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Enhancing seed germination of king chilli (*Capsicum chinense* Jacq.) using pre-treatment solutions

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Abstract
King chilli (*C. chinense* Jacq.) is an important spice crops in north eastern region of India which have numerous uses in culinary preparations as well as medicinal purpose. Presence of high amount of capsaicin compound which have good impacts on human health where it's likely to have potent effect against cancers, preclude from gastric ulcer as well as activate the immune system. However, seed borne disease like *Cucumber mosaic virus* (CMV) as well as low germination process of king chilli seeds are the most common problems faced by farmers. PCR detection of CMV and Seed priming is expected 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), were used along with water (H_2O) as a control, were evaluated on germination process. Experimental works were carried out through imbibition of king chilli seeds in the different concentrations of pre-treatment solution and imbibition period (one hour and 24 hours). Germination percentage and growing performance of chilli seeds were assessed after ten days of sowing. The germination percentage of king chilli seed were identified based on percentage of successfully germinated king chilli seeds while the growing performances were assessed through the average length of root, stem and leaf. From the experiment revealed that the most appropriate pre-treatment solution for king chilli seeds is 6% of GA_3 with one hour imbibition period with highest germination percentage (97%), and average length of root, stem and leaf of 3.5±0.1 cm, 2.6±0.1 cm and 1.7±0.2 cm, respectively and followed by 3% of H_2O_2 (80% germination) and H_2O (65% germination), respectively.

Keywords: Gibberellic acid, hydrogen peroxide, water, germination, *C. chinense* Jacq

Introduction
Chilli (*C. chinense* L.) is widely grown in Asian countries which have a lot of uses in culinary preparations that make this one of the most important vegetables (Silva, *et al.*, 2013) [16]. King chilli (*C. chinense* J.) is vastly grown in North Eastern (NE) region of India and consumed in many preparations both spice and medicines. It is known as bhut jolokia, umorok, naga king chilli and Hmarcha pui in Assam, Manipur, Nagaland and Mizoram vernacular, respectively (Chanu, *et al*., 2017) [2]. Chilli has many species and can be differentiated according to different sizes and shapes. Fresh chilli is known to have outstanding source of pro-vitamin A, vitamin C and E, carotenoids and phenolic compounds, metabolites with renowned antioxidant property which have good impacts on human health conditions where it seems to have a good property against cancers, preclude from gastric ulcer as well as trigger the immune system (Materska and Perucka, 2005; Sun, *et al.*, 2007) [9, 17]. However, production of chillies may encounter several problems due to diseases and low germination of chilli seeds which may extremely reduce the quality and yield of chillies. Low germination of chilli seeds can be enhanced through several techniques and method including seed priming. Seed priming involves hydration of seed in different ways thus improved germination rate, uniformity in emergence and germination under a wide range of environmental climatic conditions while also increase seedling vigour and growth (Venkatasubramanian and Umarani, 2010) [18]. Seed priming method is widely used in promoting germination process which involves imbibition of seed in water followed by drying process (Pulok, *et al.*, 2014) [11]. Several priming techniques have used widely including osmo priming, hydro priming, halo priming, hormone priming and others various chemical solutions (Divya and Nirmala Devi, 2015) [5]. According to Ruttanaruangboworn, *et al.*, (2017) [15] seed priming is a technique that helps rice seed to germinate better in soil under harsh conditions such as lack of moisture and unfavourable

temperature. The fundamental of seed priming is closely related on seed imbibition characteristic where it involves controlling moisture and Temperature content in seed.
Through seed priming it may enhance the germination process thus breaking the seed dormancy to initiate faster germination process.
On the other way, seed dormancy is a period where growth and development of living organisms are temporarily dropped. However, the main mechanisms which describe the seed dormancy process still remain inconclusive. According to Nonogaki (2014) [10] reported that intensive efforts have been made to investigate gibberellin and abscisic acid metabolism in seeds, which greatly bring about to the current understanding of seed dormancy mechanisms. Gibberelic acid (GA_3) is a positive adjuster of seed germination whereas abscisic acid (ABA) is essential for the establishment and continuity of seed dormancy (Finch-Savage and Leubner-Metzger, 2006) [6]. In addition, hydrogen peroxide (H_2O_2) also play beneficial roles in managing cell communication network with phytohormones like ABA, GA_3 and reactive molecules such as nitric acid and hydrogen sulfide to enable germination process (Wojtyla, *et al.,* 2016) [20].
GA_3 is a plant hormone that is stimulating plant growth which stimulate germination process. GA_3 first found in Japan as metabolic by product of the fungus *Gibberella fujikuroi*, which result in quick elongation of paddy stem result in plant collapsed (Riley, 1987) [14]. Gupta and Chakrabarty in 2013 [8] reported 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 temperature, light and water. GA_3 has an ancient 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 cytokinins and auxins (Vieira, *et al.,* 2002) [19]. 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 raised level of both salt and temperature (Covusoglu and Kabar, 2010) [3]. Germination includes development of seed where vital structures required for farther development into a plant under glowing conditions is formed (Riley, 1987) [14]. The new plant formed by sexual reproduction begins as an embryo within the developing seed, therefore, the liveliness of the young seedling is mainly influenced by the physiological and biochemical characteristics of the seed (Bewley and Black, 1994) [1]. The present study was carried out to carry out the effect of two different pre-treatment solutions; GA_3 and H_2O_2 towards germination of chilli seeds.

Materials and Methods

Seed Material

Matured fresh chilies were obtained from Khoisuman, 24°61742 Latitude, 093°77806 Longitude and 776 meter MSL, Bishnupur district of Manipuir Bishnupur and seeds were taken out by using laboratory rubber glove. Only healthy looking seeds were used as sample. Seeds were washed by using distilled water, shade dried and stored in dry place for further experiment (fig.1).

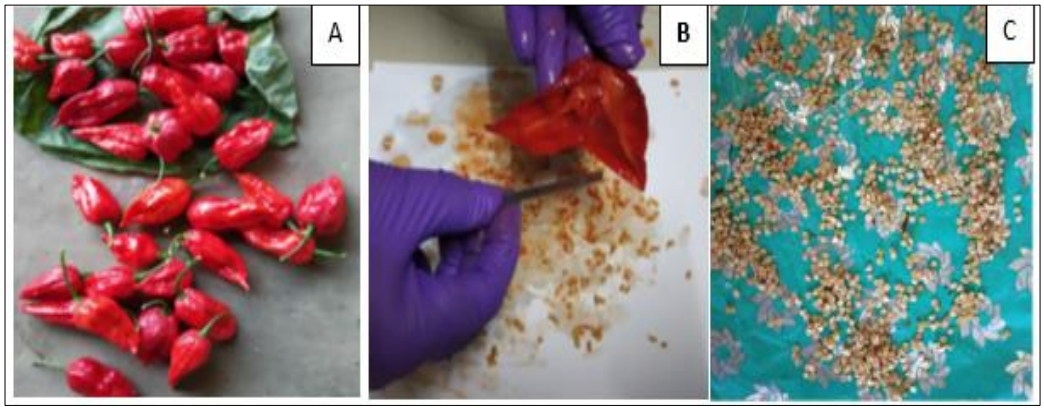

Fig 1: Extraction of king chilli seed (A, B & C) from mature fruit collected from Bishnupur district of Manipur

Preparation of Pre-treatment Solutions

Both 3% and 6% of H_2O_2 and GA_3 pre-treatment solutions were made ready freshly prior to imbibition procedure. The solvent used in the preparation of pre-treatment solution was Distilled water. Each of the pre-treatment solutions was quivered thoroughly to produce homogenous solution.

Preparation of Chili Seeds

Sort out king chilli seeds were treated with pre-treatment solutions (GA_3 and H_2O_2) before sowing procedure. Distilled water was used as a control. In this experiment, the king chilli seeds were treated in two different imbibition periods, one and 24 hours respectively. After imbibition period, all chemical treated seeds were air dried for two hours.

Germination Percentage and Seed Growing Performance

The pre-treated seeds were sown in petri dish and plastic tray containing with cotton ball (fig.2). Seeds were placed on the top of the wet cotton. The planting tray or petri dish was placed under sunlight area to enhance germination process. The seeds were watered regularly to maintain its moisture. Germination percentage of chilli seeds were identified based on numbers of successfully germinated seed. On the other hand, growth performances of chilli seeds were identified based on the average length of stem, root and leaves after seven days. Each experiment was replicate three times per treatment.

Fig 2: Comparison of growth performance of king chilli seeds among the best treated one: control (1, 2 & 3), 3% of H_2O_2 (4, 5 & 6) and 6% of GA_3 (7, 8 & 9) after ten days after sowing (1 hour imbibition).

Statistical analysis

The results of the experiment were analysed 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 that is (p<0.05) in order to determine the variation between means of the parameters that were tested.

In this experiment, the effect of different pre-treatment solutions regarding germination of king chilli seeds via seed priming technique was assessed. Seed germination percentage was made out and recorded after seven days. As said by Qureshi, *et al.*, 2016) [12] germination is defined as the radical or plumule of seed became visible on the surface of seed. In this experiment, the successfully germinated chilli seeds were noticed based on radical protrusion of seed and data obtained were arranged in Table 1.

Table 1: Germination percentage for one & 24 hour imbibition after seven days

	Germination percentage (%)	
Pre- treatment solution	**1 hour imbibition**	**24 hour imbibition**
Distilled water (control)	65	72
3% GA_3	47	62
6% GA_3	97	87
3% H_2O_2	80	62
6% H_2O_2	42	53

The imbibition period could affect the percentage of king chilli seed germination as according to Table 1, longer imbibition period (24 hours) had boosted better germination of king chilli seeds. This is the reason of all seed with 24 hours imbibition period showed more than 50% of germination. On the contrarily, 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 inability of the pre-treatment solutions to break the seed dormancy during the seed priming process.

Priming with an appropriate concentration of pre-treatment solution is critical as it provide an important factor in seed germination (Raheem *et al*., 2014). Thus, study going on by comparing between two dissimilar types of pre-treatment solutions at different concentration. Result revealed that different concentration of pre-treatments solution deliver different germination percentage. This is due to by increasing of GA_3 concentration had increased the germination percentage from 48 % to 95% (one hour) and from 62% to 87 % (24 hours). GA_3 is an appreciated for effective plant hormone regulator that is widely use to overwhelm 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 95%. Ghodrat and Rousta (2012) [7] supported this result that priming with lower concentration of GA_3 had no much effect on germination rate and germination percentage, even so at higher concentration it will give a positive effect.

Normally, chilli seed requires seven to ten days to successfully germinate by Divya and Nirmala Devi (2015) [5]. Although, non-identical result documented in this experiment as most of treated chilli seeds had successfully germinated and growth well within that period of time. In this experiment, the growing performances of chilli seeds were determined by measuring average length of root, stem and leaf. Figure 3 below illustrates the growing performance for one hour imbibition period for all pre-treatment solutions.

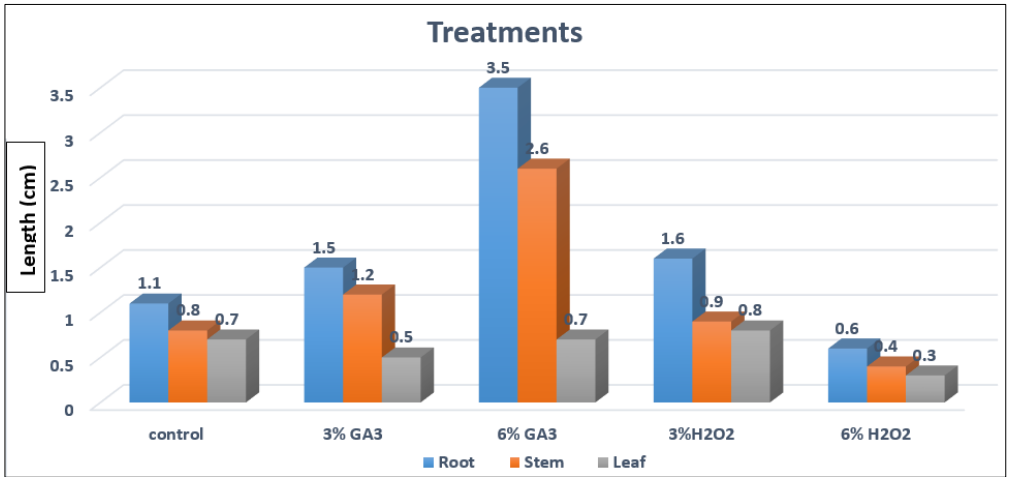

Fig 3: Growing performance of king chilli for 1 hour imbibition period

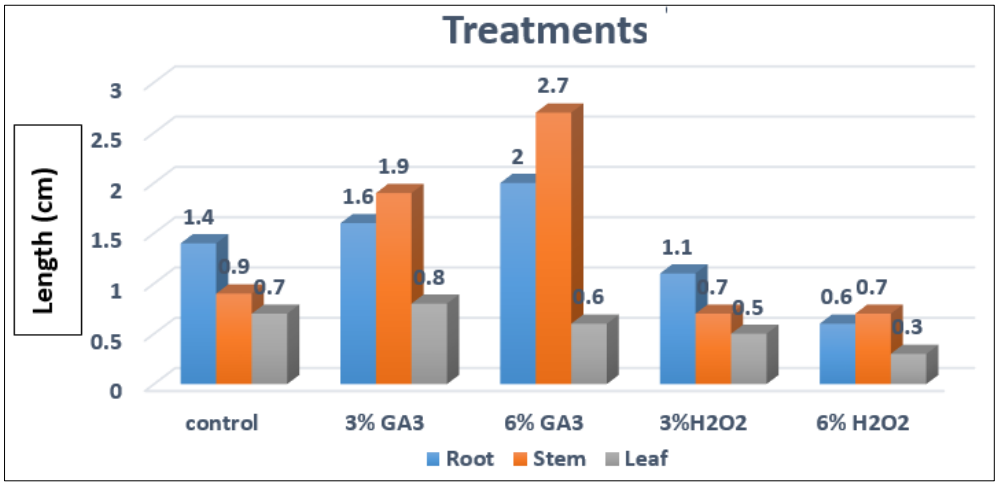

Fig 4: Growing performance of king chilli for 24 hours imbibition period

After seven days, all seeds were assessed 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 undertake germination process but did not able to assist 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 (3.1±0.1), length of stem (3.±0.1) and length of leaves (1.0±0.2). Experiment done by Vieira *et al*. (2002) [19] showed that adequate amount of GA_3 can triggered the synthesis, activation and secretion of hydrolytic enzymes that are necessary for the development of embryo within the seed. The successful growing of seeds treated with 3% H_2O_2, described by findings done by Diao *et al*. (2017) [4] that H_2O_2 is a main signal molecule which can take part in several plant physiological activities involving adaptive stress response through composite network with other plant hormones and radical species. The seeds growing performance for 24 hours imbibition period is shown in Figure 4 above.

Finding report by Riley 1987 [14], GA_3 have remarkable effect at very low concentration which can improve germination process, but too high concentration level may vanquish the germination activity. In this experiment, optimization of GA_3 concentration for seed priming was done and revealed 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 broadly used as soaking medium. Figure 5 below illustrated the performances of seed growth with 6% imbibed for one hour and 24 hours.

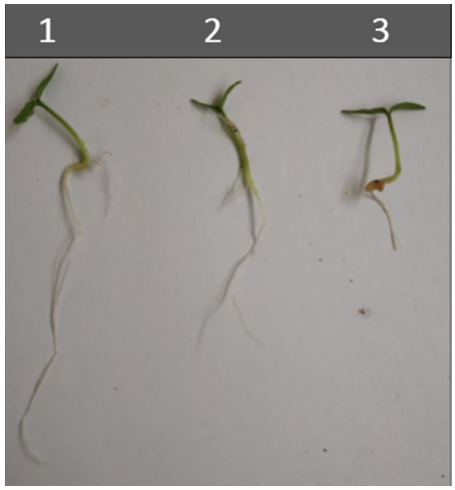

Fig 5: Growth performance of king chilli treated seeds (1) imbibed in 6% GA3, (2) imbibed in 3% H_2O_2 and (3) imbibed in H_2O (control) seven days after sowing (1 hour imbibition).

Conclusion

As a final result from the experiment, 6% GA_3 with one hour imbibition period provide the most significant result for both germination percentage and performance in term of root, stem and leaf length. It showed that this method is a fast and more

efficient technique of seed priming for breaking dormancy in king chilli seeds as well as producing better growth performance. Moreover, one hour imbibition period is far enough to provide better seed growth performance 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 king chilli seed germination as well as its growth performance.

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