STUDY OF MICRORNA IN RELATION TO SALT TOLERANCE IN BANANA *(MUSA ACUMINATA SSP. MALACCENSIS)*

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ABSTRACT

Salinity is a major abiotic stress affecting crop productivity worldwide and results in millions of dollars in lost yield and damage to infrastructure. The purpose of this study was to identify microRNAs related to NaCl stress in *Musa acuminata ssp. malaccensis* (Ridl.). Sterilization techniques were employed to reduce contamination of embryos initiated from seeds. The germination rate of *in vitro* zygotic embryo culture was observed to increase to 92 % after soaking of the seeds in water (for periods of 2 or 3 days) compared to 72 % germination when no water treatment was used. Shoot induction and multiplications were carried out and 5mg/L BAP determined as the best concentration for shoot induction and multiplication. Different concentrations of sodium chloride (60, 80, 100, 120, 140, 160, 180, 200, 220 mM NaCl and 0 mM as a control) were used to determine the lethal concentration of NaCl and 120 mM was determined as the lethal concentration of NaCl for *Musa acuminata ssp. malaccensis.* The results were used to determine the stressed conditions for analysis of microRNAs and 100 mM of NaCl, the minimal inhibitory NaCl concentration for survival of banana plantlets was used for RNA extraction and construction of a salt stress small RNA library. Consequently measurement to assess the effect of salt stress on proline and elements (Na^+, K^+, Ca^{++}, Mg^{++}) accumulation in *Musa acuminata ssp. malaccensis* showed a clear correlation between increases in salt concentration and the concentrations of the different elements. The concentration of proline increased significantly in both root and leaves at 60 and 80 mM of NaCl concentration compared to the control with the highest amount of proline accumulation in both root and leaves recorded at 80 mM of NaCl. Elevation of NaCl concentrations also resulted in increasing Na^+ and decreasing K^+, Ca^{++} and Mg^{++} concentrations in *Musa acuminata ssp. malaccensis.*

The optimized and modified RNA extraction method developed in this study resulted in the achievement of good quality and pure RNA with A260/280of 1.95. MicroRNA Discovery™ Kit (System Bio Sciences, USA) was then used for the isolation of microRNAs and a microRNA library was constructed by cloning the PCR products into PCR4-TOPO cloning kit (Invitrogen, USA). The titre of the library was estimated as 2000 recombinant clones and the DNA sequences of inserts from 45 recombinant clones were determined. Data were analyzed *via* Clustal W, BLAST and psRNA Target comparing with NCBI Genebank, miRBase and EST sequences data bases. Matches to the small library sequences included three different types of gene sequences (Salt stress, drought stress and BAP treated related), with high similarity to salt stress related gene from *Oryza sativa* and *Hordeum vulgare,* drought stress and BAP treated related genes from *Oryza sativa*. Therefore it is suggested that these small RNA library clones may be homologues from *Musa acuminata ssp. malaccensis.* Also mac-miR393 was found as a potential salt stress related miRNA homologue in *Musa acuminata ssp. malaccensis.*

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ABSTRAK

Kadar garam adalah tekanan abiotik utama yang mempengaruhi produktiviti tanaman di seluruh dunia dan menyebabkan kehilangan berjuta-juta ringgit dan kerosakan infrastruktur. Tujuan dari penelitian ini adalah untuk mengenalpasti microRNAs berkaitan dengan tekanan NaCl pada *Musa acuminata ssp. malaccensis.* Teknik sterilisasi yang digunakan untuk mengurangkan pencemaran embrio bermula dari biji benih. Kadar percambahan in vitro kultur zigotik embrio diperhatikan meningkat menjadi 92% selepas perendaman biji benih dalam air (untuk tempoh hari 2 atau 3) berbanding dengan 72% percambahan bila pemprosesan dengan air tidak digunakan. Induksi tunas dan multiplikasi yang telah dilakukan dan 5 mg/L BAP ditentukan sebagai kepekatan terbaik untuk induksi tunas dan multiplikasi. Pelbagai kepekatan natrium klorida (60, 80, 100, 120, 140, 160, 180, 200, 220 mM NaCl dan 0 mM sebagai kawalan) digunakan untuk menentukan kepekatan yang mematikan dan 120 mM NaCl ditentukan sebagai kepekatan matian bagi *Musa acuminata ssp. malaccensis*. Hasilnya digunakan untuk menentukan keadaan tertekan untuk analisis microRNAs dan 100 mM NaCl (kepekatan hambat NaCl minimum untuk kelangsungan hidup planlet pisang) digunakan untuk ekstraksi RNA dan pembangunan tekanan garam kecil perpustakaan RNA. Akibatnya pengukuran untuk mengadar pengaruh tekanan garam pada akumulasi prolin dan elemen (Na^+, K^+, Ca^{++}, Mg^{++}) di *Musa acuminata ssp. malaccensis* menunjukkan hubungan yang jelas antara peningkatan kepekatan garam dan kepakatan unsur-unsur yang berbeza. Kepekatan prolin meningkat secara signifikan di akar dan daun pada 60 dan 80 mM kepekatan NaCl berbanding dengan kawalan yang tercatat jumlah akumulasi prolin pada akar dan daun tertinggi pada 80 mM NaCl. Peningkatan kepekatan NaCl juga mengakibatkan peningkatan kepekatan Na^+ dan penurunan K^+, Ca^{++} dan Mg^{++} pada *Musa acuminata ssp. malaccensis*.

Kaedah ekstraksi RNA yang optimum dan ubahsuai telah dibangunkan dalam kajian ini mengakibatkan tercapainya kualiti yang baik dan murni RNA dengan A260/280of 1.95. MicroRNA Discovery ™ Kit (Sistem Bio Sains, USA) kemudiannya digunakan untuk isolasi microRNAs dan perpustakaan MicroRNA dibina dengan pengklonan produk PCR ke dalam kit pengklonan PCR4-TOPO (Invitrogen, USA). Titer perpustakaan dianggarkan sebagai 2000 klon rekombinan dan urutan DNA sisipan dari 45 klon rekombinan ditentukan. Data dianalisis melalui Clustal W, BLAST dan psRNA Target dibandingkan dengan pangkalan data NCBI Genebank, miRBase dan urutan EST. Perpadanan dengan urutan perpustakaan kecil itu termasuk tiga jenis urutan gen (Berkaitan dengan tekanan garam, tekanan kekeringan dan pengendalian BAP), dengan kemiripan yang tinggi untuk gen berkaitan tekanan garam dari *Oryza sativa* dan *Hordeum vulgare*, gen berkaitan tekanan kekeringan dan pengendalian BAP dari *Oryza sativa*. Oleh itu klon perpustakaan RNA kecil ini dicadangkan mungkin adalah homolog dari *Musa acuminata ssp. malaccensis.* Juga mac-miR393 dijumpai sebagai potensi homolog miRNA berkaitan tekanan garam di *Musa acuminata ssp. malaccensis*.

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ABBREVIATIONS

Most of the abbreviations used are standard. However, attention is drawn to the following.

%	: percent
~	: approximately
°C	: degree Celsius
2-ip	: 2- isopentenyl adenine
ABA	: abscisic acid
BAP	: 6-bebzylaminopurine
bp	: base pair
C/I	: chloroform/isoamylalcohol
Ca	: calcium
cDNA	: complementary DNA
cm	: centimeters
CTAB	: cetyl trimethyl ammonium bromide
DCl1	: DICER-LIKE1
DEPC	: diethyl pyrocarbonate
dH_2O	: distilled water
DNA	: deoxyribonucleic acid
DNase	: deoxyribonuclease
dNTPs	: deoxyribonucleoside triphosphates
EDTA	: ethylenediaminteteraacetate
EST	: expressed sequence tags
FAO	: Food and Agriculture Organization
g	: gram

GSS	: genomic survey sequence
h	: hour/houers
HCl	: hydrochloric acid
IAA	: indoleacetic acid
K	: potassium
kb	: kilo base pairs = 1,000 bp
KN	: kinetin
L	: liter
m	: meter
Mg	: magnesium
µg	: microgram
mg	: milligram
min	: minute
µl	: microliter
ml	: mililitre
mM	: milimolar
mRNA	: messenger RNA
MS	: Murashige and Skoog media
N_2	: nitrogen
Na	: sodium
NAA	: 1-naphthaleneacetic acid
NaCl	: sodium chloride
NaOCl	: sodium hypochlorite
NaOH	: sodium hydroxide
ng	: nanogram
nm	: nanometer

nt	: nucleotide
OD	: optical density
PCR	: polymerase chain reaction
PTGS	: Post-Transcriptional Gene Silencing
Psi	: pounds per square inch
RISC	: RNA-induced silencing complex
RNA	: ribonucleic acid
RNase	: ribonuclease
rRNA	: ribosomal RNA
RT-PCR	: reverse transcription polymerase chain reaction
SD	: standard deviation
siRNA	: Small Interfering RNAs
TBE	: Tris-Borate-EDTA
TDZ	: thidiazuron
TGS	: Transcriptional Gene Silencing
TIGS	: Transient-Induced Gene Silencing
tRNA	: transfer RNA
UV	: ultra-violet
V	: voltage
v/v	: volume per volume
Var	: variety
VIGS	: Virus-Induced Gene Silencing
ZN	: zeatin

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