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Germinability and Seedling Growth Performance of Chilli (*Capsicum annuum*) Seeds in Response to Different Gibberellic Acid Concentrations Pre-Treatment

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Abstract: Chilli seeds are always associated with physiological dormancy characteristics or endogenous dormancy that has affected their germination potential. Problems noticed on the low seed germination rate, vigour growth, further may lead to the poor seedling growth pattern of chilli crop production. Gibberellic Acid (GA_3) which is usually used as a treatment solution is not a new advent of seed dormancy breaking methods for chilli. But, looking forwards to a better GA_3 concentration is still needed for the discovery of this treatment potential effect on chilli seed germinability and seedling growth. Thus, this study aimed to evaluate the effect of different GA_3 concentrations on seed emergence and seedling growth performance in chilli. In this experiment, chilli seeds were imbibed with 25 mg/L, 75 mg/L and 125 mg/L concentration of GA_3 with three replications each; for 24 hours at room temperature (32±4°C) and the untreated seeds as a control. The data collection on final germination percentage was taken daily for 21 days after sowing. While the 15 germinated seeds were directly planted on the 5 polybags arranged in the net house of Junaidy Jonik Farm, Sabah, Malaysia. The experiment was arranged in a completely randomized design with three replications. Data were subjected to analysis of variance with SAS version 9.4 and the significant means were separated by the least significant difference test at $P<0.05$. Significant differences were observed in the seed germinability measured; germination rate index (GRI), mean germination time (MGT) and final germination percentage (FGP), as well as on seedling vigour index (SVI) between treated and control seeds. Conversely, plant height, number of internodes, number of leaves and fresh weight of seedlings showed no significant differences among treatments. It was concluded that the use of GA_3 was able to enhance chilli seeds germinability and could display a better SVI than the control. It is recommended to use GA_3 treatment at 25 mg/L of concentration, as it may give an advantage to both economic and biological importance in producing higher germinability and seedling growth performance in chilli.

Keywords: Endogenous dormancy, seed quality, imbibe seed, priming, yield

1. Introduction

In Malaysia, chilli is one of the main fruity vegetable crops that are highly demanded in the domestic or international market. It is also recorded as the highest Import Dependency Ratio (IDR) with 73.6% (Department of Statistics Malaysia, 2019). Malaysia ranked 28th on the Global Food Security (GFS) in 2019, and food security became necessary as food imports continue to rise (The Edge Malaysia, 2020). The demand had increased since the utilization of chilli in daily life increased, and the number of populations grew (Jabatan Pertanian, 2016). However, the chilli's Self-sufficiency level (SSL) in Malaysia is about 30.8% in 2019, as compared to 65.6% in 2013, which showed a significant reduction in 6 years (Jabatan Pertanian, 2020).

The agriculturists' one big concern is to attain a great seed emergence and seedling vigour (high seed quality) in a planting season for desirable chilli production. Previously, numbers of the study conducted to achieve that goal as chilli seeds are always associated with physiological dormancy characteristic or endogenous dormancy that affect its germination potential (Alcalá-Rico et al., 2019). Seed dormancy is a temporary failure of viable seeds to germinate under favourable conditions due to some properties of the seed inhibiting germination which leads to delay of germination (Baskin & Baskin, 2004; El-Keblawy, 2017). One of the promising methods to break dormancy was by GA_3 solution that used to treat seed before planting (Debbarma et al., 2018). However, researchers were still looking forwards to better

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GA_3 concentration to use. Thus, a study had been conducted to look at the response of chilli seed and seedling towards the treatment in different GA_3 concentrations. Lower concentrations of GA_3 were chosen as it demonstrated better reactions in dormancy breaking for chilli seeds (Debbarma et al., 2018), and vice versa (Muhammad et al., 2017) . ...

It is also vital for the future economic decision to save cost and its biologically able to reduce the outsource uptake by seeds to avoid the possibility of toxicity. The objective of this study is to evaluate the effect of concentrations of GA_3 solution on seed germination and seedling growth performances in chilli.

2. Literature Review

Chilli comes from the genus of Capsicum and the family of Solanaceae, comprise of 35 species and five of them are mainly planted (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*). Chilli or red chilli (*Capsicum annuum*) is the highly produced type of chilli in Malaysia (Jabatan Pertanian, 2020), for having tremendous demand as the hot flavour is consumed very often (Wong, 2017). Besides, chillies are also rich in vitamin C as used in food and drink industries and the capsaicinoids are attributed to their antioxidant and anticancer (Prasad et al., 2006). Chillies seed is produced into an essential oil that psychologically can make feelings of joy due to its strong scent (Swapan et al., 2017).

Seeds are a presence of a new tiny plant spewed out from their mother plant, it is a propagation method to ensure the species survivability. It is located at the first stage in the plant growth phases. Besides, the seed is also like a safe because the tiny plants are enclosed inside the seed coat to separate it from the outside contact. They contain high protein, starch, and oil reserves, used in the beginning stage of plant development (Government of Western Australia, 2013). At this stage, it needs the external factors from its surroundings such as temperature, moisture, air, and light correctly to germinate. Those seeds are dispersed away from their mother plant to avoid competition (Bareke, 2018). In addition, seed germination is also influenced by seed quality.

Seed quality illustrates the potential performance of a seed lot. The seed quality that is related to dormancy is the physiological quality. It consists of five elements namely germination capacity, viability, vigour, vitality and dormancy (Huda, 2001). Many reasons brought about physiological dormancy or specifically, endogenous dormancy. It is due to the chemical changes within the seed embryo such as excessive inhibitor presence like abscisic acid that act as germination inhibitor, second the immature embryos that cause germination not to occur until the embryos develop into their normal size (Sinha et al., 2017), and due to lack of enzymes, that essential for complete physiological maturation. Seed dormancy is a common attribute of temperate species (Benech-Arnold et al., 2013), crucial quality since seed storage can be prolonged even during adverse conditions such as dry weather or over moisture while still able to retain its natural quality, therefore, it is a survival mechanism in seed (Sinha et al., 2017). However, a high level of dormancy after harvest is undesirable when rapid seed germination is required on planting (Singh & Upadhyaya, 2015). Seed dormancy has affected agricultural production since resulting in no uniform germination and disturbing sowing and planting schedule, the seed could remain not germinated for a long time, and cause too hard to conserve plant population and interfere with seed testing procedure (Sinha et al., 2017). In theory, the seed germination, vigour and size of seed may influence crop yield (Ellis, 1992). Poor vigour decrease yields as derived from decreased emergence, leading to sup-optimal and seedling growth slowly (Roberts & Osei- Bonsu, 1988).

GA_3 is a plant hormone used in agriculture as the plant growth regulator and promotes growth (Weinstein, 2019). In very small concentrations, GA_3 stimulates cell division and elongation, breaks dormancy, and speeds germination. Also, GA_3 was known to induce seed germination, promote shoot growth and internode elongation, determine the sex expression of a plant, and is involved in promoting the flowering of plants (Gupta & Chakrabarty, 2013). There were various ways to apply this hormone either by imbibing, dipping, adding to media and foliar spray to crops.

3. Methodology

3.1 Preparation of Chilli Seeds

The study has been conducted in Beluran, Sabah, Malaysia. Six hundred chilli (*Capsicum annum*) seeds were assigned into 12 labelled petri dishes, control (C) and treatments (T1, T2 and T3) with 3 replications. Seeds with abnormal appearances, small, irregular shape and darken were removed and only the good seeds were allocated to be treated. Fifty seeds were placed in each of the petri dishes, as per replication (ISTA, 2019). The control seeds were directly sown into the paper towel.

3.2 Preparation of Gibberellic Acid Solutions

The homogenous GA_3 solutions at concentration 25 mg/L (T1), 75 mg/L (T2) and 125 mg/L (T3) were prepared by weighing 25 mg/L, 75 mg/L and 125 mg/L of GA_3 powder and dissolved in 1 L of distilled water respectively. The mixed solutions were then stirred using a plastic spoon until all the powder was dissolved.

3.3 Seed Imbibition Treatment

A 40 mL of GA_3 solutions were poured into each of the petri dishes content seeds for the imbibition treatment purpose. The petri dishes were then covered and sealed with parafilm and the imbibition was left for 24 hours in a closed paper box. After that, the solutions were removed and the seeds rinsed under distilled water to stop the imbibition.

3.4 Chilli Seeds Sowing and Germination

The seeds were sown in two layers of paper towel and sprayed with 3 mL distilled water. The paper towel was folded and inserted inside the labelled zip lock bag, locked and then placed inside a closed paper box for a darker condition at room temperature at 32±4°C. All seeds were watered twice a day. The data collection of germinated seeds was done for 21 days. The seed is considered germinated when a 2 mm protrusion of radicals is visible (ISTA, 2019).

3.5 Transplanting

The first 15 seeds germinated from each replication were directly transplanted into the polybags under the net house. Each of the 5 polybags was planted with three germinated seeds. The planting process was conducted early in the morning or late in the evening. The planting activity has been conducted in a net house at Junaidy Jonik's Farm, in Beluran, Sabah, Malaysia, from April until May 2021.

3.6 Data Collection on Seed Germination Characteristics and Seedling Growth Performances

The seed germinability was evaluated on final germination percentage (FGP), germination rate index (GRI) and mean germination time (MGT), and while, the seedling growth was evaluated from the plant height (H), number of internodes (I), number of leaves (L), seedling vigour index (SVI) and fresh weight (FW).

3.6.1 Final Germination Percentage (FGP) Measurement

Daily data collection for seeds germination has been conducted for 21 days after sowing (DAS). The higher the FGP value, the greater the germination of a seed population (Scott et al., 1984). FGP was calculated using the following formula:

$$\text{FGP (\%)} = \text{Final no. of seeds germinated in a seed lot} \times 100 \quad (1)$$

3.6.2 Germination Rate Index (GRI) Measurement

The GRI reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination. GRI was calculated using the following formula (Esechie, 1994):

$$\text{GRI (\% per day)} = G1/1 + G2/2 + \cdots + Gx/x \quad (2)$$

Where G1 = Germination percentage × 100 on the first day after sowing; G2 = Germination percentage × 100 on the second day after sowing.

3.6.3 Mean Germination Time (MGT) Measurement

The lower the MGT, the faster a population of seeds has germinated. MGT was calculated via the following formula (Orchard, 1977).

$$\text{MGT (day)} = \sum fx / \sum f \quad (3)$$

Where f = seeds germinated on day x

3.6.4 Seedling Growth Measurement

The activity of seedling growth data collection was done two weeks after transplanting for every 7 days. The weekly data collection was listed as follows:

- *Plant height (cm)*
  The plant height was measured from the base to the tip of the shoot using a ruler.
- *Number of internodes*
  The interval between two nodes is considered and calculated as the internodes.
- *Number of leaves*
  All leaves were counted including the small leaf on the shoot tip.

3.6.5 Seedling Vigor Index Measurement

After 6 weeks of planting, at the end of data collections, the seedlings were harvested and the remaining soil medium on the roots was cleaned carefully with water. The whole plant length from the tip of the root to the tip of the shoot were taken for every seedling. Then, the seedling vigour index (SVI) calculated using the following formula (Abdul-Baki & Anderson, 1973).

$$SVI = \text{Seedling length (cm)} \times \text{Germination percentage} \quad (4)$$

3.6.6 Seedling Fresh Weight Measurement

These seedlings were weighed on a digital weighing balance for the fresh weight data collection.

3.7 Statistical Analysis

The experiment (treatment on different concentration of GA_3 imbibition on chilli seeds) was arranged in a completely randomized design (CRD) with three replications. All the data were analyzed using the SAS statistical software and the treatment means were compared using the least significant difference at $P \leq 0.05$ level of significance.

4. Result and Discussion

4.1 Effect of Different Concentration of Gibberellic Acid Solution on Germinability

In the present study, the treated seeds with different GA_3 concentrations had greater germination characteristics (FGP, GRI and MGT) than the control (Table 1); which might be due to the GA_3 uptake during imbibition stimulates germinate metabolic process and prepared for root emergence. This indicates that GA_3 was able to break the chilli seed dormancy by fastening the germination rate per day (GRI) which makes the germination time faster per population (MGT) and eventually led to the higher seed germination (FGP) in 21 days of observation, as compared to the control.

Table 1: Mean separation for germination characteristics of chilli seeds treated with different concentrations of GA_3 solution

GA3 Concentration	FGP	GRI	MGT
C (0 mg/L)	65.33^{b}	9.08^{b}	7.67^{a}
T1 (25 mg/L)	77.33^{ab}	12.72^{a}	6.79^{b}
T2 (75 mg/L)	78.67^{a}	13.99^{a}	5.97^{b}
T3 (125 mg/L)	70.67^{ab}	11.56^{ab}	6.78^{b}

Note: FGP = Final germination percentage, GRI = Germination rate index, MGT = Mean germination time. Different superscripts (^{a,b}) indicate significant differences among treatments and control. Values are expressed as mean.

Among all, chilli seeds treated with T2 (75 mg/L) show the best performance in terms of their germinability (Table 1). Compared to the control, seeds treated with T2 increased GRI by 54 % and shortened MGT by 22 % which maximized FGP by 13 %. The significant increase in GRI (as compared to the control) for the treated seeds due to the seed vigour having been improved by the GA_3 and their dormancy might have been released, as well (Jyoti et al., 2016). Besides, the sooner in germination time also proved the GA_3 had cut the cause of dormancy to delay germination. Moreover, the increase in FGP showed the total number of viable or alive seed germinated maximized and increased germination capacity. However, the insignificant best outcome for seeds treated with T2 than the other treatments revealed that there might be some ranges of effective GA_3 concentration to be used as treatment for chilli seeds.

Besides, seeds treated with T1 (25 mg/L) are considered among the best alternatives as they have comparable GRI, MGT and FGP values to TB even though with a lower GA_3 concentration (Table 1). This further gave the economic advantages of using lower GA_3 concentration, but could still produce the same quality of seeds germination characteristics. The slightly lower GRI and MGT could be derived from differing seed vigour conditions within seeds lots. Figure 1 further indicates the wide differences (between treatments) of FGP started to show for seeds treated with T2 at 5 DAS by 9.33%, higher than the ones treated with T1. The gap patterns further widened until 14 DAS, at which those treatments recorded the insignificant values among the highest FGP. Tuan et al. (2018) stated that those seeds might have a low level of dormancy and are easy to germinate under good conditions. In this study, the use of moderate (75 mg/L; T2) GA3 concentration might fasten the dormancy-breaking in chilli seeds, but with less physio-chemical changes (cell injury) within seeds. In addition, seeds treated with the lower GA3 (25 mg/L; T1) probably caused the slower dormancy-breaking process than the ones treated with TB, but with the least physio-chemical changes within seeds. This explains the insignificant results on germination characteristics after the end of data collection.

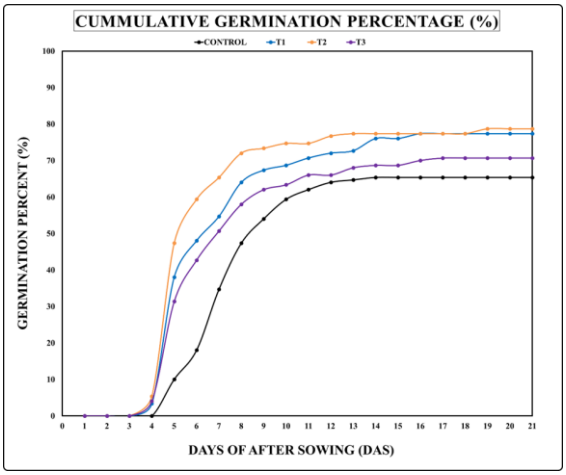

Fig. 1: Pattern of cumulative germination percentage of chilli seeds treated with different GA_3 concentrations within 21 DAS

4.2 Effect of Different Concentrations of GA_3 Solution to Seedling Growth

In the present study, the effect of treated seeds with different concentrations of GA_3 on all seedling growth variables measured was not significant (P>0.05) (Table 2). However, the treated seeds with GA_3 show slightly better seedling growth performances measured (SVI, FW, H, L and I). This showed that the GA_3 also has improved the seedling growth performance through the plant morphological appearance, as compared to the control. This might be due to GA_3 could also stimulate cell division and elongation that affect leaves and stem growth.

Table 2: Mean separation for the seedling growth pattern of chilli seeds treated with different concentrations of GA_3 solution

GA3 Concentration	H	I	L	SVI	FW
C (0 mg/L)	6.70^{a}	1.67^{a}	4.67^{a}	8.32^{b}	0.30^{a}
T1 (25 mg/L)	9.68^{a}	3.33^{a}	6.33^{a}	12.35^{a}	0.70^{a}
T2 (75 mg/L)	8.09^{a}	2.67^{a}	5.67^{a}	11.30^{ab}	0.5^{a}
T3 (125 mg/L)	7.61^{a}	2.00^{a}	6.00^{a}	9.46^{ab}	0.43^{a}

Note: SVI = H = Plant height, L = Number of leaves, I = Number of Internodes, SVI = Seedling vigour index, FW = Fresh weight. Different superscripts (^{a,b}) indicate significant differences among treatments and the control. Values are expressed as mean.

In the present study, a quite comparable effect was displayed on the SVI variable than the control, among some of the GA_3 treatments. Among all, seeds treated with T1 displayed insignificantly higher seedling performance especially for SVI variable, due to their high FGP during seed germination and eventually higher in plant length (shoot + root) values recorded. On the contrary, seeds treated with T2 exhibited insignificantly better performance only in describing their seed germination characteristics, but further, displayed insignificantly lower seedling growth, than seeds treated with T1. Similar findings in Debbarma et al. (2018), who stated that the higher GA_3 concentration may also cause poor SVI. Hence, in this study, the concentration of 25 to 75 mg/L might be the potential 'window' ranges to discover the better chilli seeds germinability with their further seedling establishment. Moreover, the insignificantly lower seedling growth for seeds treated with T2 lot might be due to the side effect of GA_3 include the increase in shoot elongation and changes in leaf shape. In turn, it after the growth pattern and might expect these effects to be non-significant or transient since GA_3 metabolized fairly quickly (Fox et al., 1995).

5. Conclusion

The use of GA_3 was able to break the dormancy of chilli seeds by displaying significantly better germination (GRI, MGT and FGP), and seedling morphological characteristics (especially for SVI variable) than the untreated seeds. Chilli seeds treated with a lower concentration of GA_3 (25 mg/L) further showed a pleasing performance. Hence, it is recommended to use GA_3 treatment at 25 mg/L of concentration, as it may give an advantage to both economic and biological importance in producing higher germinability and seedling growth performance in chilli production.

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Conflict of Interest

The authors declare no conflicts of interest.

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