STUDIES TO INVESTIGATE THE EFFECTS OF NITROGEN SOURCES ON ANDROGENESIS AND CARBON SOURCES ON SHOOT REGENERATION OF AROMATIC AND NON-AROMATIC TYPES IN *INDICA* RICE (*Oryza sativa L*).

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Dedicated to my Parents, my relatives and my friends.

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ABSTRACT

Indica rice is reported to be recalcitrant to *in vitro* techniques including anther culture. The effect of nitrogen sources in culture media at callus induction stage and sources of carbon at the regeneration stage were investigated. Two aromatic cultivars (SRI12 and Basmati 385) and two non-aromatic cultivars (SK282 and IR36) have been evaluated to androgenesis response. Aromatic cultivars responded well to androgenesis, while non-aromatic cultivars did not respond to androgenesis. The best response to callus induction rate and callus productivity have recorded of SRI12 and Basmati 385 cultivars (CR = 7.32%, 3.59%) and (CP = 4.37%, 1.88%) respectively. However, the effectiveness of androgenesis was improved in modified RZ media by using a higher level of nitrate ion ($N0_3^-$) from KNO_3 with a lower level of ammonium ion (NH_4^+) from $(NH_4)_2SO_4$. The highest value of callus induction rate, callus productivity and responsive anthers have achieved to SRI12 and Basmati 385 cultivars (CR = 14.84%, 9.52%), (CP = 8.61%, 4.62%) and (RA = 5.34%, 3.33%) respectively, when the highest level of nitrate ion ($N0_3^-$) with the lowest level of ammonium ion (NH_4^+) were utilized. The effect of saccharide (sucrose and maltose) on shoot regeneration was investigated in MS media. Sucrose media enhanced shoot induction to SRI12, The values of regeneration frequency and regeneration productivity were achieved (61.89 %, 76.14 %) respectively. However, maltose media promoted shoot induction to Basmati 385 cultivar, the values of regeneration frequency and regeneration productivity were obtained (75%, 125%) respectively. The results indicated that using the highest level of nitrate ion with the lowest level of ammonium ion is useful to enhance androgenesison response. However, the effect of saccharide on shoot regeneration is genotypes depended on recalcitrant *indica* cultivars.

ABSTRAK

Padi *Indica* dilaporkan tekalistran semua teknik *in vitro* termasuklah kultur anter. Kesan nitrogen dalam media kultur keatas pembentukan kalus dan karbon pada pertumbunan semula telah dikenalpasti. Dua jenis aromatic (SRI12 dan Basmati 385) dan 2 jenis bukan aromatic (SK282 dan IR36) telah dikenalpasti dengan tindak balas androgenisis. Jenis aromatic bertindak balas dengan baik sementara jenis bukan aromatic tidak bertindak balas dengan androgenesis. Tindak balas yang terbaik pada kadar pembentukan kalus dan penghasilan kalus telah direkodkan dengan jenis SRI12 dan Basmati 385 yang masing-masingnya (CR =7.32%, 3.59%) dan (CP = 4.37%, 1.88%). Namun, keberkesanan androgenesis di dalam media RZ yang telah dimodifikasi telah dapat dipertingkatkan dengan menggunakan paras ion nitrat (NO_3^-) yang lebih tinggi daripada kalium nitrat dan paras ion ammonia (NH_4^+) yang lebih rendah daripada ammonia sulfida. Nilai tertinggi kadar pembentukan kalus, penghasitan kalus, dan tindak balas anter telah dicapai bagi jenis SRI12 dan Basmati 385 dengan masing-masingnya (CR=14.84%, 9.52%) dan (CP=8.61% dan 4.62%) serta (RA= 5.34%, 3.33%) apabila menggunakan ion nitrat (NO_3^-) pada paras yang tertinggi dengan ion ammonia (NH_4^+) pada paras yang terendah. Kesan sakarida (sukrosa dan maltosa) pada pertumbuhan semula pucuk telah dikenalpasti dalam media MS. Media sukrosa menggalakkan induksi pucuk SRI12. Nilai-nilai frekuensi pertumbuhan semula dan pertumbuhan semula produktiviti telah dicapai dengan masing-masingnya (61.89%, 76.14%). Adapun, media maltose menggalakkan pertumbuhan semula jenis Basmati 385 dengan nilai frekuensi penjanaan semula dan penjanaan semula produktiviti yang masing-masingnya (75%, 125%). Keputusan menunjukkan bahawa dengan menggunakan paras tertinggi ion nitrat dengan paras terendah ion ammonia berhasil dalalam menggalakkan tindak balas androgenesis. Namun, kesan sakarida pada pertumbuhan semula pucuk bergantung kepada genetic sepsis *indica* tersebut.

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LIST OF ABBREVIATION

2,4D	2, 4 dichlorophynoxy acetic acid
BAP	6-benzylaminopurine
NAA	naphthalene acetic acid
K	Kinetin
CIR	Callus Induction rate
CP	Calls Productivity
CIM	Callus induction media
RA	Responsive anthers
RP	Regeneration productivity
RF	Regeneration frequency
PRM	Plant regeneration media
KNO_3	Potassium nitrate
$(NH_4)_2SO_4$	Ammonium Sulfate
MS+M	Murashige and Skoog (1962) medium + maltose
MS+S	Murashige and Skoog (1962) medium + sucrose
RZ	Raina and Zapata (1997) medium
µmol	Micromole

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Rice (*Oryza sativa L.)* is an important crop in the world which has the ability to become the food staple for half of the world's population. It has a high amount of the calories necessary for human diet;and in Asian countries makes up around 40-80 % of the normal diet. Although Asian countries have the vital climate to produce up to 90 % of rice yields worldwide, this is however not the case. Presently, the rice production in the world has been raised to 650 million tones and the area cultivation of rice is estimated at 156 million hectares. Rice is recognized as the third of the most economical crops in the world (Abdullah *et al.*,2008). Asian countries are the biggest consumers of rice with over 75% of rice yield consumed and India has the largest rate of rice cultivation of around 128 million tones for the year 2012. The rate of rice consumption has increased by 1.8 percent per year, in contrast the rate of rice yield is decreased (Hussain *et al.*, 2010).

Rice is the main food crop in the world, increasing rice production to support food security has therefore been given a high priority in Agriculture development and Biotechnology where a breeding program to encourage higher yields. Anther culture technique is used to produce new varieties of rice that have higher yields and efficient stability with genetic improvement. Biotechnology supports breeders to obtain their targets more quickly and efficiently through anther culture method through rapid fixation of homozygosity and shortening of breeding cycle that has

been successfully applied to improve and develop new rice varieties especially in China and Korea (Shahnewaz and Bari, 2004). Anther culture is the biotechnology technique that is used for the production of pure lines (Cha-um *et al.,* 2009). It is also important to produce variation because it supports the early expression of the recessive genes (Qi *et al.,* 2011). Anther culture is beneficial to the distinct acceleration of the selection process because it has a high proportion of homozygosity or pure lines which can be achieved at first regeneration in a single step (Purwoko *et al*., 2007). An important aspect of plant breeding is the induction of maximum genetic variability of germplasm sources to secure a wider scope for selection and introduction of better trait qualities in existing crop species (Razdan, 2003).

Haploid plants are recognized by the existence of only one set of chromosomes in their cells, in nature haploids occur as an irregularity when the haploid egg forms an embryo without fertilization .Haploids are sexually sterile and therefore, doubling of chromosomes is required to produce fertile plants which are called double haploids or homozygous diploids, Several scientists have successfully produced callus and haploid plants through anther culture and isolated pollen/anther from achieved plant (Davey and Anthony, 2010).

The natural occurrence of haploid plants has been reported in several species including wheat, maize, tobacco and rice and the efforts still continue to improve the frequency of haploids in plant breeding. The methods for haploid plant production are: Androgenesis which has anther culture and isolated microspore; Genogenesis when utilizing unfertile ovules; wide hybridization crosses and parthenogenesis when the embryo develops from any haploid cell of the embryo sac. But the choice of the methods of the haploid plant production depends on species, genotypes, genetic stability of doubled haploid plants and the efficiency of the generation (Donpalmer and Keller, 2005).

The sporophytes have the gamete chromosome number which is (n instead of 2n) and the producing doubled haploid plants in the comparison with diploid plants (2n=2x) are haploid plants and the androgenesis is one of the outstanding examples of the culture totipotency (Germanà, 2011). Regeneration through male gametes has

already applied for more than 200 species especially recalcitrant genotypes (Dunwell, 1986).

Using pollen parents from anther culture in rice breeding can reduce breeding time, increase selection efficiency, save space and labor in the field by allowing selection of fine strains in early generation (Hearth *et al.*, 2007).The androgenic haploid plants were achieved for the first time by Guha and Maheshwari in 1964. Haploids can be produced by different systems but most capable and successful one is microspore androgenesis (Shahnewaz and Bari, 2004).

Nowadays, anther culture tool is used commonly as a breeding tool for *Japonica* varieties while this technique still is needed to be exploited in *Indica* varieties because of the limitation of androgenesis response (Silva, 2010).

1.2 Problem Background

Indica rice includes aromatic and non aromatic types and are very common crops in India and Pakistan. They are very high priced in the markets and are therefore valuable. The famous grain quality and delicate aroma are well known throughout the world. *Indica* varieties are photoperiod sensitive and vulnerable to all common diseases by bacteria, fungi, viral, insects, pests and abiotic stress such as drought and salinity and the poor yield production. Breeding efforts to improve the yield potential of Basmati rice varieties has resulted in limited success because Basmati rice is poor combiners and their crosses with dwarf *Indica* rice parents produces poor plant type progenies (Bishnoi *et al.*, 2000).

The successful proportion of haploid production depends on some critical factors that including genotypes, physiology of donor plants, pollen grain stages, cultivation condition of plants, culture media and pre-treatments (Herath *et al.*, 2007).

The major problems of *indica* rice varieties are early anther necrosis, poor callus induction and high percentage of albino plant regeneration (Bishnoi *et al.*, 2000). Using anther culture method is to limited inbreeding and genetic research due to poor regeneration frequency of cultured anthers (Herath *et al.*, 2007). The study purposes to regulate the effects of nitrogen source in the media on the anther response of recalcitrant *indica* genotypes and show the influence of different carbon source on shoot induction of the genotypes.

The use of growth hormone regulators is damaging since the diploid or polyploid cell will be initiated; also non- genetic factors can also affect the efficiency of anther response in recalcitrant *indica* genotypes such as culture media, pre-treatment because the media makes differences in the rate of success in callus formation ability in *japonica* and *indica* genotypes (Silva and Ratnayake, 2009).

The effects of medium composition and plant hormones of the culture medium are important factors in rice tissue culture; high auxin and cytokinin concentration used for callus induction while low concentration of auxin and cytokinin used for plantlet regeneration this depends on available lectures (Rafique *et al.*, 2011). Growth of plant cultures may also be restricted in media containing high concentration of ammonium ion even higher concentration of nitrate ion presented at the same time (Mott *et al*., 1985).

The genetic control of *in vitro* response of the anther culture is multifaceted this may become a limiting possibility for improving high response through breeding. Although there are lots of non-genetic factors such as incubation temperature, light dencity and culture media can be improved to obtain high frequency of callus induction and plant regeneration (Silva *et al.*, 2010).

The presence ratios of NH_4^+ : $N0_3^-$ in media namely 20:40, 30:30 and 40:20 has the best response of cell multiplication and shoot formation while the further increase in the concentration of either NH_4^+ or NO_3^- (ratio at 10:50 and 50:10) leads to significant decrease in callus induction and shoot proliferation in rice plant. However, the ratios (0:60 and 60:0) resulted in a lower multiplication rate (Ivanova and Staden, 2009).

1.3.1 Aim of the Study

The aim of this study is to reveal the effects of nitrogen sources of media on androgenesis of aromatic and non-aromatic cultivars in *indica* rice. Also the study will be concerned with showing the response of aromatic and non-aromatic cultivars in *indica* rice to callus formation and the effects of different carbon sources on shoot induction.

1.4 Objectives of Study

- To examine the response of aromatic and non-aromatic *indica* rice types to androgenesis.
- To study the effects of nitrogen source on callus induction.
- To investigate the effects of carbon sources on shoot induction in *indica* rice.

1.5 Significant of Study

The study paves the way to finding the suitable concentration of inorganic nitrogen sources to enhance the frequency of callus induction. Moreover, the effects of carbon sources in regeneration media are investigated. Appropriate combination of inorganic sources of nitrogen at callus induction stage and carbon source at the regeneration stage will lead to enhancing the response of *indica* rice to anther culture.

1.6 Scope of Research

This research focuses on using androgenesis anther culture as a powerful tool for breeding and producing high frequency of haploid plant at shortening time. This research optimizes the concentration of NH_4^+:NO_3^-in callus induction media to enhance the response of androgenesis in *indica* rice. Nitrogen sources which consist of Potassium nitrate (KNO_3) and ammonium sulfate $(NH_4)_2SO_4$ (NH_4^+:NO_3^- : 1:9, 3:7, 5:5, 7:3) in RZ media (Raina and Zapata, 1997). However, The MS (Murashige and Skoog, 1962) media will be used for shoot induction of anther rice stage, adding sucrose and maltose separately as two treatments in MS media and evaluate the effects of each of these carbon sources on the frequency of shoot regeneration. This research will contribute by using different concentration of ammonium ion and nitrate ion as nitrogen source for callus induction stage and analyze the effects of carbon source such as sucrose and maltose on frequency of shoot regeneration. This study will also contribute to the analyzing of the response of aromatic and non-aromatic *indica* cultivars in androgenesis response in culture media.

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