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Effect of hydropriming on physiological and biochemical activities of high and low vigour seed lots of a maize hybrid and its parental lines

HEENA RASOOL MIR* AND SHIV K. YADAV
Division of Seed Science and Technology, IARI, New Delhi-110012
heenamir.hamdani@gmail.com

ABSTRACT A study was conducted to evaluate the influence of hydropriming on high and low vigour seed lots of maize hybrid (VQPM 9) and its parental lines VQL 1 (female) and VQL 2 (male). Seeds were subjected to hydropriming (30h) followed by redrying to original moisture content. The primed and unprimed seeds were assessed for physiological and biochemical seed quality parameters. The results indicated that priming increased physiological performance in terms of increased germination percentage, shoot length, root length, dry matter production and vigour index in both high (I) and low (II) vigour seed lots of VQL 1, VQL 2 and VQPM 9. Increase in percentage of germination of VQL 1 (I) and (II) by 12.6% and 3.8%; that of VQL 2 (I) and (II) by 16.1% and 3.3% whereas in VQPM 9 (I) and (II) by 4.2% and 4.8% respectively. The increase in vigour indices paralleled that of seedling length and dry weight. The increase in vigour index I ranged from 5% to 11.3% and 14.4% to 54.8% in low and high vigour seed lots, respectively. Similarly, the increase in vigour index II ranged from 3.7% to 9.5% and 10.3% to 42.7 % in low and high vigour seed lots, respectively. The priming improved germination might be due to enhanced repair of membranes, which significantly reduced leakage of electrolytes. Water-soluble sugars content of leachate from primed seeds were found significantly low as compared to unprimed seeds. The activities of hydrolytic enzymes, amylase and dehydrogenase in high and low vigour lots were greater than unprimed controls and ranged from 1.0% to 11.7% and 1.4% to 8.4%, respectively. In hydro-primed seeds of both high and low vigour seeds lots of parental lines and their hybrid, significantly increased activities of antioxidant enzymes; superoxide dismutase, peroxidase and catalase, over control was recorded. From the present study, it was concluded that priming of VQPM 9 maize hybrid and its parental line seeds with water for 30h was found to improve the physiological seed quality parameters significantly which was supported by the pattern of increased enzymatic activities. It was also recorded that the seed priming effect was more pronounced in high vigour seeds than in low vigour seed lots.

Keywords Hydro-priming, maize, germination, physiological changes, antioxidant enzymes

Maize (*Zea mays* L.) is an important food and feed crop of the world. Global maize production has almost doubled over the past two decades from 400 million tons (1990) to 817 million tones, and average yields have gone up from 1.95 t/ha (1960) to 5.12 t/ha [1]. This increase in productivity has been largely due to the introduction of single-cross hybrids. Globally, India has the fourth largest acreage and is the fifth largest corn producer.

It is grown in many states in India and in 2012-13, was planted over 8.8 million hectares with a production of 21.8 million tones [2]. Despite the high yielding potential and various advantages of hybrid maize, the yield per unit area of the crop is low in India. Delay in germination and low seed viability is the serious problems limiting the production of maize. Highly vigorous seeds germinate rapidly, uniformly and are able to withstand environmental adversity after sowing. However, the use of maize seeds of low physiological quality is a common practice under tropical and subtropical production conditions, leading to inadequate plant population in the field.

Technology that enhances germination and emergence is thus important in mitigating deleterious effects of poor crop establishment. Such technologies would

*Corresponding author

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allow farmers to achieve good crop stands and ultimately good yields. Seed hydropriming is one such technology, which has been developed to enhance germination characteristics of seeds. A low-cost approach, designated as "on farm seed priming" was proposed by Harris *et al.* [3], and involved soaking of seed in water before sowing. The pre-sowing seed treatment, known as hydropriming, allows the seed to imbibe water and go through the first phase of germination in which pre-germination metabolic activities are preceded while the latter two phases of germination are inhibited [4]. Few studies on maize are not over emphasized and are encouraging, but more information is required before its use as a routine practice in seed technology. In a series of experiments, Harris *et al.* [5] showed that hydropriming greatly improved establishment and vigour of upland rice, maize and chickpea and resulted in faster development, earlier flowering, maturity and higher yields. Primed and dried seeds normally have a more rapid and uniform germination when subsequently re-hydrated, especially under adverse environmental conditions [6].

Priming initiates metabolic activities, such as protein, RNA and DNA synthesis, DNA replication and â- tubulin accumulation [7]. It has also been suggested that priming can enhance repair of membranes, the activity of hydrolytic enzymes as well as antioxidant system [8].

Although, seed hydropriming has presented promising results for seed vigor enhancement, for many seeds including the cereal seeds, but there is dearth of information about the germination performance of primed seeds of maize hybrid and their inbred parental lines. Moreover, effect of priming on biochemical activities in maize hybrids is also sparsely reported, so it was imperative to develop suitable techniques in order to improve maize seed germination capacity. The present study was, therefore, carried out under laboratory conditions with objective to evaluate the effects of hydropriming treatment on seed germination performance and to establish a relation between the seed leachate, activity of hydrolytic enzymes (amylases and dehydrogenases) and antioxidant enzyme activity of high and low vigour seed lots of maize hybrid VQPM 9 and its parental lines Female – VQL 1, VQL 2.

MATERIALS AND METHODS

Laboratory studies were carried out at the Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi, India, to assess the effect of priming on fresh and artificially aged seed lots of a maize hybrid and its parental lines. Genetically pure fresh and high vigour (> 90% germination) seeds of maize hybrid; VQPM 9 and its parental lines (Female – VQL 1 and Male – VQL 2) obtained from VPKAS, Almora, Uttrakhand were used for this study. Half the quantity of fresh seeds having >90% germination of all the genotypes were subjected to accelerated ageing. Seeds were packed in perforated butter paper bags and placed in a desiccator containing water instead of silica gel to maintain relative humidity at 98 ± 2% that was kept inside an incubator maintaining 40 ± 1°C. The samples from aged seeds were drawn every day and subjected to germination test until the germination reduced to 80 per cent (low vigour). These seeds were shade dried to its original moisture content. Preliminary studies were carried out to standardize the duration for optimum hydropriming. Seeds were hydro-primed at 25°C for 6, 12, 18, 24, 30 and 36 h. Maximum germination percentages and vigour index of seeds were observed after 30 h hydropriming. Hence, both high (fresh) and low (aged) vigour seed lots of maize hybrid and its parental lines were subjected to hydropriming with distilled water for 30 h. After hydropriming, the seeds were removed from the distilled water, redried to original moisture content under shade at room

temperature and unprimed seeds were used as control. All the lots thus obtained were assessed for the biochemical and physiological seed quality parameters. The experiment was carried out with three replications in factorial completely randomized design. The data was analyzed using OPSTAT software package. The values in percentage were converted to arc sine values using percentage transformation for the calculation of C.D.

Seed germination was determined as per ISTA [9], with minor modifications. Three replications each of 50 seeds were placed between two layers of moist paper towel and placed in the walk-in-germinator maintained at 25°C. First and final count was taken on 4th day and 7th day respectively. Germination percentage was calculated based on number of normal seedlings on final count.

The vigour indices were determined by picking ten normal seedlings at randomly from each replication of the germination test on the final day. These were subjected to the measurement of seedling length and seedling dry weight (after 17 hrs at 80 °C oven drying). The mean values were used for computing the vigour indices adopting the method of Abdul-Baki and Anderson [10] using the following formula:

Vigour Index I = Germination (%) x Total seedling length (cm)

Vigour Index II = Germination (%) x Seedling dry weight (g)

For estimation of electrical conductivity of seed leachates four replicates of 50 seeds each were soaked in 250 ml of distilled water. Each beaker was covered with aluminum foil and placed at 20 ± 2°C for 24 h. The conductivity of the seed leachate was measured and expressed as $\mu S\ cm^{-1}g^{-1}$.

Water-soluble sugars were quantified [11] in three replications. To 0.1 ml seed leachate in a test tube, 5 ml of 5% phenol solution was added. This was followed by adding 5 ml of concentrated H_2SO_4. The mixture was mixed well and cooled at room temperature. The intensity of the colour was read at 490 nm in a spectrophotometer (Systronics, India). Activity of amylase enzyme was measured following the procedure Murata [12], by grinding 1g seeds in 10 ml of 0.05 M phosphate buffer. The homogenate was centrifuged at 12,000 rpm for 10 min at 4°C. Supernatant was collected and used as source of enzyme after diluting to 10 times with cold distilled water. To this diluted extract 1ml of starch solution was added and incubated at room temperature (27 ± 2°C) for 5 minutes. Immediately after incubation, 1ml of iodine reagent was added to stop the reaction. Optical density (OD) of resulting solution was measured at 620 nm in a spectrophotometer (Systronics, India). The enzyme activity was expressed as ìg starch hydrolyzed/g seed.

Dehydrogenase activity was estimated following the method of Kittock & Law [13] with minor modifications. Twenty seeds for each replication were preconditioned and prepared before staining in 1% TZ solution (2, 3, 5-Triphenyl tetrazolium chloride solution) for 6 hr in dark at 25°C. Excess solution was drained out and seeds were washed thoroughly. To the stained seeds, 15 ml of methyl cellosolve was added and left for 4-6 hr with occasional stirring, to extract the red coloured formazan. The intensity of decanted solution was read at 480 mm in a spectrophotometer (Systronics, India) using methyl cellosolve as blank.

For antioxidant enzyme assays the seeds were homogenized in potassium phosphate buffer (1:5 w/v) (pH 7.0) with 1% Poly vinyl pyrrolidone (PVP). The homogenate was centrifuged at 10,000 x g for 30 min at 0°C. The supernatant was collected and used for enzyme assays.

Superoxide dismutase (SOD) activity was measured according to Beauchamp and Fridovich [14] with some minor modifications by recording the enzyme induced decrease in the absorbance of formazan by nitroblue tetrazolium (NBT) by superoxide radicals. The absorbance at 560 nm was recorded in (Systronics, India) spectrophotometer. One unit of SOD was defined as the enzyme activity, which inhibited the photo reduction of NBT to blue formazan, by 50% and SOD activity of the extracts was expressed as unit/g seed/min.

Peroxidase (POX), activity was assayed as increase in optical density due to oxidation of guaiacol to tetra-guaiacol (å = 26.6 mM^{-1} cm^{-1} by following the method of Castillo *et al.* [15] with minor modifications. At 470 nm changes in absorbance due to the formation of tetra-guaiacol by using a reaction mixture containing 12 mM hydrogen peroxide and 96mM guaiacol in 0.5 mM phosphate buffer pH 7.0 were followed every 60 s. It was calculated as per the extinction coefficient of its oxidation product, tetra-guaiacol E= 26.6 nM/cm. Enzyme activity was expressed as µmoles/cm/min/gram fresh weight.

Catalase (CAT) activity was assayed by measuring the disappearance of H_2O_2 (å = 39.4 mM^{-1} cm^{1}). Three ml reaction mixture contained sodium phosphate buffer (50 mM, pH 7.0) and 10 mM H_2O_2. Absorbance was measured at 240 nm in UV/Visible spectrophotometer (Systronics, India). The activity was expressed as ì mole/min/g seed.

RESULTS AND DISCUSSIONS

Studies on the effect of seed hydropriming with water for 30 hrs revealed that the treatment significantly influenced the physiological seed quality parameters of both high (I) and low (II) vigour seed lots of maize hybrid VQPM 9 and its parental lines (Female - VQL 1 and Male - VQL 2). The influence of hydropriming on physiological characters among the genotypes was also significant. Increase in percentage of germination of VQL 1 (I) and (II) by 12.6% and 3.8%; that of VQL 2 (I) and (II) by 16.1% and 3.3% whereas in VQPM 9 (I) and (II) by 4.2% and 4.8% respectively (Fig. 1). The explanation of priming effect on higher germination might be attributed to the onset of early metabolic events during hydration leading the seed physiological state to the brink of radicle protrusion. Being retained largely after re-drying of seeds such physiological advancement of primed seeds results in faster germination upon re-hydration [7]. The faster germination of hydro-primed seeds could result in further seedling growth. Bray *et al.* [16] reported that the priming treatment abolishes the difference in germination performance of fresh and aged seeds. In the present study, the increase in shoot length, root length and dry matter production due to priming might be due to earlier start of emergence as evidenced by increased germination and vigour index (Fig. 2 & 3).The increase in vigour indices paralleled that of seedling length and dry weight. The increase in vigour index I ranged from 5% to 11.3% and 14.4% to 54.8% in low and high vigour seed lots, respectively (Fig. 4). Similarly, the increase in vigour index II ranged from 3.7% to 9.5% and 10.3% to 42.7 % in low and high vigour seeds, respectively (Fig. 5). This was in agreement with the earlier studies on maize by various workers [17-19].

The electrical conductivity (EC) of leachates from unprimed seeds was higher while hydro-primed seed leachates of all cultivars showed lower EC during imbibition (Table 1). EC is considered as an effective indicator of seed germination in sweet corn [20]. In sweet corn, priming resulted in decreased conductivity, free sugars and DNA content while RNA content increased [21]. Water soluble sugar content of leachates from primed seeds was significantly low as compared to unprimed seeds by 2.73 to 2.05 % in both high and low vigour seed lots of hybrid and its parental lines (Table 1). Simon

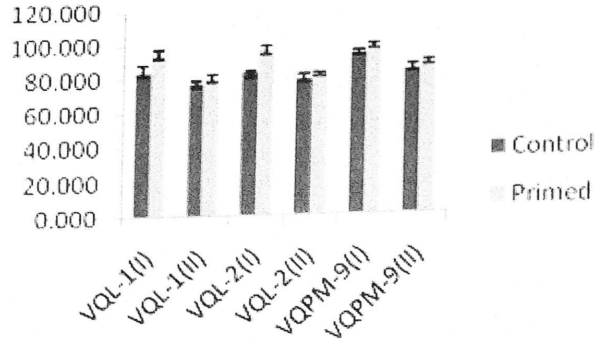

Fig. 1 Effect of hydro-priming on germination percentage of high (I) and (II) vigour lots of maize hybrid VQPM 9 and its parental lines. Values are mean of 3 measurements. Bars represent±SE(n=3)

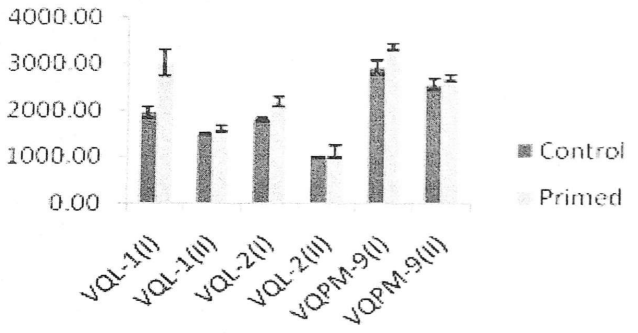

Fig. 2 Effect of hydro-priming on seedling length (cm) of high (I) and low (II) vigour lots of maize hybrid VQPM 9 and its parental lines. Values are mean of 3 measurements. Bars represent±SE(n=3)

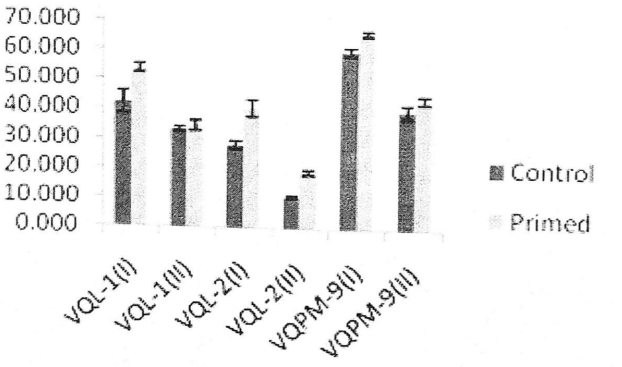

Fig. 3 Effect of hydro-priming on seedling dry weight (gm) of high (I) and low (II) vigour lots of maize hybrid VQPM 9 and its parental lines. Values are mean of 3 measuremetns. Brs represent±SE (n=3)

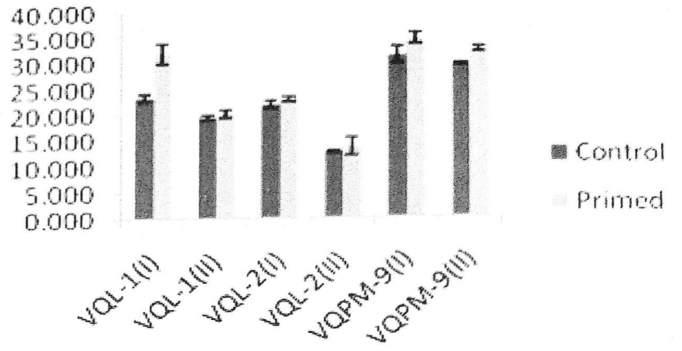

Fig. 4 Effect of hydro-priming on vigour index I of high (I) and low (II) vigour lots of maize hybrid VQPM 9 andd its parental lines. Values are mean of 3 measurements. Bars represent±SE (n=3)

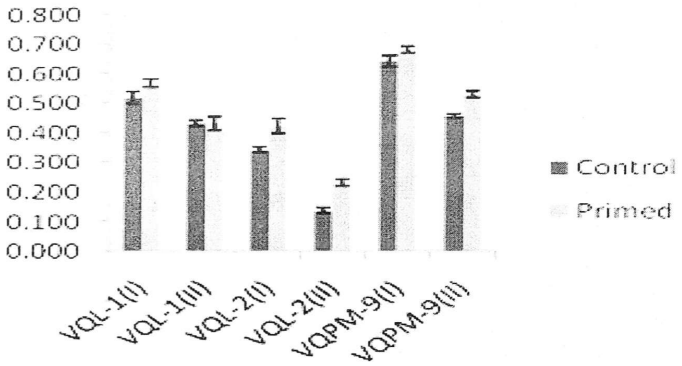

Fig. 5 Effect of hydro-priming on vigour index II of high (I) and low (II) vigour lots of maize hybrid VQPM 9 and its parental lines. Values are mean of 3 measurements. Bars represent±SE (n=3)

and Mills [22] reported that low quality seeds had poor membrane structure that allows the outward diffusion of ions during imbibition and can be detected by monitoring the electrolyte present in steep water. Hydropriming significantly reduced leakage of electrolytes than the control unprimed seeds by 1.09 to 0.55 % (Table 1). The priming improved germination might be due to priming enhanced repair of membranes [23]. All the priming treatments are known to advance the metabolism of seeds. Hydro-primed maize seeds exhibited higher activity of á-amylase as compared to control in all the seed lots and ranged from 14.7% to 19.18% and 17.84% to 34.8% in high and low vigour seed

Table 1. Effect of hydropriming on EC, WSS and hydrolytic enzyme activity of high (I) and low (II) vigour lots of maize hybrid VQPM 9 and its parental lines

Geno-type	Treatment	EC (iS/ cm/g of seed)	WSS (ìg/ ml)	Amylase activity (ìg starch hydrolysed /g seed/min)	Dehyrdogenase activity (OD units/20 seeds)
VQL 1(I)	Control	0.037±0.001	11.60±0.092	9.860±0.095	0.457±0.004
	Hydropriming	0.026±0.003	9.380±0.209	10.273±0.341	0.487±0.003
VQL 1(II)	Control	0.044±0.007	12.420±0.072	9.620±0.461	0.445±0.003
	Hydropriming	0.027±0.001	10.253±0.123	9.867±0.227	0.459±0.002
VQL 2(I)	Control	0.041±0.002	7.147±0.515	28.367±1.027	0.844±0.006
	Hydropriming	0.031±0.003	5.047±0.299	30.953±0.335	0.915±0.004
VQL 2(II)	Control	0.037±0.002	9.393±0.294	10.967±0.243	0.485±0.001
	Hydropriming	0.031±0.004	8.667±0.249	11.087±0.357	0.492±0.003
VQPM 9 (I)	Control	0.033±0.003	5.047±0.299	43.620±2.284	0.899±0.004
	Hydropriming	0.016±0.002	3.633±0.281	48.740±1.870	0.956±0.013
VQPM 9 (II)	Control	0.038±0.000	6.447±0.905	31.427±0.480	0.885±0.016
	Hydropriming	0.019±0.004	4.660±0.162	40.273±0.889	0.936±0.010
C.D. for Factor A		0.005	0.531	1.427	0.011
C.D. for Factor B		N.S.	0.434	1.166	0.009
C.D. for Factor A X B		N.S.	0.434	1.166	0.009
C.D. for Factor C		N.S.	N.S.	2.019	0.015
C.D. for Factor A X C		0.007	0.751	2.019	0.015
C.D. for Factor B X C		0.006	N.S.	N.S.	N.S.
C.D. for Factor A X B X C		0.01	1.063	N.S.	0.021

A = Variety ; B = Lot; C = Treatment
Data in the tables are the means ± SE(m) (n= 3)

lots, respectively (Table 1). Amylases are key enzymes that play a vital role in hydrolyzing the seed's starch reserve thereby supplying sugars to the developing embryo. Hence, improvement in seed germination and seedling emergence can be attributed due to enhanced supply of simple sugars to the growing embryo, which was caused by an increase in amylase activity. Similar results have been reported by Andoh and Kobata [24] while examining hydropriming effects in rice and wheat kernels. Dehydrogenase, a hydrolytic respiratory enzyme activity showed significant increase in primed seeds irrespective of the difference in vigour among the lots of hybrid and parental lines. The increase in their activity ranged from 4.1-4.8% and 6.0-8.9% in high and low vigour seed lots, respectively (Table 1).

In the present study, the activities of antioxidant enzymes of unprimed and primed seeds in hybrid and its parental lines were determined to investigate their relationship with germination characteristics. A steady increase was noted in the activities of three key antioxidant enzyme systems; superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) in both high and low vigour seed lots of hybrid and its parental lines. The SOD activity of hydro-primed seeds was significantly higher than that from unprimed seeds and ranged from 7.01% to 38.6% and 10.3% to 42.7 % in high and low vigour seeds, respectively (Table 2). A similar trend was also observed for POX and CAT enzyme activity which showed a range from 18.1 to 54.2% and 14.9 to 49.6%; 57.9 to 107.3% and 70.35 to 110.5% in high and low vigour seed lots respectively (Table 2). Enhanced SOD activity in primed seeds also helps controlling the production of malonaldehydes and haxanal, thus improving germination and vigour of primed seeds. Similar reports on quality protein maize have been reported by

Table 2. Effect of hydropriming on antioxidant enzymes activity of high (I) and low (II) vigour lots of maize hybrid VQPM 9 and its parental lines

Genotype	Treatment	SOD activity unit/g seed/min	POX activity (i moles/ cm/ min/ g seed)	CAT activity i mole/min/g seed
VQL 1(I)	Control	5.720±0.031	12.827±0.733	14.210±1.580
	Hydropriming	6.100±0.012	15.160±0.359	29.473±1.053
VQL 1(II)	Control	2.983±0.075	9.813±0.529	9.997 ±1.393
	Hydropriming	3.470±0.029	11.280±0.325	21.053±1.390
VQL 2(I)	Control	5.563±0.012	25.270±0.936	14.947±6.513
	Hydropriming	6.910±0.010	38.983±0.423	26.840±0.912
VQL 2(II)	Control	3.137±0.133	13.767±0.142	8.943±1.053
	Hydropriming	4.487±0.252	18.890±1.016	17.370 ±0.912
VQPM 9 (I)	Control	5.583±0.282	12.523±0.231	20.000 ±8.469
	Hydropriming	7.743±0.082	16.743±0.041	31.580 ±0.912
VQPM 9 (II)	Control	3.387±0.187	9.397±0.243	14.210 ±0.912
	Hydropriming	5.927±0.093	14.067±0.306	24.207±0.527
C.D. for Factor A		0.197	0.773	N.S.
C.D. for Factor B		0.161	0.631	3.868
C.D. for Factor A X B		0.161	0.631	N.S.
C.D. for Factor C		0.279	1.094	6.699
C.D. for Factor A X C		0.279	1.094	N.S.
C.D. for Factor B X C		N.S.	0.893	N.S.
C.D. for Factor A X B X C		N.S.	1.547	N.S.

A = Variety ; B = Lot; C = Treatment
Data in the tables are the means ± SE(m) (n= 3)

Anonymous [25] and Thasni [26]. This observation reiterates the principle that priming promotes the metabolic activities that take place in the II phase of imbibition in the germinating seed. Genotypic differences were significant for all the studied parameters, but low vigour lots responded better to hydropriming than high vigour lots, irrespective of genotypes.

CONCLUSIONS

From the present study, it was clearly concluded that the priming of hybrid maize; VQPM 9 and its parental line seeds with water for 30 hrs was found to improve the physiological seed quality parameters significantly. It was deduced that the seed priming effect was more pronounced in high vigour seeds than in low vigour seeds. Hence, only the marginal seed lots having relatively low vigour and germination percentage can be hydro-primed for enhanced germination, vigour and uniform field establishment. Thus, positive effects of priming on the germination performance are attributed to the induction of biochemical mechanisms of cell repair; the resumption of metabolic activity can restore cellular integrity, through the membrane repair, synthesis of hydrolytic enzymes and the improvement of the antioxidant defence system.

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