UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF A REGENERATION SYSTEM FOR SPATHOGLOTTIS PLICATA BLUME ORCHID FROM SEEDS AND ANALYSIS OF ITS BIOCHEMICAL PROPERTIES

ZALIYATUN AKHMA BT MAT YASIN

FBSB 2014 14

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By

ZALIYATUN AKHMA BT MAT YASIN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

September 2014

For Ayah, Emak, Yazid and Nabilah

Your love will shine in me forever

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

OPTIMIZATION OF A REGENERATION SYSTEM FOR *SPATHOGLOTTIS PLICATA* BLUME ORCHID FROM SEEDS AND ANALYSIS OF ITS BIOCHEMICAL PROPERTIES

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September 2014

Chairman: Professor Maziah Mahmood, PhD

Faculty: Biotechnology and Biomolecular Sciences

Spathoglottis plicata is widely used as a ground cover in landscaped garden and as potted plants. Due to its unique structure and colour this orchid is sought after by florists and orchid collectors. However, it is slow growing thus limiting the production of planting materials. Therefore, there is a need to improve its multiplication efficiency and speed up its growth. The objectives of this experiment are to improve the regeneration system of this orchid from the seeds and analyzing its biochemistry properties. The optimizations of the media for the seed germination and callus induction were carried out followed by the development of optimum medium and culture condition for the growth of plantlets and PLBs. The effect of micronutrients on the physical and biochemical changes in plantlets were investigated. In addition, the influences of amino acids and polyamines on the growth and biochemical properties of PLBs were determined followed by the investigation of total phenolic compound in the *in vivo* and *in vitro* leaves, roots and PLBs of this orchid. The optimum medium for seed germination was ½ MS augmented with 5 µM BAP. Investigation on the callus induction from seed conducted from various concentration auxin resulted in the highest callus induction were obtained from seed cultured on the medium fortified with 5 µM 2,4-D. The optimum medium and culture condition for plantlets was ½ MS without any plant growth regulator and should be transfered into fresh media after six weeks of culture. Meanwhile, the PLBs should be maintained in the ½ MS supplemented with 5 µM BAP and subcultured after four weeks of culture. Different concentrations of micronutrients give various effects on the physical and biochemical changes in the plantlets. The treatment of 50 µM Zinc had an increase of 50 % in plantlets height but for the treatment of 25 µM Manganese had 60 % increase in the total soluble protein obtained from the plantlet. The presence of micronutrient in the medium also increased the peroxidase and catalase activity but reduced the polyphenol oxidase and nitrate reductase activity in

the plantlets. The optimum amino acids concentration for the growth of PLBs was 5 mM glutamine. The addition of 5 mM glutamine and 5 µM BAP improved the growth and increased the peroxidase and catalase activities but reduced the nitrate reductase activity of PLBs. Meanwhile, the optimum polyamine concentration for the growth of PLBs was 25 µM spermidine. The 25 µM spermidine treatment cause a reduction in peroxidase and catalase but increased the nitrate reductase activity in the PLBs. Total phenolic content *in vivo* leaves and root is higher compared to *in vitro* and total phenolic content in the PLBs is the lowest.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGOPTIMUMAN SISTEM REGENERASI ORKID *SPATHOGLOTTIS PLICATA* BLUME DARI BIJI DAN ANALISA SIFAT BIOKIMIANYA

Oleh

ZALIYATUN AKHMA BT MAT YASIN

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Spathoglottis plicata banyak digunakan sebagai penutup bumi di sekitar taman dan juga sebagai pokok hiasan berpasu. Orkid ini diminati oleh pengumpul orkid dan penjual bunga kerana struktur dan warnanya yang unik. Namun begitu, sifatnya yang lambat membiak menyebabkan penghasilan bahan tanaman yang rendah. Oleh itu adalah amat perlu untuk meningkatkan kadar pembiakan dan pertumbuhan orkid ini. Objektif kajian ini dilaksanakan adalah untuk menambahbaik sistem regenerasi orkid ini dari biji dan menganalisa sifat biokimianya. Pengoptimuman media bagi percambahan biji dan pembentukan kalus dijalankan diikuti dengan membangunkan media dan keadaan kultur yang optimum untuk pembesaran anak pokok and PLBs. Kesan mikronutrien terhadap perubahan fisikal dan biokimia dalam anak pokok diselidik. Pengaruh asid amino dan poliamin terhadap pertumbuhan dan sifat biokimia PLBs dikaji seterusnya kajian mengenai kandungan fenolik dalam daun dan akar *in vivo* dan *in vitro* serta PLBs. Media optimum untuk percambahan biji ialah ½ MS ditambah dengan 5 µM BAP. Kajian bekenaan kesan pelbagai ausin terhadap pembentukan kalus menunjukan hasil tertinggi setelah dikultur dengan 5 µM 2,4-D. Medium dan keadaan kultur yang optimum untuk pertumbuhan anak pokok ialah medium ½ MS tanpa sebarang penggalak pertumbuhan dan dipindah ke medium baru selepas enam minggu dikultur. Manakala bagi PLBs, ianya perlu dikultur dalam medium ½ MS bersama 5 µM BAP dan disubkultur selepas empat minggu. Kepekatan mikronutrien yang berlainan memberi kesan yang berbeza terhadap perubahan fisikal dan biokimia anak pokok. Tinggi anak pokok yang dirawat dengan 50 µM Zink telah meningkat sebanyak 50% manakala kandungan protein terlarut dalam anak pokok yang dirawat dengan 25 µM Mangan telah bertambah sebanyak 60%. Kehadiran mikronutrien dalam media telah meningkatkan aktiviti peroksidase dan catalase tetapi belaku pengurangan dalam aktiviti polifenol oksidase dan nitrat reduktase. Kepekatan optimum asid amino bagi pertumbuhan PLBs ialah 5 mM glutamin. Penambahan 5 mM glutamin dan 5

μM BAP ke dalam media telah meningkatkan pertumbuhan dan aktiviti peroksidase serta catalase tetapi telah mengurangkan aktiviti nitrat reduktase. Poliamin pada kepekatan 25 μM spermidine adalah yang optimum bagi menambahbaik pertumbuhan PLBs dan rawatan ini telah mengurangkan aktiviti peroksidase dan catalase tetapi meningkatkan aktiviti nitrat reduktase. Kandungan fenolik dalam daun dan akar in *vivo* adalah lebih tinggi dari *in vitro* dan yang terendah adalah dari PLBs

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor, Prof. Dr. Maziah Mahmood for her guidance and support throughout this study and to complete this thesis. Appreciation also goes to my friends who have worked in the Plant Biotechnology Lab (Lab 235, 2010-2014) for sharing their knowledge, experiences, friendship and supports. Last but not least my deepest gratitude to my family, father, mother, brother and sister for their love, patience, understanding, support and courage during the time needed. May Allah bless all these people for their kindness. Thank you very much.

I certify that a Thesis Examination Committee has met on 8/9/2014 to conduct the final examination of Zaliyatun Akhma Bt. Mat Yasin on her thesis entitled "Optimization of A Regeneration System For *Spathoglottis plicata* Blume Orchid From Seeds And Analysis Of Its Biochemical Properties" in accordance with the Universities and University Colleges Act 1971 and Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

%	Percent
cm	Centimetre
uM	Micro molar
uL	Micro litre
W/V	Weight per volume
2,4-D	2,4-dichlorophenoxy acetic acid
B5	Gamborg basal media
BA	Benzyl adenine
BAP	6-benzylaminopurine
BSA	Bovine serum albumin
$CoCl_2$	Cobalt(II) chloride
$CuSO_4$	Copper(II) sulfate
Dicamba	3,6-dichloro-*o*-aniscic acid
EDTA	Ethylenediaminetetraacetic acid
FAD	Flavin adenine dinucleotide
FE EDTA	Ethylenediaminetetraacetic acid ferric sodium salt
g	Gram
g/L	Gram per litre
H_2O_2	Hydrogen peroxide
HCl	Hydrogen Chloride
IAA	Indoleacetic acid
K_2HPO_4	Potassium phosphate dibasic
$KHPO_4$	Potassium hydrogen phosphate
KI	Potassium iodide
mg/L	Miligram per litre
mM	Mili molar
$MnSO_4$	Manganese(II) sulphate
MS	Murashige and Skoog basal media
NAA	*1*-Naphthaleneacetic acid

NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
PEG	Polyethylene glycol
TDZ	Thidiazuron
ZnSO4	Zinc sulphate

CHAPTER 1

INTRODUCTION

Flowers have a special place in human society for ages. It is used by all races in the world for decoration, offerings in ceremonies and a symbol to present emotion. Some of them are use as food, medicine and colouring. Most of the flowers are cultivate and put onto the market as the potted plants and cut flowers. Orchid is one of the flowers that has high impact in human society. It has exotic shapes, variety of colours and long shelf life which has inspired devotion among orchid lovers. Some of the orchids have medicinal values and fragrances. All of these properties had caused significance evolution in the orchid industry. Orchid has evolved from a flower only for the hobbyist into high commercialized potted plants and cut flowers. Thailand, Taiwan, Japan, New Zealand, United Kingdom, Italy and Brazil are the countries that dominate the orchid exportation and America is one of the largest importers of potted orchid. (Chugh et al., 2009).

Orchid industry is a big business in Malaysia because its high demands both for local and foreign market. *Dendrobium, Cattleya*, *Vanda* and *Phalaenopsis* are highly sought after for their beautiful colours and fragrances and *Paphiopedilum* is popular due to its unique shape while *Vanilla* is cultivated for its flavour. Malaysia is a home to 3000 species of orchids that can be developed and commercialized. One of which is *Spathoglottis plicata*, a terrestrial orchid which is usually used as ground cover for the landscape and potted plants because it is easy to cultivate.

This orchid also has medicinal values (Rao, 2004). Besides, they were used as tobacco substitutes in the Philippines during the World War II. Its unique stature and charming flower have gained attention due to its high value in the ornamental market. However, *Spa. plicata* is slow growing and this plant propagates through the multiplication of the pseudobulb. (Hossain and Dey, 2013). Low multiplication efficiency produces insufficient plant materials to meet the need of the market. Deforestation and extremely indiscriminate collection have lead to extinction of many rare and wild orchids. Micropropagation of orchid especially the rare and wild type can help to conserve the germplasma and to mass produce the planting materials.

There are several studies that have been carried out on the tissue culture protocols of this orchid. Teng et al. (1997) reported that the production of plantlets from PLBs derived from nodal segments of eight month old plantlets. Sinha et al, (2009) also reported the regeneration of microshoots from the nodal explants. A study of seed germination of *Spa. plicata* was also carried out by Hossain and Dey (2013) whereby they used immature and mature seeds sown onto different media to investigate the seed germination and plantlets development. However, all these studies do not provide a complete, efficient protocol and biochemical information of this orchid. Therefore, efficient micropropagation protocols are needed to improve the germination and production of this orchid.

The optimum concentrations of certain micronutrients are also essential in tissue culture meia. According to Kowalska et al. (2012), higher concentration of copper in the media will improve the production of plantlet from the callus of *Daucus carota*. Application of additives, such as amino acids and polyamines are beneficial to increase the cell proliferation and growth of *in vitro* plant. However, one of the problems with plant tissue culture is the production of phenolic in the media which affecting the vitality of the culture. Therefore, simple preliminary experiments were also conducted as on the presence of phenolic compound and the polyphenol oxidase activity were conducted in order to determine the content of phenolic in PLBs or plantlets. *In vitro* protocols developed and the information on the biochemical changes during the growth of the species can be used for further development studies of the species and will help in conservation of this orchid. Therefore, the objectives of this study are:

1. To develop efficient protocols for seed germination and PLBs induction via callus of *Spa. plicata*
2. To examine the biochemical changes during the growth of *Spa. plicata*'s plantlets and PLBs after treated with selected micronutrient, polyamines, and amino acids.
3. To determine the phenolic content of in *in vivo* and *in vitro Spa. plicata* leaves, roots, and PLBs

REFERENCES

Abraham, S., Augustine J., & Thomas T. D. (2012). Asymbiotic seed germination and *in vitro* conservation of Coelogyne *nervosa* A. Rich. an endemic orchid to Western Ghats. *Physiology* and *Molecular Biology of Plants,* 18(3), 245–251.

Afshari, R. T., Angoshtari, R., & Kalantari, S. (2011). Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus L.*) genotypes. *Plant Omics Journal*, 4(2), 60-67.

Ahmad, I., Hussain, T., Ashraf, I., Nafees, M., Maryam, Rafay, M. & Iqbal, M. (2013). Lethal effects of secondary metabolites on plant tissue culture. *American Eurasian Journal of Agricultural and Environmental Science,* 13(4), 539 – 547.

Aktar, S., Nasiruddin, K. M. & Huq, H. (2007). *In vitro* root formation in *Dendrobium* orchid plantlets with IBA. *Journal of Agriculture & Rural Developmen,* 5(1&2), 48-51.

Ali, A., Sivakami, S. & Raghuram, N. (2007). Effect of nitrate, nitrite, ammonium, glutamate, glutamine and 2-oxoglutarate on the RNA levels and enzyme activities of nitrate reductase and nitrite reductase in rice. *Physiology and Molecular Biology of Plants,* 13(1), 17-25.

Ali, R.M. (2000). Role of putrescine in salt tolerance of *Atropa belladonna* plant. *Plant Science*, 152, 173–179.

Alvarez- Pardo, V. M., Ferreira, A. G. & Nunes, V. F. (2006). Seed disinfestations methods for *in vitro* cultivation of epiphyte orchids from Southern Brazil. *Horticultura Brasileira,* 24, 217-220.

Amin, A.A., Gharib, F. A. E., El-Awadi, M. & Rashad, E. M. (2011). Physiological response of onion plants to foliar application of putrescine and glutamine. *Scientia Horticulturae*, 129, 353–360.

Apel, K. & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology,* 55(3), 3- 99.

Arnok, P., Ruangviriyachai, C., Mahachai, R., Techawongstein, S. & Chanthai, S. (2010). Optimization and determination of polyphenol oxidase and peroxidase activities in hot pepper (*Capsicum annuum* L.) pericarb. *International Food Research Journal,* 17, 385-392.

Arnon, D. I. (1949). Copper enzyme in isolated chloroplasts.Polyphenoloxidase in *Beta vulgaris. Plant Physiology*, 24(1), 1-16.

Asad, S., Arshad, M., Mansoor, S. & Zafar, Y. (2009). Effect of various amino acids on shoot regeneration of sugarcane (*Sacchrum officinarum* L.). *African Journal of Biotechnology, 8*(7), 1214-1218**.**

Avila-Dıaz, I., Oyama , K., Go´mez-Alonso, C. & Salgado-Garciglia, R. (2009). *In vitro* propagation of the endangered orchid *Laelia speciosa*. *Plant Cell, Tissue and Organ Culture,* 99, 335–343.

Basker, S. & Bai, N.V. (2010). *In vitro* propagation of an epiphytic and rare orchid *Eria bambusifolia* Lindl. *Research in Biotechnology,* 1, 15-20.

Bernstein, N., Shoresh, M., Xu, Y. & Huang, B. (2010). Involvement of the plant antioxidative response in the differential growth sensitivity to salinity of leaves vs roots during cell development. *Free Radical Biology & Medicine*, 49, 1161–1171.

Bulpitt, C. J. (2005). The uses and misuses of orchids in medicine. *The Quarterly Journal of Medicine,* 98, 625–631.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry,* 72, 248-254.

Bythrow, J. D. (2005). Vanilla as a medicinal plant. *Seminar In Interagrative Medicine, 3,* 129- 131.

Chauhan, M. & Kothari, S.L. (2004). Optimization of nutrient levels in the medium increases the efficiency of callus induction and plant regeneration in recalcitrant Indian barley (*Hordeum vulgare* L.) *in vitro*. *InVitro Cellular & Developmental Biology. – Plant,* 40, 520 -527**.**

Chen, J. T., Chang, W. C. (2000). Efficient plant regeneration through somatic embryogenesis from callus cultures of *Oncidium* (Orchidaceae). *Plant Science,* 160, 87–93.

Chen, T. Y., Chen J. T. & Chang, W. C. (2004). Plant regeneration through direct shoot bud formation from leaf cultures of *Paphiopedilum* orchids. *Plant Cell, Tissue and Organ Culture,* 76,11–15.

Cheruvathur, M. K., Abraham, J., Mani, B. & Thomas, T. D. (2010). Adventitious shoot induction from cultured interned explants of *Malaxis acuminata* D.Don, a valuable terrestrial medicinal orchid. *Plant Cell Tissue Organ Culture,* 101, 163–170.

Chikmawati, T. (2013). Anatomical and cytological features of *Spathoglottis plicata* from Java Island. *The Journal of Tropical Life Science*, 3(2), 87 – 90.

Chinsamy, M., Finnie, J. F. & Van Staden J. (2011). The ethnobotany of South African medicinal orchids. *South African Journal of Botany,* 77, 2–9.

Chugh, S., Guha, S. & Rao, U. I. (2009). Micropropagation of orchids: A review on the potential of different explants. *Scientia Horticulturae,* 122, 507–520.

Chung, H. H, Chen, J. T. & Chang, W. C. (2007). Plant regeneration through direct somatic embryogenesis from leaf explants of *Dendrobium. Biologia Plantarum,* 51, 346-350.

Chung, J. P., Huang, C. Y., & Dai, T. E. (2010). Spectral effects on embryogenesis and plantlet growth of *Oncidium* 'Gower Ramsey. *Scientia Horticulturae,* 124, 511–516.

Chung, M. Y. & Park, C. W. (2008). Fixation of alleles and depleted levels of genetic variation within populations of the endangered lithophytic orchid *Amitostigma gracile* (Orchidaceae) in South Korea: implications for conservation. *Plant Systematic and Evolution*, 272, 119 – 130.

Das, A. & Mandal, N. (2010). Enchanced development of embryogenic callus in *Stevia rebaudiana* Bert. by additive and amino acids. *Biotechnology*, 9(3), 368 – 372.

Dearnaley, J. D. W. (2007). Further advances in orchid mycorrhizal research. *Mycorrhiza*, 17, 475–486.

De, K. K., Majumdar, S. Sharma, R. & Sharma, B. (2006), Green pod culture and rapid micropropagation of *Dendrobium chysanthum* Wall. – a horticultural and medicinal orchid. *Folia Horticulturae*, 18(1), 81 – 90.

D´eciga-Campos, M., Palacios-Espinosa, J. F., Reyes-Ramirez, A., & Mata, R. (2007). Antinociceptive and anti-inflammatory effects of compounds isolated from *Scaphyglottis livida* and *Maxillaria densa. Journal of Ethnopharmacology*, 114, 161–168.

Deb, C. R. & Pongener, A. (2011). Asymbiotic seed germination and *in vitro* plantlets development of *Cymbidium aloifolium* (L.) Sw.: a multipurpose orchid. *Journal of Plant Biochemistry and Biotechnology*, 20(1), 90–95.

De-la Pena, C., Galaz- Avolos, R. M. & Loyola – Vargas, V. M. (2008). Possible role of light and polyamines in the onset of somatic embryogenesis of *Coffea canephora*. *Molecular Biotechnology*, 39, 215-224.

Deo, B. & Nayak, P. K. (2011). Study of copper phytotoxicity on *in vitro* culture of *Musa acuminata* cv. 'Bantala'. *Journal of Agricultural Biotechnology and Sustainable Development*, 3(8), 136-140.

Dohling, S., Kumaria, S. & Tandon, P. (2012). Multiple shoot induction from axillary bud cultures of the medicinal orchid, *Dendrobium longicornu*. *AoB Plants*, 32, 1-7.

Duan, J., Li, J., Guo, S. & Kang, Y. (2008). Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *Journal of Plant Physiology,* 165, 1620—1635.

Duran, R. E., Coskun, Y. & Demırcı, T. (2013). Comparison of amino acids for their efficiency on regeneration in wheat embryo culture. *Asian Journal of Plant Science and Research*, 3(1), 115-119.

Dutra, N. T., Silveira, V., Goncalves de azevedo, I., Gomes –Neto, L. R., Facanha, A. R., Steiner, N., Guerra, M. P., Floh, E. I. S. & Santa Catarina, C. (2013). Polyamines affect the cellular growth and structure of pro-embryogenic masses in *Araucaria angustifolia* embryogenic cultures through the modulation of proton pump activities and endogenous levels of polyamines. *Physiologia Plantarum*, 148, 121–132.

El- Shiaty, O. H., El-Sharabasy, S. F. & El- Kariem, A. H. A. (2004). Effect of some amino acids and biotin on callus and proliferation of date palm (*Phoenix dactylifera* L.) Sewy cultivar. *Arab Journal of Biotechology,* 7(2), 265-272.

Fatima, N., Ahmad, N. & Anis, M. (2011). Enhanced *in vitro* regeneration and change in photosynthetic pigments, biomass and proline content in *Withania somnifera* L. (Dunal) induced by copper and zinc ions. *Plant Physiology and Biochemistry*, 49, 1465–1471.

Ganesan, M. & Jayabalan, N. (2006). Influences of cytokines, auxins and polyamines on in vitro mass multiplication of cotton (*Gossypium hirsutum* L. cv. SVPR2). *Indian Journal of Experimental Botany,* 44, 506-514.

Gantait, S., Bustam, S. & Sinniah, U.R. (2012). Alginate-encapsulation, short term storage and plant regeneration from protocorm-like bodies of *Aranda* Wan Chark Kuan 'Blue' x *Vanda coerulea* Grifft. Ex. Lindl. (Orchidaceae). *Plant Growth Regulator,* 68(2), 303-311.

Gantait, S & Sinniah, U.R. (2012). Rapid micropropagation of monopodial orchid hybrid (*Aranda* Wan Chark Kuan 'Blue' x *Vanda coerulea* Grifft. Ex. Lindl.) through direct induction of protocorm-like bodies from leaf segments. *Plant Growth Regulator*, 68, 129–140.

Gao, S., Yan, R., Cao, M., Yang, W.,Wang, S. & Chen, F. (2008). Effects of copper on growth, antioxidant enzymes and phenylalanine ammonia lyase activities in *Jatropha curcas* L. plantlets. *Plant Soil Environment*. 54(3), 117–122.

Gill, P.K., Sharma, A.D., Singh, P. & Bhullar, S.S. (2002). Osmotic stress induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. *Bulgarian Journal of Plant Physiology*, 28(3–4), 12–25.

Gill, S. S. & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909 – 930.

Godo, T., Komari, M., Nakaoki, E., Yukawa, T. & Miyoshi, K. (2010). Germination of mature seeds of *Calanthe tricarinata* Lindl., an endangered terrestrial orchid, by asymbiotic culture in vitro. *In Vitro Cellular &Developmental Biology – Plant,* 46, 323–328.

Gonzalez-Lamothe, R., El Oirdi, M., Brisson, N. & Bouarab, K. (2012). The conjugated auxin Indole-3-acetic acid–aspartic acid promotes plant disease development. *The Plant Cell*, 24, 762–777.

Govindarajan, R., Singh, D. P., & Rawat. A. K. S. (2007). High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug. *Journal of Pharmaceutical and Biomedical Analysis,* 43,527–532.

Gow, W. P., Chen, J. T. & Chang, W. C. (2008). Influence of growth regulators on direct embryo formation from leaf explants of *Phalaenopsis* orchids. *Acta Physiology Plant,* 30, 507–512.

Guo, T. R., Zhang, G. P. & Zhang, Y. H. (2007). Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. *Colloids and Surfaces Biointerfaces*, 57, 182–188.

Gutierrez, R. M. P. (2010). Orchids*:* a review of uses in traditional medicine, its phytochemistry and pharmacology. *Journal of Medicinal Plants Research,* 4(8), 592-638.

Grzesiak, M., Filek, M., Barbasz, A., Kreczmer, B. & Hartikainen, H. (2013). Relationships between polyamines, ethylene, osmoprotectants and antioxidant enzymes activities in wheat plantlets after short-term PEG- and NaCl-induced stresses. *Plant Growth Regulator*, 69, 177–189.

Hageman, R. H. & Flesher, D. (1960). Nitrate reductase in corn seedlings as affected by light and nitrate content of nutrient media. *Plant Physiology*, 35(5), 700-708.

Hajong, S., Kumaria, S. & Tandon, P. (2010). *In vitro* propagation of the medicinal orchid *Dendrobium chrysanthum*. *Proceeding of the Indian National Science Academy,* 76(1), 1-6.

Hamada, K., Shimasaki, K., Ogata, T., Nishimura, Y., Nakamura, K., Oyam Egawa, H. & Yoshida, K. (2010). Effects of spectral composition conversion film and plant growth regulators on proliferation of *Cymbidium* protocorm likes body (PLB) cultured *in vitro*. *Environment Control In Biology*, 48(3), 127-132.

Haroun, S. A., Shukry, W. M. & El-Sawy, O. (2010). Effect of glutamine on growth and metabolic changes in *Phaseolus vulgaris* under *in vitro* conditions. *Bioscience Research,* 7(1), 1-21.

Hegazi, G. A .E. & El-Lamey T. M. (2011). Callus induction and extraction of ephedrine from *Ephedra alata* Decne. Cultures. *American Eurasian Journal of Agricultural and Environmental Science,* 11(1), 19 – 25.

Hong, P. J., Chen J. T. & Chang, W. C. (2010). Shoot development and plant regeneration from protocorm-like bodies of *Zygopetalum mackayi. In Vitro Cellular & Developmental Biology – Plant.* 46, 306–311.

Hong, P. I., Chen, J. T. & Chang, W. C. (2008). Plant regeneration via protocorm like body formation and shoot multiplication from seed derived callus of a maudiae type slipper orchid. *Acta Physiology Plant,* 30, 755–759.

Hossain, M. M. & Dey, R. (2013). Multiple regeneration pathways *in Spathoglottis plicata* Blume — A study *in vitro*. *South African Journal of Botany,* 85, 56–62.

Hossain, M. M. (2011). Therapeutic orchids: traditional uses and recent advances — An overview. *Fitoterapia*, 82, 102–140.

Hossain, M. M., Sharma, M. & Pathak, P. (2009). Cost effective protocol for *in vitro* mass propagation of *Cymbidium aloifolium* (L.) Sw. – a medicinally important orchid. *Engineering in Life Science,* 9(6), 444–453.

Hossain, M. M., Sharma, M., Teixeira da Silva, J. A. & Pathak, P. (2010). Seed germination and tissue culture of *Cymbidium giganteum* Wall. ex Lindl. *Scientia Horticulturae,* 123, 479–487.

Hsiao,Y.Y., Pan, Z. J., Hsu, C. C., Yang, Y. P., Hsu,Y. C., Chuang, Y. C., Shih, H. H., Chen, W. H., Tsai W. C. & Chen, H. H. (2011). Research on orchid biology and biotechnology. *Plant and Cell Physiology*, 52(9), 1467–1486.

Huan, L. V. T., Takamura, T. & Tanaka, M. (2004). Callus formation and plant regeneration from callus through somatic embryo structures in *Cymbidium* orchid. *Plant Science*, 166(6), 1443–1449.

Husin, N., Jalil, M., Othman, R. Y., Khalid, N. (2014). Enhancement of regeneration efficiency in banana (*Musa acuminata* cv. Berangan) by using proline and glutamine. *Scientia Horticulturae,* 168, 33–37.

Ikeuchi M., Sugimoto, K. & Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The Plant Cell*, 25, 3159–3173.

Ioannidis, N.E. & Kotzabasis, K. (2007). Effects of polyamines on the functionality of photosynthetic membrane *in vivo* and *in vitro*. *Biochimica et Biophysica Acta,* 1767, 1372–1382.

Irvani, N., Solouki, M., Omidi, M., Zare, A. R. & Shahnazi, S. (2010). Callus induction and plant regeneration in *Dorem ammoniacum* D., an endangered medicinal plant. *Plant Cell Tissue Organ Culture*, 100, 293–299.

Jain, P., Kachhwaha, S. & Kothari, S. L. (2009). Improved micropropagation protocol and enhancement in biomass and chlorophyll content in *Stevia rebaudiana* (Bert.) Bertoni by using high copper levels in the culture medium. *Scientia Horticulturae*, 119, 315-319.

Jain, P., Kachhwaha, S. & Kothari, S. L. (2012). Optimization of micronutrients for the improvement of *in vitro* plant regeneration of *Stevia rebaudiana* (Bert.) Bertoni. *Indian Journal of Biotechnology*, 11, 486 490.

Jaleel, C. A., Sankar, B., Sridharan, R. & Panneerselvam, R. (2008). Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turkey Journal of Biology,* 32, 79-83.

Janarthanam, B. & Seshadri, S. (2008). Plantlet regeneration from leaf derived callus of *Vanilla planifolia* Andr. *In Vitro Cellular & Developmental Biology – Plant,* 44, 84–89.

Jermyn, M.A (1975). Increasing the sensitivity of the anthrone method for carbohydrate. *Analytical Biochemistry*, 68, 332-335.

Jiang, F., Li, W., Huang, Y., , Chen, Y., Jin, B., Chen, N., , Ding, Z & Ding, X. (2013). Antioxidant, antityrosinase and antitumor activity comparison: the potential utilization of fibrous root part of *Bletilla striata* (Thunb.) Reichb.f. *PLOS ONE*, 8(2), 1-11.

Jin, N. Y., Go, R., Nulit, R., Eng, K. H., Ching, T. M., Nordin, F. A., Nuruddin, A. A. & Sook, L. N. (2012). Orchids of cloud forest in Genting Highlands, Pahang, Malaysia. *Sains Malaysiana*, 41(5), 505–526.

Joshi, A. & Kothari S. L. (2007). High copper levels in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of *Capsicum annuum* L. *Plant Cell Tissue Organ Culture*, 88, 127–133.

Kajdzanoska, M., Petreska, J. & Stefova, M. (2011). Comparison of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. *Journal of Agricultural and Food Chemistry,* 59, 5272–5278.

Kalimuthu, K., Senthilkumar, R. & Vijayakumar, S. (2007). *In vitro* micropropagation of orchid, *Oncidium* sp. (Dancing Dolls). *African Journal of Biotechnology,* 6(10), 1171-1174.

Kalimuthu, K., Senthilkumar. R. & Murugalatha, N. (2006). Regeneration and mass multiplication of *Vanilla planifolia* Andr. – a tropical orchid. *Current Science,* 91(10), 1401 -1403.

Kartikaningrum, S. & Effendi, K. (2005). Keragaman genetic plasma nuftah anggerik *Spathoglottis*. *Journal Horticulture*, 15(4), 260-269.

Kerepesi, I. & Gabila, G. (2000). Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat plantlets. *Crop Science,* 40, 482 – 487.

Kev, N. V., Hahn, E. J., Park, S. Y., Chakrabarty, D. & Paek K. Y. (2004). Micropropagation of an endangered orchid *Anoectochilus formosanus. Biologia Plantarum,* 48(3), 339-344.

Khan, M. A., Shirazi, M. U., Khan, M. A, Mujtaba, S. M., Islam, E., Mumtaz, S., Shereen, A., Ansari, R. U. & Ashraf, M. Y. (2009). Role of proline, K/NA ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany,* 41(2), 633-638.

Khatun, M. M., Khatun, H., Khanam, D. & Al –Amin, M. (2010). *In vitro* root formation and plantlet development in *Dendrobium* orchid. *Bangladesh Journal Agriculture Research*, 35(2), 257-265.

Khatun, S., Ali., M. B., Hahn, E. & Paek, K. (2008). Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of *in vitro* grown plants. *Environmental and Experimental Botany,* 64, 279–285.

Khoddamzadeh , A. A., Sinniah, U. R., Kadir, M. A., Kadzimin, S. B., Maziah, M. & Sreeramanan, S. (2011) *In vitro* induction and proliferation of protocorm-like bodies (PLBs) from leaf segments of *Phalaenopsis bellina* (Rchb.f.) Christenson. *Plant Growth Regulator*, 65, 381–387.

Khurana–kaul, V., Kachihwaha, S. & Kothari S. L. (2010). Direct shoot regeneration from leaf explants of *Jatropha curcas* in response to thidiazuron and high copper contents in the medium. *Biologia Plantarum,* 54(2), 369-372.

Kishor, R., Khan, P .S .S .V. & Sharma G. J. (2006). Hybridization and *in vitro* culture of an orchid hybrid *Ascocenda* 'Kangla'. *Scientia Horticulturae,* 108, 66–73.

Kitsaki, C. K., Zygouraki, S., Ziobora, M. & Kintzios, S. (2004). *In vitro* germination, protocorm formation and plantlet development of mature versus immature seeds from several *Ophrys* species (Orchidaceae). *Plant Cell Report,* 23, 284–290.

Kobashi, K., Gemma, H. & Iwahori, S. (2000). Abscisic acid content and sugar metabolism of peaches grown under water stress. *Journal of the American Society for Horticultural Science,* 125(4), 425–428.

Kosir, P., Skof, S. & Luthar, Z. (2004). Direct shoot regeneration from nodes of *Phalaenopsis* orchids. *Acta agriculturae slovenica*, 83(2), 233 – 242.

Kothari–Chajer, A., Sharma, M., Kachhwaha, S. & Kothari, S.L. (2008). Micronutrient optimization results into highly improved *in vitro* plant regeneration in kodo (*Paspalum scrobiculatum* L.) and finger (*Eleusine coracana* (L.) Gaertn.) millets. *Plant Cell Tissue Organ Culture*, 94, 105 112.

Kowalska, U., Szafranska, K., Krzyzanowska, D., Kiszekzak, W., Gorecki, R.,Janas, K., Korecka, K.(2012). Effect of increased copper ion content in the medium on the regeneration of androgenetic embryos of carrot (*Daucus carota* L.). *Acta Agrobotanica*, 65(2), 73-82.

Kubis, J. (2008). Exogenous spermidine differentially alters activities of some scavenging system enzymes, H_2O_2 and superoxide radical levels in water-stressed cucumber leave. *Journal of Plant Physiology*, 165, 397 406.

Kumar, A. & Palni, L. M. S., (2013). Changes in endogenous polyamines during *in vitro* cormlet formation in *Gladious Hybridus* Hort. *Scientia Horticulturae*, 162, 260–264.

Kumar, S. P. & Kumari,B. D. R. (2010). Effect of amino acids and growth regulators on indirect organogenesis in *Artemisia vulgaris* L. *Asian Journal of Biotechnology,* 2(1), 37 – 45.

Kumar, V., Lemos, M., Sharma, M. & Shriram, V. (2013).Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. *Free Radicals and Antioxidants*, 3, 55-60.

Lang N.T. & Hang N.T. (2006). Using biotechnological approaches for *Vanda* orchid improvement. *Omonrice,* 14, 140-143.

Lauzer, D., Renaut, S., St-Arnaud, M. & Barabe, D. (2007). *In vitro* asymbiotic germination, protocorm development, and plantlet acclimatization of *Aplectrum Hyemale* (Muhl.ex Willd.) Torr. (orchidaceae). *Journal of Torrey Botanical Society,* 134(3), 344-348.

Lea, P. J., Sodek, L., Parry, M. A. J., Shewry, P.R . & Halford, N. G. (2007). Asparagine in plants. *Annals of Applied Biology,* 150, 1–26.

Lee, Y. & Lee, N. (2003). Plant regeneration from protocorm –derived callus of *Cypripedium formosanum*. *In Vitro Cellular &Developmental Biology Plant,* 39, 475–479.

Li, Z. Y. & Xu, L. (2009). *In vitro* propagation of white-flower mutant of *Rhynchostylis gigantea* (Lindl.) Ridl. Through immature seed-derived protocorm-like bodies. *Journal of Horticulture and Forestry* , 1(6), 93-97.

Liao, Y, J.,Tsai, Y. C., Sun, Y. W., Lin, R. S., & Wu, F. S. (2011). *In vitro* shoot induction and plant regeneration from flower buds in *Paphiopedilum* orchids. *In Vitro Cellular & Developmental Biology – Plant,* 47, 702–709.

Lim, Z. X., Ling, A. P. & Hussein S. (2009). Callus induction of *Ocimum sanctum* and estimation of its total flavonoids content. *Asian Journal of Agricultural Sciences*, 1(2), 55-61.

Lo, S. F., Nalawade, .S. M., Kuo, C. L., Chen, C. L., & Tsay, H. S. (2004). Asymbiotic germination of immature seeds, plantlet development and *ex vitro* establishment of plants of *Dendrobium Tosaense* Makino – A medicinally important orchid. *In Vitro Cellular & Development al Biology. – Plant, 40,* 528 – 535.

Long, B. Niemiera, A. X., Cheng, Z. & Long, C. (2010). *In vitro* propagation of four threatened *Paphiopedilum* species (Orchidaceae). *Plant Cell Tissue Organ Culture*, *101,* 151–162.

Luo, J.P., Wawrosch, C. & Kopp, B. (2009). Enhanced micropropagation of *Dendrobium huoshanense* C.Z. Tang et S.J. Cheng through protocorm like bodies: The effects of cytokinins, carbohydrate sources and cold pretreatment. *Scientia Horticulturae,* 123, 258–262.

Mahendran, G. & Bai, N. V. (2012). Direct somatic embryogenesis and plant regeneration from seed derived protocorms of *Cymbidium bicolour* Lindl. *Scientia Horticulturae*, 135, 40–44.

Malabadi, R. B., Mulgund, G. S. & Kallappa, N. (2005). Micropropagation of *Dendrobium nobile* from shoot tip sections. *Journal of Plant Physiology,* 162, 473—478.

Manner, V., Kumaria, S. & Tandon, P. (2010). *Micropropagation of Vanda coerulea Griff ex Lindl.: A Study of Regeneration Competence of Roots in vitro.* 2010 International Conference on Environmental Engineering and Applications (ICEEA 2010).

Matta-Rosas, M., Baltazar-Garcia, R. J., Moon, P., Heitz, P. & Luna Monterrojo, V. E. (2010). *In vitro* regeneration of *Lycaste aromatic* (Graham ex Hook) Lindl. (Orchidaceae) from pseudobulb section. *Plant Biotechnology Reports*, 4, 157-163.

Mayer, J. L. S., Stancato, G. C. & Appezzato-Da-Glo´ria, B. (2007). Direct regeneration of protocorm-like bodies (PLBs) from leaf apices of *Oncidium flexuosum* Sims (Orchidaceae). *Plant Cell Tissue Organ Culture,* 103, 411–416.

Mei, T. A., Danial M., Maziah, M. & Sreeramanan, S. (2012). Exquisite protocol of callus induction and protocorm-like bodies (PLBs) regeneration of *Dendrobium* 82fric-28. *Australian Journal of Crop Science,* 6(5), 793-800.

Mengxi , L., Zhigang, X., Yang, Y. & Yijie, F. (2010). Effects of different spectral lights on *Oncidium* PLBs induction, proliferation, and plant regeneration. *Plant Tissue Organ Culture*, 106, 1-10.

Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Breusegem, F. B. & Noctor, G. (2010). Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. *Journal of Experimental Botany,* 61(15), 4197 – 4220.

Mittler, R. & Poulos, T.nL., (2005). Ascorbate peroxidase. In N. Smirnoff (Eds.), *Antioxidants and Reactive Oxygen Species in Plants* (pp. 87- 100). Oxford: Blackwell Publishing Ltd.

Mittler, R., Vanderauwera, S., Gollery, M. & Breusegem, F. V. (2004). Reactive oxygen gene network of plants.*TRENDS in Plant Science,* 9(10), 490-498

Moretti, M., Cossignani, L., Messina, F., Dominici, L, Villarini, M., Curini, M. & Marcotullio M.C. (2013). Antigenotoxic effect, composition and antioxidant activity of *Dendrobium speciosum*. *Food Chemistry*, 140, 660 - 665.

Mohd- Hairul, A.R., Namasivayam, P., Lian, G. E. C. & Abdullah, J. O. (2010). Terpenoid, benzenoid, and phenylpropanoid compounds in the floral scent of *Vanda* Mimi Palmer. *Journal of Plant Biology,* 53, 358–366.

Morozkina, E. V. & Zvyagilskaya, R. A. (2007). Nitrate reductase: structure, functions, and effect of stress factors. *Biochemistry (Moscow*), 72(10), 1151-1160.

Munoz, M. & Jimenez, V.M. (2008). Capsule development, in vitro germination and plantlet acclimatization in *Phragmipedium humboldtii*, *P. longifolium* and *P. pearcei*. *LANKESTERIANA,* 8(2), 23- 31.

Murdad, R., Hwa, K. S., Seng C. K., Mariam, A. L., Zaleha, A. Z., & Rimi, R. (2006). High frequency multiplication of *Phalaenopsis gigantean* using trimmed bases protocorms technique. *Scientia Horticulturae,* 111, 73-79.

Mustafa, N. S., Taha, R. A., Hassan, S. A. M, Zaid, N. S. M. & Mustafa, E. A. (2013). Overcoming phenolic accumulation of date palm *In vitro* culture using -tochopherol and cold pre-treatment. *Middle-East Journal of Scientific Research*, 15(3), 344-350.

Nahar, S. J., Kazuhiko, S. & Haque, S. M. (2012). Effect of polysaccharides including elicitors on organogenesis in protocorm-like body (PLB) of *Cymbidium insigne in vitro. Journal of Agricultural Science and Technology*, 2, 1029-1033.

Narkhede, A., Nirmal, P., Tupei, R., Kulkarni, O., Harsulkar, A. & Jagtap, S. (2012). *In vitro* antioxidant, antiglycation and a- amylase inhibitory potential of *Eulophia ochreata* L. *Journal of Pharmacy Research,* 5(5), 2532 2537.

Nas, M. N. (2004). Inclusion of polyamines in the medium improves shoot elongation in hazelnut (*Corylus avellana* L.) micropropagation. *Turkish Journal of Agriculture and Forestry,* 28, 189 -194.

Nayak, N. R., Sahoo, S., Patnaik, S. & Rath, S.P. (2002). Establishment of thin cross section (TCS) culture method for rapid micropropagation of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* Lindl. (Orchidaceae). *Scientia Horticulturae,* 94, 107–116.

Ng, C. H., Norihan, M. S., & Faridah, Q. Z. (2010). *In vitro* multiplication of the rare and endangered slipper orchid, *Paphiopedilum rothschildianum* (Orchidaceae). *African Journal of Biotechnology*, 9(14), 2062-2068.

Niedz R. P & Evens, T. J. (2007). Regulating plant tissue growth by mineral nutrition. *In Vitro Cellular & Developmental Biology – Plant,* 43, 370–381.

Nongrum, I., Kumaria, S., & Tandon, P. (2007). Influence of *in vitro* media on asymbiotic germination, plantlet regeneration and *ex vitro* establishment of *Coelogyne ovalis* Lindl. and *Coelogyne nitida* (Wall. ex Don) Lindl. *Proceeding of the Indian National Science Academy*, 73(4), 205 – 207.

Omran, R. G. (1980). Peroxide levels and the activities of catalase, peroxidase, and indoleacetic acid oxidase during and after chilling cucumber seedlings. *Plant Physiology*, 65, 407-408

Ovono, P.O., Kevers, C. & Dommes, J., (2010). Tuber formation and development of *Dioscorea cayenensis–Dioscorea rotundata* complex in vitro effect of polyamines. *In Vitro Cellular & Developmental Biology Plant,* 46, 81–88.

Palama, T. L., Menard, P., Fock, I., Choi, Y. H., Bourdon ,E., Govinden Soulange, J., Bahut, M., Payet, B., Verpoorte, R. & Kodja, H. (2010) Shoot differentiation from protocorm callus cultures of *Vanilla planifolia* (Orchidaceae): proteomic and metabolic responses at early stage. *BMC Plant Biology*, 10(82), 1 -18.

Pandey, V. & Agrawal, V. (2009). Efficient micropropagation protocol of *Spilanthes acmella* L. possessing strong antimalarial activity. *In Vitro Cellular & Developmental Biology – Plant,* 45, 491-499.

Pant, B., Shrestha, S. & Pradhan, S. (2011). *In vitro* seed germination and plantlets development of *Phaius tancarvilleae* (L'Her) Blume. *Scientific World*, 9(9), 50 – 52.

Parab, G. V. & Krishnan, S. (2012). Rapid *in vitro* mass multiplication of orchid *Aerides maculosa* Lindl. and *Rhyncostylis retusa* Bl. From immature seeds. *Indian Journal of Biotechnology*, 11, 288 – 294.

Park, S. Y., Kong, S. S. & Paek, K .Y. (2006). Increased ethylene and decreased phenolic compounds stimulate somatic embryo regeneration in leaf thin section cultures of *Doritaenopsis* hybrid. *Journal of Plant Biology*, 49(5), 358-363.

Park, S. Y., Murthy, H. N. & Paek, K. Y. (2003). Protocorm-like body induction and subsequent plant regeneration from root tip cultures of *Doritaenopsis*. *Plant Science,* 164, 919 -923.

Paul, S., Kumaria, S. & Tandon, P. (2011). An effective nutrient medium for asymbiotic seed germination and large scale *in vitro* regeneration of *Dendrobium hookerianum*, a threatened orchid of northeast India. *AoB Plants*, 32, 1-6.

Pedroza Manrique, J. & Mican – Gutierrez, Y.(2006).Asymbiotic germination of *Odontoglossum gloriosum* Rchb. F. (Orchidacea) under *in vitro* conditions. *In Vitro Cellular & Developmental Biology – Plant,* 42, 543–547.

Popova, E., Bukhov, N., Popov, A. & Kim, H. J. (2010). Cryopreservation of protocorm like bodies of the hybrid orchid *Bratonia* (*Miltonia flavescens x Brassia longissima*). *CryoLetters*, 31(5), 426-437.

Prakash, B., Khan, S., & Bais, R. T. (2012). Effect of different media on *in vitro* seed germination and protocorm formation of *Vanda tessellata (Roxb.) Hook. Ex. G* an endangered medicinal orchid. *Researcher,* 4(12), 72-76.

Prasertsirivatna, S. & Koolpluksee, M. (2011). The study on optimization of growth conditions for *Dendrobium friedericksianum* Rchb.f. plantlets in aseptic culture. *Journal of Agricultural Technolog*, 7(3), 739-749.

Rahimizadeh, M., Habibi, D., Madani, H., Mohammadi, G. N., Mehraban, A. & Sabet, A. M. (2007). The effect of micronutrients on antioxidant enzyme metabolism in sunflower (*Helianthus annuus* L.) under drought stress. *Helia*, 30(47), 167 -174.

Rakshit, S., Rashid, Z., Sekhar, J. C., Fatma, T. & Dass, S. (2010). Callus induction and whole plant regeneration in elite Indian maize (Zea mays L.) inbreds. *Plant Cell*, *Tissue and Organ Culture,* 100, 31–37.

Rangsayatorn, N. (2009). Micropropagation of *Dendrobium draconis* Rchb. F. from thin cross-section culture. *Scientia Horticulturae,* 122, 662–665.

Rao, A. N. (2004,). Medicinal Orchid Wealth of Arunachal Pradesh. *Indian Medicinal Plants of Conservation Concern,* 1-11.

Redha, A. & Suleman, P. (2011). Effects of exogenous application of polyamines on wheat anther cultures. *Plant Cell*, *Tissue and Organ Culture,* 105, 345–353.

Rittirat, S., Te-chato, S., Kerdusuwan, N. & Kongruk, S. (2011). Micropropagation of Chang Daeng (*Rhychostylis gigantea* var, Sagarik) by embryogenic callus. *Songklanakarin Journal of Science and Technology*, 3(6), 659-663.

Robinson, J. P., Balakrishnan, V. & Britto, J. (2009). *In vitro* seed germination and protocorm development of *Dendrobium aqueum* Lindl. A rare orchid species from Eastern Ghats of Tamil Nadu. *Botany Research International*, *2(2),* 99-102.

Ronan, E. (2007). Micronutrient in agriculture. Practical Hydroponics & Greenhouse, 7(8), 39 – 48.

Rosales, E.P., Iannone, M.F., Groppa, M.D. & Benavides, M.P. (2012). Polyamines modulate nitrate reductase activity in wheat leaves: involvement of nitric oxide. *Amino Acids*, 42, 857–865.

Roy, J., & Banerjee, N. (2003). Induction of callus and plant regeneration from shoot –tip explants *of Dendrobium fimbriatum* Lindl. var. oculatum Hk. L. *Scientia Horticulturae*, 97, 333 – 340.

Roy, J., Naha, S., Majundar, M. & Banerjee, N. (2007). Direct and callus mediated protocorm-like body induction from shoot-tips of *Dendrobium chrysotoxum* Lindl. (Orchidaceae). *Plant Cell*, *Tissue and Organ Culture,* 90, 31–39.

Sakar, F. S., Arslan, H., Kirmizi, S., Guleryuz, G. (2010). Nitrate reductase activity (NRA) in *Asphodelus aestivus* Brot. (Liliaceae): distribution among organs, seasonal variation and differences among populations. *Flora,* 205, 527–531.

Santos Díaz, M. & Carranza Álvarez, C. (2009). Plant regeneration through direct shoot formation from leaf cultures and from protocorm-like bodies derived from callus of *Encyclia mariae* (Orchidaceae), a threatened Mexican orchid. *In Vitro Cellular & Developmental Biology. Plant,* 45, 162–170.

Salama, H. M. H., Al-Watban, A. A. & Al- Fughom, A. T (2011). Effect of ultraviolet radiation on chlorophyll, carotenoid, protein and proline contents of some annual desert plants. *Saudi Journal of Biological Sciences*, 18, 79–86.

Schnablova, R., Synkova, H., Vicankova, A., Burketova, L., Eder, J. & Cvikrova, M. (2006). Transgenic ipt tobacco overproducing cytokinins over accumulates phenolic compounds during in vitro growth. *Plant Physiology and Biochemistry*, 44, 526–534.

Sgarbi, E., Grimaudo, M. & Prete, C. D. (2009). *In vitro* asymbiotic germination and plantlets development of *Limodorum abortivum* (Orchidaceae). *Plant Biosystems*, 143(1), 114-119.

Shanjani, P.S. (2003). Nitrogen effect on callus induction and plant regeneration of *Juniperus excels. Internation Journal of Agriculture and Biology,* 5(4), 419-422.

Singleton, V. L. & Rossi, J. A. (1985). Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* 16, 144- 158.

Singh, J. P., Kumar, K., Katiyar P. N. & Kumar, V. (2012). Influence of zinc, iron, and copper on growth and flowering attributes in *Gladiolus* cv. Sapna. *Progressive Agriculture,* 12(1), 138 -143.

Singh, S. K., Rai, M. K., Asthana, P. & Sahoo, L. (2009). An improved micropropagation of *Spilanthes acmella* L. through transverse thin cell layer culture. *Acta Physiology Plant, 31,* 693–698.

Sinha, P., Hakim, M. L. & Alam, M. F. (2009). *In vitro* mass clonal propagation of *Spathoglottis plicata* Blume. *Plant Tissue Culture & Biotechnology*,19(2), 151-160.

Smirnoff, N. (1993) Tansley Review No. 52. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist,* 125(1), 27-58.

Steiner, N., Santa – Catarina, C., Silvera, V., Floh, E. L. S. & Guerra, M. P. (2007). Polyamine effects on growth and endogenous hormones levels in *Araucaria angustifolia* embryogenic cultures. *Plant Cell*, *Tissue and Organ Culture,* 89, 55–62.

Steinmacher, D. A., Clement, C. R. & Guerra, M. P. (2007). Somatic embryogenesis from immature peach palm inflorescence explants: towards development of an efficient protocol. *Plant Cell*, *Tissue and Organ Culture,* 89, 15–22.

Steward, S. L. & Kane, M. E. (2006). Asymbiotic seed germination and *in vitro* plantlets development of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. *Plant Cell*, *Tissue and Organ Culture*, 86, 147-158.

Suzuki, R. M., Moreira,V. C., Pescardo, R. & Ferreira, W. M. (2012). Asymbiotic seed germination and *in vitro* plantlets development of the threatened orchid *Hoffmannseggella cinnabarina*. *In Vitro Cellular & Developmental Biology -Plant,* 48, 500–511.

Swarts, N. D. & Dixon, K. W. (2009). Terrestrial orchid conservation in the age of extinction. *Annnals of Botany*, 104, 543 – 556.

Szopa, A., Ekiert, H & Muszynska, B. (2013). Accumulation of hydroxybenzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry). *Plant Cell*, *Tissue and Organ Culture,* 113, 23–329.

Takao, K., Rickhag, M., Hegardt, C., Oredsson, S. & Persson, L. (2006). Induction of apoptotic cell death by putrescine. *The International Journal of Biochemistry & Cell Biology,* 38, 621–628.

Tan, B. C., Chin, C. F., & Alderson, P. (2011). Optimisation of plantlet regeneration from leaf and nodal derived callus of *Vanilla planifolia* Andrews. *Plant Cell*, *Tissue and Organ Culture,* 105, 457–463.

Tang, W. & Newton, R. J. (2005). Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos. *Plant Physiology and Biochemistry*, 43, 760–769.

Tatiya, A., Surana, S., Bhavsar, S., Patil, D. & Patil, Y. (2012). Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. tubers (Orchidaceae). *Asian Pacific Journal of Tropical Disease*, 50-55.

Teixeira da Silva, J. (2012). New basal media for protocorm – like body and callus induction of hybrid *Cymbidium*. *Journal of Fruit and Ornamental Plant Research*, 20(2), 127-133.

Teixeira da Silva, J., Singh, N. & Michio Tanaka. (2006). Priming biotic factors for optimal protocorm-like body and callus induction in hybrid *Cymbidium* (Orchidaceae), and assessment of cytogenetic stability in regenerated plantlets. *Plant Cell*, *Tissue and Organ Culture,* 84, 135–144.

Teng, M. C. 1997. Micropropagation of *Spathoglottis plicata*. *Plant Cell Reports,* 16, 831–835.

Thiruvengadam, M., Rekha, K. T., Jayabalan, N., Praveem, N., Kim, E. H. & Chung, I. M. (2013). Effect of exogenous polyamines enhances somatic embryogenesis via suspension cultures of spine gourd (*Momordica dioica* Roxb. Ex. Willd.). *Australian Journal of Crop Science*, 7(3), 446-453.

Thomas,T. D. & Michael, A. (2007). High-frequency plantlet regeneration and multiple shoot induction from cultured immature seeds of *Rhynchostylis retusa* Blume., an exquisite orchid. *Plant Biotechnology Report,* 1, 243–249.

Tokuhara, K. & Mii, M. (2001). Induction of embryogenic callus and cell suspension culture from shoot tips excised from flower stalk bud of *Phalaenopsis* (Orchidaceae). *In Vitro Cellular & Development al Biology Plant,* 37, 457 – 461.

Tun, N. N., Santa –Catarina, C., Begum, T., Silveira, V., Handro, W., Floh, E.I.S. & Scherer, G.F.E. (2006). Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* plantlets. *Plant and Cell Physiology*, 47(3), 346–354.

Trepanier, M., Lamy, M. & Dansereau, B. (2009). *Phalaenopsis* can absorb urea directly through their roots. *Plant Soil*, 319, 95–100.

Vasanth, K., Lakshmiprabha, A. & Jayabalan, N. (2006). Amino acids enhancing plant regeneration from cotyledon and embryonal axis of peanut (*Arachis hypogaea* L.). *Indian Journal of Crop Science*, 1(1-2), 79-83.

Vasudevan, R. & Staden, J. V. (2011). Cytokinin and explant types influence *in vitro* plant regeneration of Leopard orchid (*Ansellia aficana* Lindl.). *Plant Cell, Tissue and Organ Culture,* 107, 123–129.

Vejsadova, H. (2006). Factor affecting seed germination and plantlets growth of terrestrial orchid cultured *in vitro*. *Acta Biologica Cracoviensia Series Botanica,* 48(1), 109–113.

Veitch, N. C. (2004). Structural determinants of plant peroxidase function. *Phytochemistry Reviews, 3,* 3–18.

Vujanovic, V., St- Arnaud, M., Barabe, D. & Thibeault, G. (2000). Viability testing of orchid seed and the promotion of colouration and germination. *Annals of Botany,* 86, 79-86.

Wagner A. Vendrame, & Maguire, I. (2007). *In vitro* propagation and plantlet regeneration from *Doritaenopsis* Purple Gem 'Ching Hua' flower explants. *Hortscience,* 42(5), 1256–1258.

Wang, H.J., Chen, J.T. & Chang, W.C. (2006). Somatic embryogenesis and plant regeneration from leaf, root and stem-derived callus cultures of *Areca catechu*. *Biologia Plantarum,* 50(2), 279-282.

Wang, X., Shi, G., Xu, Q. & Hu, J. (2007). Exogenous polyamines enhance copper tolerance of *Nymphoides peltatum*. *Journal of Plant Physiology*, 164, 1062—1070.

Wattanawikkit, P., Bunn, E., Chayanarit, K. & Tantiwiwat, S. (2011). Effect of cytokinins (BAP and TDZ) and auxin (2,4-D) on growth and development of *Paphiopedilum callosum*. *Kasetsart Journal. (Nat. Sci.)* 45, 12 – 19.

Waterman, R. J. & Bidartondo, M. I. (2008). Deception above, deception below: linking pollination and mycorrhizal biology of orchids. *Journal of Experimental Botany*, 59(5), 1085–1096.

Weston, P. H., Perkins, A. J. & Entwise, T. J. (2005). More than symbioses: orchid ecology, with examples from the Sydney Region, *Cunninghamia*, 9(1), 1–15.

Wu, K., Zeng, S., Teixeira da Silva, J. A., Chen, Z. Zhang, J., Yang, Y. & Duan, J. (2012).Efficient regeneration of *Renanthera* Tom Thumb 'Qilin' from leaf explants. *Scientia Horticulturae*, 135, 194–201.

Wu, J., Ma, H., Xu, X., Qiao, N., Guo, S., Liu, F., Zhang, D. & Zhou, L. (2013). Mycorrhizas alter nitrogen acquisition by the terrestrial orchid *Cymbidium goeringii*. *Annals of Botany*, 111, 1181–1187.

Xiong, Z., Liu, C. & Geng, B. (2006). Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. *Ecotoxicology and Environmental Safety*, 64, 273–280.

Yang, X., Wang, X., Wei, Min., Yang, F. & Shi, Q. (2010). Changes of Nitrate Reductase Activity in Cucumber Seedlings in Response to Nitrate Stress. *Agricultural Sciences in China*, 9(2), 216-222

Yemets, A. I., Bayer, G. Y. & Blume, Y.B. (2013). An effective procedure for *in vitro* culture of *Eleusine coracana* (L.) and its application. *ISRN Botany,* 2013, 1-4

Yin, M. & Hong, S. (2009). Cryopreservation of *Dendrobium candidum* Wall. ex Lindl. protocorm-like bodies by encapsulation –vitrification. *Plant cell and Tissue Organ Culture*, 98, 179-185.

Yiu, J., Liu, C., Fang, D. Y. & Lai, Y. (2009). Water logging tolerance of Welsh onion (*Allium fistulosum* L.) enhanced by exogenous spermidine and spermine. *Plant Physiology and Biochemistry*, 47, 710–716.

Zárate, R., Jaber-Vazdekis, N. E., Cequier-Sánchez, E, Gutierrez-Nicolás, F. & Ravelo, A. G. (2008). Biotechnology for the production of plant natural products. *Bioactive Natural Products,* 34, 309–392.

Zouine, J. & El Hadrami, I. (2007). Effect of 2,4-D, glutamine and BAP on embryogenic suspension culture of date palm (*Phoenix dactylifera* L.). *Scientia Horticulturae*, 112, 221–226.

Zhao, P., Wu, F., Feng, F. S. & Wang, W. J. (2008). Protocorm-like body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum* Wall ex Lindl. *In Vitro Cellular & Developmental Biology – Plant,* 44, 178–185.