Forage Res., **46** (4) : pp. 337-342 (2021) **http://forageresearch.in**

PRIMING EFFECT ON ENZYME ACTIVITIES IN MAIZE HYBRID (*ZEA MAYS* L.) SEEDS

ARETH KIBARAZA[1,*], AXAY BHUKER[2], V. S. MOR[2] AND S. S. JAKAHR[2]

[1]Tanzania Official Seed Certification Institute (TOSCI)
[2]Department of Seed Science & Technology
CCS Haryana Agricultural University, Hisar-125004
**(e-mail : areth.kb@gmail.com)*

(Received : 11 January 2021; Accepted : 26 March 2021)

SUMMARY

The study was conducted to assess seed priming effect on enzyme activities of five maize hybrids of Public and Private seeds sectors *viz*., PMH-1, IIMRNH 2015-3, IIMRNH 2015-4, P3396 and DKC 9144 during 2018-19 at laboratories of Department of Seed Science & Technology, CCS, Haryana Agricultural University, Hisar. The seeds were primed with KH_2PO_4 (2.0%), KNO_3 (1.0%), GA_3 (50 ppm) and hydration with water followed by dressing with vitavax power @ 2.5g/kg seed for 17 hours at room temperature and then dried to original moisture content for 24 hours at 40°C temperature. The results revealed that all priming treatments for 17 hours reduced Dehydrogenase, Catalase, Superoxide dismutase, Peroxidase activities which indicates negative effect on seed quality of maize hybrids. Hence, priming method for maize hybrids need to be standardized. Priming with KH_2PO_4 (2.0%) and KNO_3 (1.0 %) was found most effective where improvement was more over other treatments. Maximum Dehydrogenase (0.173) and Superoxide dismutase (0.623) activity was recorded in unprimed seeds while maximum Catalase activity (0.417) was recorded in seeds primed with KH_2PO_4 (2.0%) and maximum Peroxidase activities (2.574) was recorded in seeds hydrated in tap water. Among the hybrids, DKC 9144 recorded superiority over other hybrids by registering maximum enzymic activities *viz*., Dehydrogenase activity (0.210), Superoxide dismutase (0.717), Peroxidase (0.2791) and minimum electrical conductivity (87.8), so this hybrid can be used in further breeding programmes.

Key words : Maize, Priming, Dehydrogenase, Catalase, Superoxide dismutase, Peroxidase

Maize (*Zea mays* L.) is one of the major cereal crop grown worldwide. In India, it is cultivated as a dual-purpose crop, for grain as well as fodder. Maize being a C_4 plant, it has an excellent potential and able to produce the maximum carbohydrate per day (Arya *et al*., 2020). The grains of maize are affluent in starch, protein, fat, vitamins and mineral nutrients (Arya *et al*., 2015). The production of the crop depends on the availability and supply of quality seed to the farmers, therefore maintaining of quality seed has been the basic role of seed men. Maintenance of seed quality starts as far as from when the seed reach physiological maturity on parent plant until when the seed is planted again in the next season, therefore this is the continuous process and seed deterioration occurs at any time in this period if care is not taken. Seed priming is the post harvest practice applied to ameliorate physiological quality of the seed. It is the process of controlled hydration of seeds to a level that permits pre germination metabolic activity to proceed, but prevents actual emergence of the radicle (Heydecker, 1973). Seeds are soaked in variety of solutions including inorganic salts, sugars and hormones. It improves germination and seedling establishment through pre-initiation of metabolic activities before emergence of radicle (Khan *et al.,* 2005). Harris (2001) reported that priming has a potential of giving fast germination, uniform establishment, less need to resowing and maximum utilization of direct sowing. Arya *et al*. (2014) also reviewed that seed priming increases the final germination percentage of seed in pearl millet. Canak *et al.*, 2016 reported that seeds primed in dishes using water (hydropriming) and two different concentrations of KNO_3 solution (0.1% and 0.5%) at 25° C for 17 h showed positive effect on some seed germination parameters at low and mixed temperature. Possible mechanisms encountered during priming include initiation of epigenic changes, repair of membranes due to accumulation of protein signals and activation of antioxidant enzyme activities under environmental stress (Fahim *et al*., 2019). Various studies have been carried out on seed priming and have shown positive results over non-primed seeds, though the methods are not widely used (Rasool *et*

al,. 2014; Hafeez *et al.,* 2015). The effectiveness of priming is mainly affected by aeration, duration of priming process, temperature, osmotic potential, seed quality, dehydration after priming, priming method, priming agent and addition of promotive substances (Vanangamudi *et al*., 2006). Poor membrane structure and leaky cells are usually associated with deteriorating of low vigour seed. These results in a greater loss of electrolytes such as amino acid and organic acids from imbibing seeds and increase the conductivity of the soak water. Higher soak water conductivity, therefore, may indicate a low vigour of seed lot. Measurement of Electrical conductivity (seed leachates) of seeds is a rapid, precise, inexpensive and simple procedure. However, rate of solute leakage can be affected by initial seed moisture and seed size. Additionally, treatment of seeds with antibiotics may influence conductivity measurements, necessitating their removal before determinations are made. The limitation of conductivity test is that, it represents results as an average conductivity evaluation of bulk seeds which presumes that all seeds are equally deteriorated and will provide the same quantity of electrolyte leakage. Enzyme alterations, such as reduced activity of lipase, ribonuclease, acid phosphatase, protease, diastase, catalase, peroxidase, amylase, DNase and dehydrogenase enzymes. Reactive oxygen species (ROS) and hydrogen peroxides are produced from several metabolic reactions and could be destroyed by the activity of scavenger enzymes like catalase and peroxidase (Mahjabin *at el.,* 2015). Increased lipid peroxidation and reduced antioxidant enzymes activities have been associated with poorer storage of primed seeds when compared to unprimed seeds. This evidence was reported in sweet corn (Chang and Sung, 1998). Wattanakulpakin *et al*. (2012) observed that lipid peroxidation occurred during priming treatment, particularly during the imbibition step, and caused the deterioration of primed seeds of maize. Very little information is available on biochemical study of primed seeds in maize crop. Therefore, the present study was planned to assess priming effect on enzyme activities of maize hybrids.

MATERIALS AND METHODS

The present study was conducted on freshly harvested seeds of following five maize hybrids from public and private sector at laboratories of Department of Seed Science & Technology, CCS, Haryana Agricultural University, Hisar during 2018-19:

The seeds were primed (completely soaking) with KH_2PO_4 (2.0%), KNO_3 (1.0 %), GA_3 (50 ppm) and hydration with water followed by dressing with vitavax power @ 2.5g/kg seed for 17 hours at room temperature and then dried to original moisture content for 24 hours at average temperature of 34.5°C (Day temp. 40°C and night temp. 29°C). Unprimed seeds were used as control. The primed seeds were evaluated for conductivity of leachates and various enzyme activities *viz*., Dehydrogenase, Catalase, Superoxide dismutase and Peroxidase activity. Electrical conductivity was computed according to AOSA, 1983. Fifty normal and uninjured seeds of each treatment in three replications were soaked in 75 ml of distilled water in 100 ml beakers. Seed soaked totally in water and beakers were covered with parafilm to reduce evaporation at 25°C for 24 hrs. The electrical conductivity of the seed leachates was obtained through direct reading of conductivity meter and expressed in µS/cm/seed. Dehydrogenase activity (O.D $g^{-1}ml^{-1}$) was estimated according to the procedure suggested by Kittock and Law (1968). A sample of one gram seed of each hybrid and treatment replicated three times were grounded into fine powder, then 200mg of fine powder was put into centrifuge tube and 5 ml of 0.5% tetrazolium solution was added into the centrifuge tube containing 200mg of fine powder. The mixture was incubated for 3 to 4 hours at 38°C to reduce 2, 3, 5-Triphenyl Tetrazolium chloride to red formazan by dehydrogenase enzymes in the seed embryo. Then the mixture was centrifuged at 1000 rpm for 10 min and the supernatant was discarded. 10 ml of acetone was added into the centrifuge tube to extract formazan for 16 h followed by centrifugation at 10000 rpm for 10 min. Spectrophotometer was used to determine the absorbance of the solution at 520nm, acetone was used as blank.

Name of Hybrid	Source
PMH-1	Panjab Agricultural University Ludhian
IIMRNH 2015-3	Indian Institute of Maize Research (IIMR), Ludhiana
IIMRNH 2015-4	Indian Institute of Maize Research (IIMR), Ludhiana
Dekalb/DKC 9144	Bayer Crop Science (DEKALB Hybrids)
Pioneer P3396	DuPont

Extraction of Antioxidant enzymes : A gram of seed sample was put in pre-chilled pestle and mortar and grinded into fine particles with help of abrasive grass, 5 ml of cold extraction buffer containing 0.1 M phosphate buffer (pH 7.0), 2.5 mM DDT and 1 mM EDTA was added in the pestle and mortar. Enzyme extract was put into centrifuge tube, and then the homogenate was centrifuged at 10,000 rpm for 10 min. The whole procedure of preparation of enzyme extraction was carried out at 0-4^0 C. The supernatant was used for enzymatic assay for determining the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD).

Catalase Assay: Catalase activity was computed according to the procedure given by Sinha (1972). For catalase activity measurement, in a test tube 0.55ml of 0.1M potassium phosphate buffer (pH 7.0), 0.4ml of 0.2 M H_2O_2 and 50 µl of enzyme extract were added to a tube and the reaction mixture was mixed well and incubated at 37°C for 1 min. The mixture solution without enzyme extract was used as a control. The reaction was terminated by adding 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic of ratio of 1:3 v/v. Then the test tubes were kept in water bath for 10 min and cooled thereafter. Finally the absorbance was recorded at 570 nm using dichromate acetate solution as the blank. The amount of H_2O_2 in the reaction mixture was obtained by subtracting the absorbance of test samples from control. One unit of enzyme activity was explained as the amount of enzyme which catalyzed the oxidation of 1 µ mole H_2O_2 per min.

Superoxide dismutase Assay (SOD): The reaction mixture of 3 ml contained 2.5 ml of 60 mM Tris-HCl (pH 7.8), 0.1 ml of 420 mM L-methionine, 0.1 ml of 1.8mM NBT, 0.1 ml of 90 µM riboflavin, 0.1 ml of 3.0 mM EDTA and 0.1 ml of enzyme extract were mixed in the test tube and shaken well and placed 30 cm below light source consisting of three 20 W fluorescent lamps (Philips, India) along with blank which contained buffer instead of enzyme extract. The reaction was started by switching on the light and terminated after 40 min of incubation by switching off the light. After the termination of the reaction the tubes were covered with black cloth to protect them from the light. A non-irradiated reaction mixture that did not develop colour served as the blank. The reaction mixture without enzyme extract developed maximum colour and its absorbance decreased with increasing volume of extract. The absorbance was recorded at 560 nm**.** Superoxide dismutase was assayed by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) by the method of Beauchamp and Fridovich (1971).

The enzyme activity was measured in terms of unit g^{-1} FW and percent inhibition was calculated by the formula given by Asada *et al.,* (1974).

$$\text{Percent inhibition} = \frac{V - \nu}{\nu} \times 100$$

Where,
V = Rate of assay reaction in absence of SOD
v = Rate of assay reaction in presence of SOD

One enzyme unit is defined as the amount of enzyme that inhibits the NBT photo reduction by 50 percent.

Peroxidase Assay: The Peroxidase enzyme assay was estimated according to the method of Shannon *et al*, (1966). The procedure started by putting 2.75 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5% hydrogen peroxide, 0.1 ml of 0.2% O-dianisidine dye and 0.05 ml of enzyme extract were added to a tube. The assay mixture without H_2O_2 was used as a blank. Absorbance change was followed at 430 nm for 3 min and one unit of POD was defined as the amount of enzyme required to cause O.D. change per minute. The experiments were conducted in completely randomized design (CRD) and the data obtained from experiment were analyzed as per standard method suggested by Panse and Sukhatme (1985) and using the online statistical tool (OPSTAT) by Sheoran, 2010.

RESULTS AND DISCUSSION

The results of study revealed minimum electrical conductivity was recorded in T_4 (90.9) followed by T_1 (97.5) while maximum was recorded in T_3 (123.5) followed by T_2 (118.2). Among the hybrid, DKC 9144 recorded minimum electrical conductivity (87.8) as compared to other hybrids while maximum (141.9) was recorded in P3396 (Fig 1). The decrease of the electrical conductivity is strongly associated with intact of membrane integrity that prevents linkage of solutes (Pandey *et al*., 2017) by enabling reorganization of cellular membrane rapidly and completely (McDonald, 1980). Different levels of electrical conductivity in hybrids definitely represent varying quality among the hybrids in this context; DKC

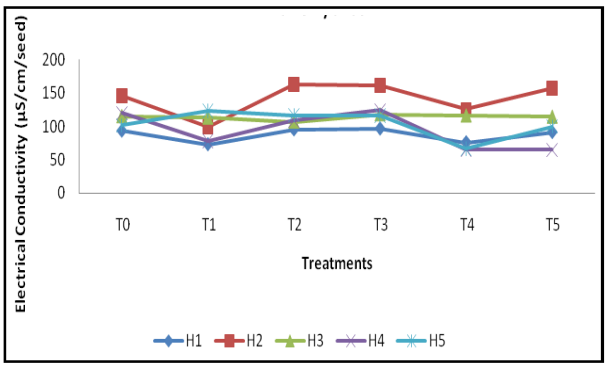

Fig. 1. Effect of treatments on Electrical conductivity of different maize hybrids.

9144 had more quality attributes than other hybrids. Increased electrical conductivity in T_3 might be associated with decrease of the membrane integrity or release of salt ions to the solution accumulated in the seed during priming.

Although priming reduced Dehydrogenase activity in all the treatments yet maximum activity was recorded in T_3 (0.172) and lowest was noted in T_5 (0.145) in all the hybrids. Among the hybrids, maximum activity was observed in DKC9144 hybrid (0.210) followed by IIMRNH-2015-4 (0.160) while minimum activity was recorded in P3396 (0.137) hybrids (Fig 2). Variation of DHA activity between hybrids is strongly linked with their viability, the more activity the higher the viability. Dehydrogenase activity is used as positive biomarker for validating seed viability (Pandey *et al.*, 2017). These results were similar to what was reported by Saha *et al.* (1990), that priming increased the activity of DHA in soybean.

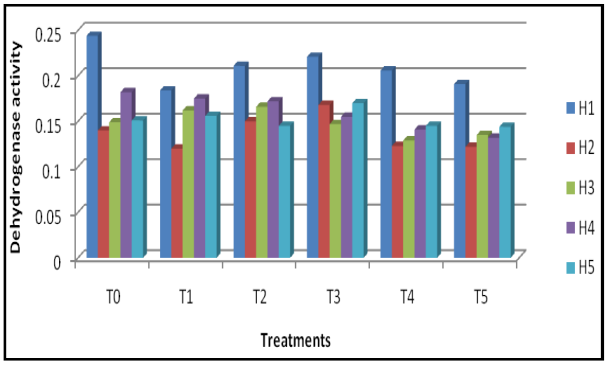

Fig. 2. Effect of treatments on Dehydrogenase activity test (O. D g/ml) of different maize hybrids.

All priming treatments enhanced Catalase activity except T_3 and T_5. Among the hybrids maximum activity was recorded in IIMRNH-2015-4 (0.445) followed by DKC 9144 (0.423) while minimum (0.355) was found in hybrid P3396 (Fig 3). Similarly, all priming treatments reduced superoxide dismutase activity in all the hybrids and maximum activity was estimated in T_0 was (0.623) which was at par with T_2 (0.611). Among the hybrids maximum was observed in DKC 9144 (0.717) followed by IIMRNH-2015-4 (0.585) while minimum (0.567) was recorded in P3396 (Fig 4). All priming treatments reduced Peroxidase activity also except T_1. It was recorded maximum in T_1 (5.574) followed by control (2.478) while minimum was estimated in T_4 (2.415). Among the hybrids, maximum peroxidase activity was recorded in DKC 9144 (2.791) while minimum (2.310) was in PMH-1 (Fig 5). Priming involves imbibition of the seed which activates metabolic activity of the seed, therefore this increases respiration activities which results to the production of reactive oxygen species (ROS). ROS especially H_2O_2 act as signalling molecules in which the seed must be empowered with ROS removing system that closely regulates its concentration (Kubala *et al.*, 2016). However the same authors demonstrated that, scavenging of ROS is enabled by antioxidant enzyme system, a driving mechanism which involves CAT and SOD and ascorbate peroxidase (APX). Kubala *et al.*, 2016 in their study considered priming process which consists of two stages that is controlled hydration and rehydration to original moisture content, changed in moisture contents, stimulated ROS production and increased activity of antioxidant enzymes such as CAT, POD, APX and SOD. In our study the repercussion of priming on activation of antioxidant enzymes differed with priming treatments and hybrids, whereby T_1 and T_2 increased the activity of CAT while all priming treatments increased SOD activity in IIMRNH-2015-3 hybrid.

Reduction in enzyme activities of primed seed might be associated with increased sensitivity of primed seeds to deteriorative factors such as temperature, oxygen and moisture content due to weakening of protective structures of the seed and reduced antioxidant enzyme activities. The effectiveness of priming is mainly affected by aeration, duration of priming process, temperature, osmotic potential, seed quality, dehydration after priming, priming method, priming agent and addition of promotive substances (Vanangamudi *et al.*, 2010).

It is concluded from the study that priming of fresh maize hybrid seeds for 17h reduces the enzyme activity of the seed and hence priming of maize should be standardized. Among the hybrids, DKC 9144 recorded superiority over other hybrids by registering maximum enzymic activities in seeds which can be

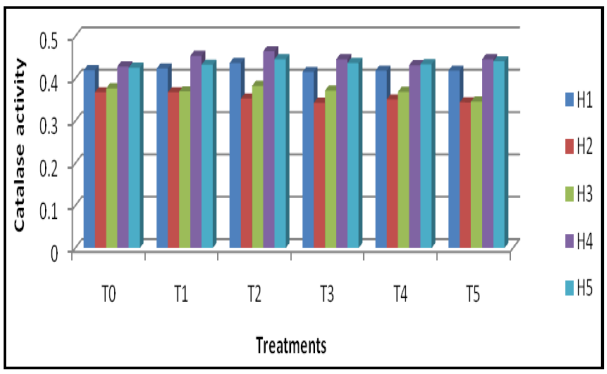

Fig. 3. Effect of treatments on Catalase (mg protein/min) of different maize hybrids.

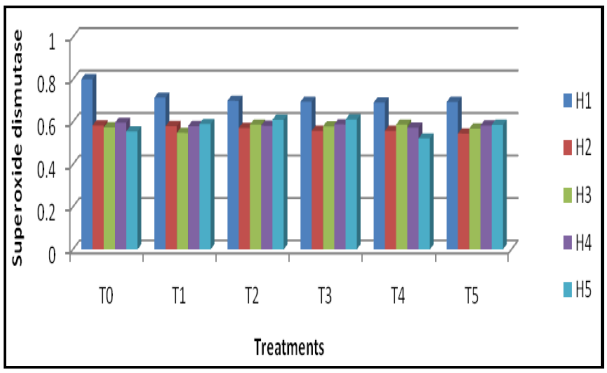

Fig. 4. Effect of treatments on Superoxide dismutase (mg protein/min) of different maize hybrids.

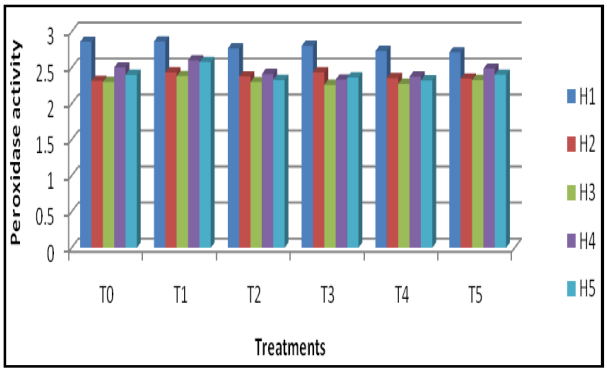

Fig. 5. Effect of treatments on Peroxidase (mg protein/min) of different maize hybrids

used in further breeding programmes. So that, the priming responsive varieties/ hybrids may be developed for commercial utilization to take the advantage of seed priming.

T_0- Control, T_1-Hydration in tap water, T_2-Hydration with KH_2PO_4 (2.0%), T_3-Hydration with KNO_3 (1.0 %), T4- Hydration with GA_3 (50 ppm), T_5-Hydration and treatment with vitavax (2.5g/kg seed). H_1- DKC 9144, H_2-P3396, H_3-PMH-1, H_4-IIMRNH-2015-4, H_5-IIMRNH-3

REFERENCES

AOSA, 1983 : Seed vigor testing handbook. East Lasing: Association of Official Seed Analysts (AOSA), 93p.

Arya R.K., M.C. Kamboj and S. Kumar, 2015: Performance of medium maturing maize hybrids under Haryana agro-climatic conditions. *Forage Res*., **41**: 130-34.

Arya R.K., M.C. Kamboj and S. Kumar, 2020: Evaluation of new extra-early maturing hybrids of maize (Zea mays L.) for grain yield and its contributing traits under humid and semi-arid conditions of Haryana. *Ekin J*., **6**(2): 23-27.

Arya, R. K., M. K. Singh, A. K. Yadav, A. Kumar and S. Kumar, 2014: Advances in pearl millet to mitigate adverse environment conditions emerged due to global warming. *Forage Res*., **40** (2): 57-70.

Asada, K., K. Kiso and K. Yoshikawa, 1974: Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. *J. Biol. Chem*. **249**: 2175–2181

Beauchamp, C. and I. Fridovich, 1971: Superoxide dismutase: improved assay and an assay applicable to acrylamide gels. *Anal Biochem*; **44** : 276-287. doi: 10.1016/0003-2697(71)90370-8.

Canak, P., M. Mirosavljević, M. Cirić, B. Vujošević, J. Kešelj, D. Stanisavljević and B. Mitrović, 2016: Seed priming as a method for improving maize seed germination parameters at low temperatures. *Ratarstvo i povrtarstvo*, 53(3), 106-110. doi:10.5937/ratpov53-10825.

Chang, S.M., and J.M. Sung, 1998: Deteriorative changes in primed sweet corn seeds during storage. *Seed Sci. Technol.*, **26**:613-626.

Fahim, N., M. Naeem, A. Akram, M.Y. Ashraf, K.S. Ahmad and B. Zulfiqar, 2019: Seed priming with KNO3 mediates biochemical processes to inhibit lead (Pb) toxicity in maize (*Zea mays* L.). *Journal of Science of Food and Agriculture,* **97**(14): 4780-4789.

Hafeez, U.R., I. Hasan, S.M.A. Basra and I. Afzal, 2015: Seed priming improves early seedling vigor, growth and productivity of spring maize. *Journal of Integrative Agriculture,* **14**(9): 1745–1754.

Harris, D., P. A. K Pathan, A. Gothkar, C. W. Joshi and P. Nyamudeza, 2001: On-farm seed priming: using participatory methods to revive and refine a key technology. *Agricultural Systems,* **69** : 151-64.

Heydecker, W., 1973: Glossary of terms. In "Seed Ecology". Ed W. Heydecker. Proceedings 19th

Easter School in Agricultural Science, University of Nottingham. Butterworths, London. 578 pp.

Heydecker, W., J. Higgiris and R.L. Gullivar 1973: Accelerated germination by osmotic seed treatment. *Nature,* **246** : 42-44.

Khan, M.M, M. J Iqbal and M. Abbas, 2005: Loss of viability correlates with membrane damage in aged turnip *(Brassica rapa*) seeds. *Seed Sci. & Technol*.**, 33** (2):517-520.

Kittock, D.L and A.G. Law, 1968: Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agron. J.,* **60** : 286-288.

Kubala, S., L. Wojtyla, K. Lechowska and M. Garnczarska, 2016: Different Modes of Hydrogen Peroxide Action During Seed Germination. *Frontiers in Plant Science,* **7** : 1-16.

Mahjabin, S. Bilal and A. B. Abidi, 2015: Physiological And Biochemical Changes During Seed Deterioration: A Review. *International Journal of Recent Scientific Research;* **6**(4):3416-3422.

McDonald, Jr. M.B., 1980: Assessment of seed quality. *Hort. Sci.*, **15**: 784-8.

Pandey, P., Bhanuprakash and K. Umesha, 2017 : Effect of Seed Priming on Biochemical Changes in Fresh and Aged Seeds of Cucumber. *Journal of Agricultural Studies*: **5**(3) : 62-73.

Panse, V. G. and P. V. Sukhatme, 1985 : Statistical methods for agricultural workers, 4th Ed., ICAR, New Delhi.

Rasool, H.M., S. Basu, M. Dadlan, S. Nagarajan and P. Aggrawal, 2014 : Bio-physical and physiological changes associated with seed enhancement treatments in specialty maize (*Zea mays*). *Indian Journal of Agricultural Sciences,* **84** (7):867–72.

Saha, R., A.K. Mandal and R. N. Basu, 1990 : Physiology of seed invigoration treatments in soybean (*Glycine max* L.). *Seed Sci. & Technol.*, **18**: 269-276.

Shannon, L. M., E. Key and J. Y. Law, 1966 : Peroxidase isoenzymes from horse radish roots : Isolation and physical properties. *J. Biol. Chem*., **241**: 2166-2172.

Sheoran, O. P. 2010 : Online statistical analysis (OPSTAT) software developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. http://www.hau.ernet.in/opstat.html.

Sinha, A. K. 1972 : Colorimetric assay of catalase. *Analytical Biochemistry,* **47**(2): 389-394.

Vanangamudi, K., S. Kalaivani, M. Vanangamudi, Djanaguiraman, K. Raja and T. Saravanan, 2006 : Seed Physiology. pp 201-213, Associated Publishing Company, New Delhi.

Wattanakulpakin, P., S. Photchanachai, S. Miyagawa and K. Ratanakhanokchai, 2012 : Loss of Maize Seed Vigor as Affected by Biochemical Changes during Hydropriming. *Crop Science*, **52**(6): 2783-2793.