

## Influence of seed priming on physiological, bio-chemical and ultra structural changes in endangered medicinal tree Bengal quince (*Aegle marmelos*)

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**ABSTRACT** An investigation was carried out to study the physiological, biochemical and ultra structural changes that occur during seed priming in the underutilized medicinal plant Bengal quince. The seeds were soaked in water and GA<sub>3</sub> solution for 6 h along with control. Among them, seed priming with 100 ppm GA<sub>3</sub> was scored as the best priming agent for Bengal quince seeds. Evaluation on the causes for the invigorative effect obtained with GA<sub>3</sub> priming expressed that seed priming with GA<sub>3</sub> enhanced the physiological performance, biochemical changes *viz.*, amylase and dehydrogenase activity, strengthened the protein profile, enhanced the protein content, improved the gibberellin content of seed and also brought ultra structural changes in embryo as analyzed through scanning electron microscope.

**Keywords:** Seed priming, bengal quince, physiological, bio-chemical and ultra structural changes

*Aegle marmelos* (L.) Corr., commonly called Bengal quince or bael, is one of the medicinal trees having tremendous therapeutic potential. It belongs to the family Rutaceae, the family of citrus fruits. It is originated from eastern ghats and central India. Hiuen Tsang, the Chinese Buddhist pilgrim who came to India in 1629 A.D. noticed the presence of this tree along with other trees in this region. It is indigenous to Indian subcontinent and mainly found in tropical and sub-tropical regions. The tree is also found as a wild tree, in lower ranges of Himalayas, up to an elevation of 500 meters. Bengal quince is found growing along foothills of Himalayas, Uttar Pradesh, Bihar, Chhattisgarh, Uttaranchal, Jharkhand, Madhya Pradesh, the Deccan Plateau and along the East Coast [1]. Bael tree was introduced in Europe in 1959. The tree is reported to be cultivated in Ceylon, northern Malaya, Java and Philippine Island where it was first fruited in 1914.

Almost all parts of the tree are used in preparing herbal medicine [2]. The roots are useful for treating diarrhea, dysentery and dyspepsia. The aqueous extracts of the stem and root bark are used to treat malaria, fever,

jaundice and skin diseases such as ulcers, urticaria and eczema [3]. In pharmacological trails, both the fruit and root showed antiamoebic and hypoglycemic activities.

The plant is rich in alkaloids, among which aegline, marmesin, marmin and marmelosin are the major ones. The compounds luvangetin and pyranocoumarin isolated from seeds showed significant anti-ulcer activity. Essential oil isolated from the leaf has antifungal activity. The tree has been identified to be a suitable wind break or wind barrier. Bengal quince tree acts as a sink for chemical pollutants as it absorbs poisonous gases from atmosphere and makes them inert or neutral. The foundation for revitalization of local health traditions (FRLHT), Bangalore, India assessed threat status of *Aegle marmelos* tree as RET (rare, endangered and threatened) species especially threatened species and importance is being given for mass multiplication through various propagation techniques. The tree is normally grown with seeds [4]. Seed priming, identified as an effective seed invigoration method, has become a common seed treatment to increase the rate and uniformity of emergence and crop

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establishments. Several studies are associated with pre-sowing treatments for seed vigour enhancement. The major problem encountered with the commercial application of seed priming is the variability among species, varieties and even seed lots, especially when treating large quantities of seeds. Since different batches of seeds are intrinsically heterogeneous with respect to germination rate, the specific treatment conditions must be optimized for each seed lot. The present study was carried out with the objective to evaluate the physiological and biochemical changes associated with primed seeds.

## MATERIALS AND METHODS

The fruits were collected from Coimbatore district, Tamil Nadu, India (76°57 E, 11°8 N and 320 MSL) and the seeds were extracted as wet extraction and were dried under shade for a week to reduce the moisture content to ten percent. Fresh seeds were subjected to the priming treatments *viz.*, soaking in water for 6 h (hydro-priming) and soaking in 100 ppm  $GA_3$  for 6 h. After priming, the seeds were removed from the solutions, rinsed in distilled water, dried to its original moisture content under shade at room temperature and assessed for the following physiological, biochemical and ultra structural changes. The unprimed seeds were used as control.

The treatment effect was evaluated by recording physiological observations (seed germination [5], root and shoot length (cm), dry matter production 10 seedling<sup>-1</sup> (mg) [6] and vigour indices [7], biochemical observations (electrical conductivity, alpha-amylase activity, dehydrogenase activity, gibberellin content and protein profile) and ultra structural observation. The data were subjected to CRD analysis to establish the effects of treatments over control.

## RESULTS AND DISCUSSION

The evaluated seed quality characters

expressed highly significant differences among the treatments in all these crops.  $GA_3$  @ 100 ppm for 6h resulted in added advantage of germination improvement concomitant with seedling quality characters of Bengal quince, which decides the success of any pre-sowing seed treatment. The results revealed that the germination percentage was enhanced with all the treatments,  $GA_3$  @ 100 ppm and hydro-priming for 6h compared to control.

Among the treatments, the seeds soaked in  $GA_3$  @ 100 ppm for 6h duration of soaking recorded the highest values for germination percent (88 %) and other seed vigour parameters (root length (14.6 cm), shoot length (11.4 cm), dry matter production (574), vigour index I (2464) and vigour index II (1285) (Table 1). The improvement in seed quality characters due to  $GA_3$  might be due to weakening of mechanical resistance of the endosperm cells around the radicle tip [8]. It was also opined that  $GA_3$  found to trigger specific enzymes which hydrolyse stored molecules and catalyse the essential reactions in energy generating cycle during seed germination [9] which provide energy and structural component for the growth and emergence of embryo as observed [10] in sugar pine and in Jack pine. The role of gibberellic acid in promoting germination of dormant seed had also been evidenced by many authors [11-12].

Bio-chemical reactions are the causes for physiological output. Hence, various biochemical changes in primed seed were evaluated along with hydroprimed and control seeds to trace the causes for seed invigoration. The electrical conductivity is the best indicator of the loss of cell membrane integrity, as it is considered as one of the primary physiological events of seed deterioration process [13]. In the present study, seeds primed with 100 ppm  $GA_3$  for 6h registered the minimum electrical conductivity of 0.048 dSm<sup>-1</sup>, while the maximum electrical conductivity (0.149 dSm<sup>-1</sup>) was registered with unprimed seeds (Fig. 1). The

Treatments	Causes for invigoration	Increase in germination (%)
GA <sub>3</sub> 100 ppm	Promoted the breakdown of starch and other substrates that induced the enzyme action that removes the metabolic block in the embryonic axis of endosperm.	20
Hydro priming	Activation of hydrolytic enzymes at endosperm converting complex stored food materials into metabolically useful chemicals that resulted in to growth of the embryo during hydration of the seeds.	8

\*Germination % increase over the control after 6 h of soaking

lower electrical conductivity of seed leachate of primed seeds might be due to the better integration of plasma membrane structure by slow hydration and the invigorative effect of priming agent as reported [14-15]. Most workers reported that the membrane become more susceptible to imbibition damage with vigour and the leakage might be due to the loss of metabolic energy for membrane transport mechanism.

The activity of the hydrolyzing enzymes such as  $\alpha$ -amylase was also found to increase with seeds primed with 100 ppm GA<sub>3</sub> compared to other treatments. Seeds primed with GA<sub>3</sub> exhibited maximum  $\alpha$ -amylase activity of 4.4 cm, while the minimum activity of 1.1 cm was obtained with unprimed seeds (Fig. 1 and 2). The enhancement in  $\alpha$ -amylase activity of primed

seeds might be due to the promotion of hydration during imbibition that increased the starch hydrolysis by the exogenously applied GA<sub>3</sub> which was not lost during the rehydration process. Dehydrogenase activity indicates the metabolic activity on the part of cells and increased the catabolization of amino acids [16]. Hence, the dehydrogenase activity increased during hydration and increases the metabolic activity during the course of germination thereby resulted in increased germination. In the present study, dehydrogenase activity that accounts for viability of seeds was also the highest in seeds primed with 100 ppm GA<sub>3</sub> for 6h (0.214 OD value).

Protein content of the seeds of the present study also exhibited significant difference among treatments. Seeds primed with

**Table 1. Influence of seed priming techniques on seed and seedling quality characters in Bengal quince (*Aegle marmelos*)**

Treatments	Germination percentage	Root length (cm)	Shoot length (cm)	Dry matter production 10 seedling <sup>-1</sup> (g)	Vigour index I	Vigour index II	Vigour index III
Unprimed seed (Control)	68 (55.551)	8.7	7.9	266	1129	592	18088
Hydro priming @ 6h	76 (60.667)	9.7	8.5	281	1383	737	21356
GA <sub>3</sub> @ 6h	88 (69.734)	14.6	11.4	574	2464	1285	50512
SEd	0.523	0.166	0.146	4.117	23.923	9.805	659.863
CD(p=0.05)	1.183	0.377	0.331	9.315	54.120	22.182	1492.748

GA<sub>3</sub> had the maximum protein accumulation (19.7 percent), while the minimum protein (18.3 percent) was with unprimed seeds. The increase in protein content due to priming was also reported [17] who indicated that seed priming induced the mobilization and solubilization of globulins and the synthesis of late embryogenesis abundant (LEA) proteins. Protein and RNA synthesis could be detected within a few hours after imbibition and this synthesis may continue for several days. It was also revealed that priming of seeds induced the *de novo* synthesis of peptides in sunflower related to lesser days taken for 50 percent germination, mean germination time, improved germination energy and final germination percentage [18]. Hence, the increase in protein content of GA<sub>3</sub> primed seeds as recorded the present study might also be due to the synthesis of new protein during hydration.

Gibberellic acid GA<sub>3</sub> is an inherent part of plant system. The variation observed in physiological seed quality characters is mainly based on the quantum of its presence either optimal or super optimal. On estimation of gibberellic content of the seed, the control seeds recorded lesser gibberellic content than with primed seeds. On application of GA<sub>3</sub> through priming an increase in gibberellins content of seed with increase in concentration was recorded, exhibiting the fact that exogenous application of GA<sub>3</sub> had increased the endogenous level of gibberellic content and improved the germination of the seeds. This was also supported in *Carica papaya* [19].

There was a significant difference in total protein profile of the primed seed in the present study, elaborating the influence of seed priming. The profiling of protein with SDS page exposed the intensity of protein banding at molecular weight of 20.1 and 20.9 KDa with seeds primed in GA<sub>3</sub> @ 100 ppm for 6h compared with corresponding protein bands of extracts from unprimed seeds. The

total protein profile of hydro primed seed was also higher than the corresponding protein bands of unprimed seeds. Similar results were also obtained in maize with primed seed [20]. Beneficial effects of priming are associated with repair and the build up of nucleic acids, increased protein synthesis as well as repair of membranes. It was reported that for a priming treatment of sugarbeet seeds to be the most efficient, there should be an increase in the level of soluble 11-S globulin  $\beta$ -subunit per seed, but contrasted that there should not be any increase in heat stable seed specific protein such as HS60 protein and SBP sugarbeet specific biotinylated protein [21]. The qualitative increase in protein profile

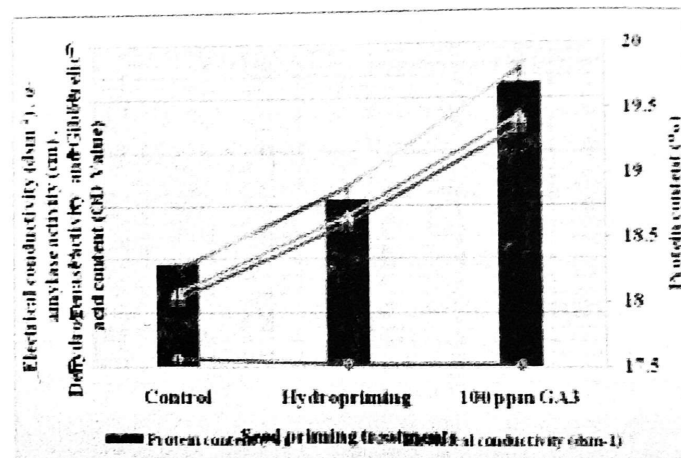


Fig.1. Evaluation of biochemical causes for the invigorative influence of seed priming techniques

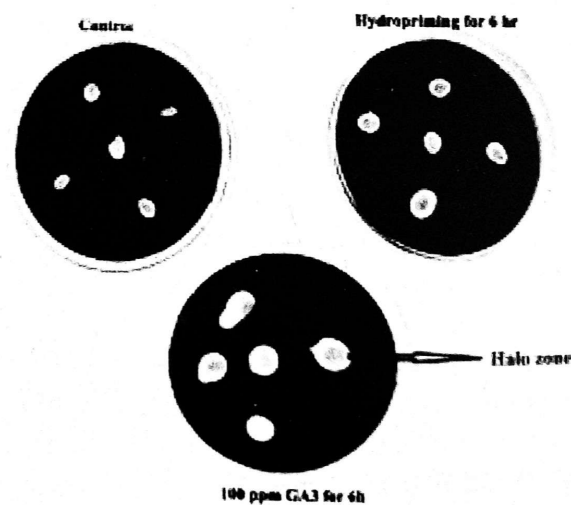


Fig. 2. Effect of seed priming on  $\alpha$ -amylase activity changes in Bengal quince

might be due to the presence of all the components necessary for resumption of protein synthesis, except polysomes within the cells of mature dry embryos. Polysomes might have been formed immediately after imbibition by combination of single ribosomes, which might have initiated the process of protein synthesis and also the activation of already stored mRNA. Further, the synthesis of new mRNA during imbibition might have also initiated the protein synthesis. The differences in protein profiles at the initial stages of imbibition were also focused to the differential rates of assemblage of polysomes [22].

A significant difference in ultra structural changes of primed seeds compared to unprimed seeds was also evident with SEM analysis. The seeds exposed to vertical sectioning of the sample were dusted on one side of the double sided adhesive carbon conducting tape conditions under scanning electron microscope on magnifying the embryo position to 200x, 400x and 1600x control, hydro primed and GA<sub>3</sub> primed seeds, respectively.

The results revealed that the initiation of radicle protrusion was observed in primed seed compared to control (Fig. 3), which was in accordance with the procedural explanation given for priming as initiation of early phases of germination but concluded before radicle emergence. Not only the embryo development but also the cell wall integration was dissipated during priming, leaving the way for embryo growth.

From the present study, it was clearly concluded that the priming of Bengal quince seeds with 100 ppm GA<sub>3</sub> for 6 h was found to improve protein content, activity of hydrolyzing enzymes such as  $\alpha$ -amylase and dehydrogenase and also reduced the seed leachate. These enhancements in the biochemical attributes of Bengal quince seeds due to priming with 100 ppm GA<sub>3</sub> for 6 h may reflect in increased germination and vigour

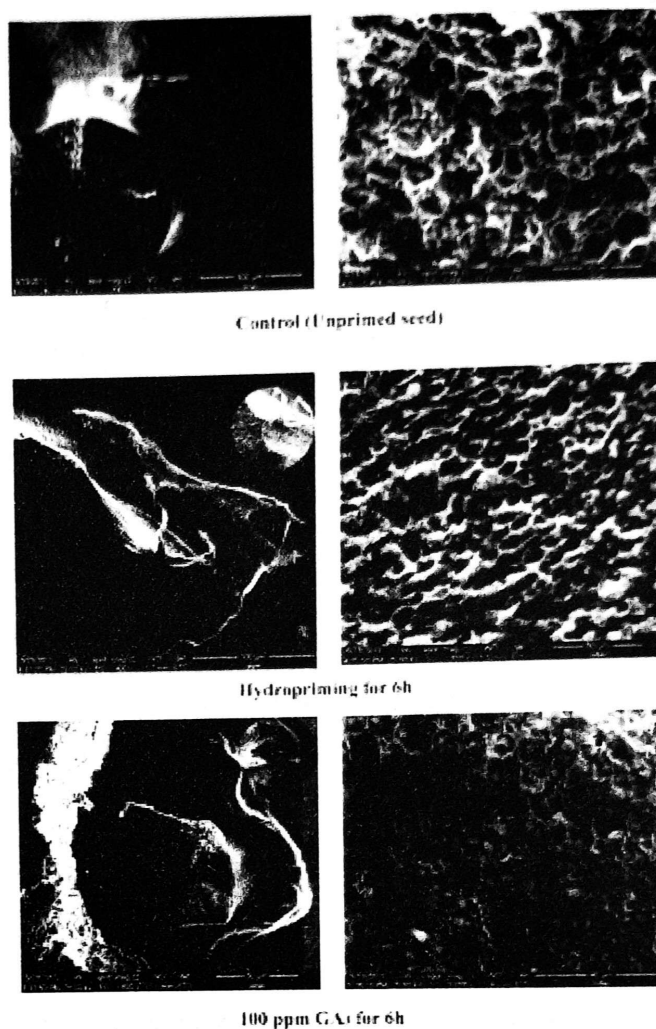


Fig. 3. Effect of seed priming on ultra structural changes in Bengal quince

and also the seed priming effect was more pronounced in seeds. Thus, the study expresses that soaking of seeds at GA<sub>3</sub> @ 100 ppm for 6h improved the germination and vigour of seeds.

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## Influence of seed priming on bengal quince

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