UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF PROTOCOL FOR IN VITRO REGENERATION IN ABELMOSCUS ESCULENTUS (L.) MOENCH (OKRA) THROUGH SHOOT APICAL MERISTEM, AND NUTRIENT AND BIOCHEMICAL COMPOSITION OF ITS LEAVES AND FRUITS

NWACHUKWU EMMANUEL CHIKA

FS 2013 81

DEVELOPMENT OF PROTOCOL FOR *IN VITRO* REGENERATION IN *ABELMOSCUS ESCULENTUS* (L.) MOENCH (OKRA) THROUGH SHOOT APICAL MERISTEM, AND NUTRIENT AND BIOCHEMICAL COMPOSITION OF ITS LEAVES AND FRUITS

By

NWACHUKWU EMMANUEL CHIKA

This Thesis is submitted to the School of Graduate Studies, Universiti Putra Malalysia, in Fulfilment of the Requirements for the Degree of Master of Science

July, 2013

DEDICATION

For His continuous love and protection, I dedicate my thesis to Almighty God and for the children killed in Syria.

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

DEVELOPMENT OF PROTOCOL FOR *IN VITRO* REGENERATION IN *ABELMOSCUS ESCULENTUS* (L.) MOENCH (OKRA) THROUGH SHOOT APICAL MERISTEM, AND NUTRIENT AND BIOCHEMICAL COMPOSITION OF ITS LEAVES AND FRUITS

By

NWACHUKWU EMMANUEL CHIKA

July 2013

Chairman: Rosimah Nulit, PhD

Faculty: Science

Okra is an important vegetable of valuable nutrient, good sources of vitamins, important antioxidants and medicinal compounds such as polyphenols and gossypol. The tissue culture was carried out by using shoot apical meristem while the nutritional and biochemical values of fresh okra leaves and fruits were evaluated. Meristematic tissues (0.4-0.6mm) of okra seedlings were excised. Shoot apical meristems (SAM) were cultured in MS medium supplemented with a range of concentrations of NAA (0.1mg/l - .0.5mg/l, BAP (0.1mg/l, 2 - 4.0mg/l), and in combination of NAA and BAP (0.1mg/l – 1.0mg/l). This present study showed the highest shoot proliferations and formation from SAM on semi-solid MS medium cultured on 1.5mg/l BAP with four shoots per culture

and best rooting on 1.0mg/l with an average of 4 roots per culture. The plantlets were transferred into pots containing soil for acclimatization and showed average of 82% survival. The proximate analysis was used to determine the percentage of protein, carbohydrate, ash, fats, fiber and moisture present in okra leaves and fruits. In addition, selected biochemical properties of okra leaves and fruits such as Chlorophyll a, b, xanthophylls and caretonoids, total phenolics and total flavonoids were measured spectrophotometrically. This study revealed the highest percentage of protein (4.82%) and ash (2.44%) in okra leaves while fruits contain highest percentage carbohydrate (11.17%), crude fiber (2.44%) and moisture (88.47%). Interestingly, this present study found that okra leaf contains higher chlorophyll, total phenolics and total flavonoid than okra fruit. Okra leaf showed highest chlorophyll a (23.66mg/0.1g), carotenoids (4.63mg/0.1g), Chlorophyll b (6.6mg/0.1g). Furthermore, the result also showed highest total phenolics (0.99mg/0.1g) and flavonoids (0.80mg/0.1g) in the leaf. This study has established standard protocol for *in vitro* regeneration of mucilaginous plant such as okra and okra leaf has shown to contain high nutritional and biochemical value compared with okra fruit.

Keywords: *Abelmoschus esculentus*, shoot apical meristem, *in vitro* regeneration, proximate analysis, chlorophyll, total phenolics, total flavonoids.

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

REGENERASI *ABELMOSCHUS ESCULENTUS* (L.) MOENCH (BENDI) SECARA *IN VITRO* MENGGUNAKAN TISU APIKAL MERISTEM, DAN KAJIAN PEMBANDINGAN KANDUNGAN, DAN NUTRIEN DAN BIOKIMIA DIANTARA DAUN DAN BUAH

Oleh

NWACHUKWU EMMANUEL CHIKA

Julai 2013

Pengerusi: Rosimah Nulit, PhD.

Fakulti: Sains

Okra (bendi) merupakan salah satu sayur-sayuran yang tinggi dengan kandungan nutrien, sumber vitamin dan mengandungi kompaun perubatan seperti folifenol, gossypol. Sehingga kini tiada kajian menggunakan tisu apikal meristem untuk regenerasi pokok okra secara *in vitro*. Oleh yang demikian, objektif pertama kajian ini adalah untuk menghasilkan prosedur untuk regenerasi tumbuhan okra secara *in vitro* menggunakan tisu apikal meristem sebagai eksplan. Okra merupakan sayur yang penting disebabkan kandungan nutrisi dan biokimia yang terdapat pada buah atau pod. Tetapi, sehingga kini tiada kajian dijalankan terhadap kandungan nutrisi dan biokimia ke atas daun okra. Oleh itu, objektif kajian ini adalah untuk menentukan kandungan nutrisi daun dan buah okra dan untuk membandingkan kandungan biokimia iaitu klorofil a, b, karotenoid dan xantofil, fenolik dan flavonoid di antara daun dan buah (muda dan matang) okra. Tisu apikal meristem (0.4-0.6mm) diisolat dari anak benih okra, kemudian dikulturkan didalam MS media yang ditambah dengan NAA dengan julat kepekatan antara 0.1 -.0.5mg/l, MS media yang ditambah dengan BAP dengan julat kepekatan antara 0.1mg - 4.0mg/l, dan MS media dengan kombinasi NAA dan BAP dengan julat

kepekatan antara 0.1–1.0mg/l. Kajian ini mendapati pertumbuhan/penggandaan dan pembentukan pucuk dari tisu apikal meristem adalah paling baik dalam MS media yang ditambah dengan 1.5mg/l BAP dengan empat pucuk per kultur. Kesemua plantlet berupaya untuk tumbuh sehingga berbunga dan berbuah. Kajian ini mendapati analisis proksimat menunjukkan daun okra mengandungi kandungan protin (4.82%) dan abu (2.44 %) lebih tinggi berbanding dengan buah okra. Manakala buah matang menunjukkan kandungan karbohidrat (11.17%) dan serat (2.44%) lebih tinggi. Kajian ini juga telah berjaya membuktikan bahawa kandungan klorofil, fenolik dan flavonoid lebih tinggi dari buah okra. Sebagai perbandingan, kandungan klorofil a dan karotenoid adalah tinggi dalam daun matang iaitu 23.66 mg/0.1g dan 4.63 mg/0.1g. Manakala kandungan klorofil b adalah tinggi (6.6 mg/0.1g) dalam daun muda. Kajian ini juga telah mendapati kandungan fenolik dan flavonoid lebih tinggi dalam daun berbanding dengan buah di mana daun muda menunjukkan kandungan fenolik yang tinggi iaitu 0.99 mg/0.1g dan kandungan flavonoid tinggi dalam daun matang (0.80 mg/0.1g). Sebagai kesimpulan, procedur untuk regenerasi secara *in vitro* pokok okra menggunakan tisu apikal meristem sebagai eksplan telah berjaya dihasilkan dan sebagai komplemen dengan kajian terdahulu. Hasil penemuan kajian ini telah berjaya membuktikan kandungan nutrisi dan biokimia daun okra lebih tinggi dari pada pucuk.

Katakunci*: Abelmoschus esculentus*, pucuk apical meristem, regenerasi secara *in vitro*, analisis proksimat, klorofil, fenolik, flavonoid

ACKNOWLEDGEMENTS

I am grateful to all that contributed to the successful completion of this thesis; Outstanding among whom is the chairman of the supervisory committee, Dr. Rosimah Nulit, for her intellectual guidance, suggestions, encouragement and advices which contributed immensely in making this research a reality.

My appreciation must go to Associate Professor Dr. Rusea Go, for her academic guidance and support throughout my research. My warm gratitude also goes to my colleagues and the staffs of the Tissue Culture laboratory, Department of Biology, Faculty Science for their technical support and kind assistance throughout my study.

My special gratitude goes to my parents; Engr. and Mrs. J. M. Nwachukwu for their emotional, moral and financial support. I want to use this opportunity to thank my sister and brothers especially my younger brother Franklin Nwachukwu for his encouragement.

My thanks also go to my friends; Nkem Okafor, Bright Iheanacho, Nicholas Ikedima.

Finally, I thank the Almighty God for His grace, mercy, love and blessings which He has shown me throughout my stay in Universiti Putra Malaysia.

I certify that a Thesis Examination Committee has met on 18th September 2013 to conduct the final examination of Emmanuel Chika Nwachukwu on his thesis entitled "Development of Protocol for *In vitro* Regeneration in *Abelmoschus esculentus* (L.) Moench (Okra) Through Shoot Apical Meristem, and Nutrient and Biochemical Composition of its Leaves and Fruits" in accordance with the Universities and University College 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.

Members of the Thesis Examination Committee were as follows:

Dr. Nor Azwady bin Abd Aziz
Jabatan Biologi,
Faculti Sains.
Universiti Putra Malaysia.
43400 UPM Serdang, Selangor.

Dr. Nur Ashikin Psyquay binti Abdullah
Jabatan Sains Tanaman,
Faculti Pertanian.
Universiti Putra Malaysia.
43400 UPM Serdang, Selangor.

Dr. Hishamuddin bin Omar
Jabatan Biologi,
Faculti Sains.
Universiti Putra Malaysia.
43400 UPM Serdang, Selangor.

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: October, 2013.

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Rosimah Nulit, PhD.
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Associate Professor Rusea Go, PhD.
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD.
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

EMMANUEL CHIKA NWACHUKWU

Date: 18th September, 2013.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
BAP	N^6 –benzylaminopurine
°C	Degree celsius
Cm	Centimeter
dH_2O	Distilled water
Ethanol	Ethyl alcohol
FAO	Food and Agriculture Organization of the United Nation
HCl	Hydrochloric acid
Hrs	Hours
I	Littre
M	Molar
Mg	Milligram
Min	Minute
mM	Milimolar
mm	Millimeter
MS	Murashiage and Skoog
NAA	I –Naphthaleneacetic acid
NaCl	Sodium Chloride
NPU	Net Protein Utilization
%	Percentage
PER	Protein Efficiency Ratio
PGR	Plant Growth Regulator

pH	Negative logarithm of hydrogen ion concentration
SAM	Shoot Apical Meristem
SE	Standard Error
t	Tones
UV	Ultraviolet
v/v	Volume over volume
w/v	Weight over volume
µg	Microgram
µl	Microliter

CHAPTER 1

INTRODUCTION

There are many ways of plant propagation either through seed, cutting and tissue culture. Tissue culture offers alternative means of propagation and helps to overcome some conventional disadvantages such as disease and seasonal problems. Other notable advantages of *in vitro* regeneration are rapid multiplication of plants; plantlets produced grow faster, healthier, stronger and have shorter production cycle and desirable yields. The use of shoot apical meristem and modifying PGRs within the culture medium are alternative ways to establish an ideal *in vitro* regeneration protocol. Plant growth regulators such as auxins and cytokinins in certain amount have demonstrated positive responds in morphogenesis of callus, organogenesis, embryogenesis as well as multiple shoot regeneration of okra. There are other factors that contribute for okra *in vitro* culture such as genotype, tissue type, pH of the media, aeration as well as other organic compounds such as vitamins and myo-inositol (Ashish *et al*., 2008; Kabir *et al*., 2008; Rahman *et al*., 2008; Ganesan *et al.,* 2007; Haider *et al*., 1993).

The use of shoot apical meristem explants as a protocol in propagation of mucilaginous plants such as okra proved an alternative potential application for development of new cultivars and encourages breeding lines (Ahmed *et al.,* 2007). Other methods of *in vitro* regeneration of okra has been used while in this present study, okra was used as a model plant for *in vitro* regeneration using shoot apical meristem to overcome some constrains such mucilage and contamination through number of sub-culturing as reported by (Ganesan *et al*., 2007). Okra (*Abelmoschus esculentus*) an ever-green

vegetable with bright yellow and black beautiful blooms of flowers belongs to the family Malvaceae. It is a cosmopolitan vegetable that dominates in South East Asia, West Africa, Turkey, Brazil, North America, India, Portugal, Cuba, and Middle East (Duzyaman, 1997; Rao, 1985).

Okra is sold as vital annual vegetable crop generally marketed fresh throughout the South East Asia regions (Rahman *et al.,* 2008). The fresh fruits are capsulated and consist of greenish to whitish round seeds that are most widely consumed in boiled or fried. The leaves are also used fresh as salad. The fresh fruits have high content of mucilage and are commonly used in Africa dishes for preparing soup (Ndunguru & Rajabu, 2004).

Okra is nutritious and is a good source of vitamin A and C, carbohydrates, proteins and important medicinal compounds such as polyphenols, gossypol, calcium and iron with low soluble fibres and pectins that lower serum cholesterol (Adetuyi *et al*., 2011; Kabir *et al.*, 2008; Ganesan *et al.,* 2007; Uda *et al.,* 1997; Basu and Kirtiker, 1984; Chopra, 1969). The fresh fruits have high content of mucilage and industrially used in confectionary for manufacturing gums (Siemonsma & Kauoma, 2004; Schalau, 2002). Thus, due to the high consumption rate, the importance of okra in agriculture significantly increased in terms of economic value. Procedures for mass *in vitro* propagation and clones/plantlets derived from meristems are genetically stable and phenotypically homogenous as well as bridging the gap in seasonal production as well as continuous availability of planting materials. The plant meristems are frequently devoid of systemic pathogen due to the absence of differentiated conducting tissues (Benchasri, 2012; Anisuzzaman *et al*., 2010).

1.1 Problems Statements and Justification of study

In the present study, there are two problems statements; I) previous studies were done on *in vitro* regeneration of okra using different parts of okra plant as explants. Previous studies by Haider *et al.* (1993) using hypocotyls as explants followed by Ganesan *et al*., (2007) using somatic embryo for *in vitro* regeneration. Kabir *et al.* (2008) used leaf and root for *in vitro* regeneration of okra and cotyledonary node was used as explants (Renu *et al*., 2008). No studies on *in vitro* regeneration of okra using shoot apical meristem have been so far reported. Thus, this study used shoot apical meristem (SAM) at seedling stage to regenerate whole okra and to overcome some problems of *in vitro* regeneration of mucilaginous plant that contaminates easily such as *Aloe vera* as reported by Ahmed *et al. (*2007). Since okra is used as a model for *in vitro* regeneration, it is also necessary to study the proximate and biochemical content as okra is regarded as important vegetable in Nigeria. However, many previous studies extensively focused on okra nutrients in the fruits/pods. Tandall (1983) & Charrier (1984) reported on the protein and oil content of okra seeds while Kumar *et al*. (2009); Lui *et al*. (2005) reported on the carbohydrate content present in the okra pods in form of mucilage. Therefore, no study was conducted to determine nutritional and biochemical composition of okra leaves. Hence, the objectives of this study are:

1. To establish a protocol for *in vitro* regeneration of green okra variety through shoot apical meristem.

2. To evaluate nutritional values of fresh okra fruits and leaves and its selected biochemical content.

REFERENCES

Adelakun, O. E., Oyelade, O. J., Ade-Omowaye, B. I., Adeyemi, A. I., Van de Venter, M. (2009a). Chemical composition and the antioxidative properties of Nigerian Okra seed (*Abelmoschus esculentus* Moench) flour. *Food Chem Toxicol*., 47(6): 1123-1126.

Adelberg, J.W, Zhang, X P, Rhodes, B. B. (1997). Micropropagation of *Citrullus lanatus* (Thumb.) Matsum. and Nakai (Watermelon). In: Bajaj YPS, ed. Biotechnology in Agriculture and Forestry. *High-Tech and Micropropagation V. Springer-Verlag Berlin Heidelberg,* 39: 60-76.

Adelusi, A. A., Makinde, A. M. and Folorunso, A. E. (2006). Comparative studies of physic-biochemical parameters in *Abelmoschus esculentus (L.)* Moench and *A. moschatus* (Moench). *Research Journal of Botany,* 1: 104-109.

Adetuyi F.O., Osagie A.U. and Adekunle A.T. (2011). Nutrient, antinutrient, mineral and zinc bioavailability of okra *Abelmoschus esculentus* (L) Moench. *American Journal of food and Nutrition,* 1(2): 49-54.

Ahmed, S., Kabir, A. H., Ahmed, M. B., Razvy, M. A., & Ganesan, S. (2007). Development of rapid micropropagation method of Aloe vera L. *Sjemenarstvo*, *24* (2): 121-128.

Aladele, S. E., Ariyo, O. J., Lapena, R. (2008). Genetic relationship among West African okra (*Abelmoschus caillei*) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. *African J. Biotechnol.* 7:1426-1431.

Alegbejo, M., Ogunlana, M., Banwo, O. (2008): Survey for incidence of okra mosaic virus in Northern Nigeria and evidence for its transmission by beetles. *Spanish J. Agric. Res.,* 6: 408-411.

Ali, A. and Deokule, S. S. (2008). Comparison of Phenolics Compounds of Some Edible Plants of Iran and India. *Pakistan Journal of Nutrition,* 7(4): 582-585.

Akintoye, H. A., Adebayo, A. G., Aina, O. O., (2011). Growth and yield response of okra intercropped with live mulches. *Asian J. Agric. Res.* 5: 146-153.

Akinyele, B. O., Temikotan, T. (2007). Effect of variation in soil texture on the vegetative and pod characteristics of okra (*Abelmoscus esculentus* (L.) Moench). *Intern. J. Agric. Res.* 2: 165-169.

Anisuzzaman, M., Kabir, A. H., Jarin, S., Kanak, K. S. and Alam, M. F. (2008). Micropropagation of *Abelmoschus esculentus* L. Moench. for disease free plantlets through meristem culture, *Phytopathology and Plant Protection,* 43(5): 460-466.

Anisuzzaman, M., Jarin, S., Naher, K., Akhtar, M. M., Alam, M. J., Khalekuzzaman, M., Alam, I. and Alam, M. F. (2008). Callus Induced Organogenesis in Okra *Abelmoschus esculents* (L) Moench. *Asian Journal of Plant Sciences*, 7: 677-681.

Arapitsas, P. (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. *Food Chem.* 101: l041-1045.

Association of Official Analytical Chemist (AOAC, 1984). Official methods of analysis. Association of official analytical chemists. 14th Ed. Washigton, D. C; U. S. A., pp.187-188.

Association of Official Analytical Chemist (AOAC- 1990). Official Method of Analysis, 15thEdition. Association of Official analytical chemist,Washington, D.C; U.S.A., pp.88-89

Ashish, S. and Sharm, R. A. (2011). Micropropagation of *Croton bonplandinum* Bali . *International Research Journal of Pharmacy*, 2(10): 82-86.

Ashish, R. W., Nandkishor H. R. and Prashant W. (2008). *In vitro* callus induction of *Abelmoschus esculentus* Medik (l) by using different hormone concentration. *International Journal of Pharmaceutical Review and Research*. 10: 1-16.

Atiri, G. I., Fayoyin, G. A. (1989). Horizontal resistance to okra leaf curl virus in okra germplasm. *Ann. Appl. Biol*., 144: 152-153.

Balaraju, K., Agastian, P., Preetamraj, J. P., Akokiyaraj, S., Ignacimuthu, S. (2008). Micropropagation of *Vitex agnus-castus* (Verbenaceae), a valuable medicinal plant. *In Vitro Cell. Dev. Biol. Plant,* 44(5): 436-441.

Benchasri, S. (2012). Screening for yellow vein mosic virus resistance and yield loss under field conditions in Southern Thailand. *J. Animal Plant Sci.* 12: 167-1686.

Bisht, I. S and Bhat, K. V (2006). Genetic Resources, Chromosome Engineering and Crop Improvement Okra (*Abelmoschus sp*.). pp.149-185.

Bogre, L. and Beemster, G. T. S. (2008). *Plant Growth Signalling (Plant Cell Monograph).* Berlin: Springer-Verlag. pp.63.

Bowman, J. L. and Eshed, Y. (2000). Formation and Maintenance of Shoot Apical Meristem. *Trends Plant Sci.* 5: 110-115.

Bradbeer, J. W. (1988). *Seed Dormancy and Germination*. Blackie and Son Ltd. Glasgow, UK. pp. 146

Chadha, K. L. (2002). Hand book of Horticulture, Indian Council of Agricultural Research. pp.422-427.

Charrier, A. (1984). Genetic resources of *Abelmoschus* (Okra). IBP-GR Secretarial, Rome, Italy. pp.61.

Chen, T. H. H., Marowitch and Thompso, B. G. (1987). Genotypic effects on somatic embryogensis amd plant regeneration from callus cultures of alfalfa*. Plant Cell Tissue and Organ Cult.* 8: 73-81.

Cheruvathur, M. K., Britto, J. and Thomas, T. D. (2010). Callus induction and shoot regeneration from epicotyls explants of ethnomedicinally important *Caesalpinia bonduc* (L) Roxb. *Iranian Journal of Biotechnology*, 8: 263-269.

Cheong, E. J. and Pooler, M. R. (2004) Factors affecting somatic embryogenesis *Prunus incise* cv. February pink. *Plant Cell Rep.* 22: 810-815.

Choi, K., Wall, C., Hanratty, R. and Keller, G. (1994). Isolation of a gene encoding a novel receptor tyrosine kinase from differentiated embryonic stem cells. *Oncogene.* 9: 1261-1266.

Compton, M. E., Gray, D. J. and Gaba., V. P. (2004). Use of Tissue Culture and Biotechnology for the genetic improvement of Watermelon. *Plant Cell Tiss. And Org. Cult.,* 77: 231-243.

Compton, M. E. (1999). Dark pretreatment improves adventitious shoot organogenesis from cotyledons of diploid watermelon. *Plant Cell Tiss. Org. Cult.,* 58: 185-188.

Compton, M. E. and Gray, D. J. (1993a). Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid and tetraploid watermelon*. J. Am. Sci., Hort. Sci.* 118: 151-157.

Cook, J. A., VanderJagt. D. J., Pastuszyn, A., Mounkaila, G., Glew, R. S., Millson, M., Glew, R. H. (2000). Nutrient and chemical composition of 13 wild plant foods of Niger. *J. Food Comp. Anal.,* 13: 83-92*.*

Cooke, M. A. (1988). M. Sc. Thesis, Concordia University Montreal, Quebec, Canada.

Crisp, P., Walkey, D. G. A. and Bellman, E. R. (1975). A mutation affecting curd colour in cauliflower (*Brassica oleracea* L. var. *botrytis* DC). *Euphytica,* 24: 173-176.

Cuenca, S. J. B., Amo-Aarco and Parra, R. (1999). Micropropagation from inflorescence stems of the Spanish endemic plant Centaurea paui Loscos ex Willk. (Composotae). *Plant Cell Rep.,* 18: 674-679.

Dan, R. D. and Gu, C. (2010). Inhibition Effect of Okra Polysaccharides on Proliferation of Human Cancer Cell lines, *Food Science, 31*(21): 353-356.

Dasgupta, I., Malathi, V. G and Mukherjee, S. K. (2003). Genetic enegineering for virus resistance*. Current Science,* 84(3): 341-354*.*

Datta, P. C., Naug, A. (1968): A few strains of *Abelmoschus esculentus* (L) Moench their karyological in relation to phylogeny and organ development. *Beitr. Biol. Pflanzen*. 45: 113-126.

Davies, P. J. W and Davies, P. J. (2005). Plant Hormones Dordrecht, the Netherlands: Springer-Verlag, Kluwer. pp.91.

Dharamvir, H. (2007*). Synthetic Plant Growth Regulators*. New Delhi:mGene-Tech Books. pp.68

Duzyaman, E. (1997). Okra: Botany of Hoticulture. *Horti. Rev.,* 21:42-68.

Dodds, J. H., and Roberts, L. W. (1995). *Experiment in Plant tissue culture*, 3rd ed. Cambridge University Press, New York, NY. pp.101-113.

Ebermann, R. Alth, G. Kreitner, M., and Kubin, A. (1996). Natural products derived from plants as potential drugs for the photodynamic destruction of tumor cells. *J Photochem Photobiol B.,* 36(2): 95-7.

El-Nahry, F. I., El-Ghorab. M. I. and Younes, R. (1978). Nutritive value of local varieties of fresh and sundried okra (*Hibiscus esculentus*) pods and seeds: *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum),* 28(3): 227-231.

Falusi, O. A., Dangana, M.C., Daudu, O. Y., and Jaime, A. (2012). Studies on morphological and yield parameters of three varieties of Nigerian okra (*Abelmoschus esculentus* (L) Moench). *J. Hortic. And Frt.,* 4(7): 126-128.

FAOSTAT, (2008) Food and Agricultural Organization of the United Nations. Online and Multilingual Database, http:/faostat.fao.org/foastat/

Fauquet, C., Thouvenel, J. C. (1987). Okra leaf curl virus. In: Plant viral Diseases in the Ivory Coast, Vol. 46. Documentations Techniques, ORSTOM, Paris, France, pp.96 - 97.

Fonnesbech, M. (1974) Temperature effects on root and shoot development from Begonia x cheimentha petiole segments grown *in vitro*. *Physiol plant,* 32: 282.

Gamborg, O. L. & Philips. (1995). Plant Cell Tissue Organ Culture. *Narosa Publishing House, New Delhi*, 978-81-7319-101-5, pp.56 - 93.

Ganesan, M., Chandrasekar, R., Kumari, B., Jayabalan, N. (2007). Somatic Embryogenesis and Plant Regeneration of *Abelmoschus esculentus* Through Suspension Culture. *Biologia plantarum,* 51 (3): 414-420.

George, E. F. (1993). Plant Propagation and Micropropagation. Plant propagation by tissue culture. Part 1. *The Technology* Wiltshire, England: Exegetics Ltd. pp.574.

Glew, R. H., VanderJagt, D. J., Lockett, C., Grivetti, L. E., Smith, G. C., Pastuszyn, A. and Millson, M. (1997). Amino acid, and mineral composition of 24 indigenous plants of Burkina-Faso. *J. Food Comp. Anal.,* 10: 205-217.

Gopalan, C., Sastri, S.B.V., Balasubramanian, S. (2007). Nutritive value of Indian foods, National Institute of Niutrition (NIN) ICMR, India.

Grubben, G. J. H and Denton, A. O. (Editors), (2004). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundations, Wageningen Netherlands/Backhuys Publishers Leiden, Netherlands/CTA, Wageningen Netherlands. pp. 668.

Gupta, S & Mahalaxmi. (2009). *In vitro* high frequency direct plant regeneration from whole leaves of blackberry. *Scientia Horticulturae,* 120- 22-26.

Gukasyan, I. A., Butenko, R. G., Petoyan, S. A. and Sevost' Yanova, T. A. (1977). Morphogensis of isolated apices remontant carnation on an artificial medium. *Sov. Plant physiol.,* 24: 130.

Haque, M. S., Wada, T., & Hattori, K. (2003). Shoot regeneration and formation from shoot and root meristem of Gerlic cv Bangladesh Local. *Asian Journal of Plant Science*, 2: 23-27.

Harborne, (1989). Phytochemical dictionary: Handbook ofbioactive compounds from plants 2nd (Edn.). Taylor and Francis, London, pp.221-234.

Haider, S. A., Islam, R., Kama, A.H.M., Rahman, S.M. and Joarder O.I. (1993). Direct and Indirect Organogenesis in Cultured Hypocotyls Explants of *Abelmoschus esculentus* L. Moench. *Plant Tissue Culture,* 3(2): 85-89.

Harran, S. and Dickinson, D.B. (1978). Metabolism of myo-inositol and growth in various sugars of suspension-cultured tobacco cells, *Planta*, 141: 77- 82.

Hopkins, W. G. and Huner, N. P. (2009). Introduction to plant physiology, 4th (ed.). Wiley, New York. pp.528.

Huang, J., Giannasi, D. E. and Price, R. A. (2005). Phylogenetic relationships in *Ephedra* (Ephedraceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution,* 35(1): 48-59.

Hussain, J., Rehman, N., Khan, A. L., Hamayun, M., Hussain, S. M., Shinwari. Z. K. (2010b). Proximate and Nutrients Evaluation of Selected Vegetables Species from Kohat Region Pakistan. *Pakistan. Pak. J. Bot.,* 42(4): 2847-2855.

Ikram-Ul-Haq. (2005). Callus Proliferation and Somatic Embryogenesis in Cotton (*Gossypium hirsutum* L.). *Afr. J. Biotchnol*., 4: 206-209.

Jain, S. M and Ishii, K (2003). Mutation breeding and micropropagation of selected mutants agronomic trait in Sri Lanka, CGIAR, ISNAR Publication, Holland. Kluwer. pp 721-731.

Jan. G., Kahan, M., Ahmad, M., Iqbal, Z., Afzal, A., Afzal, M., Shah, G. M., Majid, A., Fiaz, M., Zafar, M., Waheed, A. and Gul, F. (2011). Nutritional analysis, micronutrients and chlorophyll contents of *Cichorium intybus* L. *Journal of Medicnal Plants Research*, 5(12): 2452-2456.

Jaworski, J. M. and Compton, M. E. (1997). Plant regeneration from cotyledons of five watermelon cultivars. *HortScience,* 32: 469 - 470.

Jeffery, S. W. and Humphrey, G. F. (1975). New spectro-photometric equations for determining chloro-phylls II, b, cI and cZ in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen*, 167: 191-194.

Kabir, A.H., Sarker K.K., Sharmin, S.A., Islam,M.S. and Alam, M.F. (2008). Callus Induction and Plantlet Regeneration in *Abelmoschus esculentus*(L.) Moench. *Journal of Agricultural Technology,* 4(1): 193-204.

Kaplan, D.R. and Cooke, T. J (1997): Fundamental concepts in the embrogenesis of dicotledons: a morphological interpretation of embryo mutants. *Plant Cell*, 9: 1903-1919.

Kathriv, K., Vengedesan, G. Singer S. Steinitz, B. Paris, H. and Gaba, V. (2006). Adventitious Regeneration *In vitro* occurs across a wide spectrum of squash (*Cucurbit pepo*) genotype. *Plant Cell Tissue Org. Cult*., 85:285-295.

Khan, N.A.(2006). *Ethylene Action in Plants*. New York: Springer-Verlag, pp185-202.

Kumar, R., Patil, M. B., Patil, S. R., Paschapur, M. S. (2009). Evaluation of Abelmoschus esculentus mucilage as suspending agent in paracetamol suspension. *Intern. J. PharmTech Res*., 1: 658-665.

Kumar, S., Dagnoko, S., Haougui, S., Ratnadass, A., Pasternak, D., Kouame, C., (2010). Okra (*Abelmoschus spp*.) in West and Central Africa: potential and progress on its improvement. *African J. Agric. Res*., 5: 3590-3598.

Kyte, L. and Kleyn, J. (2003). An introduction to Micropropagation. Timber Press, Oregon. pp240.

Latham, D. S. (1973). Cytokinins from *Zea mays*. *Phytochemistry,* 12: 2445-2455.

Lawrence D. Hills (1987). *F2 and Open Pollinated Varieties*. Growing from seed. *The Seed Raising Journal*, 1(2): 1.

Lavanya, S., Rosimah, N. and Faridah, Q. Z. (2012). Effects of Plant Growth Regulators on *In vitro* Regeneration of Malaysian Indica Rice (*Oryza sativa* L.) cv. MR219 by Shoot Apical Meristem. *Asian J. of Agric. Res.*, 6(4):180-187.

Lengsfeld, C., Titgemeyer, F., Faller, G. and Hensel, A. (2004). Glycoslated Compounds from Okra Inhibit Adhesion of Helicobacter pylori to Human Gastric Muscosa, *J. Agric. Food Chem*., 52(6): 1495-1503.

Lopez, J. A., Krearon, C. and Lee, A. Y. Y. (2004). Deep Venous Thrombosis. *Hematology*, 1: 439.

Lui, I. M., Liou, S. S., Lan, T. W., Hsu, F. L., Cheng, J. T (2005). Myricetin as the active principle of *Abelmoschus moschatus* to lower plasma glucosein streptozotocin-induced diabetic rat. *Planta Medica,* 71: 617-621.

Maluszynski, M., Nichterlein, K., Zanten, L. and Hloowalia, B. S. (2002). Officially released mutant varieties - the FAO/IAEA database. *Mutate Breeding Review*, 12: 1-84.

Marinova, D., Ribarova, F. and Atanassova, M. (2012). Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. *Journal of the University of Chemical Technology and Metallurgy,* 40(3): 255-260

Mauseth, J. D. (1976) Cytokinin- and Gibberilic acid induced effects on the structure and metabolism of shoot apical meristems of *Opuntia polyacantha* (Cactacea). *American Journal of Botany,* 63: 1295-1301.

Micheal A. Dirr and Charles W. Heuser (2006). Manual of Woody Plant Propagation: From Seed to Tissue Culture, Second Edition. *Timber Press*; pp. 410.

Morel, G. and Martin, G. (1952). Guerison de dahliaa attenintes d' une maladie a virus. *Comp Ren*., 235: 1324-1324.

Murashige, T. and Skoog, F. (1962). A Revised large for Rapid Growth and Bio. Assays with Tobacco Tissue Cultures. *Physiol. Plant.,* 15: 473-497.

Myoin-Jesu, E. I. (2007). Use of plant residues for improving soil fertility pod nutrients root growth and pod weight of okra (*Abelmoschus spp.). Bioresour. Tech.,* 98: 2057-2064*.*

Naczk, M., Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence extraction and analysis*. J. Pharm. Biomed. Anal.*, 41: 1523– 1542.

Nagib, A., Hossain, S. A., Alam, M. F., 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(8): 1682-3974.

Natail, L., Sanchez, I. C. and Cavallini, A. (1990). *In vitro* culture of *Aloe barbadensis* Mill: Micropropagation from vegetative meristems*. Plant Cell, Tissue and Organ Culture,* 20: 71-74*.*

Ndunguru, J., Rajabu, A. C. (2004). Effect of okra mosaic virus disease on the above-ground morphological yield components of okra in Tanzania. *Scientia Horticulturae,* 99: 225-235.

Nelson, C. H., Nieschlag, H. J., Daxenbichler, M. E. Wolff, I. A. and Perdue, R.E. Jr. (1961). A search for new fiber crops. III. Laboratory-scale pulping studies. Tappi 44: 319-325

Nerkar, Y. S., Jambhale, N. D. (1985). Transfer of resistance to yellow vein mosaic from related species into okra (*Abelmoschus esculentus* L. Moench). *Indian J. Genet*., 45: 261-270.

N'Guessan, K. P., Fargette, D., Fauguet, C., Thoughvenel, J. C. (1992): Aspects of the epidemiology of okra leaf curl virus in "Cote d' Ivoire". *Tropical Pest Manag*., 38: 122-126

Nobre, J., Davey, M. R., Lazzerri, P. A., Cannel, M. E. (2000). Transformation of barley scutellum protoplast: regeneration of fertile transgenic plants. *Plant cell Reports,* 19(10): 1000-1005.

Noormrio, M. H., Dahot, M. U., Siddiqui, H. L., Dewani, V. K. (1996) Studies on the nutritive composition of *Phyllanthus multiforus* (Kanoo) fruit. *Scci. Sindh.*, 3: 13-19.

Novak, F. J. J and Maskova, I. (1979). Apical Shoot Tip Culture of Tomato. *Scentia Hoticulturea,* 10(4): 337-344.

Odabasi, M., (2008). "Halogenated Volatile Organic Compounds from the Use of Chlorine-Bleach- Containing Household Products", *Environmental Science & Technology,* 42: 144-145.

Oggema, N. J., Kinyua, M. G. and Ouma, J. P. (2007). Optimum 2, 4-D Concentration Suitable for Embryogeneic callus induction in local keny sweet potato cultivars. *Asian Journal of Science Plant*., 6:484-489.

Pal, S. P., Alam, I., Anisuzzaman, M., Sarker, K. K., Sharmin, S. A. and Alam. M. F. (2007). Indirect Organogenesis in Summer Squash (*Cucurbit pepo* l.). *Turkey Journa of Agriculture.,* 31:63-70.

Park, K. K., Surh, Y-J., Stewart, B. C., and Miller, J. A. (1994). Chemoprotective activities of chlorophyllin: Inhibition of mutagenicity and covalent binding of various ultimate carcinogens. Proc. *Am. Assoc. Cancer Res*., 35: 139.

Pandey, M., Abidi, A. B., Singh, S. and Singh, R. P. (2006). Nutritional evaluation of leafy vegetable paratha. *Journal of Human Ecology,* 19: 155-156.

Pant, B & Thapa, D. (2012). *In vitro* mass propagation of an epiphytic orchid, *Dendrobium primulinum* Lindl. Through shoot tip culture. *African Journal of Biotechnology*, 11(42): 1684-5315.

Pearson, D. (1970). The Chemical Analysis of Foods, 6th ed. J. & A. Churchil Living stone, London. pp. 23.

Pierik, R. L. M. and Steegmans H. H. M. (1986). Adventitious plantlet regeneration from floral stem explants of Nerine bowdenii W. Watts. *Netherland Journal of Agricultural Sci*ence, 34: 217.

Pink, D. A. C and Carter, P. J. (1987). Propagation of lettuce (*Lactuca sativa*) breeding material by tissue culture. *Annals of Applied biology,* 110(3): 611-616.

Rahman, M. T., Hossain, M. J., and Khalekuzzaman, M. (2008). *In vitro* Plantlet Regeneration from Hypocotyl segments and Cotyledonary Explant Derived Calli in Lady's Finger (*Abelmoschus esculentus* L. Monech). *Journal of Biological Sciences,* 16: 49-57.

Rao, P. U. (1985). Chemical composition and biological evaluation of Okra (*Hibiscus esculentus*) seeds and their kernels, *Plant foods for Human Nutrition (Formerly Qualitas Plantarum)*, 35(4): 389-396

Ratnadass, A., Salha, H. Maazou, A., Zakari-Moussa, O., Kadi, H., Kano, H., Ryckewaert, P., Doumma, A. (2010). Gestion agroecologique des insects ravageurs des cultures vivrieres et horticoles au Niger. In: *Proc. 18th Conference of the the African Assocition of Insect Scientists* (16-20 Nov., 2009), Oguagadougou, Burkina Faso.

Rattanpal, H. S., Gill, M. I. S., Sangwan, A. K. (2011). Micropropagation of strawberry through meristem culture. *International Society for Horticultural Science*, 890: 149-154.

Renu, S. and Anwar, S. (2008). Thidiazuran (TDZ) Induced Regeneration from Cotyledonary Node Explant of *Abelmoschus moschatus* Medik. L., (A Valuable Medicinal Plant) *World Journal of Agricultural Science,* 4(4): 449-452.

Roy, M. K. (1989). M. Sc. Thesis, Tissue culture and Plant Regeneration of Okra (Abelmoschus esculentus L.). Concordia University Montreal Quebec, Canada.

Schaeffer, G. W. and Smith, H. H. (1962). Auxin-Cytokinin Interaction in Tissue cultures of Nicotiana Species of Turmor Conditioned Hybrids. *Plant Physiology,* 38(3): 291-297.

Schalau,J.(2002).BackyardGardener.AvailableatHttp://ag.arizona.edu./yavapai/anr/hort/byg/2000.html.

Shani, E., Yanai, O. and Ori, N. (2006): The role of hormones in shoot apical meristem function. *Current Opinion in Plant Biology,* 9:484-489.

Siemonsma, J. S and Kouame, C. (2004) *Abelmoschus esculentus*. In Plant Resourses of Tropical Africa 2 Vegetable. Editors Grubben G.J.H and Denton O.A Published by PROTA foundation Netherlands. pp 21-29.

Siemonsmo, J. S. (1982). West African okra morphological and cytological indications for the existence of a natural amphiploid of *Abelmoschus esculentus* (L) Moench and *A. manihot* (L). *Medikus Euphytica,* 31: 241-252.

Skoog, F. and Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissue grown *in vitro*. *Symp. Soc. Exp. Biol.,* 11: 118.

Staba, E. J. (1982). Production of useful compounds from plant tissue cultures. In Plant Tissue Culture 1982, (ed). Fujiwara, Tokyo: Japanse Association of Plant Tissue culture. pp.25-26.

Stickle, M. B. and Obray H.F. (2005). Shoot apical meristem: A sustainable explant for genetic transformation of cereal crops*. In vitro* Cell. *Dev. Biol. Plant*, 41: 187-200.

Tindal, H. D. (1983): Vegetables in the tropics. Macmillan Education Limited, London, UK. pp. 533.

Uda, Y., Price, K. R., Williamson, G. and Rhodes, M. J. C. (1997). Induction of the anti-carcinogenic marker enzyme, quinone reductase, in murine hepatoma cells *in vitro* by flavonoids. *Cancer Lett.,* 120: 213-216*.*

Udengwu, O. S. (2009). Studies on Heterosis in *Abelmoschus esculentus* (L.) Moench *A. callei* and *A. chev*. Stevels Cultivars During Short Day Photoperiods in Eastern Nigeria, Pakistan. *Journal of Biological Sciences,* 12: 1388-1398.

Valizadeh, M., Tabar, S. K. K. and Nematzadeh, G. A. (2007). Effects of plant growth regulators on callus induction and regeneration of cumin *(Cuminum cyminum). Asian J. Agric. Res.,* 1-17-22.

Varmudy, V. (2011). Marking survey need to boost okra exports. Department of economics, Vivekananda College, Puttur, Karnataka, India. pp. 21-23

Vasil, I. K. (1991). Scale-up and Automation in Plant Propagation. Academic Press Inc., San Diego. pp. 267.

Vassade, M. Sengkhamparn, N., Verhoef, R., Delaigue, C., Oundiam, O., Vigneron, P., Voragen, A. G. J., Schols, H. A. and Nagel, M.D. (2010). Antiproliferative and

proapoptotic actions of okra pectin on B16F10 melanoma cells. *Phytotherapy Research,* 24(7): 982-989.

Waladkhani, A. R. and Clemens, M. R. (1998). Effect of dietary phytochemicals on cancer development (review). *Int. J. Mol. Med.,* 1(4):747-53.

Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y. and Tsao, R. (2011). Synergistic, Additive and Antagnostic Effects of Food Mixtures on Total Antioxidant Capacities. *J. of Agric and Food Chem.,* 59(3): 960-968.

Wang, P. J. and Hu, N. Y. (1980). Regeneration of virus-free plants through in vitro culture. In Fietcher, A. (Ed.) Advances in Biochemical Engineering: Plant Cell Culture. II. Springer, Berlin, pp. 61-99.

Weerasekar, D. (2006). Genetical analysis of yield and quality parameters in okra (*Abelmoschus esculentus* (L) Moench). Master thesis, University of Agricultural Sciences. GKVK, Bangalore. pp. 87

Welader, N. T. (1987). Propagation of *Syringa chinensis* cv Saugeana by *in vitro* culture of nodal explants. *J. Hort. Sci*., 62: 89.

Werner, T., Motyka, V., Stranad, M., & Schmulling, T. (2012). Regulation of plant growth by cytokinin. *Procedures in National Academic Science USA,* 98:10487-10492.

Wildi, E., Schaffiner, W., and Buter, B. K. (1998). Cell growth and flavonoids production in suspension culture of *Saussurea medusa. Act Bot. Sinm*., 40: 836-841.

Woolfe, M. L., M. F. Chaplin, and Otchere, G. (1977). Studies on the mucilage extract from okra fruits *Hibiscus esculentus* and baobab leaves *Adansonia digitata*. *Journal of Agricultural Food Sciences,* 28: 519-529.

Youm, O. S.S ., Vaissayre, M., Nibouche, S., Martin, t., Ochou, G. O., Monmanyi, G. (2005). Bio-ecology and management of Helicoverpa for sustainable crop production in Africa. In: Heliothis/Helicoverpa Management, Emerging Trends and Strategies for future Research,, *Sharma*, C. (ed.)., *Oxford and IBH Publishing Co. Pvt. Ld., New Delhi, India*. pp. 63-90.

Zehnder, M. (2002). "Tissue dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions". *Oral Surg Oral Med Oral Pathol Oral Radio Endodon,* 94 (6): 756–62.

Zhang, S., Zhong, H., Sticklen, M. B. (1996). Production of multiple shoots from shoot apical meristems of oat (*Avena sativa* L.). *Journal of Plant Physiology,* 148: 667- 671.

Zhenzhong, X. and Hongjun, S. (2010). Effects of okra capsule combined with valsartan in treatment of early diabetic nephropathy with microalbuminuria, *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 97(3): 447 – 452.

Zhishen, J. Mengcheng, T. and Jianming, W. (1999). The determination of flavonoids contents in mulberry and their scavenging effects and superoxide radicals. *J. Food Chemistry*, 64: 555 – 559.