per cent acetone. The optical density (O.D.) value of the extract was measured at 663, 645 and 480 mμ using 80 per cent acetone as the blank in spectrophotometer (Systsonics UV-VIS spectrophotometer 118). The amount of the pigments were calculated using the following formulae and expressed as mg of pigments $g^{-1}$ of fresh leaf.

Total chlorophyll content

$$= \frac{20.2(\text{OD at } 645) + 8.01(\text{OD at } 663) X V}{w \times 1000} \quad \text{mg g}^{-1}$$

Chlorophyll a

$$= \frac{12.7(\text{O.D. at } 663) - 2.69(\text{O.D. at } 645) X V}{w \times 1000} \quad \text{mg g}^{-1}$$

Chlorophyll b

$$= \frac{22.9(\text{O.D. at } 645) - 4.68(\text{O.D. at } 663) X V}{w \times 1000} \quad \text{mg g}^{-1}$$

Carotenoids

$$= \frac{7.6(\text{O.D. at } 480) - 1.49(\text{O.D. at } 510) X V}{w \times 1000} \quad \text{mg g}^{-1}$$

### 3.3.3.2 Estimation of protein

Protein content of leaves was estimated by using the following method developed by Lowery *et al.* (1951).

**Procedure**

Leaf samples of 500 mg each were weighed and ground well with a pestle and mortar in 5-10 ml of the phosphate buffer and centrifuged at 5000 rpm for 15 minutes. From the supernatent 1 ml of aliquot was taken and 5 ml of alkaline copper solution was added to each of the samples including the blank. After 10 minutes 0.5 ml of Folin - ciocalteaus reagent was added and incubated at room temperature in dark for 30 minutes. Blue colour developed was measured at 660 nm in a spectrophotometer (Systsonics UV-VIS spectrophotometer 118). The amount of protein present in the sample was found out by using standard graph prepared by using bovine serum albumin (fraction V). The amount of protein was expressed as micro gram albumin equivalent of soluble protein per gram on fresh weight basis.

### 3.3.3.3 Estimation of total carbohydrate

Total carbohydrate was estimated by Anthrone method (Hedge and Hofreiter, 1962). Leaf samples of 100 mg each were weighed out and hydrolysed with 5 ml of 2.5 N hydrochloric acid (HCl at 100°C in a water bath.

The hydrolysate was neutralised with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 5000 rpm for 15 minutes. From the supernatent 0.5 ml aliquot was taken and made up to 1 ml by adding distilled water. To this 4 ml of anthrone reagent was added and heated for 8 minutes at 100°C in a water bath. This was cooled rapidly and absorbance was measured at 630nm in a spectrophotometer (Systsonics UV-VIS spectrophotometer 118). Amount of carbohydrate present was calculated from standard graph using glucose and expressed in terms of mg of glucose equivalent per gram of leaf tissue on fresh weight basis.

### 3.3.4 Biometric Characters

#### 3.3.4.1 Leaf area

This was measured by using graph paper method. Area of leaf lamina expressed in sq. cm. per plant or unit area.

#### 3.3.4.2 Root length

Length of root from the collar region to the tip was measured and the mean was expressed in mm.

#### 3.3.4.3 Root: shoot ratio

This was calculated by the formula,

$$\text{Root: shoot ratio} = \frac{\text{Root Length.}}{\text{Shoot length}}$$

### 3.3.4.4 Total fresh weight and dry weight.

These parameters were taken at 15 days interval for each treatment. Fresh weight of individual plants were recorded. These plants were dried at 70°C for 48 hours and the dry weight was recorded after that.

### 3.3.5 Anatomical Characters

### 3.3.5.1 Stomatal structure and size

Stomata were observed using a 40x objective and 10x eye piece. The length and width of open stomata were measured using micrometer. The observation was recorded for tissue cultured plantlets, hardened plantlets and normal plantlets grown in green house.

### 3.3.5.2 Stomatal count :

A comparative study was made on the stomatal count of the leaves of *in vitro* plantlets, hardened plantlets and normal plants at 45 days after planting out. Leaf imprints were prepared for the purpose using "Quick fix". "Quick fix" was uniformly applied on the surface of the leaf segments and after five minutes, the dry green membrane was carefully peeled off and mounted on a microscope slide with a drop of water. The stomata were observed and counted using a 40x objective and 10x eye piece. The field of the microscope was

measured using a stage micrometer and the stomatal count per unit area was taken (Dhopte and Livera, 1989).

#### 3.3.5.3 Cuticle thickness

Cuticle thickness was observed using 40x objective and 10x eye piece and measured using micrometer. The observations were made in all three types of leaves ie. tissue cultured, hardened and normal plants.

#### 3.3.5.4 Number of mesophyll layers and type of cells

Number of mesophyll layers were counted using compound microscope with 40x objective and 10x eye piece and observations were recorded for the leaves of tissue cultured, hardened and normal plants.

# RESULTS

# 4. RESULTS

The physiological, morphological, biochemical, anatomical and biometric changes taking place during the different treatments of *in vitro* propagation and *ex vitro* establishment of tissue cultured plantlets of orchids were compared with Normal green house grown plants and the results are presented below.

## 4.1 PHYSIOLOGICAL CHARACTERISTICS :

### 4.1.1 Crop Growth Rate (CGR) mg $cm^{-2}day^{-1}$

The data on the effect of sucrose concentration of rooting medium on CGR of orchid plantlets at 45 days of *in vitro* rooting is presented in Table 1.a. Analysis of the data revealed the following results. Normal plants recorded the maximum value (0.1211) of CGR and was on par with $S_2$ (0.1038). $S_3$ recorded the minimum value (0.0443).

Table 1.b represents the effect of triazole on CGR. At $P_1$ there was significant variation among the treatments. $T_1$ (0.1761) recorded the higher value of CGR than $T_3$ (0.1162) ie, control. At $P_1$, $P_2$ and $P_3$ the mean value of $T_1$ was higher (0.1962) than $T_3$ (0.1227). The CGR of Normal plants (0.1850) was found to be highly significant when compared to tissue cultured plantlets during the initial period viz $P_1$. However during the later periods viz $P_2$ and $P_3$ the CGR of $T_1$ was on par with Normal plants.

The plantlets were subjected to different treatments of light in combination with various humidity levels. The mean value of different treatments indicates that $L_2H_2$ (0.2828) recorded the maximum value of CGR and $L_4H_1$ (0.1590) the minimum during all the three periods of study. The data presented in the Table

**Table 1a.** Effect of sucrose in the rooting medium on CGR of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | CGR (mg $cm^{-2}$ $day^{-1}$) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.0904 |
| $S_2$ | 40 | 0.1038 |
| $S_3$ | 60 | 0.0443 |
| Normal ($N_1$) | Green house grown | 0.1211 |
| Mean | | 0.0899 |
| C.D. (5%) | | 0.0208 |

**Table 1b.** Effect of triazole treatment at planting out on CGR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | CGR (mg $cm^{-2}$ $day^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $T_1$ | 5 | 0.1761 | 0.2016 | 0.2108 | 0.1962 |
| $T_2$ | 10 | 0.1254 | 0.1576 | 0.1163 | 0.1331 |
| $T_3$ | 0 (Control) | 0.1162 | 0.1152 | 0.1367 | 0.1227 |
| Normal ($N_2$) | Green house grown | 0.1850 | 0.2093 | 0.2197 | 0.2046 |
| Mean | | 0.1507 | 0.1710 | 0.1709 | 0.1642 |
| CD (1%) | | 0.002 | 0.013 | 0.009 | |

**Table 1c.** Effect of light and humidity treatment (in the hardening chamber) on CGR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | CGR ($mg\ cm^{-2}\ day^{-1}$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $L_1H_1$ | 25 | < 70 | 0.0834 | 0.2524 | 0.3470 | 0.2276 |
| $L_1H_2$ | 25 | 70 – 90 | 0.1278 | 0.1863 | 0.3523 | 0.2221 |
| $L_2H_1$ | 50 | < 70 | 0.0863 | 0.1834 | 0.2854 | 0.1850 |
| $L_2H_2$ | 50 | 70 – 90 | 0.2128 | 0.2552 | 0.3805 | 0.2828 |
| $L_3H_1$ | 75 | < 70 | 0.0979 | 0.2467 | 0.3417 | 0.2288 |
| $L_3H_2$ | 75 | 70 – 90 | 0.1919 | 0.1889 | 0.2848 | 0.2219 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.0739 | 0.1739 | 0.2291 | 0.1590 |
| $L_4H_2$ | 100 | 70 – 90 | 0.1467 | 0.2128 | 0.2854 | 0.2149 |
| Normal ($N_3$) | | | 0.2296 | 0.2871 | 0.3636 | 0.2513 |
| Mean | | | 0.1389 | 0.2207 | 0.3035 | 0.2215 |
| CD (1%) | | | 0.007 | 0.041 | 0.033 | |

1.c reveals that during the period $P_1$ there was significant difference between tissue cultured plantlets and Normal plants. But at $P_2$ the value of $L_2H_2$ (0.2552) was on par with Normal plantlets (0.2871). However towards the last stage viz. $P_3$, CGR of $L_2H_2$ (0.3805) was higher than that of Normal plants (0.3636).

### 4.1.2 Net Assimilation Rate (NAR) (mg cm$^{-2}$ day$^{-1}$)

The data presented in Table 2.a represents the effect of sucrose on NAR of orchid plantlets at 45 days of *in vitro* rooting. It was observed that NAR of Normal plants was much higher (0.0395) than tissue cultured plantlets. Among tissue cultured plantlets $S_2$ recorded the highest value (0.0313). However $S_1$ (0.0185) was also found to be on par with $S_2$ and the lowest value (0.0160) was recorded by $S_3$.

With regard to the plantlets which were subjected to different triazole treatments (Table 2.b) during planting out, it was found that there is no significant difference among treatments at the earlier stages $P_1$ and $P_2$. However in the later stage viz. $P_3$, significant difference was noted and the Normal plants recorded the maximum value (0.0244) and was on par with $T_1$ (0.0225). The minimum value was registered by $T_3$ (0.0165).

The NAR of the plantlets subjected to various light treatments in combination with different humidity levels is presented in Table 2.c. Analysis of the data revealed statistical significance at all levels. Among the treatments $L_3H_1$ (0.0651) recorded the maximum value of NAR at all the three periods and was on par with Normal plants.

**Table 2a.** Effect of sucrose in the rooting medium on NAR of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | NAR (mg $cm^{-2}$ $day^{-1}$) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.0185 |
| $S_2$ | 40 | 0.0313 |
| $S_3$ | 60 | 0.0160 |
| Normal ($N_1$) | Green house grown | 0.0395 |
| Mean | | 0.0263 |
| C.D. (5%) | | 0.0150 |

**Table 2b.** Effect of triazole treatment at planting out on NAR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | NAR (mg $cm^{-2}$ $day^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $T_1$ | 5 | 0.0159 | 0.0138 | 0.0225 | 0.0174 |
| $T_2$ | 10 | 0.0144 | 0.0128 | 0.0188 | 0.0153 |
| $T_3$ | 0 (Control) | 0.0137 | 0.0123 | 0.0165 | 0.0142 |
| Normal ($N_2$) | Green house grown | 0.0200 | 0.0146 | 0.0244 | 0.0148 |
| Mean | | 0.0160 | 0.0134 | 0.0206 | 0.0154 |
| CD (1%) | | ns | ns | 0.003 | |

ns : Not significant

**Table 2c.** Effect of light and humidity treatment (in the hardening chamber) on NAR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | NAR (mg cm$^{-2}$ day$^{-1}$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $L_1H_1$ | 25 | < 70 | 0.0454 | 0.0517 | 0.0627 | 0.0533 |
| $L_1H_2$ | 25 | 70 – 90 | 0.0577 | 0.0662 | 0.0742 | 0.0660 |
| $L_2H_1$ | 50 | < 70 | 0.0468 | 0.0574 | 0.0668 | 0.0570 |
| $L_2H_2$ | 50 | 70 – 90 | 0.0414 | 0.0538 | 0.0638 | 0.0530 |
| $L_3H_1$ | 75 | < 70 | 0.0651 | 0.0741 | 0.0791 | 0.0728 |
| $L_3H_2$ | 75 | 70 – 90 | 0.0559 | 0.0629 | 0.0702 | 0.0630 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.0413 | 0.0495 | 0.0595 | 0.0501 |
| $L_4H_2$ | 100 | 70 – 90 | 0.0508 | 0.0556 | 0.0684 | 0.0583 |
| Normal ($N_3$) | | | 0.0661 | 0.0625 | 0.0752 | 0.0679 |
| Mean | | | 0.0523 | 0.0593 | 0.0689 | 0.0602 |
| CD (5%) | | | 0.007 | 0.003 | 0.006 | |

### 4.1.3 Relative Growth Rate (RGR) (mg $g^{-1}$ $day^{-1}$)

Among the three levels of sucrose tried out, $S_2$ (0.9149) registered highest RGR on forty fifth day of rooting and it was found to be on par with $S_1$ (0.8673). The Normal plants recorded higher value of RGR (0.9833) compared to that of tissue cultured plantlets (Table 3a).

No significant difference in RGR was noticed among plantlets subjected to different triazole treatment at $P_1$. However in the later stages viz. $P_2$ and $P_3$ there was distinct variation among the treatments (Table 3b). The mean value indicates that the RGR of Normal plants (1.6922) were higher than that of tissue cultured plantlets during all the three periods and among the tissue cultured plantlets $T_1$ performed better than $T_2$ and $T_3$.

Among the different light regimes and humidity levels tried out no significant difference in RGR was noticed during $P_1$ and $P_2$. However during $P_3$, $L_2H_2$ recorded the maximum value (1.1720) of RGR and $L_4H_1$ the minimum (1.1114). At $P_3$ the RGR of Normal plants (2.1147) were found to be distinctively higher than all the tissue cultured plantlets, under different light and humidity treatments (Table 3c).

### 4.1.4 Photosynthetic rate ($\mu$ mol $m^{-2}s^{-1}$)

Analysis of data on photosynthetic rate influenced by different levels of sucrose (Table 4.a) indicates that $S_2$ (0.370) recorded the maximum value and $S_3$ (0.340) the minimum. The photosynthetic rate recorded by Normal plants (0.645) were significantly higher than that of tissue cultured plantlets.

With regard to the plantlets which were subjected to different triazole treatment during planting out, the observations recorded are shown in the Table 4b. The

**Table 3a.** Effect of sucrose in the rooting medium on RGR of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | RGR (g $g^{-1}$ $day^{-1}$) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.8673 |
| $S_2$ | 40 | 0.9149 |
| $S_3$ | 60 | 0.7640 |
| Normal ($N_1$) | Green house grown | 0.9833 |
| Mean | | 0.8824 |
| C.D. (1%) | | 0.0600 |

**Table 3b.** Effect of triazole treatment at planting out on RGR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | RGR (g $g^{-1}$ $day^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $T_1$ | 5 | 1.5150 | 1.4151 | 1.6707 | 1.5336 |
| $T_2$ | 10 | 1.3129 | 1.2058 | 1.4193 | 1.3127 |
| $T_3$ | 0 (Control) | 1.2239 | 1.0816 | 1.2898 | 1.1984 |
| Normal ($N_2$) | Green house grown | 1.6730 | 1.6137 | 1.7899 | 1.6922 |
| Mean | | 1.4312 | 1.3291 | 1.5424 | 1.4342 |
| CD (1%) | | 0.050 | ns | 0.032 | |

ns : Not significant

**Table 3c.** Effect of light and humidity treatment (in the hardening chamber) on RGR of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | RGR ($g\ g^{-1}\ day^{-1}$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 1 – 15 DAP ($P_1$) | 15 – 30 DAP ($P_2$) | 30 – 45 DAP ($P_3$) | |
| $L_1H_1$ | 25 | < 70 | 1.1015 | 1.1067 | 1.1236 | 1.1106 |
| $L_1H_2$ | 25 | 70 – 90 | 1.1135 | 1.1155 | 1.1226 | 1.1172 |
| $L_2H_1$ | 50 | < 70 | 1.1445 | 1.1519 | 1.1576 | 1.1513 |
| $L_2H_2$ | 50 | 70 – 90 | 1.1628 | 1.1698 | 1.1720 | 1.1682 |
| $L_3H_1$ | 75 | < 70 | 1.1358 | 1.1420 | 1.1453 | 1.1410 |
| $L_3H_2$ | 75 | 70 – 90 | 1.1538 | 1.1585 | 1.1626 | 1.1583 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 1.1120 | 1.1175 | 1.1114 | 1.1193 |
| $L_4H_2$ | 100 | 70 – 90 | 1.1278 | 1.1306 | 1.1382 | 1.1322 |
| Normal ($N_3$) | | | 1.1941 | 2.1007 | 2.1147 | 1.8032 |
| Mean | | | 1.1384 | 1.2437 | 1.1387 | 1.2113 |
| CD (1%) | | | ns | ns | 0.262 | |

ns : Not significant

**Table 4a.** Effect of sucrose in the rooting medium on photosynthetic rate of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Photosynthetic rate ($\mu$ mol m$^{-2}$ s$^{-1}$) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.365 |
| $S_2$ | 40 | 0.370 |
| $S_3$ | 60 | 0.340 |
| Normal ($N_1$) | Green house grown | 0.645 |
| Mean | | 0.430 |
| C.D. (5%) | | 0.229 |

**Table 4b.** Effect of triazole treatment at planting out on photosynthetic rate of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Photosynthetic rate ($\mu$ mol m$^{-2}$ s$^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.370 | 0.612 | 0.912 | 0.631 |
| $T_2$ | 10 | 0.230 | 0.562 | 0.707 | 0.500 |
| $T_3$ | 0 (Control) | 0.183 | 0.508 | 0.629 | 0.440 |
| Normal ($N_2$) | Green house grown | 1.120 | 1.185 | 1.290 | 1.198 |
| Mean | | 0.4758 | 0.717 | 0.885 | 0.692 |
| CD (1%) | | 0.191 | 0.143 | 0.139 | |

**Table 4c.** Effect of light and humidity treatment (in the hardening chamber) on photosynthetic rate of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Photosynthetic rate (μ mol $m^{-2}$ $s^{-1}$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.120 | 0.123 | 0.197 | 0.147 |
| $L_1H_2$ | 25 | 70 – 90 | 0.196 | 0.213 | 0.263 | 0.224 |
| $L_2H_1$ | 50 | < 70 | 0.277 | 0.327 | 0.387 | 0.330 |
| $L_2H_2$ | 50 | 70 – 90 | 0.400 | 0.453 | 0.477 | 0.443 |
| $L_3H_1$ | 75 | < 70 | 0.263 | 0.290 | 0.323 | 0.292 |
| $L_3H_2$ | 75 | 70 – 90 | 0.330 | 0.380 | 0.400 | 0.370 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.140 | 0.170 | 0.213 | 0.174 |
| $L_4H_2$ | 100 | 70 – 90 | 0.267 | 0.307 | 0.340 | 0.305 |
| Normal ($N_3$) | | | 0.467 | 0.490 | 0.527 | 0.495 |
| Mean | | | 0.273 | 0.306 | 0.347 | 0.308 |
| CD (5%) | | | ns | ns | 0.01 | |

mean values indicate that $T_1$ (0.631) recorded higher photosynthetic rate than $T_2$ (0.500) and $T_3$ (0.440) at all the three stages. Invariably the Normal grown plants (1.198) recorded significantly higher photosynthetic rate than tissue cultured plantlets in all cases.

The plantlets were subjected to different light treatment in combination with various humidity levels (Table 4c) and the results reveal that no significant difference in photosynthetic rate was found to exist among treatments at 15 DAP and 30 DAP. However at 45 DAP the treatment $L_2H_2$ (0.477) recorded the maximum photosynthetic rate. The mean values indicated that the plantlets under the treatment $L_2H_2$ showed higher photosynthetic rate (0.443) during all the three observations (viz 15 DAP, 30 DAP and 45 DAP). There was much variation in photosynthetic rate between Normal and tissue cultured plantlets during the earlier periods (at 15 DAP and 30 DAP). But towards the later period of plant growth the Normal grown plants as well as the tissue cultured plantlets which were subjected to the treatment $L_2H_2$ were found to exhibit higher photosynthetic rate compared to all other treatments.

### 4.1.5 Transpiration Rate (mol $m^{-2}s^{-1}$)

The data on the effect of sucrose on transpiration rate is presented in Table 5a. $S_3$ (0.578) recorded higher value and is on par with $S_1$ (0.563) and $S_2$ (0.560). The Normal plants recorded relatively lesser value (0.325) than that of the tissue cultured plantlets.

With regard to triazole treatment (Table 5b) there was significant reduction in transpiration rate at $T_1$ (0.476) and was on par with Normal plantlets (0.450) during all the three periods of analysis (viz. 15 DAP, 30 DAP and 45 DAP). $T_3$ plantlets (control) registered higher transpiration rate (0.637) than all other treatments.

**Table 5a.** Effect of sucrose in the rooting medium on transpiration rate of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Transpiration rate (mol $m^{-2}$ $s^{-1}$) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.563 |
| $S_2$ | 40 | 0.560 |
| $S_3$ | 60 | 0.578 |
| Normal ($N_1$) | Green house grown | 0.325 |
| Mean | | 0.507 |
| C.D. (5%) | | 0.132 |

**Table 5b.** Effect of triazole treatment at planting out on transpiration rate of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Transpiration rate (mol $m^{-2}$ $s^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.480 | 0.470 | 0.478 | 0.476 |
| $T_2$ | 10 | 0.572 | 0.556 | 0.560 | 0.422 |
| $T_3$ | 0 (Control) | 0.630 | 0.630 | 0.650 | 0.637 |
| Normal ($N_2$) | Green house grown | 0.454 | 0.448 | 0.448 | 0.450 |
| Mean | | 0.534 | 0.526 | 0.534 | 0.496 |
| CD (5%) | | 0.048 | 0.060 | 0.054 | |

**Table 5c.** Effect of light and humidity treatment (in the hardening chamber) on transpiration rate of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Transpiration rate (mol $m^{-2}$ $s^{-1}$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.510 | 0.517 | 0.393 | 0.473 |
| $L_1H_2$ | 25 | 70 – 90 | 0.540 | 0.513 | 0.407 | 0.473 |
| $L_2H_1$ | 50 | < 70 | 0.490 | 0.487 | 0.377 | 0.451 |
| $L_2H_2$ | 50 | 70 – 90 | 0.530 | 0.507 | 0.393 | 0.477 |
| $L_3H_1$ | 75 | < 70 | 0.500 | 0.503 | 0.383 | 0.462 |
| $L_3H_2$ | 75 | 70 – 90 | 0.520 | 0.533 | 0.407 | 0.487 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.560 | 0.550 | 0.423 | 0.536 |
| $L_4H_2$ | 100 | 70 – 90 | 0.523 | 0.537 | 0.413 | 0.480 |
| Normal ($N_3$) | | | 0.420 | 0.403 | 0.410 | 0.411 |
| Mean | | | 0.510 | 0.506 | 0.401 | 0.472 |
| CD (5%) | | | 0.018 | 0.033 | 0.010 | |

The data on the effect of different light regimes and humidity levels (Table 5.c) reveal that at 15 DAP and 30 DAP the Normal plants registered relatively lesser transpiration rate than the tissue cultured plantlets. It was observed that at 45 DAP the treatment $L_2H_2$ was found to be significant as compared to other treatments and Normal plants.

## 4.2 MORPHOLOGICAL CHARACTERS

### 4.2.1 Plant height (cm)

The effect of sucrose on plant height of orchid plantlets at 45 days of rooting is presented in the Table 6a. $S_2$ (40 g/l.) exhibited higher value (2.85) of plant height and $S_3$ (60 g/l.) was on par (2.73) with it. Sucrose treatment improved plant height. The lowest value (2.13) was recorded by $S_1$(20 g/l.). However the Normal plants recorded significantly higher value of plant height (4.55) than that of the tissue cultured ones.

With regard to triazole treatment (Table 6.b) $T_3$ (control) plantlets (4.75) recorded maximum plant height and $T_2$ (4.10) the minimum. There is a decreasing trend of plant height with increasing concentration of triazole levels.

Table 6.c represents the effect of light and humidity on height of plantlets. The analysis of data indicates that at 15 DAP, the Normal plantlets recorded the highest value (5.00). Among the various treatments $L_2H_2$ (5.50) and $L_2H_1$ (5.20) recorded higher plant height and they were on par with each other. $L_4H_1$ (4.30) recorded the minimum value. Both $L_4H_1$ and $L_4H_2$ recorded the lowest value (5.30) at 30 DAP. However at 45 DAP $L_4H_1$ recorded the minimum plant height (5.31).

**Table 6a.** Effect of sucrose in the rooting medium on plant height of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Plant height (cm) |
|---|---|---|
| $S_1$ | 20 (Control) | 2.13 |
| $S_2$ | 40 | 2.85 |
| $S_3$ | 60 | 2.73 |
| Normal ($N_1$) | Green house grown | 4.55 |
| Mean | | 3.07 |
| C.D. (1%) | | 0.39 |

**Table 6b.** Effect of triazole treatment at planting out on plant height of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Plant height (cm) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 4.18 | 4.35 | 4.68 | 4.40 |
| $T_2$ | 10 | 3.80 | 4.10 | 4.40 | 4.10 |
| $T_3$ | 0 (Control) | 4.45 | 4.75 | 5.05 | 4.75 |
| Normal ($N_2$) | Green house grown | 5.35 | 6.05 | 6.73 | 6.04 |
| Mean | | 4.195 | 4.812 | 5.215 | 4.82 |
| CD (1%) | | 0.426 | 1.044 | 0.387 | |

**Table 6c.** Effect of light and humidity treatment (in the hardening chamber) on plant height of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Plant height (cm) 15 DAP | 30 DAP | 45 DAP | Mean |
|---|---|---|---|---|---|---|
| $L_1H_1$ | 25 | < 70 | 4.70 | 5.60 | 6.38 | 5.56 |
| $L_1H_2$ | 25 | 70 – 90 | 4.80 | 5.75 | 6.47 | 5.67 |
| $L_2H_1$ | 50 | < 70 | 5.20 | 5.90 | 6.73 | 5.94 |
| $L_2H_2$ | 50 | 70 – 90 | 5.50 | 6.70 | 7.53 | 6.58 |
| $L_3H_1$ | 75 | < 70 | 4.60 | 5.40 | 6.36 | 6.45 |
| $L_3H_2$ | 75 | 70 – 90 | 4.60 | 5.50 | 6.37 | 5.49 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 4.30 | 5.30 | 6.32 | 5.31 |
| $L_4H_2$ | 100 | 70 – 90 | 4.40 | 5.30 | 6.33 | 5.34 |
| Normal ($N_3$) | | | 7.50 | 7.90 | 8.83 | 7.88 |
| Mean | | | 5.07 | 5.93 | 6.70 | 6.02 |
| CD (5%) | | | 0.417 | 0.373 | 0.580 | |

### 4.2.2 Number of Leaves

The results on the effect of sucrose on the number of leaves of orchid plantlets at 45 days of in vitro rooting is presented in the Table 7a. Among the treatments $S_2$ recorded the maximum number of leaves and was on par with Normal plants (5.25). $S_1$ (2.50) recorded the minimum number of leaves.

With regard to triazole treatment, there was no significant influence on the number of leaves at 15 DAP and 45 DAP. However at 30 DAP there was distinctly higher number of leaves noted in the Normal plants (6.40) compared to the tissue cultured plantlets. Among tissue cultured plantlets $T_1$ produced more number of leaves (5.40) than $T_2$ (4.00) and $T_3$ (3.60). The effect of triazole treatments on the production of leaves are shown in Plate 4.

Table 7c represent the number of leaves of plantlets subjected to different levels of light and humidity. Analysis of observation indicates that at 15 DAP, there was no significant variation between the different treatments, excepting that $L_4H_1$ recorded a very low value (2.3). At 30 DAP and 45 DAP, $L_2H_2$ and $L_1H_2$ exhibited higher leaf number which were on par with Normal plants. The treatment $L_4H_1$ produced minimum number of leaves at 15 DAP (2.30) and at 45 DAP (3.60).

### 4.2.3 Number of Roots.

With regard to the plantlets under sucrose treatment (Table 8a) significant difference among treatments were noticed and $S_2$ (10.60) recorded higher value and the lowest value was recorded by $S_1$ (6.00). The influence of different sucrose concentrations in the rooting medium on the number of roots produced are shown in Plate 5. However the Normal plants (14.60) recorded distinctly higher number of roots than the tissue cultured plantlets.

**Table 7a.** Effect of sucrose in the rooting medium on number of leaves per shoot of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Number of leaves per shoot |
|---|---|---|
| $S_1$ | 20 (Control) | 2.50 |
| $S_2$ | 40 | 4.25 |
| $S_3$ | 60 | 3.00 |
| Normal ($N_1$) | Green house grown | 5.25 |
| Mean | | 3.75 |
| C.D. (5%) | | 1.13 |

**Table 7b.** Effect of triazole treatment at planting out on number of leaves per shoot of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole | Number of leaves per shoot | | | |
|---|---|---|---|---|---|
| | Levels (mg/l) | 15 DAP | 30 DAP | 45 DAP | Mean |
| $T_1$ | 5 | 3.80 | 5.40 | 5.90 | 5.03 |
| $T_2$ | 10 | 3.60 | 4.00 | 4.40 | 4.00 |
| $T_3$ | 0 (Control) | 3.60 | 3.80 | 4.40 | 3.93 |
| Normal ($N_2$) | Green house grown | 5.60 | 6.40 | 6.80 | 6.27 |
| Mean | | 4.15 | 4.65 | 5.38 | 4.81 |
| CD (5%) | | ns | 0.924 | ns | |

ns : Not significant

**Plate 4. Effect of triazole treatments on number of leaves produced at 30 days after planting out**

a. **Maximum number of leaves produced by plantlets under the treatment $T_1$ (5 mg/l)**

b. **Number of leaves produced by plantlets under the treatment $T_2$ (10 mg/l)**

c. **Minimum number of leaves produced by plantlets under the treatment $T_3$ (Control)**

**Table 7c.** Effect of light and humidity treatment (in the hardening chamber) on number of leaves per shoot of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Number of leaves per shoot | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 3.00 | 3.60 | 4.60 | 3.73 |
| $L_1H_2$ | 25 | 70 – 90 | 4.50 | 5.00 | 5.30 | 4.93 |
| $L_2H_1$ | 50 | < 70 | 2.50 | 4.00 | 4.60 | 3.70 |
| $L_2H_2$ | 50 | 70 – 90 | 4.00 | 5.30 | 6.03 | 5.11 |
| $L_3H_1$ | 75 | < 70 | 3.60 | 3.00 | 4.60 | 3.73 |
| $L_3H_2$ | 75 | 70 – 90 | 2.60 | 3.30 | 4.30 | 3.40 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 2.30 | 3.30 | 3.60 | 3.06 |
| $L_4H_2$ | 100 | 70 – 90 | 2.60 | 3.60 | 4.30 | 3.50 |
| Normal ($N_3$) | | | 5.00 | 5.30 | 6.30 | 5.53 |
| Mean | | | 3.34 | 4.04 | 4.85 | 4.08 |
| CD (5%) | | | 1.513 | 1.126 | 0.952 | |

**Table 8a.** Effect of sucrose in the rooting medium on number of roots per shoot of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Number of roots per shoot |
|---|---|---|
| $S_1$ | 20 (Control) | 6.00 |
| $S_2$ | 40 | 10.60 |
| $S_3$ | 60 | 7.40 |
| Normal ($N_1$) | Green house grown | 14.60 |
| Mean | | 9.65 |
| C.D. (1%) | | 2.832 |

**Table 8b.** Effect of triazole treatment at planting out on number of roots per shoot of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Number of roots per shoot | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 15.20 | 16.80 | 16.20 | 15.73 |
| $T_2$ | 10 | 13.60 | 14.00 | 14.40 | 14.00 |
| $T_3$ | 0 (Control) | 12.80 | 13.60 | 14.20 | 13.53 |
| Normal ($N_2$) | Green house grown | 16.00 | 16.40 | 17.20 | 16.53 |
| Mean | | 14.40 | 14.95 | 15.50 | 14.95 |
| CD (1%) | | 2.30 | ns | 1.88 | |

ns : Not significant

**Plate 5. Effect of different sucrose concentrations in the rooting media on number of roots produced**

a. **Maximum number of roots produced by plantlets under the treatment $S_2$ ( 40g/l)**

c. **Minimum number of roots produced by plantlets under the treatment $S_1$ (20 g/l)**

b. **Number of roots produced by plantlets under the treatment $S_3$ (60 g/l)**

**Table 8c.** Effect of light and humidity treatment (in the hardening chamber) on number of roots per shoot of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Number of roots per shoot | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 14.10 | 15.13 | 15.40 | 14.88 |
| $L_1H_2$ | 25 | 70 – 90 | 15.10 | 15.57 | 16.10 | 15.59 |
| $L_2H_1$ | 50 | < 70 | 14.70 | 15.30 | 15.83 | 15.28 |
| $L_2H_2$ | 50 | 70 – 90 | 15.50 | 16.13 | 17.03 | 16.22 |
| $L_3H_1$ | 75 | < 70 | 15.10 | 15.43 | 16.43 | 15.65 |
| $L_3H_2$ | 75 | 70 – 90 | 14.10 | 14.83 | 15.36 | 14.76 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 15.23 | 15.60 | 16.13 | 15.65 |
| $L_4H_2$ | 100 | 70 – 90 | 14.13 | 14.80 | 15.40 | 14.83 |
| Normal ($N_3$) | | | 17.17 | 18.00 | 18.23 | 17.80 |
| Mean | | | 15.01 | 15.64 | 16.21 | 15.63 |
| CD (1%) | | | 0.359 | 0.186 | 0.284 | |

With regard to triazole treatment (Table 8b) at 15 DAP and 45 DAP the number of roots produced by $T_1$ and $T_2$ were found to be on par with Normal plants. $T_3$ (control) plantlets recorded lesser rooting (12.8) than all other treatments. No significant difference among treatments was noticed at 30 DAP.

The plantlets were subjected to different light treatment in combination with various humidity levels. Analysis of data in the Table 8c revealed the statistical significance at all the three periodic intervals among treatments. The number of roots in Normal plants (17.80) was found to be much higher than tissue cultured plantlets at all three stages of study. Among treated plantlets $L_2H_2$ (16.22) was found to have more roots at all the three stages of analysis (viz. 15 DAP, 30 DAP and 45 DAP). The minimum number of roots (14.10) were produced by both $L_3H_2$ and $L_1H_1$ at 15 DAP. At 30 DAP and 45 DAP the treatments $L_4H_2$ (14.80) and $L_3H_2$ (15.36) recorded the minimum value respectively.

### 4.2.4 Survival Percent

The data on the effect of sucrose level on survival percent is shown in Table 9a. $S_2$ (83%) recorded higher percentage of survival and $S_1$ (62%) recorded lower survival percent.

Analysis of data on Table 9b revealed that the triazole treatment has positive correlation with survival percent. The mean value in $T_1$ (74.0) was found to be the maximum as compared to $T_2$ (70.0) and $T_3$ (64.7) percent respectively.

The plantlets subjected to different light treatments in combination with variations in humidity. Among treatments $L_2H_2$ exhibited maximum survival

**Table 9a.** Effect of sucrose in the rooting medium on survival percent of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Survival percent (%) |
|---|---|---|
| $S_1$ | 20 (Control) | 62 |
| $S_2$ | 40 | 83 |
| $S_3$ | 60 | 71 |
| Mean | | 72 |

**Table 9b.** Effect of triazole treatment at planting out on survival percent of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole | Survival percent (%) | | | |
|---|---|---|---|---|---|
| | Levels (mg/l) | 15 DAP | 30 DAP | 45 DAP | Mean |
| $T_1$ | 5 | 63.0 | 78.0 | 81.0 | 74.0 |
| $T_2$ | 10 | 61.0 | 73.0 | 76.0 | 70.0 |
| $T_3$ | 0 (Control) | 60.0 | 66.0 | 68.0 | 64.6 |
| Mean | | 61.3 | 72.3 | 75.0 | 69.5 |

* Each value is the average of ten replications.

**Table 9c.** Effect of light and humidity treatment (in the hardening chamber) on survival percent of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Survival percent (%) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 54.00 | 66.00 | 60.00 | 56.67 |
| $L_1H_2$ | 25 | 70 – 90 | 59.00 | 62.00 | 64.00 | 61.67 |
| $L_2H_1$ | 50 | < 70 | 63.00 | 69.00 | 72.00 | 68.00 |
| $L_2H_2$ | 50 | 70 – 90 | 66.00 | 74.00 | 79.00 | 73.00 |
| $L_3H_1$ | 75 | < 70 | 42.00 | 46.00 | 50.00 | 46.00 |
| $L_3H_2$ | 75 | 70 – 90 | 48.00 | 49.00 | 53.00 | 50.00 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 30.00 | 34.00 | 38.00 | 34.00 |
| $L_4H_2$ | 100 | 70 – 90 | 39.00 | 42.00 | 45.00 | 42.00 |
| Mean | | | 50.13 | 54.00 | 57.63 | 47.93 |

* Each value is the average of ten replications.

percentage(73.0) and $L_4H_1$ (34.0) recorded the minimum the minimum (Table 9c.)

## 4.3 BIOCHEMICAL CHARACTERS

### 4.3.1 Photosynthetic pigments

#### 4.3.1.1 Total chlorophyll content (mg $g^{-1}$ fresh leaf)

The study on the effect of sucrose on the total chlorophyll content (Table 10a) reveals that $S_2$ (0.236) had higher value than $S_1$ (0.226) and $S_3$ (0.221). However the Normal plants(0.279) had higher total chlorophyll content than tissue cultured plantlets.

With regard to the triazole treatment presented in Table 10b, it became clear that $T_1$, $T_2$ and $T_3$ were on par with each other at 15 DAP and 30 DAP. But at 45 DAP, $T_3$ (0.1086) had lesser total chlorophyll content than $T_1$(0.1159) and $T_2$(0.1140). The Normal plants maintained relatively higher total chlorophyll content than tissue cultured plantlets throughout the three stages of the study.

The plantlets were subjected to different light treatments in combination with various humidity levels (Table 10c). The data reveals that the total chlorophyll content of Normal plants were greater than the tissue cultured plantlets at all stages of the study. During the earlier growth stages viz 15 DAP and 30 DAP there was no distinct variation between the different treatments. At 45 DAP, among the tissue cultured plantlets $L_2H_2$ (0.2732) recorded the maximum value and $L_4H_1$ (0.2524) recorded the minimum.

**Table 10a.** Effect of sucrose in the rooting medium on total chlorophyll of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Total chlorophyll (mg $g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.226 |
| $S_2$ | 40 | 0.236 |
| $S_3$ | 60 | 0.221 |
| Normal ($N_1$) | Green house grown | 0.279 |
| Mean | | 0.241 |
| C.D. (5%) | | 0.006 |

**Table 10b.** Effect of triazole treatment at planting out on total chlorophyll of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Total chlorophyll (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.1081 | 0.1140 | 0.1159 | 0.1127 |
| $T_2$ | 10 | 0.1044 | 0.1094 | 0.1140 | 0.1093 |
| $T_3$ | 0 (Control) | 0.1027 | 0.1068 | 0.1086 | 0.1060 |
| Normal ($N_2$) | Green house grown | 0.2280 | 0.2308 | 0.2339 | 0.2309 |
| Mean | | 0.1358 | 0.1403 | 0.1431 | 0.1397 |
| CD (5%) | | 0.013 | 0.011 | 0.006 | |

**Table 10c.** Effect of light and humidity treatment (in the hardening chamber) on total chlorophyll of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Total chlorophyll(mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.2523 | 0.2554 | 0.2590 | 0.2556 |
| $L_1H_2$ | 25 | 70 – 90 | 0.2557 | 0.2615 | 0.2633 | 0.2602 |
| $L_2H_1$ | 50 | < 70 | 0.2602 | 0.2654 | 0.2710 | 0.2663 |
| $L_2H_2$ | 50 | 70 – 90 | 0.2632 | 0.2676 | 0.2732 | 0.2673 |
| $L_3H_1$ | 75 | < 70 | 0.2572 | 0.2619 | 0.2650 | 0.2614 |
| $L_3H_2$ | 75 | 70 – 90 | 0.2616 | 0.2648 | 0.2687 | 0.2650 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.2443 | 0.2487 | 0.2524 | 0.2485 |
| $L_4H_2$ | 100 | 70 – 90 | 0.2485 | 0.2520 | 0.2562 | 0.2522 |
| Normal ($N_3$) | | | 0.2829 | 0.2862 | 0.2870 | 0.2854 |
| Mean | | | 0.2584 | 0.2626 | 0.2662 | 0.2624 |
| CD (5%) | | | ns | ns | 0.021 | |

**Table 11a.** Effect of sucrose in the rooting medium on chlorophyll 'a' of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Chlorophyll 'a' (mg $g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.0865 |
| $S_2$ | 40 | 0.1065 |
| $S_3$ | 60 | 0.0782 |
| Normal ($N_1$) | Green house grown | 0.1914 |
| Mean | | 0.1152 |
| C.D. (5%) | | 0.036 |

**Table 11b.** Effect of triazole treatment at planting out on chlorophyll 'a' of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Chlorophyll 'a' (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.1514 | 0.1532 | 0.1564 | 0.1537 |
| $T_2$ | 10 | 0.1463 | 0.1483 | 0.1515 | 0.1487 |
| $T_3$ | 0 (Control) | 0.1453 | 0.1462 | 0.1501 | 0.1472 |
| Normal ($N_2$) | Green house grown | 0.1940 | 0.1954 | 0.1985 | 0.1960 |
| Mean | | 0.1593 | 0.1608 | 0.1641 | 0.1614 |
| CD (5%) | | ns | 0.002 | 0.050 | |

ns : Not significant

**Table 11c.** Effect of light and humidity treatment (in the hardening chamber) on chlorophyll 'a' of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Chlorophyll 'a' (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.1532 | 0.1702 | 0.1927 | 0.1720 |
| $L_1H_2$ | 25 | 70 – 90 | 0.1582 | 0.1782 | 0.1936 | 0.1767 |
| $L_2H_1$ | 50 | < 70 | 0.1770 | 0.1970 | 0.2033 | 0.1924 |
| $L_2H_2$ | 50 | 70 – 90 | 0.1852 | 0.2122 | 0.2391 | 0.2122 |
| $L_3H_1$ | 75 | < 70 | 0.1830 | 0.2092 | 0.2226 | 0.2549 |
| $L_3H_2$ | 75 | 70 – 90 | 0.1712 | 0.1880 | 0.2391 | 0.1875 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.1628 | 0.1828 | 0.1992 | 0.1816 |
| $L_4H_2$ | 100 | 70 – 90 | 0.1732 | 0.1978 | 0.2012 | 0.1907 |
| Normal ($N_3$) | | | 0.2440 | 0.2420 | 0.2436 | 0.2422 |
| Mean | | | 0.1786 | 0.1975 | 0.2110 | 0.2011 |
| CD (5%) | | | 0.006 | ns | 0.002 | |

#### 4.3.1.2 Chlorophyll 'a' (mg $g^{-1}$ fresh leaf)

The analysis of plantlets subjected to different levels of sucrose in the rooting medium after 45 days of rooting presented in Table 11a revealed that the treatment $S_1$ (0.0865), $S_2$ (0.1065) and $S_3$ (0.0782) were on par with each other. The chlorophyll 'a' content of Normal plants (0.1065) was found to be superior than that of the tissue cultured plantlets.

With regard to triazole application (Table 11b) no significant difference was noticed among treatments at 15 DAP. At 30 DAP, $T_1$ (0.1532) recorded the maximum value and $T_3$ (control) (0.1462) the minimum. The Normal plants maintained higher chlorophyll 'a' content than tissue cultured plantlets at all three stages of analysis. However towards the later period ie, 45 DAP the tissue cultured plantlets under the treatments $T_1$, $T_2$ and $T_3$ were found to be on par with the Normal plants.

Table 11c represents the chlorophyll 'a' content under different light and humidity levels. Among the treatments $L_2H_2$ (0.1852) recorded the highest value and was on par with $L_3H_1$ (0.1830) at 15 DAP. $L_1H_1$ recorded the minimum value at all stages. No significant difference was noticed among treatments at 30 DAP. Invariably a distinctively higher chlorophyll 'a' content was noted in Normal plants compared to tissue cultured plantlets at all stages. At 45 DAP $L_2H_2$ recorded the maximum value (0.2391) and $L_1H_1$ recorded the minimum (0.1927).

#### 4.3.1.3 Chlorophyll 'b' content (mg $g^{-1}$ fresh leaf)

Analysis of data in the Table 12a revealed the statistical significance among the treatments. The Normal plants (0.1424) maintained relatively higher amount of

**Table 12a.** Effect of sucrose in the rooting medium on chlorophyll 'b' of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Chlorophyll 'b' (mg $g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.1246 |
| $S_2$ | 40 | 0.1356 |
| $S_3$ | 60 | 0.1271 |
| Normal ($N_1$) | Green house grown | 0.1424 |
| Mean | | 0.1324 |
| C.D. (5%) | | 0.0026 |

**Table 12b.** Effect of triazole treatment at planting out on chlorophyll 'b' of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Chlorophyll 'b' (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.0789 | 0.0972 | 0.1025 | 0.0929 |
| $T_2$ | 10 | 0.0687 | 0.0854 | 0.0905 | 0.0815 |
| $T_3$ | 0 (Control) | 0.0693 | 0.0792 | 0.0816 | 0.0767 |
| Normal ($N_2$) | Green house grown | 0.1305 | 0.1330 | 0.1344 | 0.1326 |
| Mean | | 0.0869 | 0.0987 | 0.1023 | 0.0959 |
| CD (5%) | | 0.009 | 0.012 | 0.016 | |

**Table 12c.** Effect of light and humidity treatment (in the hardening chamber) on chlorophyll 'b' of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Chlorophyll 'b' (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.0660 | 0.0637 | 0.0833 | 0.0710 |
| $L_1H_2$ | 25 | 70 – 90 | 0.0684 | 0.0782 | 0.0888 | 0.0785 |
| $L_2H_1$ | 50 | < 70 | 0.0824 | 0.1092 | 0.1192 | 0.1036 |
| $L_2H_2$ | 50 | 70 – 90 | 0.0977 | 0.1136 | 0.1262 | 0.1123 |
| $L_3H_1$ | 75 | < 70 | 0.0920 | 0.0947 | 0.0938 | 0.0935 |
| $L_3H_2$ | 75 | 70 – 90 | 0.0741 | 0.0987 | 0.0990 | 0.0906 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.0720 | 0.0834 | 0.0888 | 0.0814 |
| $L_4H_2$ | 100 | 70 – 90 | 0.0648 | 0.0886 | 0.0914 | 0.0816 |
| Normal ($N_3$) | | | 0.1324 | 0.1346 | 0.1350 | 0.1340 |
| Mean | | | 0.0833 | 0.0961 | 0.1028 | 0.0837 |
| CD (5%) | | | 0.013 | 0.014 | 0.002 | |

chlorophyll ‘b’ than the tissue cultured plantlets. $S_2$ (0.1356) registered the maximum value and $S_1$ (0.1246) the minimum.

With regard to triazole treatment (Table 12b) $T_1$ (0.0929) recorded higher mean value of chlorophyll compared to $T_2$ (0.0815) and $T_3$ (0.0767) at all the three stages of study. However the Normal plants (0.1326) maintained distinctively higher amount of chlorophyll ‘b’ than the tissue cultured plantlets at all stages.

The data on chlorophyll ‘b’ content of plantlets subjected to different light and humidity levels is given in Table 12c. Among the treatments $L_2H_2$ (0.1123) recorded the maximum mean value and $L_1H_1$ (0.0710) recorded the minimum. However the Normal plants (0.1340) recorded a significantly higher value of chlorophyll ‘b’ content than the tissue cultured plantlets.

#### 4.3.1.4 Carotenoid content (mg $g^{-1}$ fresh leaf)

The data on carotenoid content as influenced by sucrose treatment is given in Table 13a. Analysis of data revealed statistical significance among the treatments. $S_2$ (0.0878) recorded higher value than $S_1$ (0.0655) and $S_3$ (0.0446). The Normal plants (0.1046) registered a significantly higher value of carotenoid.

Table 13b represents the effect of triazole on the carotenoid content. Among the treatments $T_2$ (0.1113) had the maximum mean value and was on par with $T_1$ (0.0998) and $T_3$ (0.0945). The Normal plantlets (0.1466) exhibited significantly higher carotenoid content than the tissue cultured plantlets at all stages.

**Table 13a.** Effect of sucrose in the rooting medium on carotenoids of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Carotenoids (mg $g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.0655 |
| $S_2$ | 40 | 0.0878 |
| $S_3$ | 60 | 0.0446 |
| Normal ($N_1$) | Green house grown | 0.1046 |
| Mean | | 0.0756 |
| C.D. (5%) | | 0.004 |

**Table 13b.** Effect of triazole treatment at planting out on carotenoids of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole | Carotenoids (mg $g^{-1}$ fresh leaf) | | | |
|---|---|---|---|---|---|
| | Levels (mg/l) | 15 DAP | 30 DAP | 45 DAP | Mean |
| $T_1$ | 5 | 0.0939 | 0.1015 | 0.1040 | 0.0998 |
| $T_2$ | 10 | 0.1085 | 0.1128 | 0.1127 | 0.1113 |
| $T_3$ | 0 (Control) | 0.0937 | 0.0923 | 0.0974 | 0.0945 |
| Normal ($N_2$) | Green house grown | 0.1430 | 0.1451 | 0.1517 | 0.1466 |
| Mean | | 0.1098 | 0.1129 | 0.1165 | 0.1131 |
| CD (5%) | | 0.003 | 0.008 | 0.008 | |

**Table 13c.** Effect of light and humidity treatment (in the hardening chamber) on carotenoids of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Carotenoids (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.0846 | 0.0862 | 0.0927 | 0.0878 |
| $L_1H_2$ | 25 | 70 – 90 | 0.0870 | 0.0888 | 0.0898 | 0.0885 |
| $L_2H_1$ | 50 | < 70 | 0.0993 | 0.1016 | 0.1045 | 0.1018 |
| $L_2H_2$ | 50 | 70 – 90 | 0.1119 | 0.1144 | 0.1163 | 0.1142 |
| $L_3H_1$ | 75 | < 70 | 0.1082 | 0.1095 | 0.1114 | 0.1097 |
| $L_3H_2$ | 75 | 70 – 90 | 0.1034 | 0.1064 | 0.1086 | 0.1061 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.0740 | 0.0758 | 0.0824 | 0.0774 |
| $L_4H_2$ | 100 | 70 – 90 | 0.0787 | 0.0823 | 0.0879 | 0.0828 |
| Normal ($N_3$) | | | 0.1328 | 0.1384 | 0.1427 | 0.1380 |
| Mean | | | 0.0978 | 0.1004 | 0.1040 | 0.1007 |
| CD (5%) | | | 0.002 | 0.001 | 0.001 | |

With regard to the plantlets under different levels of light in combination with different regimes of humidity $L_2H_2$ (0.1142) registered the maximum value and $L_4H_1$ (0.0774) the minimum (Table 13c). In all the cases the Normal plants (0.1380) exhibited significantly higher carotenoid content than the tissue cultured plantlets.

### 4.3.2 Protein Content (mg $g^{-1}$)

The data on the effect of sucrose on protein content is presented in Table 14a. Analysis of data revealed statistical significance at all levels. $S_2$ (0.085) recorded the maximum protein content and $S_3$ (0.064) the minimum. Normal plants (0.094) maintained relatively higher protein content than tissue cultured plantlets.

With respect to triazole treatment (Table 14b) during the initial stage ie, at 15 DAP; the treatment $T_1$ (0.077) was found to be on par with Normal plants (0.087). Towards the later stages of the plantlets a slight decline in protein level was noticed.

The data on protein content of plantlets subjected to different levels of light and humidity is presented in Table 14c. Analysis of data reveals statistical significance at all levels. Among treatments, $L_3H_2$ (0.078) recorded maximum protein content at all the three stages of analysis. However other treatments viz. $L_3H_1$, $L_2H_1$, $L_2H_2$ were found to be on par with $L_3H_2$ during the earlier two stages of the study (15 DAP and 30 DAP). The treatment $L_4H_1$ (0.050), recorded the minimum value at all stages of analysis. However a distinctively higher value was recorded by Normal plants (0.093) as compared to the tissue cultured plantlets.

**Table 14a.** Effect of sucrose in the rooting medium on protein content of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Protein content (mg $g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.073 |
| $S_2$ | 40 | 0.085 |
| $S_3$ | 60 | 0.064 |
| Normal ($N_1$) | Green house grown | 0.094 |
| Mean | | 0.079 |
| C.D. (5%) | | 0.006 |

**Table 14b.** Effect of triazole treatment at planting out on protein content of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Protein content (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.077 | 0.076 | 0.075 | 0.0760 |
| $T_2$ | 10 | 0.065 | 0.065 | 0.065 | 0.0651 |
| $T_3$ | 0 (Control) | 0.062 | 0.085 | 0.083 | 0.0767 |
| Normal ($N_2$) | Green house grown | 0.087 | 0.093 | 0.094 | 0.0913 |
| Mean | | 0.072 | 0.079 | 0.079 | 0.0773 |
| CD (5%) | | 0.010 | 0.013 | 0.008 | |

**Table 14c.** Effect of light and humidity treatment (in the hardening chamber) on protein content of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Protein content (mg $g^{-1}$ fresh leaf) 15 DAP | 30 DAP | 45 DAP | Mean |
|---|---|---|---|---|---|---|
| $L_1H_1$ | 25 | < 70 | 0.067 | 0.069 | 0.074 | 0.070 |
| $L_1H_2$ | 25 | 70 – 90 | 0.062 | 0.064 | 0.062 | 0.063 |
| $L_2H_1$ | 50 | < 70 | 0.074 | 0.073 | 0.070 | 0.072 |
| $L_2H_2$ | 50 | 70 – 90 | 0.070 | 0.068 | 0.068 | 0.068 |
| $L_3H_1$ | 75 | < 70 | 0.076 | 0.076 | 0.075 | 0.075 |
| $L_3H_2$ | 75 | 70 – 90 | 0.077 | 0.078 | 0.080 | 0.078 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.050 | 0.052 | 0.046 | 0.050 |
| $L_4H_2$ | 100 | 70 – 90 | 0.054 | 0.056 | 0.054 | 0.055 |
| Normal ($N_3$) | | | 0.090 | 0.095 | 0.094 | 0.093 |
| Mean | | | 0.069 | 0.070 | 0.070 | 0.069 |
| CD (1%) | | | 0.009 | 0.015 | 0.010 | |

### 4.3.3 Carbohydrate Content (mg $g^{-1}$)

The effect of sucrose in the rooting medium on carbohydrate content of orchid plantlets at 45 days of rooting is presented in the Table 15a. The carbohydrate content of Normal plants was highly significant (0.042) and among the treated plantlets, $S_2$ (0.032) and $S_1$ (0.026) had higher value and they were on par with each other.

With regard to triazole treatment there was distinct variation among treatments at all levels (Table 15b). $T_1$ (0.048) recorded the maximum value of carbohydrate content and $T_3$ (0.038) the minimum.

As shown in Table 15c, the plantlets under the treatment $L_3H_2$ (0.0480) was found to record higher carbohydrate content and $L_1H_1$ (0.0347) the minimum carbohydrate content at all stages. Invariably the Normal plants (0.0657) recorded relatively higher carbohydrate content than tissue cultured plantlets.

## 4.4 BIOMETRIC CHARACTERS

### 4.4.1 Leaf Area ($cm^2$)

The data on leaf area of plantlets as influenced by different sucrose levels is presented in Table 16a. Among the treatments $S_2$ (4.80) recorded highest value and was on par with Normal plants (5.20).

The data on the effect of triazole on leaf area (Table 16b) reveals statistical significance at all levels. Among the treated plantlets $T_1$ (13.86) showed higher value than $T_2$ (12.55) and $T_3$ (11.62). It is quite evident from this data that the Normal plants could maintain relatively higher leaf area than tissue cultured plantlets.

**Table 15a.** Effect of sucrose in the rooting medium on carbohydrate content of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Carbohydrate content ($mg\ g^{-1}$ fresh leaf) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.026 |
| $S_2$ | 40 | 0.032 |
| $S_3$ | 60 | 0.022 |
| Normal ($N_1$) | Green house grown | 0.042 |
| Mean | | 0.031 |
| C.D. (5%) | | 0.007 |

**Table 15b.** Effect of triazole treatment at planting out on carbohydrate of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole | Carbohydrate content ($mg\ g^{-1}$ fresh leaf) | | | |
|---|---|---|---|---|---|
| | Levels (mg/l) | 15 DAP | 30 DAP | 45 DAP | Mean |
| $T_1$ | 5 | 0.044 | 0.046 | 0.054 | 0.048 |
| $T_2$ | 10 | 0.036 | 0.040 | 0.046 | 0.041 |
| $T_3$ | 0 (Control) | 0.038 | 0.040 | 0.037 | 0.038 |
| Normal ($N_2$) | Green house grown | 0.058 | 0.056 | 0.063 | 0.059 |
| Mean | | 0.044 | 0.046 | 0.050 | 0.047 |
| CD (5%) | | 0.003 | 0.004 | 0.005 | |

**Table 15c.** Effect of light and humidity treatment (in the hardening chamber) on carbohydrate content of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Carbohydrate content (mg $g^{-1}$ fresh leaf) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.031 | 0.035 | 0.038 | 0.0347 |
| $L_1H_2$ | 25 | 70 – 90 | 0.038 | 0.040 | 0.042 | 0.0400 |
| $L_2H_1$ | 50 | < 70 | 0.039 | 0.040 | 0.044 | 0.0410 |
| $L_2H_2$ | 50 | 70 – 90 | 0.042 | 0.039 | 0.041 | 0.0406 |
| $L_3H_1$ | 75 | < 70 | 0.040 | 0.044 | 0.046 | 0.0433 |
| $L_3H_2$ | 75 | 70 – 90 | 0.044 | 0.048 | 0.052 | 0.0480 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.034 | 0.039 | 0.043 | 0.0387 |
| $L_4H_2$ | 100 | 70 – 90 | 0.038 | 0.043 | 0.046 | 0.0423 |
| Normal ($N_3$) | | | 0.062 | 0.067 | 0.068 | 0.0657 |
| Mean | | | 0.049 | 0.044 | 0.047 | 0.0438 |
| CD (1%) | | | 0.013 | 0.009 | 0.010 | |

**Table 16a.** Effect of sucrose in the rooting medium on leaf area of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Leaf area ($cm^2$) |
|---|---|---|
| $S_1$ | 20 (Control) | 3.98 |
| $S_2$ | 40 | 4.80 |
| $S_3$ | 60 | 3.13 |
| Normal ($N_1$) | Green house grown | 5.20 |
| Mean | | 4.28 |
| C.D. (5%) | | 1.10 |

**Table 16b.** Effect of triazole treatment at planting out on leaf area of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Leaf area ($cm^2$) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 12.76 | 13.52 | 15.30 | 13.860 |
| $T_2$ | 10 | 11.48 | 12.30 | 13.87 | 12.550 |
| $T_3$ | 0 (Control) | 10.24 | 11.30 | 13.32 | 11.620 |
| Normal ($N_2$) | Green house grown | 15.84 | 16.40 | 18.46 | 16.900 |
| Mean | | 12.58 | 13.38 | 15.24 | 13.733 |
| CD (1%) | | 0.463 | 0.360 | 0.720 | |

**Table 16c.** Effect of light and humidity treatment (in the hardening chamber) on leaf area of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Leaf area ($cm^2$) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 14.54 | 15.24 | 16.60 | 15.46 |
| $L_1H_2$ | 25 | 70 – 90 | 15.37 | 15.67 | 16.13 | 15.72 |
| $L_2H_1$ | 50 | < 70 | 15.77 | 16.40 | 17.13 | 16.43 |
| $L_2H_2$ | 50 | 70 – 90 | 17.84 | 18.67 | 19.12 | 18.54 |
| $L_3H_1$ | 75 | < 70 | 13.67 | 15.06 | 15.90 | 14.88 |
| $L_3H_2$ | 75 | 70 – 90 | 14.37 | 14.09 | 15.24 | 14.23 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 13.20 | 14.16 | 15.46 | 14.28 |
| $L_4H_2$ | 100 | 70 – 90 | 13.64 | 14.26 | 14.20 | 14.10 |
| Normal ($N_3$) | | | 18.34 | 18.64 | 19.10 | 18.69 |
| Mean | | | 15.171 | 15.791 | 16.632 | 15.81 |
| CD (1%) | | | 0.469 | 0.516 | 0.255 | |

Among the different light regimes and humidity levels, $L_2H_2$ recorded highest value (18.54) and was on par with the Normal plants (18.69) during the later stages ie, 30 DAP and 45 DAP. The treatment $L_4H_2$ (14.10) recorded the minimum value.(Table 16c).

### 4.4.2 Root Length (cm)

The data on the effect of sucrose on root length is presented in Table 17a. Analysis of data revealed statistical significance. Among the tissue cultured plantlets the treatment $S_2$ (1.88) recorded significantly greater plant height and was on par with Normal plants (2.30). The treatment $S_3$ (1.45) recorded the minimum value.

The Table 17b represents the influence of triazole on root length of plantlets and it was noticed as highly significant. The root length of Normal plants (2.92) was found to be higher than tissue cultured plantlets at all stages. $T_1$ (1.95) exhibited the maximum value and $T_3$ (1.39) the minimum.

The plantlets were subjected to different light treatments in combination with various humidity levels. During the initial stages (15 DAP and 30 DAP) of the study, the Normal plants recorded a distinctively greater root length than all the tissue cultured plantlets subjected to treatment. But towards the later stage $L_2H_2$ (3.50) exhibited maximum value and was on par with Normal plants (3.90). (Table 17c).

**Table 17a.** Effect of sucrose in the rooting medium on root length of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Root length (cm) |
|---|---|---|
| $S_1$ | 20 (Control) | 1.43 |
| $S_2$ | 40 | 1.88 |
| $S_3$ | 60 | 1.45 |
| Normal ($N_1$) | Green house grown | 2.30 |
| Mean | | 1.765 |
| C.D. (1%) | | 0.63 |

**Table 17b.** Effect of triazole treatment at planting out on root length of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole | Root length (cm) | | | Mean |
|---|---|---|---|---|---|
| | Levels (mg/l) | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 1.62 | 1.95 | 2.28 | 1.95 |
| $T_2$ | 10 | 1.52 | 1.47 | 1.68 | 1.56 |
| $T_3$ | 0 (Control) | 1.24 | 1.37 | 1.55 | 1.39 |
| Normal ($N_2$) | Green house grown | 2.73 | 2.82 | 3.22 | 2.92 |
| Mean | | 1.78 | 1.90 | 2.18 | 1.96 |
| CD (1%) | | 0.35 | 0.32 | 0.188 | |

**Table 17c.** Effect of light and humidity treatment (in the hardening chamber) on root length of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Root length (cm) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 1.20 | 1.70 | 2.60 | 1.80 |
| $L_1H_2$ | 25 | 70 – 90 | 1.40 | 2.10 | 2.70 | 2.10 |
| $L_2H_1$ | 50 | < 70 | 2.10 | 2.10 | 2.90 | 2.40 |
| $L_2H_2$ | 50 | 70 – 90 | 2.20 | 2.50 | 3.50 | 2.70 |
| $L_3H_1$ | 75 | < 70 | 1.80 | 2.30 | 3.30 | 2.50 |
| $L_3H_2$ | 75 | 70 – 90 | 1.90 | 1.80 | 3.20 | 2.30 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 1.40 | 2.10 | 2.80 | 2.10 |
| $L_4H_2$ | 100 | 70 – 90 | 1.20 | 2.20 | 2.70 | 2.03 |
| Normal ($N_3$) | | | 2.97 | 3.80 | 3.90 | 3.56 |
| Mean | | | 1.80 | 2.29 | 3.07 | 2.39 |
| CD (5%) | | | 0.470 | 0.730 | 0.443 | |

### 4.4.3 Root : Shoot Ratio

The data on the effect of sucrose on root: shoot ratio is given in Table 18a. Among the treatments, $S_3$ (1.883) recorded the maximum value and was on par with Normal plants (1.978) and $S_1$ (0.621) recorded the minimum.

With regard to the plantlets which are subjected to different triazole treatments (Table 18b), there is no significant difference among treatments up to 30 DAP. But during 45 DAP the root : shoot ratio of tissue cultured plantlets under treatment $T_1$ (0.487) was found to be on par with Normal plants (0.485).

The root : shoot ratio of the plantlets subjected to various light regimes in combination with different humidity levels is represented in Table 18c. Analysis of the data on root : shoot ratio revealed statistical significance at all levels. Mean value reveals that among the treatments, $L_3H_1$ (0.445) recorded maximum value and $L_1H_1$ (0.322) the minimum. Normal plants (0.473), were found to be on par with the treatments $L_3H_1$ (0.519) and $L_3H_2$ (0.502) towards the later stage viz. 45 DAP.

### 4.4.4 Total Fresh Weight (g)

The data on the effect of sucrose on total dry weight is presented in Table 19a. Among the three levels of sucrose tried out $S_2$ (0.426) recorded higher value and was on par with $S_3$ (0.400). However a distinctively higher value was recorded by Normal plants (0.808) compared to the tissue cultured plantlets.

With regard to triazole treatment (Table 19b), $T_1$ (1.895) recorded the highest value and was on par with $T_3$ (1.828) and Normal plants (2.054) at

**Table 18a.** Effect of sucrose in the rooting medium on root shoot ratio of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Root shoot ratio |
|---|---|---|
| $S_1$ | 20 (Control) | 0.671 |
| $S_2$ | 40 | 1.516 |
| $S_3$ | 60 | 1.883 |
| Normal ($N_1$) | Green house grown | 1.978 |
| Mean | | 1.512 |
| C.D. (5%) | | 0.172 |

**Table 18b.** Effect of triazole treatment at planting out on root shoot ratio of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Root shoot ratio | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.388 | 0.448 | 0.487 | 0.441 |
| $T_2$ | 10 | 0.400 | 0.359 | 0.382 | 0.380 |
| $T_3$ | 0 (Control) | 0.225 | 0.288 | 0.306 | 0.273 |
| Normal ($N_2$) | Green house grown | 0.510 | 0.466 | 0.478 | 0.485 |
| Mean | | 0.380 | 0.390 | 0.413 | 0.395 |
| CD (5%) | | ns | ns | 0.045 | |

ns : Not significant

**Table 18c.** Effect of light and humidity treatment (in the hardening chamber) on root shoot ratio of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Root shoot ratio | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.255 | 0.303 | 0.408 | 0.322 |
| $L_1H_2$ | 25 | 70 – 90 | 0.292 | 0.365 | 0.417 | 0.358 |
| $L_2H_1$ | 50 | < 70 | 0.403 | 0.355 | 0.431 | 0.396 |
| $L_2H_2$ | 50 | 70 – 90 | 0.400 | 0.373 | 0.465 | 0.413 |
| $L_3H_1$ | 75 | < 70 | 0.391 | 0.425 | 0.519 | 0.445 |
| $L_3H_2$ | 75 | 70 – 90 | 0.413 | 0.327 | 0.502 | 0.414 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.325 | 0.396 | 0.426 | 0.382 |
| $L_4H_2$ | 100 | 70 – 90 | 0.272 | 0.415 | 0.426 | 0.371 |
| Normal ($N_3$) | | | 0.586 | 0.481 | 0.473 | 0.513 |
| Mean | | | 0.371 | 0.382 | 0.452 | 0.402 |
| CD (5%) | | | 0.070 | 0.093 | 0.058 | |

**Table 19a.** Effect of sucrose in the rooting medium on fresh weight of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Fresh weight (g) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.273 |
| $S_2$ | 40 | 0.426 |
| $S_3$ | 60 | 0.400 |
| Normal ($N_1$) | Green house grown | 0.808 |
| Mean | | 0.477 |
| C.D. (5%) | | 0.122 |

**Table 19b.** Effect of triazole treatment at planting out on fresh weight of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Triazole Levels (mg/l) | Fresh weight (g) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 1.849 | 1.913 | 1.923 | 1.895 |
| $T_2$ | 10 | 1.602 | 1.695 | 1.708 | 1.668 |
| $T_3$ | 0 (Control) | 1.793 | 1.836 | 1.854 | 1.828 |
| Normal ($N_2$) | Green house grown | 2.043 | 2.058 | 2.060 | 2.054 |
| Mean | | 1.822 | 1.876 | 1.886 | 1.861 |
| CD (5%) | | 0.188 | 0.232 | 0.226 | |

**Table 19c.** Effect of light and humidity treatment (in the hardening chamber) on fresh weight of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Fresh weight (g) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 1.574 | 1.663 | 1.713 | 1.6500 |
| $L_1H_2$ | 25 | 70 – 90 | 1.656 | 1.735 | 1.761 | 1.7173 |
| $L_2H_1$ | 50 | < 70 | 1.774 | 1.821 | 1.834 | 1.8097 |
| $L_2H_2$ | 50 | 70 – 90 | 1.862 | 1.924 | 1.948 | 1.9113 |
| $L_3H_1$ | 75 | < 70 | 1.775 | 1.886 | 1.852 | 1.8377 |
| $L_3H_2$ | 75 | 70 – 90 | 1.639 | 1.666 | 1.732 | 1.6790 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 1.636 | 1.676 | 1.729 | 1.6803 |
| $L_4H_2$ | 100 | 70 – 90 | 1.780 | 1.845 | 1.939 | 1.8547 |
| Normal ($N_3$) | | | 2.046 | 2.069 | 2.070 | 2.0617 |
| Mean | | | 1.7491 | 1.8094 | 1.8420 | 1.8002 |
| CD (1%) | | | 0.121 | 0.049 | 0.230 | |

the later stages viz. 30 DAP and 45 DAP. However $T_2$ (1.668) recorded the minimum value at all the three stages.

The study on the effect of different light and humidity levels (Table 19c) indicate that during the earlier stages ie, 15 DAP and 30 DAP, the Normal plants showed distinctively higher total fresh weight than the tissue cultured plantlets. But towards the later stage ie 45 DAP, the plantlets under the treatments $L_2H_2$ (1.948), $L_4H_2$ (1.939), and $L_3H_1$ (1.852) were found to be on par with the Normal plants (2.070).

### 4.4.5 Total Dry Weight (g)

The data on dry weight of plantlets under different levels of sucrose (Table 20a) reveals that $S_2$ (0.105) recorded the maximum value compared to $S_3$ (0.079) and $S_1$ (0.043). The dry weight recorded by Normal plants (0.187) was significantly higher than that of the tissue cultured plantlets.

With regard to the plantlets under triazole treatment, $T_1$ (0.8527) recorded the maximum value and $T_2$ (0.6680) the minimum (Table 20b).

The data on dry weight of plantlets subjected to different levels of light and humidity is presented in Table 20c. Analysis of data reveals statistical significance at all levels. Among the treatments $L_2H_2$ (0.8400) recorded the mean maximum value and $L_1H_2$ (0.6590) the minimum. Invariably at all stages, the dry weight of Normal plants (1.0822) was significantly higher than the tissue cultured plantlets.

**Table 20a.** Effect of sucrose in the rooting medium on dry weight of orchid plantlets at 45 days of rooting.

| Treatment | Sucrose Levels (g/l) | Dry weight (g) |
|---|---|---|
| $S_1$ | 20 (Control) | 0.043 |
| $S_2$ | 40 | 0.105 |
| $S_3$ | 60 | 0.079 |
| Normal ($N_1$) | Green house grown | 0.187 |
| Mean | | 0.1035 |
| C.D. (5%) | | 0.020 |

**Table 20b.** Effect of triazole treatment at planting out on dry weight of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

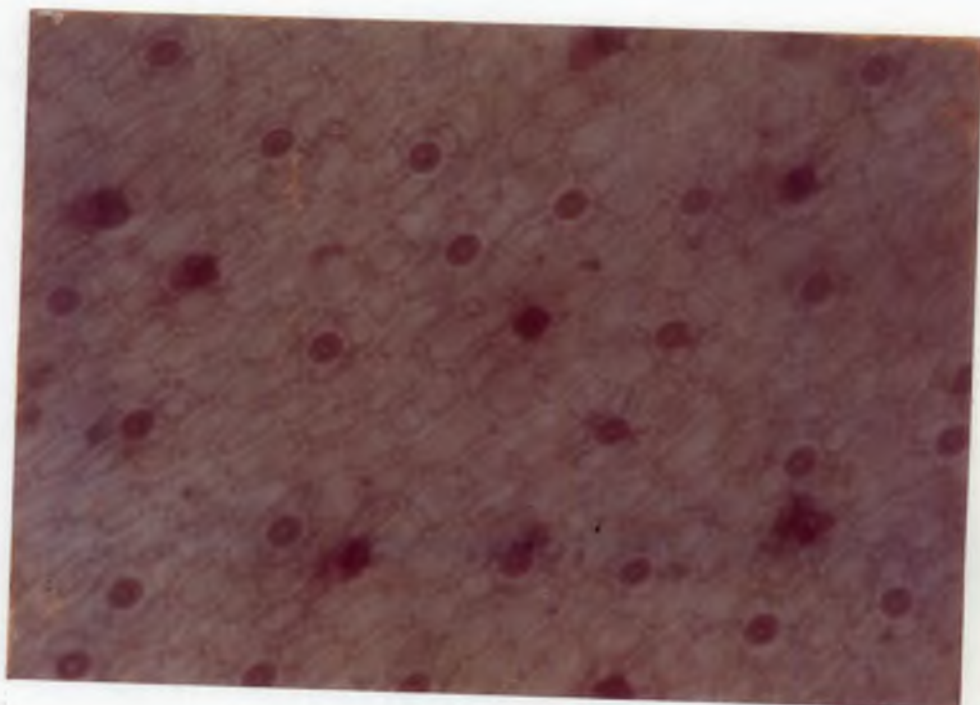
| Treatment | Triazole Levels (mg/l) | Dry weight (g) | | | Mean |
|---|---|---|---|---|---|
| | | 15 DAP | 30 DAP | 45 DAP | |
| $T_1$ | 5 | 0.730 | 0.844 | 0.984 | 0.8527 |
| $T_2$ | 10 | 0.544 | 0.692 | 0.764 | 0.6680 |
| $T_3$ | 0 (Control) | 0.633 | 0.748 | 0.885 | 0.7553 |
| Normal ($N_2$) | Green house grown | 1.026 | 1.184 | 1.326 | 1.1787 |
| Mean | | 0.7333 | 0.8670 | 0.9908 | 0.8637 |
| CD (5%) | | 0.015 | 0.047 | 0.060 | |

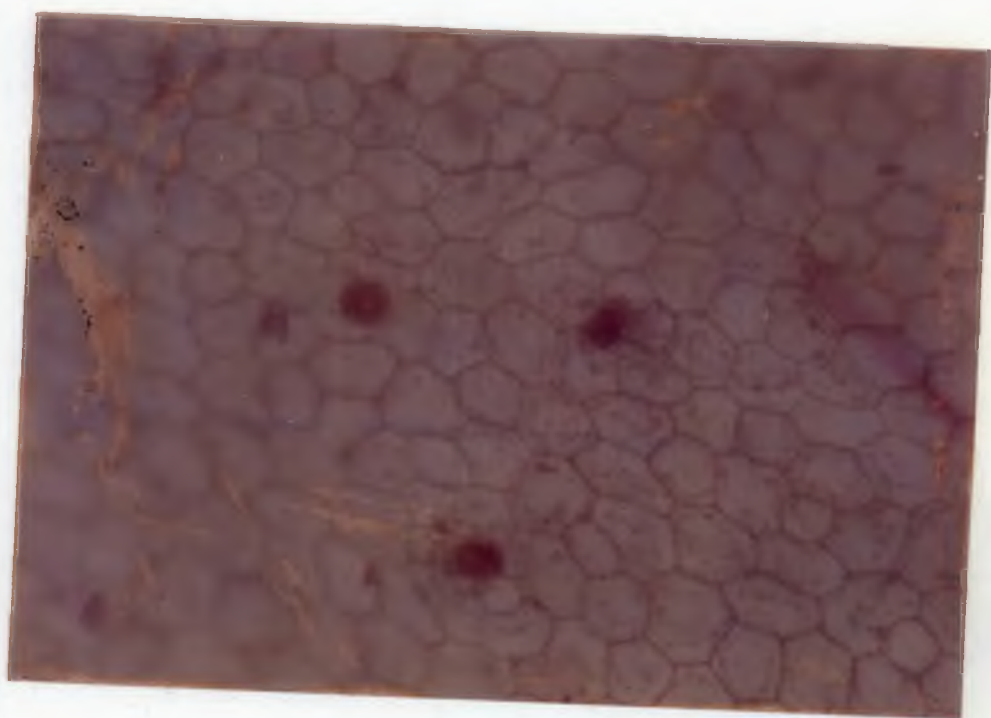
**Table 20c.** Effect of light and humidity treatment (in the hardening chamber) on dry weight of rooted ($S_2$ sucrose level) orchid plantlets at 15 days interval

| Treatment | Light Levels (%) | Humidity Levels (%) | Dry weight (g) | | | Mean |
|---|---|---|---|---|---|---|
| | | | 15 DAP | 30 DAP | 45 DAP | |
| $L_1H_1$ | 25 | < 70 | 0.616 | 0.690 | 0.782 | 0.6960 |
| $L_1H_2$ | 25 | 70 – 90 | 0.513 | 0.678 | 0.786 | 0.6590 |
| $L_2H_1$ | 50 | < 70 | 0.676 | 0.703 | 0.905 | 0.7613 |
| $L_2H_2$ | 50 | 70 – 90 | 0.732 | 0.843 | 0.945 | 0.8400 |
| $L_3H_1$ | 75 | < 70 | 0.545 | 0.693 | 0.811 | 0.6830 |
| $L_3H_2$ | 75 | 70 – 90 | 0.576 | 0.747 | 0.884 | 0.7360 |
| $L_4H_1$ | 100 (Control) | < 70 (Control) | 0.644 | 0.732 | 0.846 | 0.7407 |
| $L_4H_2$ | 100 | 70 – 90 | 0.634 | 0.695 | 0.895 | 0.7413 |
| Normal ($N_3$) | | | 1.026 | 1.072 | 1.153 | 1.0822 |
| Mean | | | 0.6620 | 0.7614 | 0.8897 | 0.7411 |
| CD (1%) | | | 0.015 | 0.018 | 0.018 | |

**Plate 6. a. Abaxial surface of field grown leaf showing more number of stomata per unit area**

**b. Abaxial surface of *in vitro* leaf showing less number of stomata per unit area**





## 4.5 Anatomical Characteristics

### 4.5.1 Stomatal Size (μm)

The data on stomatal size is given in the Table 21. The maximum stomatal width was accounted for *in vitro* leaves (9.60) and Normal leaves recorded minimum value (8.80). The length of opened stomata was found to be highest in Normal leaves (14.20) and lowest *in in vitro* leaves (10.40). However in the hardened leaves stomatal length (9.00) and width (13.20) were found to be intermediate.

### 4.5.2 Stomatal Count

The data on stomatal count (Table 22; Plate 6) reveals that the value is highest in the case of Normal green house grown plants (84.80) and lowest in the case of *in vitro* plantlets (53.30). However the hardened plantlets (72.00) recorded intermediate value.

### 4.5.3 Cuticle Thickness (μm)

A comparative study was made on cuticle thickness of *in vitro* leaves, hardened leaves and leaves of green house grown plants. With regard to cuticle thickness distinct variation was noticed among the different leaves (Table 23;

**Table 21.** Stomatal size of various leaf types of plantlets.

| Treatment | Stomatal Size (μm) | |
|---|---|---|
| | Width | Length |
| *In vitro* leaves | 9.6 | 10.40 |
| Hardened leaves | 9.0 | 13.20 |
| Normal leaves* | 8.8 | 14.20 |
| Mean | 8.33 | 12.60 |
| CD (5%) | 1.45 | 2.21 |

* Normal - Green house grown

**Table 22.** Stomatal count of various leaf types of plantlets.

| Treatment | Number of Stomata |
|---|---|
| *In vitro* leaves | 53.30 |
| Hardened leaves | 72.00 |
| Normal leaves* | 84.80 |
| Mean | 69.93 |
| CD (5%) | 3.681 |

* Normal - Green house grown

**Table 23** Cuticle thickness of various leaf types of plantlets.

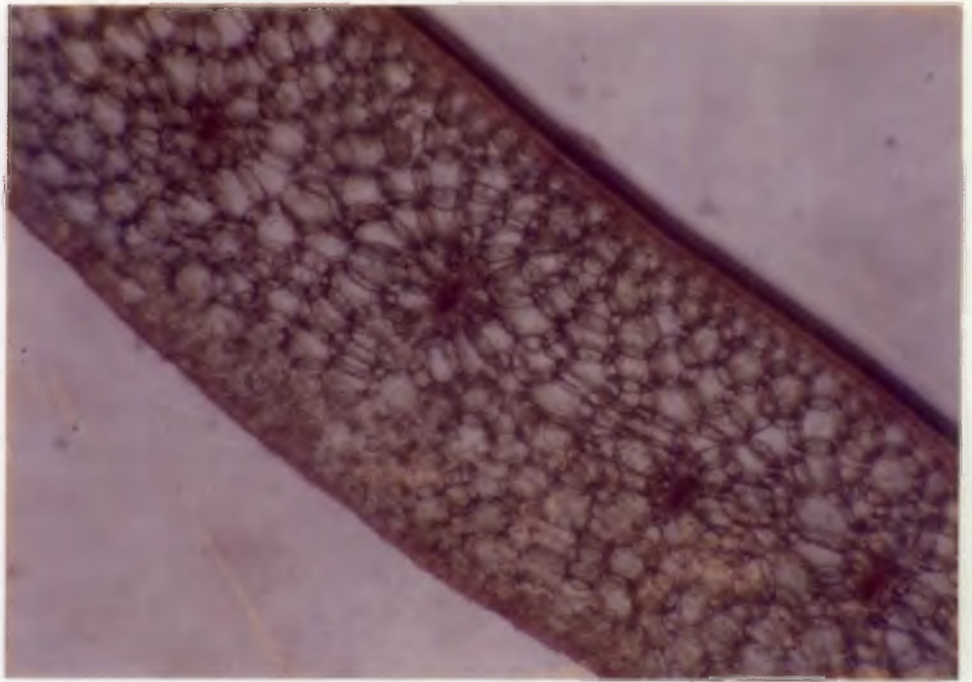
| Treatment | Cuticle Thickness (μm) |
|---|---|
| *In vitro* leaves | - |
| Hardened leaves | 0.5 |
| Normal leaves* | 2 |
| Mean | 1.25 |

* Normal - Green house grown

** Each value is average of 10 replications

**Plate 7. a. Cross section of field grown leaf showing distinct layer of cuticle above the epidermis**

**b. Cross section of *in vitro* leaf showing the absence of cuticle layer above the epidermis**



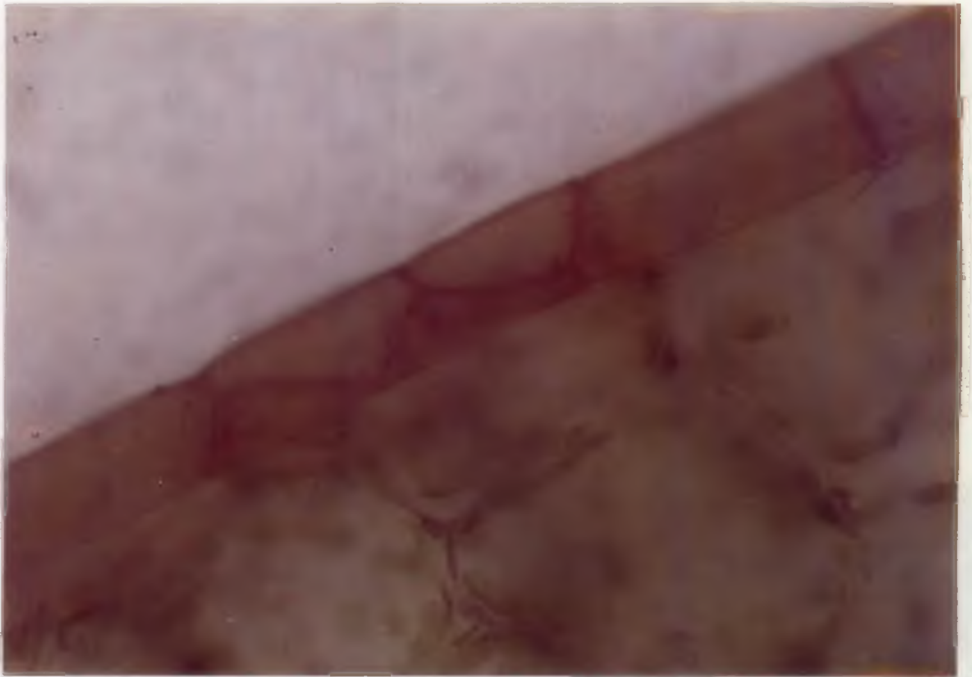


Plate 7). The leaves of tissue cultured plantlets were devoid of cuticle, whereas the Normal plants exhibited distinct cuticle thickness (2μm). However hardened plantlets possessed less layers of cuticle (0.5μm).

### 4.5.4 Number of Mesophyll layers and type of cells.

Maximum number of mesophyll layers were observed in the leaves of Normal green house grown plants (13.60), while the *in vitro* leaves had very less number of mesophyll layers (8.60) and the leaves of hardened plants had more number of mesophyll layers than *in vitro* grown leaves (Table 24).

With regard to the type of cells observed from the cross sectional view of *in vitro* leaves, they possessed chlorenchymatous paranchyma cells and a large number of inter cellular spaces or vacuoles. The ratio of pallisade cells to epidermal cells in the leaves of *in vitro* plantlets was less compared to that of green house grown plantlets. The density of chlorenchyma cells was also more in the leaves of green house grown plantlets. The cross sectional view of *in vitro* leaves also revealed that they exhibited more cytoplasmic content and have flattened chloroplast with irregularly arranged internal membrane.

**Table 24.** Number of mesophyll layers of various leaf types of plantlets.

| Treatment | Number of Mesophyll layers |
|---|---|
| *In vitro* leaves | 8.60 |
| Hardened leaves | 11.60 |
| Normal leaves* | 13.60 |
| Mean | 11.27 |
| CD (5%) | 0.71 |

* Normal - Green house grown

# DISCUSSION

# 5. DISCUSSION

The present investigation on the physiological aspects of *ex vitro* establishment of orchid plantlets was carried out at the Department of Plant Physiology, College of Agriculture, Vellayani during 1997-1999. The outcome of the study is discussed in this chapter. A critical discussion with the background material available from the literature is presented and developed in the following pages to fulfil the objective of study.

Orchids, the super ordinate among ornamental are very distinctive plants. Taxonomically they represent the most highly evolved family (*Orchidaceae)* among monocotyledons, and the largest of all flowering plant families of about 800 genera and more than 35,000 species, (Garay, 1960, and Chadha, 1992). Orchids account for 7 percent of the total species of flowering plants of the world which exhibit an incredible range of diversity in size, shape and colour of plants. The conventional methods of propagation through symbiotic seed germination and division of back bulbs, off shoots and keikis are very slow (Blower, 1964). So the use of micropropagation techniques have revolutionised the commercial orchid industry which made it possible to multiply unlimited number of desirable clones. Most of the commercially grown sympodial orchids belong to genus *Dendrobium* and they perform excellently well in the humid tropical climatic conditions of Kerala.

The *ex vitro* stage of tissue cultured plants is characterised by gradual acclimatization to the *ex vitro* environment and transformation into complete autotrophs, accompanied by anatomical and physiological changes which complement the process. Micropropagation of orchid plantlets is seriously handicapped by the high rate of field mortality during planting out. The present study was taken up with the objective of understanding physiological, morphological, biochemical and biometric changes associated with *in vitro* propagule multiplication and *ex vitro* establishment in tissue cultured plantlets of orchids. This study will help in evolving measures to overcome the field mortality and to improve propagation efficiency, not only of orchids but also of various other crops.

In the present investigation the *in vitro* rooting as well as *ex vitro* establishment of orchid plantlets were found to be influenced by sucrose concentration in the culture medium. It is reported by Corner and Thomas (1982) that lowering the sucrose level in the culture medium is advantageous for the *ex- vitro* establishment as it helps plantlets to switch over from heterotrophic to autotrophic growth. In contrast to this findings Sudeep (1994) reported that better rooting of *Dendrobium* could be obtained with an increase in the glucose level ie, 30.0 g/l. in KC (Knudson, 1946) medium. Ramesh (1990) obtained 50 percent *ex vitro* establishment in jack plantlets when MS medium supplemented with 30.0 g/l of sucrose was used. Sherly (1997) has reported that in Dendrobium, more number of roots as well as increased root

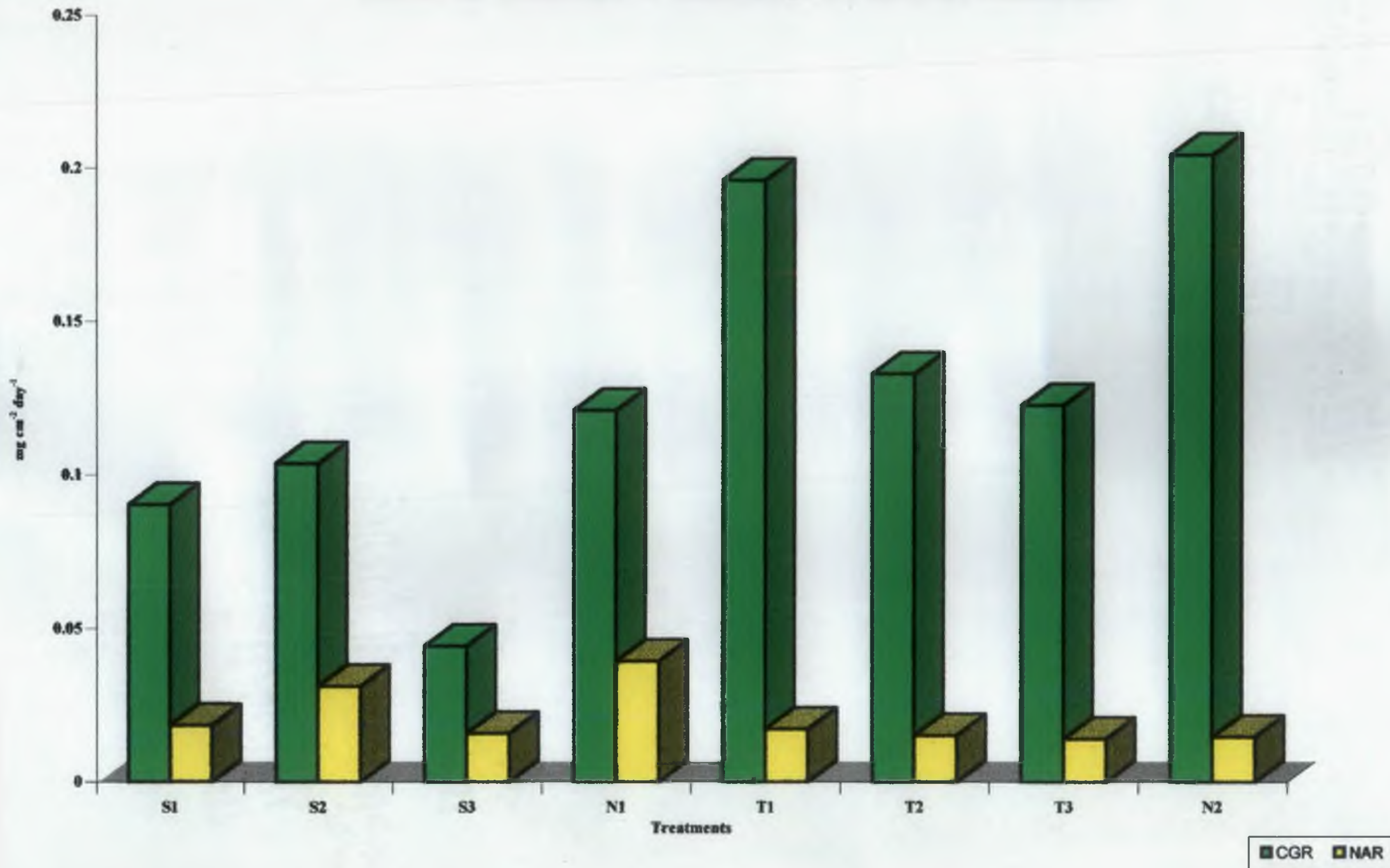
length could be obtained in minimum number of days when half strength of MS medium with 30.0 g/l. of glucose was used for culturing.

In the present investigation different levels of sucrose in the rooting medium were tried out and the half strength MS medium with 40.0 g/l of sucrose was found to perform better. Maximum number of roots per plantlets as well as increased root length could be obtained in this case. This might have helped in better *ex vitro* establishment and better survival percent of plantlets. Collins and Dixon (1992) also reported improved rooting of *in vitro* shoots on increasing sucrose concentration to 40.0 g/l. Increasing the sucrose concentration in the medium resulted in increased utilizable energy available to the plantlets during acclimatization.

**Physiological Characters**

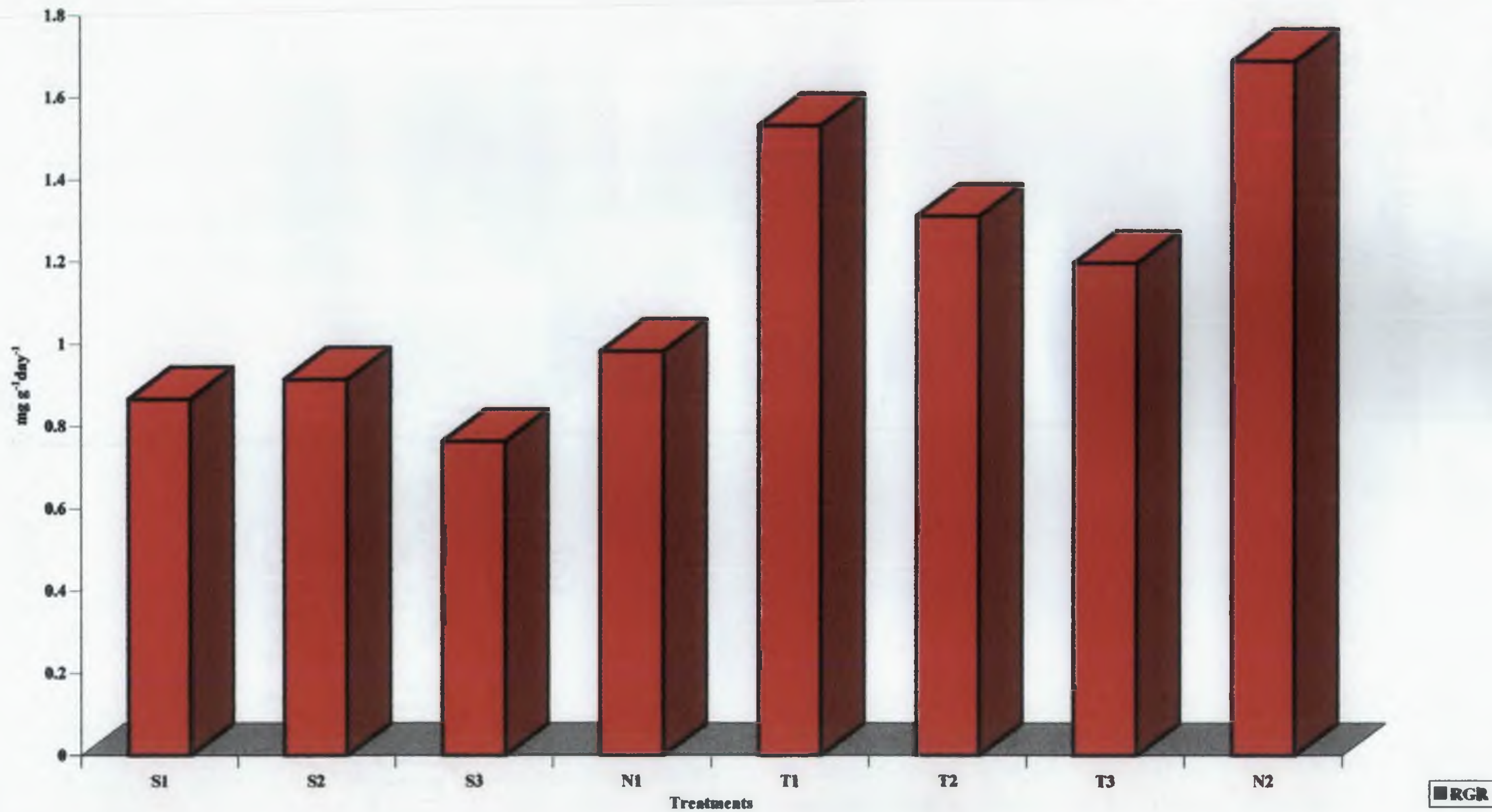
**Crop growth rate (CGR), Net assimilation rate (NAR) and Relative growth rate (RGR)**

Crop growth rate (CGR), Net assimilation rate (NAR) and Relative growth rate (RGR) of orchid plantlets was found to be maximum in the culture media containing 40 g/l level of sucrose (Fig.1 and Fig.2), which was in agreement with the findings of Kubota and Kozai (1991), who studied the effect of sucrose in the medium on the growth of *Cymbiduium* orchids during *in vitro* conditions. According to them higher concentration of sucrose in the medium @ 30 g/l would result in an increase in both the fresh and dry weight.


EFFECT OF SUCROSE (S) & TRIAZOLE (T) ON GROWTH PARAMETERS
0.25
0.2
0.15
0.1
0.05
0
mg cm$^{-2}$ day$^{-1}$
S1
S2
S3
N1
T1
T2
T3
N2
Treatments
CGR
NAR

Also an increase in RGR of cultures was observed. Bangarusamy (1988) reported that CGR, RGR and NAR were high when maximum leaf area development was observed. Increased CGR due to increased leaf area and increased dry matter production might be responsible for the increase in both RGR and NAR. Higher RGR and NAR in turn would have influenced the plantlets for better survival during *ex vitro* establishment.

Triazole was found to have an overall impact on CGR. At all three periods of observation (viz. $P_1$, $P_2$ and $P_3$), the plantlets treated with 5 mg/l of triazole ($T_1$) during planting out were found to exhibit maximum value of CGR. There was much variation in CGR between normal and tissue cultured plantlets during the earlier period (viz. 15 DAP). However towards the later period of the study ie., at 30 DAP and 45 DAP the normal plantlets and tissue cultured plantlets subjected to treatment $T_1$ were found to exhibit higher CGR. With respect to NAR the significance of triazole treatment was well established at 45 days of planting out. The tissue cultured plantlets subjected to the treatment $T_1$ exhibited higher NAR as that of normal plantlets. Plantlets which were not treated with triazole ($T_3$) exhibited very low NAR. With regard to RGR the triazole treatment was found to be significant from 30 DAP onwards. The NAR, CGR and RGR of the plantlets subjected to the treatment $T_1$ were found to be maximum at all three stages of the study. However the control plantlets without any triazole treatment recorded minimum at all stages (Fig.1 and Fig.2). The RGR of normal plantlets was found to be significantly higher than



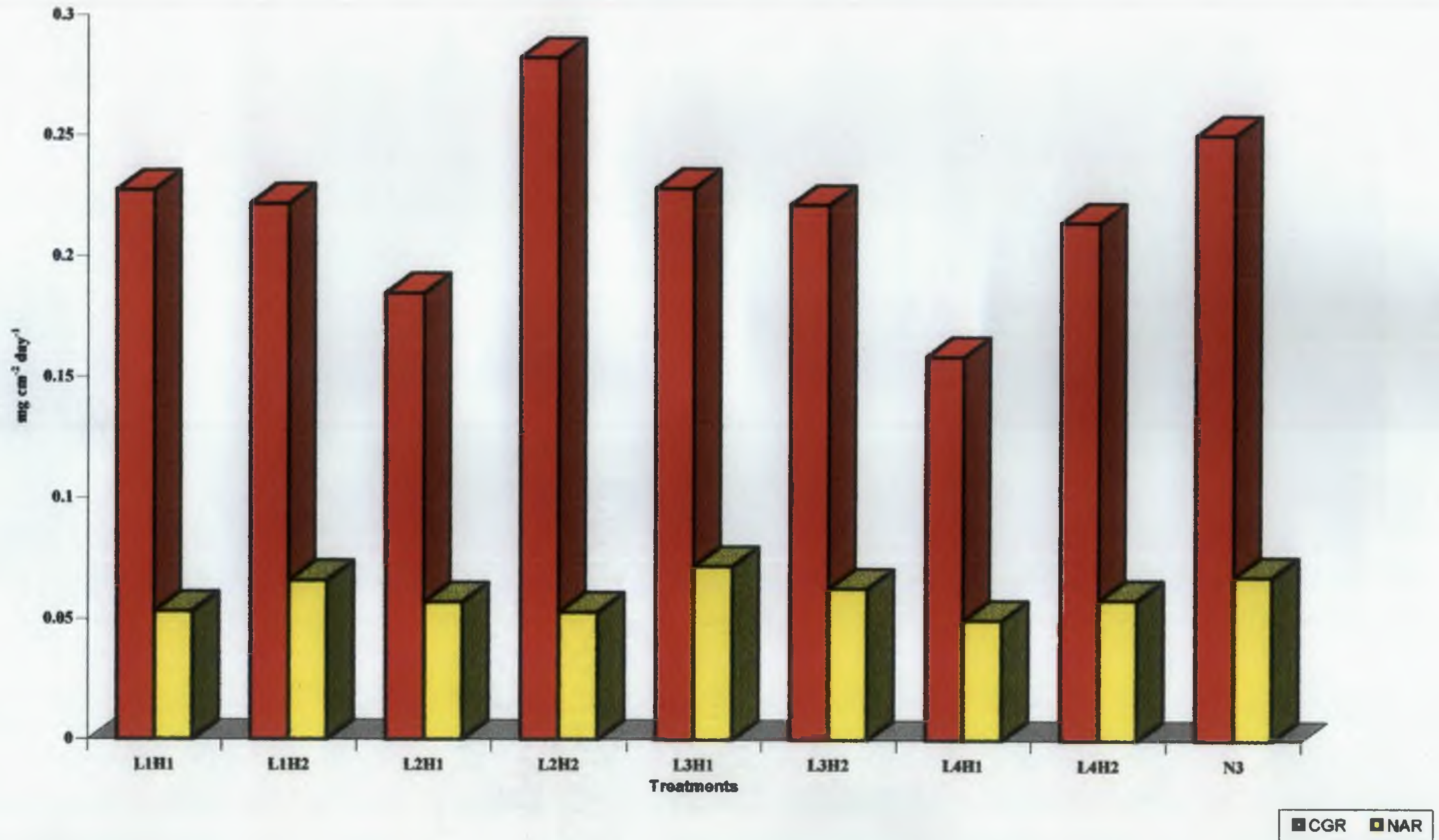
EFFECT OF SUCROSE (S) & TRIAZOLE (T) ON
RELATIVE GROWTH RATE
mg g-1day-1
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
S1
S2
S3
N1
T1
T2
T3
N2
Treatments
RGR

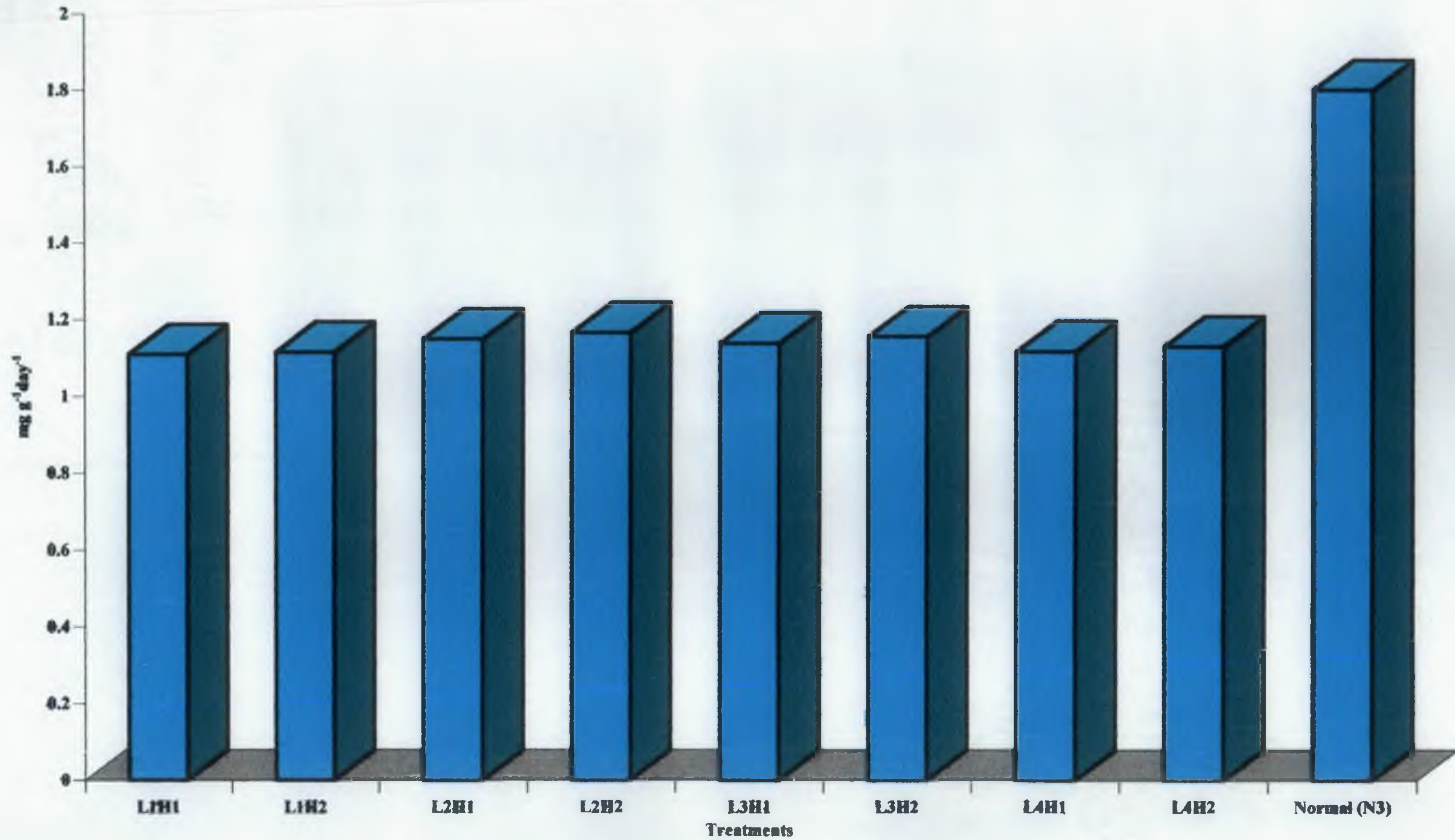
171644

tissue cultured planltes. The mechanism of influence of triazole on these growth parameters (CGR, NAR and RGR) was not clearly understood. However, it might be due to the indirect effect of increase in leaf area per plant although individual leaf size was slightly reduced by triazole application. This view was in accordance with the work of Sairam *et. al.* (1995).They have reported the effect of triazole on tolerance to moisture stress in wheat and observed a significant increase in tillers per plant , green leaves per plant and leaf area per plant in the case of triazole treated plants.

With regard to CGR of plantlets subjected to different light regimes and humidity levels, it was observed that during the initial stages, there was significant difference in CGR values between normal and tissue cultured plantlets. The plantlets subjected to the treatment with 50 percent light and below 70 percent humidity ($L_2H_2$) recorded higher CGR value as that of normal plants towards the later stages (30 DAP and 45 DAP) of the plant(Fig.3).

A similar trend was also observed in the case of NAR (Fig.3). The increased assimilation rate might be attributed to the increase in leaf area. Beringer (1978) stated that growth and yield formation of a crop are mainly based on cell division, cell enlargement and differentiation into assimilating, transporting and storage tissues. The plantlets subjected to high light intensity (100 percent) and less relative humidity (less than 70 percent) performed poorly in all the cases.


EFFECT OF LIGHT & HUMIDITY ON GROWTH PARAMETERS
mg cm-2 day-1
0.3
0.25
0.2
0.15
0.1
0.05
0
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
N3
Treatments
CGR
NAR


EFFECT OF LIGHT & HUMIDITY ON RELATIVE GROWTH RATE
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
mg g-1 day-1
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
Normal (N3)
Treatments
RGR

There was well established difference in RGR levels in the plantlets subjected to different light and humidity condition only during 45 DAP(Fig.4). Galyuon *et. al.* (1996) studied the effect of irradiance level on cocoa and found that leaf area ratio, net assimilation rate (NAR) and relative growth rate (RGR) were reduced under full sunlight. Decreasing light intensities reduced the values of NAR and total chlorophyll content in tomato, chilli and cucumber. (Shaheen *et. al.* 1995). The results of the present investigation are in confirmity with these reports. The inter related effect of high LAI, NAR, RGR and CGR with corresponding leaf area ratio reflected in final leaf yield and its quality (Tripathi and Battacharya, 1985).

**Photosynthetic Rate**

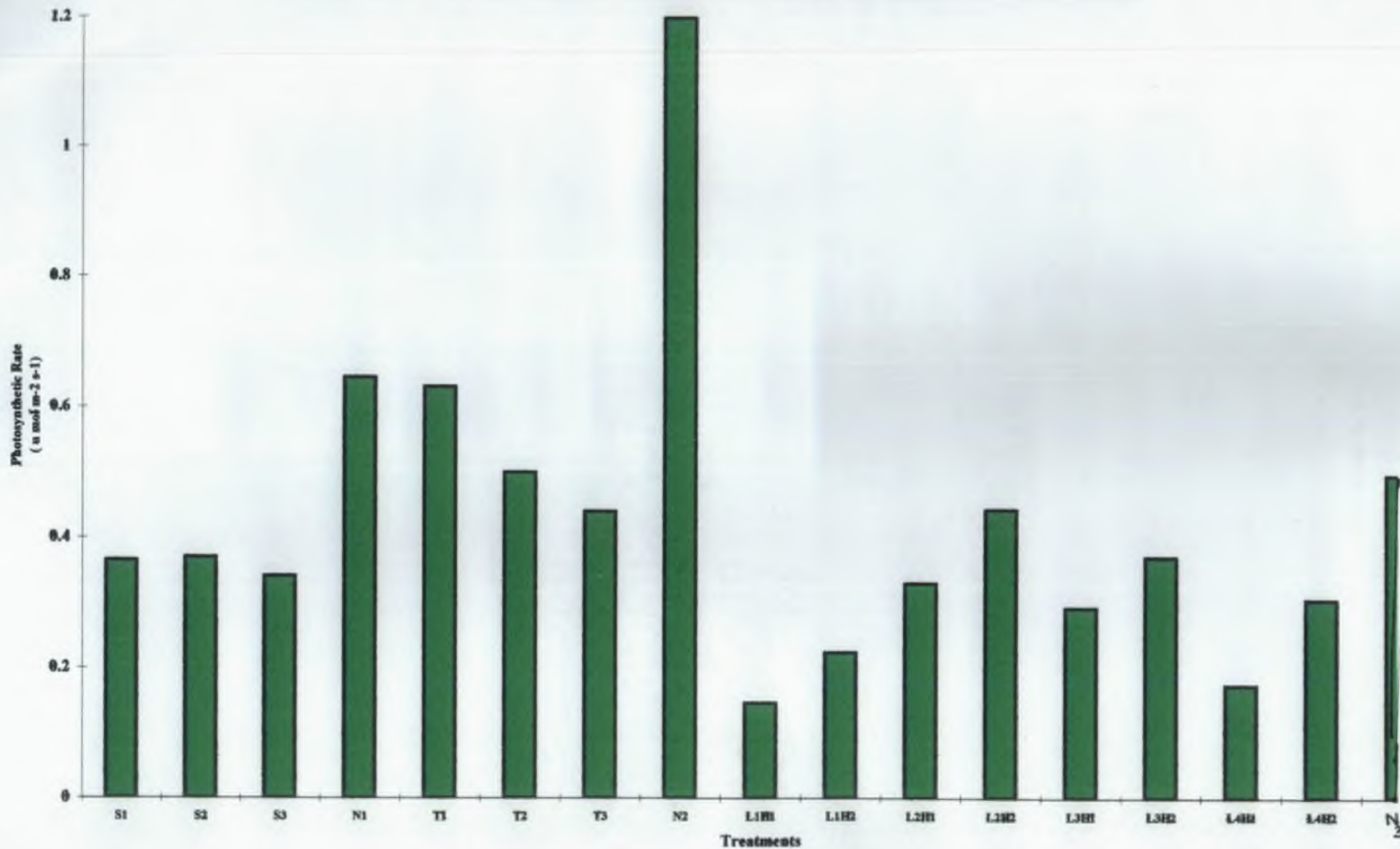
In the present study, the photosynthetic rate was found to increase with an increase in sucrose level in the treatments up to 40.0 g/l and there after a decline was noticed (Fig.5). However the normal plants recorded significantly higher value of photosynthetic rate (0.645). Wainright and Scrace (1989) reported in *Ficus lyrata* that sucrose level should be maintained at a level of 30.0 g/l or even more prior to acclimatization to maximise plant quality. The present finding supports these observations. Photosynthetic rate of the leaves of micropropagated plants was only about 50 percent of the rate found for the leaves of field grown plants. This might be due to the structural difference in the leaves, feed back inhibition from sugars in the medium, lack of $CO_2$ in the vessel and restricted development of components of photosynthetic apparatus.

These results are found to be in conformity with the reports of Mary *et. al.* (1986), Dube and Vidaver (1987) and Yue *et. al.* (1993). According to Hdider and Desjardans (1995 ) the reduced photosynthetic capacity of strawberry plantlets grown *in vitro* with sucrose was due to the reduction in the efficiency of RuBP carboxylase. In contrast, Synkova (1997) observed that during the *ex vitro* establishment of tissue cultured plantlets of *Nicotiana tabacum* cv., those plantlets grown without sucrose in the rooting medium could record higher photosynthetic rate than those grown in sucrose.

The photosynthetic rate of plantlets during *ex vitro* establishment was observed to be increasing from 15 DAP to 45 DAP. At 15 DAP the photosynthetic rate of normal plants was found to be significantly higher than that of the tissue cultured plantlets. But towards the later stage it was observed that the photosynthetic rate of *in vitro* cultured plantlets becomes higher and comparable to that of normal plants. In the earlier stages of *ex vitro* establishment photosynthesis was performed by the *in vitro* formed leaves and they have only lesser photosynthetic activity. The leaves which are formed in *ex vitro* conditions are found photosynthetically more active. This might be the reason for higher photosynthetic rate during the later stages. This result was in line with the report of Donnelly and Vidaver (1984). The treatment triazole, 0.5 mg/l. ($T_1$) shows significant effect on photosynthesis in the later stage(Fig.5). In a study conducted by Rong, *et. al.* (1995), it was found that there was an increase in the photosynthetic rate in the case of rice seedlings

when sprayed with 3ppm of multieffect triazole. Triazoles have direct effect on net photosynthetic rate (Anderson and Aldrich 1987). The present investigation supports all these findings. However the mechanism of this influence was not well understood. It might be probably due to the increase in leaf area and whole plant biomass as compared to the control plantlets. Further research should be carried out in this area to find out the mechanism of influence of triazole on photosynthesis.

It was observed that no significant difference of photosynthetic rate could be obtained during the earlier periods viz. 15 DAP, 30 DAP of light and humidity treatments. However in the later period of study the plantlets under 50 percent light intensity and 70 to 90 percent humidity ($L_2H_2$) recorded higher photosynthetic rate(Fig.5). The photosynthetic rate of green house grown normal plants were found to be higher than that of micro propagated plantlets. Nagoaka *et. al.* (1984) reported that photosynthetic rate and transpiration rate increased linearly with increasing light intensity, whereas with increase in relative humidity there was decline in transpiration rate and increase in photosynthetic rate. In the present study, plantlets under the treatment of 100 percent light intensity and below 70 percent relative humidity showed a marked reduction in photosynthetic rate. The reason attributed for this by Debergh *et. al.* (1995), that at very high light intensity the photosynthetic rate is reduced due to photooxidation of chlorophyll. Smith *et. al.* (1986) suggested that the *in vitro* plantlets are having diminished stature and reduced cell size as well as


EFFECT OF SUCROSE, TRIAZOLE, LIGHT & HUMIDITY ON PHOTOSYNTHETIC RATE
Photosynthetic Rate
( u mol m-2 s-1)
1.2
1
0.8
0.6
0.4
0.2
0
S1
S2
S3
N1
T1
T2
T3
N2
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
N3
Treatments

reduced vascular tissue, which account for the reduced rate of photosynthesis at the earlier stage of *ex vitro* establishment. Yue *et. al.* (1993) reported that high relative humidity reduced the extent of leaf damage and chlorophyll damage during the acclimatization of *Eucalyptus* plants. This resulted in a high net photosynthetic rate when relative humidity is high. The present investigation was also in confirmation with this point. Lee *et. al.* (1985) reported the effect of light acclimatization on photosynthetic activity of foliage plants and concluded that plants grown under 80 percent shade showed higher photosynthetic rate than those grown under 40 percent shade. However present investigation does not support this observation.

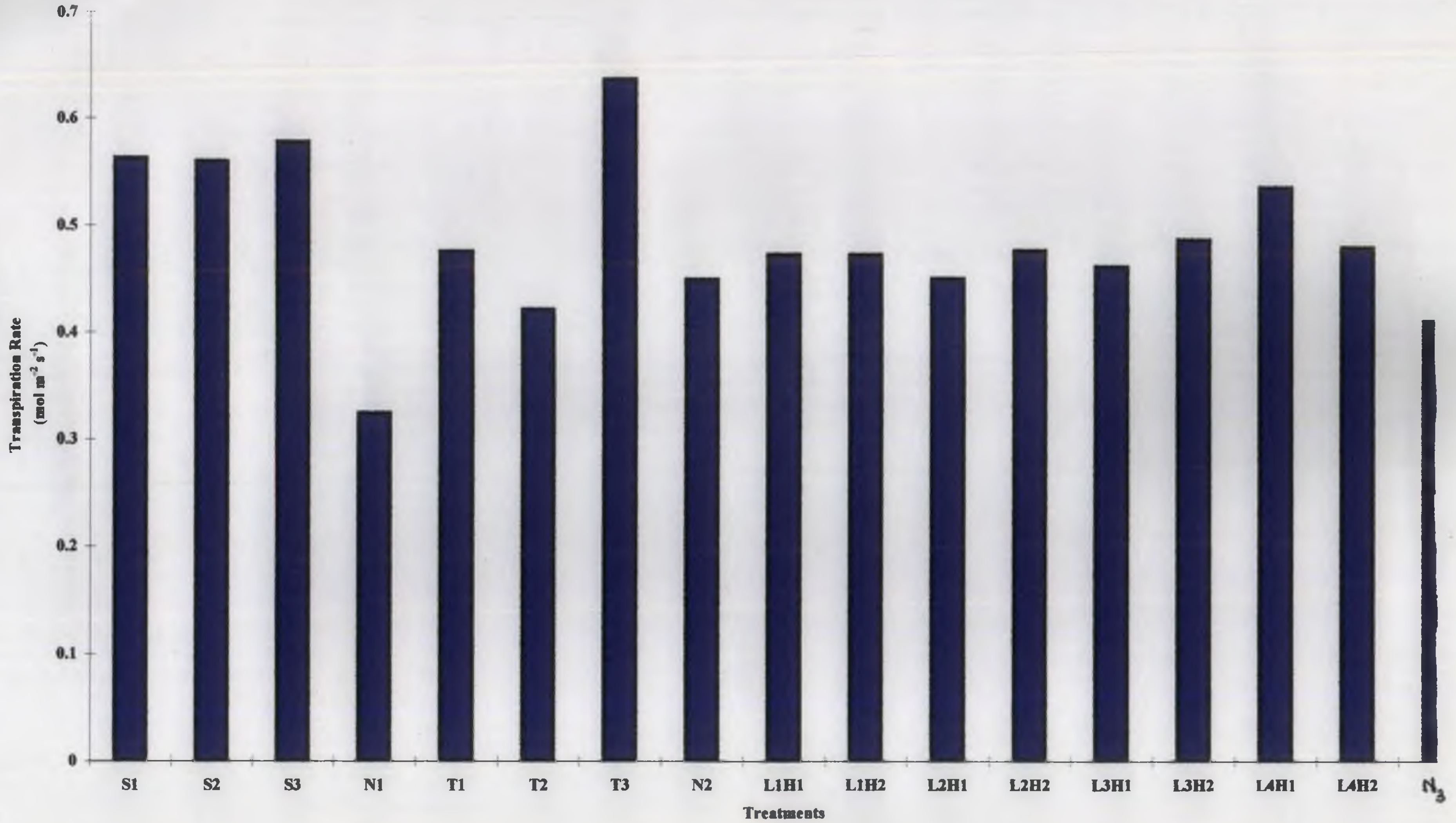
**Transpiration rate**

In the present study transpiration rate was observed to be significantly higher in the case of *in vitro* plantlets with sucrose in the rooting media as compared to the field grown plants. Sucrose at the level 60.0 g/l ($S_3$) recorded higher value and was on par with the plantlets grown at 20.0 g/l ($S_1$) and 40.0 g/l ($S_2$) of sucrose (Fig.6). De-Rick and Huylenbroeck (1982 ) reported higher transpiration rate of plantlets coming from media with higher sucrose concentration. The present study was in agreement with the above result. The transpiration rate of *in vitro* plantlets was very much higher due to the morphological peculiarity of *in vitro* formed leaves. Desjardins *et. al.* (1985) reported on photosynthesis and transpiration of *in vitro* cultured Asparagus plantlets and they observed high rate of transpiration in these plantlets during

the period of acclimatization and opined that high rate of water loss incurred by *in vitro* shoots imposes severe limitation in acclimatization.

The transpiration rate of plantlets with triazole 5.0 mg/l. was found to be minimum and was comparable with that of normal plants at all the three periods of analysis (Fig.6). The *in vitro* plantlets subjected to no triazole ($T_3$) exhibited higher rate of transpiration. Thus it was evident that triazole had a role in reducing the transpiration rate of tissue cultured plantlets, which in turn resulted in better acclimatization. Lurssen and Reiser (1985) reported a positive effect of triazole in reducing transpiration. However, the effect was only temporary. Sairam (1995) reported that a triazole derivative triadimefon reduced transpiration and increased relative water content and membrane stability in wheat under moisture stress.

Eliasson and Beyl (1993) studied the effect of triazole on *in vitro* rooting and acclimatization of *Prunus serotina* and observed that the transpiration rate of tissue cultured plantlets subjected to different regimes of light and humidity levels and was found to be significantly higher at the earlier period of study viz. 15 DAP and 30 DAP. The plantlets subjected to 100 percent light intensity and below 70 percent relative humidity ($L_4H_1$) exhibited very high rate of transpiration (Fig.6). On the otherhand the plantlets subjected to 50 percent light intensity and below 70 percent humidity ($L_2H_2$) exhibited lesser transpiration rate than normal plantlets. These results clearly indicate that *in*


EFFECT OF SUCROSE, TRIAZOLE, LIGHT & HUMIDITY ON TRANSPIRATION RATE
Transpiration Rate
(mol m-2 s-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
S1
S2
S3
N1
T1
T2
T3
N2
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
N3
Treatments

*vitro* leaves of tissue cultured plantlets subjected to high light intensity and low humidity levels are exhibiting higher transpiration losses during initial period of development. But towards the later stages the *ex vitro* formed leaves become acclimatized to the new environment, thus exhibited less transpiration rate as that of normal plants. Leonardi *et. al.* (1996) studied the effect of shading on green house grown pepper and suggested that shading reduced the transpiration rate. Nobuoka *et. al.* (1996) observed an increase in transpiration rate with increasing light intensity in tomato. Shimizu *et. al.* (1997) reported an increase of transpiration rate in maize when subjected to low relative humidity.

**Plant height**

Significant increase in shoot length was achieved during *in vitro* rooting when the medium was supplemented with different levels of sucrose. *In vitro* plantlets rooted at 40.0 g/l of sucrose ($S_2$) recorded higher plant height than at 20.0 g/l and 60.0 g/l sucrose level. As compared to tissue cultured plantlets the normal plants were found to have maximum plant height. Sunithibala *et. al.* (1998) reported that sucrose @ 10-30.0 g/l resulted in an increased plant height of micropropagated teak shoots Rahman *et. al*, (1992) reported that at sucrose level of 40.0g/l, the increment in shoot length is higher as compared to 20.0 and 60.0 g/l of sucrose in the case of *in vitro* grown rose plantlets. Schnapp and Preece (1986) observed a decline in shoot and root growth with lower level (5.0 g/l) of sucrose and the growth of shoot and root was found to be maximum at

30.0 g/l of sucrose. The review materials discussed provided ample support to the results obtained in this regard.

According to Elaisson and Beyl (1993), paclobutrazol - a triazole growth retardant is found to harden *in vitro* cultured *Prunus serotina.* At 0.6 mg/l level it was found to reduce the shoot length significantly. Menhenett (1984) reported that paclobutrazol @ 10 - 30 mg/l was found to be effective in controlling stem extension in the case of chrysanthemum. In the present study a decrease in plant height was noted with increase in concentration of triazole. Plantlets grown as control with no triazole recorded maximum plant height. Steeffens and Wang (1985) observed a reduction in plant height of apple plantlets as compared to control plantlets when treated with triazole. The present study is in confirmation with this report. In contrast to this finding, Rong *et.al.* (1995) observed increased seedling height of green house grown rice when a multi effect triazole was applied.

In the present study the plantlets which were subjected to the treatment of 50 percent light and below 70 percent humidity ($L_2H_2$) had maximum plant height. In the earlier period of the study viz. 15 DAP, the normal plants performed significantly better than tissue cultured plantlets. But towards the later stage plantlets under the treatment $L_2H_2$ were found to be comparable with the normal plants. The minimum plant height was observed in plantlets subjected to full sunlight ($L_4H_1$ and $L_4H_2$). Galyuon (1996) observed a

reduction in leaf size, leaf area, shoot length and dry matter per plant in full sunlight. Leonardi *et. al.* (1996) studied the effects of shading on green house grown pepper and found that shading increased plant height and reduced transpiraton and stomatal density. The present result supports these reports.

**Root Length :**

Ault (1994) reported a reduction in root length when sucrose concentration in the rooting medium was reduced from 50 to 25 g/l. The present investigation also showed the same trend and the maximum root length was reported at 40.0 g/l of sucrose ($S_2$) and minimum value at 20.0 g/l ($S_1$). The root length of field grown plants was found to be on par with that of $S_2$.

The plantlets subjected to 5 mg/l of triazole treatment ($T_1$) showed highest root length whereas the plantlets subjected to no triazole treatment ($T_3$) showed lesser root length. As such the normal plants recorded higher root length than tissue cultured plantlets at all stages. According to Elaison and Beyl (1993), triazole @ 0.6 mg/l could result in reduced shoot growth and shorter, thicker and more number of roots. The result of the present investigation were not in line with this report.

During the earlier periods of study viz. 15 DAP and 30 DAP root length of normal plants was significantly higher than that of tissue cultured plantlets subjected to various light and humidity treatments. But during the later stage ie 45 DAP, the length of roots produced by the micropropagated plantlets

subjected to 50 percent treatment of light and less than 70 percent humidity ($L_2H_2$) was on par with that of normal plants. This may be due to the fact that towards the later stage the plantlets acclimatized to the new environment. At high light intensity there is a tendency to produce increased root growth resulting in increased plant size (Tester *et. al*, 1993; Wiebe, 1984 and Veierskov, 1985). The result of the present study also corroborates the findings of the above workers. In contradiction to the present result, Sanden (1985) reported a reduction in root length at constant high humidity.

**Number of Leaves**

In the present investigation plantlets subjected to 40.0 g/l level of sucrose ($S_2$) produced maximum number of leaves among treatments which was on par with the normal plants. The high rate of photosynthesis of $S_2$ might be contributed by the increased number of leaves. The triazole treatment was found to have no effect on number of leaves. The plantlets subjected to the treatment, 5 mg/l of triazole ($T_1$) produced more number of leaves. But the normal plants produced significantly higher number of leaves than tissue cultured plantlets.

In all the three periods of analysis of the plantlets under light and humidity treatments, the number of leaves of tissue cultured plantlets was found to be comparable to that of normal plants. Among treatments , the plantlets subjected to 50 percent light level and less than 70 percent humidity ($L_2H_2$) as well as those subjected to 25 percent light and less than 70 percent of

humidity ($L_1H_2$) produced more number of leaves than others. Mageed *et. al.* (1998) observed an increase in the number of leaves, shoot length and dry matter in mandarin orange with an increase in humidity level. A similar trend was noticed in the present study also.

**Number of roots**

In the present study when 40.0 g/l ($S_2$) was incorporated in the rooting medium more number of roots were produced. Harvey *et. al.* (1994) reported in potato that at high sucrose concentration (0.234M) an increasing trend in number of root and fresh weight of root was observed at the *in vitro* condition . Rahman *et. al.* (1992) reported that sucrose at 40.0g/l. gave significantly more number of roots, higher rooting frequency and higher root length as compared to 20.0 g/l and 60.0 g/l during *in vitro* rooting of rose. Earlier works of Shibli *et. al.* (1990) and Tsay *et. al.* (1989) also revealed similar facts.

The effect of triazole on number of roots showed a similar trend at 15 DAP and 45 DAP. The plantlets subjected to the treatment 5 mg/l. of triazole ($T_1$) had more number of roots and was on par with that of normal plants. The plantlets with no triazole ($T_3$) produced minimum number of roots. Elaisson and Beyl (1993) viewed that paclobutrazol, a triazole derivative could produce shorter, thicker and numerous roots in *Prunus* cultured *in vitro* . ABT (1 amino benzo triazole) was reported to have the capacity of improving root primordium differentiation, root growth and number of roots of *Gerbera*

*jamesoni* (Yin-Liqing, 1996). Similar observations were also made by Rong *et. al.* (1995). The present study was in line with these reports.

Gannoun *et. al.* (1996) based on their studies on sucker proliferation and rooting *in vitro* of *Musa acuminata,* reported that very less number of roots were found at the condition of low light intensity, while intermediate light intensity favoured more root formation and dry matter accumulation. In the preset study the intermediate light intensity level of 50 percent light and below 70 percent humidity ($L_2H_2$) favoured better rooting than the lowest light intensity level of 25 percent light and below 70 percent humidity ($L_1H_1$)

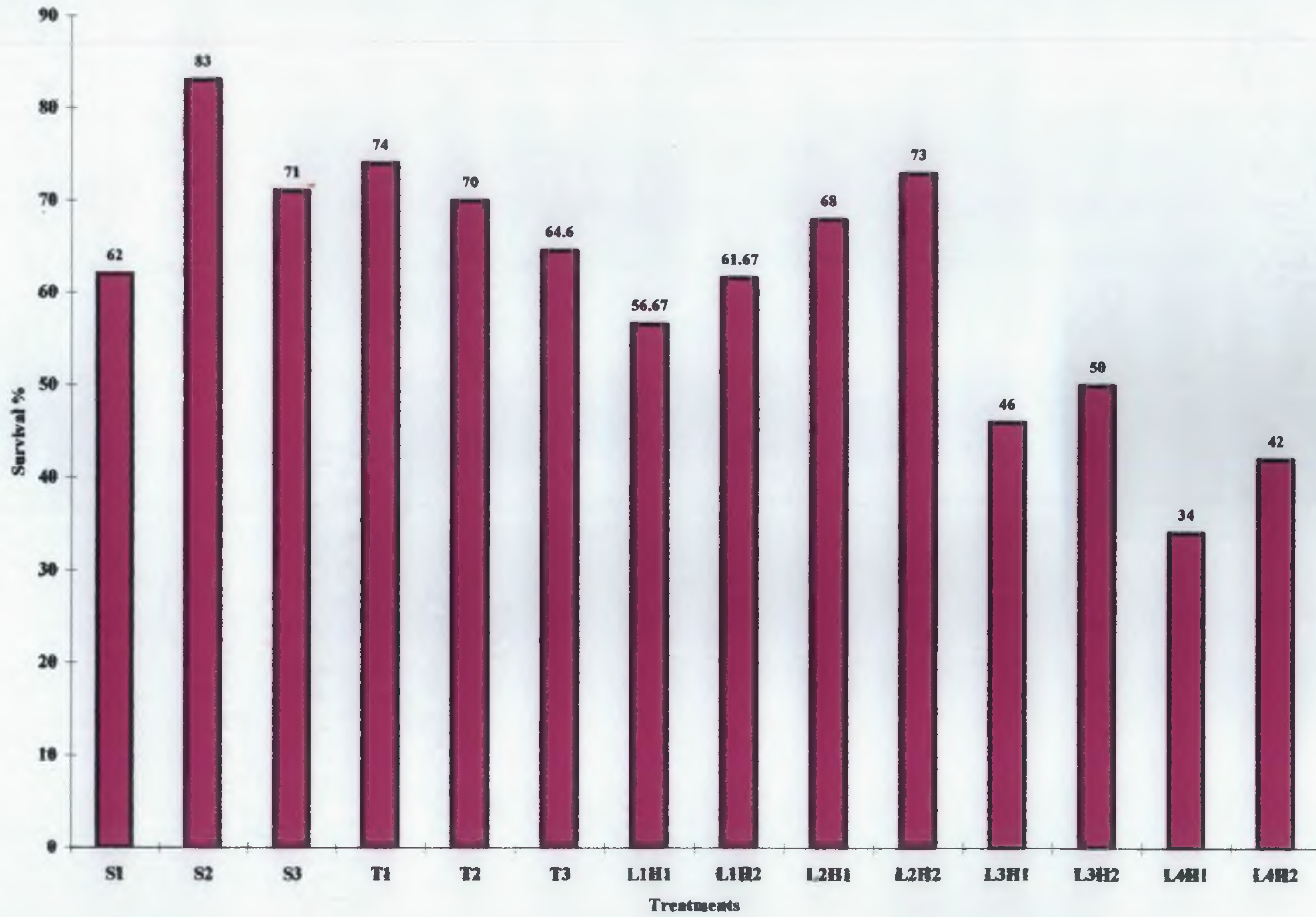
**Survival Percent**

In the present study maximum survival percent was noted in the plantlets subjected to 40 g/l. of sucrose ($S_2$) treatment compared to the control plantlets (20 g/l of sucrose) and shown in Fig.7. Berlrand and Noirot (1992) reported that the survival percent of *in vitro* cultued coffee plantlets was lesser at reduced sucrose levels (5 to 20 g/l.) in the culture media. Seneviratne (1996) reported in a shoot culture study of Hevea that better survival percent could be obtained in the media supplemented with varying sucrose concentration up to 10 % than in a medium with no sucrose. Huang *et. al.* (1995) reported that increased sucrose level to 3 percent could enhance the survival percentage of *in vitro* plantlets of apple. These results were found to be in agreement with the present investigation where better rooting was observed in the treatments with

40 g/l. of sucrose and this could have been the reason for better survival percent.

According to Elaisson and Beyl (1993), triazole treatment could enhance *in vitro* rooting and acclimatization of *Prunus sp.* favour better survival percent. Yin-Liquing (1996) reported 100 percent rooting and 98.9 percent survival by the application of ABT, a triazole derivative on Gerbera. The present investigation, where triazole treatment @ 5 mg/l. ($T_1$) and 10 mg/l. ($T_2$) recorded higher survival percentage than control plantlets with no triazole treatment was in confirmation with these reports (Fig.7).

With regard to plantlets subjected to different levels of light and humidity, higher survival percent was observed in the plantlets subjected to 50 percent light and below 70 percent relative humidity ($L_2H_2$) whereas at a still higher light intensity (100 percent light ), $L_4H_2$ recorded lesser survival percentage (Fig.7). Miranda and Fernandez (1995) reported that the acclimatization of *in vitro* produced chesnut plantlets involved a gradual reduction in relative humidity and recorded high survival percentage. The studies carried out by Kirdmanee *et. al.* (1995) also support to the present investigation. The increased survival percentage might be due to the high rooting percentage, high rate of photosynthesis and more dry matter accumulation.

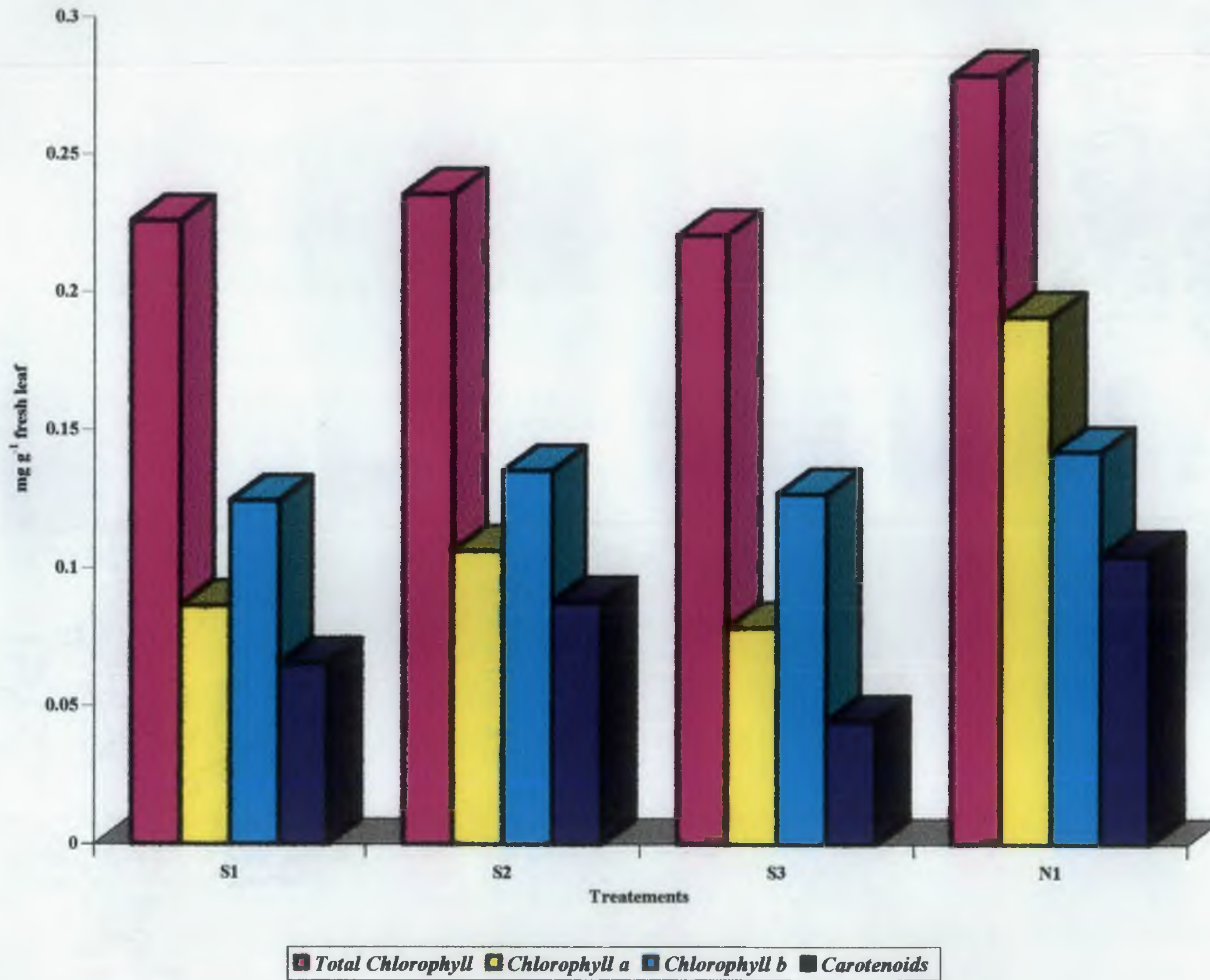

EFFECT OF SUCROSE, TRIAZOLE, LIGHT & HUMIDITIY ON SURVIVAL PERCENT
Survival %
0
10
20
30
40
50
60
70
80
90
62
83
71
74
70
64.6
56.67
61.67
68
73
46
50
34
42
S1
S2
S3
T1
T2
T3
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
Treatments

**Photosynthetic pigments (Total chlorophyll, Chlorophyll a, chlorophyll b and Catotenoids)**

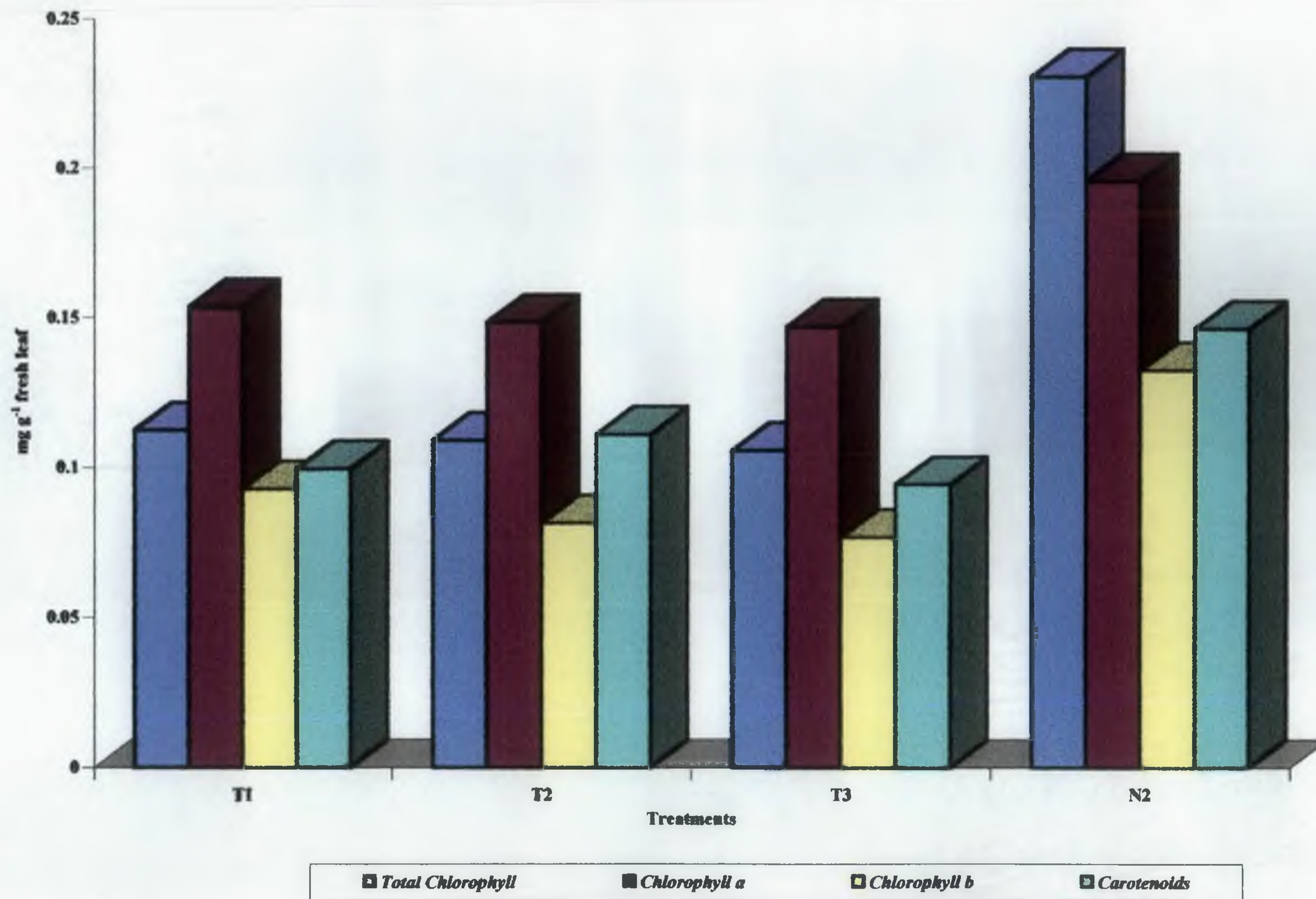
In the present investigation, incorporation of sucrose in the rooting media was found to have positive influence on the enhancement of photosynthetic pigment content. Plantlets subjected to 40 g/l of sucrose ($S_2$) performed better with regard to all photosynthetic pigments (Fig.8). In the case of chlorophyll a, the content was comparable in all the treatments. This result is in confirmity with the following results ie. Sunitibala (1998) observed an increase in chlorophyll a, chlorophyll b and total chlorophyll content of micropropagated teak plants when the medium was supplemented with 30 g/l. of sucrose; Eason (1997) opined an increase in catotenoid content in lilaceous cut flower with the presence of sucrose (2%) than that of the control; Synkova (1997) suggested that the positive effect of sucrose on photosynthetic pigment content in *Nicotiana tabacum* might be due to the improvement of continuous rising of photo-chemical efficiency of PSII, increase in the number of chloroplasts and starch grains which they contained.

With regard to plantlets treated with triazole at the time of planting out, the plantlets subjected to 5 mg/l of triazole treatment ($T_1$) showed higher value for all the photosynthetic pigments (Fig 9). However normal plants were found to have significantly higher content of photosynthetic pigment than tissue

**EFFECT OF SUCROSE ON PHOTOSYNTHETIC PIGMENT CONTENT**

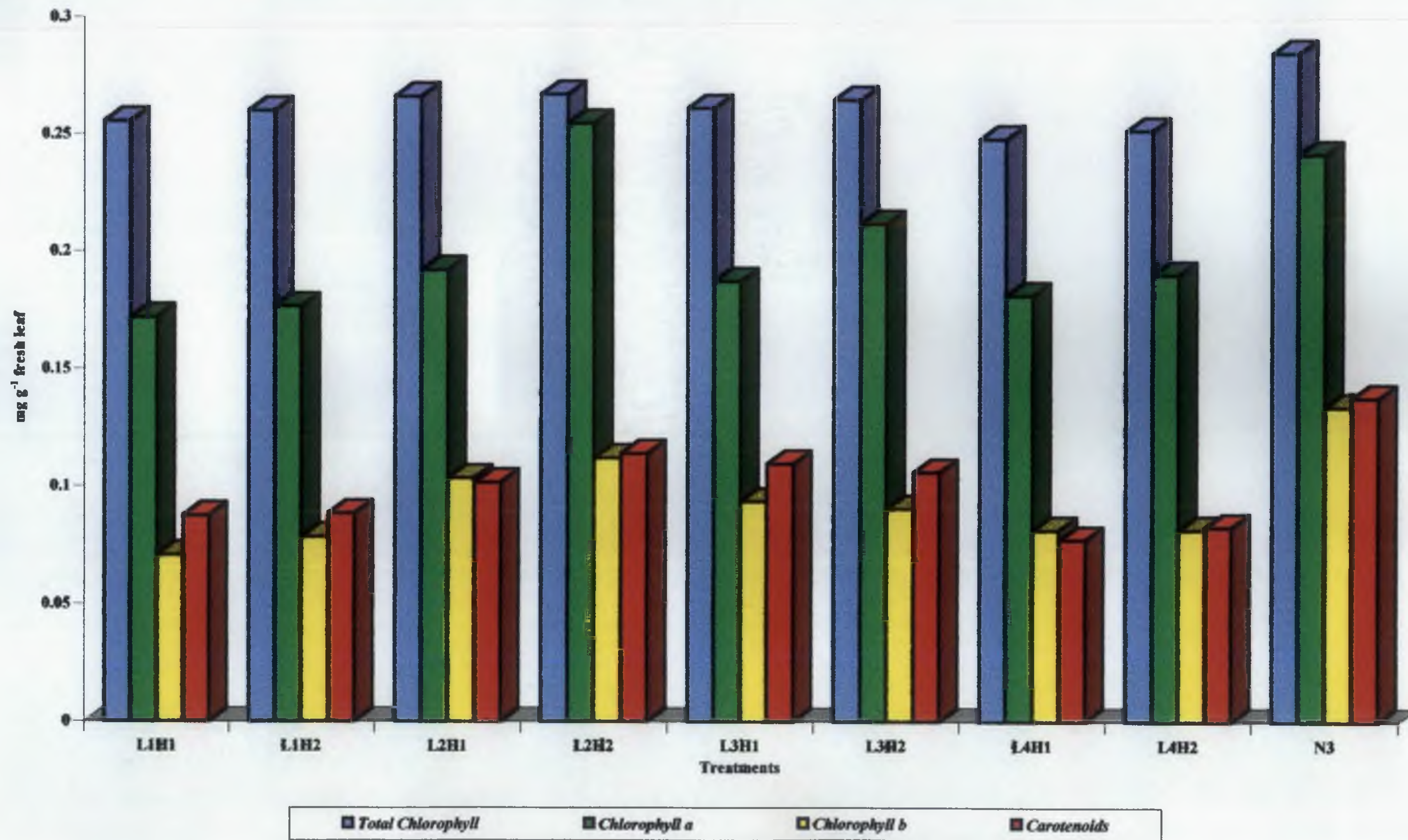


**EFFECT OF TRIAZOLE ON PHOTOSYNTHETIC PIGMENT CONTENT**



cultured plantlets. Thomas and Singh (1995) reported a marked enhancement in the accumulation of chlorophyll and carotenoid pigments by triazole treatment in cucmber cotyledons. Wang *et. al*, (1995) studied the effect of a triazole derivative on growth of wheat seedlings and observed a positive correlation between the application of uniconazole (a triazole derivative) and the chlorophyll content. Bachenauer *et. al.* (1984) studied the effect of various triazole derivatives on growth of barley seedlings. According to them the primary leaves of the treated plantlets contain more amount of chlorophyll on leaf area basis. However, the influence of triazole on pigments is not well understood. Further research in this field is needed.

In the present study there was no significant effect of light and humidiity levels on total chlorophyll content during initial stages. But towards the later stage the plantlets subjected to 50 percent light ($L_2H_1$ and $L_2H_2$) and 70 percent light ($L_3H_2$) maintained higher total chlorophyll content which is comparable to that of normal plantlets. Regarding chlorophyll a plantlets subjected to 50 percent light intensity and 70-90 percent humidity ($L_2H_2$) maintained a higher value. The normal plants maintain a distinctively higher value of chlorophyll a than tissue cultured plantlets. In the case of carotenoids also treatment with 50 percent light and 70-90 percent humidity ($L_2H_2$) was having a higher value(Fig.10). A decline in all the four photosynthetic pigments studied was observed under high light conditions (100 percent) and at low light conditions (25 percent) , invariably during all the three stages of study. Lee *et. al*, (1985)


EFFECT OF LIGHT & HUMIDITY ON PHOTOSYNTHETIC PIGMENT CONTENTS
0.3
0.25
0.2
0.15
0.1
0.05
0
mg g-1 fresh leaf
L1H1
L1H2
L2H1
L2H2
L3H1
L3H2
L4H1
L4H2
N3
Treatments
Total Chlorophyll
Chlorophyll a
Chlorophyll b
Carotenoids

reported that the chlorophyll content in the leaves of *Liquidamber styraciflua* was significantly reduced at highlight intensity and suggested that at high light intensity starch granules were dominent and associated with disrupted granal structure in the chloroplast. Atkhalifah and Alderson (1995) observed increased chlorophyll content in micropropagated *Ficus benjamina* at low light intensity.. Janous *et. al*, (1996) stated that at high relative humidity (85 ± 5 percent ) the chlorophyll content was found to be higher than at low relative humidity (55+5%). These observations were in agreement with the investigations carried out.

**Carbohydrates**

With regard to different levels of sucrose supplemented in the medium, 40 g/l($S_2$) was found to have maximum influence on carbohydrate content. The carbohydrate content of normal plants was found to be distinctly high. Carbohydrate content of plantlets treated with 5 mg/l of triazole ($T_1$) was found to be maximum during all the three stages of analysis.

In the present study, the carbohydrate content of the plantlets under the treatment of 75 percent light and below 70 percent relative humidity ($L_3H_2$) was maximum. However, in the normal plants carbohydrate content were found to be very high. Plantlets under the treatment of light ie., 25 percent light intensity ($L_1H_1$) had very less carbohydrate content. Shaheen *et. al*, (1995) studied seedling production of some vegetables under plastic houses at

different levels of light intensity and reported that under increasing light intensity total carbohydrate concentration was increased. Fukuoka *et. al*, (1996) suggested that rooting ability of cabbage seedlings after transplanting to a nursery depends largely on carbohydrate content in the roots. At low light intensity conditions, carbohydrate content causes a decrease in activity of respiratory enzymes. Diminished photosynthesis and lowered enzyme activities inhibit root respiration. Consequently a large amount of energy is consumed for basal metabolism for survival, which detracts from root formation after planting. The carbohydrate content was observed to be reduced in the case of *in vitro* plantlets of *Gypsophila paniculata* at high relative humidity of 97 percent (Hee, 1995).

**Protein content**

According to Fevereiro *et. al.* (1986), with the increase in sucrose concentration from 30 to 40 g/l, there was an increase in the intra-cellular protein production to about 60 percent in the case of callus and suspension culture of *Silybum marianum*. The present result where the treatment with 40 g/l ($S_2$) recorded higher protein content than other treatments which was in confirmity with the report.

Sairam (1995) reported an increase of protein content, chlorophyll content and yield in the case of triazole treated wheat. Treatment of a

multieffect triazole had resulted in increased seedling height, root number and protein content in rice seedlings ( Rong *et. al.*, 1995).

In the present investigation the plantlets which were subjected to 75 percent light and 70 to 90 percent humidity level ($L_3H_2$) had higher protein content than other treatments. This was comparable with most of the treatments. But the normal plantlets were found to have a distinctly higher amount of protein. At lower light intensity (up to 75 percent light), protein content is found to be higher and at light intensity beyond 75 percent level, a marked reduction in protein content was found. Kaixian and Borowitzka (1993) reported that the percent of total protein fraction is negatively correlated with irradiance and it is the result of increase in the cell content of other fractions particularly lipids. Arad *et.al.* (1986) suggested that under low light intensity, cellular metabolism is directed towards protein accumulation and under high light intensity cells direct their energy towards accumulation of starch and cell wall polysacchrides.

**Leaf area**

In the present study incoporation of sucrose at the rate of 40 g/l. ($S_2$) in the media was found to increase the leaf area of plantlets to a greater extent and any further increase in sucrose beyond this level was found to reduce the leaf area. Triazole was found to have an overall impact on leaf area. The plantlets treated with 5mg/l of triazole ($T_1$) during plantout was found to exhibit

maximum value of leaf area and was on par with normal plants. A significant increase in the leaf area and chlorophyll content of the plant was observed in the case of wheat plantlets treated with the triazole derivative - triamedefon by Sairam *et. al.* (1995). Which offer a clear support to the present investigation.

Galyuon *et. al.* (1996) reported that in cocoa, the leaf size, total leaf area and dry matter per plant were significantly reduced in full sunlight. In support of this view, in the present investigation also the plantlets subjected to the treatment with 100 percent light levels ($L_4H_1$ and $L_4H_2$) recorded lesser leaf area. On the other hand the plantlets subjected to 50 percent light intensity and below 70 percent humidity ($L_2H_2$) recorded maximum leaf area and was found comparable to the normal plants. Perez *et. al.* (1995) observed that generally the leaf area of *in vitro* produced leaves of micro propagated apple plants was less than those of field grown plants. This view is true with the present study also.

**Root: Shoot Ratio**

In the present investigation the root shoot ratio increased with increasing concentration of sucrose and sucrose @ 60 g/l ($S_3$). recorded maximum value. Root :shoot ratio of normal plants was on par with $S_3$.

The effect of triazole on root: shoot ratio was well established only during the later stage ie., 45 DAP. The root: shoot ratio of plantlets subjected

to 20 g/l triazole treatment ($T_1$) was on par with normal plants at later period. The plantlets without triazole treatment ($T_3$) exhibited a poor performance. This result clearly indicates that triazole treatment has got positive effect on root: shoot ratio.

In the present study the root: shoot ratio of plantlets subjected to different regimes of light and humidity shows statistical significance at all levels of treatment. Among them plantlets subjected to 75 percent light intensity and below 70 percent humidity showed maximum root: shoot ratio. The normal plants was on par with $L_3H_1$ during later stage viz. 45 DAP. Gannoun *et. al.* (1996) observed a higher root: shoot ratio at high light intensity which was in line with the present findings.

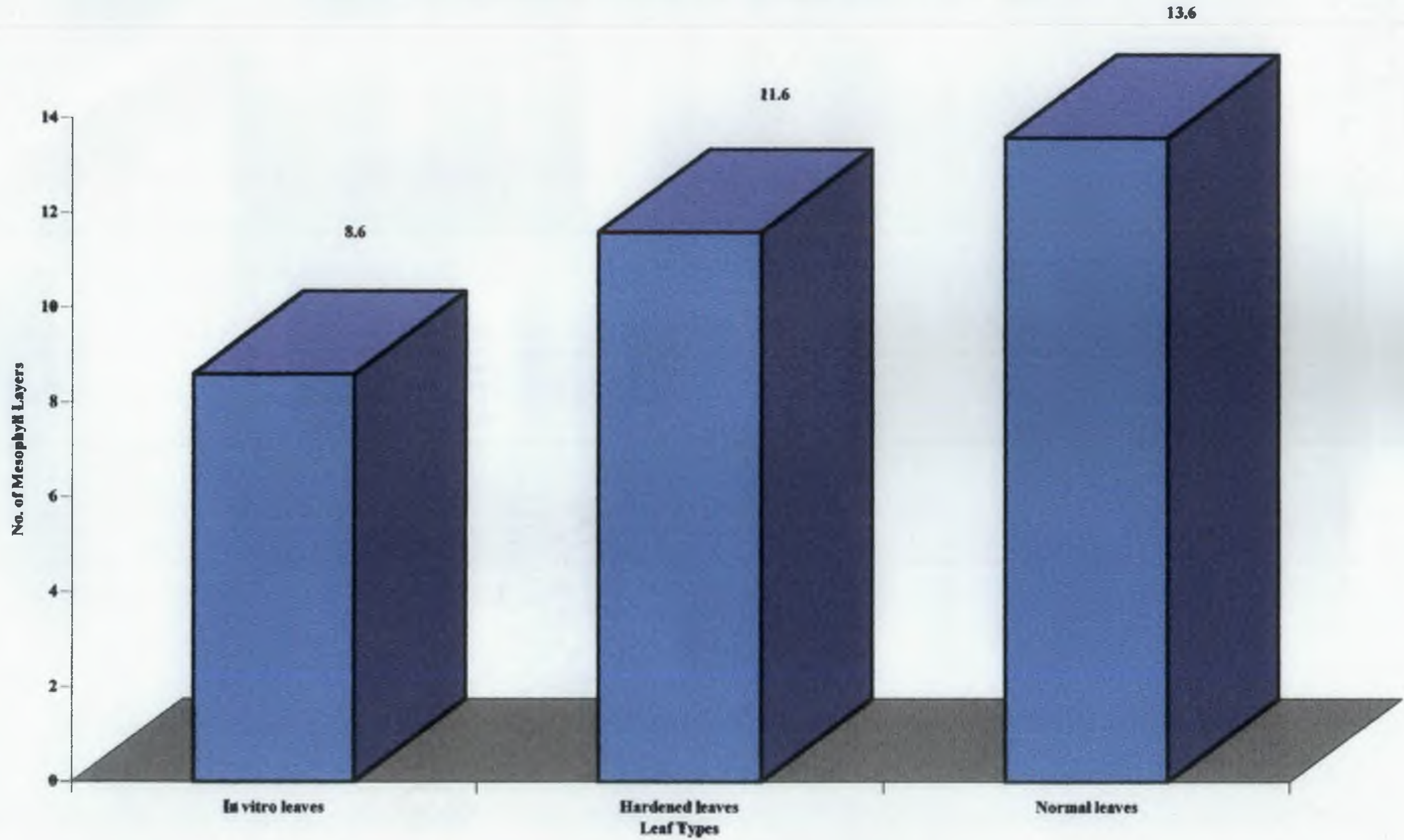
**Total fresh weight and Dry weight**

The biomass production in terms of total dry matter accumilation was worked out computing the values obtained from root, shoot and leaves. Clear evidence was envisaged that the dry matter accumilation increased with age (Watson 1971). Wareing and Patric (1975) considered high dry matter production was an important prerequisite for greater productivity in crop plants. An increase in fresh and dry weight at 3% level of sucrose was reported by Adelberg (1995) in micropropagated triploid melon. Similar results were reported in *Cymbidium* orchids by Kubota and Kozai (1991). The present investigation, where it was found that the fresh and dry weight of plantlets were

greater in case of plantlets grown at sucrose levels of 40 g/l ($S_2$) and 60 g/l ($S_3$) than at 20 g/l ($S_1$) was confirmity with these results.

Triazole treatment @ 5 mg/l. ($T_1$) was found to yield more of fresh weight and dry weight than control plantlets($T_3$). The triazole 300 ppm when sprayed on greenhouse grown rice seedling showed an increase in dry matter, protein content and plant height ( Rong *et. al.*,1995). The present result supports these reports.

The fresh and dry weight of plants under the treatment of light and humidity exhibited almost similar preformance for most of the treatments. Among the tissue cultured plantlets, those treated at 50 percent light and below 70 percent humidity ($L_2H_2$) recorded maximum value. The increased rate of assimilation might be contributed to the increased dry matter production in $L_2H_2$. Towards the later stage viz. 45 DAP, the total fresh weight and dry matter production of tissue cultured plantlets were on par with that of field grown plants. Acosta *et. al.* (1983) reported in Datura that the total fresh weight and dry weight were greater under reduced light intensity. Stoyanova *et. al.*(1997) stated that at 50-60 percent humidity level, fresh weight and dry weight were significantly increased. In contrast to this, Hee (1995) in his work on *Gysophila paniculata* reported that at 97 percent relative humidity, the ratio of fresh weight : dry weight is increased. This observation was in accordance with the present study carried out.


NUMBER OF MESOPHYLL LAYERS OF VARIOUS TYPES OF PLANTLETS
No. of Mesophyll Layers
0
2
4
6
8
10
12
14
8.6
11.6
13.6
In vitro leaves
Hardened leaves
Normal leaves
Leaf Types

**Anatomical Characters**

Generally, the *in vitro* propagated plantlets have some special anatomical characters which make their *ex vitro* establishment difficult. In the case of *in vitro* grown leaves of cauliflower the number of palisade cells and mesophyll layers were found to be limited (Grout and Aston,1978). Wetzstein and Sommer (1982) and Fabri *et. al.*(1986) have reported that the leaves of micropropagated strawberry plantlets lack well differentiated palisade parenchyma, spongy tissue and the occurence of large intercellular space. Dami and Hughes (1995) observed that the leaves of *in vitro* produced grape plantlets possess large mesophyll cells, lack normal palisade layer formation, posses greater inter cellular pore space and fewer number of chloroplasts. On the other hand, the leaves of normal plants had smaller mesophyll cells, defined palisade layer, reduced inter cellular pore space and more number of chloroplasts. In the present investigation, where it was found that the leaves of normal plantlets contain more number of mesophyll layers and the leaves of *in vitro* plants had reduced number of mesophyll layers (Fig. 11).

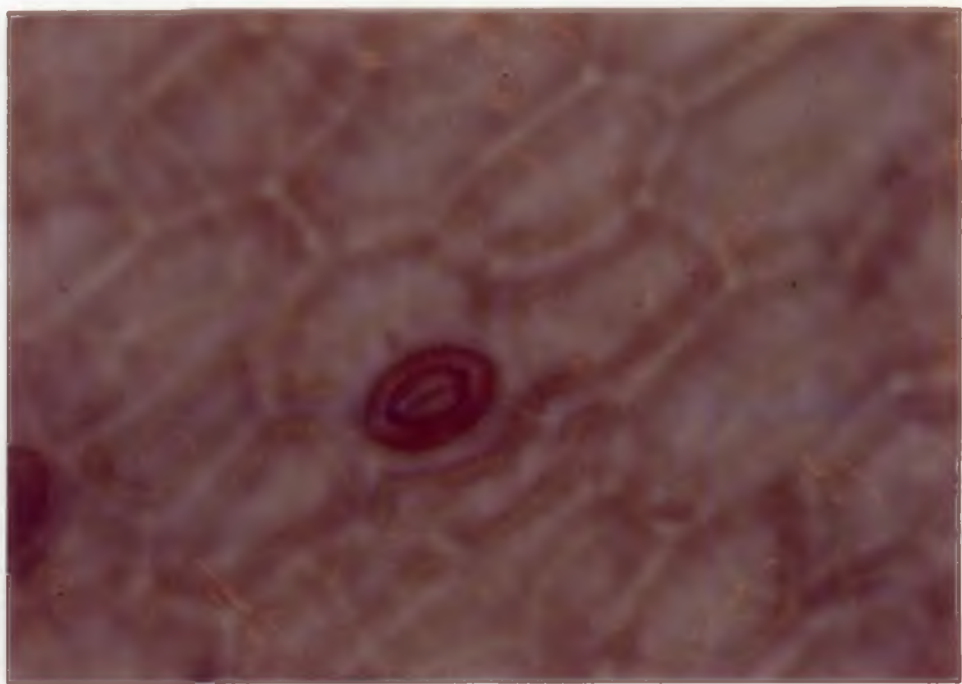
In the present study, the cuticle layer was absent in the leaves of *in vitro* plantlets whereas the leaves of normal plantlets possessed a distinct layer of cuticle. Also the hardened leaves of control plantlets were found to have a thin layer of cuticle. Velaplana and Mullins (1986) studied the substantial loss of water in grape vine during transfer to non sterile conditions and the light

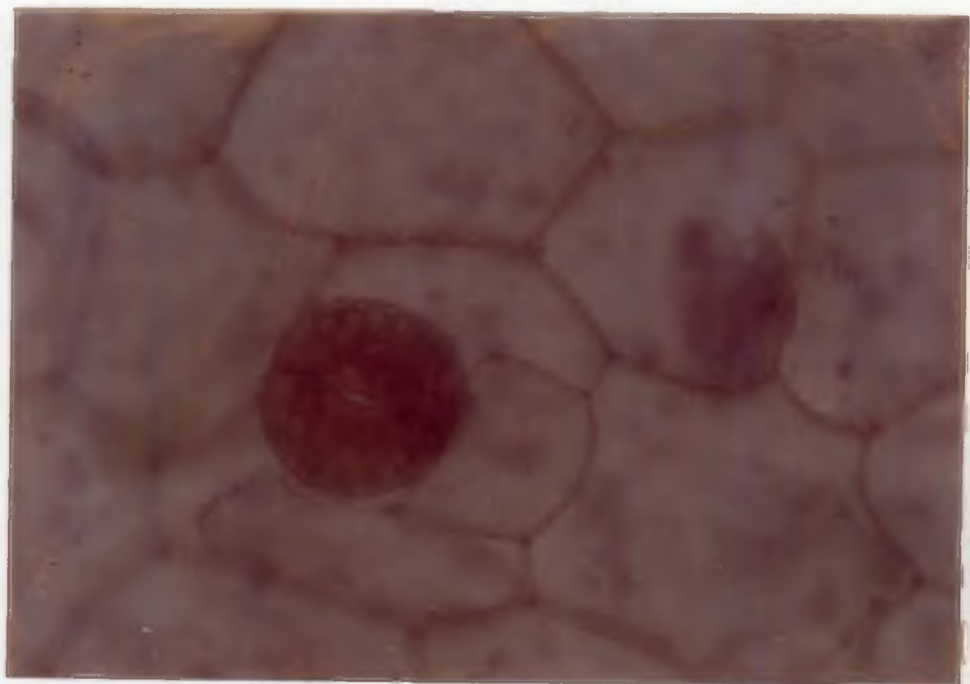
microscopic study reveals that *in vitro* leaves lack epicuticular wax. They exhibit changes in stomatal frequencies and leaf anatomy as compared to normal plantlets. They concluded that these factors are responsible for the higher water loss during hardening of the micropropagated plants. Epicuticular wax has been found to be either reduced or absent in the *in vitro* leaves, leading to high rate of water loss. The density of wax deposition was found to increase during acclimatization (Sutter and Langhani, 1982; Wardle *et. al*, (1988). Fabri *et. al*, (1986) reported that hardened leaves of tissue cultured strawberry plantlets enlarge due to increased cell size and increased epicuticular wax deposits on both adaxial and abaxial surfaces. The leaves of hardened plantlets were intermediate in morphology. The review materials hither to discussed provided ample support to the results obtained in the present study.

In the present study, stomatal frequency was found to be highest in the case of normal field grown leaves and lowest in *in vitro* leaves. The width of opened stomata was observed to be maximum in the case of *in vitro* leaves which may be due to the circular shape of stomata. The length of open stomata was found to be maximum in the leaves of normal plantlets. The ellipsoid stomata may be the reason for this. The shape of stomata of the above two types of leaves were shown in (Plate 8)The frequency and shape of the stomata of hardened leaves was found to be intermediate. Wetzstein and Sommer (1982) studied the surface morphology of *in vitro* cultured *Liquidamber styraciflua* during acclimatization and field grown leaves. They observed that

**Plate 8. a. Abaxial surface of field grown leaf showing elliptical closed stomata with small aperture**

**b. Abaxial surface of *in vitro* leaf showing fully open circular stomata with large stomatal aperture**





the *in vitro* leaves had superficial circular stomata and irregular epidermal cells. In the acclimatized leaves the stomata were ellipsoid and depressed, but the epidermal cells were irregular. In the field grown leaves, the stomata were ellipsoid and depressed, but the epidermal cells were well defined and isodiametric in shape. All these reports strengthen the results of the present study.

# 6. SUMMARY

Considering the difficulties faced during the *ex vitro* establishment of orchid plantlets, attempts have been made in the present investigation to elicit information on the physiological, morphological, biochemical, biometrical and anatomical changes which are associated with *ex vitro* establishment of orchid plantlets. The present investigation was carried out during 1997 to 1999 at the Department of Plant Physiology, College of agriculture, Vellayani. The tissue cultured plantlets of *Dendrobium* hybrid Sonia 17 was subjected to *in vitro* rooting with medium supplemented with different levels of sucrose. Based upon their rooting behaviour and survival percentage , the plantlets under the best of sucrose treatments were dipped in different concentrations of triazole and then planted out in pots. Then on the basis of survival percent and plant growth behaviour in terms of number of leaves, shoot length and number of roots per shoot, the best of triazole treatments was decided. Plantlets from the best of the triazole treatment was then subjected to hardening process by maintaining different levels of light intensity and humidity in the hardening chamber. One among the different levels of treatments viz. sucrose, triazole, light and humidity was the normal recommended level practiced in tissue culture and that was considered as the control.

The *ex vitro* establishment of tissue cultured plantlets was analysed by comparing their growth with normal green house grown plants. The protocol

# SUMMARY

developed by Sherly (1997) was adopted with necessary modification for the *in vitro* production of Dendrobium plantlets. The salient features of the study is summarised below.

The plantlets subjected to rooting media with 40 g/l of sucrose exhibited better crop growth rate (CGR), and was on par with normal green house grown plants. The net assimilation rate (NAR) and relative growth rate (RGR) were also found to be more in these plants. An increase in photosynthetic rate was noted in plantlets grown in the rooting medium supplemented with 40 g/l of sucrose. Regarding transpiration rate there was no much significant variation between treatments. However the normal green house grown plants showed very high rate of photosynthesis and lower rate of transpiration than the tissue cultured plantlets. The morphological characters of plantlets were affected by the sucrose concentration and 40 g/l of sucrose was found to enhance plant height, number of leaves and number of roots per shoot. Hence it was attributable that better *ex vitro* establishment of tissue cultured plantlets was mainly due to the improved effect of the sucrose treatment on the morphological parameters indicated above.

The *in vitro* plantlets contain relatively lesser chlorophyll content than normal plants. The *in vitro* plantlets grown in the rooting medium supplemented with different levels of sucrose exhibited varying performances with regard to photosynthetic pigments and those subjected to 40 g/l sucrose

concentration exhibited higher content of total chlorophyll, chlorophyll a chlorophyll b and carotenoids. Maximum content of protein and carbohydrate were also in plantlets with 40 g/l of sucrose.

Leaf was considered as the major site for the operation of photosynthetic mechanisms. The effect of sucrose on leaf area was amounted from the alterations obtained from the related factors viz. CGR, NAR and RGR. These growth parameters were maximum in plantlets supplemented with sucrose @40 g/l in rooting medium. The shoot and root growth were well pronounced due to sucrose treatment especially @40 g/l The root: shoot ratio was observed to be higher and positively correlated with increase in sucrose concentration. The *ex vitro* establishment potential depends on total dry matter accumulation. Plantlets @ 40 g/l of sucrose in the rooting medium , exhibited higher fresh and dry weights. However a distinctively higher value was recorded by normal green house grown plants for these parameters.

During ex vitro trials the plantlets were subjected to different levels of triazole treatment at the time of planting out. With regard to growth parameters viz. CGR, NAR and RGR of plantlets treated with 5 mg/l of triazole performed better than others. However towards the later stages (P1 and P2) these plantlets were on par with that of normal plants. The plantlets treated with 5 mg/l of triazole showed higher photosynthetic rate than plantlets under other treatments.

A decreasing trend of plant height was noticed with increase in the concentration of triazole treatment. There was distinctly higher number of leaves were noticed in normal plants than tissue cultured plantlets. Among the treated plants triazole @ 5 mg/l favoured the production of more number of leaves and roots. Survival percentage was positively influenced by the triazole treatment and maximum survival of triazole treated plantlets was at 5 mg/l concentration. The increased production of roots at 5 mg/l of triazole concentration attributed for the higher survival percentage of plantlets at the above level

The normal plants maintained distinctively higher amount of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids than tissue cultured plantlets at the initial stages. But towards the later stage, chlorophyll a, total chlorophyll and carotenoids of the plantlets under 5mg/l of triazole were found to be on par with normal plants. Regarding protein content there was not much variation between tissue cultured plantlets and normal plants at the initial stage, but towards the later stage the normal plants exhibited a distinctly higher amount of protein content. The carbohydrate content of plantlets treated with 5mg/l of triazole was significantly higher than other treatments. The root: shoot ratio and fresh weight of plantlets treated with triazole @ 5mg/l were found to be on par with normal green house grown plants towards the later stage.

In order to study the effect of different light intensity levels and humidity levels, the plantlets were grown in hardening chambers where different levels of light intensity and humidity were maintained. CGR and NAR of the plantlets grown under 50 percent light intensity and 70 to 90 percent humidity ($L_2H_2$) were found to exhibit maximum value at all the three stages of study. The influence of light treatment in combination with humidity on photosynthetic rate was significant only at the later stage ($P_3$). The photosynthetic rate recorded by $L_2H_2$ was on par with normal green house grown plants towards the later stage. With respect to morphological characters the plantlets under 50 percent light intensity and 70 to 90 percent humidity ($L_2H_2$) recorded maximum plant height, higher number of leaves and maximum number of roots per shoot, which resulted in better survival percent of these plantlets. Towards the later stages of growth the plantlets subjected to 25 percent light intensity and 70 to 90 percent humidity ($L_1H_2$) also produced more number of leaves which was on par with normal plants.

Regarding total chlorophyll, chlorophyll a, chlorophyll b and carotenoids there was no much variation noted among the treatments during the initial stage but towards the later stage, the plantlets subjected to 50 percent light intensity and 70 to 90 percent humidity had maximum content of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids. However normal plants maintained distinctively higher content of these pigments. The plantlets

subjected to 75 percent light intensity and 70 to 90 percent humidity ($L_3H_2$ ) recorded maximum protein content and carbohydrate content.

The root: shoot ratio of normal plants were on par with that of plantlets subjected to 75 percent light level ($L_3H_1$ and $L_3H_2$ ) irrespective of humidity level. However the total dry weight of normal plants was much higher than that of tissue cultured plantlets.

The anatomical characters associated with *in vitro* leaves, hardened leaves and normal leaves of green house grown plants was compared. The stomata of *in vitro* leaves were round where as that of normal leaves were elliptical in shape. The width of stomatal aperture was maximum in *in vitro* leaves, but the length was maximum in normal leaves. The number of stomata per unit area of leaf was higher in normal leaves compared to the *in vitro* leaves and hardened leaves. In the case of *in vitro* leaves no cuticle layer was formed where as in normal leaves a distinct layer of cuticle was observed and the hardened leaves also possessed a thin layer of cuticle. The number of mesophyll layers was higher in normal leaves than *in vitro* leaves. These anatomical peculiarities of in vitro cultured orchid plantlets were a major limitation for their *ex vitro* establishment

171644

# REFERENCES

# REFERENCES

*Acosta, L., Lerch, G. and Galvez, M. 1983. Effect of some climatic factors on the growth, yield and total alkaloid content of *Datura candida* growing with and without cover. *Agrotecnia-de-Cuba* **15**(2): 63-78

Adelberg, J., Fujiwara, K., Kirdmanee, C, and Kozai, T. 1995. Photoautotrophic micropropagation of triploid melon. *Hort. sci.* **30** (4): 752-753

Al.Khalifah, N.S. and Alderson, P.G. 1995. The effect of culture physiological conditions, ex plant type and source on the growth and multiplication of *Ficus benjamina in vitro.* *Hort. sci.* **30** (4): 873

Anderson, P.C. and Aldrich, J.H. 1987. Effect of soil applied paclobutrazole on "Cheyenne pecans". *Hort. sci.* **22:** 79-82

*Arad, S., Adda,M. and Cohen, E. 1986. Effect of growth conditions on the production of the cell wall polysacchrides in porphyridium. *Nova Hedwigia* **83:** 75-78

Arnon, D.I. 1949. Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris.* *Plant Physiol.* **24:** 1-15

Arne, S., Trygve, K. and Maigull, A. 1995. Light quality effects photosynthesis and leaf anatomy of birch plantlets *in vitro.* *Plant Cell Tiss. Org. Cult.* **41:** 177-185

Ault, J.R., 1994. In vitro propagation of *Eriostemon myoporoids* and *Eriostemon stardust. Hort. sci.* **29**(6): 686-688

*Bachenauer, H., Kutzner, B. and Koths, T. 1984. Effect of various triazole fungicides on growth of cereal seedlings and tomato plants as well as on gibberlin content and lipid metabolism in barley seedlings. *Zeitschriftfur Pflanzenkran Kheiten undpflanzenschutz*. **91**(5): 506-524

Bangarusamy, U. 1988. Physiological studies on water relations in *Sorgum*. Ph. D. thesis. Tamil Nadu Agricultural University. Coimbatore.

Bausher, M.G. and Yelenosky, K. 1987. Morphological changes in citrus associated with relatively high concentration of paclobutrazol. *J. Plant Growth Regul.* **5:** 139-147

Beringer, H. 1978. Functions of potassium in plant, metabolism with particular reference to yield. In: *Potassium in Soils and Crops.* (Ed.) Sekher, G.S., New Delhi, PR 2.**47**: 6-9 1978

Bertrand, D.A., Noirot, M. and Charriea, A. 1992. Slow growth *in vitro* conservation of coffee, influence of reduced concentration of sucrose and low temperature. *Plant Cell Tissue Organ Culture* **31**(2:) 105-110

Blanke, M.M. and Belcher, A.R. 1989. Stomata of apple leaves cultured *in vitro. Plant Cell Tiss, Org. Cult.* **19:** 85-89

Blower, J.W. 1964. Meristem propagation and possible effects. *Orchid Rev.* **72:** 407-408

Brainerd, K.E. and Fuchigami, I.H. 1981. Acclimatization of aseptically cultured apple plants to low relative humidity. *J. Amer. Soc. Hort. Sci.* **106**(4): 515-518

Byoung, R.J., Kazuhiro, F. and Toyoki, K. 1995. Environmental control and photoautotrophic micropropagation. *Hort. Rev.* **17:** 125-171

Capellades, M., Fontarnau, R., Carula, C. and Debergh, P. 1990. Environment influences anatomy of stomata and epidermal cells in tissue cultured *Rosa multiflora. J. Amer. Hort. Sci.* **115**(1): 141-145

Chadha, K. L. 1992. The Indian Orchid Senario. *J. Orchid Soc. India.* **6**(1): 1-4

Clarkson, D. T. 1986. Regulation of the absorption and release of nitrate by plant cells. A review of current ideas and methodology. In : *Fundamental, ecological and agricultural aspects of N metabolism in higher plants.* Nyhoff Publishers, The Netherlands. p. 3-27.

Collins, M. T., and Dixon, K. W. 1992. Micro propagation of an Australian terrestrial orchid *Diuris longifolia Australian journal of experimental agriculture* **32**(1) : 131-135

Corner, A. J. and Thomas, M. B. 1982. Re-establishing plantlets from tissue culture. *A review Int. Plant Propag. Soc. Comb. Proc.* **31** : 342 - 357

Dami, I. and Hughes, H. 1995. Leaf anatomy and water loss of in vitro PEG treated "Valiant grape" *Plant Cell Tiss. Org. Cult.* **42** (2): 179 - 184

Das, D. R. and Mukherji, S. 1995. Chlorophyll and carotenoid contents of light and dark grown seedlings of *Vigna radiata.* L. after heat shock during early imbibition. *Plant Physiol. and Biochem.* **22**(2) : 192 - 194

Davis, T. D. and Sankhla, N. 1988. Effect of shoot growth retardants and inhibitors on adventitious root formation in cuttings. In "*Adventitious root formation in cuttings*". Dioscorides Press, Portland. p. 174 - 184

Davis, T. D., Walser, R. H., Soerenson, K. and Sankhla, N. 1986. Rooting and subsequent growth of cuttings treated with paclobutrazol. *J. Am. Soc. Hort. Sci.* **109** : 876 - 882

Debergh, P. and Maene, H. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. *Scientia Hort.* **14** : 335 - 345

De bergh, P., Van, H. B. and Huygens, H. 1995. Photo inhibition during acclimatization of micro propagated *Spathiphyllum* plantlets. *In vitro plant.* **31** (3): 162 - 164

*De Rick, J. and Van, H. J. 1992. Acclimatization of micro propagated roses in multi layer cells, effect of different stage three conditions and $CO_2$ enrichment. *Meded. Fac. Landbouwet. Rijksnuiv. Gent.* **57** (4): 1549 - 1552

Desjardins, Y. and Tiessen, H. 1985. The effect of sucrose concentration and ancymidol on the rooting of asparagus *in vitro. Proceedings of the sixth international asparagus symposium.*

Desjardins, Y., Gosselin, A. and Yelle, S. 1987. Acclimatization of *ex vitro* strawberry plantlets in CO2 enriched environment and supplementry lighting. *J. Amer. Soc. Hort. Sci.* **112**(5): 846-851

Dhawan, V. and Bhojwani, S. S. 1987. Hardening *in vitro* and morpho physiological changes in the leaves during acclimatization of micro propagated plants of *Leucaena leucocephala. Plant Sci*. **53**: 65-72

Dhopte, A.M. and Livera, M.M. 1989. *Useful techniques for plant scientists.* Published from Foum for Plant Physiologist, RDG College, Hostel.1, Akola, (M.S.), India

Diaz, P.J., Sutter, E. G. and Shackel, K. A. 1995. Acclimatization and subsequent gas exchange water relations, survival and growth of micro cultured apple plantlets after transplanting them in soil. *Physiologica Plantarum.* **95**(2): 225-232

Donnelly, D. J. and Vidaver, W. E. 1984. Leaf anatomy of red raspberry transferred from culture to soil. *J. Amer. Soc. Hort. Sci.* **109** : 172 - 176

Donnelly, D. J. and Skelton, F. E. 1989. Comparison of hydrathode structure in micro propagated plantlets and green house grown "Queen Elizabath" rose plantlets. *J. Amer. Soc. Hort. Sci.* **114**(5) : 841 – 846

Donnelly, D. J. and Vidaver, W. E. and Lee, K. Y. 1985. The anatomy of tissue cultured red raspberry prior to and after transfer to soil. *Plant cell tissue organ culture.* **4** : 43 - 50

Dube, S. and Vidaver, W. E. 1987. *Adverse effects or benefits of air feeding in plant tissue cultures.* Plant Physiol. **83**(4) : 36

Dustan, D. I. and Turner, K. E. 1984 The acclimatization of micro propagated plants. In : *Cell culutre and somatic cell genetics of plants.* Academic press, New York (1) : 123 - 129.

Eason, J. R., Vre-LA de, Somerfield, S. D., Hayes, J. A. and De-Vre LA. 1997. Physiological changes associated with *Sander sonia aurantiaca* flower senescence in response to sugar. *Post. harvest. Biology and Technology* **12**(1) : 43 - 50

Eliasson, M. K. and Beyl, C. A. 1993. *In vitro* rooting and acclimatization of *Prunus serotina* using paclobutrazole. *Hort. Science* **28**(5) : 545

*Fabbri, A. and Bartocini, G. 1985. Anatomical observations on the roots of vegetatively propagated paradox plantlets. *Rivista de Frietticoltura edi orto florecultura* **47**(11) : 43 – 46

Fabbri, A., Sutter, E. and Duston, S.K. 1986. Anatomical changes in persistent leaves of tissue cultured strawberry plants after removal from culture. *Scientia Hort.* **28 :** 331-337

Fevereiro, P., Cabrel, J. M. S., Fonseca, M. N. R., Novais, J. M. and Pais, M. S. 1986. Callus and suspension culture of *Silybum marianum.* biosynthesis of proteins with clotting activity. Biotechnol. Lett. **8**(1) : 19 - 24

Fletcher, R. A., Hofsta, G. and Gao, J. 1986. Comparative fungi toxic and plant growth regulating properties of triazole derivatives. *Plant cell Physiol.* **27** : 367 - 371

Fukuoka, N., Yoshioka, H., Shimizu, E. and Fujiwara, T. 1996. Effect of shading in cabbage seedlings on their physiological process and rooting ability after transplanting to the nursery. *Plant physiology* **18**(6) : 715 - 721

Galyuon, I. K. A., Mc. David, C. R., Lopez, F. B. and Spence, J. A. 1996. The effect of irradiance level on cocoa, its growth and leaf adaptation. *Tropical Agriculture* **73**(1) : 23 - 28

Gannoun, S., Lionakis, S. M. and Gerasopoulos, D. 1996. Effects of light intensity on sucker proliferation and rooting *in vitro* of *Musa accuminata. Agricultura Mediterranea* **126**(4) : 405 - 407

Garay, L. 1960. On the origin of *Orchidaceae. Procedings of third world orchid conference* 172 - 195.

Gent, M. P. N. 1986. Carbohydrate level and growth of tomato plants, the effect of irradiance and temperature. *Plant Physiology* **81**(4) : 1075 - 1079.

Gregory. G. F. 1917. Physiological condition in cucumber houses. In : *Plant physiology a Treatise,* Acadamic Press New York. p. 19 - 28

Grout, B. W. W. 1975. Wax development on leaf surface of *Brassica oleraceae.* var. currawong regenerated from meristem culture. *Plant Science Lett.* **5** : 401 - 405

Grout, B. W. W. and Aston, M. J. 1977. Transplanting of cauliflower plants regenerated from meristem culture, carbondioxide fixation and the development of photosynthetic ability. *Hort. Res.* **17** : 1 - 7

Grout, B. W. W. and Aston, M. J. 1978. Modified leaf anatomy of cauliflower plantlets regenerated from meristem culture. *Ann. Bot.* **42** : 993 - 995

Grout, B. W. W. and Crisp, P. 1977. Practical aspects of the propagation of cauliflower by meristem culture. *Acta. Hort.* **78** : 289 - 296

Grout, B. W. W. and Millam, S. 1988. Photosynthetic development of micro propagated strawberry plantlets following transplanting. *Ann. Bot.* **55** : 129 - 131

Hammerschlag, F. A., Bauchan, G. R. and Scorza, R. 1987. Factors influencing in vitro multiplication and rooting of peach cultivars. *Plant cell tissue organ culture.* **8** : 235 - 242

*Han, B. H., Joung, H. Y. and Han, C. B. 1995. Effect of sealing materials and relative humidity of culture vessels on growth vitrification and nutrient contents of *in vitro* plantlets of *Gypsophila paniculata. Journal of the Korean Society for Horticulture* **36**(6) : 886 - 892

Harvey, B. M. R., Bowden, G., Reavey, C. and Selby, C. 1994. Stimulation of *in vitro* root and shoot growth of potato by increasing sucrose concentration in the presence of fluridone, an inhibitor of ABA synthesis. *Plant cell tissue organ culture* **37**(3) : 271 - 296

Hdider, C. and Desjardins, Y. 1995. Reduction of RuBP carboxlase efficiency by the presence of sucrose during tissue culture of strawberry plantlets. *In vitro* Plant. **31**(3) : 165 - 170

Hedge, J. E. and Hofreiter, B, T. 1962. In : Methods in carbohydrate chemistry ( eds. whister, R. L. and Be Miller, J. N. ) Academic press, New York. 14 - 16

Hess, S. R., Hasegawa, P. R. and Bressan, R. A. 1986. Sucrose utilization during adaptation to salinity. *Int. Congr. Plant tissue cell culture 6 Meet,* 5

Honjo, T., Futaya, Y. and Takakura, 1988. Effect of $CO_2$ enrichment on the growth of *Cymbidium* PLB *in vitro. Acta. Horticulture.* **230** : 185 - 188

Honmode, R and Sehgal, O. P. 1991. Micro propagation of *Rhyncostylis arundina and Dendrobium* Paper presented for National Seminar on *Biology Improvement propagation and commerilation of Indian Orchids* at I. I. H. R. Bangalore.

Hu, C. Y. and Wang, P. Y. 1983. Meristem, shoot tip and bud cultures. In : *Hand book of Plant cell culture*, Macmillian, New York. **1** : 127 - 227

Isiah, J. W. and Rao, M. P. 1992. Vegetative anatomy of *Dendrobium jenkensy*. *J. Orchid Soc. India* **6**(1) : 63 - 69

Janous, D., Dvorak, V. and Kalina, J. 1996. Chamber effect and response of trees in the experiment using open top chambers. *Journal of Plant Physiology* **148** : 332 - 338

Kaixian, Q. and Borowitzka, M. A. 1993. Light and nitrogen deficiency effects on the growth and composition of *Phaeodactylum tricornutum*. *Appl. Biochem. Biotechnol.* **38**(1) : 93 -103

Kane, R. T. and Smiley, R. W. 1983. Plant growth regulating effects of systemic fungicides applied to Kentwiky blue grass. *Agron. J.* **75** : 469 - 473

Kirdmanee, C., Kitaya. Y. and Kozai, T. 1995. Rapid acclimatization of *Eucalyptus* plantlets by controlling photosynthesis photon flux density and relative humidity. *Environment control in Biology*. **33**(2) : 123 - 132

Knudson, C. 1946. A new nutrient solution for the germination of orchid seeds. *Am. Orchid Soc. Bull.* **15** : 214 - 217

Kortessa, D. T. and Artemios, M. B. 1997. Effect of light, Mg and sucrose on leaf anatomy, photosynthesis, starch and total sugar accumilation in kiwi fruit cultured *in vitro*. *Plant cell tiss. org. cult.* **47** : 127 - 134

Kubota, C. and Kozai, T. 1991. Effect of initial amount of sugar in the media on the growth of *Cymbidium* orchid *in vitro*. *Hort. Sci.* **26**(6) : 766

Kumar, P. K. S. 1991. Post transplantation growth of *Dendrobium* hybrid seedlings in community pots. *Paper presented for National seminar on Biology*. Improvement, propagation and commercialisation of Indian orchids at I. I. H. R.

Lakshmidevi, S. 1992. Standardisation of ex plant for *in vitro* propagation in *Dendrobium* species. M.Sc. thesis submitted to Kerala Agricultural University, Vellanikara, Thrissur, Kerala.

Lalithabhai, E. K. 1981. Shade response of five common inter crops of coconut garden. M.Sc. thesis submitted to Kerala Agricultural University, Vellanikara, Thrissur, Kerala.

Langford, P. J. and Wainwright, H. 1987. Effect of sucrose concentration on the photosynthetic ability of rose shoots *in vitro*. *Ann. Bot.* **60** : 633 - 640

Lee, N., Wetzstein, H. Y. and Sommer, H. E. 1985. Effect of quantum flux density on photosynthesis and chloroplast ultrastructure in tissue culture plantlets and seedlings of *Liquidamber styraciflua*. L. towards improved acclimatization and field survival. *Plant Physiology* **78**(3) : 637 - 641

*Leonardi, C. 1996. Effect of shading on green house peppers. *Colture Protette* **25**(7) : 61 - 65

Lim, L. Y., Hew, Y. C., Wong, S. C. and Hew, C. S. 1992. Effect of light intensity, sugar and carbondioxide concentration on growth and mineral up take of *Dendrobium* plantlets. *J. Hort. Sci.* **67**(5) : 601 - 611

Lowry, O. H., Rosebrough, N. J., Faward, A. L., and Rawall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** : 266 - 275

Lurssen, K. and Reiser, W. 1985. Chemistry and physiological properties of the new plant growth regulator, RSW 0411. Proceedings of British crop protection conference, *Weeds.* **1** : 121 - 128

Madhuri, S. and Vasundhara, G. 1990. Micro propagation of *Dendrobium Joannie. J. Orchids Soc. India* **1** : 145 - 148

Mageed, K. J. A., Sharma, B. and Balakrishnan, K. A. 1988. Association of growth characters in mandarin orange with consumptive water use. *Indian Journal of Agricultural Sciences.* **55**(6) : 406 - 408

Marini, R. P. 1987. Growth and cropping of peach trees following soil application of paclobutrazole. *J. Am. Soc. Hort. Sci.* **112** : 241 - 246

Marin, J. A., Gella, R. and Herrero, M. C. 1988. Stomatal structure and functioning as a response to environmental changes in acclimatized micro propagated *Prunus Cerasus.* L. *Annals of Botany* **62** : 663 - 670

Mary, A. L. S., Jiwan, P. P. and Brant, H. M. 1986. Comparative anatomy and physiology of micro cultured seedling and green house grown Asian White Birch. *J. Amer. Hort. Sci.* **111**(3) : 437- 442

Novello, V. and Roberts, A. V. 1992. Effects of paclobutrazole and reduced humidity on stomatal conductance of micropropagated grape vines. *Acta. Hort.* **319** : 65 - 70

Park, S. H., Lee, Y. B. and Park, N. G. 1997. Effect of light acclimatization on photosynthetic activity of foliage plants. *Journal of Korean Society for Horticultural Science.* **38**(1) : 71 - 76

Poole, R. T. and Conover, C. A. 1983. Establishment and growth of *in vitro* cultured *Diffenbachia. Hort. Sci.* **18**(2) : 185 - 187

Raghava, S. P. S. and Dadlani, N. K. 1997. Untapped commercial potential. *The Hindu survey of Indian Agriculture.* 139 - 143

Rahman, S. M., Hossain, M., Refiul, I. and Joarder, O. I. 1992. Effects of media composition and culture conditions on *in vitro* rooting of rose. *Sci. Hort.* **52**(1-2) : 163 - 169

Rajmohan, K. 1985. Standardisation of tissue / meristem culture techniques in important horticultural crops. Ph.D. thesis submitted to the Kerala Agricultural University, Vellanikara, Thrissur

Ramesh, B. 1990. *Ex vitro* establishment of jack (*Artocarpus heterophyllus* Lann.) plantlets. M.Sc. thesis submitted to the Kerala Agricultural University, Vellanikara, Thrissur

Reuther, G. 1988. Comparative anatomical and physiological study with ornamental plants under *in vitro* and green house conditions. *Acta. Hort.* **226** : 91 - 97

Mathur, J., Ahuja, P.S., Mathur, A., Kulreja, A.K. and Sham, W.C. 1988. *In vitro* pripagation of *Valeriana wallichi. Planta medico* **54** (1): 82-83

Menhenett, R. 1984. Comparison of new triazole retardant paclobutrazol with amcymidol, chlorophonium chloride daminosi de and peproctanyl bromide on stem extension and infloresence development in *Chrysanthmum morifolium. Scientea. Horticulture.* **24**(3) : 349 - 358

Midmore, D. J. and Prange, R. K. 1992. Growth response of two solanum species to contrasting temperature and irradiance levels. Relation to photosynthesis, dark respiration and chlorophyll fluorescence. *Annals of Botany* **69 :** 13 - 20

Miranda, F.M.E. and Fernandex, L.J. 1995. Acclimatizati on, nursery cultivation and plant quality of micro propagated chesnut. *ITEA. Production Vegetab.* **91**(3) 149 - 156

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plantarum.* **15**: 4 73-497

Nagoaka, M., Takahashi, K. and Arai, K. 1984. Effect of environmental factors on photosnthesis and transpiration of tomato and cucmber plants. *Bull. Vegetable and ornamental crops Research Station* **12**: 97-117

Nobuoka, T., Oda, M. and Sasaki, H. 1996. Effect of relative humidity, light intensity and leaf temperature on transpiration of tomato scions. *Journal of the Japanese for Horticultural Science* **64**(4) : 859 - 865

*Rong, C.G., Xiao, L., Wan, K.J. and Li, Y.M. 1995. The physiological and yield increasing effects of multi effect triazole treatment of greenhouse rice seedlings *Henan Nongye Kexue* **2:** 1-4

Saha, K. and Gupta, K. 1998. Effect of triazole and CCC on growth, yield and metabolism of mung bean plants under salinity stress. *Indian Journal of Plant Physiology* **3**(2) : 107 - 111

Sairam, R. K., Shukla, D. S. and Deshmukh, P. S. 1995. Effect of triazole - triadimefon on tolerance to moisture stress in wheat. *Indian Journal of Agricultural Sciences* **65**(7) : 483 - 489

Salisberry, F. B. and Ross, C. W. 1992. *Plant Physiology,* Fourth edition, Wadsworth Publishing Co., Belmont, U.S.A. p. 457

Sanden, P. V. 1985. Effect of humidity on growth and water exchange of cucumber seedlings, preliminary report. *Acta. Hort.* **174** : 259 - 267

Sankhla, N., Davis, T. D., Jolley, V. D. and Upadhyaya, A. 1986. Effect of paclobutrazol on the development of iron chlorosis in soya beans. *J. Plant Nutr.* **9** : 923 - 934

Schnapp, S. R. and Preece, J. E. 1986. *In vitro* growth reduction of tomato and carnation micro plants. *Plant cell tissue organ culture.* **6**(1) : 3 - 8

Seneviratne, P., Fleqmann, A. W. and Wijesekera, G. A. S. 1996. The effects of the basic medium and carbohydrate content on shoot cultures of *Hevea brasiliensis. Journal of the Rubber Institute of Srilanka* **78** : 60 - 68

*Shaheen, A. M., Helal, R. M., Omar, N. M. and Mahmoud, A. R. 1995. Seedling production of some vegetables under plastic houses at different levels of light intensity. *Egyptian Journal of Horticulture* **22**(2) : 175 - 192

Sharma, J. and Chauhan, Y. S. 1995. Establishment of *in vitro* raised seedlings of *Dendrobium* and *Paphiopedilum*. *J. Orchid. Soc. India* **9** : 1 - 2

Sharma, S. K. and Tandon, P. 1990. Asymbiotic germination and seedlings growth of *Cymbidium* as influenced by different carbon sources. *J. Orchid Soc. of India* **4**(12) : 149 - 159

Sheela, K. R. and Alexander, V. T. 1995. Physiological response of rice varieties as influenced by soil moisture and seed hardening. *Indian Journal of Plant Physiology* **38**(3) : 269 - 271

Sherly, Kuriakose. 1997. Standardisation of *in vitro* techniques for mass multiplication of *Aranthera* and *Dendrobium*. Ph.D. thesis submitted to the Kerala Agricultural University, Vellanikara, Thrissur

Shibli, R., Spomer, L. A. and Smith, M. A. L. 1990. Osmotic adjustment and growth response of three *in vitro* grown *Chrysanthimum* cultivars to osmotic stress. *Hort. Sci.* **25**(9) : 125

Shimizu, H., Fujinuma, Y. and Omasa, K 1997. Effect of carbon dioxide and relative humidity on the growth and transpiration of wheat. International symposium on plant production in closed ecosystems. *Acta. Hort.* **440** : 175 – 180

Short, K.C., Warborton, J. and Roberts, A.V. 1987. *In vitro* hardening of cultured cauliflower and chrysanthemum plantlets to humidity. *Acta. Hort.* **212**(1): 329-334

Smith, M. A., Patta, J. P. and Mc Down, B. H. 1986. Comparative anatomy and physiology of microcultured seedlings and green house grown Asian white birch. *J. Amer. Soc. Hort. Sci.* **111** : 437 - 442

Steffens, G. L. and Wang, S. Y. 1985. Persistence of several triazole GA bio synthesis inhibitor for reducing growth of young apple plants. *Proceedings of 12$^{th}$ annual meeting of Plant Growth Regulatory Society of America* p. 248

Stoyanova, J. S. 1997. Growth, nitrogen fixation and transpiration in soya bean plants as affected by humidity. *Russian J. of Plant Physiol.* **44**(3) : 363 - 367

Sudeep, R. 1994. Standardisation of medium supplements for shoot proliferation in *Dendrobium* M.Sc. thesis submitted to the Kerala Agricultural University, Vellanikara, Thrissur

Sunitibala, Y., Gupta, S. and Mukherjee, V. B. 1998. Effect of sucrose on growth and chlorophyll synthesis of teak shoots in mixotrophic culture. *J. Plant Biochem. Biotech.* **7**(1) : 57 - 59

Sutter, E. 1988. Morphological, physical and chemical characteristics of epicuticular wax on ornamental plants regenerated *in vitro*. *Ann. Bot.* **55** : 321 - 329

Sutter, E. and Langhani, R. W. 1979. Epicuticular wax formation of carnation plantlets regenerated from shoot tip culture. *J. Amer. Soc. Hort. Sci.* **164** : 493 - 496

Swapna, S. 1996. Environmental effects on the growth of *Philodendron* "Wendlandi" M.Sc. thesis submitted to the Kerala Agricultural University, Vellanikara, Thrissur

Synkova, H. 1997. Sucrose affects the photosynthetic apparatus and the acclimation of transgenic tobacco to *ex vitro* culture. *Photosynthetica* **33** : 403 - 412

Tester, M., Smith, S. E., Smith F. A. and Walker, N. A. 1986. The effects of photon irradiance on the growth of shoots and roots on the rate of initiation of mytorrhizal infection and on the growth of infection units in *Trifolium subterraneum. New phytologist* **103**(2) : 375 - 390

Thomas, R. M. and Singh, V. P. 1995. Effects of three triazole derivatives on mercury induced inhibition of chlorophyll and carotenoid accumilation in cucumber cotyledons. *Indian Journal of Plant Physiology* **38**(4) : 313 - 316

Tripathi, S. L. and Battacharya, B. 1985. Physiology of growth in tobacco. *Indian Journal of Agricultural Sciences* **55**(5) : 342 - 346

Tsay, H. G., Gau, T. G. and Chen, T. C. 1989. Rapid clonal propagation of *Pinelliaternata* by tissue culture. *Plant Cell Report* **8(8)** : 450 - 454

Vacin, E. and Went, F. 1949. Some pH changes in nutrient solution. *Bot. Giz.* **110** : 605 - 613

*Veierskov, B. 1985. Pea seedling growth and development regulated by cotyledons and modified by irradiance. *Physiologia Plantarum* **65**(1) : 79 - 84

Velaplana, M.M. and Mullins, M.G. 1986. Establishment of grapevines from somatic embryos. *Hort. Sci.* **21**(3): 734

Vij, S. P. and Promila, Pathak. 1989. Micropropagation of *Dendrobium, Chrysamthemum* through pseudo bulb segments. *Journal of the Orchid Society of India* **3**(1-2) : 25 - 28

Wainwright, H. and Scrace, J. 1989. Influence of *in vitro* preconditioning with carbohydrates during the rooting of microcuttings on *in vitro* establishment. *Scientia Horticulturae* **38** : 261 - 267

Wang, Xi., Yu- Mei Yu, Tao-Long Xing, Hung- Xiaolin. 1995. Controlling effect of unicanazole on growth of wheat seedlings. *Acta. Agriculturae* **10**(2) : 50 - 56

Wardle, K., Dobbs, E. B. and Short, K. C. 1983. *In vitro* acclimatization of aseptically cultured plantlets to humidity. *J. Amer. Soc. Hort. Sci.* **108**(3) : 386 - 389

Wareing, P.F. and Patric, J. 1975. Source sink relations and the partition of assimilates in the plant. In: *Photosynthesis and productivity in different environments.* Cambridge University Press, London. p. 82

Watson, D.J. 1971. Size, structure and activity of the productive system of crops. In: *Potential crop production.* Educational Books, London. p.271

Welander, A. M. 1985. *In vitro* shoot and root formation in apple cultivar. *Anals of Botany* **55** : 249 - 261

Wetzstein, H.Y. and Sommer, H.E. 1982. Leaf anatomy of tissue cultured *Liquidambar styraciflua* during acclimatization. *J. Amer. Soc. Hort. Sci.* **108** (3): 475-480

Wiebe, H. J. 1984. Premature bolting of black radish. *Genuse* **20**(12) : 426 - 429

Williams, R. F. 1946. The phenology of plant growth with special reference to the concept of net assimilation rate. *Ann. Bot.* **10** : 41 - 72

Williamson, J. G., Coston, D. C. and Grimes, L. W. 1986. Growth responses of peach roots and shoots to soil and foliar applied paclobutrazol. *Hort. Sci.* **21** : 1001 - 1003

Yin - LiQing., Zhang - Jian Jun, Yin, L. Q. and Fan, K. H. 1996. Effect of ABT on rapid propagation of *Gerbera jamesoni. Acta. Agriculturae* **12**(4) : 55 - 58

Yoshida, S. 1972. Physiological aspects of grain yield. *Ann. Rev. Plant Physiol.* **23** : 437 - 464

Yue, D., Desjardins, V. Lamarre, M. and Gosselin, A. 1993. Photosynthesis and transpiration of *in vitro* cultured asparagus plantlets. *Sci. Horti.* **49** : 9 - 16

Zimmerman, R. H. 1984. Rooting apple cultivars *in vitro* - Interactions among light, temperature, phloroglycinol and auvin. *Plant Cell Tiss. Org. Cult.* **3** : 301 - 311

* Originals not seen

# APPENDIX

# APPENDIX - I

**Weather data during the *Ex vitro* establishment of orchid plantlets for the period of 45 days (08.02.1999 - 25.03.1999)**

| Date | Max. Temp. (°C) | Min. Temp. (°C) | Relative humidity (%) | Rainfall (mm) | Evaporation (mm) |
|---|---|---|---|---|---|
| 08.02.99 | 30.2 | 21.9 | 98 | 1.80 | 3.3 |
| 09.02.99 | 31.0 | 22.5 | 96 | 0.80 | 3.9 |
| 10.02.99 | 30.8 | 22.2 | 96 | 0.00 | 3.6 |
| 11.02.99 | 30.9 | 24.7 | 92 | 0.00 | 3.3 |
| 12.02.99 | 31.4 | 22.3 | 88 | 0.00 | 3.7 |
| 13.02.99 | 31.3 | 22.3 | 84 | 0.00 | 3.9 |
| 14.02.99 | 31.8 | 23.2 | 84 | 0.00 | 4.0 |
| 15.02.99 | 31.6 | 23.3 | 81 | 0.00 | 4.0 |
| 16.02.99 | 32.0 | 23.4 | 78 | 0.00 | 3.9 |
| 17.02.99 | 31.8 | 23.4 | 82 | 0.00 | 3.7 |
| 18.02.99 | 31.5 | 24.0 | 76 | 0.00 | 3.6 |
| 19.02.99 | 31.9 | 24.0 | 76 | 0.00 | 3.8 |
| 20.02.99 | 31.5 | 24.0 | 85 | 0.00 | 3.6 |
| 21.02.99 | 31.5 | 23.0 | 97 | 0.00 | 2.6 |
| 22.02.99 | 32.0 | 22.5 | 92 | 0.00 | 2.6 |
| 23.02.99 | 32.4 | 21.0 | 86 | 0.00 | 5.2 |
| 24.02.99 | 31.6 | 23.1 | 78 | 0.00 | 4.0 |
| 25.02.99 | 31.5 | 23.6 | 78 | 0.00 | 3.6 |
| 26.02.99 | 32.2 | 22.3 | 82 | 0.00 | 4.0 |
| 27.02.99 | 31.0 | 23.3 | 86 | 0.00 | 4.0 |
| 28.02.99 | 32.1 | 22.8 | 79 | 0.00 | 3.5 |
| 01.03.99 | 32.1 | 23.4 | 76 | 0.00 | 4.3 |
| 02.03.99 | 33.0 | 23.3 | 76 | 0.00 | 4.3 |
| 03.03.99 | 33.2 | 23.3 | 86 | 0.00 | 4.2 |
| 04.03.99 | 32.0 | 23.3 | 76 | 0.00 | 5.2 |
| 05.03.99 | 32.3 | 24.6 | 74 | 0.00 | 4.0 |

| Date | Max. Temp. (°C) | Min. Temp. (°C) | Relative humidity (%) | Rainfall (mm) | Evaporation (mm) |
|---|---|---|---|---|---|
| 06.03.99 | 31.4 | 23.3 | 75 | 0.00 | 4.3 |
| 07.03.99 | 32.3 | 22.4 | 76 | 0.00 | 4.0 |
| 08.03.99 | 32.5 | 23.3 | 74 | 0.00 | 4.5 |
| 09.03.99 | 32.5 | 23.5 | 72 | 0.00 | 4.0 |
| 10.03.99 | 32.5 | 23.5 | 76 | 0.00 | 4.4 |
| 11.03.99 | 32.5 | 23.6 | 81 | 1.00 | 4.4 |
| 12.03.99 | 32.9 | 24.1 | 74 | 0.00 | 4.0 |
| 13.03.99 | 32.0 | 25.0 | 86 | 0.80 | 4.4 |
| 14.03.99 | 32.0 | 25.0 | 82 | 0.60 | 4.4 |
| 15.03.99 | 33.6 | 25.5 | 78 | 0.00 | 4.0 |
| 16.03.99 | 32.9 | 22.2 | 74 | 0.00 | 3.9 |
| 17.03.99 | 32.9 | 25.5 | 76 | 0.00 | 4.8 |
| 18.03.99 | 32.0 | 22.2 | 74 | 0.00 | 5.5 |
| 19.03.99 | 32.7 | 28.7 | 79 | 0.00 | 4.7 |
| 20.03.99 | 32.2 | 24.7 | 77 | 0.00 | 4.0 |
| 21.03.99 | 32.3 | 24.9 | 83 | 0.00 | 4.0 |
| 22.03.99 | 32.9 | 25.2 | 76 | 0.00 | 4.0 |
| 23.03.99 | 32.7 | 25.4 | 76 | 0.00 | 4.6 |
| 24.03.99 | 33.3 | 25.4 | 78 | 0.00 | 4.0 |
| 25.03.99 | 33.4 | 25.0 | 75 | 0.00 | 4.0 |

# PHYSIOLOGICAL ASPECTS OF *EX VITRO* ESTABLISHMENT OF TISSUE CULTURED ORCHID (*Dendrobium* sp. var. SONIA 17) PLANTLETS

By

**SAMASYA K. S.**

## ABSTRACT OF THESIS

*Submitted in partial fulfilment of the requirement for the degree*

# Master of Science in Agriculture

*Faculty of Agriculture*
*Kerala Agricultural University*

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI
THIRUVANANTHAPURAM

**2000**

# ABSTRACT

The present investigation was undertaken to elicit information on the physiological, morphological, biochemical, biometric and anatomical changes during *in vitro* propagule multiplication and *ex vitro* establishment in tissue cultured plantlets of orchids. *Dendrobium* hybrid Sonia 17 was the variety used for the study. The rooting media was supplemented with different levels of sucrose. At the time of planting the plantlets were subjected to triazole treatment. During the process of hardening the plantlets were maintained in hardening chambers with different levels of light and humidity.

Among growth parameters the crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR) were found to be high at 40 g/l of sucrose concentration. The CGR of these plantlets were on par with normal green house grown plantlets. The photosynthetic rate was found to increase and the transpiration rate was found to decrease at 40 g/l of sucrose concentration.

The maximum survival percentage of the *in vitro* plantlets occur when 40 g/l of sucrose incorporated in the rooting medium. This may be attributed to the influence of the sucrose concentration on morphological characters studied viz. plant height, number of leaves per shoot and number of roots per shoot. With regard to photosynthetic pigments an increase in the content of total chlorophyll, chlorophyll a chlorophyll b and carotenoids occurred in plantlets

treated with 40 g/l of sucrose. Also the protein content and carbohydrate content was maximum at the above sucrose level. The leaf area, root length, total fresh weight and dry weight of the plantlets maintained at 40 g/l of sucrose level were higher than other treatments. These effects ultimately lead to better survival percentage.

Triazole treatment of plantlets during planting out helped in better survival percentage. With regard to the growth parameters the effect was distinct towards the later stage of growth. CGR, NAR and RGR were maximum on the triazole treated (5 mg/l) plantlets and was comparable to normal green house grown plants. There was marked increase in the photosynthetic rate and decrease in transpiration rate of plantlets treated with 5 mg/l of triazole. However the photosynthetic rate of normal green house grown plants were much more than the tissue cultured plantlets.

Regarding morphological characters, increasing concentration of triazole had negative influence on plant height. However plant height of normal green house grown plants were distinctively higher than tissue cultured plantlets. With regard to number of leaves per shoot, triazole treatment showed significant effect only after 30 days of planting out, whereas the number of roots per shoot was very much influenced by triazole. The maximum number of roots was produced at 5mg/l of triazole treatment and these effects in turn influenced higher survival percentage of plantlets.

Regarding the biochemical aspects total chlorophyll, chlorophyll a, chlorophyll b and carotenoids contents of plantlets treated with triazole (5 mg/l) were higher and found to be on par with that of green house grown normal plants towards the later stage. The protein content was also positively influenced by triazole treatment (5 mg/l) and the value was comparable to that of normal plantlets. In the case of carbohydrate content the treatment becomes statistically significant and the normal green house grown plants exhibited distinctively higher value. The plantlets treated with 5 mg/l of triazole were found to have higher leaf area, root length, root: shoot ratio, total fresh weight and dry weight than other treatments. However the root shoot ratio and total fresh weight of treated plantlets were on par with the normal green house grown plants towards the later stage viz, 45DAP.

The physiological, morphological, biochemical and biometric characters of the plantlets were also found to be influenced by different levels of light intensity and humidity maintained in the hardening chamber. Among the different treatments, plantlets grown at 50 percent light intensity and 70 to 90 percent relative humidity exhibited higher CGR, NAR and RGR. Also these plantlets exhibited a marked increase in photosynthetic rate and decrease in transpiration rate. However CGR and NAR of green house grown normal plants were distinctively higher during the later period. However the normal plants had distinctively higher CGR, RGR and photosynthetic and very less

transpiration rate than other treatments. The plantlets grown under 50 percent light intensity and 70 to 90 percent humidity produced maximum plant height, number of leaves and number of roots per shoot. The survival percent of the tissue cultured plantlets in the field condition (green house) was found to be superior under appropriate environment of light and humidity (50 percent light intensity and 70 to 90 percent relative humidity)

There was not much significant difference among the treatments of light and humidity in the case of pigment content. However the plantlets grown in the hardening chamber maintained at 50 percent light intensity and 70 to 90 percent of relative humidity recorded maximum value of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids among treatments and the normal green house grown plants exhibited distinctively higher pigment content. The normal plantlets recorded marked increase in protein content and carbohydrate content than the tissue cultured plantlets. In the present study, an environment of 50 percent light intensity and 70 to 90 percent relative humidity was found to be superior and could favour enhanced leaf area, total fresh weight, total dry weight and root length of plantlets which ultimately resulted in better survival percentage.

The in vitro plantlets observed to have anatomical characters as compared to the normal green house grown plants and hardened plantlets. The stomata remained open and less number of stomata per unit area of leaf was observed as the *in vitro* leaves were concerned. Another peculiarity of the in vitro plantlets

was the absence of cuticle layer. The mesophyll layers were also found to be less compared to normal plantlets. These anatomical characters were one of the severe limitations of the micropropagated orchid plantlets during *ex vitro* establishment which ultimately resulted in high rate of field mortality.