



UNIVERSITI PUTRA MALAYSIA

***IN VITRO AND IN VIVO REGENERATION OF ORYZA SATIVA L. cv. MR219
AND ZEA MAYS L. var. RUGOSA BY APICAL MERISTEM TISSUES***

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By

LAVANYA SILVARAJAN

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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

***IN VITRO* REGENERATION OF *ORYZA SATIVA* L. cv. MR219 AND *ZEA MAYS* L. var. *RUGOSA* BY APICAL MERISTEM TISSUES**

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Conventional propagation of important cereal crops such as corn and rice are vulnerable to unpredictable climatic changes. To ensure the constant availability of these crops, it is important to look towards alternative propagation methods such as *in vitro* regeneration. To date, there are limited reports of an ideal *in vitro* regeneration protocol of both rice and corn from shoot apical meristem (SAM) and no reports on root apical meristem (RAM) as explant. Thus, the objective of the present study is to establish an ideal *in vitro* regeneration system for Malaysian *indica* rice MR219 and Thai Super Sweet corn. Regeneration was successfully achieved by means of shoot and root apical meristem (SAM and RAM) obtained from 4-day old germinating seedlings through tissue culture. The study found that primary shoot was successfully induced from SAM and RAM of MR219 rice on liquid MS medium supplemented with 0.1 mg L⁻¹ KIN (kinetin) and 0.2 mg L⁻¹ KIN respectively. Vigorous primary shoots were induced from SAM and RAM of Thai Super Sweet corn on liquid MS medium supplemented with 0.15 mg L⁻¹ KIN and only liquid MS medium respectively. Following this, shoot multiplication from SAM of MR219 rice was highest on solid MS medium supplemented with a combination of 1.5 mg L⁻¹ KIN and 0.05 mg L⁻¹ IAA (indole-3-acetic-acid) with an average of 8.8 ± 0.22 shoots. RAM of

MR219 rice required solid MS medium supplemented with a combination of 2.0 mg L⁻¹ KIN and 0.05 mg L⁻¹ IAA with an average of 5.7± 0.17 shoots. Shoot multiplication of Thai Super Sweet corn was highest for SAM and RAM on solid MS medium supplemented with a combination of 3.0 mg L⁻¹ KIN and 0.01 mg L⁻¹ IAA and solid MS medium supplemented with a combination of 3.0 mg L⁻¹ KIN and 0.15 mg L⁻¹ IAA with an average of 13.1 ± 0.16 and 6.3 ± 0.19 shoots. In both species, shoot multiplication was in concomitant with root formation for both explants on ideal treatments. Regenerated plantlets survived greenhouse conditions upon acclimatization with a satisfying survival rate of more than 80% and 60% for plantlets produced from SAM and RAM of both species respectively. In conclusion, the highly efficient and economic protocol suggested in this study can be applied as an alternative to conventional propagation method for the large-scale production of MR219 rice and Thai Super Sweet corn throughout the year.

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

REGENERASI *IN VITRO* ORYZA SATIVA L. cv. MR219 DAN ZEA MAYS L. var. RUGOSA MELALUI TISU EPIKAL MERISTEM

Pembiakan konvensional padi dan jagung menghadapi pelbagai kekangan seperti kekangan biotik dan abiotik. Untuk memastikan ketersediaan bekalan bijirin-bijirin ini bagi memenuhi permintaan pengguna, kaedah pembiakan alternatif seperti regenerasi *in vitro* adalah penting. Laporan regenerasi *in vitro* padi dan jagung melalui kaedah tisu kultur menggunakan tisu meristem (SAM dan RAM) adalah terhad. Oleh itu, objektif kajian ini adalah untuk menghasilkan sistem regenerasi *in vitro* untuk padi Malaysia MR219 dan jagung “Thai Super Sweet” melalui kultur tisu menggunakan tisu SAM dan RAM yang telah diperoleh daripada plumul dan radikel anak benih yang bercambah sebagai eksplan. Pucuk utama berjaya dihasilkan daripada SAM dan RAM padi MR219 dalam media cecair MS yang ditambah 0.1 mg L^{-1} KIN and 0.2 mg L^{-1} KIN masing-masing. Manakala, bagi jagung “Thai Super Sweet”, pucuk utama telah berjaya dihasilkan daripada SAM and RAM dalam media cecair MS yang ditambah 0.15 mg L^{-1} KIN dan media tanpa zat pengatur tumbuhan. Pertumbuhan dan pemanjangan pucuk daripada SAM padi MR219 paling optimum (8.8 ± 0.22 pucuk/daun) dalam media pepejal MS media yang ditambah kombinasi 1.5 mg L^{-1} KIN dan 0.05 mg L^{-1} IAA. Manakala, RAM padi MR219 memerlukan media pepejal MS yang ditambah kombinasi 2.0 mg L^{-1} KIN dan 0.05 mg L^{-1} IAA dengan purata 5.7 ± 0.17 pucuk/daun. Pertumbuhan pucuk daripada SAM jagung “Thai Super Sweet” paling optimum dalam media pepejal MS yang ditambah kombinasi 3.0 mg L^{-1} KIN dan 0.01 mg L^{-1} IAA, Manakala, RAM memerlukan media pepejal MS ditambah kombinasi 3.0 mg L^{-1} KIN dan 0.15 mg L^{-1} IAA. Pertumbuhan pucuk daripada eksplan kedua-dua spesies berlaku seiring formasi dan pertumbuhan akar. Anak

pokok yang berjaya diregenerasi menunjukkan pertumbuhan normal setelah diaklimatisasi. Purata kadar mandiri anak pokok yang dihasilkan daripada SAM melebihi 80%, manakala anak pokok yang dihasilkan daripada RAM melebihi 60% bagi kedua-dua spesies. Maka, secara keseluruhan, SAM merupakan eksplan yang lebih efisien berbanding RAM. Sebagai kesimpulan, protocol regenerasi *in vitro* yang telah dihasilkan dalam kajian ini boleh digunakan sebagai alternatif kepada pembiakan konvensional untuk meningkatkan pengeluaran padi MR219 dan jagung “Thai Super Sweet” bagi memenuhi permintaan pengguna sepanjang tahun.

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God can repay all of your kindness.



DECLARATION

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

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LIST OF ABBREVIATIONS

2,4-D	2,4-Dichlorophenoxyacetic acid
2ip	6-(gamma,gamma-Dimethylallylamino) purine
ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
BERNAS	Padiberas Nasional Berhad
CPA	Cyproterone acetate
cv.	Cultivar
cm	Centimeter
DOA	Department of Agriculture
FAO	Food and Agriculture Organization
g	grams
g L ⁻¹	grams per liter
ha	Hectare
IAA	Indole-3- acetic acid
IBA	Indole-3- butyric acid
KIN	Kinetin
L	Liter
LAF	Laminar Air Flow
MADA	MUDA Agricultural Development Authority
MARDI	Malaysian Agricultural Research and Development Institute

mg L ⁻¹	Miligrams per liter
Min	Minutes
mm	Milimeter
mL	Mililiter
MT	Metric Ton
NAA	1-naphthaleneacetic-acid
PGR	Plant Growth Regulators
ppm	Parts per million
RAM	Root Apical Meristem
SAM	Shoot Apical Meristem
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
USDA	United States Department of Agriculture
var.	Variety
°C	Degree celcius
(v/v)	Volume over volume
%	Percent

CHAPTER 1

INTRODUCTION

Cereal crops are important worldwide as a major food source for human and their domesticated animals. Among these, rice serves as a staple food in many countries throughout the world, whereas corn is one of the most important cereal crops in the world. In Malaysia, MR219 is currently the most popular rice variety grown since its release in 2001 while Thai Super Sweet corn is increasing in popularity due to its delicious taste, high sugar content and long shelf life.

Presently, Malaysia's rice self-sufficiency stands at 72% while domestic corn production is insignificant. Hence, Malaysia still depends on imports to meet any further increase in consumer demand. In addition to this, conventional propagation of these crops is vulnerable to climatic uncertainties. Moreover, rice and corn are subjected to diseases especially in temperate and tropical regions such as Asia. This poses a major biological restriction on production of these important cereal crops. Due to this, the current rice self-sufficiency rate is expected to decline and dependency on corn imports is expected to increase in Malaysia.

With the increasing population, it is vital to improve the production of local crop varieties, as loss in production may lead to hunger and famine, especially in developing countries such as Malaysia. As such, it is imperative for Malaysia to look towards alternative methods of propagation to achieve rice self-sufficiency and decrease dependency on corn imports. The alternative method proposed should overcome disadvantages faced by conventional propagation methods, hence, should be independent of limitations such as environmental factors, availability of land,

disease and seasonal constraints. The high potential alternative method proposed in this study is *in vitro* plant regeneration.

Generally, *in vitro* regeneration has superiority over conventional propagation methods due to production of disease free plants at high multiplication rate and plantlets produced establish faster, healthier, and stronger, have shorter production cycle and higher yields (Darvari *et al.*, 2010). However, the establishment of a highly ideal protocol depends on suitable explants, plant growth regulators (PGRs), media composition as well as appropriate physical and chemical environments.

Preceding studies available on the *in vitro* regeneration of rice and corn involved laborious and time-consuming methods due to the long intermediate callus phase, which required sub-culturing. Moreover, there are no reports of an ideal regenerative protocol for rice and corn that bypasses the intervening callus phase. A protocol such as this could minimize somaclonal variation.

Using meristem tissue as an explant and modifying PGRs within the culture medium are two most effective ways to establish an ideal *in vitro* regeneration protocol (George *et al.*, 2008) that not only avoids intermediate callus formation but is also efficient, economic and time saving. Organogenesis (shoot induction) can be achieved directly from meristem tissue (Alam *et al.*, 2010) due to its high sustainability, totipotency and plasticity.

Two important meristematic regions of a plant are the shoot apical meristem (SAM) and root apical meristem (RAM). Although many tissue culture studies thus far have

utilized SAM as an explant, RAM is a less popular choice of explant in comparison. However, RAM is a potentially efficient source of explant for regeneration if shoot can be regenerated from root meristem. This can be achieved through the modification of PGRs within growth medium.

To date, there are limited reports of an ideal *in vitro* regeneration protocol of both rice and corn from SAM and no reports on RAM as explant. Therefore, the present study was undertaken to research avenues based on the following objectives.

- 1) To establish an efficient *in vitro* regeneration protocol for *indica* rice (*Oryza sativa* L. cv. MR219) and Thai Super Sweet Corn (*Zea mays* L. var. *rugosa*) by shoot and root apical meristem tissue.
- 2) To study the effects of plant growth regulators towards the *in vitro* regeneration of *indica* rice (*Oryza sativa* L. cv. MR219) and Thai Super Sweet corn (*Zea mays* L. var. *rugosa*) by shoot and root apical meristem tissue.

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