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## Effect of seed priming with salicylic acid and methyl jasmonate on germination and primary root length of cowpea genotypes

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### Abstract

Under this investigation we studied about minimization of weekly data through stratification and The study was carried out to assess the optimum incubation time for cowpea seed priming and determining the optimum concentration of salicylic acid (SA) and methyl jasmonate (MJ) for cowpea seed priming. We have observed that the optimum time for cowpea seed priming with methyl jasmonate are 6hr. Further we have analysed the effect of different concentration of SA and MJ on germination percentage and root length of cowpea genotypes. We have observed that SA was inhibiting germination percentage while MJ was inhibiting primary root length.

**Keywords:** Cowpea seed priming, salicylic acid, Jasmonic acid, germination and root length

### 1. Introduction

Cowpea is one of the most important multifunction legume crops. It is commonly grown in semi-arid tropical areas of India and other countries. Each part from the bottom of root to tip of shoot including root, stem, leaves, flowers, pods and seeds of cowpea plant is beneficial to environment and environmental components including soil, humans and animals (Singh, 2005<sup>[10]</sup>; Timko *et al.*, 2007)<sup>[12]</sup>. The ripened seed of cowpea contains an average of 22-35% protein, 1.4% fat, 59.1% carbohydrate, and 3.7% ash. Cowpea seeds contain 340kcal energy per 100 g of seeds. It can fix up to 25-179 pounds of nitrogen per hectare (Elawad and Hall, 1987<sup>[2]</sup>; Sprent *et al.*, 2010). Due to high protein content, it is often called as “poor man’s meat” in many under developing countries in which animal meat is expensive (Singh, 2005)<sup>[10]</sup>. Additionally, Cowpea is often recommended due to its high nutritional values to pregnant and nursing mothers. It is well known for its ability to grow up in drought, high temperature and wide range of pH stress condition (Hall *et al.*, 2002<sup>[6]</sup>; Hall, 2004<sup>[5]</sup>, Singh, 2005<sup>[10]</sup>; Timko *et al.*, 2007)<sup>[12]</sup>. Beside this national, environmental and social importance, cowpea is considered as orphan crop, because, it received very little attention from a research standpoint as compared to chickpea, pigeon pea and green beans. Cowpea is an herbaceous and warm-seasonal annual crop (Ehlers and Hall, 1996; Craufurd *et al.*, 1997)<sup>[1]</sup>. It is diploid with 22 number of chromosome pairs containing 640M base pairs of genome size (Lonardi *et al.*, 2013). Cowpea is well known as abiotic stress tolerant crop especially in high drought and high temperature condition but it is susceptible to many biotic stresses such as bacterial, fungal, insect pest and virus diseases (Singh, 2005<sup>[10]</sup>; Timko *et al.*, 2007)<sup>[12]</sup>. Various breeding programmes are already in pipeline for screening cowpea genotypes tolerant to these biotic stresses especially for insect infestation. But the exact mechanism underlines the insect stress tolerance is still unknown. Recently various elicitors as priming agents have been used to improve host plant resistance against different stress condition (Farooq *et al.*, 2020<sup>[3]</sup>; Nabi *et al.*, 2020)<sup>[9]</sup>. In this study we have determined the optimum incubation time for cowpea seed priming. Further we have determined the effect of salicylic acid and methyl jasmonate on germination percentage of cowpea.

### 2. Material and Method

#### 2.1 Seed Priming Treatment

The experimental work was carried out in the department of Biochemistry, G.B. Pant University of Agriculture & Technology, Pantnagar.

Seeds of cowpea genotypes were procured from the department of Genetics and Plant Breeding, G.B. Pant University of Agriculture & Technology, Pantnagar. Seeds of cowpea genotypes were surface sterilized with 0.1%  $\text{HgCl}_2$  for 3 min then all toxic  $\text{HgCl}_2$  was removed out by rinsing with distilled water. This rinsing process was carried out 5-6 times to remove all remaining toxic  $\text{HgCl}_2$ . Further we have primed cowpea seeds (of PL-3 and PL-4) with 2mM concentration of methyl jasmonate (MJ) in the ratio of 1:5 ( $\text{g mL}^{-1}$ ) of weight of cowpea seed to the volume of priming solution (Wang *et al.*, 2016) [13]. Unprimed seed was considered as control treatment. Cowpea seeds were incubated for 0, 2, 4, 6, 8 and 10 hr. to determine optimum time. After incubating for particular time primed cowpea seed were dried in shadow to their original weight. Further cowpea seeds of PL-3 and PL-4 were primed with 0.01mM, 0.1mM and 1mM concentration of MJ. Additionally, cowpea seeds of PL-4 (which had lowest seed germination percentage) were primed 1mM-5mM different concentration of salicylic acid (SA). Priming was carried out in same way as describe above. Primed seeds was placed on germination paper and incubated at 37°C in incubator for germination.

## 2.2 Germination and root length measurement

Germination was recorded as radicle protruded and the percentage of germination was determined by using the formula:

$$\text{Percentage germination} = (\text{Number of seeds that germinated} / \text{Total seeds sown}) \times 100$$

Standard meter rule was used to measure the root length after 4 days from the date of seeds placed for germination in incubator.

## 2.3 Statistical Analysis

Mean and standard error was calculated using the statistical package available in Microsoft - Excel Version-2010.

## 3. Results and Discussions

### 3.1. Seed Germination and optimum incubation time

Seed priming of each of PL-3 and PL-4 with 1mM MJ showed that the germination percentage varied with different incubation time of priming solution. The germination percentage of PL-3 was 97.77%, 92.10%, 91.67%, 76.31%, 75.00% and 73.80% at 0, 2, 4, 6, 8 and 10hr of incubation time, respectively. Additionally, the germination percentage of PL-4 was 88.00%, 73.07%, 65.38%, 54.83%, 32.43% and 18.18% at 0, 2, 4, 6, 8 and 10hr of incubation time, respectively. We observed that the germination percentage of PL-4 was decreased below 50%

after incubating priming seeds for 8hr, while the germination percentage of PL-3 was more than 50%. We conclude that 6hr is the optimum incubation time for cowpea seed priming at 37 °C (Fig. 1). Our results are similar to the finding obtained by Singh *et al.*, 2014.

### 3.2 Seed germination and optimum priming concentration of SA

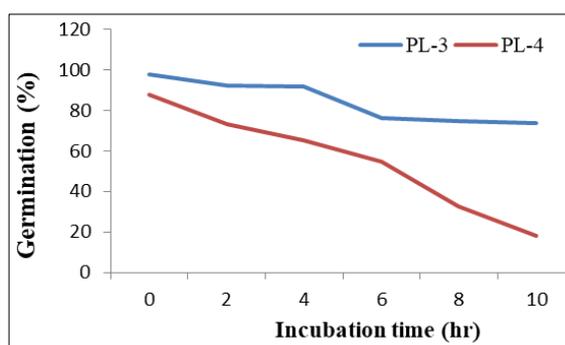
Seed priming of PL-4 with 1mM-5mM concentration of SA for 6hr of incubation time showed that SA was inhibiting cowpea seed germination. Priming of PL-4 seeds with 1mM SA showed 88.88% of seed germination. Further priming of PL-4 seeds with 2mM SA was found to have reducing germination percentage to 44.44%, which is below 50% of seed germination. Additionally, we observed that the germination percentage was reduced and it was as 22.22% and 11.12% in 3mM SA and 4mM SA primed seeds. There was 100% reduction in germination percentage of cowpea seeds primed with 5mM SA. We found that 1mM concentration of SA was optimum concentration for priming cowpea seed (Fig. 2). Further we observed that increase of SA concentration inhibits seed germination. Our result is similar to the finding of Xie *et al.*, (2007) [14]. However, it is also reported that SA inhibits seed germination in maize (Guan and Scandalios, 1995) [4] and triangle orache (*A. triangularis*) (Khan and Ungar, 1986) [7]. There might be the SA represses GA induced  $\alpha$ -amylase production in aleurone cells (Xie *et al.*, 2007) [14].

### 3.3. Seed Germination and Priming effect of MJ

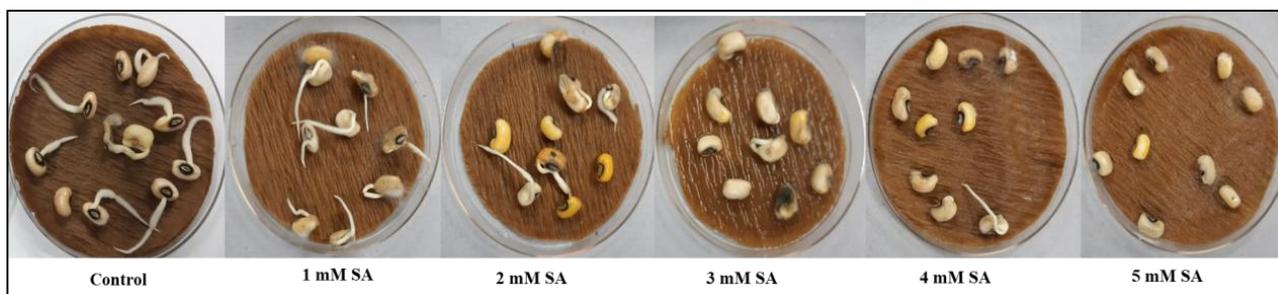
Seed priming of PL-3 and PL-4 with 0.01mM, 0.1mM and 1mM concentration of MJ for 6hr of incubation time showed that MJ have differential effect on PL-3 and PL-4 genotypes. We have observed that MJ was not affecting on germination percentage but it was reducing root length. Root length of PL-4 was decreased more as compared to the root length of PL-3 (Table. 1 and Fig. 3). Similar result was observed by Staswick *et al.*, (1992) [11], they stated that primary root growth of wild-type *Arabidopsis thaliana* seedlings was inhibited by 50% when seedlings were treated with 0.1M methyl jasmonate. Xu *et al.*, (2020) also reported that Jasmonic acid inhibited seed germination of *Arabidopsis thaliana*.

**Table 1:** Effects of seed priming on root length of PL-3 and PL-4 cowpea genotypes. Root length was measured in cm (Centimeter)

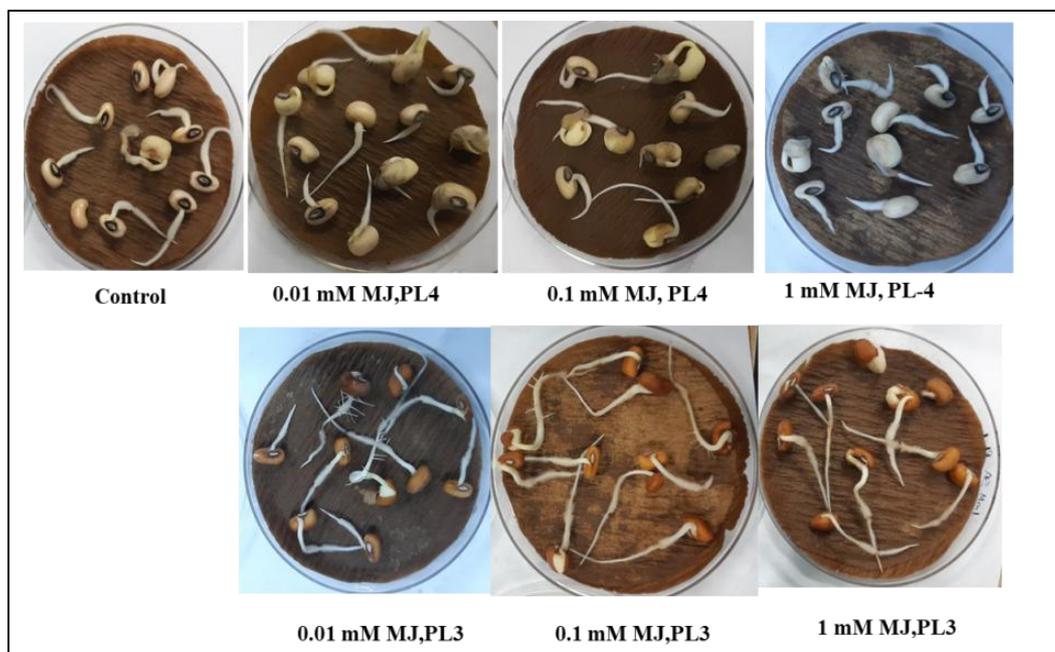
Cowpea	Root Length (cm)		
	0.01mM MJ	0.1mM MJ	1mM MJ
PL-3	5.45	4.85	3.7
PL-4	3.01	2.71	1.81



**Fig 1:** Effect of different incubation time of seed priming with 2mM MJ on germination percentage of PL-3 and PL-4



**Fig 2:** Effects of different concentrations of SA on germination of PL-4



**Fig 3:** Effect of 0.01mM, 0.1mM and 1mM concentration of MJ on primary root length of PL-3 and PL-4 genotypes

#### 4. Conclusion

In conclusion, the study performed on cowpea suggested that cowpea seeds can be primed with SA and MJ for improving host plant resistance in plant. However, the optimum incubation time for seed priming with 2mM MJ could be 6hr. The optimum concentration for SA priming of cowpea could be 1mM. Further research is needed for finding the exact role of methyl jasmonate on root length of cowpea.

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