Original Research Article

Development of liquid enhancer containing KCl, Thiourea, Gibberellic acid and Salicylic acid for germination of drought-stressed Malaysian Indica rice seed cv. MR284

Running title: Development of liquid enhancer for drought-stressed rice germination

Abstract

Application of chemical solution is one of the practical way to manage drought stress in crop production. Hence, this study was conducted to develop liquid enhancer containing KCl, TU, GA, and SA for germination of drought-stressed Malaysian Indica rice cv. MR284 seed. The sterilised rice seed cv. MR284 was stressed in the -1.2 Mpa PEG 6000 solution for three days and germinated in the KCl, TU, GA, and SA solution in a series of concentration. Two steps were involved in the development process which are to select ideal concentration for each KCl, TU, GA, and SA, and to combine each of the ideal concentration treatments and select the best combination treatments to form the liquid enhancer. In the first step, drought-stressed rice seed showed the best germination performance in the 30 mM of KCl, 2.0 mM of TU, 0.24 mM GA, and 0.5 mM SA. Meanwhile, in the second step, the drought-stressed rice seed showed the best germination performance in the combination of 30 mM KCl + 2.0 mM TU + 0.24 mM GA + 0.5 mM SA. The best germination performance was evaluated by the highest germination percentage (%), germination index, seed vigor, leaf length, root length and biomass. Therefore, the combination treatments of 30 mM KCl + 2.0 mM TU + 0.5 mM SA was found to be the most effective and simplest liquid enhancer formula that has an ability to enhance seed germination of drought-stressed rice cv. MR284 seed.

Keywords: Liquid enhancer; drought-stressed seed; germination; seed vigor

Introduction

Drought is one of the abiotic stresses that known as scarcity of water resources and related to high degree of temperature. It can occur in all climatic zones with the characteristics that varies significantly based on the regions. In agriculture industries, insufficiency of water is a creeping phenomenon that will become a severe limiting factor, since it affecting the plant growth, plant development and crop stability (Zargar *et al*., 2017).

There are a few strategies to overcome drought stress in crop production such as selection drought tolerance cultivars, water irrigation treatment, and also application of chemical solution treatment. This study has chosen the application of chemical formulation and plant growth regulators since it has been practised by the rice growers in Malaysia in the rice planting process. According to the previous research, potassium chloride (KCl), thiourea (TU), gibberellic acid (GA) and salicylic acid (SA) was found to have potential to increase plant growth of plant species in stress condition (Vieira *et al.,* 2002; Sheykhbaglou *et al.,* 2014; Sharma *et al.* 2015; Al-Shaheen *et al.,* 2016).

Recently, drought is no longer an uncommon phenomenon in Malaysia. In early 2019, almost all states in Malaysia has been reported to face high degree of temperature or drought from January to March and affected rice plantation in Kelantan (Razali, 2019). Malaysian Indica rice is grown in several areas in Malaysia including Kedah, Perak, Johor, Melaka and Kelantan. Rice is a water-thirsty plant. Hence, they are usually cultivated in flooded area, and drought stress can definitely disrupt their germination and growth process. Germination is a crucial stage to ensure the survival of the plant. Miles and Brown (2007) stated that the basic needs for the plant such as suitable temperature and soil moisture must be presented to ensure the seed germination process to occur. Hence, drought stress will give a great impact on the seed growth because seed embryo need a sufficient amount of water for the expansion and elongation process. Usually, under drought, there will be low rate of germination in most plant because plant do not receive an adequate amount of water to survive. According to Liu, Li, Liu, and Siu, (2015), drought has significantly reduced the germination potential, germination index, shoot length and root length of seven days-old maize seedling. Therefore, in effort to increase the successful germination and growth rate of rice seed germination in low amount of water, this study was developing a liquid enhancer that contained KCl, TU, GA and SA for germination of drought-stressed rice seed cv. MR284.

Materials and methods

Seed material and sterilization

Rice seeds of MR284 were obtained from Malaysian Agriculture Research and Development Institute (MARDI), Parit, Perak. Seed sterilization method is according to (Jisha & Puthur, 2016) with minor modification. Rice seeds were surface sterilized with 10% Sodium hypochlorite and few drops of Tween 20 for 10 minutes to remove dirt. Then, seeds were rinsed with sterilized distilled water for 2-3 times. The surface of the seeds were air-dried for a couple of minutes prior to be used.

Priming of MR284 rice seed in PEG 6000

The rice seeds were osmoprimed with -1.2 MPa (severe drought stress) of Polyethylene Glycol (PEG 6000) according to Pirdashti, Sarvestani, Nematzadeh, and Ismail, (2003) for 3 days before germination to induce stress in the MR284 seeds.

Development of liquid enhancer

Step 1: To select ideal concentration for KCl, TU, GA and SA

KCl, TU, SA, and GA were prepared in a range of concentration based on previous studies as in table 1. For TU and GA, concentrations used were altered and modified slightly according to the availability and compatibility to the plant of interest in this study.

Table 1: Concentrations of KCl, TU, GA, and SA

Treatment	Concentration	Proposed by
KCl	0, 10, 20, 30 and 40 mM	(Mohammed, 2016)
SA	0, 0.25, 0.50, 0.75 and 1.0 mM	Arfan, Artar, & Ashraf (2006) (Mohammed, 2016)
TU	0, 1.0, 2.0, 3.5, and 5.0 mM	Hassanein, Amin, Rashad, and Ali (2014)
GA	0, 0.06, 0.12, 0.18 and 0.24 mM	Vieira, Fraga, Oloveira & Santos (2002). De Mello, Streck, Blankenship & Paparozzi (2009).

Step 2: To select the best combination treatments for the liquid enhancer

The ideal concentration from step 1 for each chemical and phytohormones was combined to each other to observe the effect of combination treatments on the seed germination of drought-stressed rice seed. The combination treatments were listed in the table 2.

Table 2: The combination treatments of ideal concentration of KCl, TU, GA, and SA

Group	Combination treatment
1.	Control
2.	KCl+SA
3.	KCl+TU
4.	KCl+GA
5.	SA+TU
6.	SA+GA
7.	GA+TU
8.	KCl+SA+TU
9.	KCl+SA+TU+GA

Seed germination

Seven healthy stressed seeds were placed in two layers of Whatmann No.1 filter paper in a petri dish containing 8 ml of KCl, TU, GA, and SA solution in different concentrations and distilled water as control. The petri dishes were arranged in CRD and kept at room temperature (26 ± 1°C). The germination process was monitored daily for 10 days. Seeds were considered germinated when the radicle had extended at least 2 mm from the seed coat. All the parameters needed to measure the germination process were recorded. Each treatment has five replicates and repeated thrice.

Germination parameters

Root length, leaf length, and biomass were measured at day 10 of seed germination. All data were analysed by using One-way ANOVA, SPSS version 22 at confidence level $p \leq 0.05$. Germination percentage, seed vigor and germination index were calculated according to these formula:

1. Germination percentage % (GP) (Ellis and Roberts, 1981)

$$\text{GP} = \frac{\text{Number of germinated seed}}{\text{Total number of seed sown}} \text{ x } 100$$

2. Seed vigor (SV) (Abdul-Baki and Anderson, 1973)

$$\text{SV} = (\text{Average shoot length} + \text{Average root length}) \text{ x GP}$$

3. Germination index (Anchalee, 2011)

$$\text{Germination index} = \sum \frac{\text{Number of germinated seed}}{\text{Number of days}}$$

Results

Selection of ideal concentration for KCl, TU, GA, and SA

This study found that most of the concentration used in all chemicals showed better results compared to control treatment. They gave higher germination percentage, germination index, seed vigor, leaf length, root length and biomass. The best concentration for KCl, TU, GA, and SA were as shown in table 3-6. From the table, 30 mM KCl, 2.0 mM TU, 2.4 mM GA, and 0.5 mM SA were selected as the ideal concentration and used in the second step.

Table 3: Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought stressed of MR284 seeds in different concentrations of KCl

Concentration (mM)	Germination Percentage (%)	Germination Index	Seed Vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
0	88.1^{a}±2.4	11.5^{ab}±0.3	8.9^{a}±0.1	6.0^{a}±0.2	4.0^{a}±0.3	0.064^{a}±0.0005
10	95.2^{a}±3.0	11.6^{ab}±0.3	12.2^{c}±0.3	6.7^{ab}±0.3	3.9^{a}±0.5	0.070^{ab}±0.0002
20	97.6^{a}±2.4	11.6^{ab}±0.3	12.1^{c}±0.2	6.6^{ab}±0.3	5.4^{a}±0.3	0.072^{ab}±0.0032
30	95.2^{a}±3.0	12.3^{b}±0.2	12.7^{c}±0.3	7.8^{b}±0.6	8.8^{b}±0.5	0.076^{b}±0.0027
40	85.7^{a}±5.2	11.0^{a}±0.3	10.2^{b}±0.4	6.0^{a}±0.4	7.1^{b}±0.3	0.066^{ab}±0.0023

Values are the mean and standard errors of measurement made on six replicates. Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means according to Tukey HSD test, $p<0.05$.

Table 4: Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought stressed of MR284 seeds in different concentrations of TU

Concentration (mM)	Germination Percentage (%)	Germination Index	Seed Vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
0	88.1^{a}±4.4	11.1^{a}±0.3	9.5^{a}±0.5	5.0^{ab}±0.4	6.1^{bc}±0.2	0.074^{ab}±0.0050
1.0	95.2^{a}±3.0	11.9^{a}±0.4	10.6^{ab}±0.3	5.9^{bc}±0.3	6.5^{bc}±0.2	0.095^{bc}±0.0065
2.0	97.6^{a}±2.4	12.7^{a}±0.3	11.9^{b}±0.5	6.6^{c}±0.2	7.1^{c}±0.1	0.100^{c}±0.0070
3.5	95.2^{a}±3.0	11.2^{a}±0.4	11.3^{ab}±0.4	4.6^{a}±0.3	5.9^{ab}±0.2	0.083^{abc}±0.0046
5.0	88.1^{a}±4.4	11.0^{a}±0.6	10.4^{ab}±0.6	4.3b^{a}±0.2	4.8^{a}±0.5	0.066^{a}±0.0028

Values are the mean and standard errors of measurement made on six replicates. Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means according to Tukey HSD test, $p<0.05$.

Table 5: Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought stressed of MR284 seeds in different concentrations of SA

Concentration (mM)	Germination Percentage (%)	Germination Index	Seed Vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
0	$95.2^{a}\pm3.0$	$10.5^{abc}\pm0.6$	$10.1^{abc}\pm0.5$	$5.5^{ab}\pm0.5$	$5.5^{b}\pm0.5$	$0.070^{ab}\pm0.0028$
0.25	$97.6^{a}\pm2.4$	$11.9^{bc}\pm0.5$	$11.6^{bc}\pm1.5$	$6.1^{ab}\pm0.4$	$6.8^{bc}\pm0.8$	$0.073^{bc}\pm0.0010$
0.50	$97.6^{a}\pm2.4$	$12.2^{c}\pm0.4$	$13.3^{c}\pm0.7$	$6.5^{b}\pm0.5$	$8.2^{c}\pm0.5$	$0.083^{c}\pm0.0022$
0.75	$92.9^{a}\pm3.2$	$10.1^{ab}\pm0.3$	$8.2^{ab}\pm0.5$	$5.0^{ab}\pm0.3$	$4.4^{ab}\pm0.5$	$0.060^{a}\pm0.0024$
1.0	$92.9^{a}\pm4.9$	$9.9^{a}\pm0.3$	$6.9^{a}\pm0.6$	$4.6^{a}\pm0.1$	$2.9^{a}\pm0.5$	$0.067^{ab}\pm0.0030$

Values are the mean and standard errors of measurement made on six replicates. Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means according to Tukey HSD test, $p<0.05$.

Table 6: Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought stressed of MR284 seeds in different concentrations of GA

Concentration (mM)	Germination Percentage (%)	Germination Index	Seed Vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
0	$92.9^{a}\pm3.0$	$11.2^{a}\pm0.9$	$10.2^{a}\pm0.6$	$5.0^{a}\pm0.3$	$8.5^{a}\pm0.4$	$0.063^{a}\pm0.0007$
0.06	$88.1^{a}\pm5.7$	$12.1^{a}\pm0.3$	$18.3^{b}\pm1.1$	$15.3^{b}\pm0.4$	$11.1^{b}\pm0.7$	$0.064^{a}\pm0.0014$
0.12	$95.2^{a}\pm3.0$	$12.2^{a}\pm0.2$	$20.6^{bc}\pm1.1$	$15.7^{bc}\pm0.8$	$8.3^{a}\pm0.4$	$0.065^{a}\pm0.0011$
0.18	$92.9^{a}\pm3.2$	$12.5^{a}\pm0.4$	$20.3^{bc}\pm0.5$	$16.2^{bc}\pm1.2$	$7.5^{a}\pm0.6$	$0.069^{ab}\pm0.0009$
0.24	$97.6^{a}\pm2.4$	$12.9^{a}\pm0.3$	$23.6^{c}\pm1.1$	$18.7^{c}\pm0.8$	$7.7^{a}\pm0.3$	$0.074^{b}\pm0.0023$

Values are the mean and standard errors of measurement made on six replicates. Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means according to Tukey HSD test, $p<0.05$.

Combination treatments to produce liquid enhancer

Some of the combination treatments resulted in a better seedling growth of drought-stressed MR284 seed compared to control treatment. It showed a significant higher germination performances and seedling growth. Table 7 showed the effects of combination treatments on germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought-stressed MR284. From the table, some of the treatments are not significant to each other. Figure 1 shows the comparison of 10 days-old MR284 rice seedling in different combination treatments. The last two treatments showed better growth of rice seedlings compared to others. The best combination treatment was selected according to the simplest combination that give the best performance and lower cost of production. Therefore, it was found that the combination treatment consist of 30 mM KCl + 2.0 mM TU + 0.5 mM SA was the best to enhance germination and seedling growth of drought-stressed MR284 rice seed.

Table 7: Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought stressed of MR284 seedlings in different combination treatments

Combination	Germination Percentage (%)	Germination index	Seed Vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
Control	$90.5^{a}\pm4.8$	$10.4^{a}\pm0.5$	$14.5^{ab}\pm0.6$	$6.2^{a}\pm0.2$	$5.9^{bc}\pm0.1$	$0.125^{a}\pm0.0031$
30 mM KCl+0.50 mM SA	$95.2^{a}\pm3.0$	$12.0^{ab}\pm0.3$	$16.5^{abc}\pm1.4$	$6.3^{a}\pm0.3$	$8.0^{de}\pm0.8$	$0.132^{ab}\pm0.0032$
30 mM KCl+0.20 mM TU	$92.9^{a}\pm4.9$	$10.8^{ab}\pm0.4$	$14.5^{ab}\pm0.6$	$6.5^{a}\pm0.3$	$7.2^{cd}\pm0.5$	$0.130^{a}\pm0.0029$
30 mM KCl + 0.24 mM GA	$90.5^{a}\pm3.0$	$11.8^{ab}\pm0.4$	$15.9^{ab}\pm0.5$	$13.5^{c}\pm0.4$	$3.0^{a}\pm0.2$	$0.135^{ab}\pm0.0022$
0.50 mM SA+0.20 mM TU	$95.2^{a}\pm4.8$	$11.6^{ab}\pm0.4$	$20.9^{cd}\pm0.4$	$11.0^{b}\pm0.3$	$9.3^{e}\pm0.6$	$0.133^{ab}\pm0.0018$
0.50 mM SA+0.24 mM GA	$95.2^{a}\pm4.9$	$10.2^{a}\pm0.3$	$13.1^{a}\pm0.9$	$10.1^{b}\pm0.3$	$3.1^{a}\pm0.5$	$0.124^{a}\pm0.0019$
2.0 mM TU+ 0.24 mM GA	$95.2^{a}\pm3.0$	$10.8^{ab}\pm0.3$	$17.8^{bc}\pm1.5$	$17.2^{d}\pm0.8$	$3.8^{ab}\pm0.2$	$0.135^{ab}\pm0.0034$
30 mM KCl+0.50 mM SA+ 2.0 mM TU	$97.6^{a}\pm2.4$	$12.0^{ab}\pm0.5$	$23.8^{d}\pm1.5$	$16.1^{d}\pm0.7$	$8.8^{de}\pm0.3$	$0.137^{ab}\pm0.0028$
30 mM KCl+0.50 mM SA+ 2.0 mM TU+0.24 mM GA	$97.6^{a}\pm2.4$	$12.5^{b}\pm0.7$	$24.4^{d}\pm0.8$	$20.0^{e}\pm0.4$	$5.6^{bc}\pm0.3$	$0.144^{b}\pm0.0039$

Values are the mean and standard errors of measurement made on six replicates. Superscripts within the means of each column (a-b) with different letters indicate significant differences among the means according to Tukey HSD test, $p<0.05$.

Figure 1: Ten days old of drought-stressed MR284 seeds germinated in different combination treatments of KCl, SA, TU and GA

Discussion

The aim of this study was to develop a liquid enhancer that contains KCl, TU, GA and SA to enhance germination and seedling growth of drought-stressed MR284 rice seed. There were two step involved in developing the liquid enhancer. In the first step, the ideal concentration of KCl, TU, GA and SA was determined. From the result, low concentration of TU, GA and SA were used except for KCl.

In the step one, 30 mM was the ideal concentration for KCl. K plays a critical role in plant growth and metabolism, and give significant effects in plants survival under various biotic and abiotic stresses through morphological, physiological and biochemical mechanisms alterations (Farooq, Irfan, Aziz, Ahmad and Cheema, 2013; Wang, Zheng, Shen and Guo, 2013; Prajapati & Kalavati, 2012). Application of K fertilizer mitigated the adverse effect of plant growth under drought stress by increasing plant height. Beside, Cakmak (2005) also reported that plant under drought stress seems required more K supply compared to plant under normal environment.

The ideal concentration for TU is 2.0 mM. TU has been recognized as an effective plant regulator with two functional group which were 'thiol' and 'imino' which have the function in oxidative stress response and fulfills the nitrogen requirement respectively. Amin, Abd El-Kader, Shalaby, Gharib, Rashad and Da Silva, (2013), reported that TU was found to be more effective than SA due to its ability in enhancing photosynthetic activity, accumulating dry matter and increased the translocation and accumulation of certain metabolites in Maize. Beside, photosystem efficiency in *Brassica juncea*, vegetative growth and regulation of the source-sink were also improved when TU was used (Pandey, Srivastava, D'Souza, & Penna, 2013).

GA is very popular due to its effectiveness in alleviating drought-imposed adverse effects on plants at varying development stages. The development of drought-stressed MR284 seeds in germination and early seedlings growth were influenced by GA application as shown by higher germination and early growth results compared to the control treatment with more than double increment in seed vigor, hypocotyl, and seedling length especially in 0.24 mM GA. GA application with appropriate concentration was able to induce the plant height and internode length during drought stress in maize and alleviated the drought stress effects (Akter, Islam, Karim, and Hossain, 2014). This was well explained by the fact that GA has the ability in improving impaired cell division and cell elongation in the plant as an escape strategy under environmental stresses.

This study found that SA alleviated the inhibitory effect of drought stress on seed germination of MR284. It is shown that 0.25 mM and 0.50 mM SA significantly increased the germination of drought-stressed MR284 seeds, while 0.50 mM was the ideal concentration that highly increased the germination parameters. In contrast, the high concentration of SA which are 0.75 mM and 1.0 mM decreased the germination of drought-stressed MR284 seeds. Shakirova, Shakhbutdinova, Bezrukova, Fatkhutdionova and Fatkhutdionova, (2003) reported that different concentrations of SA gave different hormonal effects, either inhibit or enhance the growth of plant. Salicylic acid is a naturally occurring phytohormone that contains phenolic compound. It involves in many defence-related functions such as increased responses to biotic and abiotic stress factors, induced tolerance toward environmental stress and regulated growth and development of plants (Miura & Tada, 2014).

The combination of these chemicals were found to have better germination and seedling growth compared to single treatment. The best combination treatment was 30 mM KCl + 2.0 mM TU + 0.5 mM SA. The synergistic effect of these chemicals and phytohormones gave a positive respond and alleviated drought stress in the seed and enhanced the germination process and the seedling growth of MR284 seed. Based on previous studies, many combinations of different types of plant growth regulators had been used on different plant types to understand on how a plant survive under stress conditions. In 2007, a study that was conducted by Cavusoglu and Kabar to determine the effect of combination of GA, cytokinin (Kin) and ethylene (EBR) on barley and radish seeds under high-temperature stress, had found that the combination of growth regulators used removed the adverse effect on germination more successfully than single application of a plant growth regulator only.

Conclusion

Drought is one of the abiotic stress that caused a reduction in the germination rate and seedling growth of many plant species including rice. A drought-stressed seed is facing problem to start germinate due to the inadequate of water content. One of the strategies to aid in the germination of drought-stressed seed is by the application of liquid enhancer. This study found that the combination of 30 mM KCl + 2.0 mM TU + 0.5 mM SA was the simplest and most effective treatment to enhance germination and early seedling growth of drought-stressed MR284 rice seed.

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