



**UNIVERSITI PUTRA MALAYSIA**

***SUITABILITY OF SHOOT APICAL MERISTEM AS EXPLANT  
TO MINIMIZE VARIATION OF TOBACCO  
(NICOTIANA TABACUM L. CV. VIRGINIA) PLANTLETS***

**AZIMAH BINTI CHE AZIZ**

**FS 2015 4**



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(*NICOTIANA TABACUM* L. CV. VIRGINIA) PLANTLETS**

By

**AZIMAH BINTI CHE AZIZ**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**February 2015**

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

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**February 2015**

**Chair : Rosimah binti Nulit, PhD**  
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A protocol for *in vitro* regeneration using shoot apical meristem (SAM) of tobacco (*Nicotiana tabacum* L. cv. Virginia) has been established and entire tobacco plantlets were produced. The explants were isolated from 5-days-old germinating tobacco seedlings. Shoot development and root formation were shown in all treatments but the most effective treatment is control with 73% of explant survival, followed by 0.5 mg/l IAA with 60% of explant survival. However, before acclimatization, plantlets from medium supplemented with 0.5 mg/l KIN showed the highest number of leaves and plantlet height with an average of  $6.0 \pm 0.37$  leaves and  $0.7 \pm 0.04$  cm height, followed by 1.0 mg/l KIN with an average of  $5.4 \pm 0.37$  leaves and  $0.6 \pm 0.04$  cm height. Number of roots was found highest in medium supplemented with 0.5 mg/l IAA with an average of  $4.9 \pm 0.18$  roots followed by control medium with an average of  $3.5 \pm 0.23$  roots. After 12 weeks of acclimatization, only plantlets from treatment with 0.5 mg/l IAA survived in the environmental condition. To detect the variation, the morphology, anatomy and primary metabolites between tobacco plants and its plantlets were compared. Both morphology (plant height, number of leaves and leaf morphology) and anatomy (midrib of leaf, stem and root) of tobacco plantlets were similar to tobacco plants. Besides, there is also no significant difference in the total chlorophyll, carbohydrate and protein content of both tobacco plants and plantlets. The secondary metabolites and anti-oxidative profiling between tobacco plants and its plantlets were also studied. Total flavonoid and carotenoid content were found higher than phenolic compounds in both plants. However, the antioxidant activity varied between both plants. Plantlets of tobacco have slightly higher antioxidant activity than tobacco plants. This was indicated by the significant difference ( $p < 0.05$ ) in the  $IC_{50}$  values between both plants in which plantlets have a lower  $IC_{50}$  value ( $16.82 \pm 0.21$ ) than tobacco plants ( $18.97 \pm 0.28$ ). In conclusion, no variation was expressed in tobacco plantlets regenerated from SAM *in vitro* except for a slightly different in the  $IC_{50}$  values. Tobacco plants and its plantlets can also be considered as good sources of natural antioxidant.

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

**KESESUAIAN MERISTEM APIKAL PUCUK SEBAGAI EKSPLAN UNTUK  
MENGURANGKAN VARIASI PADA POKOK KLON TEMBAKAU  
(*NICOTIANA TABACUM* L. CV. VIRGINIA)**

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Protokol untuk regenerasi pokok klon tembakau secara *in vitro* dengan menggunakan meristem apikal pucuk (SAM) tembakau (*Nicotiana tabacum* L. cv. Virginia) sebagai eksplan telah dihasilkan. Eksplan diisolat daripada biji benih tembakau yang sedang bercambah pada hari ke-5. Pertumbuhan pucuk dan pembentukan akar pada klon-klon ditunjukkan dalam semua medium selepas 2 minggu, walaubagaimanapun medium kawalan menunjukkan peratusan yang paling tinggi dengan 73% eksplan yang hidup, diikuti oleh medium yang mengandungi 0.5 mg/l IAA dengan 60% eksplan yang hidup. Walaubagaimanapun, sebelum proses aklimatisasi, pokok klon daripada medium yang mengandungi 0.5 mg/l KIN menunjukkan bilangan daun dan ketinggian pokok yang paling tinggi dengan purata  $6.0 \pm 0.37$  helai daun dan  $0.7 \pm 0.04$  sm ketinggian, diikuti oleh 1.0 mg/l KIN dengan purata  $5.4 \pm 0.37$  helai daun dan  $0.6 \pm 0.04$  sm ketinggian. Bilangan akar yang paling tinggi telah ditunjukkan dalam medium yang mengandungi 0.5 mg/l IAA dengan purata  $4.9 \pm 0.18$  akar diikuti oleh medium kawalan dengan purata  $3.5 \pm 0.23$  akar. Selepas 12 minggu proses aklimatisasi, hanya pokok klon daripada MS medium yang mengandungi 0.5 mg/l IAA berupaya untuk hidup dan menyesuaikan diri dalam persekitaran. Morfologi, anatomi dan metabolit primer antara pokok tembakau dan pokok klonnya telah dibandingkan untuk mengkaji kewujudan variasi. Kedua-dua morfologi (ketinggian pokok, bilangan daun dan morfologi daun) dan anatomi (urat tengah daun, batang dan akar) pokok klon tembakau adalah sama dengan pokok tembakau. Selain itu, tiada juga perbezaan yang signifikan pada jumlah kandungan klorofil, karbohidat dan protin dalam kedua-dua pokok tembakau dan pokok klon. Kajian perbandingan metabolit sekunder dan profil anti-oksidatif antara pokok tembakau dan pokok klonnya juga telah dijalankan. Kajian menunjukkan jumlah kandungan flavonoid dan karotenoid didapati sedikit lebih tinggi daripada sebatian fenolik dalam kedua-dua pokok. Walau bagaimanapun, aktiviti antioksidan adalah berbeza antara kedua-dua pokok. Pokok klon tembakau mempunyai aktiviti antioksidan yang sedikit lebih tinggi daripada pokok tembakau. Ini telah ditunjukkan oleh perbezaan yang signifikan ( $p < 0.05$ ) pada nilai-nilai  $IC_{50}$  antara kedua-dua pokok di mana pokok klon mempunyai nilai  $IC_{50}$  ( $16.82 \pm 0.21$ ) yang lebih rendah daripada pokok tembakau ( $18.97 \pm 0.28$ ). Kesimpulannya, tiada variasi ditunjukkan pada pokok klon tembakau yang diregenerasi daripada SAM secara *in*

*vitro* kecuali sedikit perbezaan pada nilai-nilai IC<sub>50</sub>. Pokok tembakau dan pokok klonnya juga boleh dianggap sebagai sumber antioksidan semula jadi yang baik.



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I certify that a Thesis Examination Committee has met on 13 February 2015 to conduct the final examination of Azimah binti Che Aziz on her thesis entitled “Suitability of Shoot Apical Meristem as Explant to Minimize Variation of Tobacco (*Nicotiana tabacum* L. cv. Virginia) Plantlets” in accordance with the Universities and University Colleges Act 1971 and the 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

%	percentage
°C	degree Celcius
µl	microliter
AlCl <sub>3</sub>	Aluminium chloride
ANOVA	Analysis of Variance
BSA	Bovine serum albumin
cm	centimeter
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DPX	diputal petroleum xylene
g	gram
gL <sup>-1</sup>	gram per liter
IAA	Indole-3-Acetic Acid
KIN	Kinetin
L	liter
mg	milligram
mg/µl	milligram per microliter
mg/l	milligram per liter
mg/ml	milligram per milliliter
min	minute
ml	milliliter
mm	millimeter
MS	Murashige and Skoog
Na <sub>2</sub> CO <sub>3</sub>	Sodium carbonate
nm	Nanometer
PGR	Plant Growth Regulator
pH	Negative logarithm of hydrogen ion concentration

SAM	shoot apical meristem
SE	standard error
t	time
v/v	volume per volume



## CHAPTER 1

### INTRODUCTION

#### 1.1 General Introduction

One of the most significant content of cell engineering is the tissue culture, which is the *in vitro* culture of cells, tissues or organs. Plant tissue culture has undergone an interesting development because it provides knowledge about totipotency, cell nutrition, cell division, differentiation, cell preservation, metabolism, radiobiology and mutations (Ganapathi *et al.*, 2004).

History has proved that by tissue culture technology, new variety of plantlets has been produced. Leva *et al.* (2012) also mentioned that somaclonal variation provides a valuable source of genetic variation for the plant improvement by selecting the novel variants that show resistance to disease, higher yield or improved quality. Yet, *in vitro* regeneration has an advantage over conventional propagation to produce disease free plants at high multiplication rate. According to Ganapathi *et al.* (2004), this technology also shows high potential to produce many biologically active compounds, such as alkaloids, phenolics, vitamins, steroids and other useful chemicals.

One of the advantages of the *in vitro* tissue culture is for the conservation of endangered plant species. Tissue culture technology has been successful to conserve endangered species (Sudharsan *et al.*, 2003). Thus, tissue culture technique can be used to replace the conventional method in order to produce plants that have been considered as rare and endangered. In order to conserve the endangered species, it is necessary to produce true-to-type plantlets and to eliminate somaclonal variation of plantlets. Therefore, the plantlets must have similar characteristics in the morphology, anatomy and physiology, as well as the genetics. By manipulation or optimizing in tissue culture technique, the characteristics of plantlets can be retained.

Clone is genetically identical copy of an organism produced by asexual reproduction while the term somaclone refer to plants produced from *in vitro* culture and somaclonal variation refer to the genetic variation among that plants (Leva *et al.*, 2012). This somaclonal variation usually results in off-types that reduce the commercial value of the regenerated plants (Chuang *et al.*, 2009). Factors that caused somaclonal variation are changes in chromosome number (polyploidy or aneuploidy), chromosome damage (insertions, deletions, mutations, translocation) or changes in methylation of chromatin (Biswas *et al.*, 2009). According to Bordallo *et al.* (2004), four critical variables for somaclonal variation are genotype, explant origin, cultivation period and culture condition. The conditions of *in vitro* culture can be mutagenic and regenerated plants derived from calli, organ cultures, somatic embryos and protoplast sometimes can lead to phenotypic and genotypic variation (Orbovic *et al.*, 2008). Because the goal of plant

conservation is to obtain clonal identity, it is necessary to control the somaclonal variation (Bordallo *et al.*, 2004).

## 1.2 Problem Statements, Justification and Objectives of Study

*In vitro* regeneration through tissue culture is one of the methods for the conservation of endangered plant species (Lynch, 2002). However, tissue culture can cause somaclonal variation in the plantlets produced. The sources of explant for tissue culture such as stem, root, shoot or leaf, the application of exogenous plant growth regulators and the culture condition are the factors that determine the existence of somaclonal variation of the plantlets. The sources of explant are considered as the main factor for somaclonal variation because explant tissue can affect the frequency and nature of somaclonal variation (Leva *et al.*, 2012). Explants may present dissimilar regeneration rates, so the selection procedures can differ among different explant types (Bordallo *et al.*, 2004). Therefore, to minimize the variation, suitable explants must be selected for the *in vitro* regeneration. According to Leva *et al.* (2012), the use of meristematic tissues as starting materials for tissue culture can reduce the possibility of variation. In the present study, shoot apical meristem (SAM) tissue was selected as explant due to its lower tendency to generate somaclonal variation as it avoids the formation of callus (Duncan 1997).

In the present study, tobacco plant was chosen because it has been considered as the model plants in the field of tissue culture (Ganapathi *et al.*, 2004). Previous studies on *in vitro* regeneration of tobacco have used several different types of explants, such as shoots (Sun and Kang, 2003), young leaf (Yang *et al.*, 1995) and stem (Smith and Murashige, 1970; da Silva, 2005). There are reports published on the use of SAM in other plant species such as chrysanthemum (Kumar *et al.*, 2009), red pepper (Peddaboina *et al.*, 2006), rice (Duong *et al.*, 2000; Silvarajan *et al.*, 2012) and lily (Chen *et al.*, 2011). However, there are no reports on using SAM of tobacco's germinating seed as explants in *in vitro* tissue culture so far.

SAM used in this study was obtained from the plumule of germinated tobacco seedlings. This is because the response of plumules to *in vitro* culture conditions is better than that of other tissues (Fernando *et al.*, 2004). The roots also develop at a high rate and in a large numbers from plumules as a response to the production of endogenous auxin by the plumules (Vesperinas, 1998). According to Perez-Nunez *et al.* (2006), the use of plumule explants, consisting of the shoot meristem and surrounded by leaf primordia, has allowed the development of a reproducible micropropagation protocol. Seed was not used for starting material for the *in vitro* regeneration of tobacco because the objective of the present study is to use SAM as the explants, not seeds.

Tobacco is well known to be harmful to human health and environment. However, a few studies reported that it also have medicinal values and insecticide properties. There

are also reports published on the antioxidant properties of tobacco plant. In the present study, the secondary metabolites (antioxidant properties) and antioxidant activities between tobacco plant and its plantlet were evaluated and compared. The purpose of the comparison is to detect whether the variation among the plantlets exists in term of secondary metabolites and antioxidant activities. Therefore, the objectives of this study are:

1. To regenerate entire tobacco plantlets *in vitro* by using shoot apical meristem (SAM),
2. To compare the morphology, anatomy and primary metabolites between tobacco plant and its plantlet and
3. To study the secondary metabolites and anti-oxidative profiling of tobacco plant and its plantlet.

## REFERENCES

- Abdellatif, K. F., Hegazy, A. E., Aboshama, H. M., Emara, H. A., and El-Shahed, A. A. (2012). Morphological and molecular characterization of somaclonal variations in tissue culture-derived banana plants. *Journal of Genetic Engineering and Biotechnology*. 10: 47-53.
- Akik, N. E. (2014). Antioxidants properties and free radical scavenging activity of cherry tomato (*Solanum lycopersicum*). Final Year Project Thesis, Universiti Putra Malaysia.
- Alam, I., Sharmin, S. A., Naher, M. K., Alam, M. J., Anisuzzaman, M., and Alam, M. F. (2010). Effect of growth regulators on meristem culture and plantlet establishment in sweet potato (*Ipomoea batatas* (L.) Lam). *Plant Omics Journal*. 3: 35-39.
- Alam, I., Sharmin, S. A., Naher, M. K., Alam, M. J., Anisuzzaman, M., and Alam, M. F. (2013). Elimination and detection of viruses in meristem-derived plantlets of sweet potato as a low-cost option toward commercialization. *Biotechnology*. 3: 153-164.
- Alam, M. F., Banu, M. L. A., Swaraz, A. M., Parvez, S., Hossain, M., Khalekuzzaman, M., and Ahsan, N. (2004). Production of virus free seeds using meristem culture in tomato plant under tropical conditions. *Journal of Plant Biotechnology*. 6: 221-227.
- Alameda, D., Anten, N. P. R., and Villar, R. (2012). Soil compaction effects on growth and root traits of tobacco depend on light, water regime and mechanical stress. *Soil and Tillage Research*. 120: 121-129.
- Ali, G., Hadi, F., Ali, Z., Tariq, M., and Khan, M. A. (2007). Callus induction and *in vitro* complete plant regeneration of different cultivars of tobacco (*Nicotiana tabacum* L.) on media of different hormonal concentrations. *Biotechnology*. 6: 561-566.
- Amensour, M., Sendra, E., Abrini, J., Bouhdid, S., Perez-Alvarez, J.A., and Fernandez-Lopez, J. (2009). Total phenolic content and antioxidant activity of myrtle (*Myrtus communis*) extracts. *Journal of National Production Community*. 4: 819-824.
- Andreu, P. and Marin, J. A. (2005). *In vitro* culture establishment and multiplication of the Prunus rootstock 'Adesoto 101' (*P. insititia* L.) as affected by the type of propagation of the donor plant and by the culture medium composition. *Scientia Horticulturae*. 106: 258-267.
- Bag, N., Chandra, S., Palni, L. M. S., and Nandi, S. K. (2000). Micropropagation of Dev-ringal (*Thamnocalamus spathiflorus* (Trin.) Munro) – a temperate bamboo, and comparison between *in vitro* propagated plants and seedlings. *Plant Science*. 156: 125-135.

- Bairu, M. W., Fennell, C. W., and Staden, J. V. (2006). The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. 'Zelig'). *Scientia Horticulturae*. 108: 347-351.
- Bandonien, D., Murkovic, M., Pfannhauser, W., Venskutonis, P.R. and Gruzdien, D. (2002). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. *European Food Research and Technology*. 214: 143-147.
- Berg, L. R. (2008). *Introductory Botany: Plants, People and the Environment*, 2<sup>nd</sup> edition. Thomson Brooks/Cole. (pp. 116-159).
- Berlyn, G. P. and Miksche, J. P. (1976). *Botanical microtechnique and cytochemistry*. Ames, Iowa: The Iowa State University Press. (pp. 326).
- Bidabadi, S.S., Meon, S., Wahab, Z., and Mahmood, M. (2010). Study of genetic and phenotypic variability among somaclones induced by BAP and TDZ in micropropagated shoot tips of banana (*Musa* spp.) using RAPD marker. *Journal of Agriculture Science*. 2: 49-60.
- Bindler, G., Plieske, J., Bakaher, N., Gunduz, I., Ivanov, N., Van der Hoeven, R., Ganal, M., and Donini, P. (2011). A high density genetic map of tobacco (*Nicotiana tabacum* L.) obtained from large scale microsatellite marker development. *Theoretical and Applied Genetics*. 123: 219-230.
- Biswas, M. K., Dutt, M., Roy, U. K., Islam, R., and Hossain, M. (2009). Development and evaluation of *in vitro* somaclonal variation in strawberry for improved horticultural traits. *Scientia Horticulturae*. 122: 409-416.
- Bordallo, P. N., Silva, D. H., Maria, J., Cruz, C. D., and Fontes, E. P. (2004). Somaclonal variation on *in vitro* callus culture potato cultivars. *Horticultura Brasileira*. 22: 300-304.
- Bouiamrine, E. H., Diouri, M., and El Halimi, R. (2012). Assessment of somaclonal variation in regenerated plants from immature embryos culture of durum wheat. *International Journal of Agriculture and Biology*. 14: 941-946.
- Bradford, 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.
- Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Journal of Lebensmittel-Wissenschaft u-Technology*. 28: 25-30.
- Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*. 56: 317-333.



- Brennan, K. A., Laugesen, M., and Truman, P. (2014). Whole tobacco smoke extracts to model tobacco dependence in animals. *Neuroscience and Biobehavioral Reviews*. 47: 53-69.
- Chang, C., Moll, B. A., Evenson, K. B., and Gultinan, M. J. (1996). *In vitro* plantlet regeneration from cotyledon, hypocotyl and root explants of hybrid seed geranium. *Plant Cell, Tissue and Organ Culture*. 45: 61-66.
- Charlton, A. (2004). Medicinal uses of tobacco in history. *Journal of the Royal Society of Medicine*. 97: 292-296.
- Chen, J., Leng, H., Duan, Y., Zhao, W., Yang, G., Guo, Y., Chen, Y., and Hu, Q. (2013). Three new flavonoids from the leaves of oriental tobacco and their cytotoxicity. *Phytochemistry Letters*. 6: 144-147.
- Chen, X. L., Li, J. H., Xin, X., Zhang, Z. E., Xin, P. P., and Lu, X. X. (2011). Cryopreservation of *in vitro*-grown apical meristems of *Lilium* by droplet-vitrification. *South African Journal of Botany*. 77: 397-403.
- Chuang, S. J., Chen, C. L., Chen, J. J., Chou, W. Y., and Sung, J. M. (2009). Detection of somaclonal variation in micro-propagated *Echinacea purpurea* using AFLP marker. *Scientia Horticulturae*. 120: 121-126.
- Close, K. R. and Ludeman, L. A. G. (1989). Structure-activity relationships of auxin-like plant growth regulators and genetic influences on the culture induction response in maize (*Zea mays* L.). *Plant Science*. 61: 245-252.
- Da Silva, J. A. T. (2005). Simple multiplication and effective genetic transformation (four methods) of *in vitro*-grown tobacco by stem thin cell layers. *Plant Science*. 169: 1046-1058.
- Dewey, R. E. and Xie, J. (2013). Molecular genetics of alkaloid biosynthesis in *Nicotiana tabacum*. *Phytochemistry*. 94: 10-27.
- Dey, T., Saha, S., and Ghosh, P. D. (2015). Somaclonal variation among somatic embryo derived plants – evaluation of agronomically important somaclones and detection of genetic changes by RAPD in *Cymbopogon winterianus*. *South African Journal of Botany*. 96: 112-121.
- Divakaran, M., Babu, K. N., and Peter, K. V. (2006). Conservation of *Vanilla* species, *in vitro*. *Scientia Horticulturae*. 110: 175-180.
- Droual, A. M., Hamdi, S., Creche, J., Kevers, C., and Rideau M. (1998). Autonomy to plant growth regulators and gene expression in periwinkle cultures *in vitro*. *Journal of Plant Physiology*. 153: 623-630.
- Duncan, R.R. (1997). Tissue culture-induced variation and crop improvement. *Advance in Agronomy*. 58: 201-240.

- Duong, T. N., Bui, V. L., and Van, K. T. T. (2000). Somatic embryogenesis and direct shoot regeneration of rice (*Oryza sativa* L.) using thin cell layer culture of apical meristematic tissue. *Journal of Plant Physiology*. 157: 559-565.
- Duthie, G. G., Gardner, P. T., and Kyle, J. A. M. (2003). Plant polyphenols: are they a new magic bullet. *Proceedings of the Nutrition Society*. 62: 599-603.
- Fernando, S. C., Weerakoon, L. K., and Gunathilake, T. R. (2004). Micropropagation of coconut through plumule culture. *Cocos*. 16: 1-10.
- Freytag, A. H., Rao-Arelli, A. P., Anand, S. C., Wrather, J. A., and Owens, L. D. (1989). Somaclonal variation in soybean plants regenerated from tissue culture. *Plant Cell Reports*. 8: 199-202.
- Gaikwad, S.A., Kamble, G. S., Devare, S., Deshpande, N. R., and Salvekar, J. P. (2011). *In vitro* evaluation of free radical scavenging potential of *Cassia auriculata* L. *Journal of Chemical and Pharmaceutical Research*. 3: 766-772.
- Gamborg, O. L., Murashige, T., Thorpe, T. A., and Vasil, I. K. (1976). Plant tissue culture media. *In Vitro*. 12: 473-478.
- Ganapathi, T. R., Suprasanna, P., Rao, P. S., and Bapat, V. A. (2004). Tobacco (*Nicotiana tabacum* L.)- A model system for tissue culture interventions and genetic engineering. *Indian Journal of Biotechnology*. 3: 171-184.
- Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., and Thorpe, T. A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cellular & Developmental Biology-Plant*. 32: 272-289.
- George, E. F., Hall, M. A., and Klerk, G. J. D. (2008). *Plant Propagation by Tissue Culture, Volume 1*. The Background, Springer, Dordrecht. (pp. 1-28).
- GMO Compass: Tobacco. (2010). Retrieved 8 April 2015 from <http://www.gmo-compass.org/eng/database/plants/304.tobacco.html>
- Gonzalez, A. G., Cuadros, F., Celma, A. R., and Rodriguez, F. L. (2013). Potential application of anaerobic digestion to tobacco plant. *Fuel*. 113: 415-419.
- Gould, J., Devey, M., Hasegawa, O., Ulian, E. C., Peterson, G., and Smith, R. H. (1991). Transformation of *Zea mays* L. using *Agrobacterium tumefaciens* and the shoot apex. *Plant Physiology*. 95: 426-434.
- Harborne, J. B. (1989). General procedure and measurement of total phenolics. In Dey, P. M. and Harborne, J. B. (eds.). *Methods in plant biochemistry, Vol 1* (pp. 1-28). Academic Press Limited, London.
- Havel, L. and Novak, F. J. (1985). Meristem-tip culture of *Allium cepa* L. *Scientia Horticulturae*. 27: 209-214.

- Helgeson, J. P., Kemp, J. D., Haberlach, G. T., and Maxwell, D. P. (1972). A tissue culture system for studying disease resistance: the black shank disease in tobacco callus culture. *Phytopathology*. 62: 1439-1443.
- Hopkins, W. G. and Huner, N. P. A. (2009). *Introduction to Plant Physiology*, 4<sup>th</sup> edition. Wiley, John and Sons, Incorporated. (pp. 96-108).
- Hsu, T. W., Tsai, W. C., Wang, D. P., Lin, S., Hsiao, Y. Y., Chen, W. H., and Chen, H. H. (2008). Differential gene expression analysis by cDNA-AFLP between flower buds of *Phalaenopsis* Hsiang Fei cv. H. F. and its somaclonal variant. *Plant Science*. 175: 415-422.
- Javanmardi, J., Stushnoff, C., Locke, E., and Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry*. 83: 547-550.
- Jemon, J. P. (2014). Antioxidative properties of leaves, ripe fruits and unripe fruits of *Phaleria macrocarpa*. Final Year Project Thesis, Universiti Putra Malaysia.
- Kartha, K. K. and Gamborg, O. L. (1975). Elimination of cassava mosaic disease by meristem culture. *Phytopathology*. 65: 826-828.
- Kartha, K. K., Mroginski, L. A., Pahl, K., and Leung, N. L. (1981). Germplasm preservation of coffee (*Coffea arabica* L.) by *in vitro* culture of shoot apical meristems. *Plant Science Letters*. 22: 301-307.
- Khan, S., Al-Qurainy, F., and Nadeem, M. (2012). Biotechnological approaches for conservation and improvement of rare and endangered plants of Saudi Arabia. *Saudi Journal of Biological Sciences*. 19:1-11.
- Kochert, G. (1978). Carbohydrate determination by the phenol sulfuric acid method. In Helebust, J. A. and Craig, J. S. (eds.). *Hand book of phycologia method* (pp. 56-97). Cambridge University Press, Cambridge.
- Kukavica, B., Mitrovic, A., Mojovic, M., and Jovanovic, S. V. (2007). Effect of Indole-3-acetic acid on pea root growth, peroxidase profiles and hydroxyl radical formation. *Archives of Biological Science*. 59: 319-326.
- Kumar, S., Khan, M. S., Raj, S. K., and Sharma, A. K. (2009). Elimination of mixed infection of *Cucumber mosaic* and *Tomato aspermy virus* from *Chrysanthemum morifolium* Ramat. cv. Pooja by shoot meristem culture. *Scientia Horticulturae*. 119: 108-112.
- Laccourreye, O., Werner, A., Laccourreye, H., and Bonfils, P. (2014). Tobacco and otorhinolaryngology: Epic and disaster. *European Annals of Otorhinolaryngology, Head and Neck Diseases*. 131: 183-188.
- Lagrimini, L. M. and Rothstein, S. (1987). Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. *Plant Physiology*. 84: 438-442.

- Lanata, S. C. (2005). Tobacco from medicinal use to substance abuse. *Seminars in Integrative Medicine*. 3: 132-138.
- Leitch, I. J., Hanson, L., Lim, K. Y., Kovarik, A., Chase, M. W., Clarkson, J. J., and Leitch, A. R. (2008). The ups and downs of genome size evolution in polyploidy species of *Nicotiana* (Solanaceae). *Annals of Botany*. 101: 805-814.
- Leva, A. R., Petrucelli, R., and Rinaldi, L. M. R. (2012). Somaclonal variation in tissue culture: A case study with olive. *Recent Advances in Plant in vitro Culture*. 7: 123-150.
- Li, H. P., Huang, T., Wang, C. X., and Liao, Y. C. (2009). An efficient regeneration system of barley cultivars from leaf base segments. *Biologia Plantarum*. 53: 733-736.
- Li, Y., Shi, X., Strabala, T. J., Hagen, G., and Guilfoyle, T. J. (1994). Transgenic tobacco plants that overproduce cytokinins show increased tolerance to exogenous auxin and auxin transport inhibitors. *Plant Science*. 100: 9-14.
- Lichtenthaler, H.K. and Wellburn, A.R. (1985). Determination of Total Carotenoids and Chlorophylls A and B of Leaf in Different Solvents. *Biochemical Society Transactions*. 11:591-592.
- Lynch, P. T. (2002). Tissue culture techniques in *in vitro* plant conservation. In Benson, E. (ed.). *Plant Conservation Biotechnology* (pp. 41-62). CRC Press.
- Maisuthisakul, P., Suttajit, M., and Pongsawatmanit, R. (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chemistry*. 100: 1409-1418.
- Malda, G., Suzan, H., and Backhaus, R. (1999). *In vitro* culture as a potential method for the conservation of endangered plants possessing crassulacean acid metabolism. *Scientia Horticulturae*. 81: 71-87.
- Mallaya, N. P. and Ravishankar, G. A. (2013). *In vitro* propagation and genetic fidelity study of plant regenerated from inverted hypocotyl explants of eggplant (*Solanum melongena* L.) cv. Arka Shirish. *Biotechnology*. 3: 45-52.
- Mangat, B. S. and Roy, M. K. (1986). Tissue culture and plant regeneration of okra (*Abelmoschus esculentus*). *Plant Science*. 47: 57-61.
- Manwell, C. and Ann Baker, C. M. (2012). Protein polymorphism in domesticated species: evidence or hybrid origin? In Karlin, S. (ed.). *Population Genetics and Ecology* (pp. 106). Elsevier.
- Marshall, J. G. and Staba, E. J. (1976). Hormonal effects on diosgenin biosynthesis and growth in *Dioscorea deltoidea* tissue cultures. *Phytochemistry*. 15: 53-55.

- Mauseth, J. D. (2003). *Botany: An Introduction to Plant Biology*, 3<sup>rd</sup> edition. Jones and Bartlett Publishers, Incorporated. (pp. 130-195).
- Mazvimba, M. T., Yu, Y., Cui, Z. Q., and Zhang, Y. (2012). Optimization and orthogonal design of an ultra-sonic-assisted aqueous extraction process for extracting chlorogenic acid from dry tobacco leaves. *Chinese Journal of Natural Medicines*. 10: 0311-0320.
- Minano, H. S., Benito, M. E. G., and Martin, C. (2009). Molecular characterization and analysis of somaclonal variation in chrysanthemum cultivars using RAPD markers. *Scientia Horticulturae*. 122: 238-243.
- Modgil, M., Guleria, N., Ghani, M., and Sharma, J. N. (2012). Identifying somaclonal variants of the apple rootstock Malling 7 resistant to white root rot. *Scientia Horticulturae*. 137: 148-155.
- Mohamad, N., Mahmood, M., and Mansor, H. (2009). Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. *Journal of Medicinal Plant Research*. 3: 217-223.
- Montoro, P., Braca, A., Pizza, C., and De Tommasi, N. (2005). Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chemistry*. 92: 349-355.
- Moyo, M., Aremu, A. O., Gruz, J., Subrtova, M., Szucova, L., Dolezal, K., and Van Staden, J. (2013). Conservation strategy for *Pelargonium sidoides* DC: Phenolic profile and pharmacological activity of acclimatized plants derived from tissue culture. *Journal of Ethnopharmacology*. 149: 557-561.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*. 15: 473-479.
- Nagib, A., Hossain, S. A., Alam, M. F., Hossain, M. M., Islam, R., and Sultana, R. S. (2003). Virus free potato tuber seed production through meristem culture in tropical Asia. *Asian Journal of Plant Sciences*. 2: 616-622.
- Nakano, M., Nomizu, T., Mizunashi, K., Suzuki, M., Mori, S., Kuwayama, S., Hayashi, M., Umehara, H., Oka, E., Kobayashi, H., Asano, M., Sugawara, S., Takagi, H., Saito, H., Nakata, M., Godo, T., Hara, Y., and Amano, J. (2006). Somaclonal variation in *Tricyrtis hirta* plants regenerated from 1-year-old embryonic cultures. *Scientia Horticulturae*. 110: 366-371.
- Negrel, J. (1989). The biosynthesis of cinnamoylputrescines in callus tissue cultures of *Nicotiana tabacum*. *Phytochemistry*. 28: 477-481.
- Ng, Y. J., Go, R., Nulit, R., Khor, H. E., Tan, M. C., Nordin, F. A., Nuruddin, A. A., and Lee, N. S. (2012). Orchids of cloud forest in Genting Highlands, Pahang, Malaysia. *Sains Malaysiana*. 41: 505-526.

- Nobre, J., Davey, M. R., Lazzeri, P. A., and Cannel, M. E. (2000). Transformation of barley scutellum protoplasts: regeneration of fertile transgenic plants. *Plant Cell Reports*. 9: 1000-1005.
- Novak, F. J. and Maskova, I. (1979). Apical shoot tip culture of tomato. *Scientia Horticulturae*. 10: 337-344.
- Nwachukwu, E. C., Nulit, R., and Go, R. (2014). Nutritional and biochemical properties of Malaysian okra variety. *Advancement in Medicinal Plant Research*. 2: 16-19.
- Ohtsu, K., Smith, M. B., Emrich, S. J., Borsuk, L. A., Zhou, R., Chen, T., Zhang, X., Timmermans, M. C. P., Beck, J., Buckner, B., Buckner, D. J., Nettleton, D., Scanlon, M. J., and Schnable, P. S. (2007). Global gene expression analysis of the shoot apical meristem of maize (*Zea mays* L.). *The Plant Journal*. 52: 391-404.
- Orbovic, B., Calovic, M., Vilorio, Z., Nielsen, B., Gmitter, F., Castle, W., and Grosser, J. (2008). Analysis of genetic variability in various tissue culture-derived lemon plant populations using RAPD and flow cytometry. *Euphytica*. 161: 329-335.
- Othman, A., Ismail, A., Abdul Ghani, N., and Adenan, I. (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry*. 100: 1523-1530.
- Ozel, C. A., Khawar, K. M., and Arslan, O. (2008). A comparison of the gelling of isubgol, agar and gelrite on *in vitro* shoot regeneration and rooting of variety Samsun of tobacco (*Nicotiana tabacum* L.). *Scientia Horticulturae*. 117: 174-181.
- Ozudogru, E. A., Kirdok, E., Kaya, E., Capuana, M., De Carlo, A., and Engelmann, F. (2011). Medium-term conservation of redwood (*Sequoia sempervirens* (D. Don.) Endl.) *in vitro* shoot cultures and encapsulated buds. *Scientia Horticulturae*. 127: 431-435.
- Paly, G. P., Franck, T., Brisson, L., Kevers, C., Chenieux, J. C., and Rideau, M. (1999). Cytokinin modulates catalase activity and coumarin accumulation in *in vitro* cultures of tobacco. *Journal of Plant Physiology*. 155: 9-15.
- Patel, A. K., Agarwal, T., Phulwaria, M., Kataria, V., and Shekhawat, N. S. (2014). An efficient *in vitro* plant regeneration system from leaf of mature plant of *Leptadenia reticulata* (Jeevanti): A life giving endangered woody climber. *Industrial Crops and Products*. 52: 499-505.
- Pati, P. K., Rath, S. P., Sharma, M., Sood, A., and Ahuja, P. S. (2006). *In vitro* propagation of rose – a review. *Biotechnology Advances*. 24: 94-114.
- Peddaboina, V., Thamidala, C., and Karampuri, S. (2006). *In vitro* shoot multiplication and plant regeneration in four *Capsicum* species using thidiazuron. *Scientia Horticulturae*. 107: 117-122.

- Perez-Nunez, M. T., Chan, J. L., Saenz, L., Gonzalez, T., Verdeil, J. L., and Oropeza, C. (2006). Improved somatic embryogenesis from *Cocos nucifera* (L.) plumule explants. *In Vitro Cellular and Developmental Biology – Plant*. 42: 37-43.
- Phelan, S., Hunter, A., and Douglas, G. C. (2009). Effect of explants source on shoot proliferation and adventitious regeneration in 10 *Buddleia* cultivars. *Scientia Horticulturae*. 120: 518-524.
- Polanco, C. and Ruiz, M. L. (2002). AFLP analysis of somaclonal variation in *Arabidopsis thaliana* regenerated plants. *Plant Science*. 162: 817-824.
- Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L., and Debeaujon, I. (2006). Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Plant Science*. 12: 29-36.
- Prammanee, S., Thumjamras, S., Chiemsombat, P., and Pipattanawong, N. (2011). Efficient shoot regeneration from direct apical meristem tissue to produce virus-free purple passion fruit plants. *Crop Protection*. 30: 1425-1429.
- Qiao, M. R., Li, J. W., Wei, M. L., Jing, L. W., and Yu, T. D. (2012). *In vitro* antioxidant properties of flavonoids and polysaccharides extract from tobacco (*Nicotiana tabacum* L.) leaves. *Molecules*. 17: 11281-11291.
- Radhakrishnan, R. and Ranjithakumari, B. D. (2008). Morphological and agronomic evaluation of tissue culture derived Indian soybean plants. *Acta Agriculturae Slovenica*. 91: 391-396.
- Rahman, M. A., Alam, M. A., Hossain, M. R., Hossain, A., and Afroz, R. (2010). *In vitro* regeneration of popular tobacco varieties of Bangladesh from leaf disc. *Bangladesh Journal of Agricultural Research*. 35: 125-134.
- Rauh, V. A., Whyatt, R. M., Garfinkel, R., Andrews, H., Hoepner, L., Reyes, A., Diaz, D., Camann, D., and Perera, F. P. (2004). Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. *Neurotoxicology and Teratology*. 26: 373-385.
- Ray, T., Dutta, I., Saha, P., Das, S., and Roy, S. C. (2006). Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant Cell, Tissue and Organ Culture*. 85: 11-21.
- Razdan, M. K. (2003). *Introduction to Plant Tissue Culture, 2<sup>nd</sup> edition*. Science Publishers, Enfield.
- Rodu, B. and Ou, B. (2000). The antioxidant properties of tobacco. *Tobacco Science*. 44: 71-73.
- Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R., and Mulatsih, W. (2010). Antioxidant activity, total phenolic, and total flavonoid of extracts and

- fractions of red fruit (*Pandanus conoideus* Lam). *International Food Research Journal*. 17: 97-106.
- Rong, L., Frontera Jr., A. T., and Benbadis, S. R. (2014). Tobacco smoking, epilepsy, and seizures. *Epilepsy and Behavior*. 31: 210-218.
- Roopadarshini, V. and Gayatri, M. C. (2012). Isolation of somaclonal variants for morphological and biochemical traits in *Curcuma longa* (turmeric). *Research in Plant Biology*. 2: 31-37.
- Roulette, C. J., Mann, H., Kemp, B. M., Remiker, M., Roulette, J. W., Hewlett, B. S., Kazanji, M., Breurec, S., Monchy, D., Sullivan, R. J., and Hagen, E. H. (2014). Tobacco use vs. helminths in Congo basin hunter-gatherers: self-medication in humans? *Evolution and Human Behaviour*. 35: 397-407.
- Rout, G. R., Mohapatra, A., and Jain, S. M. (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. *Biotechnology Advances*. 24: 531-560.
- Roy, M. K. and Mangat, B. S. (1989). Regeneration of plants from callus tissue of okra (*Abelmoschus esculentus*). *Plant Science*. 60: 77-81.
- Saker, M., El-Demerdash, M., and Allam, M. A. (2011). *In vitro* propagation and genetic characterization as effective tools for conservation of *Silene leucophylla*, grown in St. Katherine protected area, Sinai, Egypt. *Journal of Genetic Engineering and Biotechnology*. 9: 21-27.
- Sato, M., Hosokawa, M., and Doi, M. (2011). Somaclonal variation is induced *de novo* via the tissue culture process: a study quantifying mutated cells in *Saintpaulia*. *PLoS ONE*. 6: 1-7.
- Schnablova, R., Synkova, H., Vicankova, A., Burketova, L., Eder, J., and Cvikrova, M. (2006). Transgenic *ipt* tobacco overproducing cytokinins overaccumulates phenolic compounds during *in vitro* growth. *Plant Physiology and Biochemistry*. 44: 526-534.
- Silvarajan, L. (2013). *In vitro* regeneration of *Oryza sativa* (MR219) and *Zea mays* (Thai Super Sweet) by SAM and RAM. Master Thesis, University Putra Malaysia.
- Silvarajan, L., Nulit, R., and Qamaruz Zaman, F. (2012). Effects of plant growth regulators on *in vitro* regeneration of Malaysian Indica rice (*Oryza sativa* L.) cv. MR219 by shoot apical meristem. *Asian Journal of Agricultural Research*. 6: 180-187.
- Simonovska, B., Srbinska, M., and Vovk, I. (2006). Analysis of sucrose esters – insecticides from the surface of tobacco plant leaves. *Journal of Chromatography A*. 1127: 273-277.



- Smith, R. H. and Murashige, T. (1970). *In vitro* development of the isolated shoot apical meristem of angiosperms. *American Journal of Botany*. 57: 562-568.
- Solomon, E. P., Berg, L. R., and Martin D. W. (2011). *Biology*, 9<sup>th</sup> edition. Brooks/Cole, Cengage Learning. (pp. 719).
- Sticklen, M. B. and Obray, H. F. (2005). Invited review: Shoot apical meristem – a sustainable explant for genetic transformation of cereal crops. *In Vitro Cellular & Developmental Biology*. 41: 187-200.
- Sudharsan, C., Nil, M. A., and Hussain, J. (2003). Tissue culture technology for the conservation and propagation of certain native plants. *Journal of Arid Environments*. 54: 133-147.
- Sun, E. J. and Kang, H. W. (2003). Tobacco clones derived from tissue culture with supersensitivity to ozone. *Environmental Pollution*. 125: 111-115.
- Tabata, M., Yamamoto, H., Hiraoka, N., Marumoto, Y., and Konoshima, M. (1971). Regulation of nicotine production in tobacco tissue culture by plant growth regulators. *Phytochemistry*. 10: 723-729.
- Tan, M., Yan, M., Wang, L., Yan, X., Fu, C., and Wang, L. (2013). Replication of pistillate plants of *Ricinus communis* L. and investigation of the sex stability and genetic variation of the somaclones. *Industrial Crops and Products*. 50: 50-57.
- Thomas, J., Kumar, R. R., and Mandal, A. K. A. (2006). Metabolite profiling and characterization of somaclonal variants in tea (*Camellia* spp.) for identifying productive and quality accession. *Phytochemistry*. 67: 1136-1142.
- Turner, S., Krauss, S. L., Bunn, E., Senaratna, T., Dixon, K., Tan, B., and Touchell, D. (2001). Genetic fidelity and viability of *Anigozanthos viridis* following tissue culture, cold storage and cryopreservation. *Plant Science*. 161: 1099-1106.
- Upadhyay, R. and Mishra, H. N. (2014). Antioxidant activity measurement of oleoresin from rosemary and sage. *Industrial Crops and Products*. 61: 453-459.
- Valizadeh, M., Kazemi Tabar, S. K., and Nematzadeh, G. A. (2007). Effect of plant growth regulators on callus induction and regeneration of cumin (*Cuminum cyminum*). *Asian Journal of Agricultural Research*. 1: 17-22.
- Van Hoof, L. (2011). Tobacco (*Nicotiana tabacum*). Retrieved 20 August 2014 from [https://bioweb.uwlax.edu/BIO203/2011/vanhoof\\_log/classification.htm](https://bioweb.uwlax.edu/BIO203/2011/vanhoof_log/classification.htm)
- Vesperinas, E. S. (1998). *In vitro* root induction in hypocotyl and plumule explants of *Helianthus annuus*. *Environmental and Experimental Botany*. 39: 271-277.
- Viehmánová, I., Bortlova, Z., Vitamvas, J., Cepkova, P. H., Eliasova, K., Svobodova, E., and Travnickova, M. (2014). Assessment of somaclonal variation in somatic embryo-derived plants of yacon (*Smallanthus sonchifolius* (Poepp.

- Endl.) H. Robinson) using inter simple sequence repeat analysis and flow cytometry. *Electronic Journal of Biotechnology*. 17: 102-106.
- Vijayaraghavan, M., Pierce, J. P., White, M., and Messer, K. (2014). Differential use of other tobacco products among current and former cigarette smokers by income level. *Addictive Behaviors*. 39: 1452-1458.
- Vinson, J. A., Dabbagh, Y. A., Serry, M. M., and Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*. 43: 2800-2802.
- Wang, H. and Holl, F. B. (1988). *In vitro* culture and the incidence of somaclonal variation in regenerated plants of *Trifolium pratense* L. *Plant Science*. 55: 159-167.
- Wang, W. C., Myers, J. R., and Collins, G. B. (1989). Establishment of stable long-term photomixotrophic cell cultures of tobacco (*Nicotiana tabacum* L.). *Plant Science*. 61: 145-151.
- Werner, T., Motyka, V., Strnad, M., and Schmulling, T. (2001). Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences USA*. 98: 10487-10492.
- Wysokinska, H. and Lisowska, K. (2000). *In vitro* propagation of *Catalpa ovata* G. Don. *Plant, Cell, Tissue and Organ Culture*. 60: 171-176.
- Xin, W., Liu, Z., Song, Y., Hou, T., and Xiang, F. (2012). Direct shoot regeneration from *Arabidopsis thaliana* shoot apical meristems. *Biologia Plantarum*. 56: 601-606.
- Yang, Y. S., Chu, D. C., Aizawa, M., Kim, M., and Yamamoto, T. (1995). Ethanol metabolism by tobacco callus *in vitro*. *Plant Science*. 104: 147-151.
- Yuan, Z. L., Xiong, S. P., Li, C. M., and Ma, X. M. (2011). Effects of chronic stress of cadmium and lead on anatomical structure of tobacco roots. *Agricultural Sciences in China*. 10: 1941-1948.
- Zaiton, S., Sariah, M., and Zainal Abidin, M. A. (2006). Isolation and characterization of microbial endophytes from oil palm roots: Implication as biocontrol agents against *Ganoderma*. *The Planter*. 82: 587-597.
- Zazimalova, E., Brezinova, A., Holik, J., and Opatrny, Z. (1996). Partial auxin deprivation affects endogenous cytokinins in an auxin-dependent, cytokinin-independent tobacco cell strain. *Plant Cell Reports*. 16: 76-79.
- Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*. 64: 555-559.

Zhong, J. J., Bai, Y., and Wang, S. J. (1996). Effects of plant growth regulators on cell growth and ginsenoside saponin production by suspension cultures of *Panax quinquefolium*. *Journal of Biotechnology*. 45: 227-23.



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