



## Germination, vigour and physiological responses of hybrid maize (*Zea mays* L.) to pre-sowing seed-hardening practices

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### Abstract

Maize (*Zea mays* L.) serves a significant role in ensuring food and nutritional security and livelihoods of millions of resource-poor smallholders. In India, most of the traditional varieties are replaced with hybrids. However, hybrid maize productivity in the rainfed agro-ecosystems are vulnerable to various climate-induced abiotic stresses, especially drought, which often severely impact crop yield. Physiological pre-conditioning of the seed by hydration, known as seed hardening, is believed to withstand moisture stress under rainfed conditions. Hence, lab experiments were conducted at Department of Agronomy, Annamalai University, Annamalai Nagar, Tamil Nadu during Kharif 2018 to assess the effect of seed hardening treatments on germination, growth and physiological conditions of hybrid maize. Fourteen treatments viz., T<sub>1</sub> – Absolute control, T<sub>2</sub> – Distilled water soaked, T<sub>3</sub> – Cycocel @ 250 ppm, T<sub>4</sub> – Cycocel @ 500 ppm, T<sub>5</sub> – CaCl<sub>2</sub> @ 1%, T<sub>6</sub> – CaCl<sub>2</sub> @ 2%, T<sub>7</sub> – KCl @ 1%, T<sub>8</sub> – KCl @ 2%, T<sub>9</sub> – KH<sub>2</sub> PO<sub>4</sub> @ 1%, T<sub>10</sub> – KH<sub>2</sub> PO<sub>4</sub> @ 2%, T<sub>11</sub> – ZnSO<sub>4</sub> @ 100 ppm, T<sub>12</sub> – ZnSO<sub>4</sub> @ 200 ppm, T<sub>13</sub> – MgSO<sub>4</sub> @ 100 ppm and T<sub>14</sub> – MgSO<sub>4</sub> @ 200 ppm with three replications were given to hybrid maize seeds. Each seed-hardening treatment caused a significant variation in germination, seedling vigour and physiological responses. Among them CaCl<sub>2</sub> treatment gave significantly favourable effect on seed germination (96.00 %), germination energy (94.67 %), time to reach 50 % germination (2.3 days), germination value (1.86), speed of germination (6.8 per day), mean emergence time (3.2 days), coefficient of velocity (167) and germination index (38.5), compared to the control. Likewise, the same treatment appreciably influenced the growth and physiological parameters such as shoot length (21.20 cm), root length (13.82 cm), collar thickness (7.67 mm), seedling fresh weight (4.22 g), seedling dry weight (0.46 g), R/S (length) ratio (0.65), R/S (dry weight) ratio (0.74), moisture content (89.10 %), RWC (95.92 %), seedling vigour (Length) index (3360) and seedling vigour (Mass) index (44.16). Thus, CaCl<sub>2</sub> at two per cent concentration may be used to seed hardening of hybrid maize.

**Keywords:** best management practice, maize hybrid, resilience, relative water content, vigour index

### Introduction

Maize (*Zea mays* L.) is the third most important yielding cereal crop after rice and wheat. It can be grown twice a year; hence, it is capable of fulfilling the upcoming dietary needs of ever growing global demographic and livestock pressure and solves the future biofuel crises. Generally, under irrigated conditions, traditional maize varieties are replaced by hybrid maize and at present, cultivation with hybrids are also occupied the major rainfed areas especially during Kharif season. However, its yield potential is extremely limited due to abiotic factors such as drought induced moisture stress. In Tamil Nadu, monsoon failures, erratic rainfall and decline in availability of irrigation water on time replaced the rice-rice-pulse cropping system in to maize– rice-pulse cropping system (Rex Immanuel *et al.*, 2021) [29].

Maize is generally more resistant to drought during its early stages of growth. The stress at germination or early growth can cause an irreversible decrease in yield potential (Anosheh *et al.*, 2012) [3]. It can weaken morphological, physiological and metabolic processes, reduce maize plant source size, leaf area growth and reducing the yield markedly. Maize grain yield reduction, caused by moisture stress, ranges from 10 to 76 % depending on the severity and stage of occurrence (Bolaos *et al.*, 1993) [9]. Cultivation of hybrid maize under rainfed conditions severally affected due to drought induced moisture stress.

Inducing early seedling vigour is considered as one of the management option to survive under drought stress situations. Among them, seed hardening is one of the several agronomic techniques used to cope with the adversities of drought induced losses and improve maize resilience to drought stress. Seed hardening is a repeated soaking and control seed hydration in solution containing organic and or inorganic solutes followed by redrying that allows pre-germinative metabolic activities but prevent radical emergence. Seed-hardening appears to be significantly superior in improving seed vigour than conventional water immersion (Manjunath *et al.*, 2011) [23].

Seed hardening enhances seeds vigor by protecting structure of the plasma membrane against injury during stress environment. Seed hardening treatment increases seed germination rates, coleoptile emergence percentages,

radicle and coleoptile elongation and fresh weights of the seedlings. Hardening seeds in chemicals induces enzymes activity and play a significant role on naturally over-expressing stress responses and signal activation at biochemical level.

Cycocel (CCC) inhibits the plant hormone biosynthesis and is primarily helpful in regulating the growth properties in plants. Priming with CCC at suitable concentration mitigated the stress-induced ill effects on crop plants (Anosheha *et al.*, 2014)<sup>[3]</sup>. It can make in thickening the stems and make the plant stronger, leaves darker and thicker and this enhances the plant stability in drought and cold stresses tolerance (Shekoofa and Emam, 2008). CCC application typically depends on the concentration and climatic conditions and its application increased the growth and yield of maize (Soltanbeigi, 2019)<sup>[31]</sup>.

Studies indicated that calcium chloride (CaCl<sub>2</sub>) has been used to ameliorate the adverse effects of drought stress on several plants. CaCl<sub>2</sub> osmo-hardening improved germination and emergence (Rehman *et al.*, 2011)<sup>[28]</sup>. Under drought conditions, CaCl<sub>2</sub> pre-treatments were found to enhance the above and below ground fresh and dry biomass (Xu *et al.*, 2013)<sup>[35]</sup>. CaCl<sub>2</sub> seed priming mitigated the damaging effects of moisture stress due to drought on the root system in maize (Bismillah *et al.*, 2015)<sup>[7]</sup>. CaCl<sub>2</sub> preconditioning interacts significantly with genetically determined drought tolerance (Kaczmarek *et al.*, 2017)<sup>[19]</sup>.

KCl supports the plants for tolerance of drought by stimulating root growth, enhancing root to shoot ratio and water absorbing capacity (Kalita *et al.*, 2002)<sup>[20]</sup>. The increased root length and vigour index was reported due to the application of KH<sub>2</sub>PO<sub>4</sub> (Chauhan *et al.*, 2016)<sup>[10]</sup>. Seed priming with ZnSO<sub>4</sub> improves germination of crops (Choudhary *et al.*, 2021)<sup>[11]</sup>. ZnSO<sub>4</sub> seed priming also caused early germination and enhancing the growth condition than in non-primed (Mahmood *et al.*, 2019)<sup>[22]</sup>. Maximum percent of germination was observed in MgSO<sub>4</sub> primed seeds (Swatoleena and Bose, 2017)<sup>[32]</sup>. There is very scanty information on effects of seed hardening on hybrid maize and thus the current study was conducted to determine the ability of chemicals responsible for increasing germination and seedling vigor of hybrid maize.

### Materials and Methods

The present investigation was carried out at the Agronomy Experimental lab, Annamalai University, Annamalai Nagar, Tamil Nadu, during *Khaif*, 2018. It is located at 11°24' North latitude, 79°44' East longitude and at an altitude of +5.79 m above the mean sea level and 10 km west from the Bay of Bengal. The weather of Annamalai Nagar is moderately warm with hot summer months. The mean annual rainfall received was 1450 mm with a distribution of 80% during North East monsoon, 15% during the South West monsoon and 5% during hot weather period. During Kharif season the maximum temperature ranges between 30.9° and 38.0°C with a mean of 34.79°C while the minimum temperature fluxgates between 17.8° to 27.4°C with a mean of 24.29°C. The average RH varies from 78 to 91% with a mean of 83.67%.

The maize hybrid SMH 7930 was used to the experiment. The experiment was laid out in Randomized Block Design (RBD) and replicated thrice. The treatments consisted of 14 combinations *viz.*, T<sub>1</sub> – Absolute control, T<sub>2</sub> – Distilled water soaked, T<sub>3</sub> – Cycocel @ 250 ppm, T<sub>4</sub> – Cycocel @ 500 ppm, T<sub>5</sub> – CaCl<sub>2</sub> @ 1%, T<sub>6</sub> – CaCl<sub>2</sub> @ 2%, T<sub>7</sub> – KCl @ 1%, T<sub>8</sub> – KCl @ 2%, T<sub>9</sub> – KH<sub>2</sub>PO<sub>4</sub> @ 1%, T<sub>10</sub> – KH<sub>2</sub>PO<sub>4</sub> @ 2%, T<sub>11</sub> – ZnSO<sub>4</sub> @ 100 ppm, T<sub>12</sub> – ZnSO<sub>4</sub> @ 200 ppm, T<sub>13</sub> – MgSO<sub>4</sub> @ 100 ppm and T<sub>14</sub> – MgSO<sub>4</sub> @ 200 ppm.

Respective analytical grade substances were obtained from the Agronomy laboratory and required concentrations were prepared using standard procedures. As per the treatment, seeds were soaked for 12 hours at 1/3 volume of seed and dried for under the shade at normal room temperature until the original seed moisture level reaches.

### Seed Germination Test

For germination studies, each treatment was performed with a total of 25 seeds and placed in petri dish on one layer of filter paper and covered with a second layer. The seeds were moistured daily with uniform quantity of distilled water which lasted fourteen days. All petri dishes were kept under controlled condition with normal light.

Counts of germinating seeds were started from the first day of imbibitions and every morning at 8.30 am. The seeds were considered to have sprouted when the tip of the radicle appeared to have free of seed coat. The germination period was determined as the number of days from the first observed germination to the time when there was no germination. Germination per cent was calculated using the following equation and expressed as percentage.

$$\text{Germination Percentage} = \frac{N_i}{N} \times 100$$

Where, N= Total number of seeds and N<sub>i</sub>= germinated seeds at the end of counting days.

Germination energy defined as the percentage by number of seeds in a given sample which germinate within a definite period under optimum or stated condition (Willan, 1993)<sup>[34]</sup>. Germination energy was determined on 7 day after sowing. The time to reach 50 per cent germination (T<sub>50</sub>) was calculated according to the equation outlined by Coolbear *et al.* (1984)<sup>[12]</sup>. Germination value is calculated using the equation proposed by Hartmann *et al.* (1997)<sup>[16]</sup>.

The germination rate is expressed as (final) mean daily germination (MDG), calculated as a cumulative percentage of full seed germination at the end of the test divided by the number of days from sowing to the end of the test period. Speed of germination (S) was determined and expressed as peak value, which is the maximum mean germination reached at any time during the period of the experiment (Willan, 1993) [34].

Mean emergence time (MET) was calculated using the following equation and expressed as day (Ellis and Roberts, 1982) [13]. The coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination (Jones and Sanders, 1987) [18]. Its value increases as the number of germinated seeds increases and the time required for germination is reduced. The CVG was calculated using the equation of Al-Mударis (1998) [2].

The germination index (GI) is defined as a weighted sum of the daily numbers of germinated seeds (Benech *et al.*, 1991) [6]. Germination index (GI) was calculated according to ISTA (2009) [17] equation. The maximum weightage is given to the seeds germinated on the first day, while lesser weightage was given to the seeds germinated at later dates, with the lowest weight being assigned to seeds germinated on the last day. GI therefore emphasizes both the germination percentage and its speed. Higher GI values mean higher percentages and a higher rate of germination.

### Growth, vigour and physiological attributes

For growth studies, each treatment was performed with a total of 10 seeds and dibbled at 0.5 cm depth in poly coated disposable cup containing native soil (clay loam in texture with low in available nitrogen (169 kg/ha), medium in available phosphorus (9.91 kg/ha) and available potassium (279 kg/ha)). The soil was moistened daily with uniform quantity of distilled water which lasted eighteenth day.

On 21<sup>st</sup> day the biometric observations were taken for the study. Root and shoot length is measured with the help of scale. Immediately after harvest fresh weight of seedling was recorded. The seedlings used for measuring turgid weight were kept in a petri dish containing distilled water. After two hours, the seedlings were taken out and the surface was blotted gently to remove excess water. The weight of the turgid seedlings was recorded and expressed in gram. Micrometer (0-25 mm) is used to measuring the collar thickness. Plant samples were oven dried at 65°C for 24 hours to get constant weight.

The root shoot ratio on seedling length basis per seedling was calculated by using the following equation.

$$\text{Root Shoot Ratio (Seedling Length basis)} = \frac{\text{Root Length}}{\text{Shoot Length}}$$

The root shoot ratio on dry weight basis per seedling was calculated by the using following equation.

$$\text{Root Shoot Ratio (Dry Weight basis)} = \frac{\text{Root Dry Wt}}{\text{Shoot Dry Wt}}$$

The moisture content per seedling is calculated by the following formula and expressed in percentage.

$$\text{Moisture Content (\%)} = \frac{\text{Seedling Fresh Wt} - \text{Seedling Dry Wt}}{\text{Seedling Fresh Wt}} \times 100$$

The relative water content (RWC) was estimated by the method of Barrs and Weatherley (1962) and expressed in percentage.

The vigour level of each treated seed lot was calculated using the following procedure suggested by Abdul-Baki and Anderson (1973) [11] and expressed in whole number. Seedling Vigour Index<sub>(Length)</sub> in terms of length was determined by multiplication of germination percentage with seedling length.

$$\text{Seedling vigour index (Length)} = \text{Germination \%} \times \text{Seedling Length (cm)}$$

Seed Vigour Index<sub>(Mass)</sub> in terms of mass was determined by multiplication of germination percentage with seedling dry weight.

$$\text{Seedling vigour index (Mass)} = \text{Germination \%} \times \text{Seedling Dry Wt (g)}$$

The data on the germination and growth parameters recorded during the investigation was analyzed statistically (Gomez and Gomez, 1984) [14]. Wherever the results were found significant ('F' test), the critical differences (CD) were arrived at 5% probability level (P=0.05).

### Results and Discussion

The seed hardening substances notably enhanced the germination %, germination energy (%), time to reach 50 % germination and germination value, compared with the untreated and water soaked control seeds of hybrid maize (Table 1). Among the treatments tested, CaCl<sub>2</sub> @ 2% concentration (T<sub>6</sub>) registered the maximum germination percentage (96.00 %), germination energy (94.67 %), time to reach 50 % germination (2.3 days) and

germination value (1.86), followed by Cycocel @ 500 ppm (T<sub>4</sub>). Similarly, the same treatment (Table 2) also exhibited the maximum speed of germination (6.8 per day), mean emergence time (3.2 days), coefficient of velocity (167) and germination index (38.5). In all the parameters, absolute control has exhibited the least values.

**Table 1:** Effect of pre-sowing seed hardening treatments on germination of hybrid maize

Treatments	Germination %	Germination energy (%) (7 DAS)	Time to reach 50 % germination (days)	Germination value
T <sub>1</sub> – Absolute control	84.00 (66.42)	62.67 (52.34)	6.0	1.09
T <sub>2</sub> – Distilled water soaked	84.00 (66.42)	66.67 (54.74)	5.7	1.12
T <sub>3</sub> – Cycocel @ 250 ppm	88.00 (69.73)	81.33 (64.40)	4.3	1.49
T <sub>4</sub> – Cycocel @ 500 ppm	94.67 (77.08)	93.33 (75.03)	2.7	1.71
T <sub>5</sub> – CaCl <sub>2</sub> @ 1%	89.33 (70.63)	82.67 (65.40)	3.3	1.51
T <sub>6</sub> – CaCl <sub>2</sub> @ 2%	96.00 (78.46)	94.67 (76.65)	2.3	1.86
T <sub>7</sub> – KCl @ 1%	86.67 (68.87)	77.33 (61.56)	5.0	1.30
T <sub>8</sub> – KCl @ 2%	93.33 (74.66)	89.33 (70.93)	3.0	1.70
T <sub>9</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 1%	85.33 (67.21)	73.33 (58.91)	5.3	1.24
T <sub>10</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 2%	92.00 (73.57)	88.00 (69.73)	3.3	1.63
T <sub>11</sub> – ZnSO <sub>4</sub> @ 100 ppm	84.00 (66.42)	69.33 (56.37)	5.3	1.24
T <sub>12</sub> – ZnSO <sub>4</sub> @ 200 ppm	86.67 (68.87)	80.00 (63.44)	4.7	1.42
T <sub>13</sub> – MgSO <sub>4</sub> @ 100 ppm	84.00 (66.42)	66.67 (54.74)	5.7	1.18
T <sub>14</sub> – MgSO <sub>4</sub> @ 200 ppm	85.33 (67.21)	76.00 (60.67)	5.3	1.26
SEm±	0.39	0.41	0.17	0.05
C.D. (P = 0.05)	1.28	1.35	0.49	0.12
C.V %	4.94	4.57	4.65	5.76

**Table 2:** Performance of pre-sowing seed hardening treatments on speed of germination, MET, coefficient of velocity and germination Index of hybrid maize

Treatments	Speed of Germination (per day)	Mean emergence time (days)	Coefficient of velocity (12 DAS)	Germination index
T <sub>1</sub> – Absolute control	4.3	7.3	102	14.5
T <sub>2</sub> – Distilled water soaked	4.6	6.4	109	16.0
T <sub>3</sub> – Cycocel @ 250 ppm	5.6	4.9	139	23.4
T <sub>4</sub> – Cycocel @ 500 ppm	6.3	3.8	159	32.2
T <sub>5</sub> – CaCl <sub>2</sub> @ 1%	5.9	4.7	145	25.2
T <sub>6</sub> – CaCl <sub>2</sub> @ 2%	6.8	3.2	167	38.5
T <sub>7</sub> – KCl @ 1%	5.1	5.2	130	20.0
T <sub>8</sub> – KCl @ 2%	6.2	4.2	152	31.7
T <sub>9</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 1%	4.9	5.7	124	18.0
T <sub>10</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 2%	5.9	4.2	147	27.0
T <sub>11</sub> – ZnSO <sub>4</sub> @ 100 ppm	4.8	6.1	119	16.2
T <sub>12</sub> – ZnSO <sub>4</sub> @ 200 ppm	5.6	5.2	131	21.5
T <sub>13</sub> – MgSO <sub>4</sub> @ 100 ppm	4.6	6.1	115	16.2
T <sub>14</sub> – MgSO <sub>4</sub> @ 200 ppm	5.0	5.7	125	18.7
SEm±	0.11	0.11	1.62	1.28
C.D. (P = 0.05)	0.32	0.37	5.04	3.92
C.V %	7.4	6.9	4.8	5.2

The pre-sowing seed-hardening treatments exhibited significant effect on seedling growth, physiological attributes and vigour index of hybrid maize (Table 3&4). Among the treatments tested, the pre sowing treatment with CaCl<sub>2</sub> @ 2% concentration (T<sub>6</sub>) registered maximum seedling physical parameters viz., shoot length (21.20 cm), root length (13.82 cm), collar thickness (7.67 mm), seedling fresh weight (4.22 g), seedling dry weight (0.46 g), root-shoot ratio on seedling length basis (0.65) and root-shoot ratio on dry weight basis (0.74) and the lowest characters were observed under the control (T<sub>1</sub>) treatment. The same treatment, CaCl<sub>2</sub> @ 2% concentration recorded maximum relative water content of 95.92 %, seedling vigour index<sub>(length)</sub> of 3360 and Seedling vigour index<sub>(Mass)</sub> of 44.16, while it was registered the least moisture content of 89.10 %.

**Table 3:** Performance of pre-sowing seed-hardening treatments on growth parameters of hybrid maize

Treatments	Shoot length (cm)	Root length (cm)	Collar thickness (mm)	Seedling fresh weight (g)	Seedling dry weight (g)	R/S Ratio	
						(Seedling length basis)	(Dry weight basis)
T <sub>1</sub> – Absolute control	11.53	05.63	2.90	2.13	0.15	0.49	0.36

T <sub>2</sub> – Distilled water soaked	12.30	06.23	3.37	2.40	0.18	0.51	0.43
T <sub>3</sub> – Cycocel @ 250 ppm	17.13	09.07	5.47	3.30	0.30	0.53	0.60
T <sub>4</sub> – Cycocel @ 500 ppm	19.53	11.77	6.73	4.05	0.39	0.60	0.68
T <sub>5</sub> – CaCl <sub>2</sub> @ 1%	17.77	09.53	5.83	3.47	0.31	0.54	0.62
T <sub>6</sub> – CaCl <sub>2</sub> @ 2%	21.20	13.82	7.67	4.22	0.46	0.65	0.74
T <sub>7</sub> – KCl @ 1%	15.80	09.03	4.47	2.97	0.25	0.57	0.55
T <sub>8</sub> – KCl @ 2%	18.80	10.43	6.50	3.90	0.35	0.55	0.67
T <sub>9</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 1%	14.17	08.20	3.80	2.80	0.21	0.57	0.52
T <sub>10</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 2%	18.20	10.83	6.17	3.67	0.32	0.59	0.63
T <sub>11</sub> – ZnSO <sub>4</sub> @ 100 ppm	14.07	07.00	3.77	2.67	0.21	0.50	0.51
T <sub>12</sub> – ZnSO <sub>4</sub> @ 200 ppm	16.80	09.43	4.83	3.07	0.29	0.56	0.57
T <sub>13</sub> – MgSO <sub>4</sub> @ 100 ppm	14.00	07.37	3.47	2.57	0.20	0.52	0.48
T <sub>14</sub> – MgSO <sub>4</sub> @ 200 ppm	15.20	08.53	4.03	2.83	0.24	0.56	0.55
SEm±	0.26	0.34	0.31	0.03	0.02	0.06	0.09
C.D. (P = 0.05)	0.71	0.98	0.64	0.11	0.06	0.21	0.29
C.V %	7.6	6.1	5.3	5.5	4.8	5.7	4.2

**Table 4:** Performance of pre-sowing seed hardening treatments on physiological attributes and vigour index of hybrid maize

Treatments	Moisture Content (%)	Relative water content (%)	Seedling vigour index (Length)	Seedling vigour index (Mass)
T <sub>1</sub> – Absolute control	92.96	92.52	1441	12.60
T <sub>2</sub> – Distilled water soaked	92.50	93.28	1557	15.12
T <sub>3</sub> – Cycocel @ 250 ppm	90.90	94.94	2305	26.40
T <sub>4</sub> – Cycocel @ 500 ppm	90.37	95.31	2963	36.92
T <sub>5</sub> – CaCl <sub>2</sub> @ 1%	91.06	94.61	2439	27.69
T <sub>6</sub> – CaCl <sub>2</sub> @ 2%	89.10	95.92	3360	44.16
T <sub>7</sub> – KCl @ 1%	91.58	94.44	2152	21.67
T <sub>8</sub> – KCl @ 2%	91.03	94.66	2728	32.67
T <sub>9</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 1%	92.50	93.84	1908	17.92
T <sub>10</sub> – KH <sub>2</sub> PO <sub>4</sub> @ 2%	91.28	94.90	2670	29.44
T <sub>11</sub> – ZnSO <sub>4</sub> @ 100 ppm	92.13	93.89	1770	17.64
T <sub>12</sub> – ZnSO <sub>4</sub> @ 200 ppm	90.55	94.23	2273	25.13
T <sub>13</sub> – MgSO <sub>4</sub> @ 100 ppm	92.21	94.05	1825	16.80
T <sub>14</sub> – MgSO <sub>4</sub> @ 200 ppm	91.52	94.18	2025	20.48
SEm±	0.17	0.16	62	2.27
C.D. (P = 0.05)	0.56	0.48	197	6.82
C.V %	5.3	4.9	6.2	5.6

Seed vigor determines the potential for rapid and uniform emergence of plants (Wen *et al.*, 2018) [33]. The hardening of seeds with CaCl<sub>2</sub> at two per cent concentration was found to effective in germination indices of hybrid maize. The progress in germination by CaCl<sub>2</sub> hardened seeds may be attributed to stimulation of hydrolytic enzyme activity. CaCl<sub>2</sub> solutions have proved effective in improving germination rate of crops. Calcium in CaCl<sub>2</sub> improves cell water status and also acts as cofactors in the activities of diversified enzymes during reserve metabolization and radical extension. CaCl<sub>2</sub> also prevent damage from cellular dehydration by balancing the osmotic strength of the cytoplasm (Arshi *et al.*, 2006) [4].

Calcium ions have essential structural and signaling roles in plants. Seed hardening generally altering the protoplasmic characters and increases the embryo physiological activity associated with the structure development responsible for seed germination (Bhadane *et al.*, 2021) [7]. If the hydration of seeds that equals, however does not exceed, the lag phase of hardening allows early DNA replication, more ATP availability, elevated RNA and protein synthesis, quicker embryo growth, decreased leakage of metabolites and restore of deteriorated seed components than untreated control (Maheswari *et al.*, 2020) [21].

In the present study, observed increases in root length, shoot length, collar diameter, seedling fresh and dry weight, root-shoot ratio on both biomass values were greatest in the 2% CaCl<sub>2</sub> pre-treatment. Fast growing roots act as strong sink for food reserves and grow at the expense of shoot growth. Under stressful environments, rapid and uniform germination of crops is necessary to achieve enhanced yield potential (Hamidi and Pirasteh-Anosheh, 2013) [15]. It can be achieved through good root proliferation during the early stages. Seed hardening has been found to be associated with enhanced nutrient reserves through increased physiological activities and root proliferation.

The treated seedlings exhibited lower moisture content compared to control. Generally the treated seedlings produce more compact plant parts like roots which help to hold less moisture than the other plant parts. The highest percentage of moisture content in the control was due to immature cell and cell wall to hold more water

within the cell. The oven dried seedlings lose their moisture content very rapidly because their cells are not as compact as in treated seedlings. The obtained results revealed that 2% CaCl<sub>2</sub> pre-treatment enhanced the vigour index in comparison with untreated seed. This might be due to that treated seeds enhanced the germination percentage, produced more shoot and root length and more seedling dry weight ultimately vigour of seedlings. The results obtained from this study were in accordance with those reported by Prakash *et al.* (2013)<sup>[26]</sup> and Bhadane *et al.* (2021)<sup>[7]</sup>.

### Conclusion

Seedling survival and establishment is a significant factor and principally depends on the seed germination and vigour. Seed hardening is a simple best management practice which has the potential to enhance the seed vigour and germinability of seeds and also has the tremendous ability to improve the germination power over a wide range of abiotic stress conditions. Since, seed hardening is found to be an effective technology there is a necessity to standardize this technology in every crop species particularly including. In the present study, CaCl<sub>2</sub> seed hardening at two per cent concentration at 12 hours exhibited significant beneficial effect on germination, seedling growth and vigour index of hybrid maize.

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