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PERFORMANCE OF CROSSES AND DEVELOPMENT OF DOUBLED HAPLOID LINES IN WHEAT (Triticum aestivum L.) FOR SALT TOLERANCE

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By

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February 2018

Chairman : Professor Siti Nor Akmar Abdullah, PhD
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Wheat (*Triticum aestivum* L.) is an important staple food crop for the world population. Despite its importance, the crop productivity is often affected by salinity stress. This study aimed to develop sodium chloride (NaCl) tolerant doubled haploid wheat using anther culture technique. The effect of different concentrations of NaCl (0, 50, 100, 150 and 250 mM) on seed germination and selected seedling growth traits of eight wheat genotypes was evaluated. The G1, G2, G6, G8, and G10 were classified as salt tolerant while Abo- Graib, Forat and, Dijla genotypes were salt susceptible. Increasing concentration of NaCl solution led to the gradual reduction in the seed germination percentage, and seedling growth traits. The three salt susceptible and one high salt tolerant genotypes (G8) were selected for crossing to obtain a total of six crosses. The agronomic performance of the six F_1 crosses was investigated under 0, 50, 100, 150 mM of NaCl. For all, grain yield exhibited positive correlation with spikes number/plant, grains number per spike and weight of 1000 grain. Abo-Graib×G8, Forat×G8, and Dijla×G8 showed superiority in grain yield (7.3 g/plant, 7.4 g/plant and 6 g/plant, respectively) under high concentration of NaCl (100 mM) related to the spikes number/plant, grains number per spike and the 1000 grain weight. Investigation on callus induction from anther culture of the F_1 progenies was performed. All F_1 produced a high percentage of callus (47.9%) when subjected to 33°C + chemical pre-treatment for 48 hours followed by culturing on CHB_3 induction medium containing Myo-inositol (300 mg/L), L-glutamine (993.5 mg/L) and 2 mg/L of both 2, 4-D and kinetin. The F_1 produced good percentage (45.6%) of green plants and the lowest percentage (6.8%) of albino plants after growing on R9 regeneration medium containing 0.076 mg/L $CuSO_4 \cdot 5H_2O$ and 90000 mg/L maltose following the pre-treatment of the anther with heat + chemical and culturing on CHB_3 callus induction medium. Abo-Graib×G8 displayed the highest ability for callus induction and green plants production (82.3% and 81.3%, respectively) and the lowest

percentage of albino plants (2%). The 883 regenerated plants from the F_1, 646 were green and 237 were albino plants. Among the green plants, 281 grew to maturity. Thirty-nine lines were fertile and their seeds were grown for developing salt tolerant doubled haploid lines. Haploid plant's chromosomes were doubled under colchicine treatment (0.2%) with dimethyl sulfoxide (DMSO) (2%) and 2-3 drops of Tween-20 for 4 hours at room temperature. The doubling of chromosomes was validated using a fluorescent microscope. Thirty-nine doubled haploid lines were evaluated under 0, 50, 100 and 150 mM of NaCl in the greenhouse. The doubled haploid lines DH6 obtained from (Abo-Graib×G8), DH8 (Forat×G8) and DH6 (Dijla×G8) showed a high performance due to their superiority in leaves K^+ content, K^+/Na^+ ratio, the spikes number, grains number, and the decrease in leaves Na^+ content. Taken together, it can be concluded that through anther culture, doubled haploid lines that displayed high yielding trait were developed for future exploitation.

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

PRESTASI KACUKAN DAN PEMBANGUNAN TITISAN HAPLOID GANDA DUA RINTANG NATRIUM KLORIDA DALAM GANDUM (*Triticum aestivum* L.)

Oleh

ABBAS LATEEF ABDULRAHMAN

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Gandum (*Triticum aestivum* L.) merupakan makanan ruji utama kepada populasi dunia. Walaupun penting, produktiviti tanaman sering dipengaruhi oleh tekanan kemasinan. Penyelidikan ini telah dilakukan untuk menghasilkan jenis gandum yang toleran pada natrium klorida melalui teknik kultur anter. Kesan perbezaan kepekatan NaCl (0, 50, 100, 150, 200, dan 250 mM) pada percambahan biji benih dan pada sebahagian ciri-ciri pertumbuhan anak benih dari lapan genotip gandum telah dikaji. Genotip G1, G2, G6, G10 telah dikelasifikasikan sebagai toleran garam tahap sederhana manakala Abo- Graib, Forat, dan Dijla sensitif garam. Peningkatan kepekatan larutan NaCl membawa kepada pengurangan secara beransur-ansur pengambilan air, peratus percambahan biji benih dan ciri-ciri pertumbuhan anak benih. Tiga varieti gandum sensitif garam dan satu kultivar toleran garam (G8) telah dipilih untuk kacukan untuk mendapatkan sejumlah 6 kacukan. Pencapaian agronomik enam kacukan telah dikaji pada tiga kepekatan (0, 50, 100, 150 mM) NaCl. Kesemuanya menunjukkan korelasi positif dengan bilangan/tumbuhan, bilangan bijian setiap spike dan berat 1000 bijian. Abo-Graib×G8, Forat×G8, dan Dijla×G8 menunjukkan keunggulan di dalam hasil bijian (7.3 g/tumbuhan, 7.4 g/tumbuhan dan 6 g/tumbuhan) di bawah kepekatan tinggi NaCl (100 mM) berkaitan dengan bilangan spike/tumbuhan, bilangan bijian pada setiap spike dan berat 1000 bijian. Kajian mengenai induksi kalus dari kultur anter progeni F_1 telah dijalankan. Semua kacukan F_1 yang dikaji telah menghasilkan peratusan kalus yang tinggi (47.9%) apabila dikenakan suhu 33°C + pra-rawatan kimia selama 48 jam diikuti dengan pengkulturan pada media induksi kalus CHB_3 yang mengandungi Myo-inositol (300 mg/L), L - glutamine (993,5 mg/L) dan 2 mg/L kedua-dua 2, 4-D dan kinetin. F_1 telah menghasilkan peratusan tumbuhan hijau yang baik (45.6%) dan peratus terendah (6.8%) tumbuhan albino selepas tumbuh di media regenerasi R9 yang mengandungi 0.076 mg/L $CuSO_4 \cdot 5H_2O$ dan 90000 mg/L maltosa diikuti dengan pra-rawatan anter

dengan haba + kimia dan pengkulturan pada media induksi kalus CHB_3. Abo-Graib×G8 mempamerkan keupayaan tertinggi bagi induksi kalus dan penghasilan tumbuhan hijau (masing masing 82.3% dan 81.3%) serta peratusan terendah tumbuhan albino (2.0%). Daripada 883 tumbuhan yang tergenerasi dari F_1, 646 adalah tumbuhan hijau dan 237 adalah tumbuhan albino. Di antara tumbuhan hijau, 281 tumbuhan sahaja yang membesar hingga matang. Tiga puluh sembilan tumbuhan didapati subur dan menghasilkan anak benih titisan haploid ganda dua tahan garam. Kromosom tumbuhan haploid ini telah meningkat dua kali ganda di bawah rawatan colchicine (0.2%), DMSO (2%) dan 2-3 titis tween-20 selama 4 jam pada suhu bilik. Pengesahan jumlah kromosom titisan haploid ganda dua ditentukan dengan mengira bilangan kromosom di bawah mikroskop fluoresen. Tiga puluh sembilan DH telah dinilai di bawah 0, 50, 100 dan 150 mM NaCl di dalam rumah hijau. Titisan DH6 (Abo-Graib×G8), DH8 (Forat×G8) dan DH6 (Dijla×G8) menunjukkan prestasi yang tinggi dan melampaui batas untuk kandungan K^+ pada daun, nisbah K^+/Na^+, bilangan spike setiap tumbuhan, jumlah bijirin setiap spike, dan penurunan dalam kandungan Na^+ pada daun. Secara keseluruhannya, ia boleh disimpulkan bahawa melalui kultur anter, titisan haploid ganda dua yang memaparkan hasil yang tinggi telah berjaya dihasilkan untuk dieksploitasi dimasa hadapan.

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I certify that a Thesis Examination Committee has met on 30 January 2018 to conduct the final examination of Abbas Lateef Abdulrahman on his thesis entitled "Performance of Crosses and Development of Doubled Haploid Lines in Wheat (*Triticum aestivum* L.) for Salt Tolerance" 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 Philosophy.

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ANOVA	Analyses of Variance
BAP	Benzylaminopurine
bp	base pair
C1	The initial Conc. (mM) of stock solution
C2	Final concentration
CHB3	Callus induction medium
2, 4-D	2, 4-Dichlorophenoxyacetic acid
DAPI	4'-6-diamidino-2-phenylindole HCl staining
df	Degree of freedom
DH	Doubled haploid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC	Electrical Conductivity
ETOH	Ethyl Alcohol
FDA	Fluorescein diacetate
FGP	Final germination percentage of seeds
GY	Grain yield
HIR	High haploid induction rate
2-HNA	2-Hydroxynicotinic acid
K^+	Potassium ion
K^+/Na^+	Ratio of Potassium ion to Sodium ion
LSD	Least significant difference
mg/L	Milligrams per liter

mM	Mill molar
Mpa	Megapascal
MS	Murashige and Skoog medium
N	Total number of seeds
Na^+	Sodium ion
NAA	Naphthalene acetic acid
NaCl	Sodium Chloride
Ni	Number of germinated seed
NG	Number of grain
NS	Number of spikes
PCR	Polymorphism chain reaction
PH	Plant height
pH	Negative logarithm of the hydrogen ion concentr ation
QTL	Quantitative trait locus
RCBD	Randomized Complete Block Design
S.O.V	Source of variance
STTIs	Salt tolerance traits indices
TBE	Tris/Borate/EDTA buffer
V1	Initial Volume
V2	Final volume
W1	Initial weight of seed
W2	Weight of seed after water uptake at a particular time
WG	Weight of grain

CHAPTER 1

INTRODUCTION

In the developing countries, around 2.5 billion people depend on wheat (*T. aestivum*) as their food source (FAOSTAT, 2010). For them, wheat is a staple commodity whose production involves around 200 million farmers and their families. Production of wheat and its yield directly impact human existence in developing nations and quality of life in industrial economies. From 1995 to 2013, a 1.1% (Dixon et al., 2009) to 0.9% (Ray et al., 2013) yearly rise in productivity was noted in around 85% of 20 wheat producing nations. Projections based on this growth rate indicated that wheat productivity would rise 17% by 2025, which is lower than the required 25% to feed the projected populace at that time (William et al., 2008). More improvements in wheat productivity can be accomplished by utilizing the land resources that are at present unfeasible to produce crops such as wheat because of the surplus of salts and other abiotic stresses.

It is projected that around 60% rise in cereal production (Rosegrant and Cline, 2003; Long et al., 2015) and 38% in wheat production (Ray et al., 2013) is necessary to meet the global demand by the year 2050. This situation highlights that food security is still the most fundamental concern despite advancement and deployment of advanced technologies. Selection of varieties using conventional technique needs many generations of selection which may take more than ten years to produce a variety (Mansouri et al., 2005; Breseghello and Coelho, 2013). However, through the procedure of *in vitro* culture, the time required for the creation of new lines can be significantly shortened by taking benefits of existing genetic variation (Maheswary and Mak, 1993; Tadesse et al., 2012). In addition to conventional breeding techniques used for wheat development (Su et al., 1992; Wilkinson et al., 2012), the anther culture technique could be used as an untraditional supplementary technique (Santra et al., 2012). *In vitro* anther culture exhibits a fast technique for production of true breeding lines in the next generation from any segregated species (Marassi et al., 1993; Yan et al., 2017) and promoting homozygosity (Smith and Drew, 1990; Asif et al., 2014). Breeding researchers showed that the development of doubled haploid (Mohiuddin et al., 2006; Ma'arup et al., 2012) to achieve homozygosity allows the possibility for crop improvement in producing the wanted trait at a faster time through minimizing the breeding processes (Hassawi et al., 2005; El-Hennawy et al., 2011).

For a successful breeding programme using the microspores for an increased doubled haploid production, it is important to use genotypes having higher regeneration capacity. Several studies stated that the anther culture is heritable through crossing (Grauda et al., 2010; Nielsen et al., 2015). However, since the varieties are derived from several genetic sources, it is possible to find good genotypes in unselected crosses or genetic lines (Govindaraj et al., 2015).

Stress treatment for inducing embryogenesis is a prerequisite for the success of anther culture. Different stresses have been applied including physical, physiological and chemical stresses of excised spikes, anthers or microspores for inducing androgenesis (Zoriniants et al., 2005; Echavarri and Cistue, 2016). In general, it seems that stress performances by applying the pretreatment lead to the change from the gametophytic pathway to the saprophytic pathway (Rakha et al., 2012).

Production of albino plants often limits the capability of anther culture from cereals such as wheat (Lantos et al., 2013). Therefore, it is important to provide a suitable environment which is necessary for the development and proliferation of the embryogenic microspores and production of embryos. Auxiliary factors during this stage may include improved physical conditions and appropriate elements in the media particularly glutamine (amino acid), myo-inositol, sulfate pentahydrate and maltose (Ekhveh et al., 2013). For the development of high grain yielding-salt tolerant wheat genotypes, it is hypothesized there would be tolerant genotypes for different concentrations of sodium chloride for selection. With some chosen wheat genotypes known to be high grain yield- or salt tolerant, it is hypothesized that F_1 crosses that are both high yielding and tolerance to salinity could be developed by crossing among these selected genotypes. Development of pure lines using self-pollinating crops needs many generations after making a cross to obtain homozygosity and uniformity to be able to evaluate of inherited traits such as yield and quality. However, doubled haploids can acquire uniformity in short generations. Therefore, it is hypothesized that high grain yielding-salt tolerant doubled haploid lines of wheat using anther culture technique can be developed from F_1 crosses. The objectives of this study were to

1- Study the effect of salinity on seed germination percentage and seedling growth traits of eight bread wheat genotypes (*Triticum aestivum* L.) and select the best genotypes for the development of sodium chloride tolerant progenies
2- Obtain F_1 crosses by crossing among genotypes based on their high yielding and salt tolerant traits and evaluate their growth and grain yield performance under different concentrations of NaCl
3- Develop high grain yielding-salt tolerant homozygous pure lines derived from segregating populations of heterozygous F_1's and evaluate their agronomic performance under salt stress conditions to select the superior salt tolerant high grain yielding lines

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LIST OF PUBLICATIONS

Abbas Lateef Abdulrahman, Siti Nor Akmar Abdullah, Mohd Rafii Yusop, Mohd Razi Ismail and Mahdi Moradpour. 2016. Seed germination and seedling growth of hexaploid wheat (*Triticum aestivum* L.) varieties as influenced by different levels of sodium chloride. *Res. on Crops* 17 (3): 445-453 (2016).

Abbas Lateef Abdulrahman, Siti Nor Akmar Abdullah, Mohd Rafii Yusop and Mohd Razi Ismail. 2016. Role of wheat germplasm, anther pre-treatments, and medium on the production of doubled haploid plants. *Transactions of Persatuan Genetik of Malaysia*, No 3, pp. 113-122, 2016.

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