

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



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DOUBLED HAPLOIDS SYNTHETIC SEED PRODUCTION
IN LOCAL THAI RICE GENOTYPES BY
ANTHER CULTURE

PIYACHAI PREMVARANON

DOCTOR OF PHILOSOPHY
IN AGRONOMY

THE GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
FEBRUARY 2012

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**A THESIS SUMMITTED TO THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
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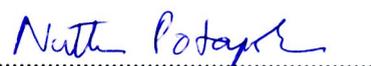
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28 February 2012

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อาหารเหลว LS ซึ่งประกอบด้วย KNO_3 ความเข้มข้น 10 ไมโครโมล 2,4-D ความเข้มข้น 2 มิลลิกรัมต่อลิตร NAA ความเข้มข้น 2 มิลลิกรัมต่อลิตร น้ำมะพร้าว 200 มิลลิลิตร และผงถ่านกัมมันต์ ความเข้มข้น 1 มิลลิกรัมต่อลิตร พบว่าสามารถชักนำให้เกิดเอมบริโอเจนิคแคลัสที่มีขนาด 4-5 มิลลิเมตรเป็นจำนวนมาก นอกจากนี้การเติมโคลชิซินที่ความเข้มข้น 0.2 กรัมต่อลิตร ร่วมกับ 2,4-D ความเข้มข้น 100 ไมโครโมลลงในอาหารสูตร LS มีประสิทธิภาพดีที่สุดในการผลิตต้นอ่อนที่เป็นดับเบิลแฮฟลอยด์ให้มีชีวิตรอดในปริมาณที่สูง (มากกว่า 70 %) ในระยะเวลา 8 สัปดาห์ และทำการเปลี่ยนอาหารเพาะเลี้ยงเพียง 2 ครั้ง โดยปราศจากการแปรปรวนทางพันธุกรรม เปรียบเทียบกับการเพาะเลี้ยงเนื้อเยื่ออับละอองเกสรแบบเดิมซึ่งใช้ระยะเวลาในการเพาะเลี้ยงมากกว่า 12 สัปดาห์ และต้องทำการเปลี่ยนอาหารเพาะเลี้ยงเนื้อเยื่อมากกว่า 4 ครั้ง หลังจากเคลือบโซมาติกเอมบริโอด้วย sodium alginate ความเข้มข้น 3% และ calcium chloride ความเข้มข้น 75 มิลลิโมล และทำการระเหยน้ำออกจากเมล็ดสังเคราะห์จนมีระดับการสูญเสียน้ำ 80 % พบว่าเปอร์เซ็นต์ความมีชีวิตยังสูงถึง 74 % หลังจากเก็บรักษาไว้ที่อุณหภูมิ $25 \pm 2^\circ\text{C}$ ในสภาพมีแสง 16 ชั่วโมงเป็นระยะเวลา 2 สัปดาห์ และสามารถงอกได้ภายใน 1 สัปดาห์

ดังนั้นงานวิจัยนี้จึงสามารถนำไปประยุกต์ใช้ในการผลิตต้นอ่อนดับเบิลแฮฟลอยด์ข้าวในปริมาณมากในระยะเวลาที่น้อยลงได้ด้วย

Thesis Title Doubled Haploids Synthetic Seed Production in Local Thai Rice Genotypes by Anther Culture

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Degree Doctor of Philosophy (Agronomy)

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ABSTRACT

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This research demonstrates the improve *in vitro* technique of double haploid production in *indica* hybrid rice by combining anther culture, hormone shock and doubling chromosome techniques to avoid somaclonal variation during culturing and reduce culturing time. The anthers of KDML 105 x SPR 1 (*indica x indica*) were cultured in developed Linsmaier and Skoog (LS) medium which contained macro nutrient concentration (KNO_3 , NH_4NO_3) growth regulators (2,4-D, NAA) and other organic compounds and then subcultured by inducing embryo-like structure (ELS) LS media . During 4 weeks used LS media supplemented with $10\mu\text{M KNO}_3 + 2\text{ mg L}^{-1}$ of 2,4-D + 2 mg L^{-1} of NAA + 20% coconut water + 1 mg L^{-1} of activated charcoal had induced high embryogenic frequent callus with length was 4-5 mm. Moreover, the supplements of 0.2 g L^{-1} colchicine and $100\mu\text{M 2,4-D}$ was the most

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had induced high embryogenic frequent callus with length was 4-5 mm. Moreover, the supplements of 0.2 g L^{-1} colchicine and $100 \text{ }\mu\text{M}$ 2,4-D was the most efficient in LS media. High number of viable double haploid ELS were produced (over 70 %) in 8 weeks and subcultured only twice without somaclonal variation compared with conventional anther cultured which take time more than 12 weeks and more than 4 times subcultured. After encapsulated somatic embryos with 3% sodium alginate and 75 mM calcium chloride and dehydration until they lost 80% of their moisture contents. The survival reaches 74 % after storage at $25 \pm 2^\circ\text{C}$, with 16 hours photoperiod for 2 weeks and germinate within 1 week.

This research can therefore be applied to produce a large amount haploid rice plantlet in shorten time to produce higher number of double haploid plantlets.

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ABBREVIATIONS AND SYMBOLS

ABA	=	Absciscic acid
BA	=	6-benzylaminopurine
° C	=	Degree of Celsius
2,4-D	=	2,4-Dichlorophenoxyacetic acid
cm	=	Centimeter
cv.	=	Cultivar
DW	=	Dry weight
EDTA	=	Ethylenediaminetetraacetate
e.g.	=	Exempli gratia (for example)
g	=	Gram
GA	=	Gibberellic acid
hrs	=	Hours
IAA	=	Indole-3-acetic acid
IBA	=	Indole-3-butyric acid
i.e.	=	Id est (it is or that is)
L	=	Liter
LS	=	Linsmaier and Skoog
mg	=	Milligram
mg/L	=	Milligram per liter
ml	=	Milliliter

mm	=	Millimeter
mM	=	Millimole
MS	=	Murashige and Skoog
NAA	=	α -Naphthaleneacetic acid
rpm	=	Revolutions per minute
UV	=	Ultraviolet (light)
v/v	=	volume/volume (concentration)
w/v	=	weight/volume (concentration)
%	=	Percent
μ M	=	Micromole