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OPTIMIZATION OF VEGETATIVE AND IN VITRO PROPAGATION FOR GIANT REED (ARUNDO DONAX L.)

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OPTIMIZATION OF VEGETATIVE AND *IN VITRO* PROPAGATION FOR GIANT REED (*ARUNDO DONAX* L.)

By

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

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

OPTIMIZATION OF VEGETATIVE AND *IN VITRO* PROPAGATION FOR GIANT REED (*ARUNDO DONAX* L.)

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June 2019

Chair : Franklin Ragai Kundat, PhD
Faculty : Agricultural and Food Science (Bintulu)

Arundo donax L. or also known as giant cane or giant reed is well known as the source of musical reeds and industrial cellulose. It is potential for phytoextraction of arsenic from synthetic wastewater, and also considered as a promising energy crop due to its high biomass production. It yields three times as much ethanol per acre as corn therefore qualifies it as a cellulosic renewable fuel. This sustainable *energy crop* for second generation ethanol is also found in Malaysia, but was never known as native nor invasive. The fact that it can grow in Malaysia into full flowering stage brings possibilities that it can be cultivated for biofuel purposes. *A. donax L.* has been cultivated for biofuel in countries such as Italy but never in Malaysia. Prior to cultivation, their agronomic practices are required. A study was conducted to determine suitable techniques in producing seedlings of *A. donax L.* for its cultivation. Two methods were approached, vegetative propagation and *in vitro* propagation. For vegetative propagation, the effects of root hormone, Sadex ® and IBA, at concentration of 0-5 mg L^{-1} Sadex ®, 0-5 g L^{-1} Sadex ® and 0-5 mg L^{-1} IBA respectively were studied on soft and hard wood cuttings. When using hardwood cuttings, cutting should firstly be treated with 1g L^{-1} Sadex ® for root induction then followed by 4g L^{-1} Sadex ® for elongation. When using softwood cuttings, 1g L^{-1} Sadex ® was the optimum concentration. For *in vitro* propagation, the effects of BAP, IBA, and NAA for multiple shoots induction of *A. donax L.* were studied. The optimum BAP concentration for shoot regeneration of *A. donax L.* was 0.2 mg L^{-1} and optimum IBA was 0.4 mg L^{-1}. Both concentrations resulted with an average of 1.8 shoot per explants. Callus was induced on explant cultured in MS media supplemented 5mg L^{-1} NAA. This study suggest that vegetative propagation was more economical for seedlings production of *Arundo donax* L. as the time for shoot regeneration and the number of shoots was similar to those produced *in vitro*. However, *in vitro* cultures provide better techniques for mass production of seedlings through

indirect callus regenerations. The outcomes of this study were suitable and could be applied for the production of this high potential energy crop.

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

PROPAGASI OPTIMISASI TEBU GERGASI (*ARUNDO DONAX* L.) SECARA VEGETATIF DAN *IN VITRO*

Oleh

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Arundo donax L. atau juga dikenali sebagai rotan atau raksasa gergasi terkenal sebagai sumber buluh muzik dan selulosa industri. Ia berpotensi untuk fitopengekstakan arsenik daripada air buangan sintetik, dan juga dianggap sebagai tanaman tenaga yang menjanjikan kerana pengeluaran biojisim yang tinggi . Ia menghasilkan tiga kali lebih banyak etanol per ekar daripada jagung, yang mana memenuhi syarat sebagai bahan bakar selulosa boleh diperbaharui. Tanaman tenaga mampan untuk etanol generasi kedua ini juga dijumpai di Malaysia, tetapi tidak pernah dikenali sebagai tumbuhan asli atau invasif. Fakta bahawa ia boleh tumbuh di Malaysia ke peringkat penuh bunga membawa kemungkinan bahawa ia boleh ditanam untuk tujuan biofuel. *A. donax L.* Telah ditanam untuk biofuel di negara-negara seperti Itali tetapi tidak pernah di Malaysia. Sebelum penanaman, amalan agronomi mereka diperlukan. Satu kajian telah dijalankan untuk menentukan teknik yang sesuai dalam menghasilkan anak benih *A. donax L.* untuk penanamannya. Dua kaedah telah didekati, penyebaran vegetatif dan propagasi *in vitro*. Untuk penyebaran vegetatif, kesan hormon akar, Sadex ® dan IBA, pada kepekatan 0-5 mg L^{-1} Sadex ®, 0-5 g L^{-1} Sadex® dan 0-5 mg L^{-1} IBA masing-masing dikaji atas keratan kayu lembut dan keras. Apabila menggunakan keratan kayu keras, keratan harus terlebih dahulu dirawat dengan 1g L^{-1} Sadex ® untuk induksi akar kemudian diikuti dengan 4g L^{-1} Sadex ® untuk pemanjangan. Apabila menggunakan keratan kayu lembut, 1g L^{-1} Sadex ® adalah kepekatan optimum. Untuk propagasi *in vitro*, kesan BAP, IBA dan NAA untuk induksi pucuk *A. donax L.* telah ditelidi. Kepekatan BAP optimum untuk penanaman *A. donax* ialah 0.2 mg L^{-1} dan IBA optimum adalah 0.4 mg L^{-1}. Kedua-dua kepekatan ini menghasilkan purata 1.8 pucuk setiap explan. Callus telah diinduksi pada media mengandungi MS media yang ditambah dengan 5mg L^{-1} NAA. Kajian ini mencadangkan bahawa penyebaran vegetatif lebih ekonomik untuk pengeluaran benih *Arundo donax* L. sebab masa dan jumlah pucuk adalah serupa dengan yang dihasilkan secara *in vitro*. Walau bagaimanapun,

propagasi *in vitro* memberikan teknik-teknik yang lebih baik untuk pengeluaran besar-besaran benih melalui regenerasi kalus. Hasil kajian ini sesuai digunakan untuk pengeluaran tanaman tenaga berpotensi tinggi ini.

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This thesis was 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:

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LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
UPMKB	Universiti Putra Malaysia Kampus Bintulu
PGR	Plant Growth Regulator
BAP	6-bezylaminopurine
IBA	Indole Butyric acid
NAA	1-naphtaleneacetic acid
IAA	indole-3-acetic acid
2,4-D	dichlorophenoxyacetic acid
MS	Murashige and Skoog
TDZ	Thidiazuron

CHAPTER 1

INTRODUCTION

The current energy infrastructure of the world remained highly dependent on fossil fuels. However, human activities related to burning of fossil fuels are the main contributor to greenhouse gas emissions and the change to global climate (McMichael et al., 2004). Burning of fossil fuels is active in agriculture, heavy industry and transportation triggered by human population boom. It was expected that the depletion of fossil fuel will occur over the years and therefore, strategies were developed to replace fossil fuel with others sustainable sources. The current scenario is also one of the major concerns in Malaysia, as Malaysia is one of the oil producing countries. According to Muda and Tey (2012), the rate of petroleum production has reached the maximum level in 2004 and started to decline since that time and predicted that petroleum will be spent over in 2020, followed by natural gas in 2058 and coal around the year 2066.

The concern of depletion in fossil fuels and their impact on climate since then has propel new strategies to find alternative renewable fuel and energy from different sources such as biomass. Crops such as maize, soybean and oil palm have oil extract that can be converted to biofuel or biodiesel. However, with strict policies governing food crops or forage in related to the world food crisis, the cultivation of these plants are restricted as food crops. Therefore, other plant species are sought after for biodiesel production such as *Jatropha curcas* (Pandey et al., 2012). Another source known for its biomass for biofuel is *Arundo donax* (Corno, Pilu and Adani, 2014).

Arundo donax or also known as giant cane or giant reed is native to Mediterranean Basin and middle-east Asia. It is deride for being aggressive invasive species, has the ability to reproduce quickly, allowing it to out-compete native plant species and efficient in absorbing water establishing itself as one of the primary threats to native riparian habitats (Coffman, Ambrose and Rundell, 2010). However, *A. donax* yields three times as much ethanol per acre as corn therefore qualifies it as a cellulosic renewable fuel. It is found in Malaysia, but was never known as native nor invasive. The fact that it can grow in Malaysia into full flowering stage brings possibilities that it can be cultivated for biofuel purposes. *A. donax* has been cultivated for biofuel in countries such as Italy but never in Malaysia. Prior to cultivation, their agronomic practices are required.

A. donax developed complete panicle type inflorescence but flower are sterile. Their most effective mode of reproduction is through underground rhizomes which enable them to spread rapidly (Mariani et al., 2010). However, without fertile flowers, viable seeds are not available for seedlings production.

A. donax was reported to be successfully propagated through *in vitro* (Takahashi et al., 2010, Cavallaro et al., 2014, Gubisova et al., 2016) and shoot cutting (Ceotto and Candillo, 2010). However, there are no reports on the vegetative propagation for *A. donax* in Malaysia as it was never cultivated. Therefore, a study was conducted to determine suitable techniques in producing seedlings of *A. donax* for its cultivation. Two approaches were used, *ex vivo* vegetative using stem cuttings and *in vitro* propagation. The optimization of plant growth hormone was carried out in both approaches. This study was conducted to test a hypothesis that seedling growths produced from vegetative propagules are affected by different type and concentration of growth hormones.Vegetative or asexual propagations are used for producing seedlings that are true to type. Clones tend to be genetically similar hence possessed the same vigorousity and yield. Poaceae culm is hollow and divided into visible nodes and internodes. The active meristematic cells in the pro-cambium region in the nodal area are responsible for secondary growth and also differentiation of side shoots (Rudall, 2007).

The number of shoots developing from the node relies on the mobilization of indigenous hormones and the cell totipotency in this region (Wareing and Philips, 1981). To promote in root development, nursery used plant growth hormones (PGR) such as the commercial Seradix® and Sadex®, applied by brushing the tip of stem with PGR. To ensure mass propagation in seedlings production, the node region is required to develop as many shoots as possible. Alternatively, mass propagation can be achieved through micropropagation. Although micropropagation are more costly and requires trained and skill staff, this technique are usually used when it is difficult to obtain seed, having non-viable seeds, vegetative propagation are slow, low percentage in seed and shoot germination *ex vivo* and when seedlings are required in mass number in a short period of time. Because there is no report on the propagation of *Arundo donax* in the Malaysian environment, this study proposed to test both techniques. In both techniques, different type of PGR was compared and optimized.

The objectives of this study were:

(1) to optimized concentration of root hormone for root development on *Arundo donax* stem cuttings and,

(2) to optimized concentration of BAP, IBA, and NAA for *Arundo donax in vitro* cultures.

REFERENCES

Agusti, J., Herold, S., Schwarz, M., Sanchez, P., Ljung, K., Dun, E. A., ... & Greb, T. (2011). Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. *Proceedings of the National Academy of Sciences*, *108*(50), 20242-20247.

Angelini, L. G., Ceccarini, L., & Bonari, E. (2005). Biomass yield and energy balance of giant reed (Arundo donax L.) cropped in central Italy as related to different management practices. *European Journal of Agronomy*, *22*(4), 375-389.

Antal, G., Kurucz, E., Fári, M. G., & Popp, J. (2014). Tissue culture and agamic propagation of winter-frost tolerant 'Longicaulis' Arundo donax L. *Environmental Engineering and Management Journal*, *13*(11), 2709-2715.

Atkinson, C. J. (2009). Establishing perennial grass energy crops in the UK: A review of current propagation options for Miscanthus. *Biomass and bioenergy*, *33*(5), 752-759.

Babaei, N., Abdullah, N. A. P., Saleh, G., & Abdullah, T. L. (2013). Control of contamination and explant browning in Curculigo latifolia in vitro cultures. *Journal of Medicinal Plants Research*, *7*(8), 448-454.

Balat, M., & Balat, H. (2010). Progress in biodiesel processing. *Applied energy*, *87*(6), 1815-1835.

Bell, G. P. (1997). Ecology and management of Arundo donax and approaches to riparian habitat restoration in southern California.

Bourne, J. K., & Clark, R. (2007). Biofuels: Boon or Boondoggle? Producing fuel from corn and other crops could be good for the planet-if only the process didn't take a significant environmental toll. New breakthroughs could make a difference. *National Geographic*, *212*(4), 38.

Braatne, J. H., Rood, S. B., & Heilman, P. E. (1996). Life history, ecology, and conservation of riparian cottonwoods in North America. *Biology of Populus and its Implications for Management and Conservation*, (Part I), 57-85.

Bukowska, B. (2006). Toxicity of 2, 4-Dichlorophenoxyacetic Acid--Molecular Mechanisms. *Polish Journal of Environmental Studies*, *15*(3).

Carlquist, S., & Schneider, E. L. (2011). Origins and Nature of Vessels in Monocotyledons. 13. Scanning Electron Microscopy Studies of Xylem in Large Grasses. *International Journal of Plant Sciences*, *172*(3), 345-351.

Cavallaro, A., Mierczynska, A., Barton, M., Majewski, P., & Vasilev, K. (2016). Influence of immobilized quaternary ammonium group surface density on antimicrobial efficacy and cytotoxicity. *Biofouling*, *32*(1), 13-24.

Cavallaro, V., Patanè, C., Cosentino, S. L., Di Silvestro, I., & Copani, V. (2014). Optimizing in vitro large scale production of giant reed (Arundo donax L.) by liquid medium culture. *Biomass and Bioenergy*, *69*, 21-27.

Cavallaro, V., Tringali, S., & PatanÈ, C. (2011). Large-scale in vitro propagation of giant reed (Arundo donax L.), a promising biomass species. *The Journal of Horticultural Science and Biotechnology*, *86*(5), 452-456.

Ceotto, E., & Di Candilo, M. (2010). Shoot cuttings propagation of giant reed (Arundo donax L.) in water and moist soil: The path forward?. *Biomass and Bioenergy*, *34*(11), 1614-1623.

Coffman, G. C., Ambrose, R. F., & Rundel, P. W. (2010). Wildfire promotes dominance of invasive giant reed (Arundo donax) in riparian ecosystems. *Biological invasions*, *12*(8), 2723-2734.

Corno, L., Pilu, R., & Adani, F. (2014). Arundo donax L.: a non-food crop for bioenergy and bio-compound production. *Biotechnology advances*, *32*(8), 1535-1549.

da Costa Sousa, L., Chundawat, S. P., Balan, V., & Dale, B. E. (2009). 'Cradle-to-grave'assessment of existing lignocellulose pretreatment technologies. *Current opinion in biotechnology*, *20*(3), 339-347.

D'Antonio, C. M., & Vitousek, P. M. (1992). Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual review of ecology and systematics*, *23*(1), 63-87.

Daud, N. H., Jayaraman, S., & Mohamed, R. (2012). Methods Paper: An improved surface sterilization technique for introducing leaf, nodal and seed explants of Aquilaria malaccensis from field sources into tissue culture. *Aspac J Mol Biol Biotechnol*, *20*, 55-58.

Davies, P. J. (2010). The plant hormones: their nature, occurrence, and functions. In *Plant hormones* (pp. 1-15). Springer, Dordrecht.

Denchev, P. D., & Conger, B. V. (1995). In vitro culture of switchgrass: influence of 2, 4-D and picloram in combination with benzyladenine on callus initiation and regeneration. *Plant cell, tissue and organ culture*, *40*(1), 43-48.

Dragoni, F., Ragaglini, G., Corneli, E., o Di Nasso, N. N., Tozzini, C., Cattani, S., & Bonari, E. (2015). Giant reed (Arundo donax L.) for biogas production: land use saving and nitrogen utilisation efficiency compared with arable crops. *Italian Journal of Agronomy*, *10*(4), 192-201.

Dudley, T. (1998). Exotic plant invasions in California riparian areas and wetlands. *Fremontia*, *26*(4), 24-29.

Dudley, T. L. (2000). Arundo donax L. *Invasive plants of California's wildlands*, 53-58.

El-Bakry, A. A., & Abdel-Salam, A. M. (2012). Regeneration from embryogenic callus and suspension cultures of the wild medicinal plant Cymbopogon schoenanthus. *African Journal of Biotechnology*, *11*(43), 10098-10107.

Evert, R. F. (2006). *Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development*. John Wiley & Sons.

Ezekiel, A. (2010). Low Cost Vegetative Propagation of Tropical Trees. *International Journal of Botany*, *6*(2), 187-193.

Fahn, A. (1967). *Plant anatomy*. Pergamon Press New York.

Fay, M. F. (1992). Conservation of rare and endangered plants using in vitro methods. *In Vitro Cellular & Developmental Biology-Plant*, *28*(1), 1-4.

Fiore, V., Scalici, T., & Valenza, A. (2014). Characterization of a new natural fiber from Arundo donax L. as potential reinforcement of polymer composites. *Carbohydrate polymers*, *106*, 77-83.

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., & Thorpe, T. A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cellular & Developmental Biology-Plant*, *32*(4), 272-289.

Gehlot, A., Gupta, R. K., Tripathi, A., Arya, I. D., & Arya, S. (2014). Vegetative propagation of Azadirachta indica: effect of auxin and rooting media on adventitious root induction in mini-cuttings. *Advances in Forestry Science*, *1*(1), 1-9.

George, E. F., Hall, M. A., & De Klerk, G. J. (2008a). Plant tissue culture procedure-background. In *Plant propagation by tissue culture* (pp. 1-28). Springer, Dordrecht.

George, E. F., Hall, M. A., & De Klerk, G. J. (2008b). Plant growth regulators I: introduction; auxins, their analogues and inhibitors. In *Plant propagation by tissue culture* (pp. 175-204). Springer, Dordrecht.

Gubišová, M., Čičková, M., Klčová, L., & Gubiš, J. (2016). In vitro tillering—An effective way to multiply high-biomass plant Arundo donax. *Industrial Crops and Products*, *81*, 123-128.

Gucel, S. (2010). Arundo donax L.(Giant reed) Use by Turkish Cypriot. *Ethnobotany Research and Applications*, *8*, 245-248.

Hardion, L., Verlaque, R., Saltonstall, K., Leriche, A., & Vila, B. (2014). Origin of the invasive *Arundo donax* (Poaceae): A Trans-Asian Expedition in Herbaria. *Annals of Botany*, *114*(3), 455-462.

Hartmann, H. T., & Kester, D. E. (1963). Plant propagation: principles and practice. *Soil Science*, *95*(1), 89.

Herrera-Alamillo, M. Á., & Robert, M. L. (2012). Liquid *in Vitro* Culture for the Propagation of Arundo donax. In *Plant Cell Culture Protocols* (pp. 153-160). Humana Press, Totowa, NJ.

Huetteman, C. A., & Preece, J. E. (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant cell, Tissue and Organ Culture*, *33*(2), 105-119.

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The Plant Cell*, tpc-113.

Imadi, S. R., & Kazi, A. G. (2015). Extraction of lignin from biomass for biofuel production. In *Agricultural Biomass Based Potential Materials* (pp. 391-402). Springer International Publishing.

Jan, A., Bhat, K. M., Bhat, S. J. A., Mir, M. A., Bhat, M. A., Imtiyaz, A., & Rather, J. A. (2013). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture. *African Journal of Biotechnology*, *12*(39).

Kebrom, T. H., Spielmeyer, W., & Finnegan, E. J. (2013). Grasses provide new insights into regulation of shoot branching. *Trends in Plant Science*, *18*(1), 41-48.

Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, *12*(3), 307-310.

Kyozuka, J. (2007). Control of shoot and root meristem function by cytokinin. *Current Opinion in Plant Biology*, *10*(5), 442-446.

Lansdown, R.V. 2013. *Arundo donax*. The IUCN Red List of Threatened Species 2013: e.T164340A1043245. http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T164340A1043245.en. Downloaded on **16 April 2018**.

Lemons e Silva, C. F., Artigas Schirmer, M., Nobuyuki Maeda, R., Araújo Barcelos, C., & Pereira, N. (2015). Potential of giant reed (*Arundo donax L.*) for second generation ethanol production. *Electronic Journal of Biotechnology*, *18*(1), 10-15.

Lewandowski, I., Scurlock, J. M., Lindvall, E., & Christou, M. (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, *25*(4), 335-361.

Mabizela, G. S., Slabbert, M. M., & Bester, C. (2017). The effect of rooting media, plant growth regulators and clone on rooting potential of honeybush (Cyclopia subternata) stem cuttings at different planting dates. *South African Journal of Botany*, *110*, 75-79.

Mariani, C., Cabrini, R., Danin, A., Piffanelli, P., Fricano, A., Gomarasca, S., ... & Soave, C. (2010). Origin, diffusion and reproduction of the giant reed (Arundo donax L.): a promising weedy energy crop. *Annals of Applied Biology*, *157*(2), 191-202.

McMichael, A. J., Campbell-Lendrum, D., Kovats, S., Edwards, S., Wilkinson, P., Wilson, T., ... & Schlesinger, M. (2004). Global climate change.

Mirza, N., Mahmood, Q., Pervez, A., Ahmad, R., Farooq, R., Shah, M. M., & Azim, M. R. (2010). Phytoremediation potential of Arundo donax in arsenic-contaminated synthetic wastewater. *Bioresource Technology*, *101*(15), 5815-5819.

Mishra, R., & Rao, G. J. N. (2016). In-vitro androgenesis in rice: advantages, constraints and future prospects. *Rice Science*, *23*(2), 57-68.

Monteiro, A., Teixeira, G., & Moreira, J. F. (2015). Relationships between leaf anatomical features of Arundo donax and glyphosate efficacy. *Revista de Ciências Agrárias*.

Muda, N., & Pin, T. J. (2012). On prediction of depreciation time of fossil fuel in Malaysia. *J Math Stat*, *8*, 136-143.

Nackley, L. L., Vogt, K. A., & Kim, S. H. (2014). Arundo donax water use and photosynthetic responses to drought and elevated CO2. *Agricultural water management*, *136*, 13-22.

Naqvi, M., & Yan, J. (2015). *First‐Generation Biofuels*. John Wiley & Sons, Ltd.

o Di Nasso, N. N., Bosco, S., Di Bene, C., Coli, A., Mazzoncini, M., & Bonari, E. (2011). Energy efficiency in long-term Mediterranean cropping systems with different management intensities. *Energy*, *36*(4), 1924-1930.

Pandey, V. C., Singh, K., Singh, J. S., Kumar, A., Singh, B., & Singh, R. P. (2012). Jatropha curcas: A potential biofuel plant for sustainable environmental development. *Renewable and Sustainable Energy Reviews*, *16*(5), 2870-2883.

Papazoglou, E. G., Karantounias, G. A., Vemmos, S. N., & Bouranis, D. L. (2005). Photosynthesis and growth responses of giant reed (Arundo donax L.) to the heavy metals Cd and Ni. *Environment International*, *31*(2), 243-249.

Perdue, R. E. (1958). Arundo donax—source of musical reeds and industrial cellulose. *Economic Botany*, *12*(4), 368-404.

Pierik, R. L. M. (1988). *In vitro* culture of higher plants. Bibliography.

Pilu, R., Manca, A., Landoni, M., Barrière, Y., & Motto, M. (2013). Arundo donax as an energy crop: pros and cons of the utilization of this perennial plant. *Maydica*, *58*(1), 54-59.

Ram, Arun T & M.K., Shareef & K, Prasool. (2014). Development of a simple, reproducible and efficient *in vitro* culture protocol for callus proliferation under varying concentrations of 6-benzylaminopurine (BAP) via leaf and nodal segments. *International Journal of Current Biotechnology*. 2. 1-4.

Ramgareeb, S., Watt, M. P., & Cooke, A. J. (2001). Micropropagation of Cynodon dactylon from leaf and nodal segments. *South African journal of botany*, *67*(2), 250-257.

Rudall, P. J. (2007). *Anatomy of flowering plants: an introduction to structure and development*. Cambridge University Press.

Sassi, M., & Vernoux, T. (2013). Auxin and self-organization at the shoot apical meristem. *Journal of experimental botany*, *64*(9), 2579-2592.

Seca, A. M., Cavaleiro, J. A., Domingues, F. M., Silvestre, A. J., Evtuguin, D., & Neto, C. P. (2000). Structural characterization of the lignin from the nodes and internodes of Arundo donax reed. *Journal of agricultural and food chemistry*, *48*(3), 817-824.

Sharber, C., & County, C. (1957). Plant Propagation.

Shatalov, A. A., & Pereira, H. (2002). Influence of stem morphology on pulp and paper properties of Arundo donax L. reed. *Industrial Crops and Products*, *15*(1), 77-83.

Shatalov, A. A., Quilho, T., & Pereira, H. (2001). Arundo donax L. reed: New perspectives for pulping and bleaching: I. Raw material characterization. *Tappi Journal*, *84*(1).

Singh A. (2015) Micropropagation of Plants. In: Bahadur B., Venkat Rajam M., Sahijram L., Krishnamurthy K. (eds) Plant Biology and Biotechnology. Springer, New Delhi

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured. In *Vitro, Symp. Soc. Exp. Biol* (No. 11).

Su, Y. H., Liu, Y. B., & Zhang, X. S. (2011). Auxin–cytokinin interaction regulates meristem development. *Molecular plant*, *4*(4), 616-625.

Sugimoto, K., & Meyerowitz, E. M. (2013). Regeneration in Arabidopsis tissue culture. In *Plant Organogenesis* (pp. 265-275). Humana Press, Totowa, NJ.

Sun, T. P. (2010). Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. *Plant physiology*, *154*(2), 567-570.

Takahashi, W., Takamizo, T., Kobayashi, M., & Ebina, M. (2010). Plant regeneration from calli in giant reed (Arundo donax L.). *Grassland science*, *56*(4), 224-229.

Tan, K. T., Lee, K. T., & Mohamed, A. R. (2008). Role of energy policy in renewable energy accomplishment: the case of second-generation bioethanol. *Energy policy*, *36*(9), 3360-3365.

Triana, F., Nassi o Di Nasso, N., Ragaglini, G., Roncucci, N., & Bonari, E. (2015). Evapotranspiration, crop coefficient and water use efficiency of giant reed (Arundo donax L.) and miscanthus (Miscanthus× giganteus Greef et Deu.) in a Mediterranean environment. *Gcb Bioenergy*, *7*(4), 811-819.

Tye, Y. Y., Lee, K. T., Abdullah, W. N. W., & Leh, C. P. (2011). Second-generation bioethanol as a sustainable energy source in Malaysia transportation sector: status, potential and future prospects. *Renewable and Sustainable Energy Reviews*, *15*(9), 4521-4536.

Verdeil, J. L., Alemanno, L., Niemenak, N., & Tranbarger, T. J. (2007). Pluripotent versus totipotent plant stem cells: dependence versus autonomy?. *Trends in plant science*, *12*(6), 245-252.

Vernoux, T., Besnard, F., & Traas, J. (2010). Auxin at the shoot apical meristem. *Cold Spring Harbor Perspectives in Biology*, *2*(4), a001487.

Ververis, C., Georghiou, K., Christodoulakis, N., Santas, P., & Santas, R. (2004). Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production. *Industrial crops and products*, *19*(3), 245-254.

Vysotskaya, L. B., Timergalina, L. N., Simonyan, M. V., Veselov, S. Y., & Kudoyarova, G. R. (2001). Growth rate, IAA and cytokinin content of wheat seedling after root pruning. *Plant Growth Regulation*, *33*(1), 51-57.

Wareing, P. F., & Phillips, I. D. J. (1981). *Growth and differentiation in plants* (Vol. 3). Oxford: Pergamon Press.

Watts, D. A., & Moore, G. W. (2011). Water-use dynamics of an invasive reed, Arundo donax, from leaf to stand. *Wetlands*, *31*(4), 725-734.

Welker, C. M., Balasubramanian, V. K., Petti, C., Rai, K. M., DeBolt, S., & Mendu, V. (2015). Engineering plant biomass lignin content and composition for biofuels and bioproducts. *Energies*, *8*(8), 7654-7676.

Williams, C. M. J., Biswas, T. K., Black, I. D., Marton, L., Czako, M., Harris, P. L., ... & Virtue, J. G. (2008, March). Use of poor quality water to produce high biomass yields of giant reed (Arundo donax L.) on marginal lands for biofuel or pulp/paper. In *International Symposium on Underutilized Plants for Food Security, Nutrition, Income and Sustainable Development 806* (pp. 595-602).

Xiao, Y., Tang, J., Qing, H., Zhou, C., Kong, W., & An, S. (2011). Trade-offs among growth, clonal, and sexual reproduction in an invasive plant Spartina alterniflora responding to inundation and clonal integration. *Hydrobiologia*, *658*(1), 353-363.

Yang, Y. Y., & Kim, J. G. (2016). The optimal balance between sexual and asexual reproduction in variable environments: a systematic review. *Journal of Ecology and Environment*, *40*(1), 12.