



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF PHLOROGLUCINOL AND RESORCINOL ON *IN VITRO*  
VEGETATIVE GROWTH ENHANCEMENT AND LETHAL BROWNING  
REDUCTION IN LANG BANANA**

**NURHANA NADIA RAMLAN**

**FBSB 2019 12**



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REDUCTION IN LANG BANANA**

**By**

**NURHANA NADIA BINTI RAMLAN**

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

**May 2019**

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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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**May 2019**

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Browning is one of the major problems in banana tissue culture industry. The formation of browning is due to the formation of dark exudates around explants, in which it can inhibit explant proliferation. Phenolic compounds such as phloroglucinol (PG) and resorcinol are known to have an ability to reduce browning in food and cosmetic products. Hence, both phenolics were used in this study to determine their capability to reduce browning and at the same time to enhance growth of *in vitro* Lang banana. The total soluble protein content, gene expression level of antioxidant (*catalase (CAT)*, *ascorbate peroxidase (APX)* and *peroxidase (POD)*), browning-inducing (*phenylalanine ammonia lyase (PAL)* and *polyphenol oxidase (PPO)*) and lignin-biosynthetic (*MYB31*, *MYB58*, *cinnamoyl coA reductase (CCR)*, *catechol-O-methyltransferase (COMT)*, *cinnamyl aldehyde dehydrogenase (CAD)* and *p-coumarate hydroxylase (C3H)*) genes as well as their enzymes activities were also observed on the early stage of browning of Lang banana explants grown on selected media. To achieve the objectives, Murashige and Skoog (MS) basal media supplemented with B5 vitamin, different concentrations of phenolics (PG or resorcinol), different concentrations of cytokinins (6-benzylaminopurine (BAP) or kinetin) and auxin (*α*-naphthalene acid (NAA)) were used. The growth of each explant was recorded by determining the number of shoots, number of buds and number of roots every week for 12 weeks. The visual degree of browning was also recorded. The highest percentage of explants belong to mild browning was observed from media containing NAA (0 to 0.013 mM) + resorcinol at range of concentrations of 1.0 to 2.5 mM (60 to 100%). Meanwhile, the combination of NAA (0 to 0.013 mM) + PG at range of concentrations from 1.0 to 2.5 mM showed lower percentage of mild browning (0 to 40%) compared to NAA + resorcinol media. The medium containing 0.023 mM kinetin + 0.5 mM resorcinol produced the highest mean number of shoots (6.2±1.20). Meanwhile, the medium containing 0.022 mM BAP + 0.5 mM PG showed the highest bud proliferation (7.8±0.45). The medium containing 0.005 mM NAA + 1.5 mM PG produced the highest number of roots (30.4±1.43). Among the media tested six media were categorized into mild (MS medium containing 0.008 mM NAA + 0.5 mM PG (MBPG) and MS medium containing 0.005 mM NAA + 2.0 mM resorcinol (MBR)

media), intermediate (MS medium containing 0.023 mM kinetin + 0.011 mM NAA + 0.5 mM PG (IBPG) and MS medium containing 0.023 mM kinetin + 0.5 mM resorcinol (IBR)) and severe browning inducing media (MS medium containing 0.022 mM BAP + 0.013 mM NAA + 2.5 mM PG (SBPG), and MS medium containing 0.022 mM BAP + 0.013 mM NAA + 2.5 mM resorcinol (SBR)). The highest total soluble protein content was observed in SBPG explant (40.46±0.01 mg/g FW). The highest CAT (356.26±0.02 µmol/min/mg) and APX (5893.18±0.20 µmol/min/mg) activities were observed in MBPG explant. Meanwhile, the highest POD, PPO, C3H and PAL activities were determined in SBR explants (13849.88±0.06, 2639.52±0.02, 1332.38±0.00 and 132.57±0.01 µmol/min/mg, respectively). Among the genes tested, the highest *PAL* expression was found in SBPG explant (225.31-fold). Meanwhile, the highest expression of *APX* and *PPO* was observed in IBR explants (4887.9- and 37.33-fold, respectively). The *POD*, *COMT* and *CCR* expression were up-regulated in MSO9 explant (49.57-, 302.49- and 89.30-fold, respectively), while the highest expression of *C3H*, *CAD* and *CAT* (44.36-, 11.22- and 29.33-fold, respectively) were observed in MBR explant. The expression of *MYB31* was highly expressed in MBPG explant (9.07-fold) while the expression of *MYB58* was found the highest in MSO7 explant (19.39-fold). Hence, the incorporation of the PG and resorcinol into different media formulations is proven can reduce browning and improve proliferation of *in vitro* Lang banana. The presence of antioxidant, browning-inducing and lignin-biosynthetic enzymes as well as their mRNA transcripts in the early browning explants suggest their involvement in scavenging free radical, reducing browning and enhancing lignin production. Therefore, the best media produced from this study can help plant tissue culture laboratories to mass produce Lang banana seedlings. The analyzed genes can be used as biomarker to develop browning resistant Lang banana in the near future.

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

**KESAN FLOROGLUSINOL DAN RESORSINOL DALAM PENGGALAKAN  
PERTUMBUHAN VEGETATIF *IN VITRO* DAN PENGURANGAN  
KEPERANGAN MAUT PADA PISANG LANG**

Oleh

**NURHANA NADIA RAMLAN**

**Mei 2019**

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Keperangan adalah salah satu masalah utama dalam industri tisu kultur. Pembentukan keperangan adalah disebabkan pembentukan rembesan gelap di sekeliling eksplan di mana ia akan menghalang percambahan eksplan. Sebatian fenolik seperti floroglusinol (PG) dan resorsinol adalah diketahui berupaya untuk mengurangkan keperangan pada produk makanan dan juga kosmetik. Maka, kedua-dua fenolik ini telah digunakan di dalam kajian ini untuk mengenalpasti kebolehan mereka untuk mengurangkan keperangan dan pada masa yang sama untuk meningkatkan pertumbuhan pisang Lang secara *in vitro*. Jumlah kandungan protein larut, ekspresi gen antioksidan (*katalase* (CAT), *askorbat peroksidase* (APX) and *peroksidase* (POD)), merangsang-keperangan (*phenylalanine ammonia lyase* (PAL) and *polyphenol oxidase* (PPO)) dan biosintesis lignin (*MYB31*, *MYB58*, *cinnamoyl coA reductase* (CCR), *catechol-O-methyltransferase* (COMT), *cinnamyl aldehyde dehydrogenase* (CAD) and *p-coumarate hydroxylase* (C3H)) serta aktiviti enzim juga dikaji pada peringkat awal keperangan eksplan pisang Lang yang ditanam di atas media yang terpilih. Untuk mencapai objektif, media asas Murashige dan Skoog (MS) yang ditambah dengan vitamin B5, kepekatan berbeza fenolik (PG atau resorcinol), kepekatan berbeza sitokinin (*6-benzylaminopurine* (BAP) atau kinetin) dan auksin (*α-naphthalene acid* (NAA)) telah digunakan. Pertumbuhan setiap eksplan telah direkodkan dengan mengira bilangan pucuk, tunas dan akar setiap minggu selama 12 minggu. Visual tahap keperangan juga telah direkodkan. Peratusan tertinggi eksplan yang mengalami keperangan sederhana telah didapati dari media yang mengandungi NAA (0.003 sehingga 0.013 mM) + resorcinol pada lingkungan kepekatan dari 1.0 kepada 2.5 mM (60 sehingga 100%). Manakala, kombinasi NAA (0.003 sehingga 0.013 mM) + PG pada lingkungan kepekatan dari 1.0 kepada 2.5 mM telah menunjukkan peratusan keperangan sederhana yang lebih rendah (0 sehingga 40%) berbanding dengan media NAA + resorcinol. Medium yang mengandungi 0.023 mM kinetin + 0.5 mM resorcinol telah menghasilkan purata bilangan pucuk tertinggi ( $6.2 \pm 1.20$ ). Manakala, medium yang mengandungi 0.022 mM BAP dan 0.5 mM PG telah menunjukkan percambahan tunas yang tertinggi ( $7.8 \pm 0.45$ ). Medium yang mengandungi 0.005 mM NAA dan 1.5 mM PG telah menghasilkan percambahan akar yang tertinggi ( $30.4 \pm 1.43$ ). Di antara media yang diuji, enam media telah dikategorikan kepada media sederhana (MS

medium yang mengandung 0.008 mM NAA + 0.5 mM PG (MBPG) dan MS medium yang mengandung 0.005 mM NAA + 2.0 mM resorcinol (MBR)), pertengahan (MS medium yang mengandung 0.023 mM kinetin + 0.011 mM NAA + 0.5 mM PG (IBPG) dan MS medium media yang mengandung 0.023 mM kinetin + 0.5 mM resorcinol (IBR)) dan teruk merangsang keperangan (MS medium yang mengandung 0.022 mM BAP + 0.013 mM NAA + 2.5 mM PG (SBPG) dan MS medium yang mengandung 0.022 mM BAP + 0.013 mM NAA + 2.5 mM resorcinol (SBR)). Jumlah protein larut yang tertinggi telah didapati di dalam eksplan SBPG (40.46±0.01 mg/g FW). Aktiviti CAT (356.26±0.02 µmol/min/mg) dan APX (5893.18±0.20 µmol/min/mg) tertinggi telah didapati di dalam eksplan MBPG. Manakala, aktiviti POD, PPO, C3H dan PAL yang tertinggi telah didapati di dalam eksplan SBR (13849.88±0.06, 2639.52±0.02, 1332.38±0.00 dan 132.57±0.01 µmol/min/mg/g FW, masing-masing). Di kalangan gen yang dikaji, ekspresi *PAL* yang tertinggi telah didapati di dalam eksplan SBPG (225.31 kali ganda). Manakala, ekspresi tertinggi *APX* dan *PPO* telah didapati di dalam eksplan IBR (4887.90 dan 37.33 kali ganda, masing-masing). Ekspresi *POD*, *COMT* dan *CCR* telah dinaikkawal di dalam MSO9 eksplan (49.57, 302.49 and 89.30 kali ganda, masing-masing), manakala ekspresi tertinggi *C3H*, *CAD* dan *CAT* (44.36-, 11.22- dan 29.33-kali ganda, masing-masing) telah didapati di dalam eksplan MBR. Ekspresi *MYB31* telah diekspres tertinggi di dalam eksplan MBPG (9.07 kali ganda) manakala ekspresi *MYB58* telah didapati tertinggi di dalam eksplan MSO7 (19.39 kali ganda). Maka, penambahan PG dan resorsinol ke dalam formulasi media yang berbeza terbukti boleh mengurangkan keperangan dan menambah baik percambahan pisang Lang secara *in vitro*. Kehadiran enzim antioksidan, enzim merangsang keperangan dan enzim yang terlibat di dalam biosintesis lignin serta transkrip mRNA mereka di dalam tindak balas awal keperangan eksplan mencadangkan penglibatan mereka di dalam menghapus radikal bebas, mengurangkan keperangan dan meningkatkan penghasilan lignin. Oleh itu, media yang terbaik yang telah dihasilkan dari kajian ini boleh membantu makmal kultur tisu tumbuhan menghasilkan anak benih pisang Lang dengan banyak. Gen yang dianalisa boleh digunakan sebagai biopenanda untuk membangunkan pisang Lang yang mempunyai daya tahan keperangan pada masa hadapan.



## ACKNOWLEDGEMENTS

Alhamdulillah, praise to Allah S.W.T. for His mercy and guidance I manage to complete my research and dissertation.

Utmost gratitude and appreciation go to my main supervisor Dr. Azzreena Mohamad Azzeme and my co-supervisors, Assoc. Prof. Dr. Noor Azmi Shaharuddin and Prof. Datin Dr. Siti Nor Akmar Abdullah for their guidance, inspiration and motivation during the entire period of study, either in conducting research works or thesis writing. Not to forget, to all my labmates at Plant Biochemistry and Biotechnology Laboratory, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, UPM, and all my friends for their never-ending supports, advices and suggestions for me to complete this research.

Finally, I would like to express my deepest appreciation to all my family members for their moral supports especially my parents Mr. Ramlan Mahmud and Mrs. Sakina Arshad, and also my siblings. Without their endless love, support and encouragement, I probably would not have this strength to complete the research.



This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment 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

APX	Ascorbate peroxidase
BAP	6-Benzylaminopurine
C3H	<i>p</i> - Coumarate Hydroxylase
CAD	Cinnamyl Aldehyde Dehydrogenase
CAT	Catalase
CCR	Cinnamoyl CoA reductase
COMT	Catechol-O-Methyltransferase
MBT	Meristem Block Tissue
MS	Murashige and Skoog
MSO	MS basal media
IAA	Indole-3-Acetic Acid
IBA	Indole-Butyric Acid
NAA	1-Naphthaleneacetic acid
PAL	Phenylalanine Ammonia-Lyase
PG	Phloroglucinol
PGR	Plant Growth Regulator
POD	Peroxidase
PPO	Polyphenol Oxidase
PVP	Polyvinylpyrrolidone
mg	milligram
μmol	micromole
min	minute
FW	fresh weight
g	gram
mL	milliliter
L	liter
μL	microliter
mM	millimolar
M	molar
μM	micromolar
mm	millimeter
cm	centimeter
v/v	volume per volume
w/v	weight per volume
ε	epsilon
°C	degree celcius
nm	nanometer
V	volt
x g	g force
mmol	millimoles
PCR	Polymerase Chain Reaction
C <sub>T</sub>	threshold cycle
C <sub>q</sub>	quantitation cycle

## CHAPTER 1

### INTRODUCTION

In 2015, Malaysia was reported to spend RM1.55 billion for importation of banana fruits from different countries like Africa, Australia, India, Thailand and China (Nieman et al. 2012; Department of Agriculture, 2015) due to the low production of banana fruits from local farms. The dissemination of diseases such as *Fusarium* wilt, Moko wilt, banana bract mosaic virus (BBMV), banana streak disease (BSV) and infectious chlorosis (CMV) is among the reasons of shortage banana fruit production in many countries including Malaysia (Mostert et al. 2017). Thus, to fulfill the need of Malaysians, the banana fruits have to be imported from other countries.

Lang banana (genome AAB) is a plaintain (*Musa paradisiaca*) belongs to the genus *Musa* and family Musaceae. Lang banana fruit is similar to Tanduk banana, which is commonly used to produce chips. Further, the Lang banana fruit is cheaper and smaller in size compared to Tanduk (Abdullah et al. 2011). Lang banana fruits are difficult to obtain in the market due to the less cultivation of this cultivar. This is probably due to the limited seedlings that are available for big scale plantation. Hence, the mass propagation of Lang banana through tissue culture is a practical way to increase the production of disease-free and true-to-type seedlings.

Tissue culture is a proven method that can be used to overcome the limited production of banana seedlings compared to vegetative method or natural regeneration using banana sucker (Singh, 2015). In banana tissue culture, propagation of banana seedlings using meristem culture can ensure rapid propagation and mass production of disease-free seedlings (Ahmed et al. 2014; Al-Amin et al. 2009). However, the tissue culture technique or *in vitro* propagation of banana still faces a lot of problems, especially browning of explant tissues. Browning occurs when the tissue is bruised, cut or peeled during propagation (Saltveit, 2016). The injured tissues gradually turn dark when exposed to the air due to the oxidation of phenolic compounds including chrysin, quercetin and catechin to brown pigments (Olubunmi, 2013). These undesirable exudates can form a barrier around the tissue, thus preventing nutrient uptake and hindering explant growth (Kishor et al. 2017).

There are various treatments that have been used to overcome browning problem in tissue culture including the use of ascorbic acid (Safwat et al. 2015; Olunbumi, 2013; Ngomuo et al. 2014b; Ko et al. 2009), activated charcoal (Safwat et al. 2015; Ahmad et al. 2013), polyvinylpyrrolidone (PVP) (Ko et al. 2009) and citric acid (Safwat et al. 2015; Titov et al. 2006). Previous study by Ko et al. (2009) on *Musa* cv. Formosana stated that the modification of culture medium by adding adsorptive materials such as activated charcoal and PVP did not solve the browning problem. However, both of these chemical compounds have an ability to adsorb phenol-like substances. Also, some of the antibrowning chemicals used tend to oxidize or absorb the nutrients in the media due to adsorptive properties of antibrowning chemicals.

The incorporation of phenolics such as PG and resorcinol into nutrient media can be one of the approaches to reduce oxidative browning and to improve growth of Lang banana explants. A study by Londe et al. (2017) reported that the combination of PG and 6-benzylaminopurine (BAP) in nutrient medium of Grand Naine banana had increased production of shoots and roots compared to control. Meanwhile, resorcinol is able to inactivate tyrosinase activity, and act as polyphenol oxidase (PPO) inhibitor (Sinha et al. 2012). Thus, resorcinol might be a potential compound that can be used to reduce browning in plant tissues.

Browning reaction occurs due to oxidative stress, and the process involves different types of enzymes such as PAL and PPO (Chuanjun et al. 2015). The PAL is the first enzyme involved in phenolics and lignin production in plants through phenylpropanoid pathway. Other phenylpropanoid enzymes such as C3H also important (Liu et al. 2018). During oxidative stress, the phenolics produced from phenylpropanoid pathway might be a substrate for PPO enzyme, which later can cause browning due to polymerization of quinone. The oxidative stress induces overproduction of reactive oxygen species (ROS), which the process can further activate antioxidant enzymes to detoxify the ROS (Bose et al. 2014). The CAD, COMT and CCR are important enzymes involved in lignin biosynthesis. Interruption of lignin production in plants can mediate browning reaction (Toivonen and Brummell, 2008). Hence, at biochemical level, there are many enzymes and genes involved in browning reaction that have to be elucidated their involvement in lethal browning of Lang banana.

Therefore, the objectives of this study were:

1. To determine the most effective treatment that can minimize browning and enhance growth of *in vitro* Lang banana seedlings
2. To compare antioxidant enzymes activity (peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT)), browning-inducing enzymes activity (phenylalanine lyase (PAL) and polyphenol oxidase (PPO)) and lignin-biosynthetic enzymes activity (*p*-coumarate hydroxylase (C3H)) in the control and treated Lang banana explants
3. To compare relative gene expression of antioxidant genes (*POD*, *APX* and *CAT*), browning-inducing genes (*PAL* and *PPO*), lignin-biosynthetic genes (*C3H*, *cinnamyl aldehyde dehydrogenase (CAD)*, *catechol-O-methyltransferase (COMT)* and *cinnamoyl coA reductase (CCR)*) and lignin-associated transcription factors (*MYB31* and *MYB58*) in the control and treated Lang banana explants.



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Nurhana Nadia Ramlan was born on December 28<sup>th</sup>, 1993. She attended her primary school at Sekolah Kebangsaan Pendang, Kedah from 2000 to 2005. She continued her secondary education at SMK Seri Tualang, Pahang (2006 – 2008) and MARA Junior Science College, Gemencheh, Negeri Sembilan (2009 – 2010). Later, in 2011, she went to Pahang Matriculation College, Gambang, Pahang for one-year matriculation program. After completion of her matriculation program, she enrolled her First Degree in Bachelor of Science (Honours) Biochemistry, a four-year program at Universiti Putra Malaysia and graduated in 2016. Then at the same year of her graduation, she enrolled Masters of Science in the field of Plant Biotechnology at the same university.



## LIST OF PUBLICATIONS

### Paper publication:

Nurhana Nadia Ramlan, Azzreena Mohamad Azzeme, Khairul Naim Md Padzil, Maziah Mahmood (2017). Influence of different extraction solvent on phytochemical content and antioxidant capacity extracted from banana pulp and flower of dessert and cooking bananas, *Malaysian Journal of Biochemistry and Molecular Biology*, 2&3: 10-16.

Nurhana Nadia Ramlan, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Siti Nor Akmar Abdullah. Effect of phloroglucinol and resorcinol supplemented nutrient media in controlling lethal browning and enhancing growth of in vitro Lang banana. Manuscript will be submitted to journal.

Nurhana Nadia Ramlan, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Siti Nor Akmar Abdullah. enzyme activity and gene expression of antioxidant, lignin- biosynthetic and browning-inducing enzymes in early browning symptom of Lang banana explants treated with resorcinol and phloroglucinol. Manuscript will be submitted to journal.

### Abstract in conference/symposium:

Nurhana Nadia Ramlan, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Siti Nor Akmar Abdullah. Phloroglucinol and resorcinol reduces lethal browning and enhances vegetative growth of in vitro Lang banana seedlings. In Third International Conference for Crop Improvement 2017 (ICCI2017), University of Reading Malaysia, Iskandar Puteri, Johor, Malaysia. (Poster Presenter)

Nurhana Nadia Ramlan, Azzreena Mohamad Azzeme, Noor Azmi Shaharuddin, Siti Nor Akmar Abdullah. The use of phenolics reduced lethal browning and enhanced vegetative growth of in vitro Lang banana seedlings. In International Conference on Biochemistry, Molecular Biology and Biotechnology 2018 (ICBMBB2018), Puchong, Selangor, Malaysia. (Poster Presenter)

Azzreena Mohamad Azzeme, Nurhana Nadia Ramlan, Noor Azmi Shaharuddin, Siti Nor Akmar Abdullah. Phloroglucinol and resorcinol reduces lethal browning and enhances vegetative growth of in vitro Lang banana seedlings. In International Association for Plant Biotechnology 2018 (IAPB2018), Dublin, Ireland.



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