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Effect of seed priming with Phytohormones on physiological traits of maize

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Abstract
Maize is one of the most important crops being cultivated as a cereal grain in India. High temperature stress affects the growth and development of crops by a series of morphological, physiological and biochemical changes. Seed priming with phytohormones helps in protecting the plants from terminal heat stress. The present study was conducted to evaluate the influence of seed priming with various phytohormones *viz*., salicylic acid, brassinolides and sodium nitro-prusside on performance of seed quality parameters such as speed of germination, germination (%), and root and shoot length, dry matter production and vigour index. These studies were carried out in the maize seeds COH (M) 8 for identifying the suitable phytohormones and for optimizing the concentration to improve the germination and vigour of maize seeds. Seed priming treatments were carried out in various concentrations *viz*., salicylic acid 50, 75 and 100 ppm, brassinolides 0.1, 0.2 and 0.5 ppm, sodium nitroprusside 50 and 75 and 100 µM for the soaking duration of 9 hrs along with hydropriming and control. The concentrations were standardized and seedlings were tested under different temperature regimes at 40 and 42 ℃ in plant growth chamber with 65% relative humidity and ambient temperature (25 ℃) with 95% relative humidity. The results of the study revealed that the seeds treated with sodium nitroprusside 50 µM performed well by recording highest germination and vigour under both ambient temperature (25 ℃) and at 40 and 42 ℃ in the plant growth chamber when compared to control, hydropriming and the other treatments.

Keywords: Maize, heat stress, seed priming, phytohormones

Introduction
In India, maize is one of the leading crops being cultivated as a cereal grain. It is the third important and highly versatile food crop with wider adaptability. Because of its higher genetic yield potential, it is called as the “Queen of cereals.” Annually, maize contributes maximum of about 40% to the global food production among the cereal crops.

Nowadays, the air temperature has risen as a consequence of climatic variability. Climate change will apparently increase the effect of high temperature stress on the crop yields. Rising temperature represents severe food insecurity hazards. The extremely high temperature causes heat stress and serious injuries to the plants. It is one of the destructive abiotic stresses and the major growth limiting factor at various developmental stages. It also causes over production of reactive oxygen species which causes cellular damage to the plants. The plant productivity gets decreased due to the changes in photosynthesis and respiration. Mitigation strategies are essential to increase the productivity and quality of maize. µ

Plant hormones as a seed treatment, mainly through seed priming is a good option for mitigating the heat stress. Priming the seeds with plant hormones provides protection to the plants against heat stress and it results in improved seed germination and vigour. Phytohormones got an important position in the plant reaction to heat stress. It is stated that phytohormones enriched seeds performs well even under the unfavourable environmental conditions. They play a vital role in the plant growth, development and also respond to several abiotic stresses. So, there is a need to optimize the concentration of phytohormones for seed priming of maize. With this context, the current study was formulated to optimize the concentration of seed priming treatments with various phytohormones for maize seeds based on their different seed quality parameters.

Materials and Methods

The freshly harvested hybrid maize seeds COH (M) 8 obtained from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore has formed the base materials for the present study. The laboratory studies were carried out in the Dept. of Seed Science and Technology, TNAU, Coimbatore. The concentration of the phytohormones were optimized and tested under the plant growth chamber at different temperature regimes of 40 ℃ and 42 ℃ and at ambient condition (25 ℃). The maize seeds were primed with salicylic acid 50, 75 and 100 ppm, brassinolides 0.1, 0.2 and 0.5 ppm, sodium nitroprusside 50 and 75 and 100 µM for 9 hours soaking duration with 1:2 seed solution ratio. The hydropriming treatments were also done for maize seeds.

After the soaking duration of 9 hours, the primed seeds were kept for shade drying to bring back the seeds to original moisture content. The germination test was conducted for maize seeds and seed quality parameters were observed and evaluated. The experiment was conducted with four replications in Completely Randomized Design (CRD).

Speed of germination

Four replications with 100 seeds in each treatments were taken and the seeds were germinated in the petri plates by using top of the paper method as per the ISTA guidelines (2011). The seeds showing the protrusion of the radicle was counted from the sowing date until the completion of total germination of the seedlings. Speed of germination was calculated by calculating the number of seeds germinated on each day.

Speed of germination $= \frac{G_1}{D_1} + \frac{G_2}{D_2} + \frac{G_3}{D_3} + \cdots \frac{Gn}{Dn}$ Where, G_1, G_2, ……G_n are the number of seeds germinated on D_1, D_2, ……D_n day.

Root length

Ten normal seedlings from each replication were chosen at random during the time of final count and root lengths were measured for each seedlings. It was measured from the seed attachment point to the primary root tip and the average values were expressed in centimeter.

Shoot length

The shoot length was measured using the same set of seedlings used for measuring the root length. It was measured from the seed attachment point to the leaf tip and the average values were expressed in centimeter.

Germination (%)

According to the seed testing rules of ISTA, the germination tests were conducted in the germination room with 25±2 ℃ and 95% relative humidity by using the roll towel method. After the final count (i.e., 7 days after sowing) the normal seedling count was taken for each replication and the germination percentage was calculated.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Dry matter production

During the time of final count, ten normal seedlings were chosen randomly for measuring the root and shoot length of the seedlings. These selected seedlings were shade dried for 24 hours and kept in an hot air oven at 103±2 ℃ for 16±1 hour. After the drying period, the seedlings were cooled in a desiccator and weighed in a balance and the mean is expressed as g seedlings^{-10}.

Vigour index

Vigour index (VI) was calculated by using the method given by Abdul-Baki and Anderson (1973) and the values were expressed in the whole number.

Vigour index = Germination (%) x [Root length (cm) + Shoot length (cm)]

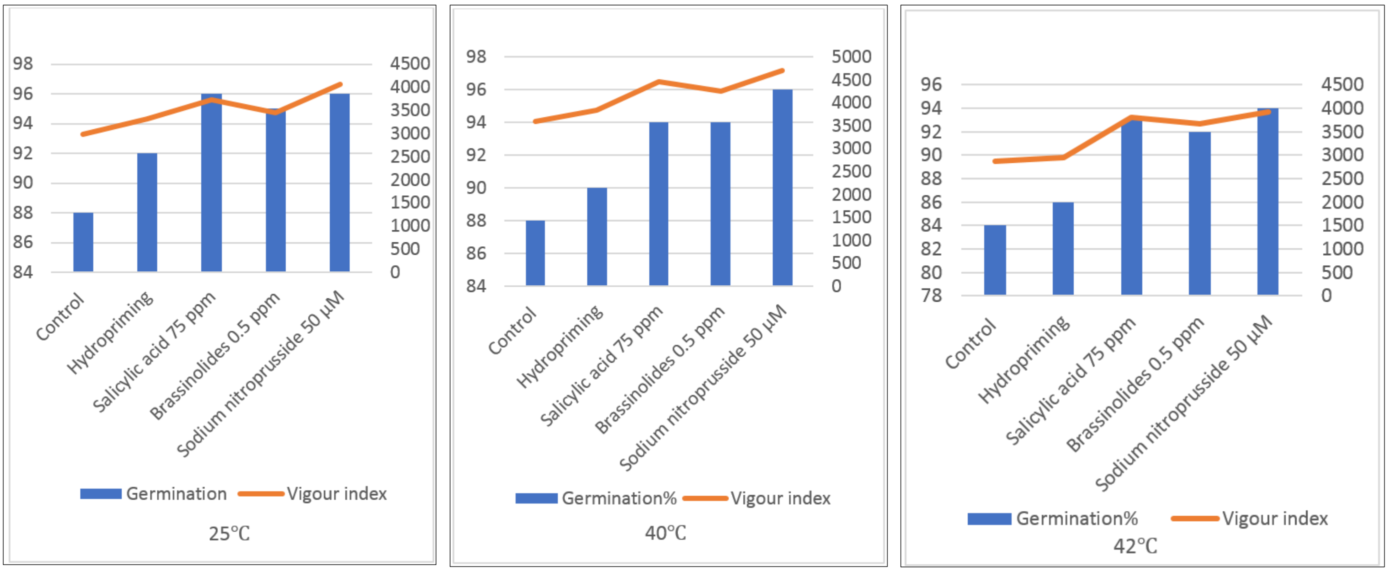
Table 1: Effect of phytohormones on Physiological Traits of Maize

Treatments	Speed of germination	Germination (%)	Root length (cm)	Shoot length (cm)
T_0 – Control	5.52	84 (66.42)	24.55	17.71
T_1 – Hydropriming	5.81	87 (66.87)	25.81	19.46
T_2 – Salicylic acid 50 ppm	6.22	93 (74.66)	28.45	20.53
T_3 – Salicylic acid 75 ppm	6.72	94 (75.82)	28.79	21.43
T_4 – Salicylic acid 100 ppm	6.32	93 (74.66)	27.87	20.26
T_5 – Brassinolides 0.1 ppm	5.68	89 (70.63)	25.64	16.00
T_6 – Brassinolides 0.2 ppm	5.82	88 (69.73)	25.04	16.20
T_7 – Brassinolides 0.5 ppm	6.10	95 (77.08)	26.61	18.13
T_8 – Sodium nitroprusside 50 µM	6.70	95 (77.08)	29.16	21.60
T_9 – Sodium nitroprusside 75 µM	6.57	91 (72.54)	27.69	19.71
T_{10} – Sodium nitroprusside 100 µM	6.08	90 (71.57)	27.77	20.11
MEAN	6.14	91 (72.54)	27.03	19.19
SEd	0.12	2.04	0.76	0.73
CD @ 5%	0.24	4.16	1.55	1.49

Table 2: Effect of phytohormones on Physiological Traits of Maize

Treatments	Dry matter production (g/10 seedlings)	Vigour index
T_0 – Control	0.83	3548
T_1 – Hydropriming	1.05	3941
T_2 – Salicylic acid 50 ppm	2.50	4553
T_3 – Salicylic acid 75 ppm	2.95	4700
T_4 – Salicylic acid 100 ppm	2.58	4474
T_5 – Brassinolides 0.1 ppm	1.15	3692

T_6 – Brassinolides 0.2 ppm	1.30	3629
T_7 – Brassinolides 0.5 ppm	1.63	4252
T_8 – Sodium nitroprusside 50 µM	3.00	4792
T_9 – Sodium nitroprusside 75 µM	2.03	4314
T_{10} – Sodium nitroprusside 100 µM	2.10	4304
MEAN	1.92	4200
SEd	0.19	151.80
CD @ 5%	0.38	308.80



(a) (b) (C)

Fig 1: Effect of Phytohormones on Physiological Traits of Maize

Results

Soaking seeds in the phytohormones significantly influenced the physiological traits of maize seedlings. Among the various concentrations used, seeds treated with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM recorded the maximum speed of germination *viz.*, 6.72, 6.10 and 6.70 respectively whereas the speed of germination was delayed for the non primed seeds (5.52) (Table 2).

Statistically, germination was significant due to the priming treatments. Among the various concentrations used, germination was found to be maximum for the seeds primed with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM *viz.*, 94% (75.82), 95% (77.08) and 95% (77.08) respectively. The poor performance was recorded in the non primed seeds (84%) (Table 1).

Variation in root length was observed due to the priming treatments. Among the various concentrations used, maximum root length was observed for the seeds primed with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM *viz.*, 28.79 cm, 26.61 cm and 29.16 cm respectively. The poor performance was recorded in the non primed seeds (24.55 cm) (Table 1).

Shoot length was significant due to the priming treatments. Among the various concentrations used, maximum shoot length was recorded in salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM *viz.*, 21.43 cm, 18.13 cm and 21.60 cm respectively. Brassinolides 0.1 ppm showed the poorest performance by recoding the shortest shoot length of 16.00 cm (Table 1).

Due to the priming treatments, highly significant difference was observed for the dry matter production of 10 seedlings. Among the various concentrations used, the dry matter production was recorded maximum for the seeds treated with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM *viz.*, 2.95, 1.63 and 3.00 g/10 seedlings. The minimum dry matter production was observed for the non primed seeds (0.83 g/10 seedlings) (Table 2).

The vigour index showed highly significant difference for the priming treatments. Among the various concentrations used, seeds treated with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM recorded the maximum vigour index of 4700, 4252 and 4792 respectively. The minimum vigour index was observed in the non primed seeds (3548) (Table 2).

Maximum germination was successful for the seeds treated with sodium nitroprusside 50 µM at 25, 40 and 42 °C in the plant growth chamber *viz.*, 96% (88.61), 96% (88.62) and 94% (75.82) respectively, among the optimal concentrations. At 25, 40 and 42 °C in the plant growth chamber, the non-primed seeds had the lowest germination percentages *viz.*, 88% (69.73), 88% (69.73) and 84% (66.42), respectively (fig.1).

Among the optimal concentrations, the vigour index was found to be highest for seeds treated with sodium nitroprusside 50 µM at 25, 40 and 42 °C in the plant growth chamber *viz.*, 4062, 4706 and 3916 respectively (fig.1).

Discussion

The main focus area of our study was to check the feasibility of the hormones over heat stress detrimental effects. This implicates soaking seeds in hormone solution as an effective method for heat stress and in terms of costing as well. Our data also gives us a heads up on growth promoter hormones at optimum concentration which can be used to mitigate the high temperature stress when compared to the growth retarding hormones. The current findings gives us the impact of thermotolerance in the exogenously applied plant hormones.

Farooq *et al.*, (2008) [5] stated that seed priming with phytohormones improves the performance of crops under heat stress. Harris *et al*., (2001) [7] reported that in maize, rice and chickpea, seed priming treatment led to the better germination and growth, early flowering thus resulting in higher production under semi- arid conditions. Shakirova *et al*., (2003) [15] stated that seed priming improved cell growth and division of apical meristem, resulting in the vigorous growth of wheat. He also stated that SA pre-treatment at 0.05µM increased the cell division levels of seedling roots which resulted in the significant plant growth and expedited wheat productivity. Chabbra *et al.*, (2009) reported that in chickpea, phytohormone application induces heat tolerance by improving the activities of anti-oxidant and stability of the membrane.

Upreti and Murti (2004) [18] reported that brassinosteroids (BRs) treatment at 5 µM under the heat stress uprooted the growth and yield of French beans. Kumar *et al*., 2012 [10] studied the variable epibrassinolide (24-EBL) concentration on mustard under heat stress. He found that the exogenous application of epibrassinolide in mustard induced high temperature stress tolerance by improving the antioxidant system activities. Sirhindi *et al*., (2009) [16] reported that pre-treatment of BR's with varied concentrations resulted in significant growth improvement when compared to control. He also stated that 24 Epi-BR's pre-treatment enhanced the enzyme activities such as SOD, CAT, APOX. The regulation of different enzyme activities with respect to cellular level in plants from germination till the maternity stage were observed when they were treated with BR. Bassiony *et al*., (2012) [3] reported that BR's at 25 and 50 ppm concentration has a pivot impact in the vegetative growth, quality and yield of the pods in the bean plants.

Kawano *et al*., (1998) [9] reported that salicylic acid is involved in the systemic acquired resistance (SAR) and hyper sensitive responses. Wang and Li (2006) [20] stated that salicylic acid induced the tolerance to heat stress through involvement in both antioxidant system and Ca^{2+} homeostasis. In variety of species the phytohormone salicylic acid (SA) significantly showcases increased stress tolerance levels. Wang *et al*., (2010) [19] stated that seed treatment with SA rectified the defects caused by high temperature stress in grapevine seedlings. Similar study was reported in some species with efficiency in heat tolerance levels by Larkindale and Huang (2004) [11]. Saleh *et al*., (2007) [13] stated that in the mung bean seedlings which was observed under heat stress, the SA pre-treatment improved thermostability and decreased lipid peroxidation and boosted the antioxidant activity. The pea plants respond to heat stress with efficient levels of SA was observed by Pan *et al*., (2006) [12]. Similar findings were reported by Senaratna *et al*., (2000) [14] that SA at 0.1-0.5 mM levels applied in common beans and tomato had improved results under heat stress. Ananieva *et al*., (2004) [1] stated that seed priming with different substances protects the photosynthetic system from heat stress damage by improving the anti-oxidant system activities. He also stated that, salicylic acid pre-treatment increased the enzyme activities like catalase with respect to the chloroplast and other modules of the cell.

Waraich *et al*., (2012) [21] reported that nitric oxide is involved in various plant physiological processes and it improves the performance of crop against heat stress. Song *et al*., (2006) [17] stated that the exogenous application of nitric oxide significantly reduces the damage caused by heat stress by reducing ion leakage and improving the various antioxidant enzyme system activities. Fancy *et al*., (2017) [4] reported that nitric oxide (NO) is a redox signalling molecule and important concentration dependent to be considered. Hasanuzzaman *et al*., (2012) [8] stated that NO has a significant role in controlling tolerance in plants and has a regulation of various physiological processes under heat stress. Yang *et al*., (2006) [22] reported that mung bean plants under the treatment of heat stress with NO assisted in the stability maintenance of chlorophyll as fluorescence, hydrogen peroxide content, membrane integrity and antioxidant enzyme activity. Lamattina *et al*., (2001) [6] observed that the heat damage got reduced when with NO pre-treatment in rice seedlings and this also expedited their survival rate significantly.

Conclusion
Seed priming has been regarded as a suitable and useful technology in promoting stress tolerance against heat stress. Seed priming treatment is economically cheap and requires little investment. The present study revealed that among various concentrations used, pre-treatment with salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM performed well by recording the maximum seed quality parameters. Seeds treated with sodium nitroprusside 50 µM outperformed the other treatments by recording the maximum seed quality parameters when the seedlings are exposed to higher temperatures *viz*., 40 and 42 °C in the plant growth chamber. Thus, exogenous application of phytohormones at optimized concentrations *viz.*, salicylic acid 75 ppm, brassinolides 0.5 ppm and sodium nitroprusside 50 µM can be recommended for protecting the seedlings from heat stress and to improve the quality and also growth of the plant.

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