UNIVERSITI PUTRA MALAYSIA

MOLECULAR AND PHYSIOLOGICAL RESPONSES OF RECALCITRANT INDICA RICE TO PLURONIC F-68 DURING CALLUS REGENERATION

ANDREW KOK DE XIAN

FBSB 2021 34

MOLECULAR AND PHYSIOLOGICAL RESPONSES OF RECALCITRANT *INDICA* RICE TO PLURONIC F-68 DURING CALLUS REGENERATION

By

ANDREW KOK DE XIAN

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

June 2021

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

MOLECULAR AND PHYSIOLOGICAL RESPONSES OF RECALCITRANT *INDICA* RICE TO PLURONIC F-68 DURING CALLUS REGENERATION

By

ANDREW KOK DE XIAN

June 2021

Chair : Janna Ong Binti Abdullah, PhD
Faculty : Biotechnology and Biomolecular Sciences

Pluronic F-68 (PF-68) is a non-ionic surfactant commonly used as a growth additive in plant tissue culture. However, there are limited studies on the effects of PF-68 in rice. Therefore, this study was undertaken to evaluate the growth promoting effects of PF-68 on callus proliferation, shoot growth and root growth of recalcitrant MR219 rice. MR219 calli and shoot apices were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations [0 %, 0.02 %, 0.04 %, 0.06 %, 0.08 %, 0.10 % (v/v)] of PF-68. Calli grown on MS medium supplemented with 0.04 % PF-68 improves callus proliferation rate by 1.59-fold (fresh weight), 1.24-fold (dry weight) and enhanced root induction from the calli by 1.29-fold. However, increasing frequency of brown and black calli was observed when 0.10 % PF-68 was used. In shoot growth study, PF-68 did not exhibit any growth promoting effects on MR219. On the other hand, optimum root growth was observed in shoot apices treated with 0.04 % PF-68. Growth of the roots was increased significantly by 1.43-fold and root length by 1.19-fold compared to the control. In order to evaluate the underlying mechanism of growth promoting effects of PF-68, callus was used as a study model and three different concentrations were selected for further analysis; namely control, optimum (0.04 % PF-68) and high concentration (0.10 % PF-68). Biochemical analyses revealed high accumulation of sugar (1.77 mg/mL) and protein (0.17 mg/mL) contents in 0.04 % PF-68-treated calli. Similarly, quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) also revealed that high expressions of *sucrose synthase* (2.65-fold) and *NADH-dependent glutamate synthase* (1.86-fold) transcripts, which correlated with the high sugar and protein contents detected in 0.04 % PF-68-treated calli. Besides, calli treated with high concentration of PF-68 (0.10 %) recorded increased accumulation of phenolic (0.74 mg/mL), flavonoid (0.08 mg/mL), and phenylalanine ammonia lyase (PAL) activity (0.28 U/µg protein), which implied enhanced secondary metabolites biosynthesis in 0.10 % PF-68-treated calli. Further gene expression quantification also recorded an increased in *4-coumarate:CoA ligase 3* (1.28-fold) and c*halcone-flavonone isomerase* (1.65-fold) transcripts in 0.10 % PF-68-treated calli. Subsequent biochemical analyses revealed high H_2O_2 activity (0.10 mg/mL), malondialdehyde content (0.024 U/µg protein) and peroxidase activity (0.15 U/µg protein) in 0.10 % PF-68-treated calli. Consistently, high expression level of *ascorbate peroxidase* (1.61-fold) was observed in 0.10 % PF-68-

treated calli, suggesting activation of the plant defense mechanism against increasing stress induced from high concentration of PF-68. However, a decrease in esterase activity (34,204.50 nmol/ng protein) was recorded at 0.10 % PF-68, which implied increasing stress induced by PF-68 to trigger programmed cell death. Further comparative proteomic analysis revealed an upregulation of alpha-amylase and NADH-dependent glutamate synthase proteins detected in 0.04 % PF-68-treated calli. This indicates PF-68 enhances callus proliferation via enhanced carbon and nitrogen metabolism in 0.04 % PF-68-treated calli. In contrast, upregulation of PAL protein was detected in 0.10 % PF-68-treated calli. These results suggest that secondary metabolite biosynthesis was enhanced in 0.10 % PF-68-treated calli. In addition, nutrient ion analysis revealed an increased uptake of K, Mg, Ca, Fe, Zn, Cu and Mn ions were also observed in 0.04 % PF-68-treated calli. Among these nutrient ions, K had the highest increment of nutrient content detected in 0.04 % PF-68-treated calli. The increased K uptake plays an important role in plant growth and development such as protein synthesis and carbohydrate metabolism. Overall, the results from this study showed that the growth promoting effects of PF-68 on *in vitro* MR219 rice cultures were concentration dependent. Taken together, at optimum concentration, PF-68 improves recalcitrant rice callus proliferation via enhanced sugar metabolism, amino acid biosynthesis and nutrient uptake which are crucial towards plant growth and development. However, at high concentration, PF-68 induces stress response in plant as evidenced by the increased secondary metabolites content, H_2O_2 activity, malondialdehyde content and peroxidase activity. Hence, optimum concentration of PF-68 has potential to be utilized as an additive for plant growth and development in tissue culture of rice cultivars.

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

PERUBAHAN MOLEKUL DAN FISIOLOGI DALAM BERAS *INDICA* REKALSITRAN TERHADAP PLURONIC F-68 SEMASA REGENERASI KALUS

Oleh

ANDREW KOK DE XIAN

Jun 2021

Pengerusi : Janna Ong Binti Abdullah, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Pluronic F-68 (PF-68) adalah surfaktan bukan ionik yang biasa digunakan sebagai aditif pertumbuhan dalam kultur tisu tumbuhan. Walau bagaimanapun, terdapat kajian terhad mengenai kesan PF-68 dalam beras. Oleh sebab itu, kajian ini dilakukan untuk menilai kesan peningkatan pertumbuhan PF-68 terhadap percambahan kalus, pertumbuhan pucuk dan pertumbuhan akar beras MR219 kultivar. Kalus MR219 dan pucuk apeks dikultur di dalam media Murashige dan Skoog (MS) ditambah dengan PF-68 pada kepekatan yang berbeza [0 %, 0.02 %, 0.04 %, 0.06 %, 0.08 %, 0.10 % (v/v)]. Dalam kajian ini, kalus yang tumbuh pada 0.04 % PF-68 meningkatkan percambahan kalus secara signifikan sebanyak 1.56-kali ganda (berat segar), 1.24-kali ganda (berat kering) dan meningkatkan pertumbuhan akar kalus sebanyak 1.29-kali ganda. Walau bagaimanapun, peningkatan frekuensi kalus coklat dan hitam diperhatikan pada 0.10 % PF-68. Dalam kajian pertumbuhan pucuk, PF-68 tidak menunjukkan kesan peningkatan pada pertumbuhan pucuk MR219. Sebaliknya, dalam kajian pertumbuhan akar, pertumbuhan akar optimum diperhatikan pada pucuk apeks yang ditambah dengan 0.04 % PF-68. Pada 0.04 % PF-68 peningkatan yang signifikan dalam pertumbuhan akar dari segi jumlah akar (1.43-kali ganda) dan panjang akar (1.19-kali ganda) telah dicatatkan berbanding dengan kawalan. Untuk menilai mekanisme yang mendasari kesan mempromosikan pertumbuhan PF-68, kalus yang ditambah dengan PF-68 digunakan sebagai model kajian dan tiga kepekatan yang berbeza dipilih untuk analisis yang selanjutnya; iaitu kawalan, 0.04 % PF-68 dan 0.10 % PF-68. Analisis biokimia menunjukkan pengumpulan kandungan gula (1.77 mg/mL) dan protein (0.17 mg/mL) yang tinggi dalam 0.04 % PF-68. Selanjutnya, quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) mendedahkan bahawa ekspresi tinggi transkrip *sucrose synthase* (2.65-kali ganda) dan *NADH-dependent glutamate synthase* (1.86-kali ganda), yang berkorelasi dengan kandungan gula dan protein yang tinggi dikesan pada 0.04 % PF-68. Sementara itu, kalus yang ditambah dengan kepekatan tinggi PF-68 (0.10 %) memerhatikan pengumpulan fenolik (0.74 mg/mL), flavonoid (0.08 mg/mL), dan aktiviti phenylalanine ammonia lyase (PAL) (0.28 U/µg protein), menunjukkan peningkatan biosintesis metabolit sekunder pada 0.10 % PF-68. Kuantifikasi ekspresi gen selanjutnya menunjukkan peningkatan dalam transkrip *4-coumarate: CoA ligase 3* (1.28-kali ganda) dan *chalcone-flavonone isomerase* (1.65-kali

ganda) dalam 0.10 % PF-68. Analisis biokimia seterusnya mendedahkan kandungan H_2O_2 (0.10 mg/mL), malondialdehid (0.024 U/µg protein) dan aktiviti peroksidase (0.15 U/µg protein) yang tinggi dijumpai pada 0.10 % PF-68. Secara konsisten, tahap ekspresi tinggi *ascorbate peroxidase* (1.61-kali ganda) diperhatikan pada 0.10 % PF-68, menunjukkan pengaktifan mekanisme pertahanan tanaman terhadap peningkatan tekanan yang disebabkan oleh kepekatan tinggi PF-68. Walau bagaimanapun, penurunan aktiviti esterase (34,204.50 nmol/ng protein) dicatat pada 0.10 % PF-68, menunjukkan peningkatan tekanan yang disebabkan oleh PF-68 yang memicu kematian sel. Analisis proteomik selanjutnya menunjukkan peningkatan regulasi protein alpha-amylase dan NADH-glutamate synthase yang dikesan dalam 0.04 % PF-68. Ini menunjukkan PF-68 meningkatkan percambahan kalus melalui metabolisme karbon dan nitrogen dalam 0.04 % PF-68. Sebaliknya, peningkatan regulasi PAL dikesan pada 0.10 % PF-68. Hasil ini menunjukkan bahawa peningkatan biosintesis metabolit sekunder ditingkatkan pada 0.10 % PF-68. Sementara itu, analisis ion nutrien mendedahkan peningkatan pengambilan ion K, Mg, Ca, Fe, Zn, Cu dan Mn juga diperhatikan pada 0.04 % PF-68. Di antara ion nutrien ini, K merupakan kenaikan kandungan nutrien tertinggi yang dikesan pada 0.04 % PF-68 berbanding dengan kawalan. Peningkatan pengambilan K memainkan peranan penting dalam pertumbuhan dan perkembangan tanaman seperti sintesis protein dan metabolisme karbohidrat. Secara keseluruhan, hasil kajian ini menunjukkan bahawa kesan pertumbuhan PF-68 terhadap penanaman padi *in vitro* MR219 bergantung kepada jumlah kepekatan. Secara bersama, pada kepekatan optimum, PF-68 meningkatkan percambahan kalus beras yang cepat melalui peningkatan metabolisme gula, biosintesis asid amino dan pengambilan nutrien yang penting terhadap pertumbuhan dan perkembangan tanaman. Walau bagaimanapun, pada kepekatan tinggi, PF-68 mendorong tindak balas tekanan pada tanaman seperti yang dibuktikan oleh peningkatan kandungan metabolit sekunder, aktiviti H_2O_2, kandungan malondialdehid dan aktiviti peroksidase. Oleh itu, kepekatan optimum PF-68 berpotensi untuk digunakan sebagai bahan tambahan untuk pertumbuhan dan pengembangan tanaman dalam kultur tisu kultivar padi.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank everyone who has helped me throughout the project. Additionally, I would like to thank my supervisory committees: Dr. Lai Kok Song, Assoc. Prof. Dr. Janna Ong Abdullah, Dr. Rogayah Sekeli and Dr. Nur Fatihah Mohd Yusoff. Without the expertise and support from the supervisory committees, the completion of this study may not be possible. Besides, I would like to express my gratitude to Dr Wee Chien Yeong from MARDI, Serdang, for her expertise and guidance in the project.

I would like to thank my family and friends for their endless supports and motivation throughout this study. In addition, I would like to thank the members of Floral Biotechnology Laboratory: Wan Muhamad Asrul Nizam Bin Wan Abdullah, Yang Shun Kai, Lee Siew Yi, Teh Kah Yee, Low Lee Yoon, Moo Chew Li, Thye Kah Lok and Tang Chu Nie for their helps throughout this project.

Lastly but not least, I would like to acknowledge the Graduate Research Fellowship and Putra Grants (GP-IPS/2017/9572000) from Universiti Putra Malaysia for providing financial supports in this research project.

Thank you.

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:

Janna Ong binti Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Nur Fatihah binti Mohd Yusoff, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Lai Kok Song, PhD
Assistant Professor
Health Sciences Division
Higher Colleges of Technology
(Member)

Rogayah binti Sekeli, PhD
Senior Research Officer
Biotechnology and Nanotechnology Research Centre
Malaysian Agricultural Research and Development Institute (MARDI)
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 November 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Andrew Kok De Xian

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Janna Ong binti Abdullah

Signature: _____
Name of Member of Supervisory Committee: Nur Fatihah binti Mohd Yusoff

Signature: _____
Name of Member of Supervisory Committee: Lai Kok Song

Signature: _____
Name of Member of Supervisory Committee: Rogayah binti Sekeli

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetic acid
ANOVA	Analysis of variance
BAP	Benzylaminopurine
DW	Dry weight
FDA	Fluorescein diacetate
FC	Folin-Ciocalteu
FW	Fresh weight
GOGAT	Glutamate synthase
H_2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MDA	Malondialdehyde
MARDI	Malaysian Agricultural Research and Development Institute
MS	Murashige and Skoog
NAA	1-Naphthaleneacetic acid
PF-68	Pluronic F-68
PMSF	Phenylmethylsulfonyl fluoride
PAL	Phenylalanine ammonia-lyase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEO	Polyethylene oxide
PPO	Polypropylene oxide
PCD	Programmed cell death

ROS	Reactive oxygen species
RT-qPCR	Real-time reverse transcription polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
TDZ	Thidiazuron

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

Rice is one of the major staple food and Asia countries being the top producer and consumer. In Malaysia alone, a total land area of 671 679 hectares has been utilized for growing paddy with the highest production occurred in Kedah, Perak, Perlis and Penang (FAO, 2017). However, the rice production in Malaysia is still unable to sustain the demand of the ever-increasing population. Therefore, rice crop improvement, such as increasing yield and quality are crucial. Malaysian Agricultural Research and Development Institute (MARDI) is the leading research institute conducting research related to livestock, food, and crops, including rice. MARDI has released 45 rice varieties for planting through plant breeding (MARDI, 2017). Among these rice varieties, MR219 cultivar is one of the most widely cultivated rice cultivars in Malaysia.

MR219 cultivar is well known for its high yielding, increased grain weight, and resistance towards leaf blight disease (Liew et al., 2012). Despite having these desirable traits, MR219 cultivar is sensitive towards environmental stresses (Tan et al., 2017). For instance, production of rice suffers when it is grown under drought or excess soil salinity conditions. These environmental stresses cause the reduction in water use efficiency in plants, which in turn, impairs plant growth and development. Thus, crop improvement via genetic manipulation is required in order to overcome these limitations. However, genetic manipulation in MR219 cultivar remains a challenge. This is because of its recalcitrant trait towards *in vitro* regeneration responses which include poor callus proliferation, low regeneration efficiency and long regeneration period (Amandeep Kaur et al., 2014). For instance, a total of eight weeks is required to produce embryogenic callus in MR219 (Zuraida et al., 2012), as compared to two weeks for *japonica* rice to produce embryogenic callus prior to shoot regeneration (Duan et al., 2012). Therefore, improving *in vitro* response of MR219 cultivar, such as callus proliferation, would be the first step towards the success of genetic manipulation on MR219 cultivar (Low et al., 2018).

In order to improve the *in vitro* responses of MR219 cultivar, optimization of plant growth medium is required. In this case, exogenous plant growth hormones and additives are the key components in improving the *in vitro* responses of MR219 cultivar (Abiri et al., 2017). Numerous studies were undertaken in order to establish an efficient tissue culture system for *indica* rice using different combinations of plant growth hormones such as benzylaminopurine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), thidiazuron (TDZ), 1-naphthaleneacetic acid (NAA), and kinetin (Abiri et al., 2017; Wani, Sanghera, & Gosal, 2011; Zuraida et al., 2011; Shahsavari et al., 2010). Among these studies on plant growth hormones, application of 2,4-D and kinetin were observed to be effective in callus proliferation of MR219 cultivar (Zuraida et al., 2011). Nevertheless, to achieve a desirable amount of callus in a short period remains to be a challenge for recalcitrant rice cultivar like MR219.

Pluronic F-68 (PF-68) is a non-ionic, co-polymer surfactant that has been employed as an additive in both *in vitro* animal and plant cultures (Barbulescu et al., 2011; Meier et al., 1999). PF-68 has been widely used in animal cell suspension culture to protect and repair damaged cells from constant sparging and agitation (Meier et al., 1999). Besides, studies have found that application of PF-68 in animal cell culture was able to enhance plasma membrane permeability that in turn, enhanced growth in animal cell culture (Shelat et al., 2013). On the other hand, application of PF-68 in plant tissue culture was found to enhance shoot regeneration in *Citrus sinensis* (Curtis & Mirkov 2012), *Pyrus communis* (Dashti et al., 2012), *Ricinus communis* (Kulathuran & Narayanasamy 2015) and *Abelmoschus esculentus* (Irshad et al., 2018). Notably, PF-68 was demonstrated to improve shoot regeneration of recalcitrant *Brassica napus* embryos (Barbulescu et al., 2011). This implies that PF-68 could be a good candidate for plant cell growth and regeneration improvement of the recalcitrant cultivar. Despite its successful growth enhancement in various plant species, application of PF-68 at higher concentrations was found to retard plant growth (Curtis & Mirkov 2012). Therefore, in order to maximize the usage of PF-68 as a growth additive, concentration optimization and understanding the mode of its action is crucial.

To date, there are no reported studies on the growth promoting effects of PF-68 on recalcitrant rice cultivar. Besides, the underlying growth promoting mechanism of PF-68 in plant cell remains largely unknown. Hence, this study aimed to improve *in vitro* cultivation of recalcitrant MR219 cultivar through supplementation of PF-68. In addition, proteomic profiling, biochemical assays and gene expression analysis were also performed to shed light on the possible mechanism of PF-68 in promoting rice callus growth.

The objectives of this study were:

1. to determine the suitable concentration of PF-68 on callus growth, shoot induction, shoot growth, and root growth of recalcitrant MR219 cultivar,

2. to assess the effects of PF-68 on MR219 callus proliferation through biochemical assays and gene expression analysis, and

3. to determine the differentially expressed proteins between callus treated with PF-68 and untreated callus

REFERENCES

Abiri, R., Maziah, M., Shaharuddin, N. A., Yusof, Z. N. B., Atabaki, N., Hanafi, M. M., Sahebi, M., Azizi, P., Kalhori, N., & Valdiani, A. (2017). Enhancing somatic embryogenesis of Malaysian rice cultivar MR219 using adjuvant materials in a high-efficiency protocol. *International Journal of Environmental Science and Technology*, *14*(5), 1091–1108.

Agostini, E., De Forchetti, S. M., & Tigier, H. A. (1997). Production of peroxidases by hairy roots of *Brassica napus*. *Plant Cell, Tissue and Organ Culture*, 47(2), 177–182.

Ahmad, I., Mian, A., & Maathuis, F. J. M. (2016). Overexpression of the rice AKT1 potassium channel affects potassium nutrition and rice drought tolerance. *Journal of Experimental Botany*, 67(9), 2689–2698.

Ahmad, N., Ahmad, N., & Rab, A. (2016). Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus cultures of *Stevia rebaudiana* (Bert). *Journal of Photochemistry and Photobiology B: Biology*, 154, 51–56.

Ahsan, N., Lee, D. G., Alam, I., Kim, P. J., Lee, J. J., Ahn, Y. O., Kwak, S. S., Lee, I. J., Bahk, J. D., Kang, K. Y., & Renaut, J. (2008). Comparative proteomic study of arsenic-induced differentially expressed proteins in rice roots reveals glutathione plays a central role during As stress. *Proteomics*, 8(17), 3561–3576.

Amandeep Kaur, S. K. S., Am, Kaur, E., Kaur, G., & Cheema, G. S. (2014). Genetic transformation of rice: Problems, progress and prospects. *Rice Research*, 3(1), 1–10.

Amano, T., Hirasawa, K. I., O'Donohue, M. J., Pernolle, J. C., & Shioi, Y. (2003). A versatile assay for the accurate, time-resolved determination of cellular viability. *Analytical Biochemistry*, 314(1), 1–7.

Amemiya, A., Akemine, H., & Toriyama, K. (1956). Culture condition and growth of immature embryo culture in rice plant. *Bulletin of the National Institute of Agricultural Sciences*, D6, 1–40.

Anthony, P., Jelodar, N. B., Lowe, K. C., Power, J. B., & Davey, M. R. (1996). Pluronic F-68 increases the post-thaw growth of cryopreserved plant cells. *Cryobiology*, 33(5), 508–514.

Araya, T., Noguchi, K., & Terashima, I. (2006). Effects of carbohydrate accumulation on photosynthesis differ between sink and source leaves of *Phaseolus vulgaris* L. *Plant and Cell Physiology*, 47(5), 644–652.

Ashwood-Smith, M. J., Voss, W. A. G., & Warby, C. (1973). Cryoprotection of mammalian cells in tissue culture with pluronic polyols. *Cryobiology*, 10(6), 502–504.

Bañuelos, M. A., Garciadeblas, B., Cubero, B., & Rodríguez-Navarro, A. (2002). Inventory and functional characterization of the HAK potassium transporters of rice. *Plant Physiology*, 130(2), 784–795.

Barbulescu, D. M., Burton, W. A., & Salisbury, P. A. (2011). Pluronic F-68: An answer for shoot regeneration recalcitrance in microspore-derived *Brassica napus* embryos. *In Vitro Cellular and Developmental Biology - Plant*, 47(2), 282–288.

Ben Henda, M., Ghaouar, N., & Gharbi, A. (2013). Rheological properties and reverse micelles conditions of PEO-PPO-PEO Pluronic F68: Effects of temperature and solvent mixtures. *Journal of Polymers*, 2013(1), 1–7.

Binte Mostafiz, S., & Wagiran, A. (2018). Efficient callus induction and regeneration in selected *indica* rice. *Agronomy*, 8(5), 77.

Biswas, A., & Mandal, A. B. (2007). Plant regeneration in different genotypes of *indica* rice. *Indian Journal of Biotechnology*, 6(4), 532–540.

Brukhin, V., & Morozova, N. (2011). Plant growth and development - Basic knowledge and current views. *Mathematical Modelling of Natural Phenomena*, 6(2), 1–53.

Bunai, K., & Yamane, K. (2005). Effectiveness and limitation of two-dimensional gel electrophoresis in bacterial membrane protein proteomics and perspectives. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 815, 227–236.

Campo, S., Baldrich, P., Messeguer, J., Lalanne, E., Coca, M., & Segundo, B. S. (2014). Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. *Plant Physiology*, 165(2), 688–704.

Cangahuala-Inocente, G. C., Steiner, N., Maldonado, S. B., & Guerra, M. P. (2009). Patterns of protein and carbohydrate accumulation during somatic embryogenesis of *Acca sellowiana*. *Pesquisa Agropecuária Brasileira*, 44(3), 217–224.

Cánovas, F. M., Dumas-Gaudot, E., Recorbet, G., Jorrin, J., Mock, H. P., & Rossignol, M. (2004). Plant proteome analysis. *Proteomics*, 4(2), 285–298.

Capicciotti, C. J., Kurach, J. D. R., Turner, T. R., Mancini, R. S., Acker, J. P., & Ben, R. N. (2015). Small molecule ice recrystallization inhibitors enable freezing of human red blood cells with reduced glycerol concentrations. *Scientific Reports*, 5(1), 1–10.

Chen, X. Y., Kim, S. T., Cho, W. K., Rim, Y., Kim, S., Kim, S. W., Kang, K. Y., Park, Z. Y., & Kim, J. Y. (2009). Proteomics of weakly bound cell wall proteins in rice calli. *Journal of Plant Physiology*, 166(7), 675–685.

Cheng, M., Fry, J. E., Pang, S., Zhou, H., Hironaka, C. M., Duncan, D. R., Conner, T. W., & Wan, Y. (1997). Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. *Plant Physiology*, 115(3), 971–980.

Chew, Y. C., Halim, M. H. A., Wan Abdullah, W. M. A. N., Abdullah, J. O., & Lai, K. S. (2018). Highly efficient proliferation and regeneration of protocorm-like bodies (PLBS) of the threatened orchid, *Phalaenopsis bellina*. *Sains Malaysiana*, 47(6), 1093–1099.

Chichkova, S., Arellano, J., Vance, C. P., & Hernández, G. (2001). Transgenic tobacco plants that overexpress alfalfa NADH-glutamate synthase have higher carbon and nitrogen content. *Journal of Experimental Botany*, 52(364), 2079–2087.

Choi, W. C., Hon, W. M., Mohamad, M., Kok, A. D. X., Lai, K. S., & Yap, W. S. (2019). Antioxidant properties of crude and cold ethanol precipitated protein from palm kernel cake (PKC) As potential cosmeceutical agent. *Journal of Oil Palm Research*, 31(1), 159–164.

Chou, T.-S., Chao, Y.-Y., & Kao, C. H. (2012). Involvement of hydrogen peroxide in heat shock- and cadmium-induced expression of ascorbate peroxidase and glutathione reductase in leaves of rice seedlings. *Journal of Plant Physiology*, 169(5), 478–486.

Cui, S., Huang, F., Wang, J., Ma, X., Cheng, Y., & Liu, J. (2005). A proteomic analysis of cold stress responses in rice seedlings. *Proteomics*, 5, 3162–3172.

Curtis, I. S., & Mirkov, T. E. (2012). Influence of surfactants on growth and regeneration from mature internodal stem segments of sweet orange (*Citrus sinensis*) cv. Hamlin. *Plant Cell, Tissue and Organ Culture*, 108(2), 345–352.

Dashti, S., Habashi, A. A., Azghandi, A. V., Abdollahi, H., & Chamani, M. (2012). Effects of Pluronic F-68 on regeneration and rooting of two pear cultivars (*Pyrus communis* cvs Dar Gazi and Bartlett). *International Research Journal of Applied and Basic Sciences*, 3(1), 190–196.

Datta, S. K., Peterhans, A., Datta, K., & Potrykus, I. (1990). Genetically engineered fertile *indica* rice recovered from protoplasts. *BioTechnology*, 8,736–740.

Daud, N. S., Zaidel, D. N. A., Lai, K. S., Muhamad, I. I., & Mohd Jusoh, Y. M. (2016). Antioxidant properties of rice bran oil from different varieties extracted by solvent extraction methods. *Jurnal Teknologi*, 78(6–12), 107–110.

Daud, N. S. M., Zaidel, D. N. A., Lai, K. S., Jusoh, Y. M. M., Muhamad, I. I., & Ya'akob, H. (2017). Microwave-assisted stabilisation and storage stability study of rice bran oil from different varieties. *Chemical Engineering Transactions*, *56*, 1285–1290.

Daud, N. S. M., Zaidel, D. N. A., Lai, K. S., Khairuddin, N., Jusoh, Y. M. M., & Muhamad, I. I. (2018). Crude oil yield and properties of rice bran oil from different varieties as affected by extraction conditions using soxhterm method. *Arabian Journal for Science and Engineering*, 43(11), 6237–6244.

Duan, Y., Zhai, C., Li, H., Li, J., Mei, W., Gui, H., Ni, D., Song, F., Li, L., Zhang, W., & Yang, J. (2012). An efficient and high-throughput protocol for Agrobacterium-mediated transformation based on phosphomannose isomerase positive selection in *Japonica* rice (*Oryza sativa* L.). *Plant Cell Reports*, 31(9), 1611–1624.

Epstein, E., Rains, D. W., & Elzam, O. E. (1963). Resolution of dual mechanisms of potassium absorption by barley roots. *Proceedings of the National Academy of Sciences*, 49(5), 684–692.

Ertani, A., Francioso, O., Tugnoli, V., Righi, V., & Nardi, S. (2011). Effect of commercial lignosulfonate-humate on *Zea mays* L. metabolism. *Journal of Agricultural and Food Chemistry*, 59(22), 11940–11948.

Eveland, A. L., & Jackson, D. P. (2012). Sugars, signalling, and plant development. *Journal of Experimental Botany*, 63, 3367–3377.

Faust, F., & Schubert, S. (2016). Protein synthesis is the most sensitive process when potassium is substituted by sodium in the nutrition of sugar beet (*Beta vulgaris*). *Plant Physiology and Biochemistry*, 107, 237–247.

Food and Agriculture Organization. (2017). GIEWS - Global information and early warning system. The United Nation, accessed 10 June 2020, http://www.fao.org/giews/countrybrief/country.jsp?code=MYS.

Fuller, D. Q., Sato, Y. I., Castillo, C., Qin, L., Weisskopf, A. R., Kingwell-Banham, E. J., Song, J., Ahn, S.-M., & van Etten, J. (2010). Consilience of genetics and archaeobotany in the entangled history of rice. *Archaeological and Anthropological Sciences*, 2(2), 115–131.

Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*, 50(1), 151–158.

Gao, L. Z., & Innan, H. (2008). Nonindependent domestication of the two rice subspecies, *Oryza sativa* ssp. *indica* and ssp. *japonica*, demonstrated by multilocus microsatellites. *Genetics*, 179(2), 965–976.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S., & McCouch, S. (2005). Genetic structure and diversity in *Oryza sativa* L. *Genetics*, 169(3), 1631–1638.

Geng, P., La, H., Wang, H., & Stevens, E. J. C. (2008). Effect of sorbitol concentration on regeneration of embryogenic calli in upland rice varieties (*Oryza sativa* L.). *Plant Cell, Tissue and Organ Culture*, 92(3), 303–313.

Grant, V., & Oka, H. I. (1988). Origin of cultivated rice. *Taxon*, 37(4), 935.

Gupta, H. S., Pattanayak, A., Bhuyan, R. N., & Pandey, D. K. (1989). Cytokinin mediated induction of embryogenic calli and plant regeneration in *indica* rice (*Oryza sativa*). *Indian Journal of Agricultural Sciences*, 59(8), 526–528.

Hamamoto, S., Horie, T., Hauser, F., Deinlein, U., Schroeder, J. I., & Uozumi, N. (2015). HKT transporters mediate salt stress resistance in plants: From structure and function to the field. *Current Opinion in Biotechnology*, 32, 113–120.

Hansen, H., & Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiology*, 124(3), 1437–1448.

Hasanuzzaman, M., Bhuyan, M., Nahar, K., Hossain, M., Mahmud, J., Hossen, M., Masud, A. M., & Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*, 8(3), 31.

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I. S., & White, P. (2011). Functions of macronutrients. In P. Marschner (Ed.), *Marschner's Mineral Nutrition of Higher Plants: Third Edition* (pp. 135–189). London, United Kingdom: Elsevier.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125(1), 189–198.

Hiei, Y., & Komari. T. (2008). *Agrobacterium*-mediated transformation of rice using immature embryos or calli induced from mature seed. *Nature Protocals*, 3, 824–834.

Hu, W., Berdugo, C., & Chalmers, J. J. (2011). The potential of hydrodynamic damage to animal cells of industrial relevance: Current understanding. *Cytotechnology*, 63, 445–460.

Hymes, A. C., Safavian, M. H., Arbulu, A., & Baute, P. (1968). A comparison of Pluronic F-68, low molecular weight dextran, mannitol, and saline as priming agents in the heart-lung apparatus. I. Pluronic F-68: First use as a plasma substitute. *The Journal of Thoracic and Cardiovascular Surgery*, 56(1), 16–22.

Irshad, M., Rizwan, H. M., Debnath, B., Anwar, M., Li, M., Liu, S., He, B., & Qiu, D. (2018). Ascorbic acid controls lethal browning and Pluronic F-68 promotes high-frequency multiple shoot regeneration from cotyldonary node explant of Okra (*Abelmoschus esculentus* L.). *HortScience*, 53(2), 183–190.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research*, 52, 39.

Jain, R. K., Jain, S., & Wu, R. (1996). Stimulatory effect of water stress on plant regeneration in aromatic *Indica* rice varieties. *Plant Cell Reports*, 15(6), 449–454.

Javed, F., & Ikram, S. (2008). Effect of sucrose induced osmotic stress on callus growth and biochemical aspects of two wheat genotypes. *Pakistan Journal of Botany*, *40*, 1487–1495.

Jiang, L., He, L., & Fountoulakis, M. (2004). Comparison of protein precipitation methods for sample preparation prior to proteomic analysis. *Journal of Chromatography A*, 1023(2), 317–320.

Jin, Z. L., Zhang, F., Ahmed, Z. I., Rasheed, M., Naeem, M. S., Ye, Q. F., & Zhou, W. J. (2010). Differential morphological and physiological responses of two oilseed *Brassica* species to a new herbicide ZJ0273 used in rapeseed fields. *Pesticide Biochemistry and Physiology*, 98(1), 1–8.

Jorrín-Novo, J. V, Maldonado, A. M., Echevarría-Zomeño, S., Valledor, L., Castillejo,

M. A., Curto, M., Valero, J., Sghaier, B., Donoso, G., & Redondo, I. (2009). Plant proteomics update (2007-2008): Second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfill MIAPE standards, increase plant proteome coverage and expand biological knowledge. *Journal of proteomics*, 72(3), 285–314.

Jun, S. Y., Sattler, S. A., Cortez, G. S., Vermerris, W., Sattler, S. E., & Kang, C. H. (2018). Biochemical and structural analysis of substrate specificity of a phenylalanine ammonia-lyase. *Plant Physiology*, 176(2), 1452–1468.

Karpiuk, U. V., Al Azzam, K. M., Mahmoud Abudayeh, Z. H., Kislichenko, V., Naddaf, A., Cholak, I., &Yemelianova, O. (2016). Qualitative and quantitative content determination of macro-minor elements in *Bryonia alba* l. roots using flame atomic absorption spectroscopy technique. *Advanced Pharmaceutical Bulletin*, 6(2), 285–291.

Khatun, A., Laouar, L., Davey, M. R., Power, J. B., Mulligan, B. J., & Lowe, K. C. (1993). Effects of Pluronic F-68 on shoot regeneration from cultured jute cotyledons and on growth of transformed roots. *Plant Cell, Tissue and Organ Culture*, 34(2), 133–140.

Kim, D. W., Rakwal, R., Agrawal, G. K., Jung, Y. H., Shibato, J., & Jwa, N. S. (2005). A hydroponic rice seedling culture model system for investigating proteome of salt stress in rice leaf. *Electrophoresis*, 26, 4521–4539.

King, A. T., Davey, M. R., Mulligan, B. J., & Lowe, K. C. (1990). Effects of Pluronic F-68 on plant cells in suspension culture. *Biotechnology Letters*, 12(1), 29–32.

Koller, A., Washburn, M. P., Lange, B. M. Andon, N. L., Deciu, C., Haynes, P. A. Hays, L., Schieltz, D., Ulaszek, R., Wei, J., Wolters, D., & Yates, J. R. (2002). Proteomic survey of metabolic pathways in rice. *Proceedings of the National Academy of Sciences of the United States of America*, 99(18), 11969–11974.

Kong, W., Liu, F., Zhang, C., Zhang, J., & Feng, H. (2016). Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. *Scientific Reports*, 6(1), 1–8.

Konieczny, R., Banaś, A. K., Surówka, E., Michalec, Ż., Miszalski, Z., & Libik-Konieczny, M. (2014). Pattern of antioxidant enzyme activities and hydrogen peroxide content during developmental stages of *rhizogenesis* from hypocotyl explants of *Mesembryanthemum crystallinum L. Plant Cell Reports*, 33(1), 165–177.

Konishi, N., Ishiyama, K., Matsuoka, K., Maru, I., Hayakawa, T., Yamaya, T., & Kojima, S. (2014). NADH-dependent glutamate synthase plays a crucial role in assimilating ammonium in the *Arabidopsis* root. *Physiologia Plantarum*, 152(1), 138–151.

Kosová, K., Vítámvás, P., Prášil, I. T., & Renaut, J. (2011). Plant proteome changes under abiotic stress - Contribution of proteomics studies to understanding plant stress response. *Journal of Proteomics*, 74, 1301–1322.

Kruger, N. J. (1994). The Bradford method for protein quantitation. *Methods in Molecular Biology (Clifton, N.J.)*, *32*. 9–15.

Kulathuran, G. K., & Narayanasamy, J. (2015). Evaluation of Pluronic F68 and PGR's for high frequency somatic embryogenesis and plant regeneration in castor (*Ricinus communis* L.) through solid culture. *International Journal of Current Biotechnology*, 3(8),1–10.

Kumar, V., Laouar, L., Davey, M. R., Mulligan, B. J., & Lowe, K. C. (1990). Effects of Pluronic F-68 on growth of transformed roots of *Solanum dulcamara*. *Biotechnology Letters*, 12(12), 937–940.

Kumar, V., Laouar, L., Davey, M. R., Mulligan, B. J., & Lowe, K. C. (1992). Pluronic F-68 stimulates growth of *Solanum dulcamara* in culture. *Journal of Experimental Botany*, 43(4), 487–493.

Lai, K. S. (2016). Analysis of *EXO70C2* expression revealed its specific association with late stages of pollen development. *Plant Cell, Tissue and Organ Culture*, 124(1), 209–215.

Lai, K. S., Abdullah, P., Yusoff, K., & Mahmood, M. (2011). An efficient protocol for particle bombardment-mediated transformation of *Centella asiatica* callus. *Acta Physiologiae Plantarum*, 33(6), 2547–2552.

Lai, K. S., & Masatsugu, T. (2013). Isolation and characterization of an *Arabidopsis thaliana* self-incompatibility mutant induced by heavy-ion beam irradiation. *Acta Biologica Cracoviensia Series Botanica*, 55(2), 146–152.

Lai, K. S., Yusoff, K., & Mahmood, M. (2013). Functional ectodomain of the hemagglutinin-neuraminidase protein is expressed in transgenic tobacco cells as a candidate vaccine against Newcastle disease virus. *Plant Cell, Tissue and Organ Culture*, 112(1), 117–121.

Lai, K. S., Yusoff, K., & Mahmood, M. (2014). Morphological and physiological characterization of embryogenic and non-embryogenic tissue of *Centella asiatica*. *Plant Tissue Culture and Biotechnology*, 24(1), 125–129.

Lai, K. S., Yusoff, K., & Maziah, M. (2011). Extracellular matrix as the early structural marker for *Centella asiatica* embryogenic tissues. *Biologia Plantarum*, 55(3), 549–553.

Lai, Kok Song, & Yusoff, K. (2013). Production of tENDO1 in stably transformed tobacco cell cultures for mismatch detection. *Plant Cell, Tissue and Organ Culture*, 114(2), 287–294.

Lee, D. G., Ahsan, N., Lee, S. H., Kang, K. Y., Bahk, J. D., Lee, I. J., & Lee, B. H. (2007). A proteomic approach in analyzing heat-responsive proteins in rice leaves. *Proteomics*, 7(18), 3369–3383.

Lee, H.-T., & Huang, W.-L. (2019). Cross talk among phytohormone signal and

carbohydrate metabolism involving regenerable calli induction under osmotic treatment. In A. Gonzalez, M. Rodriguez, N.G. Sağlam (Eds.), *Plant Science - Structure, Anatomy and Physiology in Plants Cultured in vivo and in vitro* (pp.1–12). Norderstedt, Germany: IntechOpen.

Lehmeier, C. A., Wild, M., & Schnyder, H. (2013). Nitrogen stress affects the turnover and size of nitrogen pools supplying leaf growth in a grass. *Plant Physiology*, 162(4), 2095–2105.

Leong, K. Y. B., Chan, Y. H., Abdullah, W. M. A. N. W., Lim, S. H. E., & Lai, K. S. (2018). The CRISPR/Cas9 system for crop improvement: Progress and prospects. In Y.O. Çiftçi (Ed.), *Next Generation Plant Breeding* (pp. 129–150). Norderstedt, Germany: IntechOpen.

Li, W., Xu, G., Alli, A., & Yu, L. (2018). Plant HAK/KUP/KT K+ transporters: Function and regulation. *Seminars in Cell and Developmental Biology*, 74, 133–141.

Li, Y., Kim, J. I., Pysh, L., & Chapple, C. (2015). Four isoforms of *Arabidopsis* 4-coumarate:CoA ligase have overlapping yet distinct roles in phenylpropanoid metabolism. *Plant Physiology*, 169(4), 2409–2421.

Liew, Y., Omar, S. R. S., Husni, M. A., Zainal, A. M. A., & Ashikin, P. A. N. (2012). Effects of foliar applied copper and boron on fungal diseases and rice yield on cultivar MR219. *Pertanika Journal of Tropical Agricultural Science*, 35(2), 339–349.

Lim, Y. Y., & Lai, K. S. (2017). Generation of transgenic rice expressing cyclotide precursor Oldenlandia affinis kalata B1 protein. *Journal of Animal and Plant Sciences*, 27(2), 667–671.

Lin, D. G., Chou, S. Y., Wang, A. Z., Wang, Y. W., Kuo, S. M., Lai, C. C., Chen, L. J., & Wang, C. S. (2014). A proteomic study of rice cultivar TNG67 and its high aroma mutant SA0420. *Plant Science*, 214, 20–28.

Liu, Y., Pan, T., Tang, Y., Zhuang, Y., Liu, Z., Li, P., Li, H., Huang, W., Tu, S., Ren, G., Wang, T., &Wang, S. (2020). Proteomic analysis of rice subjected to low light stress and overexpression of *OsGAPB* increases the stress tolerance. *Rice*, 13(1), 30 –40.

Liu, C. W., Chang, T. S., Hsu, Y. K., Wang, A. Z., Yen, H. C., Wu, Y. P., Wang, C. S., & Lai, C. C. (2014). Comparative proteomic analysis of early salt stress responsive proteins in roots and leaves of rice. *Proteomics*, 14(15), 1759–1775.

Liu, Y. H., Lu, S., Liu, K., Wang, S., Huang, L., & Guo, L. (2019). Proteomics: A powerful tool to study plant responses to biotic stress. *Plant Methods*, 15, 1–20.

Liu, Y. F., Liu, Y., He, H., & Wang, J. (2004). Factors influencing callus induction and plantlet regeneration from mature embryos in *indica* rice. *Plant Physiology Communications*, 40(3), 319–322.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using

real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, 25(4), 402–408.

Low, L.-Y., Ong-Abdullah, J., Wee, C.-Y., Sekeli, R., Tan, C.-K., Loh, J.-Y., & Lai, K.-S. (2019). Effects of lignosulfonates on callus proliferation and shoot induction of recalcitrant *indica* rice. *Sains Malaysiana*, 48(1), 7–13.

Low, L. Y., Yang, S. K., Kok, A. D. X., Ong-Abdullah, J., Tan, N. P., & Lai, K.S. (2018). Transgenic plants: Gene constructs, vector and transformation method. In Y.O. Çiftçi (Ed.), New Visions in Plant Science (pp. 41–61). Norderstedt, Germany: IntechOpen.

Lowe, B. A., Shiva Prakash, N., Way, M., Mann, M. T., Spencer, T. M., & Boddupalli, R. S. (2009). Enhanced single copy integration events in corn via particle bombardment using low quantities of DNA. *Transgenic Research*, 18(6), 831–840.

Lowe, K. C., Anthony, P., Davey, M. R., & Power, J. B. (2001). Beneficial effects of Pluronic F-68 and artificial oxygen carriers on the post-thaw recovery of cryopreserved plant cells. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology*, 29(4), 297–316.

Lu, B. R., Zheng, K. L., Qian, H. R., & Zhuang, J. Y. (2002). Genetic differentiation of wild relatives of rice as assessed by RFLP analysis. *Theoretical and Applied Genetics*, 106(1), 101–106.

Lu, Y., Liu, Z., Lyu, M., Yuan, Y., & Wu, B. (2019). Characterization *of JsWOX1* and *JsWOX4* during callus and root induction in the shrub species *Jasminum sambac*. *Plants*, 8(4), 79.

Ludwig-Müller, J. (2011). Auxin conjugates: Their role for plant development and in the evolution of land plants. *Journal of Experimental Botany*, 62(6), 1757–1773.

Ma, B., Yin, C.-C., He, S.-J., Lu, X., Zhang, W.-K., Lu, T.-G., Chen, S. Y., & Zhang, J.-S. (2014). Ethylene-induced inhibition of root growth requires abscisic acid function in rice (*Oryza sativa* L.) seedlings. *PLoS Genetics*, 10(10), e1004701.

Mäser, P., Thomine, S., Schroeder, J. I., Ward, J. M., Hirschi, K., Sze, H., Talke, I. N., Amtmann, A., Maathuis, F. J., Sanders, D., Harper, J. F., Tchieu, J., Gribskov, M., Persans, M. W., Salt, D. E., Kim, S. A., & Guerinot, M. L. (2001). Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiology*, 126(4), 1646–1667.

Meier, S. J., Hatton, T. A., & Wang, D. I. C. (1999). Cell death from bursting bubbles: Role of cell attachment to rising bubbles in sparged reactors. *Biotechnology and Bioengineering*, 62(4), 468–478.

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

Moloney, M. M., Elliott, M. C., & Cleland, R. E. (1981). Acid growth effects in maize roots: Evidence for a link between auxin-economy and proton extrusion in the control of root growth. *Planta*, 152(4), 285–291.

Munene, R., Changamu, E., Korir, N., & Joseph, G.-O. (2017). Effects of different nitrogen forms on growth, phenolics, flavonoids and antioxidant activity in amaranth species. *Tropical Plant Research*, 4(1), 81–89.

Muñoz-Nortes, T., Pérez-Pérez, J. M., Sarmiento-Mañús, R., Candela, H., & Micol, J. L. (2017). Deficient glutamate biosynthesis triggers a concerted upregulation of ribosomal protein genes in *Arabidopsis*. *Scientific Reports*, 7(1), 1–14.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497.

Naik, N., Rout, P., Umakanta, N., Verma, R. L., Katara, J. L., Sahoo, K. K., Singh, O. N., & Samantaray, S. (2017). Development of doubled haploids from an elite *indica* rice hybrid (BS6444G) using anther culture. *Plant Cell, Tissue and Organ Culture*, 128(3), 679–689.

Nguyen, Q. A., Luan, S., Wi, S. G., Bae, H., Lee, D.-S., & Bae, H.-J. (2015). Pronounced phenotypic changes in transgenic tobacco plants overexpressing sucrose synthase may reveal a novel sugar signaling pathway. *Frontiers in Plant Science*, 6, 1 −15.

Nieves-Cordones, M., Martínez-Cordero, M. A., Martínez, V., & Rubio, F. (2007). An NH4+-sensitive component dominates high-affinity K+ uptake in tomato plants. *Plant Science*, 172(2), 273–280.

Noël, N., Leplé, J. C., & Pilate, G. (2002). Optimization of in vitro micropropagation and regeneration for *Populus* x *interamericana* and *Populus* x *euramericana* hybrids (*P. deltoides*, *P. trichocarpa*, and *P. nigra*). *Plant Cell Reports*, 20(12), 1150–1155.

Ntwampe, S. K. O. O., Williams, C. C., & Sheldon, M. S. (2010). Water-immiscible dissolved oxygen carriers in combination with Pluronic F-68 in bioreactors. *African Journal of Biotechnology*, 9(8), 1106–1114.

Oinam, G. S., & Kotharii, S. L. (1995). Totipotency of coleoptile tissue in *indica* rice (*Oryza sativa* L. cv. Ch1039). *Plant Cell Reports*, 14, 245–248.

Pabuayon, I. M., Yamamoto, N., Trinidad, J. L., Longkumer, L., Raorane, M. L., & Kohli, A. (2016). Reference genes for accurate gene expression analyses across different tissues, developmental stages and genotypes in rice for drought tolerance. *Rice*, 9(1), 32 – 40.

Paque, S., & Weijers, D. (2016). Auxin: The plant molecule that influences almost anything. *BMC Biology*, *14*(1), 67 −72.

Park, S. H., Lee, C. W., Cho, S. M., Lee, H., Park, H., Lee, J., & Lee, J. H. (2018). Crystal structure and enzymatic properties of chalcone isomerase from the Antarctic vascular plant *Deschampsia antarctica* Desv. *PLoS ONE*, 13(2), e0192415.

Plataki, M., Lee, Y. D., Rasmussen, D. L., & Hubmayr, R. D. (2011). Poloxamer 188

facilitates the repair of alveolus resident cells in ventilator-injured lungs. *American Journal of Respiratory and Critical Care Medicine*, 184(8), 939–947.

Pyo, Y. J., Gierth, M., Schroeder, J. I., & Cho, M. H. (2010). High-affinity K^+ transport in *Arabidopsis*: *AtHAK5* and *AKT1* are vital for seedling establishment and postgermination growth under low-potassium conditions. *Plant Physiology*, 153(2), 863–875.

Radić, S., & Pevalek-Kozlina, B. (2010). Differential esterase activity in leaves and roots of *Centaurea ragusina* L. as a consequence of salinity. *Periodicum Biologorum*, 112(3), 253–258.

Rafiq, M. A., Ali, A., Malik, M. A., & Hussain, M. (2010). Effect of fertilizer levels and plant densities on yield and protein contents of autumn planted maize. *Pakistan Journal of Agricultural Sciences*, 6(1), 201–208.

Raghavendra, G., Kumaraswamy, G. K., Ramya, B., Sandesh, H. S., Yogendra, K. N., Deepak, N., & Gowda, P. H. R. (2010). Direct multiple shoot regeneration of *indica* rice (*Oryza sativa*) var. 'Rasi'. *Asian and Australasian Journal of Plant Science and Biotechnology*, 4(1), 71–73.

Rayle, D. L., & Cleland, R. (1970). Enhancement of wall loosening and elongation by acid solutions. *Plant Physiology*, 46(2), 250–253.

Reid, R., & Hayes, J. (2003). Mechanisms and control of nutrient uptake in plants. *International Review of Cytology*, 229, 73–114.

Rohde, A., Morreel, K., Ralph, J., Goeminne, G., Hostyn, V., De Rycke, R., Kushnir, S., Doorsselaere, J. V., Joseleau, J.-P., Vuylsteke, M., Driessche, G. V., Beeumen, J. V., Messens, E., & Boerjan, W. (2004). Molecular phenotyping of the *pal1* and *pal2* mutants of *Arabidopsis thaliana* reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism. *Plant Cell*, 16(10), 2749–2771.

Ruan, S. L., Ma, H. S., Wang, S. H., Fu, Y. P., Xin, Y., Liu, W. Z., Wang, F., Tong, J.-X., Wang, S.-Z., &Chen, H. Z. (2011). Proteomic identification of *OsCYP2*, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when overexpressed. *BMC Plant Biology*, 11, 34 –49.

Ryschka, S., Ryschka, U., & Schulze, J. (1991). Anatomical studies on the development of somatic emhryoids in wheat and barley explants. *Biochemie Und Physiologie Der Pflanzen*, 187(1), 31–41.

Sah, S. K., Kaur, A., Kaur, G., & Cheema, G. S. (2014). Genetic transformation of rice: Problems, progress and prospects. *Rice Research: Open Access*, 3(1), 132.

Sahoo, K. K., Tripathi, A. K., Pareek, A., Sopory, S. K., & Singla-Pareek, S. L. (2011). An improved protocol for efficient transformation and regeneration of diverse *indica* rice cultivars. *Plant Methods*, 7(1), 49 –60.

Sahu, A., Kasoju, N., Goswami, P., & Bora, U. (2011). Encapsulation of curcumin in Pluronic block copolymer micelles for drug delivery applications. *Journal of Biomaterials Applications*, 25(6), 619–639.

Santa-María, G. E., Oliferuk, S., & Moriconi, J. I. (2018). KT-HAK-KUP transporters in major terrestrial photosynthetic organisms: A twenty years tale. *Journal of Plant Physiology*, 226, 77–90.

Schaller, G. E., Bishopp, A., & Kieber, J. J. (2015). The yin-yang of hormones: Cytokinin and auxin interactions in plant development. *Plant Cell*, 27(1), 44–63.

Shafi, A., Chauhan, R., Gill, T., Swarnkar, M. K., Sreenivasulu, Y., Kumar, S., Kumar, N., Shankar, R., Ahuja, P. S. & Singh, A. K. (2015). Expression of *SOD* and *APX* genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in *Arabidopsis* under salt stress. *Plant Molecular Biology*, 87(6), 615–631.

Shahsavari, E., Maheran, A. A., Akmar, A. S. N., & Hanafi, M. M. (2010). The effect of plant growth regulators on optimization of tissue culture system in Malaysian upland rice. *African Journal of Biotechnology*, 9(14), 2089–2094.

Sharma, C., Kaur, M. P., Kaur, A., & Gosal, S. S. (2012). *In vitro* plant regentration studies in three *indica* rice varieties. *International Journal of Agriculture, Environment and Biotechnology*, 5(4), 309–313.

Shelat, P. B., Plant, L. D., Wang, J. C., Lee, E., & Marks, J. D. (2013). The membrane-active tri-block copolymer Pluronic F-68 profoundly rescues rat hippocampal neurons from oxygen-glucose deprivation-induced death through early inhibition of apoptosis. *Journal of Neuroscience*, 33(30), 12287–12299.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology*, 11, 118–130.

Soeno, K., Goda, H., Ishii, T., Ogura, T., Tachikawa, T., Sasaki, E., Yoshida, S., Fujioka, S., Asami, T., &Shimada, Y. (2010). Auxin biosynthesis inhibitors, identified by a genomics-based approach, provide insights into auxin biosynthesis. *Plant and Cell Physiology*, 51(4), 524–536.

Sofo, A., Scopa, A., Nuzzaci, M., & Vitti, A. (2015). Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *International Journal of Molecular Sciences*, 16, 13561–13578.

Song, X., Wang, Y., & Lv, X. (2016). Responses of plant biomass, photosynthesis and lipid peroxidation to warming and precipitation change in two dominant species (*Stipa grandis* and *Leymus chinensis*) from North China Grasslands. *Ecology and Evolution*, 6(6), 1871–1882.

Stein, O., & Granot, D. (2019). An overview of sucrose synthases in plants. *Frontiers in Plant Science*, 10, 95 −127.

Su, H., Golldack, D., Zhao, C., & Bohnert, H. J. (2002). The expression of HAK-Type K^+ transporters is regulated in response to salinity stress in common ice plant. *Plant Physiology*, 129(4), 1482–1493.

Sugimoto, K., Gordon, S. P., & Meyerowitz, E. M. (2011). Regeneration in plants and animals: Dedifferentiation, transdifferentiation, or just differentiation? *Trends in Cell Biology*, 21(4), 212–218.

Swedlund, B., & Locy, R. D. (1993). Sorbitol as the primary carbon source for the growth of embryogenic callus of maize. *Plant Physiology*, 103(4), 1339–1346.

Swim, H. E., & Parker, R. F. (1960). Effect of Pluronic F68 on growth of fibroblasts in suspension on rotary shaker. *Experimental Biology and Medicine*, 103(1), 252–254.

Takatsuka, H., & Umeda, M. (2014). Hormonal control of cell division and elongation along differentiation trajectories in roots. *Journal of Experimental Botany*, 65(10), 2633–2643.

Talbi, S., Romero-Puertas, M. C., Hernández, A., Terrón, L., Ferchichi, A., & Sandalio, L. M. (2015). Drought tolerance in a Saharian plant *Oudneya africana*: Role of antioxidant defences. *Environmental and Experimental Botany*, 111, 114–126.

Talei, D., Valdiani, A., Maziah, M., & Mohsenkhah, M. (2013). Germination response of MR219 rice variety to different exposure times and periods of 2450 MHz microwave frequency. *The Scientific World Journal*, 2013, 1–8.

Tamás, L., Huttová, J., Mistrík, I., Šimonovičová, M., & Široká, B. (2005). Aluminium induced esterase activity and isozyme pattern in barley root tip. *Plant, Soil and Environment*, 51(5), 220–225.

Tan, L. W., A. Rahman, Z., Goh, H.-H., Hwang, D.-J., Ismail, I., & Zainal, Z. (2017). Production of transgenic rice (*indica* 1 cv. MR219) Overexpressing *Abp57* gene through *Agrobacterium*-mediated transformation. *Sains Malaysiana*, 46(5), 703–711.

Teh, C. Y., Ho, C. L., Shaharuddin, N. A., Lai, K. S., & Mahmood, M. (2019). Proteome of rice roots treated with exogenous proline. *3 Biotech*, 9(3), 101.

Terzi, R., Kadioglu, A., Kalaycioglu, E., & Saglam, A. (2014). Hydrogen peroxide pretreatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. *Journal of Plant Interactions*, 9(1), 559–565.

Townsend, D. W., Turner, I., Yasuda, S., Martindale, J., Davis, J., Shillingford, M., Kornegay, J. N., & Metzger, J. M. (2010). Chronic administration of membrane sealant prevents severe cardiac injury and ventricular dilatation in dystrophic dogs.

Journal of Clinical Investigation, 120(4), 1140–1150.

Vallejo, A. J., Peralta, M. L., & Santa-Maria, G. E. (2005). Expression of potassium-transporter coding genes, and kinetics of rubidium uptake, along a longitudinal root axis. *Plant, Cell and Environment*, 28(7), 850–862.

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science*, 151(1), 59–66.

Víteček, J., Wünschová, A., Petřek, J., Adam, V., Kizek, R., & Havel, L. (2007). Cell death induced by sodium nitroprusside and hydrogen peroxide in tobacco BY-2 cell suspension. *Biologia Plantarum*, 51(3), 472–479.

Walls, P. L. L., McRae, O., Natarajan, V., Johnson, C., Antoniou, C., & Bird, J. C. (2017). Quantifying the potential for bursting bubbles to damage suspended cells. *Scientific Reports*, 7(1), 1–9.

Wang, C.-H., Yu, J., Cai, Y.-X., Zhu, P.-P., Liu, C.-Y., Zhao, A.-C., Lü, R.-H., Li, M.-J., Xu F.-X., & Yu, M.-D. (2016). Characterization and functional analysis of *4-coumarate:CoA ligase* genes in mulberry. *PloS ONE*, 11(5), e0155814.

Wang, J. C., Bindokas, V. P., Skinner, M., Emrick, T., & Marks, J. D. (2017). Mitochondrial mechanisms of neuronal rescue by F-68, a hydrophilic Pluronic block co-polymer, following acute substrate deprivation. *Neurochemistry International*, 109, 126–140.

Wang, M., Zheng, Q., Shen, Q., & Guo, S. (2013). The critical role of potassium in plant stress response. *International Journal of Molecular Sciences*, 14, 7370–7390.

Wang, P., Xu, D.Y., Wang, G. and Ji, J. (2007). Comparison study on tissue culture and regeneration ability of mature embryo in *indica-japonica* rice two subspecies. *Seed*, 26(10), 66–67.

Wang, X., Han, F., Yang, M., Yang, P., & Shen, S. (2013). Exploring the response of rice (*Oryza sativa*) leaf to gibberellins: A proteomic strategy. *Rice*, 6(1), 17.

Wang, X., Li, Y., Fang, G., Zhao, Q., Zeng, Q., Li, X., Gong, H., & Li, Y. (2014). Nitrite promotes the growth and decreases the lignin content of *indica* rice calli: A comprehensive transcriptome analysis of nitrite-responsive genes during *in vitro* culture of rice. *PLoS ONE*, 9(4), e95105.

Wani, S. H., Sanghera, G. S., & Gosal, S. S. (2011). An efficient and reproducible method for regeneration of whole plants from mature seeds of a high yielding *Indica* rice (*Oryza sativa* L.) variety PAU 201. *New Biotechnology*, 28(4), 418–422.

Watanabe, N., & Lam, E. (2008). BAX inhibitor-1 modulates endoplasmic reticulum stress-mediated programmed cell death in *Arabidopsis*. *Journal of Biological Chemistry*, 283(6), 3200–3210.

Wee, W. C., Lai, K. S., Kong, C. L., & Yap, W. S. (2018). Impact of within-row plant spacing and fixed fruit setting on yield and quality of rockmelon fruit cultivated by drip irrigation in a greenhouse. *Horticultural Science and Technology*, 36(2), 172–182.

Weise, S. E., Schrader, S. M., Kleinbeck, K. R., & Sharkey, T. D. (2006). Carbon balance and circadian regulation of hydrolytic and phosphorolytic breakdown of transitory starch. *Plant Physiology*, 141(3), 879–886.

White, P. J., & Karley, A. J. (2010). Potassium. *Plant Cell Monographs*, 17, 199–224.

Wong, C. K. F., Lai, K. S., Wong, M. Y., & Maziah, M. (2016). Heat and hydrolytic enzymes treatment improved the *Agrobacterium*-mediated transformation of recalcitrant *indica* rice (*Oryza sativa* L.). *Plant Cell, Tissue and Organ Culture*, 125(1), 183–190.

Wong, C. K. F., Lai, K. S., Wong, M. Y., & Mahmood, M. (2015). Efficient regeneration and *Agrobacterium*-mediated transformation protocol for recalcitrant *indica* rice (*Oryza sativa* L.). *Emirates Journal of Food and Agriculture*, 27(11), 837–848.

Wong, C. K. F., Lai, K. S., Wong, M. Y., & Mahmood, M. (2016). Minimal inhibitory concentration of common selectable agents on recalcitrant Malaysian rice cultivar. *Asian Journal of Agricultural Research*, 10(2), 126–131.

Wu, C., Wan, Y., Xu, J., Su, J., & Fang, X. (2002). Establishment of a rice transformation system by regeneration of embryonic callus induced from mature embryo scutum. *Chinese Journal of Tropical Crops*, 23(3), 88–94.

Xu, H., Zhang, W., Gao, Y., Zhao, Y., Guo, L., & Wang, J. (2012). Proteomic analysis of embryo development in rice (*Oryza sativa*). *Planta*, 235(4), 687–701.

Yamaya, J., Yoshioka, M., meshi, T., Okada, Y., & Ohno, T. (1988). Cross protection in transgenic tobacco plants expressing a mild strain of tobacco mosaic virus. *MGG Molecular & General Genetics*, 215(1), 173–175.

Yang, H., Meng, Y., Chen, B., Zhang, X., Wang, Y., Zhao, W., & Zhou, Z. (2016). How integrated management strategies promote protein quality of cotton embryos: High levels of soil available N, N assimilation and protein accumulation rate. *Frontiers in Plant Science*, 7, 1–14.

Yang, S. K., Yusoff, K., Ajat, M., Thomas, W., Abushelaibi, A., Akseer, R., Lim, S. H. E., & Lai, K. S. (2019). Disruption of KPC-producing Klebsiella pneumoniae membrane via induction of oxidative stress by cinnamon bark (*Cinnamomum verum* J. Presl) essential oil. *PLoS ONE*, 14(4), 1–20.

Yap, W.-S., & Lai, K.-S. (2017). Biochemical properties of twelve malaysia rice cultivars in relation to yield potential. *Asian Journal of Agricultural Research*, 11(4), 137–143.

Yildirim, A. B., & Turker, A. U. (2014). Effects of regeneration enhancers on micropropagation of *Fragaria vesca* L. and phenolic content comparison of field-

grown and in vitro-grown plant materials by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS). *Scientia Horticulturae*, 169, 169–178.

Yildiz, M. (2011). Evaluation of the effect of *in vitro* stress and competition on tissue culture response of flax. *Biologia Plantarum*, 55(1), 541–544.

Yin, L., Tao, Y., Zhao, K., Shao, J., Li, X., Liu, G., Liu, S., & Zhu, L. (2007). Proteomic and transcriptomic analysis of rice mature seed-derived callus differentiation. *Proteomics*, 7(5), 755–768.

Yuan, J. F., Feng, G., Ma, H. Y., & Tian, C. Y. (2010). Effect of nitrate on root development and nitrogen uptake of *Suaeda physophora* under NaCl salinity. *Pedosphere*, 20(4), 536–544.

Zeeman, S. C., Kossmann, J., & Smith, A. M. (2010). Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annual Review of Plant Biology*, 61(1), 209–234.

Zelelew, D. Z., Lal, S., Kidane, T. T., & Ghebreslassie, B. M. (2016). Effect of potassium levels on growth and productivity of potato varieties. *American Journal of Plant Sciences*, 7(12), 1629–1638.

Zhang, X., & Liu, C. J. (2015). Multifaceted regulations of gateway enzyme phenylalanine ammonia-lyase in the biosynthesis of phenylpropanoids. *Molecular Plant*, 8, 17–27.

Zhang, Z., Zhao, H., Tang, J., Li, Z., Li, Z., Chen, D., & Lin, W. (2014). A proteomic study on molecular mechanism of poor grain-filling of rice (*Oryza sativa* L.) inferior spikelets. *PloS ONE*, 9(2), 1–19.

Zhao, Y., Hu, Y., Dai, M., Huang, L., & Zhou, D. X. (2009). The *WUSCHEL-Related homeobox* gene *WOX11* is required to activate shoot-borne crown root development in rice. *Plant Cell*, 21(3), 736–748.

Zhou, F., Wang, J., & Yang, N. (2014). Growth responses, antioxidant enzyme activities and lead accumulation of *Sophora japonica* and *Platycladus orientalis* seedlings under Pb and water stress. *Plant Growth Regulation*, 75(1), 383–389.

Zubair Rafique, M., Rashid, H., Chaudhary, M. F., Chaudhry, Z., & Cheema, N. M. (2011). Study on callogenesis and organogenesis in local cultivars of rice (*Oryza sativa* L.). *Pakistan Journal of Botany*, 43(1), 191–203.

Zuraida, A. R., Naziah, B., Zamri, Z., & Sreeramanan, S. (2011). Efficient plant regeneration of Malaysian *indica* rice MR219 and 232 via somatic embryogenesis system. *Acta Physiologiae Plantarum*, 33(5), 1913–1921.

Zuraida, A. R., Zulkifli, A. S., Habibuddin, H., Naziah, B., Rahman, Z. A., Seman, Z. A., Hashim, H., & Basirun, N. (2012). Regeneration of Malaysian rice variety MR219 via somatic embryogenesis. *Journal of Tropical Agriculture and Food Science*, 39(2), 167–177.