Hydropriming associated physiological and biochemical changes responsible for the enhanced planting value of maize hybrid and its parental line seeds

Heena Rasool MIR, Shiv Kumar YADAV* skysst@gmail.com, **Sangita YADAV**

Division of Seed Science and Technology, ICAR- Indian Agricultural Research Institute, New Delhi India

ORCiDs of the authors:

Heena Rasool Mir - https://orcid.org/0000-0003-0408-227X

Shiv Kumar Yadav - https://orcid.org/0000-0003-3586-8128

Sangita Yadav - https://orcid.org/0000-0002-3791-7424

Mir HR, Yadav SK, Yadav S. Hydropriming associated physiological and biochemical changes responsible for the enhanced planting value of maize hybrid and its parental line seeds. Turkish Journal of Agriculture and Forestry. doi: 10.3906/tar-2006-77

Abstract: Hydropriming has the potential to ameliorate the stress-induced ill effects on yields of the crop plants and quality of seed produced. In the present study, half of the fresh seeds of maize hybrid, PEHM 5 and its parental lines (Female – CM 150, Male - CM 151) were subjected to accelerated aging test for bringing down the viability and vigour, thereby creating low vigour lots while high vigour lots consisted of fresh seeds which were hydroprimed (30 h at 25°C). Half of the hydroprimed seeds were surface dried while the other half were redried back to the original moisture contents. All the seed lots after treatments were evaluated for performance of physiological and biochemical parameters viz.; germination test, vigour index, electrical conductivity of seed leachates and antioxidant enzyme activities. Results divulged significant improvement of germination and the seedling growth parameters in both the treatments; surface dried and redried after hydropriming over untreated seeds. Significant improvement was observed in root and shoot lengths, dry weight of seedlings and vigour indices of surface dried maize seeds and this treatment also resulted in earlier and synchronized germination. A steady increase was also noted in the activities of four key antioxidant enzyme systems; peroxidase (POX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in both high and low vigour seed lots of hybrid and its parental lines upon hydropriming irrespective of surface dried or redried strategies. It is therefore suggested that hydro priming could improve the seed vigour by protecting the seed from oxidative damage by reactive oxygen species by the increasing activities of antioxidant enzymes. The surface dried seed showed all the benefits of seed priming in terms of seed quality parameters and enhanced antioxidant defense mechanism as compared to redried seeds. Therefore, surface drying could be promulgated as an effective and simple technique to mitigate the ill effects while sowing maize seeds under unfavorable growing conditions.

Keywords: Antioxidant enzymes, hydration, maize, MGT, priming, redried, ROS, surface dried, viability, vigour

1. Introduction

Maize (*Zea mays* L.) is an important global food and feed crop. India is the 5th largest corn producer and has the 4th largest acreage in the world. It is grown in many states in India and in 2018–19, was planted over 9.47 million hectares with a production of 28.72 million tonnes. The increase in productivity from 547kg/ha in 1950 to 3032 kg/ha of maize has been largely due to the introduction of single-cross hybrids (DES, 2018), despite the climatic changes causing serious reductions in yield and seed quality. Though, hybrid maize has the advantage of high yielding potential, but the productivity of the crop in India is still far below the international average. Good quality seed alone can ensure a minimum of 10-12% higher yield. However, seed with low viability and vigour resulting in poor and delayed germination is one of the factors resulting in lower yields of maize (Mondo et al., 2013). Sub-optimal plant population in maize fields, especially in tropical and subtropical areas is mainly attributed to use of lower physiological quality maize seeds (Narayanan et al., 2019). On the other hand, high vigour seeds may withstand environmental adversities by rapid and uniform germination after sowing (Finch-Savage and Bassel, 2015).

Any technology that enhances germination and emergence is therefore important in mitigating deleterious effects of the poor crop establishment. Seed priming is one such technology which was developed to enhance germination characteristics of seeds under stress conditions. Hydropriming is a low-cost and simple approach where the pre-germination metabolic activities start with imbibition of water and seeds complete the first phase and not the latter two phases of germination (Ali and Elozeiri, 2017). The process of hydropriming is complete when seeds are redried back to the original moisture and subsequent re-hydration upon sowing typically resulted in the more rapid and uniform

emergence, particularly under unfavourable environmental conditions. An analysis of 20 years experiments on 'on-farm' seed priming revealed significant effects on performance of crops that resulted in 22% faster emergence, up to 11% increase in final emergence and 21% higher yields than the conventional methods of sowing (Carrillo-Reche et al., 2018). Seed priming of pepper (*Capsicum annuum* L.) conducted under temperature stress (low 15°C and high 35°C) for two consecutive runs revealed enhanced germination even in stressful conditions. However, it was noticed that priming decreased sucrose content, whereas the fatty acid composition remained unchanged. In both the runs, increased enzymatic activities were observed and most importantly activity of catalase which was enhanced significantly (Kaya et al., 2010).

Responses to environmental stress vary significantly amongst the plant species and genotypes within a crop species. Higher levels of antioxidants, constitutive or stimulated, reported to have superior resistance to oxidative damage in plants (Siringam et al., 2011). Priming was found to enhance repair of membranes, the activities of hydrolytic enzymes and antioxidant system (Wang et al., 2003). Enhanced planting value of primed seeds has been attributed to possibly many changes at physiological, biochemical and molecular levels. Chilling tolerance in maize was found to be improved with KCl priming because of activation of antioxidants and other metabolic indicators of stress like; soluble sugars and α-amylase activity (Farooq et al., 2008).

Even though, the numerous works done previously point towards the beneficial effects on seed vigour linked with pre-sowing enhancement treatments specifically drying followed by soaking. To know if the effect of priming can be realised only by surface drying the maize seed which could be a practical proposition for the marginal farmers, we compared surface dried seeds with seeds that were dried back to original moisture content after imbibition. Additionally, the reasons to fill the gap in understanding about the augmented accomplishments of primed seeds were first time explored in the maize hybrid

and its parental lines. The information that was lacking on comparative analysis of enhanced physiological and biochemical processes especially between seeds of maize hybrids and their male and female parents was to be generated. This study was conducted with an ultimate objective to come up with an appropriate technique to improve the planting value of seeds of parental lines as well as hybrid in maize and elucidate the reasons for enhanced performance.

2. Materials and methods

2.1. Experimental details

To evaluate the effect of hydropriming on two seed lots with difference in viability or vigour , studies were carried out at the laboratories of Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi, India. Genetically pure and fresh seeds of maize hybrid, PEHM-5 and its parental lines (Female, CM-150 and Male, CM-151) were obtained from Sampoorna Seeds and Seed Production Unit, ICAR-IARI, New Delhi, respectively. Before starting the experiment, the moisture content of all the genotypes was assessed using standard hot air oven method (ISTA, 2013). For initiating the experimentation, total seeds of all the genotypes were divided in two equal lots; the first lot (Lot I) was of aged seed whose germination was brought down after subjecting seeds to accelerated ageing test (AAT) and the other lot (Lot II) comprised of the fresh seeds of high vigour . The AAT was conducted by placing seeds packed in perforated butter paper bags over the wire mess in a desiccation glass jar that contained water to maintain 98±2% relative humidity. The desiccation glass jar was then kept in an incubator at maintained 40±1°C. Observations on percent germination of seeds were recorded till it fell to ≤80%; afterwards the seeds were fan redried in shade to bring to their initial moisture content levels. The initial status of seed quality parameters and viability after accelerated test of different genotypes used for this study has been given (Table 1).

Both fresh and aged seed lots of maize hybrid, PEHM 5 and its parental lines, Female – CM 150 and Male - CM 151 were subjected to the following treatments *viz*.; (T_0) unsoaked seed (control), (T_1) Hydropriming with distilled water for 30 h at 25°C followed by surface drying only (seeds surface dried with blotting papers and used immediately) and (T_2) Hydropriming with distilled water for 30 h at 25°C and dried back to the original moisture content (the seeds were removed from the distilled water and redried under the shade at room temperature at 25 ±2°C). All the lots, thus, obtained were assessed for physiological and biochemical seed quality parameters.

2.2. Seed Germination (%)

Standard method (ISTA, 2013), with minor modifications was adapted to determine the seed germination by placing 50 seeds of each genotype in 3 replications. The seeds were rolled between two layers of moist paper towel and placed in the walk-in-germinator maintained at 25°C. The first and final counts were taken on the 4th day and 7th day, respectively. Germination percentage was calculated based on the number of normal seedlings on the day of final count. Normal seedlings were those that showed the potential for continued development into satisfactory plants when grown in good quality soil and under favourable conditions of moisture, temperature and light. The intact seedlings, seedlings with slight defects and even seedlings with secondary infection were counted towards the normal ones.

Seeds that were put for germination were counted daily till 7th of putting for calculation of mean germination time (MGT). The formula ($n \times \frac{d}{N}$) was used for estimating the MGT as suggested (Ellis and Roberts, 1980). In formula, 'n, d and N' represents the number of seeds germinated daily, days since the test began and seeds germinated in total at the end of test, respectively.

2.3. Vigour Indices

On the day of final count for the calculation of germination percentage 10 normal seedlings from each replication were randomly picked up to measure the root and shoot lengths of seedling. The same seedlings were redried by keeping them for 17 h in oven set at 80°C to record the dry weight (after cooling in a desiccator for half an hour) of seedlings. The mean values were used for computing the vigour indices by using the following formula (Abdul-baki and Anderson, 1973):

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

Vigour Index II = Germination (%) × Dry weight of seedlings (gm)

2.4. Electrical Conductivity of Seed Leachate

Three replicates of fifty seeds each were soaked in 250 ml of distilled water. Each beaker was covered with aluminium foil and placed at 20 ± 2°C for 24 h (ISTA, 2013). The conductivity of the seed leachates was measured at room temperature and calculated as:

$$\text{Electrical Conductivity } (\mu Scm^{-1}g^{-1}) = \frac{\text{Conductivity Reading - Background Reading}}{\text{Weight of seeds (in grams)}}$$

2.5. Enzyme Extraction and Antioxidant Enzyme Assay

For the antioxidant enzyme assays 1 g seeds were homogenized in 15 ml of potassium phosphate buffer (1:5 W/V) (pH 7.0) with 1% Polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000 x *g* for 30 min at 4°C. The supernatant was collected and used for enzyme assays.

2.5.1. Superoxide dismutase (SOD) (EC 1.15.1.1)

Superoxide dismutase activity was measured as per the previously reported procedure (Beauchamp and Fridovich, 1971) with minor modifications. 1 ml of 100 mM potassium phosphate buffer (pH 7.8); 100 µl of 2.25 mM nitroblue tetrazolium (NBT); 100 µl of 3 mM Ethylene Diamine Tetraacetic Acid (EDTA); 200 µl of 200 mM L-methionine and 1.75 ml distilled water; 200 µl enzyme extract and 150 µl 0.075 mM riboflavin were mixed in test tubes and shaken properly. Glass test tubes containing the reaction mixture were then

placed at a distance of 30 cm below two 15W fluorescent lamps for 15 min. switching off the light stopped the reaction and the tubes were immediately covered with a black cloth. A non-irradiated reaction mixture containing enzyme extract, which does not develop color, was used as blank. Control was lacking enzyme in the reaction mixture and developed maximum color. 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 recorded in units/gram of seed fresh weight.

2.5.2. Catalase (CAT) (EC 1.11.1.6)

Catalase activity was measured at 25°C was assayed using previous report of (Aebi, 1984) with some minor modifications as mentioned further. The ultra violet (UV) light absorbance of hydrogen peroxide solution can be measured between 230 and 250nm. 3 ml of reaction mixture contained 50 µl enzyme extract, 1.5 ml of 100 mM Phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2 and 950 µl of distilled water. Control contained enzyme extract and phosphate buffer devoid of H_2O_2. Catalase activity was estimated by the decrease in absorbance of H_2O_2 (ε = 39.4 mM^{-1} cm^{1}) at 240 nm and was recorded in µmol H_2O_2 decomposed/min/gram of seed fresh weight.

2.5.3. Glutathione reductase (GR) (EC 1.6.4.2)

Glutathione reductase activity was assayed at 25°C using previous report of (Mavis and Stellwagen, 1968), by following the rate of NADPH oxidation at 340 nm. 1.5 ml of 100 mM potassium phosphate buffer (pH 7.6) prepared with 3.4 mM EDTA; 0.10 ml of 30 mM glutathione substrate solution (GSSG) prepared in deionised water; 0.35 ml of 0.8 mM β nicotinamide adenine dinucleotide phosphate prepared in 5 ml cold water; 0.30 ml of 1.0% (W/V) bovine serum albumin (BSA) prepared in 100 ml 100 mM potassium phosphate buffer (pH 7.6) and 0.65 ml of deionised water was mixed in a cuvette by inversion and incubated at 25°C. The thermostat spectrophotometer was monitored at 340 nm until constant. To this, 0.1 ml of enzyme extract or 1.0% (W/V) BSA (in blank) was added. The

contents were mixed by inversion and the decrease was recorded at 340 nm for approximately five minutes. The maximum linear rate (ΔA 340 nm/minute) for both the test and blank was calculated. GR activity was expressed as µmoles NADPH oxidized/min/gram of seed fresh weight.

2.5.4. Peroxidase (POX) (EC 1.11.1.7)

Peroxidase (POX), activity was assayed as per the previously reported procedure (Castillo et al., 1994) with minor modifications. 3 ml reaction mixture contained one ml of 100 mM phosphate buffer (pH 7.0), 0.5 ml each of 96 mM guaiacol and 12 mm H_2O_2, 50 µl of enzyme extract and 950 µl of distilled water. Changes in absorbance due to the formation of tetra-guaiacol was recorded at 470 nm after every 60s for 5 minutes and enzyme activity was calculated as per extinction coefficient of its oxidation product, tetra-guaiacol ε = 26.6 nM/cm. Enzyme activity was expressed as µmoles/cm/min/gram fresh weight.

2.6. Statistical analyses

The data recorded in 3 replications for each treatment combination were analyzed for LSD and linear regression using SPSS 10.0 software for windows. The values in percentage were converted to arc sine values using percentage transformation for analysis of variance.

3. Results and discussion

Both the priming, seeds soaked in distilled water for 30 h at 25°C, techniques; surface dried (T_1) and redried (T_2) revealed remarkable effectiveness over un-primed/control (T_0) seeds in improving the planting value physiologically as well as the activities of the enzymes studied. Moreover, both T_1 and T_2 treated seeds significantly influenced the seed quality parameters and biochemical aspects of both high and low vigour seed lots of maize hybrid, PEHM-5 and its parental lines (Female, CM-150; Male, CM-151).

3.1. Seed Germination (%) and Mean Germination Time (MGT)

Significant increase in germination of surface dried (T_1) and redried (T_2) seeds of CM 150 (I) and (II) by 5.2% and 3.9%; 11.7% and 9.4%; that of CM 151 (I) and (II) by 7.0% and 5.6%; 7.5% and 8.7% whereas in PEHM 5 (I) and (II) by 5.2% and 3.7%; 3.4% and 2.3% respectively, in comparison with un-primed (T_0) seeds was noticed. The interaction effects for germination were found to be non-significant (Table 2). The higher germination after priming might be attributed to the onset of early metabolic events during hydration leading the seed physiological state to the brink of radicle protrusion (Hussain et al. 2016). Such physiological advancements of primed seeds were retained even after surface drying of seeds resulted in faster germination upon rehydration. Rapid germination of hydro-primed seeds for T_1 and T_2 strategies appeared to be related to prior and uniform germination and subsequent growth of seedlings.

Both the treatments; T_1 (surface redried) and T_2 (redried) significantly reduced the MGT as compared with T_0 (untreated) seeds (Figure 1). The MGT was found to be significantly reduced to 2.8 d in T_1 seeds from 4.9 d, as was recorded in T_0 seeds.

Significantly higher MGT was noticed in T_2 seeds (3.3 d) than the T_1 seeds. Among all the genotypes, the male line (CM 151) took significantly longer time (4.5 d) than the hybrid (PEHM 5) as well as the female line (CM 150). The faster germination of treated seeds for both the strategies appeared to be related to earlier and uniform germination and subsequent seedlings growth. Seed priming in onion also resulted in increased germination rate, enhanced enzymatic activities, reduced MGT and non significant effect on the soluble protein content (Sivritepe and Demirkaya, 2012).

3.2. Seedling length, Dry weight and Vigour Indices

The vigour indices are manifestation of germination, seedling length and dry weight. The increase in vigour indices paralleled that of lengths of seedlings and dry weight. Increase in vigour index I ranged from 12.6% to 23.4% in surface dried (T_1) treatment and 8.2% to 17.9% in redried (T_2) treatment; and 23.8% to 26.9% in T_1 treatment and 13.9% to 15.0% in T_2 treatment of low and high vigour seed lots, respectively. Significant interactions effects of genotypes with lots and lots with treatments on vigour indices were observed. Significantly higher vigour index I was noticed in T_1 seeds (3907.2) of the high vigour lots of maize hybrid and the T_0 seeds of low vigour lots of male line (CM-151) recorded significantly lower (1438.1) vigour index I (Table 3). Similarly, the increase in vigour index II ranged from 9.4% to 22.4% in T_1 treatment and 8.5% to 41.5% in T_2 treatment; and 6.5% to 15.1% in T_1 treatment and 5.2% to 31.1% in T_2 treatment in low and high vigour seeds, respectively. Significantly higher vigour index II was noticed in T_1 seeds (59.77) of the high vigour lots of maize hybrid and T_0 seeds of low vigour lots of male line (CM-151) recorded significantly lower (20.57) vigour index II (Table 4). Effect of seed priming treatments was found more pronounced under suboptimal (15°C and 15/25°C) on maize seed and seedlings vigour traits than at optimal temperature conditions (Čanak et al., 2016).

Significantly higher seedling length (32.56 cm) was recorded in surface redried (T_1) than the redried (T_2) seeds (30.84 cm). Seedling from untreated seed (T_0) recorded significantly lower (28.57 cm) length than T_1 and T_2 treatments. Significantly higher seedling length (40.70 cm) was observed in T_1 seeds of high vigour lots of PEHM 5 followed by non-significant decreased seedling length (38.02 cm) in seeds of same lot of same genotypes (Table 5). Significantly higher dry weight (0.491 gm) was recorded in T_1 seeds than the T_2 seeds (0.475 gm).Seedling from untreated seed (T_0) recorded significantly lower (0.437 gm) dry weight than both the treatments. Significantly higher dry weight (0.623 gm) was observed in T_1 seeds of high vigour lots of PEHM 5 followed by non-significant decreased seedling dry weight (0.612 gm) in seeds of same lot of same genotypes (Table 6). In context of this study, hydropriming resulted in an early start of germination that might have been induced by the seedling (shoot + root lengths in cm) enlargements and uniform emergence as evidenced by improvement in dry matter (seedling dry weight in gm) production as expressed in both the vigour indices (Table 3 and 4). These findings support the prior work in maize (Sathish et al., 2012) and rice (Farooq et al., 2006) who explained superior rate and percentage of seed germination because of hydro-priming. Increased germination percentage, seedling growth and dry weight appeared to be related to the proficient mobilization and exploitation of seed reserves, thereby guiding to an early start of germination events (Basra et al., 2005).

3.3. Electrical Conductivity of Seed Leachate

The electrical conductivity (EC) of leachates in hydro-primed and surface dried (T_1) seeds was significantly higher while hydro-primed and redried (T_2) seed leachates of all genotypes showed lower EC than the unprimed (T_0) dry seeds in both high and low vigour lot seeds of all genotypes during imbibition. Electrical conductivity revealed significant interaction effects of genotypes with lots and genotypes with treatments; however, EC resulted in non-significant interactions of lots with treatments and with all three factors

combine. Significantly higher EC (0.071 µS/cm/g of seed) was detected in T_1 seeds of the low vigour lots of male line (CM-151) and T_2 seeds evidenced significantly lower EC (0.020 µS/cm/g of seed) in high vigour lots of maize hybrid. Moreover, the conductivity of low vigour seed lots was significantly higher than high vigour seed lots in all the genotypes (Table 7). The seed should imbibe water to start the germination process. Imbibition by the dry seed is rapid and could damage the internal cell membrane structure and contributes to substantial cytoplasmic leakage upon hydration (Ishibashi et al., 2013). Thus, rapid water inflow may result in imbibitional injuries of the seed membrane which are reflected in EC measurement of solute leakages. Imbibitional injuries could be slowed down by priming by avoiding the membrane phase transition, resulting in flexibility of plasma membrane and thus, the expanding protoplast could be accommodated without damage (Buitink et al., 2001). Moreover, the hydropriming might have allowed completion of cellular repair processes in the maize seeds. Therefore, significantly decreased conductivity of seed leachates was observed in T_2 seeds as compared to T_1 seeds and T_0 seeds. The age dependent increase in malondialdehyde (MDA) content, EC and mean germination time (MGT) was recorded that were negatively correlated with loss of viability in pepper seeds (Demirkaya, 2013). Significantly reduced leakage of electrolytes in the T_2 seeds was noticed that ranged from 0.026 µS/cm/g seed to 0.041 µS/cm/g seed in low vigour seed lots whereas it ranges from 0.020 µS/cm/g of seed to 0.031 µS/cm/g of seed in case of high vigour lot seeds. EC is considered as an effective indicator of seed quality in sweet corn (Fessel et al., 2006).

The regression analysis of EC with vigour index I (Germination % X Seedling length) and vigour index II (Germination % X Seedling dry weight) was also carried out. The R^2 revealed 34% variation in vigour index I and 25% variation in vigour index II that could be explained by EC value (Figure 1), which is quite understandable as seed vigour indices are complex physiological trait involving many biochemical and physiological processes. The overall conductivity of the male line CM 151 was higher for control

followed by female line CM 150 while hybrid PEHM 5 seeds showed the lowest level of conductivity. Poor quality seeds have a membrane which could be deprived of structures that allow the obvious diffusion of ions during imbibition. Measurement of EC for detecting the electrolyte present in seed steep water was successfully used to evaluate seed vigour and field emergence potential of soybean seed lots (Vieira et al., 2004). It was also reported that the priming improved germination might be due to enhanced repair of membranes (Chiu et al., 2006). Numerous changes in metabolic processes occur due to priming which could possibly contribute to the seed quality enhancement and subsequent performance (Varier et al., 2010). Priming could help membranes to repair and return to their normal arrangement that might be disrupted during seed maturation drying. Therefore, it reduced imbibitional leakage in primed and redried seeds. Predictably, membrane re-configuration would lead to re-activation of enzymes associated with membrane and consequently result in the seed enhancement.

3.4. Antioxidant enzymes

The variations resulted in physiological attributes of seedlings in both the strategies (T_1 and T_2) could be traced back to biochemical changes occurred during priming of seeds. The improvement in germination and vigour of primed maize seeds is ascribed to a range of changes like, control of lipid peroxidation and increased activity of enzymes (Girolamo and Barbanti, 2012). 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 four key antioxidant enzyme systems; superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in both high and low vigour seed lots of hybrid and its parental lines upon hydro-priming, irrespective of surface dried (T_1) or redried (T_2) strategies.

3.4.1. Superoxide dismutase (SOD)

The SOD activity of hydro-primed seeds (T_1 and T_2) was significantly higher than that from unprimed (T_0) seeds. In low vigour seed lots SOD activity ranged from 3.47 unit/g seed/ min to 5.93 unit/g seed/ min in T_2 strategy whereas for T_1 it ranged from 4.05 unit/g seed/ min to 7.24 unit/g seed/ min. In case of high vigour seed lots SOD activity ranged from 6.09 unit/g seed/ min to 7.74 unit/g seed/ min in T_2 strategy whereas for T_1 it ranged from 6.87 unit/g seed/ min to 8.97 unit/g seed/ min (Table 8). Superoxide dismutase activity divulged significant interactions effects of genotypes with lots and genotypes with treatments; whereas, rest of interactions were found to be non-significant. Significantly higher SOD activity was detected in T_1 seeds of the high vigour lots in maize hybrid and T_0 seeds evidenced significantly lower SOD activity (2.98 unit/g seed/min) in low vigour lots of female line of maize hybrid, PEHM 5.

3.4.2. Catalase (CAT)

Catalase (CAT) activity of T_1 seeds was significantly higher than that of T_0 seeds. In low vigour seed lots CAT activity ranged from 17.37 µmole/min/g seed to 24.21 µmole/min/g seed in T_2 strategy whereas for T_1 it ranged from 30.0 µmole/min/g seed to 37.37 µmole/min/g seed. In case of high vigour seed lots, CAT activity ranged from 26.84 µmole/min/g seed to 31.58 µmole/min/g seed in primed redried (T_2) strategy whereas for primed and surface dried (T_1) it ranged from 36.84 µmole/min/g seed to 48.95 µmole/min/g seed (Table 9). Catalase activity gave away significant effects of all two factors; genotypes and lots, whereas non-significant interactions were evidenced in all of the factor combinations. Numerically highest CAT activity (48.95µmol/min/g seed) was perceived in T_1 seeds of the high vigour lots in female line (CM-150) and T_0 seeds in low vigour lots of male line (CM-151) divulged lowest CAT activity (8.94 µmol/min/g seed).

3.4.3. Glutathione reductase (GR)

GR also has an important role in scavenging the free radicals produced in primed and germinating seeds. GR activity disclosed significant effects of all three factors and all

interactions. GR activity was recorded highest in high vigour T_1 treated seeds of female line CM-150 *i.e.* 15.45 NADH µmole/min/g fresh seed weight over T_0 *i.e.* 5.85 NADH µmole/min/g fresh seed weight. GR activity like other antioxidant enzymes, stayed higher in primed seeds compared to T_0 seeds. In low vigour seed lots GR activity ranged from 7.54 NADH µmole/min/g fresh seed weight to 11.97 NADH µmole/min/g fresh seed weight in T_2 strategy whereas for T_1 it ranged from 8.98 NADH µmole/min/g fresh seed weight to 13.99 NADH µmole/min/g fresh seed weight. In case of high vigour seed lots GR activity ranged from 10.82 NADH µmole/min/g fresh seed weight to 14.6 NADH µmole/min/g fresh seed weight in T_2 strategy whereas for T_1 it ranged from 13.81 NADH µmole/min/g fresh seed weight to 17.97 NADH µmole/min/g fresh seed weight (Table 10). Significantly higher GR activity was detected in T_1 of the high vigour seed lots of maize hybrid (PEHM 5) and T_0 evidenced significantly lower GR activity (3.73 NADH µmol/min/g of seed fresh weight) in low vigour seed lots of female line.

3.4.4. Peroxidase (POX)

Significant effects on peroxidase activity were revealed in genotypes and lots but not in treatments. Effects of all the interactions also significantly affected the peroxidase activity. Primed and surface dried (T_1) seeds noticed significantly higher POX activity in both the vigour lots of all 3 genotypes. Among all the genotypes and vigour lots, the low vigour lot of PEHM-5 showed highest percent increase in POX activity over control (T_0). It was 24.34 µmoles/cm/min/g seed and 14.07 µmoles/cm/min/g seed in T_1 and T_2 seeds, respectively (Table 11). Significantly higher POX activity was detected in T_1 seeds (25.84 µmol/cm/min/g seed) of the high vigour seed lots of the hybrid and T_0 seeds divulge significantly lower POX activity (9.39 µmol/cm/min/g seed) in low vigour seed lots of female line of PEHM 5. It could be related to the positive effect of priming on enhancement of viability through the elimination process of hydrogen peroxide (H_2O_2). For effective control of seed deterioration removal of H_2O_2 is must and the antioxidant enzymes, catalase

(CAT) and peroxidase (POD) can scavenge oxo intermediates and free radicals (Jaleel et al., 2009; Bailly et al. 2000). Hydration repairs the damage done by lipid peroxidation via assembly of antioxidants and repair enzymes. Biswas et al. (2020) revealed that the membrane repair could be credited to evoked actions of enzymes that are scavenging free radicals.

Priming is believed to the stress for seeds and results in the production of ROS. Hydropriming could also induce oxidative stress and generate free radical-scavenging enzyme like catalase (CAT) thus minimize the cell damage. Superoxide radicals that emerge as a result of stress in the plant tissues are transformed into hydrogen peroxide (H_2O_2) by the SOD enzyme (Dixit et al., 2001; Mittiova et al., 2002). The hydrogen peroxide is then effectively neutralised by POX and CAT. Protection against naturally occurring lipid peroxidation by the increased activities of these enzymes during seed priming have been noticed that might protect cell membranes in various crops (Bailly et al., 2000; Swami et al., 2016; Hussain et al., 2017). The observations reiterate that metabolic activities in the phase II of imbibition are promoted in the germinating seed by priming. Significant genotypic differences were perceived for all the studied parameters. But irrespective of genotypes, overall enhancement of germination and vigour of hydro-primed seeds (T_1 and T_2) was better in high vigour lots than low vigour lots, whereas the augmentation of antioxidant enzymes trend was reverse and was high in low vigour lots. Antioxidant enzymes; POX, CAT and SOD were found positively correlated with loss of seed viability of pepper seeds (Demirkaya, 2013). The activity of most of the antioxidant enzymes under stress conditions was observed to be enhanced (Jaleel et al., 2009).

4. Conclusion

The genotypes, lots, treatments and their interactions; especially genotypes with lots and genotype with treatments had a bearing on the performance of maize seeds. The physiological effects of hydropriming as pre-treatment on the improvement of seed planting

value during germination were associated with the significantly lower level of seed leachates and higher seed vigour indexes. Seed priming triggers significantly increased SOD, POD, and CAT activities during the seedling stage of the plants thus increase the antioxidant capability of seeds under unfavorable conditions. In conclusion, hydropriming with distilled water for 30 h at 25°C of maize seeds proved to be an effective treatment for seed invigour ation of both high and low vigour seeds, while the surface drying (T_1) proved to be more effective than re-drying (T_2). Although both strategies of hydropriming were effective, the former was more efficient and can be preferable to attain better planting value of maize hybrid and its parental lines. It is believed that hydropriming lessens the storability therefore; T_2 is advocated for prolonged storage, if required. However, hydropriming is a pre-sowing treatment and primed seeds need to be sown immediately after the treatment. Therefore, in the long run it could not only save the labour cost but also decrease the energy requirements thus could help in reducing carbon footprints on earth.

Acknowledgement

The authors are thankful to the Indian Council of Agricultural Research, New Delhi for providing required funds for smooth conduct of research work and Division of Seed Science and Technology, ICAR- Indian Agricultural Research Institute, New Delhi for providing the necessary facilities for the studies.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Abdul-Baki AA, Anderson JD (1973). Vigour determination in soybean seed by multiple 4 criteria. Crop Science 13 (6): 630-633.

Aebi H (1984). Catalase in vitro. Methods in Enzymology 105: 121-126.

Ali A, Elozeiri AA (2017). Metabolic processes during seed germination. In: Jimenez-Lopez JC (ed.) Agricultural and Biological Sciences: Advances in Seed Biology 2 (1): 21–29.

Bailly C, Benamar A, Corbineau F, Come D (2000). Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Science Research 10: 35–42.

Basra SMA, Farooq M and Tabassum R (2005). Physiological and biochemical aspects of seed vigour enhancement treatments in fine rice (*Oryza sativa* L.). Seed Science and Technology, 33: 623-628.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gel. Annals of Biochemistry, 44: 276-287.

Biswas N, Yadav S, Yadav SK, Choudhary R, Saini N et al. (2020). Vigour difference during storage and germination in Indian mustard explained by reactive oxygen species and antioxidant enzymes. Turkish Journal and Agriculture and Forestry 44: 577-588. doi:10.3906/tar-1909-48

Čanak P, Mirosavljević M, Ćirić M, Košelj J, Vujošević B et al. (2016). Effect of seed priming on seed vigour and early seedling growth in maize under optimal and suboptimal temperature conditions. SELEKCIJA I SEMENARSTVO 22: 17-24. 10.5937/SelSem1601017C

Carrillo-Reche J, Vallejo-Marín M, Quilliam RS (2018). Quantifying the potential of 'on-farm' seed priming to increase crop performance in developing countries- A meta-

analysis. Agronomy for Sustainable Development 38 (64): 1-14. https://doi.org/10.1007/s13593-018-0536-0

Castillo MP, Stenström J, Ander P (1994). Determination of Manganese Peroxidase activity with 3-Methyl-2-benzothiazolinone Hydrazone and 3-(Dimethylamino) benzoic acid. Annals of Biochemistry, 218: 399-404.

Chiu KY, Chuang SJ, Sung JM (2006). Both anti-oxidation and lipid-carbohydrate conversion enhancements are involved in priming-improved emergence of *Echinacea purpurea* seeds that differ in size. Scientia Horticulturae 108: 220-226.

Demirkaya M (2013). Relationships between antioxidant enzymes and physiological variations occur during ageing of pepper seeds. Horticulture, Environment and Biotechnology 54 (2): 97-102. 10.1007/s13580-013-0187-7

DES (2018). Agricultural Statistics at a Glance. Directorate of Economics and Statistics, Department of Agriculture Cooperation and Farmers' Welfare, Ministry of Agriculture and Farmers' Welfare, Government of India, New Delhi. Source: http://agricoop.gov.in/sites/ default/files/agristatglance2018.pdf.

Dixit V, Pandey V, Shyam R (2001). Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). The Journal of Experimental Biology 52: 1101–1109.

Ellis RH, Roberts EH (1980). Improved Equations for the Prediction of Seed Longevity. Annals of Botany 45 (1): 13-30.

Farooq M, Aziz T, Cheema ZA, Hussain M, Khaliq A (2008). Activation of Antioxidant System by KCl Improves the Chilling Tolerance in Hybrid Maize. Journal of Agronomy and Crop Science 194 (6): 438-448. https://doi.org/10.1111/j.1439-037X.2008.00334.x

Farooq M, Basra SMA, Khalid M, Tabassum R, Mehmood T (2006). Nutrient Homeostasis, Reserves Metabolism and Seedling Vigour as Affected by Seed Priming in Coarse rice. Canadian Journal of Botany 84: 1196–1202.

Fessel SA, Vieira RD, da Cruz MCP, de Paula RC, Panobianco M (2006). Electrical conductivity testing of corn seeds as influenced by temperature and period of storage. Pesquisa Agropecuária Brasileira 41 (10): 1551-1559. 10.1590/S0100-204X2006001000013

Finch-Savage WE, Bassel GW (2015). Seed vigour and crop establishment: extending performance beyond adaptation. Journal of Experimental Biology 67 (3): 567-591.

Girolamo G, Barbanti L (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. Italian Journal of Agronomy 7: 178-188.

Hoekstra FA, Golovina E, Buitink J (2001). Mechanisms of desiccation tolerance. Trends in Plant Science 6 (9): 431-438. 10.1016/S1360-1385(01)02052-0

Hussain M, Farooq M, Lee DJ (2017). Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice. Journal of Agronomy and Crop Science, 203: 269–276.

Hussain S, Khan F, Hussain HA, Nie L (2016). Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Frontier Plant Science 7 (116):116.

Ishibashi Y, Koda Y, Zheng SH, Yuasa T, Iwaya-Inoue M (2013). Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. Annals of Botany 111 (1):95-102. 10.1093/aob/mcs240.

ISTA (2013). International Rules for Seed Testing. Proceeding, International Seed Testing Association-ISTA, Zurich, Switzerland.

Jaleel CA, Riadh K, Gopi R, Manivannan P, Inès J et al. (2009). Antioxidant defense responses: Physiological plasticity in higher plants under abiotic constraints. Acta Physiologiae Plantarum 31 (3): 427–436.

Kaya G, Demir I, Tekin A, Yaşar F, Demir K (2010). Effect of priming treatment on germination at stressful temperatures, fatty acid, sugar content and enzymatic activity of pepper seeds. Journal of Agricultural Sciences 16 (1): 9-16.

Mavis RD, Stellwagen E (1968). Purification and Subunit Structure of Glutathione Reductase from Bakers' Yeast. The Journal of Biological Chemistry 243 (4):809-14.

Mittiova V, Tal M, Volokita M, Guy M (2002). Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Physiologia Plantarum 115 (3): 393–400.

Mondo VHV, Cicero SM, Dourado-Neto D, Pupim TL, Dias MAN (2013). Effect of Seed Vigour on Intraspecific Competition and Grain Yield in Maize. Agronomy Journal 105 (1): 222-228.

Narayanan GS, Suvarna G, Baradhan G, Kumar SB (2019). Standardization of chemical seed priming treatment to improve seed quality in maize cv. CO 1. Plant Archives 19 (1): 372-376.

Sathish S, Sundareswaran S, Senthil N, Ganesan KN (2012). Biochemical changes due to seed priming in maize hybrid [COH (M) 5]. Research Journal of Seed Science 5: 71-83.

Siringam K, Juntawong N, Cha Um S, Kirdmanee C (2011). Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. indica) roots under iso-osmotic conditions. African Journal of Biotechnology 10: 1340–1346.

Sivritepe ÖH, Demirkaya M (2012). Does humidification technique accomplish physiological enhancement better than priming in onion seeds? Acta Horticulturae 960: 237-244.

Swami S, Yadav S, Yadav Shiv K, Dahuja A, Yadava DK (2016). Imbibition behaviour and germination response in conventional and quality of Indian mustard (*Brassica juncea*) seeds. Indian journal of Agricultural Science 86 (12): 1625–1629.

Varier A, Vari AK, Dadlani M (2010). The sub-cellular basis of seed priming. Current Science 99 (4): 450-456. https://www.jstor.org/stable/24109568

Vieira RD, Neto AS, de Bittencourt SRM, Panobianco M (2004). Electrical conductivity of the seed soaking solution and soybean seedling emergence. Scientia Agricola (Piracicaba, Braz.) 61 (2): 164-168. http://dx.doi.org/10.1590/S0103-90162004000200007

Wang XB, Cai D, Zhang S (2003). Effects of some water retention materials as soil conditioners on improving emergence of grass seedlings under dry and saline soil conditions. Plant Nutrition and Fertilizer Science 9 (4): 462-466.

Table 1. Initial status of seed quality parameters of different genotypes

Genotype	100 seed weight (gm)	Moisture (%)	Germination (%)	
			Before AAT	After AAT
CM-150 (Female)	17.46	10.4	85.0	76.0
CM-151(Male)	18.35	10.2	80.0	71.0
PEHM-5 (Hybrid)	17.84	10.2	92.0	80.5
Mean	17.88	10.3	85.7	75.8
SEm (±)	0.26	0.07	3.48	2.74

Table 2. Effect of different hydropriming treatment strategies on germination (%) of low (I) and high (II) vigour seed lots of maize hybrid, PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	76.0	80.0	79.0	78.3
		(60.7)*	(63.5)	(62.8)	(62.3)
	(II)	85.0	95.0	93.0	91.0
		(67.2)	(77.1)	(74.7)	(72.6)
Mean		80.5	87.5	86.0	84.7
		(63.8)	(69.3)	(68.1)	(67.0)[a]
CM 151	(I)	71.0	76.0	75.0	74.0
		(57.4)	(60.7)	(60.0)	(59.4)
	(II)	80.0	86.0	87.0	84.3
		(63.5)	(68.1)	(68.9)	(66.7)
Mean		75.5	81.0	81.0	79.2
		(60.4)	(64.2)	(64.2)	(62.9)[b]
PEHM 5	(I)	81.0	84.0	84.0	83.0
		(64.2)	(66.5)	(66.5)	(65.7)
	(II)	92.0	96.0	95.0	94.3
		(73.6)	(78.5)	(77.1)	(76.3)
Mean		86.5	90.0	89.5	88.7
		(68.5)	(71.6)	(71.1)	(70.4)[c]
Mean (Genotypes)		81.8	86.2	85.5	
		(64.1)#[a]	(68.2)[b]	(67.7)[b]	
Mean (Lots)		(I): 78.4 (62.4)[a]		(II): 89.9 (71.5)[b]	

C.D. (p=0.05)		Genotype (A)	2.12
Lot (B)	1.73	Treatment (C)	2.13
Interaction (A X B)	NS	Interaction (A X C)	NS
Interaction (B X C)	NS	Interaction (A X B X C)	NS

*Figures in parentheses are transformed values

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 3. Effect of different hydropriming treatment strategies on vigour index I of low (I) and high (II) vigour seed lots of maize hybrid, PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	2146.7	2419.2	2323.1	2296.4
	(II)	2809.0	3552.9	3230.8	3197.6
Mean		2477.9	2986.1	2777.0	2747.0[a]
CM 151	(I)	1438.1	1775.5	1644.9	1619.5
	(II)	2704.3	3431.9	3101.1	3079.1
Mean		2071.2	2603.7	2373.0	2349.3[b]
PEHM 5	(I)	1639.1	1994.2	1932.8	1855.3
	(II)	3155.4	3907.2	3597.1	3553.2
Mean		2397.2	2950.7	2764.9	2704.3[a]
Mean (Genotypes)		2315.4#[a]	2846.8[b]	2638.3[c]	
Mean (Lots)		(I): 1923.7[a]		(II): 3276.6[b]	

C.D. (0.05)		Genotype (A)	111.6
Lot (B)	91.2	Treatment (C)	111.7
Interaction (A X B)	157.9	Interaction (A X C)	NS
Interaction (B X C)	157.9	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 4. Effect of different hydropriming treatment strategies on vigour index II of low (I) and high (II) vigour seed lots of maize hybrid, PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	32.17	36.41	35.37	35.72
	(II)	39.26	55.59	51.49	48.78
Mean		35.71	46.00	43.44	41.72[a]
CM 151	(I)	20.57	25.19	23.67	23.15
	(II)	40.52	50.61	49.03	46.71
Mean		30.55	37.90	34.93	34.46[b]
PEHM 5	(I)	27.78	30.41	29.61	29.26
	(II)	55.04	59.77	57.95	57.59
Mean		41.41	45.09	43.43	43.31[a]
Mean (Genotypes)		35.89#[a]	43.00[b]	41.19[b]	
Mean (Lots)		(I): 29.02[a]		(II): 51.03[b]	

C.D. (p=0.05)		Genotype (A)	1.87
Lot (B)	1.53	Treatment (C)	1.87
Interaction (A X B)	2.65	Interaction (A X C)	NS
Interaction (B X C)	2.65	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 5. Effect of different hydropriming treatment strategies on seedling length (cm) of low (I) and high (II) vigour lots of maize hybrid PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	28.27	30.37	29.50	29.38
	(II)	32.91	37.40	34.61	34.97
Mean		30.59	33.88	32.05	32.18[a]
CM 151	(I)	20.20	23.39	22.03	21.87
	(II)	33.80	39.74	35.76	36.43
Mean		27.00	31.57	28.89	29.15[b]
PEHM 5	(I)	20.13	23.74	23.01	22.29
	(II)	34.25	40.70	38.02	37.66
Mean		27.19	32.56	30.51	29.98[b]
Mean (Genotypes)		28.57#[a]	32.56[b]	30.84[c]	
Mean (Lots)		(I): 24.51[a]		(II): 36.35[b]	

C.D. (p=0.05)		Genotype (A)	1.01
Lot (B)	0.82	Treatment (C)	1.01
Interaction (A X B)	1.41	Interaction (A X C)	NS
Interaction (B X C)	1.41	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 6. Effect of different hydropriming treatment strategies on seedling dry weight (gm) of low (I) and high (II) vigour lots of maize hybrid PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	0.421	0.457	0.450	0.443
	(II)	0.460	0.585	0.551	0.532
Mean		0.441	0.521	0.500	0.488[a]
CM 151	(I)	0.291	0.332	0.317	0.313
	(II)	0.506	0.586	0.565	0.553
Mean		0.399	0.459	0.441	0.433[b]
PEHM 5	(I)	0.342	0.362	0.352	0.352
	(II)	0.598	0.623	0.612	0.611
Mean		0.470	0.492	0.482	0.482[c]
Mean (Genotypes)		0.437#[a]	0.491[b]	0.475[c]	
Mean (Lots)		(I): 0.369[a]		(II): 0565[b]	

C.D. (p=0.05)		Genotype (A)	0.025
Lot (B)	0.021	Treatment (C)	0.021
Interaction (A X B)	0.036	Interaction (A X C)	NS
Interaction (B X C)	0.029	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 7. Effect of different hydropriming treatment strategies on electrical conductivity (µS/cm/g of seed) of low (I) and high (II) vigour seed lots of maize hybrid, PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	0.042	0.055	0.026	0.041
	(II)	0.036	0.049	0.031	0.039
Mean		0.039	0.052	0.029	0.041[a]
CM 151	(I)	0.056	0.071	0.041	0.056
	(II)	0.037	0.063	0.024	0.041
Mean		0.047	0.067	0.032	0.049[b]
PEHM 5	(I)	0.035	0.060	0.032	0.042
	(II)	0.025	0.055	0.020	0.033
Mean		0.030	0.057	0.026	0.038[a]
Mean (Genotypes)		0.039#[a]	0.059[b]	0.029[c]	
Mean (Lots)		(I): 0.046[a]		(II): 0.038[a]	

C.D. (p=0.05)		Genotype (A)	0.004
Lot (B)	0.003	Treatment (C)	0.004
Interaction (A X B)	0.005	Interaction (A X C)	0.006
Interaction (B X C)	NS	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 8. Changes in superoxide dismutase activity (unit/g seed/min) in response to hydropriming (surface dried and redried) treatment strategies of low (I) and high (II) vigour seed lots of maize hybrid, PEHM-5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	2.98	4.05	3.47	3.49
	(II)	5.72	6.87	6.09	6.23
Mean		4.35	5.46	4.78	4.86[a]
CM 151	(I)	3.14	5.71	4.48	4.44
	(II)	5.56	8.18	6.91	6.88
Mean		4.35	6.95	5.69	5.66[b]
PEHM 5	(I)	3.38	7.24	5.93	5.52
	(II)	5.58	8.97	7.74	7.43
Mean		4.48	8.11	6.84	6.48[c]
Mean (Genotypes)		4.39#[a]	6.84[b]	5.77[c]	
Mean (Lots)		(I): 4.49[a]		(II): 6.85[b]	

C.D. (p=0.05)		Genotype (A)	0.14
Lot (B)	0.11	Treatment (C)	0.14
Interaction (A X B)	0.20	Interaction (A X C)	0.24
Interaction (B X C)	NS	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 9. Changes in catalase activity (µmol/min/g seed) in response to hydropriming (surface dried and redried) treatment strategies of low (I) and high (II) vigour seed lots of maize hybrid, PEHM 5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	9.97	34.21	21.05	21.75
	(II)	14.21	48.95	29.47	30.88
Mean		12.10	41.58	25.26	26.31[a]
CM 151	(I)	8.94	30.00	17.37	18.77
	(II)	14.95	36.84	26.84	26.21
Mean		11.95	33.42	22.11	22.49[b]
PEHM 5	(I)	14.21	37.37	24.21	25.26
	(II)	20.00	47.89	31.58	33.16
Mean		17.11	42.63	27.89	29.21[c]
Mean (Genotypes)		13.72#[a]	39.21[a]	25.09[a]	
Mean (Lots)		(I): 21.93 [a]		(II): 30.08[b]	

C.D. (p=0.05)		Genotype (A)	3.18
Lot (B)	2.60	Treatment (C)	NS
Interaction (A X B)	4.51	Interaction (A X C)	NS
Interaction (B X C)	NS	Interaction (A X B X C)	NS

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 10. Changes in Glutathione reductase activity (NADH µmol/min/g of seed fresh weight) in response to hydropriming (surface dried and redried) treatment strategies of low (I) and high (II) vigour seed lots of maize hybrid, PEHM 5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	3.73	8.98	7.54	6.75
	(II)	5.85	15.45	13.52	11.60
Mean		4.79	12.22	10.53	9.18[a]
CM 151	(I)	4.81	11.15	8.74	8.24
	(II)	6.55	13.81	10.82	10.39
Mean		5.68	12.48	9.78	9.31[a]
PEHM 5	(I)	5.95	13.99	11.97	10.64
	(II)	8.31	17.97	14.60	13.63
Mean		7.13	15.99	13.29	12.14[b]
Mean (Genotypes)		5.86#[a]	13.56[b]	11.19[c]	
Mean (Lots)		(I): 8.54[a]		(II): 11.88[b]	

C.D. (p=0.05)		Genotype (A)	0.54
Lot (B)	0.44	Treatment (C)	0.54
Interaction (A X B)	0.77	Interaction (A X C)	0.94
Interaction (B X C)	0.77	Interaction (A X B X C)	1.33

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

Table 11. Changes in peroxidase activity (µmol/cm/min/g seed) in response to hydropriming (surface dried and redried) treatment strategies of low (I) and high (II) vigour seed lots of maize hybrid, PEHM 5 and its parental lines

Genotypes	Lots	Control (T_0)	Primed (T_1) (surface redried)	Primed (T_2) (redried)	Mean (Treatments)
CM 150	(I)	9.82	20.63	11.28	13.91
	(II)	12.82	19.51	15.16	15.83
Mean		11.32	20.07	13.21	14.87[a]
CM 151	(I)	8.16	18.27	11.28	12.57
	(II)	10.49	20.63	12.35	14.49
Mean		9.33	19.45	11.82	13.53[b]
PEHM 5	(I)	9.39	24.34	14.07	15.93
	(II)	12.52	25.84	16.74	18.37
Mean		10.96	25.09	15.41	17.15[c]
Mean (Genotypes)		10.54#[a]	21.54[a]	13.48[a]	
Mean (Lots)		(I): 14.14[a]		(II): 16.23[b]	

C.D. (p=0.05)		Genotype (A)	0.67
Lot (B)	0.54	Treatment (C)	NS
Interaction (A X B)	0.94	Interaction (A X C)	1.15
Interaction (B X C)	0.94	Interaction (A X B X C)	1.63

Figures with same alphabet don't statistically differ significantly ($\alpha \leq 0.05$).

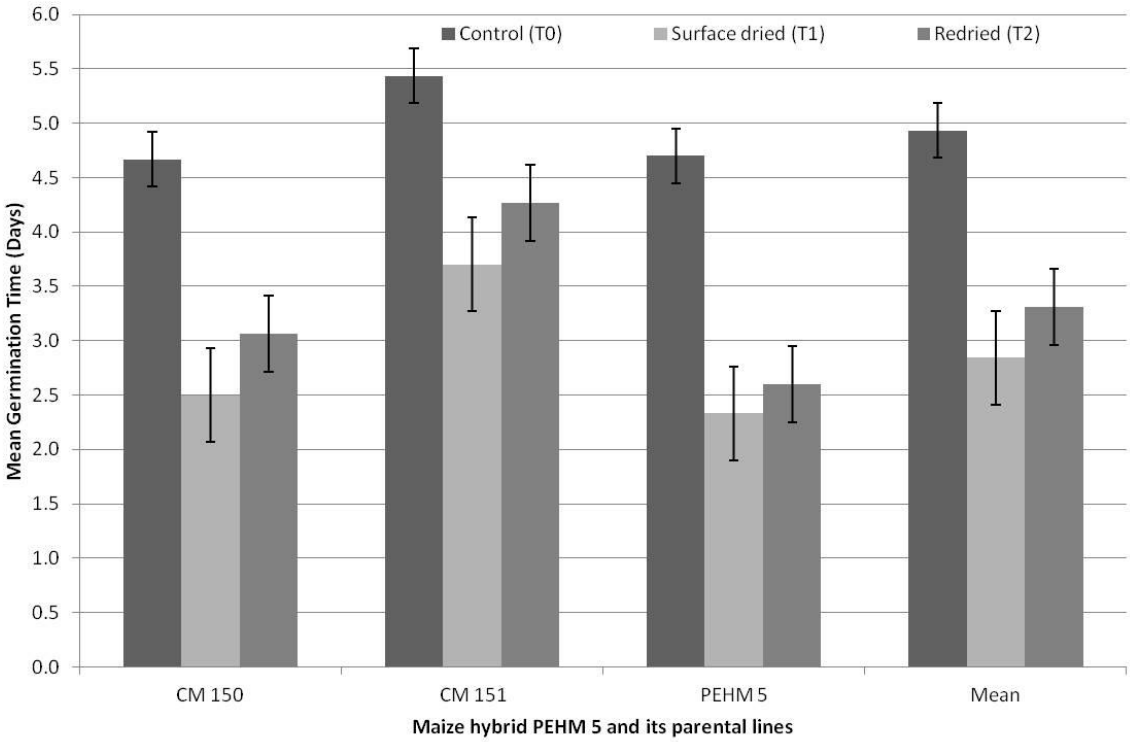

Figure 1. Effect of different hydropriming treatment strategies on mean germination time (MGT) in seeds of maize hybrid PEHM 5 and its parental lines

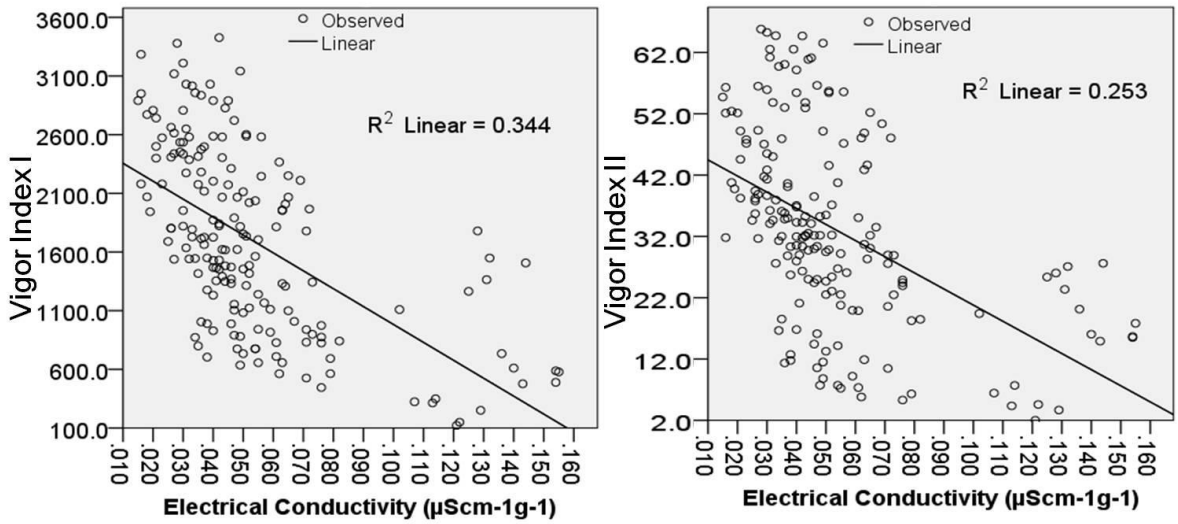

Figure 2. Linear regression analysis of EC with vigour index I and vigour index II of hydroprimed seed lots of maize hybrid, PEHM 5 and its parental lines