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A PRELIMINARY STUDY ON PRE-TREATMENT SOLUTIONS TOWARDS CHILI SEEDS GERMINATION

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ABSTRACT

Capsicum annuum L. is an important crop due to its large scale consumption in many countries. However, low germination process of chili seed is the common problem encountered by planters. Seed priming is believed able to overcome this problem by stimulate imbibition of seed using pre-treatment solution. In this study effect of two pre-treatment solutions; gibberellic acid (GA_3) and hydrogen peroxide (H_2O_2) on germination process of chili seeds were evaluated. Experimental works were carried out through imbibition of chili seeds in the different concentrations of pre-treatment solution and imbibition period (one hour and 24 hours). After imbibition procedure, chili seeds were air dried for one hour before sowing process. Germination percentage and growing performance of chili seeds were assessed after seven days of sowing. The germination percentage of chili seed were identified based on percentage of successfully germinated chili seed while the growing performances were assessed through the average length of root, stem and leaf. Study has found that the most suitable pre-treatment solution for chili seeds is 6% of GA_3 with one hour imbibition period with highest germination percentage (92%) and average length of root, stem and leaf of 3.2±0.1 cm, 3.1±0.1 cm and 1.0±0.2 cm respectively. GA_3 significantly speed up germination process by regulating protein synthesis, hence suitable to be used as pre-treatment solution.

Keywords: *Gibberellic acid, hydrogen peroxide, germination, chili seed*

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1. INTRODUCTION

Chili (*Capsicum annuum L.*) is widely cultivated in Asia, Africa, and Mediterranean countries which have numerous uses in culinary preparations that make this one of the most important vegetables (Silva, *et al.*, 2013). Chili has several species and can be characterized according to different sizes and shapes. Fresh chili is known to have outstanding source of vitamin C and E, provitamin A, carotenoids and phenolic compounds, metabolites with renowned antioxidant activity which have good impacts on human health where it likely to have potent effect against cancers, preclude from gastric ulcer as well as activate the immune system (Materska and Perucka, 2005; Sun, *et al.*, 2007). However, production of chilies may encounter several obstacles due to disease and low germination of chili seeds which may drastically reduce the quality and yield of chilies. Low germination of chili seeds can be enhanced through several techniques including seed

priming. Seed priming involves hydration of seed in several ways thus improved germination rate, uniformity in emergence and germination under a wide range of environmental climates while also enhanced seedling vigor and growth (Venkatasubramanian and Umarani, 2010). Seed priming technique is widely used in promoting germination process which involves imbibition of seed in water followed by drying process (Pulok, *et al.,* 2014). Several priming techniques have used widely including osmo priming, halo priming, hydro priming, hormone priming and others various chemical solutions (Divya and NirmalaDevi, 2015). Based on study by Ruttanaruangboworn, *et al.*, (2017) seed priming is a technique that helps rice seed to germinate better in soil under harsh conditions such as lack of moisture and unsuitable temperature. The fundamental of seed priming is closely related on seed imbibition characteristic where it involves controlling temperature and moisture content in seed. Through seed priming it may enhance the germination process hence breaking the seed dormancy to initiate faster germination process.

On the other hand, seed dormancy is a period where growth and development of living organisms are temporarily stopped. However, the fundamental mechanisms which describe the seed dormancy process still remain inconclusive. According to Nonogaki in 2014, intensive efforts have been made to investigate gibberellin and abscisic acid metabolism in seeds, which greatly contributed to the current understanding of seed dormancy mechanisms. Gibberelic acid (GA_3) is a positive regulator of seed germination whereas abscisic acid (ABA) is essential for the establishment and maintenance of seed dormancy (Finch-Savage and Leubner-Metzger, 2006). In addition, hydrogen peroxide (H_2O_2) also play beneficial roles in managing cell communication network with phytohormones like GA_3, ABA and reactive molecules such as nitric acid and hydrogen sulfide to facilitate germination process (Wojtyla, *et al.*, 2016).

GA_3 is a plant hormone stimulating plant growth which promotes germination process. GA_3 first found in Japan as metabolic byproduct of the fungus *Gibberella fujikuroi*, which result in rapid elongation of paddy stem result in plant collapsed (Riley, 1987). Gupta and Chakrabarty in 2013 stated that, GA_3s are endogenous plant growth regulators, having tetracyclic, diterpenoid compounds function in stimulating seed germination as well as triggering plant development together with an interaction of various environment elements such as light, temperature and water. GA_3s have a primordial role as their exogenous application that counter balance the inhibition effect of ABA which plays significant function in initiating seed germination as compared to others growth regulators like auxins and cytokinins (Vieira, *et al.*, 2002). As for H_2O_2, it can react as catalyst in seed development like elongation of radicle, coleoptile and fresh weight of the seedling by reducing the germination-delaying and inhibit the effects of elevated level of both salt and temperature (Covusoglu and Kabar, 2010). Germination involves development of seed where vital structures required for further development into a plant under favorable conditions is formed (Riley, 1987). The new plant formed by sexual reproduction starts as an embryo within the developing seed, therefore, the vitality of the young seedling is mainly influenced by the physiological and biochemical features of the seed (Bewley and Black, 1994). The present study was carried out to investigate the effect of two different pre-treatment solutions; GA_3 and H_2O_2 towards germination of chili seeds.

2. MATERIAL AND METHODS

2.1 Seed Material

Fresh chilies were obtained from local market and seeds were taken out. Only seeds with good condition were used as sample. Seeds were washed by using distilled water, dried and stored in dry place.

2.2 Preparation of Pre-treatment Solutions

3% and 6% of both H_2O_2 and GA_3 pre-treatment solutions were prepared freshly prior to imbibition procedure. Distilled water was used as solvent in preparation of pre-treatment solution. Each of the pre-treatment solutions was shake thoroughly to produce homogenous solution.

2.3 Preparation of Chili Seeds

Selected chili seeds were treated with pre-treatment solutions (GA_3 and H_2O_2) before sowing procedure. Distilled water was used as control. The chili seeds were treated in two different imbibition periods, one and 24 hours respectively. After imbibition period, all treated seeds were air dried for one hour.

2.4 Germination Percentage and Seed Growing Performance

The pre-treated seeds were sown in planting tray containing pith moist soil. Seeds were placed approximately 1 cm depth from the base of planting tray. Then, approximately 1 cm of soil was poured on top to cover the seeds planted. The planting tray was placed under direct sunlight to enhance germination process. The seeds were watered twice a day to maintain its moisture. Germination percentage of chili seeds were identified based on numbers of successfully germinated seed. On the other hand, growth performances of chili seeds were identified based on the average length of stem, root and leaves after seven days. Each experiment was replicate three times with approximately 25 seeds per treatment.

2.5 Statistical analysis

The results of the study were analyzed by using the Statistical Package for the Social Sciences (SPSS) Enterprise IBM SPSS version 20 for Window 7. Two – way ANOVA has used to test at 5% less than level of significance which is ($p < 0.05$) in order to determine the variation between means of the parameters that were tested.

3. RESULT AND DISCUSSION

In this study, the effect of different pre-treatment solutions towards germination of chili seeds via seed priming technique was evaluated. Seed germination percentage was observed and documented after seven days. As mentioned by Qureshi, *et al.,* 2016) germination is defined as the radical or plumule of seed became visible on the surface of seed. In this experiment, the successfully germinated chili seeds were observed based on radical protrusion of seed and data obtained were tabulated in Table 1.

Table 1: Germination percentage for all treatments after seven days

Pre-treatment solution	Germination percentage (%)	
	1 hour imbibition	24 hours imbibition
Distilled water (control)	68	76
3% GA_3	48	52
6% GA_3	92	84
3% H_2O_2	80	64
6% H_2O_2	44	56

Imbibition period could affect the seed germination percentage as according to Table 1, longer imbibition period (24 hours) had promote better germination of chili seeds. This is because all seed with 24 hours imbibition period showed more than 50% of germination. On the other hand, seeds imbibed for one hour had showed less than 50% germination as for 3% GA_3 and 6% H_2O_2. This may due to incapability of the pre-treatment solutions to break the seed dormancy during the seed priming procedure.

Priming with appropriate concentration of pre-treatment solution is crucial as it provide an important role in seed germination (Raheem *et al*., 2014). Thus, study proceeds by comparing between two different types of pre-treatment solutions at different concentration. Result showed that different concentration of pre-treatments solution give different germination percentage. This is because the by increasing of GA_3 concentration had increased the germination percentage from 48 % to 92% (one hour) and from 52% to 84 % (24 hours). GA_3 is known as an effective plant hormone regulator in overcome seed dormancy and enhance rapid seed germination (Riley, 1987). According to study done by Raheem *et al.* (2014), higher concentration of GA_3 (10^{-4} M) has significantly enhanced germination of sponge gourd. In contrast, for H_2O_2 the germination percentage decreases as the concentration increase.

Among all treatment, 6% GA_3 with one hour imbibition period showed a remarkable germination percentage at 92%. The result is supported by finding from Ghodrat and Rousta (2012) that priming with lower concentration of GA_3 had no effect on germination rate and germination percentage, however at higher concentration it will give a positive effect.

Normally, chili seed requires seven to ten days to successfully germinate (Divya and NirmalaDevi, 2015). However, different result documented in this experiment as most of treated chili seeds had successfully germinated and growth well within that time period. In this study, the growing performances of chili seeds were determined by measuring average length of root, stem and leaf. Figure 1 below illustrates the growing performance for one hour imbibition period for all pre-treatment solutions.

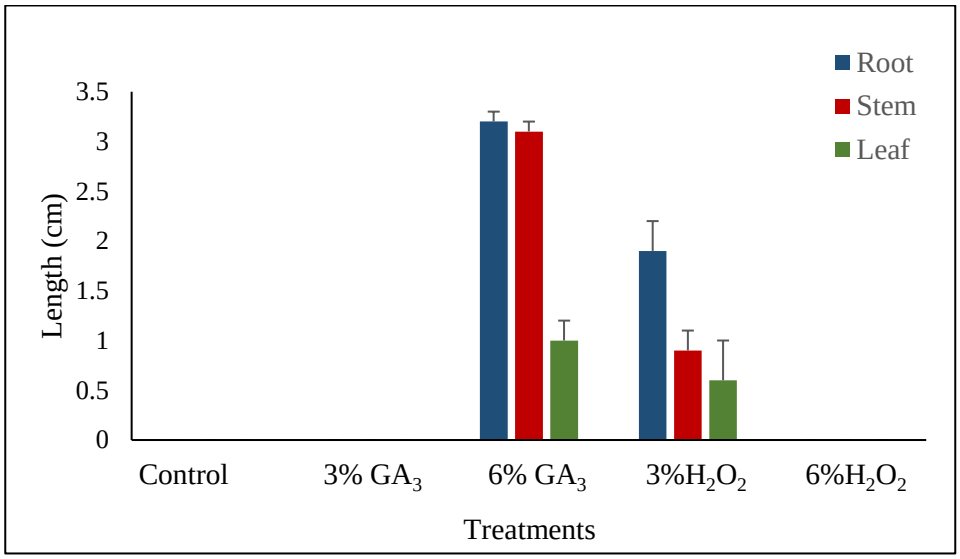

Figure 1: Growth performance of chili seeds for one hour imbibition period

After seven days, all seeds were evaluated and data observed showed that several pre-treatment solutions (control, 3% GA_3 and 6% H_2O_2) did not promote any seed growth after one hour imbibition. Seeds only undergo germination process but did not able to promote growth in seven days. According to Figure 1, 6% GA_3 showed significantly highest growth performance of chili seeds ($p<0.05$) for all measured variable length of root (3.2±0.1), length of stem (3.1±0.1) and length of leaves (1.0±0.2). Study done by Vieira *et al*. (2002) proved that adequate amount of GA_3 can triggered the synthesis, activation and secretion of hydrolytic enzymes that are need for development of embryo within the seed. The successful growing of seeds treated with 3% H_2O_2, explained by findings done by Diao *et al.* (2017) that H_2O_2 is an important signal molecule which can participate in several plant physiological activities involving adaptive stress response through complex network with other radical species and plant hormones. Figure 2 below showed the seeds growing performance for 24 hours imbibition period.

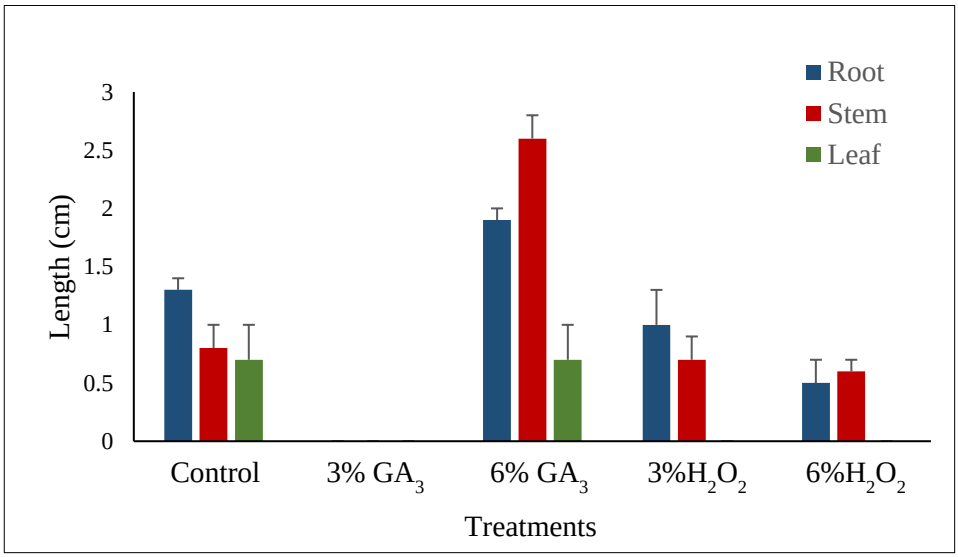

Figure 2: Chili seeds growing performance for 24 hours imbibition period

According to Riley 1987, GA_3 have significant effect at very low concentration which can boost germination process, however too high concentration level may suppress the germination process. In this study, optimization of GA_3 concentration for seed priming was done and discovered that 6% was the best concentration for seed growth after 24 hours imbibition period (Figure 2). Then, the growth performance for 24 hours imbibition was followed by control, 3% H_2O_2 and 6% H_2O_2. Control treatment showed comparable growth performance with 3% and 6% H_2O_2 as the water is a universal imbibition medium that is widely used as soaking medium. Figure 3 below illustrated the seed growth performances imbibed with 6% for one hour and 24 hours.

Figure 3: Growth performance of chili seeds imbibed in 6% GA_3 after seven days sowed (a) 1 hour imbibition period (b) 24 hours imbibition period.

4. CONCLUSION

As a conclusion, 6% GA_3 with one hour imbibition period provide the most significant result for both germination percentage and performance. It proved that this technique is a quick and efficient method of seed priming for breaking dormancy in chili seeds as well as producing better growth performance. Furthermore, one hour imbibition period is enough to provide better seed growth as the average length of root, stem and leaf recorded are greater than 24 hours. For future research, some improvement can be done by increase the concentration for GA_3 and test on the ideal imbibition period for each of the pre-treatment solution to optimize the chili seed germination as well as its growth performance.

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