FLOWERING SYNCHRONIZATION AND PANICLE ELONGATION OF CYTOPLASMIC MALE STERILE RICE LINES DURING SEED MULTIPLICATION

By

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Chair : Associate Professor Phebe Ding, PhD
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Rice (*Oryza sativa* L.) is a staple food of most Malaysia because it has good source of energy that contains nutrients, vitamins and minerals. It is estimated that Malaysia population will increase to 43 million in 2050 thus; rice production needs to be increased too. Producing hybrid rice is one of the options that can be considered because it has been successfully adopted in most of rice producer countries in Asia such as China, Thailand, Vietnam and India. Hybrid rice needs to be produced for growers to plant. However, poor cross-pollination or out-crossing between parental lines is the major constraint in hybrid rice seed production. The low out-crossing is due to poor flowering synchronization between parental lines and also poor panicle exsertion of cytoplasmic male sterile (CMS) line. Improper flowering synchronization may reduce number of seed set because probabilities of floret to be out-crossed during flowering period are low. Hence, the objectives of this study was to improve the out-crossing rate and increase the grain yield of the multiplied CMS during CMS seed multiplication through utilization of seeding date interval for proper flowering synchronization and application of exogenous gibberellic acid to improve the panicle exsertion rate of the CMS. In the first study, floral traits and flowering behaviours of two locally developed CMS and maintainer lines were observed namely 0025A/0025B and 0047A/0047B. The observation shows that the female reproductive parts of 0025A CMS lines such as stigma length, stigma breadth and stigma area were 30.32-69.50% longer, 80-140% wider and 135.1-217.4% larger than 0025B maintainer. Stigma length, stigma breadth and stigma area of 0047A CMS were also 83.3-100% longer, 147.6-152.6% wider and 8.7-31.6% larger than 0047B maintainer. These characteristics are essential because more pollen grains are expected to be deposited on the stigma of the CMS. The 0025B maintainer had 34.0-177.86% longer

filament than their respective CMS thus indicating that long filament increased the possibilities of pollen grains to deposit on the stigma. The on-set of flowering of 0025A CMS and 0025B maintainer was 63-69 and 63-67 days after transplanting (DAT), respectively, thus indicating that the flowering period of both parental lines may be synchronized and the seed can be sown concurrently. However, the on-set of flowering between 0047A CMS and 0047B maintainer was significantly different ($P<0.05$). Hence, staggered sowing needs to be done for these two parental lines in order to get proper flowering synchronization. Six seeding date intervals were implemented on the maintainer lines in order to get proper flowering synchronization between parental lines. Result shows that sowing the 0025B maintainer at 2 days prior and 6 days after the CMS was sown significantly reduced the number of tillers per sq m by 18.99-20.39 and 13.17-17.94%, respectively. The grain yield was also decreased by 20.59-45.83 and 127.78-150.00% when the 0025B maintainer was sown at 2 days prior and 6 days after the 0025A CMS was sown, respectively. The 0047B maintainer has to be sown at 4 and 6 days after the 0047A CMS was sown because these seeding date interval significantly affected the number of tillers per sq m, out-crossing rate, grain yield and harvest index of the 0047A CMS ($P<0.05$). Four concentrations of gibberellic acid (GA_3) were applied to both CMS and maintainer lines when 10% of the panicles emerged out from the sheath. Result shows that application of GA_3 at the concentrations of 15, 30 and 45 mg/L significantly improved the plant height of 0025A CMS by 7.8-13.7, 10.8-17.0 and 13.9-16.5%, respectively. The grain yield of 0025A CMS was also increased by 71.4-433.3, 450.0-500.0 and 116.5-125.0%, respectively. Selected seed qualities such as germination rate and germination index of 0025A CMS were also improved by 48.77-97.53 and 100-150%, respectively, as GA_3 at the concentrations of 15, 30 and 45 mg/L were applied to the plants. Application of GA_3 was found affecting some of the changes in the internode of the CMS. Forty five mg/L GA_3 is not recommended to be used in CMS seed multiplication because it is related to lodging incidence. Information on the floral traits and flowering behaviours of the CMS and maintainer lines may be useful for the breeders and rice physiologist to improve certain traits of the parental lines. An appropriate concentration of GA_3 found in this study may be adopted by industry takers such as seed producer in order to minimize the cost with better yield performance.

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

PENYERAGAMAN PEMBUNGAAN DAN PEMANJANGAN TANGKAI TITISAN-TITISAN PADI SITOPLASMA MANDUL JANTAN SEMASA PENGGANDAAN BENIH

Oleh

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Padi (*Oryza sativa* L.) adalah makanan asas kebanyakan rakyat Malaysia kerana ia merupakan sumber tenaga yang mengandungi nutrien, vitamin dan mineral. Populasi rakyat Malaysia dijangka meningkat sehingga 43 juta orang menjelang tahun 2050; maka pengeluaran padi perlu juga ditingkatkan. Penghasilan padi hibrid merupakan satu pilihan yang perlu dipertimbangkan kerana ia telah berjaya digunapakai di kebanyakan negara-negara pengeluar padi di Asia seperti China, Thailand, Vietnam dan India. Benih padi hibrid perlu dihasilkan kepada penanam untuk ditanam. Bagaimanapun, kelemahan kacuk silang di antara titisan-titisan baka induk merupakan halangan utama di dalam pengeluaran benih padi hibrid kerana ia mempengaruhi hasil. Kacukan silang yang rendah adalah disebabkan oleh kelemahan penyeragaman berbunga di antara baka induk dan juga juluran tangkai sitoplasma mandul jantan (CMS) yang rendah. Ketidakaturan penyeragaman berbunga boleh mengurangkan pembentukan benih kerana kebarangkalian floret untuk dikacuk semasa pembungaan adalah rendah. Maka, objektif kajian ini adalah untuk menambahbaik kadar kacuk silang titisan CMS semasa penggandaan benih CMS melalui penggunaan selang hari pembenihan untuk penyeragaman berbunga dan aplikasi hormon giberelik luaran untuk meningkatkan juluran tangkai CMS. Di dalam kajian pertama, ciri-ciri bunga dan sifat pembungaan dua titisan CMS dan baka penyara tempatan iaitu 0025A/0025B dan 0047A/0047B diperhatikan. Pemerhatian menunjukkan bahagian reproduktif betina CMS 0025A seperti panjang stigma, lebar stigma dan luas stigma adalah masing-masing 30.32-69.50% lebih panjang, 80-140% lebih lebar dan 135.1-217.4% lebih besar daripada induk penyenggara 0025B. Ciri-ciri ini adalah penting kerana dijangka lebih banyak debunga akan mendarat di stigma induk CMS. Induk penyenggara 0025B mempunyai 34.0-177.86% filamen yang lebih panjang berbanding induk CMS yang berkenaan justeru menunjukkan filamen yang panjang meningkatkan kebarangkalian pendaratan debunga di stigma. Permulaan pembungaan induk CMS 0025A dan induk penyenggara 0025B adalah masing-masing 63-69 dan 63-67 hari selepas tanam, justeru

menunjukkan tempoh berbunga bagi kedua-dua induk mungkin seragam dan benih kedua-dua induk boleh ditanam serentak. Bagaimanapun, permulaan berbunga di antara induk CMS 0047A dan penyenggara 0047B adalah berbeza secara bererti ($P<0.05$). Maka, taburan benih secara berperingkat perlu dilakukan bagi kedua-dua induk bagi mendapatkan pembungaan seragam. Enam selang masa pembenihan diuji pada induk penyara bagi mendapatkan masa pembungaan yang seragam. Keputusan kajian menunjukkan penaburan benih induk penyenggara 0025B pada dua hari sebelum dan 6 hari selepas benih induk CMS ditabur menurunkan secara signifikan bilangan anak per meter masing-masing sebanyak 18.99-20.39 dan 13.17-17.94%. Hasil juga menurun sebanyak 20.59-45.83 dan 127.78-150.00% apabila induk penyenggara 0025B ditabur masing-masing pada 2 hari sebelum dan 6 hari selepas induk CMS 0025A ditabur. Induk penyenggara 0047B perlu ditabur pada hari ke-4 dan 6 selepas induk CMS 0047A ditabur kerana kedua-dua sela masa ini memberi kesan bererti terhadap bilangan anak per meter persegi, kadar kacuk-silang, hasil dan indeks tuaian induk CMS 0047A ($P<0.05$). Empat konsentrasi asid giberelik (GA_3) diberi kepada kedua-dua induk CMS dan penyenggara apabila 10% daripada tangkai muncul dari seludang. Keputusan kajian menunjukkan aplikasi GA_3 pada konsentrasi 15, 30 dan 45 bahagian per sejuta (bsj) meninggikan induk CMS 0025A secara bererti masing-masing sebanyak 7.8-13.7, 10.8-17.0 and 13.9-16.5%. Hasil induk CMS 0025A juga meningkat masing-masing sebanyak 71.4-433.3, 450.0-500.0 and 116.5-125.0%. Kualiti benih terpilih seperti kadar percambahan dan indeks percambahan CMS 0025A juga meningkat masing-masing sebanyak 48.77-97.53 and 100-150% apabila 15, 30 dan 45 bsj GA_3 disembur pada pokok. Semburan GA_3 didapati memberi perubahan terhadap ruas CMS. Asid giberelik pada konsentrasi 45 bsj tidak disyorkan untuk penggandaan benih CMS kerana ia menjurus kepada kejadian pokok rebah. Maklumat mengenai ciri-ciri bunga dan sifat pembungaan induk CMS dan penyara mungkin berguna kepada ahli pembaikbaka fisiologi bagi menambahbaik ciri-ciri tertentu induk tersebut. Konsentrasi GA_3 yang tepat yang didapati daripada kajian ini mungkin boleh digunapakai oleh pemain industri padi seperti pengeluar benih dalam meminimakan kos dan prestasi hasil yang baik.

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

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This is to confirm that:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

of treated CMS could be seen due to its more number of lumens (L) as compared to the untreated CMSCross section of the large vascular bundles (LVBs) of CMS 0047A treated with (a) 0 ppm/ha GA_3 and (b) 30 ppm/ha GA_3 viewed under scanning electron microscope (500x). The orientation of the metaxylem vassels (MX) and phloem (P) of both CMS internodes looked similar and the phloem was sorrounded with a number of metaxylem vessels. A layer which sorrounded the cells of vascular bundle is known as mestome sheath (M) and it was thicker in untreated CMS internode than the treated CMS. A compact look of treated CMS could be seen due to its more number of lumens (L) as compared to the untreated CMS

LIST OF ABBREVIATIONS

EAT	Effective accumulated temperature
WA-CMS	Wild-abortive cytoplasmic male sterile
SER	Stigma exsertion rate
PER	Panicle exsertion rate
OCR	Out-crossing rate
I-KI	Iodine potassium iodide
FAA	Formaldehyde-acetic acid-alcohol
ANOVA	Analysis of variance
S.D.	Standard deviation
DAT	Days after transplanting
PGR	Plant growth regulator
bsj	*bahagian per sejuta*

CHAPTER 1

INTRODUCTION

Rice is an important staple dietary requirement for most Malaysian where adult population consumed an average of 76.5 kg of rice per year (MOA, 2018). Bryan (2015) estimated that by 2050, the demand of rice in Malaysia will soar by an estimation of 908%. The value seems huge but the information maybe plausible as Malaysian population will continue to increase as projected by FAO (2019) and it is expected to reach out 43 million in 2050. In order to meet the domestic demand of the increasing population, the current production of 1.11 million metric tons (2018) has to increase to 2.91 million metric tons by the year 2050 or 14.12% within 40 years (FAO, 2019). It is difficult to achieve this target because the yield of current inbred rice varieties is plateauing. Therefore, to sustain the self-sufficiency in rice, additional production of 1.3% is needed every year (Bryan, 2015). Hybrid rice technology is one of the limited options and currently available for significantly stepping up the rice production. The adoption of hybrid rice technology has made wonders in rice production in China (Yuan and Fu, 1995) and Malaysia is also working towards this technology since the last 10 years. It was proven that the hybrid rice give 10-15 t/ha additional yield over the inbred rice varieties (about 15-20% increase), thereby contributing towards higher on-farm productivity (FAO, 2018).

The technology of hybrid rice seed production is totally different from the inbred rice seed production. Currently, the three-line system is being adopted in our local hybrid rice seed production (Guok, 1994; Elixon et al., 2014). Three different lines namely a cytoplasmic male sterile line (CMS/A line/female parent), a maintainer (B line/male parent) and a restorer (R line) are utilized. Two major steps involve in this system for commercializing hybrid seeds which are 1) multiplication of CMS seed and 2) production of hybrid (F_1) seed. The multiplication of the CMS must be carried out before the production of hybrid seed.

Failure to obtain proper synchronization in flowering between the parental lines is the most commonly encountered problem in hybrid rice seed production. This is because seed yield on the female parents depended largely on the amount of pollen supplied by the male parent during flowering period and as well as the out-crossing rate of this male parents (Virmani, 1994; Biradarpatil and Shekhargouda, 2006). It is essential to know the exact difference in days to flowering between the parents. If the flowering gap is more, the problem of non-synchronization could be overcome by staggered sowing of male parent based on the information on days to flowering. Sometimes in spite of adjusting the sowing date, the parents do not flower at a time because of the differential response of the parents to the change in environmental conditions (Biradarpatil and Shekhargouda, 2006). Therefore, it is essential to adjust the flowering of parental lines after observing the difference at primordial development stage. If the difference in flowering is marginal, it can be manipulated to some extent by adopting a few techniques to achieve synchronization in flowering (Maurya, 1998; Mall and Vishwakarma, 2013).

Incomplete panicle exsertion or natural deficiency of 'panicle enclosure' of almost CMS is the other impediments in hybrid rice seed production. Almost 30-40% of the panicles are partially or fully enclosed in the flag leaf sheath and this character hinders pollination between the A and B or R lines and consequently decreases seed yield (Yin et al, 2007). Yin et al. (2007) also found that the decreased of gibberellins A_1 (GA_1) shortened the uppermost internode and therefore, the panicle failed to push out from the flag leaf sheath. Gibberellic acid (GA_3) is an exogenous hormone that commonly use in hybrid rice seed production. In China, hybrid rice seed growers use a very high concentrations of GA_3 (150-300 g/ha) to get high seed yield. However, outside China, the high cost of GA_3 limits seed growers to use only 45-50 g/ha of GA_3. In Malaysia, 30-60 g/ha of GA_3 have been recommended by Kato (1997) to be used in hybrid rice seed production. However, appropriate concentration(s) of GA_3 need to re-identify for local consumption because the recommended concentration used was 20 years ago for imported hybrid rice lines.

Understanding the flower characters and other flowering behaviour of our locally developed parental lines is also crucial (Elixon et al., 2014). The floral traits that influence out-crossing in rice include stigma size, style length, stigma exsertion (in seed parent) and anther length, filament length and pollen number/anther (in pollen parent). While the flowering behaviour traits that influence out-crossing in rice are number of days of flowering, time of flowering, duration of flowering, duration of floret opening and angle of floret opening (Virmani, 1994).

With this background, the general objective of this study was to improve the out-crossing rate and grain yield of Malaysian CMS during CMS seed multiplication. The good out-crossing rate between the CMS and maintainer lines is derived from good synchronization between these parental lines during flowering period. Hence, the specific objectives of this study were:

(i) to identify the floral traits, flowering behaviours and pollination of Malaysian CMS and maintainer lines;
(ii) to synchronize flowering between Malaysian CMS and maintainer lines; and
(iii) to improve panicle elongation of Malaysian CMS by using exogenous gibberellic acid.

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