
**UNIVERSITI PUTRA MALAYSIA**

# *ENHANCEMENT OF GERMINATION PERFORMANCE AND EARLY SEEDLING GROWTH OF MALAYSIAN INDICA RICE (Oryza sativa L.) cv. MR219 BY HORMONAL PRIMING*

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By

**MOHAMAD RASYID SUKIFTO**

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**September 2020**

**Chair:** **Rosimah Nulit, PhD**
**Faculty:** **Science**

Seed germination and early seedling establishment is a critical stage of rice life cycle that determines its survival and productivity. The success of germination and seedling establishment is affected by both abiotic and biotic factors and the seed physiological attributes. Enhancing seed quality for direct seeding method of rice cultivation by priming under unfavourable conditions is commonly practised. However, improving poor germination and seedling establishment of Malaysia's indica rice (*Oryza sativa* L.) MR219 under normal growth condition by priming has yet to be conducted. Therefore, this study aimed to increase germination performance and early seedling growth of MR219 rice cultivar by determining the optimum priming time and ideal concentration of hormones under normal growth conditions. MR219 seeds were primed with salicylic acid (SA) and gibberellic acid ($GA_3$) in ranges of concentrations (20-100 mg $L^{-1}$) for 12 and 18 hours separately. Unprimed seed was used as control. Germination was conducted using completely randomized design (CRD) with four replicates for eight cycles and seeds were allowed to germinate for ten days at room temperature (25±1°C). MR219 seeds were also primed with combination of SA+$GA_3$ at 12 hours. Following this, cultivation of primed MR219 seed was carried out in the pots in the field conditions using randomised complete block design (RCBD) with 8 replicates for 4 weeks. Germination and early seedling growth parameters were measured and statistically analysed using Analysis of Variance (ANOVA) at $p<0.05$ followed by Duncan Multiple Range Test (DMRT) at $p<0.05$ for mean comparison. Results showed 12 hours hormonal priming with SA and $GA_3$ between 60-100 mg $L^{-1}$ enhanced germination performance and early seedling growth of MR219 with germination percentage achieved 80-100%, germination index increased 2-fold, seed vigour increased 3-fold, coefficient of velocity of germination increased exceeding 2%, 100% increase in seedling height and reduced the mean germination time by 24 hours. Germination performance of MR219 seeds primed with $GA_3$ showed the best germination

performance compared to unprimed seed. However, experiment using a combination of SA+$GA_3$ priming showed reduction in germination performance and early seedling growth compared to single priming. Contrarily, cultivation of primed MR219 seeds in pots showed better establishment and early seedling growth under SA priming at 12 hours, exhibiting better morpho-physiological characteristics with seedling height increased a 2-fold and total fresh weight a 3-fold accompanied by increased in cell membrane integrity compared to unprimed seedlings. In addition, SA priming significantly improved the biochemical properties of MR219 seedlings with increased in carbohydrate content, protein content and 4-fold in total soluble sugar compared to unprimed seedling. As a conclusion, hormonal priming with SA (100 mg $L^{-1}$) at 12 hours determined to be the best priming method to enhance germination performance and early seedling growth of MR219 rice cultivar.

Keywords: *Oryza sativa*; hormonal priming; salicylic acid (SA); gibberellic acid ($GA_3$); germination performance; early seedling growth

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENAMBAHBAIKAN PRESTASI PERCAMBAHAN DAN PERTUMBUHAN AWAL ANAK BENIH BERAS INDICA MALAYSIA (*Oryza sativa* L.) KULTIVAR MR219 DENGAN PERANGSANGSEDIAAN HORMON**

Oleh

**MOHAMAD RASYID SUKIFTO**

**September 2020**

**Pengerusi:** **Rosimah Nulit, PhD**
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Percambahan dan pertumbuhan awal anak benih adalah peringkat yang sangat penting dalam kitar hidup padi di mana ia menentukan kemandirian dan jumlah hasil pengeluaran. Kejayaan percambahan dan pertumbuhan awal anak benih dipengaruhi oleh kedua-dua faktor abiotik dan biotik serta atribut fisiologi bijih benih. Meningkatkan kualiti biji benih padi bagi kaedah penanaman secara langsung menggunakan perangsangsediaan di bawah kondisi yang kurang memuaskan selalu diamalkan. Walaubagaimanapun, kajian untuk meningkatkan percambahan dan pertumbuhan anak benih padi MR219 menggunakan perangsangsediaan masih belum dijalankan setakat ini. Oleh yang demikian, kajian ini bertujuan untuk meningkatkan prestasi percambahan dan pertumbuhan awal anak benih padi MR219 dengan menentukan masa perangsangsediaan yang optimum dan kepekatan ideal hormon. Biji benih MR219 dirangsangsedia dengan asid salisilik (SA) dan asid giberelik ($GA_3$) dalam julat kepekatan (20-100 mg $L^{-1}$) untuk 12 dan 18 jam secara berasingan. Biji benih yang tidak dirangsangsedia digunakan sebagai kawalan. Percambahan dijalankan menggunakan rekabentuk ujikaji rawak lengkap (CRD) dengan empat replikat untuk lapan kitaran dan biji benih dibiarkan bercambah selama sepuluh hari pada suhu bilik (25±1°C). Biji benih MR219 juga dirangsangsedia dengan gabungan SA dan $GA_3$ pada 12 jam. Sejurus itu, penanaman biji benih MR219 yang telah dirangsangsedia dilakukan di dalam 8 pasu di lapangan menggunakan reka bentuk ujikaji blok rawak lengkap (RCBD) dengan lapan replikat selama empat minggu. Parameter percambahan dan pertumbuhan anak benih diukur dan dianalisa menggunakan ANOVA pada aras keertian $p<0.05$ diikuti oleh ujian julat berganda Duncan (DMRT) pada aras keertian $p<0.05$ untuk perbandingan purata. Keputusan menunjukan perangsangsediaan hormon dengan SA dan $GA_3$ di antara 60-100 mg $L^{-1}$ untuk 12 jam meningkatkan prestasi percambahan dan pertumbuhan awal anak benih MR219 dengan peratusan percambahan menjangkau 80-100%, indeks

percambahan meningkat dua kali ganda, kesuburan biji benih meningkat tiga kali ganda, pekali halaju percambahan meningkat melebihi 2%, 100% peningkatan dalam ketinggian anak benih dan mengguragkan purata masa percambahan untuk 24 jam. Prestasi percambahan biji benih MR219 yang dirangsangsedia dengan $GA_3$ menunjukan prestasi percambahan terbaik berbanding dengan biji benih yang tidak dirangsangsedia. Walaubagaimanapun, gabungan perangsangsediaan SA dan $GA_3$ menunjukkan pengurangan dalam prestasi percambahan dan pertumbuhan awal anak benih berbanding dengan perangsangsediaan tunggal. Sebaliknya, penanaman biji benih yang dirangsangsedia di dalam pot menunjukan kestabilan dan pertumbuhan awal anak benih lebih baik dengan perangsangsediaan SA pada 12 jam, ciri-ciri morfo-fisiologi didapati lebih baik dengan ketinggian anak benih meningkat dua kali ganda dan berat basah meningkat tiga ganda disertai dengan peningkatan integriti membran sel berbanding dengan anak benih yang tidak dirangsangsedia. Kajian juga mendapati, kandugan karbohidrat dan protein anak benih yang diberi rawatan perangsangsediaan SA adalah lebih tinggi dan kandungan gula terlarut adalah empat kali ganda berbanding dengan anak benih yang tidak dirangsangsedia. Sebagai kesimpulannya, perangsangsediaan hormon dengan SA (100 mg $L^{-1}$) pada 12 jam didapati terbaik untuk meningkatkan prestasi percambahan dan pertumbuhan awal anak benih MR219.

Kata kunci: *Oryza sativa*; perangsangsediaan hormon; asid salisilik (SA); asid giberelik ($GA_3$); prestasi percambahan; pertumbuhan awal anak benih

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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 Master of Science. The members of the Supervisory Committee were as follows:

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| | |
|---|---|
| ABA | Abscisic acid |
| AUX | Auxin |
| B | Boron |
| BR | Brassinosteroid |
| Cd | Cadmium |
| $Cd^{2+}$ | Cadmium ion |
| cm | Centimeter |
| CVG | Coefficient of velocity of germination |
| CRD | Completely Randomised Design |
| CK | Cytokinin |
| °C | Degree Celsius |
| DMRT | Duncun Multiple Range Test |
| EC | Electrical conductivity |
| EL | Electrolyte leakage |
| ET | Ethylene |
| FW | Fresh weight |
| GI | Germination index |
| GP | Germination percentage |
| $GA_3$ | Gibberellic acid |
| g | Gram |
| *g* | Gravitational force |
| h | Hours |
| HCl | Hydrochloric acid |
| JA | Jasmonic acid |
| $R^2$ | Coefficient of determination |
| L | Liter |
| Mn | Manganese |
| MGT | Mean germination time |
| mt/ha | Metric ton per hectare |
| µM | Micromolar |
| µS | Micro Siemens |

| | |
|---|---|
| µl | Microliter |
| mM | Millimolar |
| mg | Milligram |
| mg/g | Milligram per gram |
| mg $L^{-1}$ | Milligram per liter |
| ml | Milliliter |
| mm | Millimeter |
| nm | Nanometer |
| N | Nitrogen |
| *n* | Number of sample |
| ppm | Part per million |
| % | Percentage |
| P | Phosphorus |
| PGR | Plant growth regulator |
| PEG | polyethylene glycol |
| $K^+$ | Potassium ion |
| RCBD | Randomised Complete Block Design |
| ROS | Reactive oxygen species |
| SA | Salicylic acid |
| SV | Seed vigour |
| SHR | Seedling height reduction |
| Si | Silicon |
| NaOCl | Sodium hypochlorite |
| SE | Standard error |
| $H_2SO_4$ | Sulfuric acid |
| w/v | Weight by volume |
| Zn | Zinc |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

The United Nations estimated the global population is reaching 8 billion by 2022 with the largest proportion is Asia (United Nations, 2017). As most countries in Asia, rice is the staple food with regional consumption more than 80% of the world's rice production. The world's five largest rice producers and largest rice consumers viz. China, India, Indonesia, Bangladesh and Vietnam. Therefore, rice demand is expected to rise as high level of rice consumption with growing population (Bellemare, 2015).

Germination is a critical phase in plant life cycle affected by an array of environmental factors and viability of seed in determination of the potential growth of the embryo. The viability of seed in the field will be determined to a large degree from its germination potential and vigour (Keller & Kollmann, 1999; Koorneef, Bentsink & Hilhorst, 2002). Seed vigour is a major factor that often results in poor stand establishment. Seeds with low vigour will mostly produce weak seedlings that are susceptible to environmental stresses whereas high seed vigour offer early and synchronize stands which provide the growing seedlings an advantage against wide environmental constraint (IRRI, 2013).

High seed vigour is donate by good quality seed which can increase yield by 5-20%. Seed quality is defined as the summation of all factors that contribute to seed performance (Shaban, 2013). Seed quality is determined by numbers of genetics and physiological characteristics. Rice planters often face problems in poor germination especially in arid and semi-arid area (Ruttanaruangboworn et al., 2017). With high quality seed, enable planters to attain crops which have higher percentage of germination, a vigorous and more uniform stand, faster growth rate and greater resistance to stress and diseases (IRRI, 2013).

Deterioration of seed is a serious problem in tropical and subtropical regions where high temperature and humidity accelerate seed aging. As seed aged, germination become slower and more susceptible to disease (Thejeshwini, Rao, Nayak & Sultana, 2019). Deterioration in seed quality could have started at any point in a plant's development stage. Seed quality depends on the physical conditions of mother's plant during growth stage, aside from harvesting, processing, storage and planting (IRRI, 2013). Use of quality seed is important to ensure better germination and stand establishment. Therefore, invigoration of seed to increase seed quality leading to enhance germination and stand establishments is crucial in direct-seeding method and in maintaining the rice production sustainability (Matsushima & Sakagami, 2013).

## 1.2 Problem Statement and Justification of Study

Over six decades, direct seeding of rice was the primary cultivation method. It is estimated 23% of global rice cultivation is through direct seeding (Rao, Johnson, Sivaprasad, Ladha & Mortimer, 2007). In recent years, there has been a shift from transplanting to direct seeding rice cultivation in few Southeast Asia countries (Pandey & Velasco, 2005). The shifting was mainly caused by large water inputs, expensive labour cost and shortage for transplanting which have reduced profit margins (Joshi et al., 2013).

One of the techniques used to improve poor germination and stand establishment of direct-seeded rice is by seed priming. Seed priming techniques are a promising solution to poor germination and stand establishment in direct-seeded rice (Farooq, Basra & Wahid 2006; Wang et al., 2016). Priming is a seed technology that has been applied to commercially cultivated crops to enhance seed quality by increasing germination rates and stand establishment which results in higher tolerance and crop yields (Jisha, Vijayakumari & Puthur, 2013). In addition, seed priming is a low-risk technology (Harris et al., 1999) and a low-cost solution for poor germination and stand establishment (Farooq, Basra, Cheema & Afzal, 2006).

Priming in direct-seeded rice is an effective, practical and easy technique to improve rate of germination (1 to 3 days) and synchronize seed emergence resulting in more uniform and vigorous seedling growth (Paparella et al., 2015). Germination of primed rice seeds reduced emergence rate and time from planting to 50% germination in 0.9-3.7 days less than unprimed seeds (Farooq, Basra, Tabassum & Afzal, 2006). Healthier and synchronized germination of primed seeds mainly as a result of reduction in the lag time of imbibition (Brocklehurst & Dearman, 2008; Paparella et al., 2015).

Different strategies of seed priming could be used to improve germination and stand establishment, but likely to be species- and dose-dependent (Xiao, Fu-lai & Dong, 2017). In this present study, use of hormonal priming – priming mixture of bioactive molecules was performed by adding phytohormones which are salicylic acid (SA) and gibberellic acid ($GA_3$). These phytohormones were selected as it regulates major biochemical processes during seed germination, maturation and throughout plant development (Iqbal et al, 2017).

The use of $GA_3$ was selected in this study as $GA_3$ known to break seed dormancy and significantly enhance seed germination in many species through activation of embryonic growth, mobilization of reserves and weakening the endosperm wall (Pallaoro et al., 2016; Ma et al., 2018). Meanwhile, seed priming with SA induces defensive mechanism and resistance towards various stresses through increase in antioxidant enzymes activity (Somata, et al., 2017).

As many previous studies reported small quantity of hormone used increase cost-effectiveness. In addition, the use of phyohormones containing active substances will have no adverse effect on human or animal health or on groundwater or any inappropriate environmental effects (EFSA, 2012). Seed priming is environmentally safe and effective technology which can be practiced by resource-poor farmers and benefited them in many ways (Raj & Raj, 2019).

In present study, Malaysian indica rice (*Oryza sativa* L.) cv. MR219 were studied using approach of hormonal priming with SA and $GA_3$. MR219 rice was selected as it is popular for high potential yield and lasted for 20 years and still receive demands from farmers (MARDI, 2017). In addition, some unofficial reports comply with the existence of germination problem of the MR219 rice variety (Talei, Valdiani, Maziah & Mohsenkhah, 2013). Therefore, optimizing the priming method using SA and $GA_3$ for MR219 variety is important to increase maximum germination potential and stand establishments in direct-seeded MR219 rice.

### 1.3 Objectives of Study

The following are the objectives of the present study:

I. To determine the optimum concentration of SA and $GA_3$ and priming time on the germination performance and early seedling growth of MR219.

II. To investigate the effects of priming with combination of SA and $GA_3$ on the germination performance and early seedling growth of MR219.

III. To investigate the effects of SA and $GA_3$ priming at optimum priming time on the early seedling growth and biochemical contents of MR219.

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