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Biochemical Changes Due to Seed Priming in Maize Hybrid COH(M) 5

[1]S. Sathish, [2]S. Sundareswaran, [3]N. Senthil and [2]K.N. Ganesan
[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[2]Seed Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[3]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author: S. Sathish, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

ABSTRACT

Nowadays seed priming was proved to be an effective seed invigouration treatment. However, firm evidence on the molecular basis underlying priming treatments is either sparse or lacking. The objective of the present study was to ascertain the changes in seed protein profile viz., total, soluble and heat stable protein due to priming and the changes in DNA, phytate and minerals viz., B, Cu, Mg, Mn, Fe, Zn and K content immediately after priming and on subsequent germination of seeds of maize hybrid COH(M) 5. Seeds of maize hybrid were primed with water and 1% KH_2PO_4 for 6 h and the result revealed that seed priming with 1% KH_2PO_4 for 6 h can undoubtedly increase germination potential and vigour of maize hybrid COH(M) 5 and attributed reason was, increase in new protein synthesis, advancement of DNA replication and cell division, absorption of K ions due to priming with potassium salt and rapid utilization of food reserves like phytate, minerals viz., B, Cu, Mg, Mn, Fe Zn, K and imbibed K ions. The result of decrease in phytate content due to priming can be used in poultry sector to reduce the phytate content in poultry feed. However, further research is required to identify the specific proteins synthesised and characterization of molecular marker to standardise priming treatment for each batch of seed lots of maize hybrid COH(M) 5.

Key words: Seed priming, KH_2PO_4, protein profile, phytate, minerals content, biochemical changes, maize hybrid COH(M) 5

INTRODUCTION

Maize (*Zea mays* L.) is the third important cereal next to rice and wheat, in the world as well as in India. In any variety or hybrid, higher production and productivity is possible only through use of good quality seeds and proper management practices. Good quality seeds imply vigour, uniformity and structural soundness besides its genetic and physical purity. Thorough study of available literature suggests seed priming as an effective seed invigouration treatment, to increase the rate and uniformity of emergence and crop establishments in most agricultural crops (Khalil *et al*., 2001; Murungu *et al*., 2004; Ismail *et al*., 2005; Rashid *et al*., 2006; Windauer *et al*., 2007; Adebisi *et al*., 2011; El-Saidy *et al*., 2011; Sathish *et al*., 2011; Patade *et al*., 2011).

A major problem encountered with the commercial application of seed priming is the variability among species, varieties and even seed lots, especially when treating large quantities of seeds (Bradford, 1986). Since batches of seeds are intrinsically heterogeneous with respect to germination rate, the specific treatment conditions must be optimized for each new seed lot which is tedious and can only yield a posteriori indications on the priming conditions. Earlier investigations suggest characterization of molecular marker that is easy to detect and monitor priming treatment would allow standardization of priming treatment for each batch of seed lots (Job *et al*., 1997; Chareyre *et al*., 1998; Carpon *et al*., 2000). However, for effective use of biochemical and molecular tools related to seed quality and optimization of priming techniques for several species, interactive mechanism on cellular and molecular events that takes place during priming and on subsequent germination need to be understood. It has been demonstrated that the onset of germination is associated with a rapid resumption of RNA and protein biosynthesis while DNA replication is often delayed for several hours, possibly to allow repair processes which might be operative during the first few hours of germination to be completed prior to resumption of growth (Blower *et al*., 1980; Osborne, 1983).

Maize seeds are rich in phytate (Raboy, 1997; Lott *et al*., 2000) and minerals such as K, B, Cu, Mg, Mn, Fe and Zn (FAO, 1992). Phytate is the main storage form of phosphorous and inositol in seeds where it frequently accounts for 1% or more of the dry weight and 50-80% of the total phosphorous (Lott *et al*., 2000). Phytates are regarded as important for non-germinating seeds in storage and homeostasis of both phosphorous and other mineral elements until needed for germination (Raboy, 1997). Reduction in reserve materials due to soaking and germination was reported in several crop species (Gupta and Sehgal, 1991; Alonso *et al*., 2000; Ejigui *et al*., 2005). However, firm evidence concerning these suggestions on the molecular basis underlying priming treatments is either sparse or lacking. With this background present study was carried out with the objective to assess changes in protein profile of three different proteins viz., total protein, soluble protein and heat stable protein due to priming and changes in DNA, phytate and mineral content due to priming and on subsequent germination of maize hybrid COH(M) 5 seeds.

MATERIALS AND METHODS

Seed material: Genetically pure fresh seeds of hybrid maize COH(M) 5 were obtained from Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore formed the base material for this study. The priming treatments were conducted at the Department of Seed Science and Technology and the biochemical studies were carried out at the Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2007-2009.

Seed priming: Seeds of maize hybrid COH(M) 5 were primed with 1% KH_2PO_4 for 6 h as standardised by Sathish and Sundareswaran (2010). Seeds were also primed with water for 6 hr for comparison in order to identify absolute biochemical changes due to priming with 1% KH_2PO_4 and unprimed seeds were used as control. At the end of priming duration, seeds were removed, rinsed in distilled water, shade dried at room temperature and subjected to germination test for evaluation of following seedling quality parameters.

Germination: Germination test was conducted with four replicates of hundred seeds each using sterilised sand medium in the germination room maintained at 25±2°C and 95±2% RH. The

germination percentage was calculated based on the normal seedlings evaluated on 7th day and it expressed in percentage (International Seed Testing Association, 1999).

Shoot length: All the normal seedlings were selected and length of the shoot was measured from the collar region to tip of the primary leaf and the mean value was expressed in cm seedling^{-1}.

Root length: From the above seedlings, the length of the root was measured from the collar to tip and the mean value was expressed in cm seedling^{-1}.

Vigour index: Vigour index value was computed by multiplying germination of seeds in percentage and total seedling length in centimetre as described by Abdul-Baki and Anderson, (1973) and expressed as whole number.

Biochemical analysis: Changes in protein profile of three different proteins viz., total protein, soluble protein and heat stable protein were assessed immediately after priming along with control and to ascertain the changes in content of DNA, phytate, minerals such as boron (B), copper (Cu), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn) and potassium (K) due to priming and on subsequent germination, primed and unprimed seeds were subjected to germination by using between paper method. Three replicates of 25 seeds each were germinated in an ambient room condition. The germinating seeds were taken randomly at 0, 6, 12, 24 and 48 h of germination from each treatment in required quantities and used for the analysis as follows. Primed and unprimed seeds taken at zero hour of germination represent ungerminated seeds or seeds taken immediately after priming.

Protein profile analysis: Protein profile analysis was done according to the procedure outlined by Job *et al.* (1997). Seeds from each treatment were ground separately with bio-homogenizer and a quantity of 4.5 g of seed powder was taken in a 40 mL centrifuge tube and 12 mL of extraction buffer A, consisting of 50 mM HEPES pH 8.0 and 1 mM EDTA, were added and stirred at 5°C for 15 min and was then centrifuged at 10000 rpm for 10 min at 5°C and an aliquot of 8 mL of the supernatant was withdrawn and subjected to a second, clarifying centrifugation at 10000 rpm for 20 min at 5°C. This fraction corresponded to the soluble protein extracts. Heat stable protein fraction was obtained by incubating the aliquot of soluble protein extract at 90°C for 10 min followed by chilling on ice and then centrifugation (12,000 rpm, 15 min, 5°C). For the extraction of total proteins, SDS 2% (v/v) was added to the remaining suspension in buffer A, following 15 min of incubation at 5°C and this suspension was centrifuged as described for soluble protein extracts and the aliquots were kept at -20°C. The protein concentrations in the various extracts were measured according to the method outlined by Bradford (1976). Bovine serum albumin was used as a standard and the colour intensity was read at 595 nm.

SDS-PAGE of the protein extracts was carried out using Hoefer SE600 Ruby standard vertical electrophoresis unit. Samples were mixed with the load buffer containing 10 mM tris-HCl, pH 7.8, 1 mM EDTA, 2.5% (v/v) SDS, 50 mM dithiothreitol (DTT) and 0.01% (w/v) bromophenol blue and heated to 100°C for 5 min and then loaded on to gel (15% homogeneous polyacrylamide gels). Electrophoresis was conducted at a constant voltage intensity of 100 V until the bromophenol blue dye reaches the bottom. Proteins were revealed by staining with Coomassie blue R250.

DNA content: DNA was extracted by following the protocol described by Gawel and Jarret (1991). The DNA quantity was determined using the nanodrop spectrophotometer with one µL of DNA sample and expressed in µg g^{-1} of sample.

Phytate content: phytate content was estimated calorimetrically as suggested by Wheeler and Ferrel (1971) and expressed in g per 100 g of sample.

Minerals content: Minerals content were estimated by following the method described by McQuaker *et al.* (1979). Finely ground seed samples weighing 0.5 g was digested in 15 mL of triple acid containing concentrated nitric acid, sulphuric acid and perchloric acid in the ratio of 9:2:1 using kel plus infra digestion system (Model: KES 12IL) at low temperature initially and later increased to 300°C till the solution turns colourless. To the digested solution 100 mL of double distilled water was added and filtered through Whatman's filter paper number 40 to obtain a clear colourless solution and minerals content were quantified using inductively coupled plasma spectrometer (ICP) and iTEVA software and the minerals content were expressed in ppm. The ICP multi-element standard solution VIII (24 elements in dilute nitric acid) obtained from Merck chemicals, Germany was used as standard.

Statistical analysis: All the experiments were conducted using completely randomized block design and the data obtained for different parameters were analysed by the 'F' test of significance following the methods described by Panse and Sukhatme (1985). The Critical Differences (CD) were calculated at 5% probability level. If the F test is non-significant it was indicated by the letters NS.

RESULTS

Seedling quality parameters: All the seedling quality parameters were significantly influenced by priming treatment. Seeds primed with 1% KH_2PO_4 for 6 h recorded the maximum germination (98%), shoot length (23.45 cm), root length (26.09 cm) and vigour index (4839) followed by seeds primed with water for 6 h while unprimed seeds recorded the lowest value for all the parameters (Table 1).

Protein profile: There was no change in the protein profile of the maize seeds due to priming. However, quantitative increase in the intensity was observed for all the three protein profile. Protein bands of seeds primed with water and 1% KH_2PO_4 were higher in all the three types of protein viz., total protein, soluble protein and heat stable protein when compared with corresponding protein bands of extracts from unprimed seeds. Specifically, the intensity of protein bands at molecular weight of 20.1, 26.7 and 50.6-kDa in total and soluble protein profile (lane 2, 3, 5 and 6 in Fig. 1) and 66-kDa in heat stable protein profile (lane 8 and 9 in Fig. 1) of water and

Table 1: Effect of seed priming on seedling quality parameters of maize hybrid COH(M) 5

Priming treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
Unprimed control	92 (73.57)	18.23	20.55	3569
Water for 6 h	94 (75.82)	20.32	23.04	4123
1% KH_2PO_4 for 6 h	98 (81.87)	23.45	26.09	4839
Mean	95 (77.08)	20.67	23.35	4177
SEd	0.817	00.982	00.973	5.564
CD at 5%	1.998**	02.404**	02.380**	13.616**

Values in parenthesis are arc sine values, ** Significant at 1% level

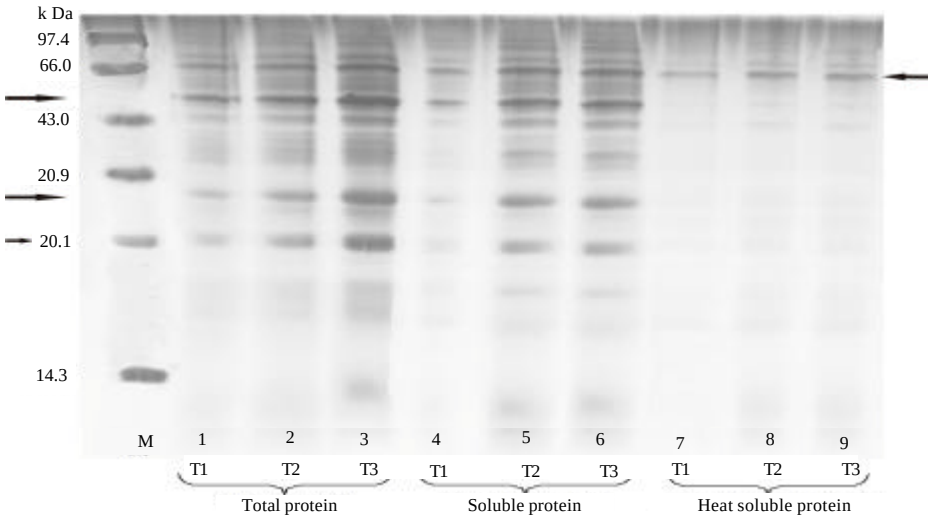

Fig. 1: Changes in protein profile due to seed priming. M: Protein marker, T1: Control, T2: Seeds primed with water for 6 h and T3: Seeds primed with 1% KH_2PO_4 for 6 h. Molecular weight at which increase in intensity of protein bands observed were indicated using arrow

1% KH_2PO_4 primed seeds were much higher than the corresponding protein bands of unprimed seeds.

DNA content: There was no significant difference in DNA content among the treatments immediately after priming. However, significant difference was observed during course of germination and the DNA content of water and 1% KH_2PO_4 primed seeds were on par with each other throughout the course of germination. At 6 h of germination, there was significant increase in DNA content of seeds primed with water (66.3 μg) and 1% KH_2PO_4 (66.8 μg) when compared with unprimed seeds (53.9 μg). Interestingly, at 12 h of germination, nearly two fold increase in DNA content was found in both the seeds primed with water (124.2 μg) and 1% KH_2PO_4 (125.3 μg) than unprimed seeds (65.1 μg). Increase in DNA content was also found at 48 h of germination in seeds primed with water (259.1 μg) and 1% KH_2PO_4 (259.5 μg) over control (249.0 μg). But the rate of increase in DNA content of primed seeds at 48 hr was not much higher than the increase at 12 h of germination (Fig. 2).

Phytate content: Immediately after priming there was a significant reduction in the phytate content of seeds primed with water (1.02 g) and 1% KH_2PO_4 (1.02 g) when compared with unprimed seeds (1.12 g). However, phytate content of water and 1% KH_2PO_4 primed seeds were on par with each other during priming and on subsequent germination. During subsequent germination, there was a rapid and significant decline in the phytate content in seeds primed with water and 1% KH_2PO_4 than unprimed seeds. At 24 h of germination, three fourth of the phytate content was reduced in seeds primed with water (0.28 g) and 1% KH_2PO_4 (0.26 g); whereas only half of the phytate content was reduced in unprimed seeds (0.55 g). After 48 hr of germination, 90 and 91% of phytate was reduced in seeds primed with water (0.11 g) and 1% KH_2PO_4 (0.10 g), respectively whereas only 76% of phytate content was reduced in unprimed seeds (0.27 g) (Fig. 3).

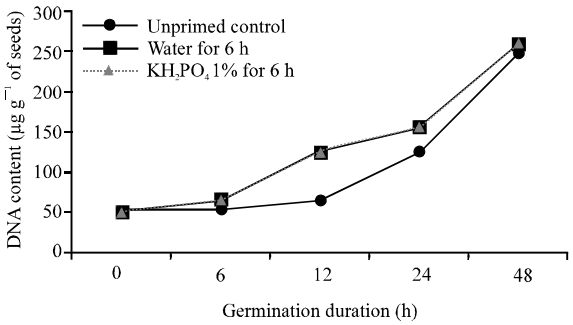

Fig. 2: Changes in DNA content immediately after priming and on subsequent germination

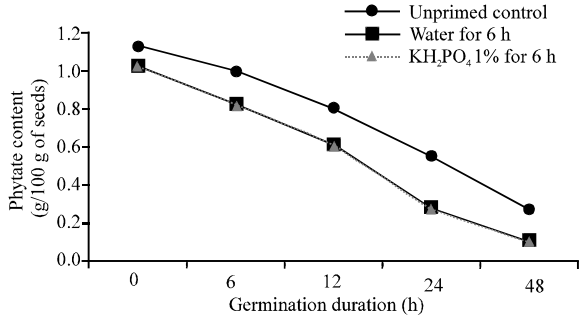

Fig. 3: Changes in phytate content immediately after priming and on subsequent germination

Minerals content: Expect K content reduction in all the other minerals were observed immediately after priming (0 h) but it was not significant when compared with unprimed seeds. During the course of germination viz., 6, 12, 24 and 48 h all the minerals including K content declined rapidly and the decline was significantly at higher rate in primed seeds than unprimed seeds. However, all the minerals excluding K content of water and 1% KH_2PO_4 primed seeds were on par with each other at the end of priming and on subsequent germination (Table 2 and 3). In primed seeds, boron content declined significantly throughout the course of germination. At 6 h of germination half of the initial B content was reduced in seeds primed with water (9.89 ppm) and 1% KH_2PO_4 (9.78 ppm), respectively while it was only one fourth in unprimed seeds (13.96 ppm) and at 48 h of germination 97% of initial B content was declined both in water (0.68 ppm) and 1% KH_2PO_4 (0.53 ppm) primed seeds while it was only 93% in unprimed seeds (1.37 ppm) (Table 2). Cu content declined significantly only from 12 h of germination, at which 87% of initial Cu content was declined both in water (1.27 ppm) and 1% KH_2PO_4 (1.24 ppm) primed seeds while it was only 55% in unprimed seeds (4.46 ppm) and at 48 h of germination almost all Cu content (99%) was declined both in seeds primed with water (0.13 ppm) and 1% KH_2PO_4 (0.09 ppm) whereas only 89% of initial Cu content was reduced in unprimed seeds (1.47 ppm) (Table 2). Similar to B content, Mg content too declined significantly throughout the course of germination. However, at 12 h of germination 79 and 80% of initial Mg content was declined in water (108.50 ppm) and 1% KH_2PO_4 (107.50 ppm) primed seeds, respectively whereas only one fourth of the initial Mg content was reduced in unprimed seeds (399.80 ppm). At 48 h of germination 89% of initial Mg content was declined both in seeds primed with water (59.88 ppm) and 1% KH_2PO_4 for 6 h (59.08 ppm) while it was 81% in unprimed seeds (99.78 ppm) (Table 2). Decline in Mn content was significant only from 24 h or

Table 2: Changes in B, Cu and Mg content immediately after priming and on subsequent germination

	Boron (ppm)					Copper (ppm)					Magnesium (ppm)				
	0	6	12	24	48	0	6	12	24	48	0	6	12	24	48
Priming treatment	(h)					(h)					(h)				
Unprimed control	19.45	13.96	5.39	2.42	1.37	9.84	6.23	4.46	2.73	1.47	528.9	427.8	399.8	139.84	99.78
Water for 6 h	18.26	9.89	3.42	1.02	0.68	9.23	4.32	1.27	0.97	0.13	519.8	312.6	108.5	87.21	59.88
KH_2PO_4 1% for 6 h	18.25	9.78	3.21	0.99	0.53	9.2	4.21	1.24	0.92	0.09	519.78	312.4	107.5	86.74	59.08
Mean	18.65	11.21	4.01	1.48	0.86	9.42	4.92	2.32	1.54	0.56	522.83	350.93	205.27	104.60	72.91
SEd	0.117	0.063	0.072	0.048	0.025	0.290	0.177	0.131	0.071	0.038	1.639	1.526	1.442	1.248	0.926
CD	NS	0.155	0.176	0.118	0.060	NS	NS	0.291**	0.164*	0.093*	NS	3.734**	3.528**	3.053**	2.266**

Table 3: Changes in Mn, Fe and Zn content immediately after priming and on subsequent germination

	Manganese (ppm)					Iron (ppm)					Zinc (ppm)				
	0	6	12	24	48	0	6	12	24	48	0	6	12	24	48
Priming treatment	(h)					(h)					(h)				
Unprimed control	19.56	18.24	10.25	4.58	1.98	69.84	62.1	59.7	38.71	7.01	58.25	51.89	34.85	19.68	3.86
Water for 6 h	18.46	12.41	3.56	0.81	0.41	69.47	55.68	32.17	15.56	0.98	57.95	48.57	18.89	4.84	0.97
KH_2PO_4 1% for 6 h	18.41	12.38	3.21	0.72	0.39	69.42	55.49	32.09	15.52	0.98	57.96	48.47	18.58	4.47	0.89
Mean	18.81	14.34	5.67	2.04	0.93	69.58	57.76	41.32	23.26	2.99	58.05	49.64	24.11	9.66	1.91
SEd	0.874	0.822	0.412	0.066	0.042	0.127	0.175	0.101	0.086	0.065	1.119	1.063	0.987	0.624	0.102
CD	NS	NS	NS	0.161**	0.104*	NS	0.429**	0.247**	0.210**	0.158**	NS	NS	NS	1.226**	0.249**

germination at which 96% of initial Mn content was declined both in seeds primed with water (0.81 ppm) and 1% KH_2PO_4 (0.72 ppm) whereas only 77% of the initial Mn content was reduced in unprimed seeds (4.58 ppm). At 48 h of germination 98% of initial Mn content was declined both in seeds primed with water (0.41 ppm) and 1% KH_2PO_4 for 6 h (0.39 ppm) while it was 90% in unprimed seeds (1.98 ppm) (Table 3). Fe content declined significantly right from 6 h and extended up to 48 h of germination as in B and Mg content. However, at 12 h of germination half of the initial Fe content was significantly declined in seeds primed with water (32.17 ppm) and 1% KH_2PO_4 (32.09 ppm) while it was only 15% in unprimed seeds (59.70 ppm). Whereas at 24 h of germination three fourth of initial Fe content was declined in seeds primed with water (15.56 ppm) and 1% KH_2PO_4 (15.52 ppm) while it was only 45% in unprimed seeds (38.71 ppm) and at 48 h of germination almost all the Fe content (99%) was declined in seeds primed with water (0.98 ppm) and 1% KH_2PO_4 (0.98 ppm) while it was 90% in unprimed seeds (7.01 ppm) (Table 3). Similar to Mn content, decline in Zn content was also significant only from 24 h of germination at which 92% of initial Zn content was declined both in water (4.84 ppm) and 1% KH_2PO_4 (4.47 ppm) primed seeds. While it was only 66% in unprimed seeds (19.68 ppm) and at 48 hr of germination 98% of initial Zn content was declined in seeds primed with water (0.97 ppm) and 1% KH_2PO_4 (0.89 ppm) while it was 93% in unprimed seeds (3.86 ppm) (Table 3).

In exceptional to all other minerals, K content showed a significant increase in seeds primed with 1 % KH_2PO_4 over water and unprimed seeds. An increase of 22.6% in K content was observed in seed primed with 1% KH_2PO_4 (3770.0 ppm) over unprimed seeds (3074.7 ppm) while 3.3% decline in K content was observed in water primed seeds (2972.5 ppm) over unprimed seeds. In spite of increase in K content due to priming with 1% KH_2PO_4, significant reduction was observed from 6 h and extended up to 48 h of germination. However, up to 12 h of germination K content of 1% KH_2PO_4 (1992.3 ppm) primed seeds was on par with unprimed seeds (2010.3) and significantly higher than water primed seeds (1680.2 ppm) and at 24 h of germination it was significantly lower in 1% KH_2PO_4 primed seeds (1092.4 ppm) than unprimed seeds (1486.1 ppm) and higher than

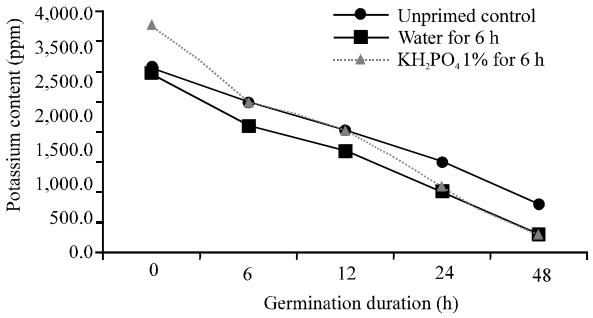

Fig. 4: Changes in potassium content immediately after priming and on subsequent germination

water primed seeds (1010.5 ppm). While at 48 h of germination K content of 1% KH_2PO_4 primed seeds (292.4 ppm) was on par with water primed seeds (292.5 ppm) and significantly lower than unprimed seeds (789.9 ppm) (Fig. 4).

DISCUSSION

The results of present investigation revealed that seed priming with 1% KH_2PO_4 showed its superiority in terms of germination and vigour improvement over water primed and unprimed seeds. There were 6 and 36 % increase in germination and vigour, respectively due to priming with 1% KH_2PO_4 over control (Table 1). These findings was also in confirmation with Basra *et al.* (1988) who found that priming of corn seed using K salts (K_2HPO_4 or KNO_3) resulted in accelerated germination at a chilling temperature (10°C). Sathish and Sundareswaran (2010) in maize reported that seed priming with 1% KH_2PO_4 had advanced the germination, emergence and vigour of the seedlings. Berchie *et al.* (2010) proved that seed priming with water improved seedling emergence of groundnut land races and suggested it as cheap and easy technology to improve final stand and yield of Bambara groundnut.

Beneficial effects of priming are associated with repair and build up of nucleic acids, increased proteins synthesis as well as repair of membranes (McDonald, 2000). The increase in the intensity of total, soluble and heat stable protein profile of both water and 1% KH_2PO_4 primed seeds indicate that new proteins were synthesised during priming. However, similarity in protein profiles of water and 1% KH_2PO_4 primed seeds reveals that there was no additional influence of KH_2PO_4 on protein synthesis than water. These results was also in agreement with Sung and Chang (1993) who reported that new polypeptides band were synthesised at 62, 17 and 14 kDa in soluble protein profile of sweet corn seeds primed with water. Haroun and Hussien (2003) reported that seed priming with algal extract increased the intensities of protein bands from 20-32 kDa. Job *et al.* (1997) found that 11-S globuhn corresponding to 22-kDa in soluble protein profile was synthesised abundantly during priming of sugar beet seeds. Carpon *et al.* (2000) also found that, for a priming treatment of sugarbeet seeds to be most efficient, there should be an increase in the level of soluble 11-S globuhn **β**-subunit per seed but contrasted that there should not be any increase in heat stable seed specific protein such as HS60 protein and SBP sugarbeet seed specific biotinylated protein. The quantitative increase in protein profile might be due to the presence of all the components necessary for resumption of protein synthesis except polysomes within the cells of mature dry embryos. However, polysomes might have been formed immediately after imbibition by combination of single ribosomes which might have initiated the process of protein synthesis as suggested by Bewley (1997) and also due to the activation of already stored mRNA as reported by Marcus and

Feeley (1965). Further, the synthesis of new mRNA during imbibition as reported by Dommes and Van De Walle (1983) might have also been initiated the protein synthesis. The differences in protein profiles at the initial stages of imbibitions could be due to the differential rates of assemblage of polysomes (Jain *et al.*, 2008).

From the results of DNA content, it was obvious that there was no significant changes in DNA content due to priming and also up to 6 h of germination. However, the DNA repair mechanism might have taken place during priming and also at the initial stages of germination as suggested by McDonald (2000). Two fold increase of DNA content in primed seeds over unprimed seeds at 12 h of germination implies the advancement of DNA replication and cell division due to priming and rapid increase in unprimed seeds thereafter, indicated that in primed seeds DNA replication and cell division was attained its maximum at 12 h of germination while it starts only after 12 h of germination in unprimed seeds. Obviously, this could be related to the earlier seedling emergence, increased speed of germination and high vigour of primed seeds. Bray *et al.* (1989) reported that the DNA content and DNA synthesis patterns in leek embryos showed little change throughout priming period, but exhibited increases of several fold upon germination after priming. Ashrafa and Bray (1993) found that DNA synthesis in leek embryo tissue during priming was of both repair and replicative types of DNA and also revealed that both nuclei and mitochondria were the sites of DNA synthesis during priming period.

From the result it was obvious that the rate of decrease in phytate content during the course of germination was 12 and 13% higher in seeds primed with water and 1% KH_2PO_4, respectively, than unprimed seeds. It infers that seed priming declined phytate content and enhanced its utilization during the course of germination and it may corresponds to the energy supply for advancement in DNA replication and cell division. This might also adds to the cause for vigour improvement and higher germination due to priming. This result was in agreement with West *et al.* (1994) who reported that 0.01 to 0.5% of phytic acid was leaked during soaking of soybean seeds in water for 6 hour. Six days of germination of castor bean seeds resulted in 87% phytate digestion (Azarkovich *et al.*, 1999). Andriotis *et al.* (2005) reported that phytic acid levels in embryonic axes of hazel seeds were reduced by 60% within the first 3 weeks of chilling treatment and also indicated that phytate was mobilized by phytase enzyme. Gernah *et al.* (2011) also reported that germination of maize seeds significantly reduced phytate content. It was suggested that early phytate turnover is important for metabolic activity of resting tissue by means of supplying Pi and minerals that could reflect physiological and metabolic demands, for example, starch enzymology as has been postulated by Ogawa *et al.* (1979). It has also been suggested that pyrophosphate-containing inositol phosphates, compounds related to phytate and linked to the biochemical pathways involving inositol phosphate, may serve as Pi donors for ATP synthesis (Raboy, 2003).

Though significant decline in minerals content viz., B, Mg, Fe starts from 6 h of germination, Cu from 12 h of germination and Mn and Zn only from 24 h of germination, the rate of decline during course of germination was rapid as like that of phytate content of primed seeds than the unprimed seeds, indicating rapid utilization of minerals content due to priming. Nithya *et al.* (2006) revealed that mineral content such as Fe, Zn, Mn, Cu, Ca, Mg, K and total phosphorous in pearl millet seeds were reduced due to soaking and sprouting treatments and also proposed that the reduction was due to the metabolic losses and transfer of nutrients to the growing embryo. Significant increase in K content in 1% KH_2PO_4 primed seeds and its drastic reduction during the course of germination, reaching on par with water primed seeds at 48 h of germination concluded

that K^+ ions were absorbed during seed priming with 1% KH_2PO_4 solution and it was utilised rapidly during the course of germination. This might be one of the reason for germination enhancement and production of longer root, shoot and heaviest seedlings by seeds primed with 1% KH_2PO_4 for 6 hr than the seeds primed with water and unprimed seeds. These findings were in agreement with findings of Alvarado *et al.* (1987) who indicated that embryonic K^+ content of tomato seeds primed in KNO_3 was increased by approximately 50% as compared to that of unprimed embryos, undoubtedly contributing to osmotic absorption of water by the seeds and the ion absorption during priming may be responsible for the faster germination. Harris *et al.* (1999) also reported that halopriming with NaCl, KCl, and $CaCl_2.2H_2O$ increased the concentration of Na^+, K^+ and Ca^{2+} significantly in germinating seeds of maize. Harris *et al.* (2007) found that maize seeds primed for 16 h with 1% Zn solutions increased the seed Zn content significantly and produced heavier and taller seedlings than unprimed seeds which was also resulted in significant increase in mean grain yield. Potassium is required for every major step of protein synthesis. The reading of the genetic code in plant cells to produce proteins and enzymes that regulate all growth processes would be impossible without adequate K and it activates at least 60 different enzymes involved in plant growth. Potassium salts had been reported to raise the ambient oxygen level by making less oxygen available for the citric acid cycle (Bewley and Black, 1982). However, K is not involved in protein synthesis immediately after priming as indicated earlier.

The present investigation concluded that seed priming with 1% KH_2PO_4 for 6 h can undoubtedly increase germination potential and vigour of maize hybrid COH(M) 5 and attributed reason was, increase in new protein synthesis, advancement of DNA replication and cell division, absorption of K ions due to priming with potassium salt and rapid utilization of food reserves like phytate, minerals viz., B, Cu, Mg, Mn, Fe Zn, K and imbibed K ions. The result of decrease in phytate content due to priming can be used in poultry sector to reduce the phytate content in poultry feed. However, further research is required to identify the specific proteins synthesised and characterization of molecular marker to standardise priming treatment for each batch of seed lots of maize hybrid COH(M) 5.

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