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Effect of halo priming and hormonal priming on seed germination and seedling vigour in maize (*Zea mays* L) seeds

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Abstract
The experiment was conducted in Post Graduate Laboratory, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.), in order to standardize the suitable method of priming for Maize seeds (var. SHIATS MS 3). Three methods of priming viz., T_0-Unprimed (Control), T_1-Hydropriming with Distilled water, T_2-Hormonal priming with GA_3 100ppm, T_3-Hormonal priming with Salicylic Acid 100ppm, T_4-Hormonal priming with Ascorbic Acid 100ppm, T_5- Halo priming with KNO_3 1% and T_6-Halo priming with $CaCl_2$ hydration for 12 hours. It was found that among all the priming showed significance difference with the control and the highest germination percentage (%), germination index, energy of emergence, seedling length (cm), seedling fresh weight (g), seedling dry weight (g) and vigour indices were observed in GA_3 priming for 12 hours. This study also showed that Seed priming with Salicylic Acid and $CaCl_2$ were found to increase the seedling characters. The study helps to improve the seedling character, growth of seeds with the help of seed priming treatments which are cost effective, economic, non-toxic and eco-friendly sources.

Keywords: Maize, Priming Methods, GA_3, Salicylic Acid, Ascorbic Acid, KNO_3, $CaCl_2$

Introduction
Maize (*Zea mays* L.) 2n=20 is the world's leading crop and is widely cultivated as cereal grain that was domesticated in Central America. It is one of the most versatile emerging crops having wider adaptability. Globally, maize is known as queen of cereals because of its highest genetic yield potential. Maize is the only food cereal crop that can be grown in diverse seasons, ecologies and uses. Beside this maize have many types like normal yellow/ white grain, sweet corn, baby corn, popcorn, waxy corn, high amylase corn, high oil corn, quality protein maize, *etc*. Apart from this, maize is an important industrial raw material and provides large opportunity for value addition. Maize grain has greater nutritional value as it contains 72% starch, 10% protein, 4.8% oil, 8.5% fibre, 3.0% sugar and 1.7% ash (Chaudhary, 1983) [9]. Area under maize crop is 9258000 ha and the average yield and production is about 2257 kg/ha and 25673 tonnes in India (Directorate of Economics and statistics, Ministry of Agriculture 2014-15) [10].

The theory of seed priming was proposed by Heydecker in 1973 [19]. Seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigour, leading to better stand establishment and yield (Harris *et al*. 2007) [18]. Various seed priming techniques have been developed which include hydro-priming, halo-priming, osmo-priming and hormonal priming etc. Hydro priming generally enhances seed germination and seedling emergence. Hydropriming defined as soaking of seeds in water (Ghassemi-Golezani *et al.* 2008) [16]. Halopriming is a pre-sowing soaking of seeds in salt solutions, which enhances germination and seedling emergence uniformly under adverse environmental conditions and normal condition. NaCl, KCl, $KN0_3$, and $CaCl_2$ is used. Evaluated the effects of NaCl priming with KNO_3 on the germination traits and seedling growth of four *Helianthus annuus* L. cultivars under salinity conditions and reported that germination percentage of primed seeds was greater than that of un-primed seeds Bajehbaj, (2010) [6]. Osmopriming is the most widely used type of seed priming in which seeds are soaked in aerated low water potential solution. Priming of seeds in osmoticums has been reported to be an economical, simple and a safe technique for seedling establishment and crop production under stressed conditions (Guzman and Olave, 2006) [17]. Hormonal priming is soaking of seed in hormone solution is referred as hormonal priming. GA_3, Salicylic acid, Ascorbic acid, Cytokinins etc can be used for this. Investigated the primed seeds of carrot, onion and tomato showing that priming these seeds with GA_3 increased the germination percentage and rate.

Afzal *et al.* (2006) [2] evaluated the effect of hormonal priming with ABA, salicylic acid, or ascorbic acid on Wheat germination and seedling growth under normal and saline conditions. Bahrani and Pourreza, (2012) [5] studied the Gibberellic Acid and Salicylic Acid effects on seed germination and seedlings growth of wheat (*Triticum aestivum* L.) under salt stress conditions.

The present study was evaluated the effect of different priming treatment on seed vigour and germination in Maize and assess the best priming method for Maize.

Material and Methods

The experiment was conducted in Post Graduate Laboratory of Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and sciences, Allahabad (U.P.) using Maize variety SHIATS MS3. The treatments used at different concentrations for priming were T_0-Unprimed (Control), T_1-Distilled water, T_2-Gibberellic acid (GA_3) 100ppm, T_3–Salicylic acid (SA) 100ppm, T_4–Ascorbic acid (AsA) 100ppm, T_5-Potassium Nitrate (KNO_3) 1% and T_6-Calcium Chloride 1%.

For the preparation of solution one gram of each chemical was taken in a beaker. These chemical was added separately in 1000 ml. of distilled water with constant stirring. The volume of solution will finally constituted to one litter, then it become 1000 ppm stock solution of each chemical. The flasks containing chemicals was covered with muslin cloth to avoid any contamination. For the preparation of GA_3 (100ppm), Salicylic acid(100ppm) and ascorbic acid (100ppm) solution 100 mg of GA_3, Salicylic acid and ascorbic was taken in a measuring flask and made up to 1000 ml. distilled water, while for (1%) Calcium chloride ($CaCl_2$) solution 10 (g) $CaCl_2$ salt was taken in a measuring flask and made up to 1000 ml with distilled water and 1% Potassium nitrate (KNO_3) solution 10 (g) was taken in a measuring flask and made up to 1000 ml with distilled.

After preparation of solution of GA_3, Salicylic acid, ascorbic acid, KNO_3 and $CaCl_2$ maize seeds was soaked in required solution for 12 hour at 25 ^{0}C temperature. After 12 hr of soaking the solution was drained out from the beaker and pre-soaked air dried to original weight and then placed for germination in laboratory under controlled condition and then placed four replication in completely randomized design (CRD) in between paper method for germination in laboratory under controlled condition.

The observation on the characters *viz.*, Germination per cent (ISTA 2004) [20], Germination index Kader and Jutzi, (2004) [21], Energy of emergence (Ruan *et al.* 2002) [28], Root length (cm), Shoot length (cm), Seedling length (cm), seedling Fresh weight (g), seedling dry weight (g), Seedling vigour index I, vigour index II (Baki and Anderson, 1973) [7] were recorded. The experimental data recorded were subjected to statistical analysis for calculating analysis of variance, range, mean, critical difference and coefficient of variation (R.A. Fisher, 1936) [15].

Results and discussion

According to the results, all studied traits were affected by the treatments and there was completely significant difference between control (non-primed seeds) and primed seeds (Table-1) and (Table-2). All seedling characters *viz.* Germination per cent, Germination index, Energy of emergence, Root length (cm), Shoot length (cm), Seedling length (cm), seedling fresh weight (g), seedling dry weight (g), Seedling vigour index I, Vigour index II were affected by GA_3 100ppm concentration and significantly recorded maximum.

Significantly higher germination per cent (98.00%) reported in treatment T_2 GA_3 100ppm followed by T_3 (96.25) primed with SA 100ppm and T6 (95.00%) primed with $CaCl_2$ 1%. Minimum germination per cent recorded by T_0 (91.00%) with unprimed control (Table 2). Several hormones, such as GA_3, SA, have been shown to have positive effects on germination capability Afrigan *et al.* 2013 [1]. Reported that maize seeds subjected to hormonal priming with 100mg L^{-1} GA_3 for 24 hours resulted in a higher germination per cent and germination index, lower mean germination time and mean emergence time.

Significantly highest germination index (34.80) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (32.81) primed with Salicylic Acid (SA) 100ppm and T_6 (32.50) primed with Calcium chloride ($CaCl_2$) 1%. Minimum germination index was recorded by T_0 (26.63) with unprimed control. Khan *et al.* (2011) [23] reported that wheat seeds primed with GA_3 (20 ppm) recorded minimum mean germination and emergence time as compared to control.

Significantly highest energy of emergence (80.25%) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppmand it was followed by T_3 (78.75%) primed with Salicylic Acid (SA) 100ppm and T_6 (78.00%) primed with Calcium chloride ($CaCl_2$) 1%. Minimum energy of emergence was recorded by T_0 (70.5%) with unprimed control. Bassi *et al*. (2011) [8] reported that priming with GA_3 50 ppm for 2 hour enhanced emergence, germination and speed of germination in soybean as compared to non-primed seed lots.

Significantly highest seedling root length (19.15 cm) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (17.93 cm) primed with Salicylic Acid (SA) 100ppm and T_6 (17.40 cm) primed with Calcium chloride ($CaCl_2$) 1%. Minimum seedling root length was recorded by T_0 (13.62 cm) with unprimed control. Ashraf *et al*. (2001) [4] found that GA_3 treatment enhanced the vegetative growth of two wheat cultivars.

Significantly highest seedling shoot length (22.40 cm) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (20.72 cm) primed with Salicylic Acid (SA) 100ppm and T_6 (19.57 cm) primed with Calcium chloride ($CaCl_2$) 1%. Minimum seedling shoot length was recorded by T_0 (14.90 cm) with unprimed control. GA_3 stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and thus speeds germination by promoting seedling elongation growth of cereal seeds (Rood *et al*. 1990) [27].

Significantly highest seedling length (41.55 cm) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (38.65 cm) primed with Salicylic Acid (SA) 100ppm and T_6 (36.97 cm) primed with Calcium chloride ($CaCl_2$) 1%. Minimum seedling length was recorded by T_0 (28.52 cm) with unprimed control. Priming with GA_3 and NaCl can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment Sedghi, 2010 [30].

Significantly highest seedling fresh weight (12.25 g) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (11.75 g) primed with Salicylic Acid (SA) 100ppm and T_6 (11.56 g) primed with Calcium chloride ($CaCl_2$) 1%. Minimum seedling fresh weight was recorded by T_0 (8.92 g) with unprimed control.

Significantly highest seedling dry weight (2.72 g) was reported in the primed with T_2 Gibberellic Acid (GA_3)

100ppm followed by T_3 (2.42 g) primed with Salicylic Acid (SA) 100ppm and T_6 (2.31 gm) primed with Calcium chloride ($CaCl_2$) 1%.Minimum seedling dry weight was recorded by T_0 (1.03 gm) with unprimed control. Pourazar and Mirshekari (2015) [26] reported seed priming with higher doses of gibberellic acid and cytokinin may improve germination and vigorous performance of lentil verified that the final emergence per cent, coefficient of uniformity of emergence and seedling dry weight had a marked increasing effect on seedling vigor indices of lentil.

Significantly highest seedling vigour index I (4071.90) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppm followed by T_3 (3720.06) primed with Salicylic Acid (SA) 100ppm. Minimum seedling vigour index I was recorded by T_0 (2595.32) with unprimed control. Sarika *et al*. (2013) [29] reported that chemo priming with GA_3 and Ethrel improved the seed quality and showed improved seedling length, seedling dry weight which in turn improved higher seedling vigour index, germination speed and mean germination time. Ansari *et al*. (2012) [3] reported that priming with concentrations of 25 ppm GA for 12 and 15h at 10 and for 12h at 15°C could consider as optimal treatment for priming of secale montanom of seeds in cold stress conditions. Yogananda *et al*. (2004) [33] noticed that bell pepper seeds invigorated with GA_3 (200 ppm) recorded higher germination, root and shoot length seedling dry weight, rate of germination and seedling vigour indices over control. Reported that bitter gourd seeds primed with 100 ppm GA_3 for 24 hours gave better germination, field emergence, speed of emergence, seedling length and vigour index-I over the control.

Significantly highest seedling vigour index II (266.56) was reported in the primed with T_2 Gibberellic Acid (GA_3) 100ppmfollowed by T_3 (232.92) primed with Salicylic Acid (SA) 100ppm. Minimum vigour index II was recorded by T_0 (93.73) with unprimed control. Yarnia *et al.* (2012) [32] concluded that seed priming with auxin, kinetin and gibberellin increase germination attributes.

Table 1: Analysis of variance for seedling traits in Maize.

S. No.	Characters	Mean sum of squares	
		Treatments (df=6)	Error (df=21)
1.	Germination	22.23**	5.19
2.	Germination Index	40.67**	2.81
3.	Energy of Emergence	54.50**	6.85
4.	Seedling Root length	17.11**	1.66
5.	Seedling Shoot length	28.79**	4.11
6.	Seedling Length	90.14**	9.85
7.	Seedling Fresh Weight	7.10**	0.30
8.	Seedling Dry Weight	1.58**	0.08
9.	Seedling Vigour Index I	1127960.02**	94187.43
10.	Seedling Vigour Index II	16278.56**	741.47

* And ** significant at 5% and 1% level of significance, respectively.

Table 2: Mean Comparison of Germination and Vigour Traits in Maize.

Treatment	Germination %	Germination Index	Energy of emergence (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Vigour index I	Vigour index II
T0	91.00	26.63	70.5	14.90	13.62	28.52	8.92	1.03	2595.32	93.73
T1	92.25	27.09	72.00	16.15	14.22	30.37	9.02	1.29	2801.63	119.002
T2	98.00	34.80	80.25	22.40	19.15	41.55	12.25	2.72	4071.90	266.56
T3	96.25	32.81	78.75	20.72	17.93	38.65	11.75	2.42	3720.06	232.92
T4	93.75	27.89	73.25	16.72	14.85	31.57	9.98	1.56	2959.68	146.25
T5	94.25	30.22	75.75	17.82	15.77	33.59	10.45	1.77	3165.85	166.82
T6	95.00	32.50	78.00	19.57	17.40	36.97	11.56	2.31	3512.15	219.45
G mean	94.35	30.28	75.5	18.32	16.13	34.46	10.56	1.87	3260.94	177.81
SE(d)	1.61	1.19	1.85	1.43	0.91	2.21	0.38	0.20	217.010	19.25
SEM±	1.13	0.84	1.30	1.014	0.64	1.56	0.27	0.14	153.45	13.61
CD@5%	3.35	2.47	3.85	2.98	1.89	4.61	0.80	0.42	451.29	40.04

Conclusion

It is concluded that the present investigation of different concentration of priming treatments showed significant effect on seed germination and seed vigour parameters. Priming with GA_3 (100ppm) increased the germination (%) and seed vigour in Maize. Soaking of seed with GA_3 solution is advantageous to obtain healthy seedlings. The second best option for priming is hormonal priming with Salicylic acid and halopriming with $CaCl_2$.

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