UNIVERSITI PUTRA MALAYSIA

GENETIC TRANSFORMATION OF RICE CULTIVARS USING PARTICLE BOMBARDMENT FOR SALINITY TOLERANCE

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GENETIC TRANSFORMATION OF RICE CULTIVARS USING PARTICLE BOMBARDMENT FOR SALINITY TOLERANCE

By

NWE NWE HTWE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

July, 2011

DEDICATED TO;

MY LOVELY FATHER, U KHIN MAUNG HTWE AND MOTHER,

DAW KHIN SAW CHIT,

BROTHERS AND SISTERS,

ALSO ALL MY TEACHERS AND FRIENDS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

GENETIC TRANSFORMATION OF RICE CULTIVARS USING PARTICLE BOMBARDMENT FOR SALINITY TOLERANCE

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July, 2011

Chairman: Professor Maziah Mahmood, PhD

Faculty: Institute of Bioscience

Rice is one of the major cereal crops and stable food for feeding more than half of world population. Now we are facing with some problems for rice production and it must be increased by 60% for next 20 years. Also 30% of rice growing area in the world contains high salt to allow normal yield. Salinity stress is severely limited the plant growth and productivity. The main problem in rice growth and productivity are unfavourably affected by salt stress. Na^+ and Cl^- derived from NaCl salts contaminate the soils, which are well known as the toxic ions that damage the plant cells in ion homeostatis and osmotic effect. Genetic transformation has widely been considered as a tool for crop improvement. Transient gene expression system is a critical requirement for target gene into cell and easy for analysing the function of a particular gene. This study was mainly aimed to establish a suitable *in vitro* culture system under salinity condition of selected five Malaysin rice genotypes as well as to introduce the delta 1 Pyrroline five carboxylase (P5CS) cDNA into rice genome using particle

bombardment. This present study has highlighted some interesting findings as described below:

Callus induction is one of the significant steps for selection of suitable genotypes and identifies the most suitable medium. In this study, five selected Malaysian rice genotypes were used and investigated for *in vitro* salt stress responses. All genotypes showed that the callus-growth capacities were significantly affected by the genotypes and the culture medium. Markedly, callus was best induced on the MS medium added with 10µM 2,4-D and 0.4gm/L casein hydrolysate. The shoot regeneration capacity was the most effective in half MS strength with 10µM BAP. The genotype, MR219-4, consistently performed the best in callus induction (93.5%) as well as in plant regeneration (27%). However under salinity condition, it showed a decline in callus growth, regeneration capacity and proline accumulation. This genotype can be a good model system for studying the genetic transformation.

For genetic transformation, antibiotic resistance genes are routinely used as powerful markers for selection of transformed cells. The minimun inhibitory level of hygromycin concentration was optimized for selected mutant rice line and it was showed that 21.3% calli survived on the medium containing 45 mg/L hygromycin. Transient expression of *gfp* and *gus* gene mediated by particle bombardment is rapid and provide useful approach for visual monitoring of genetic transformation. All the results indicated that *gfp* gene expression is superior to *gus* gene expression. The optimised conditions were bombardment once at a helium pressure of 1100psi, 6cm target tissue distance, 27 inHg vacuum pressure, 1µm gold particle size and 1.5µg DNA concentration per bombardment. These optimized conditions have been used to

obtain stably transformed explants for subsequent regeneration. Sixteen transgenic rice plants expressing *gfp gus* and *hptII* transgene were obtained from MR219-4 and transformation efficiency was 2.5% for *hptII, gfp* and *gus*. The pBIP5CS plasmid includng P5CS (Δ 1-pyrroline-5-carboxylate synthetase catalyses the first two steps of proline biosynthesis) cDNA encoding *hptII* gene conferring resistance to hygromycin was transformed to rice callus. The resultant primary transgenic plants showed more proline accumulation than non-transformed plant and transformation efficiency was 2.1%. Nine plants regenerated from hygromycin containing medium and were molecularly characterized by using PCR, RT PCR (Reverse transcriptase) and Southern Blot analysis. The result showed that P5CS cDNA had been integrated into six transgenic rice lines and proline level was increasing nine fold compared with non-transformed plants in 250mM NaCl stress. All these lines will be integrated into breeding programs for further assessment of their benefits.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TRANSFORMASI GENETIK KULTIVAR PADI MENGGUNAKAN PENEMBAKAN PARTIKEL UNTUK TOLERANSI SALINITI

Oleh

NWE NWE HTWE

July 2011

Pengerusi: Profesor Maziah Mahmood, PhD

Fakulti: Institut Biosains

Padi merupakan salah satu tanaman bijirin utama dan makanan ruji kepada lebih dari separuh populasi dunia. Kini, kita sedang menghadapi krisis dalam penghasilan padi di mana penghasilan padi perlu dipertingkatkan sebanyak 60% dalam masa 20 tahun yang akan datang. Dalam masa yang sama, 30% tempat penanaman padi di seluruh dunia mengandungi garam yang terlampau tinggi sehingga menjejaskan penghasilan. Tekanan saliniti akan menghadkan pertumbuahan pokok dan penghasilannya. Pertumbuhan padi dan produktivitinya dipengaruhi oleh tekanan saliniti. Na^+ dan Cl^- yang terhasil daipada NaCl mencemarkan tanah, merupakan ion-ion toksik yang boleh merosakkan sel tumbuhan dari segi homoestasis ionik dan juga osmotik. Oleh itu, transformasi genetik telah menjadi pilihan utama untuk proses penambahbaikan tanaman. Sistem ekspresi gen transien merupakan keperluan kritikal untuk merangsang pergerakan gen sasaran ke dalam sel dan mempermudahkan analisis fungsi gen tertentu. Kajian ini dijalankan untuk menghasilkan system kultur *in vitro* di bawah kondisi saliniti di dalam lima genotaip padi dari Malaysia dan juga untuk

memperkenalkan gen delta 1 Pyrroline five carboxylase gene (P5CS) ke dalam genom padi dengan menggunakan kaedah penembakan partikel. Kajian ini telah menunjukkan penemuan yang menarik seperti yang diterangkan di bawah:

Induksi kalus merupakan satu langkah yang signifikan untuk memilih genotaip dan medium yang paling sesuai. Dalam kajian ini, lima genotaip padi Malaysia digunakan dalam kajian tekanan saliniti secara *in vitro*. Kesemua genotaip menunjukkan kapasiti pertumbuhan kalus dipengaruhi oleh jenis genotaip dan jenis kultur media. Kadar induksi kalus didapati paling tinggi dalam medium MS yang dibekalkan dengan 10µM 2,4-D dan 0.4gm/L kasein hidrolisat. Kadar regenerasi pucuk pula didapati paling efektif di dalam media ½ MS yang dibekalkan dengan 10µM BAP. Genotaip MR219-4 menunjukkan kadar pembentukan kalus (93.5%) dan regenerasi pucuk (27%) yang tertinggi. Walaubagaimanapun, kedua-dua genotaip ini menunjukkan kadar pembentukkan kalus, kapasiti regenerasi dan pembentukan prolina yang rendah di bawah tekanan saliniti. Genotaip ini sesuai dijadikan sistem model bagi tujuan kajian transformasi genetik.

Dalam transformasi genetik, gen yang tahan terhadap antibiotik sering digunakan sebagai penanda yang kuat bagi memilih sel yang sudah mengalami transformasi. Kadar rencatan minimum kepekatan higromisin dioptimumkan untuk menentukan padi mutan terpilih dan menunjukkan 21.3% kalus yang rentan di dalam media yang mengandungi 45 mg/L higromisin. Ekspresi transien gen (*gfp*) dan (*gus*) yang dibantu oleh penembakan partikel merupakan kaedah yang cepat dan berguna bagi memerhatikan transformasi gen secara visual. Kesemua keputusan telah menunjukkan

gen *gfp* lebih efektif daripada gen *gus.* Keadaan yang optimum ialah penembakan sekali di bawah tekanan helium sebanyak 1100 psi, dengan jarak sasaran 6cm, tekanan vakum sebanyak 27 mmHg, saiz partikel emas 1µm dan 1.5µg DNA untuk setiap tembakan. Keadaan optimum ini telah digunakan untuk memperolehi eksplan yang sudah mengalami transformasi yang stabil untuk regenerasi berikutnya. Enam belas tanaman padi yang mengekspres *gfp*, *gus* dan transgen *hptII* telah diperolehi daripada MR219-4 dan keberkesanan transfomasi adalah 2.5% untuk *hptII*, *gfp* dan *gus*. Plasmid pBIP5CS mengandungi P5CS (enzim utama delta 1- pyrroline-5-carboxylate synthetase menjadi katalis dua langkah pertama sintesis prolin), gen yang mengkod *hptII* tahan terhadap higromisin ditransfom kepada kalus padi. Tanaman transgenik primer yang terhasil menunjukkan akumulasi prolin yang lebih berbanding tanaman yang tidak ditransfom di mana keberkesanan transformasi direkod sebanyak 2.1%. Sembilan tanaman telah diperolehi daripada medium yang mengandungi higromisin telah diperhatikan secara molekular dengan cara PCR, RT PCR dan analisa Southern Blot. Kandungan prolina didapati lebih di dalam tanaman transgenik di bawah tekanan saliniti sebanyak 250mM NaCl. Hasil keputusan menunjukkan cDNA P5CS telah diintegrasikan ke dalam enam padi transgenik dan kandungan prolin menunjukkan kenaikan sebanyak sembilan kali ganda berbanding dengan tanaman yand tidak ditransfom di dalam 250 mM tekanan NaCl. Kesemua tanaman ini akan diserapkan ke dalam program pembiakbakaan dengan tujuan penilaian faedah yang selanjutnya.

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APROVAL

I certify that an Examination Committee has met on 13.7.2011 to conduct the final examination of NWE NWE HTWE on her Doctor of Phyliosopy thesis entitled "GENETIC TRANSFORMATION OF RICE CULTIVARS FOR SALINITY TOLERANCE BY PARTICLE BOMBARDMENT." in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Phyliosopy.

Members of the Thesis Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Phyliosopy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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NWE NWE HTWE

Date: 13 July 2011

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