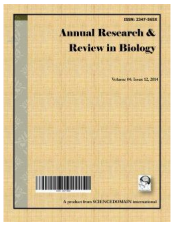

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Development of Liquid Enhancer for Germination of Drought-stressed *Oryza sativa* subsp. *indica* Seed cv. MR284

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SNM and RN designed the study and wrote the protocol. Authors SNM and FZ performed the statistical analysis and wrote the first draft of the manuscript. Authors RN and MHI managed the analyses of the study. Author NIAG managed the literature searches. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was conducted to develop liquid enhancer containing KCl, TU, GA, and SA for germination of drought-stressed *Oryza sativa* subsp. *indica* cv. MR284 seed.
Study Design: All experiments were conducted in a completely randomized design. Two steps were involved in the development process which are to select an ideal concentration for each KCl, TU, GA, and SA, and to find an ideal combination of chemicals from the selection of ideal concentrations acquired in step 1 to form liquid enhancer. There were 20 treatments for step 1 and 9 treatments for step 2. All of these treatments with 6 replicates.
Place and Duration of Study: Department of Biology, Faculty of Science, University Putra Malaysia, between June 2018 and December 2018.
Methodology: The sterilized rice seed cv. MR284 was stressed in the -1.2 Mpa PEG 6000

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solution for three days and germinated in the KCl, TU, GA, and SA solution in a series of concentration for 10 days, in a controlled room. Seed germination was observed daily.
Results: 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.
Conclusion: 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; Oryza sativa subsp; indica cv; MR284.

1. INTRODUCTION

Drought is one of the abiotic stresses that is known as scarcity of water resources and related to a high degree of temperature. It can occur in all climatic zones with the characteristics that vary significantly based on the regions. In agriculture industries, insufficiency of water is a crippling phenomenon that will become a severe limiting factor, since it is affecting the plant growth, plant development and crop stability [1].

There are a few strategies to overcome drought stress in crop production such as the selection of drought tolerant 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 practiced 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 [2,3,4,5].

Recently, drought is no longer an uncommon phenomenon in Malaysia. In early 2019, almost all States in Malaysia has been reported to face a high degree of temperature or drought from January to March and affected rice plantation in Kelantan [6]. 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 a 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 [7] 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 seed growth because seed embryo needs a sufficient amount of water for the expansion and elongation process. Usually, under drought, there will be a low rate of germination in most plants because plants do not receive an adequate amount of water to survive. According to Liu et al. [8], 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 developed in a liquid enhancer that contained KCl, TU, GA and SA for germination of drought-stressed rice seeds cv. MR284.

2. MATERIALS AND METHODS

2.1 Seed Material and Sterilization

Seeds of *Oryza sativa* subsp. *indica* cv. MR284 were obtained from the Malaysian Agriculture Research and Development Institute (MARDI), Parit, Perak. Seed sterilization method is according to Jisha & Puthur [9] 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 was air-dried for a couple of minutes prior to being used.

2.2 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 et al. [10] for 3 days before germination to induce stress in the *Oryza sativa* subsp. *indica* cv. MR284 seeds.

2.3 Development of Liquid Enhancer

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

KCl, TU, GA, and SA 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.

2.3.2 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 with each other to observe the effect of combination treatments on the seed germination of drought-stressed rice seed cv. MR284. The combination treatments were as listed in Table 2.

2.4 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 a control. The petri dishes were arranged under a completely randomized design (CRD) of 9 treatments with 6 repetitions, totalling 54 seedlings in all 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.

2.5 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 $p< 0.05$. Germination percentage, seed vigor and germination index were calculated according to these formulae:

1. Germination percentage % (GP) [15]

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

2. Seed vigor (SV) [16]

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

3. Germination index [17]

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

4. Biomass = Weight of germinated seeds (g)

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

Treatment	Concentration	Proposed by
KCl	0, 10, 20, 30 and 40 mM	Mohammed [11]
TU	0, 1.0, 2.0, 3.5 and 5.0 mM	Hassanein et al. [13]
GA	0, 0.06, 0.12, 0.18 and 0.24 mM	Vieira et al. [2] De Mello et al. [14]
SA	0, 0.25, 0.50, 0.75 and 1.0 mM	Arfan et al. [12] Mohammed [11]

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

Group	Combination treatment
1.	Control (Distilled water)
2.	30 mM KCl+0.5 mM SA
3.	30 mM KCl+2.0 mM TU
4.	30 mM KCl+0.24 mM GA
5.	0.5 mM SA+2.0 mM TU
6.	0.5 mM SA+0.24 mM GA
7.	0.24 mM GA+2.0 mM TU
8.	30 mM KCl+2.0 mM TU+0.5 mM SA
9.	30 mM KCl+2.0 mM TU+ 0.24 mM GA +0.5 mM SA

Table 3. Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought-stressed rice cv. 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±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 rice cv. 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±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 rice cv. 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}±3.0	11.2^{a}±0.9	10.2^{a}±0.6	5.0^{a}±0.3	8.5^{a}±0.4	0.063^{a}±0.0007
0.06	88.1^{a}±5.7	12.1^{a}±0.3	18.3^{b}±1.1	15.3^{b}±0.4	11.1^{b}±0.7	0.064^{a}±0.0014
0.12	95.2^{a}±3.0	12.2^{a}±0.2	20.6^{bc}±1.1	15.7^{bc}±0.8	8.3^{a}±0.4	0.065^{a}±0.0011
0.18	92.9^{a}±3.2	12.5^{a}±0.4	20.3^{bc}±0.5	16.2^{bc}±1.2	7.5^{a}±0.6	0.069^{ab}±0.0009
0.24	97.6^{a}±2.4	12.9^{a}±0.3	23.6^{c}±1.1	18.7^{c}±0.8	7.7^{a}±0.3	0.074^{b}±0.0023

Values are the mean±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 rice cv. 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}±3.0	10.5^{abc}±0.6	10.1^{abc}±0.5	5.5^{ab}±0.5	5.5^{b}±0.5	0.070^{ab}±0.0028
0.25	97.6^{a}±2.4	11.9^{bc}±0.5	11.6^{bc}±1.5	6.1^{ab}±0.4	6.8^{bc}±0.8	0.073^{bc}±0.0010
0.50	97.6^{a}±2.4	12.2^{c}±0.4	13.3^{c}±0.7	6.5^{b}±0.5	8.2^{c}±0.5	0.083^{c}±0.0022
0.75	92.9^{a}±3.2	10.1^{ab}±0.3	8.2^{ab}±0.5	5.0^{ab}±0.3	4.4^{ab}±0.5	0.060^{a}±0.0024
1.0	92.9^{a}±4.9	9.9^{a}±0.3	6.9^{a}±0.6	4.6^{a}±0.1	2.9^{a}±0.5	0.067^{ab}±0.0030

Values are the mean±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 7. Germination percentage, germination index, seed vigor, leaf length, root length and biomass of drought-stressed rice cv. MR284 seedlings in different combination treatments

Treatments	Germination percentage (%)	Germination index	Seed vigor	Leaf length (cm)	Root length (cm)	Biomass (g)
Control	90.5^{a}±4.8	10.4^{a}±0.5	14.5^{ab}±0.6	6.2^{a}±0.2	5.9^{bc}±0.1	0.125^{a}±0.0031
30 mM KCl+0.50 mM SA	95.2^{a}±3.0	12.0^{ab}±0.3	16.5^{abc}±1.4	6.3^{a}±0.3	8.0^{de}±0.8	0.132^{ab}±0.0032
30 mM KCl+0.20 mM TU	92.9^{a}±4.9	10.8^{ab}±0.4	14.5^{ab}±0.6	6.5^{a}±0.3	7.2^{cd}±0.5	0.130^{a}±0.0029
30 mM KCl + 0.24 mM GA	90.5^{a}±3.0	11.8^{ab}±0.4	15.9^{ab}±0.5	13.5^{c}±0.4	3.0^{a}±0.2	0.135^{ab}±0.0022
0.50 mM SA+0.20 mM TU	95.2^{a}±4.8	11.6^{ab}±0.4	20.9^{cd}±0.4	11.0^{b}±0.3	9.3^{e}±0.6	0.133^{ab}±0.0018
0.50 mM SA+0.24 mM GA	95.2^{a}±4.9	10.2^{a}±0.3	13.1^{a}±0.9	10.1^{b}±0.3	3.1^{a}±0.5	0.124^{a}±0.0019
2.0 mM TU+ 0.24 mM GA	95.2^{a}±3.0	10.8^{ab}±0.3	17.8^{bc}±1.5	17.2^{d}±0.8	3.8^{ab}±0.2	0.135^{ab}±0.0034
30 mM KCl+2.0 mM TU+ 0.5 mM SA	97.6^{a}±2.4	12.0^{ab}±0.5	23.8^{d}±1.5	16.1^{d}±0.7	8.8^{de}±0.3	0.137^{ab}±0.0028
30 mM KCl+0.50 mM SA+ 2.0 mM TU+0.24 mM GA	97.6^{a}±2.4	12.5^{b}±0.7	24.4^{d}±0.8	20.0^{e}±0.4	5.6^{bc}±0.3	0.144^{b}±0.0039

Values are the mean±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, $p<0.05$

Fig. 1. Ten days old of drought-stressed rice cv. MR284 seeds germinated in different combination treatments of KCl, TU, GA and SA

3. RESULTS

3.1 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 shown in Tables 3-6. From Tables 3-6, 30 mM KCl, 2.0 mM TU, 0.24 mM GA, and 0.5 mM SA were selected as the ideal concentration and used in the second step.

3.2 Combination Treatments to Produce Liquid Enhancer

Some of the combination treatments resulted in better seedling growth of drought-stressed cv. MR284 seed compared to control treatment. It showed a significantly 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 rice cv. MR284. In Table 7, some of the treatments did not differ p>0.05 from each other. Fig. 1 shows a comparison of 10 days-old rice cv. MR284 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 consisting of 30 mM KCl + 2.0 mM TU + 0.5 mM SA was the best to enhance germination and seedling growth of drought-stressed rice cv. MR284 seed.

4. 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 cv. MR284 rice seed. There were two steps 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 was used except for KCl.

In step one, 30 mM was the ideal concentration for KCl. Potassium (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 [18,19,20]. Application of K fertilizer mitigated the adverse effect of plant growth under drought stress by increasing plant height. Beside, Cakmak [21] 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 groups which were 'thiol' and 'imino' which have the function in oxidative stress response and fulfills the nitrogen requirement respectively. Amin et al. [22], reported that TU was found to be more effective than SA due to its ability in enhancing the 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 [23].

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 rice cv. 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 [24]. 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 rice cv. 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 rice cv. MR284 seeds. Shakirova et al. [25] reported that different concentrations of SA gave different hormonal effects, either inhibit or enhance the growth of the plant. Salicylic acid is a naturally occurring phytohormone that contains phenolic compound. It involves in many defense-related functions such as increased responses to biotic and abiotic stress factors, induced tolerance toward environmental stress and regulated growth and development of plants [26].

The combination of these chemicals were found to have better germination and seedling growth compared to a 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 response and alleviated drought stress in the seed and enhanced the germination process and the seedling growth of rice cv. MR284 seed. Based on previous studies, many combinations of different types of plant growth regulators had been used on different plant types to understand how a plant survive under stress conditions. In 2007, a study that was conducted by Cavusoglu and Kabar [27] 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.

5. 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, due to the inadequate of water content. One of the strategies to aid in the germination of the 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 rice cv. MR284 seed. This liquid enhancer was found to have an ability to increase the germination performance and early seedling growth compared to other treatments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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