



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 2085-2088
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www.thepharmajournal.com
Received: 18-04-2022
Accepted: 26-05-2022

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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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DOI: <https://doi.org/10.22271/tpi.2022.v11.i6Sz.13380>

Abstract

The study was carried out to assess the optimum incubation time for cowpea seed priming and to determine 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 is 6 hrs. Furthermore, we have analysed the effects of different concentrations of SA and MJ on germination percentage and root length of cowpea genotypes. We saw that SA decreased the number of seeds that sprouted, while MJ decreased the length of the first roots.

Keywords: Cowpea, seed priming, salicylic acid, jasmonic acid, root length

1. Introduction

Cowpea is one of the most important multifunctional 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; Timko *et al.*, 2007) [10, 12]. 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 340 kcal of energy per 100 g of seeds. It can fix up to 25–179 pounds of nitrogen per hectare (Elawad and Hall, 1987; Sprent *et al.*, 2010) [2]. Due to its high protein content, it is often called "poor man's meat" in many underdeveloped countries in which animal meat is expensive (Singh, 2005) [10]. 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 in drought, high temperatures, and a wide range of pH stress conditions (Hall *et al.*, 2002; Hall, 2004, Singh, 2005 [10]; Timko *et al.*, 2007 [12]) [6, 5, 10, 12]. Besides this national, environmental and social importance, cowpea is considered an orphan crop because it has 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) [1]. It is diploid with 22 pairs of chromosomes 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; Timko *et al.*, 2007) [10, 12]. Various breeding programmes are already in the pipeline for screening cowpea genotypes tolerant to these biotic stresses, especially for insect infestation. But the exact mechanism that underlies the insects stress tolerance is still unknown. Recently, various elicitors as priming agents have been used to improve host plant resistance to different stress conditions (Farooq *et al.*, 2020; Nabi *et al.*, 2020) [3, 9]. 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 the 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 (PL-3 and PL-4) 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% HgCl₂ for 3 min, and then all toxic HgCl₂ was removed by rinsing with distilled water. This rinsing process was carried out 5–6 times to remove all residual toxic HgCl₂. Further, we have primed cowpea seeds (of PL-3 and PL-4) with a 2 mM concentration of methyl jasmonate (MJ) in the ratio of 1:5 (g mL⁻¹) of the weight of cowpea seed to the volume of priming solution (Wang *et al.*, 2016) [13]. Unprimed seed was considered as a control treatment. Cowpea seeds were incubated for 0, 2, 4, 6, 8, and 10 hrs. to determine the optimum time. After incubating for a particular time, primed cowpea seeds were dried in shadow to their original weight. Further, cowpea seeds of PL-3 and PL-4 were primed with 0.01 mM, 0.1 mM, and 1 mM concentrations of MJ. Additionally, cowpea seeds of PL-4 (which had the lowest seed germination percentage) were primed with 1 mM – 5 mM of different concentrations of salicylic acid (SA). Priming was carried out in the same way as described above. Primed seeds were placed on germination paper and incubated at 37 °C in an incubator for germination.

2.2 Measurement of Germination and Root Length

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

$$\text{Germination (\%)} = (\text{Total number of germinated seeds} / \text{Total seeds sown}) \times 100$$

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

2.3 Statistical Analysis

The mean and standard error were 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 the different incubation times of the 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 10 hrs. 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 10 hr. of incubation time, respectively. We observed that the germination percentage of PL-4 was decreased below 50% after incubating seeds for 8 hrs, while the germination percentage of PL-3 was more than 50%. We conclude that 6 hrs is the optimum incubation time for cowpea seed priming at 37 °C (Fig. 1). Our results are similar to the findings obtained by Singh *et al.*, 2014.

3.2 Seed Germination and Optimum Priming Concentration of SA

Seed priming of PL-4 with 1 mM – 5 mM concentration of

SA for 6 hrs of incubation time showed that SA was inhibiting cowpea seed germination. Priming of PL-4 seeds with 1 mM SA showed 88.88% seed germination. Further priming of PL-4 seeds with 2 mM SA was found to have reduced the 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 low as 22.22% and 11.12% in 3 mM SA and 4 mM SA primed seeds. There was a 100% reduction in the germination percentage of cowpea seeds primed with 5 mM SA. We found that a 1 mM concentration of SA was the optimum concentration for priming cowpea seeds (Fig. 2). Further, we observed that an increase in 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 or ache (*A. triangularis*) (Khan and Ungar, 1986) [7]. There might be the SA represses GA induced α -amylase production in aleurone cells (Xie *et al.*, 2007) [14].

3.3 Seed Germination and Priming Effects of MJ

Seed priming of PL-3 and PL-4 with 0.01 mM, 0.1mM, and 1mM concentrations of MJ for 6 hrs of incubation time showed that MJ has a differential effect on PL-3 and PL-4 genotypes. We have observed that MJ was not affecting germination percentage but it was reducing root length. The root length of PL-4 decreased more as compared to the root length of PL-3 (Table. 1 and Fig. 3). A 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 Genotypes	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

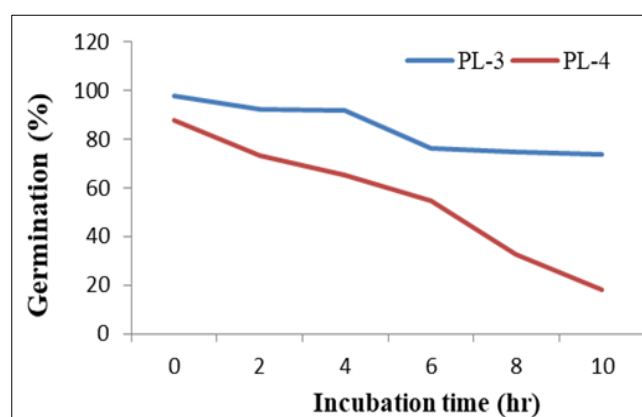


Fig 1: Effect of different incubation time of seed priming with 2mM MJ on germination percentage of PL-3 and PL-4.

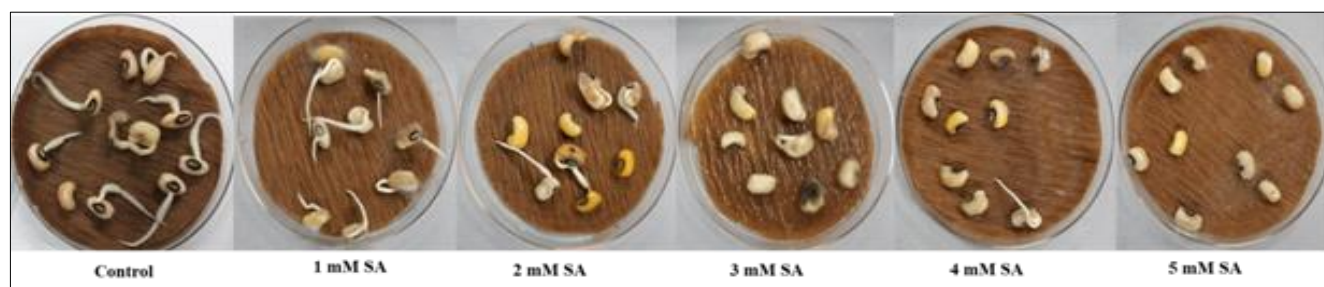


Fig 2: Effects of different concentrations of SA on germination of PL-4

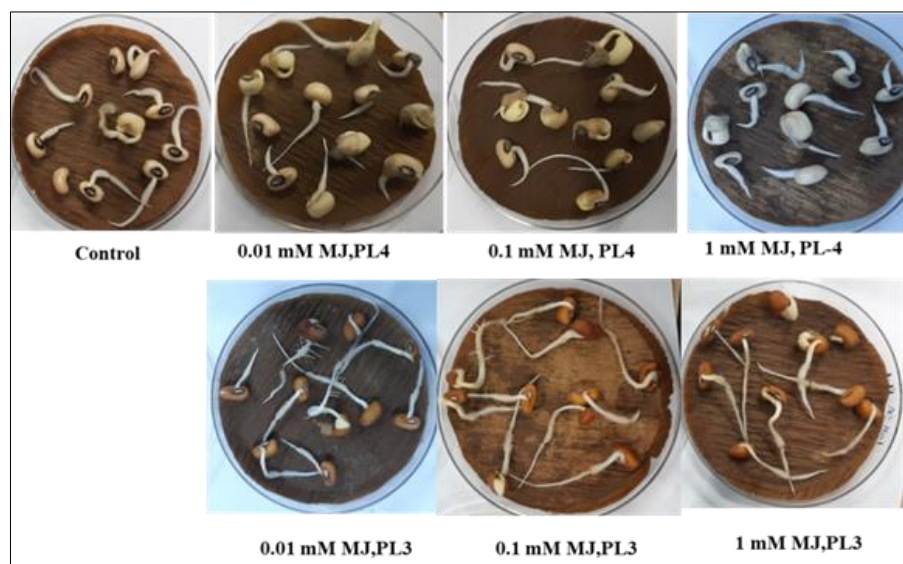


Fig 3: Effect of 0.01mM, 0.1mM and 1mM concentration of MJ on primary root length of PL-3 and PL-4 genotypes

4. Conclusion

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

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