EFFECT OF DIFFERENT SEED PRIMING TECHNIQUES ON ENDOGENOUS NUTRIENT ACCUMULATION AND GRAIN VITAMIN CONTENTS IN SOFT AND HARD WHEAT SPECIES

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Abstract

The Experiments were conducted to modulate the effect of different priming agents on endogenous nutrient accumulation and grain vitamin contents in two wheat species viz, *Triticum aestivum* (Soft) and *Triticum durum* (Hard). There were six priming treatments viz., water, Salicylic acid, Gibberellic acid, Ascorbic acid, Calcium chloride, Potassium nitrate along with unprimed control treatment. The experiments were conducted in completely randomized design (CRD) with three factor factorial arrangements. LSD test were applied for the comparison on means of different parameters. The findings of present study suggested that different priming agents behaved differently in both wheat species. Some priming agents brought enhancement in mineral nutrients such as nitrate-N, phosphate-P, K, Ca, Mg and sulphate-S. contents while some priming agents caused decrease. However, vitamins such as ascorbic acid, riboflavin and niacin have been found to be increased following all priming treatments. All these changes might be due to the modified biochemical activities taking place within endosperm cells of hard and soft grains which differ greatly in structure and chemical composition.

Key words: Wheat, Seed priming, Mineral nutrients, Vitamins.

Introduction

Cereal grains possess different characteristics, such as shape, size, hardness etc. and there is much of the intra-specific differences in this regard. The extent of seed hardness, designated as 'endosperm vitreousness' has much influence on the grinding and baking quality of flour (Samson *et al*., 2005; Pasha *et al*., 2010). Kernel vitreousness is an apparent standard for hardness and is the characteristic assessed during the grading process of grains (Gooding *et al*., 2003) to determine the end-use quality characteristics of cereals (Neethirajan *et al*., 2006). The degree of vitreousness of kernels is associated with the hardness of the kernel, and the quantity of protein and starch within the kernel (Ghassan *et al*., 2006). Vitreous endosperm contains more proteins and less starch whereas mealy (non-vitreous) endosperm is rich in starch contents as compared to proteins (Holding, 2014). Vitreous endosperm contains greater amounts of insoluble proteins with greater protein cross linking resulting in compact structure with continuous matrix (Baasandorj *et al*., 2016). Starchy or non-vitreous grains have been shown to have a discontinuous type of endosperm with many air spaces and appear white in color (Turnbull & Rahman, 2002). Vitreous grains are hard while non vitreous grains are soft or starchy. For milling and bakery use of wheat, kernel texture is an important factor. Wheat has been classified into different hard and soft categories based on kernel vitreousness (Samson *et al*., 2005; Pasha *et al*., 2010). This classification is important for differentiating the global marketing of wheat. Farmers, millers and bakers also rely on this wheat grading for their intentional final utilization (Morris, 2002). *Triticum aestivum* is hexaploid and has soft textured kernel while *T. durum* is tetraploid and possesses harder or vitreous kernels due to which both these species have different physiological properties and different uses (Zarroug *et al*., 2015). Hardness or vitreousness is an important end use quality feature of wheat with regards to

milling process (El-Khayat *et al*., 2006) because it affects semolina yield, granulation and protein contents of the grains (Ghassan *et al*., 2006).

Seed priming is a technique widely used for improved germination and uniform and vigorous establishment of seedlings of different crops (Rehman *et al*., 2015) including wheat and purslane which showed improved performance of lower quality grains (Ruttanaruangboworn *et al*., 2017; Akram *et al.*, 2023). Process of priming involves controlled seed imbibition to initiate metabolic activities, but are stopped before radical emergence (Hadinezhad *et al*., 2013. Different priming agents have different effects on seed germination and stand establishment. Water as priming agent shows better effect than other salts (halopriming) for different crops (Moghanibashi *et al*., 2012; Hadinezhad *et al*., 2013). Seed germination involves the weakening of endosperm and radical elongation brought about by the activity of certain enzymes such as cellulase (Chen *et al*., 2016) and endo-β-mannanase which is found to be increased following hydropriming (Raj & Raj, 2019). It has been reported that priming with ascorbic acid, salicylic acid and $CaCl_2$ induced high salt tolerance in wheat and other crops (Martinez-Ballesta *et al*., 2020; Afzal *et al*., 2006; Junaid *et al.*, 2023) along with greater accumulation of K^+ and decrease in Na^+ contents of leaves (Martinez-Ballesta *et al*., 2020; Ashraf *et al.*, 2023). Halopriming with KNO_3 and $CaCl_2$ resulted in increased activation of enzymes such as alpha amylase essential for metabolic reactions during germination (Singh *et al*., 2013). K^+ activates many enzymes needed for manufacturing of proteins and starch (Hadinezhad *et al*., 2013). Hormonal priming with gibberellic acid has been reported to enhance tiller density in wheat (Dhillon *et al*., 2021), seedling vigor, seedling length and fresh and dry matter (Kumari *et al*., 2017; Abnavi & Ghobadi, 2012). Priming with gibberellic acid has been found to increase dry matter yield and grain yield in wheat (Kalpana *et al*., 2013).

Material and Methods

The present study was conducted in Botanical Garden of University of Agriculture Faisalabad Pakistan. For experimentation, seeds of two wheat species i.e., *Triticum aestivum* (Soft) var. Galaxy 13 and *T. durum* (Hard) var. D.97 were obtained from Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Following treatments along with hydropriming (HP) were applied for the purpose of priming.

Priming agent	Without shaking	With shaking
Salicylic acid (SA)	0.4 mM	0.8 mM
Gibberellic acid (GA)	1 mM	3 mM
Ascorbic acid (AsA)	15 mM	20 mM
CaCl$_2$	12 mM	16 mM
KNO$_3$	15 mM	20 mM

Fifteen seeds of both the wheat species were soaked for 7 h in 10 mL of aerated solution of each priming agent. Unprimed seeds were considered as control. There were two sets of treatments i.e., priming without shaking and priming with shaking. For shaking treatment, the seeds were shaken using an orbital shaker at 110 rpm. After priming, seeds of both species were sown in plastic pots with a hole at bottom for leaching. Eight kg of sun dried, and thoroughly mixed loamy soil were used in each pot. After three days of germination, the seedlings were thinned to maintain five uniform seedlings in each pot. The experiments were conducted in completely randomized design (CRD) with factorial arrangement and three replications (Steel *et al*., 1996).

Mineral nutrients analyses: To estimate minerals ions (K, Ca, Mg), nitrate-N and phosphate-P, seed samples were digested with concentrated H$_2$SO$_4$ and H$_2$O$_2$ according to the method of Wolf (1982). For estimation of sulfate-S, samples were digested in concentrated HNO$_3$ (Tendon, 1993).

Flame photometer (Jenway PEP 7) was used to estimate the concentration of K$^+$, Ca^{2+} and Hitachi Polarized Zeeman atomic absorption spectrophotometer was used to determine Mg^{2+} in the samples. A graded series of standards (10, 20, 30, 40 and 50 ppm) of K and Ca^{2+} were run and a standard curve was constructed to quantify these ions in sample solutions.

Nitrate-N were estimated according to the method described by Kowalenko & Lowe (1973). 3 mL of the extract obtained after acid digestion were taken and 7 mL of 0.01% chromotropic acid (CTA) solution was added and briefly vortexed. After 20 min, yellow color intensity was measured at 430 nm by a spectrophotometer. A standard series of 10, 20, 30, 40, 50 and 100 mg/L KNO$_3$ was prepared to determine final amounts of nitrates in the samples.

Phosphate-P contents were determined following method of Yoshida (1976). To 1 mL of the extract obtained after acid digestion, 2 mL of 2 N HNO$_3$ was added and diluted to 8 mL with distilled water. Then 1 mL of molybdate-vanadate reagent was added, and volume was made up to 10 mL with distilled water; vortexed and allowed to stand for 20 minutes. Absorbance was measured at 420 nm using water as blank. A series of standards (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg/L) was prepared by using monobasic phosphate (KH$_2$PO$_4$). Phosphate-P contents of samples were estimated by comparing standard values.

Amount of sulfate-S was determined using the method of Tendon (1993). To 1 mL of extract, 1 mL of 6 N HCl and 1 mL of 0.5% gum acacia solution were added and shaken. Then 0.5 g BaCl$_2$ crystals were added and shaken until the crystals were dissolved. Transmission of the solution was taken at 440 nm using a spectrophotometer. A graded series of K$_2$SO$_4$ standards (0, 4, 8, 12, 16 and 20 ppm) was prepared to construct a standard curve to quantify sulfate-S contents of the samples.

Vitamins: Vitamins such as ascorbic acid, riboflavin and niacin were determined according to available procedures. For ascorbic acid quantification, method described by Mukherjee & Choudhuri (1983) was used. Seed sample (0.25 g) was extracted in 10 mL of 6% TCA solution. Then to 4 mL of extract, 2 mL of 2% acidic solution of dinitrophenyl hydrazine were added followed by addition of 1 drop of 10% thiourea solution. The mixture was heated in a boiling water bath for 20 minutes. After cooling to room temperature, 5 mL of 80% H$_2$SO$_4$ were added. Absorbance was taken at 530 nm. A standard curve of ascorbic acid was constructed by series of concentrations (5, 10, 15, 20 and 25 ppm) and ascorbic acid contents of samples were calculated.

Riboflavin was determined using the method of Okwu & Josiah (2006). A 5 g seed sample was extracted in 100 mL of 50% ethanol; shaken for 1 h and then filtered. To 10 mL of extract, 10 mL of 5% potassium permanganate and 10 mL of 30% H$_2$O$_2$ were added and allowed to stand in a water bath at 50°C for 30 minutes. Then 2 mL of 40% sodium sulfate is added and volume was made up to 50 mL with distilled water. The absorbance was measured at 510 nm. Riboflavin contents of samples were determined by comparing with standard curve of riboflavin.

To estimate niacin, method of Okwu & Josiah (2006) was followed. A 5 g seed sample was treated with 50 mL of 1N H$_2$SO$_4$, shaken for 30 minutes followed by addition of one drop of ammonia solution and then filtered. Then to 10 mL of filtrate, 5 mL of potassium cyanide solution were added followed by addition of 5 mL of 0.02N H$_2$SO$_4$. Absorbance was taken at 470 nm. Final quantities of niacin in samples were measured from a standard curve of known niacin concentrations.

Statistical analyses

The designs of all the experiments were completely randomized (CRD) factorial with three replications (Steel *et al*., 1996). The data were statistically analyzed for analysis of variance (ANOVA) using Statistix8.1 software, while the comparisons of means were done using least significant difference (LSD) test. The Excel 365 was used for the graphical presentation of data throughout the manuscript.

Results

Following priming, the seeds of both hard and soft wheats were analyzed for six macronutrients namely nitrate-N, phosphate-P, K, Ca, Mg and sulphate-S.and vitamins namely ascorbic acid, riboflavin and niacin. ANOVA are presented in (Tables 1 & 2) while (Figs. 1 & 2) show the comparison of means.

Table 1. ANOVA showing the efficacy of different priming agents on the kernel nutrient contents in the wheat species differing in kernel hardness.

Source of variation	DF	NO_3^--N	PO_4^{3-}-P ($\times 10^{-3}$)	K^+	Ca^{2+} ($\times 10^{-3}$)	Mg^{2+} ($\times 10^{-3}$)	SO_4^{2+}-S
Priming (Pr)	6	0.363^{**}	2.38^*	1.023^{**}	0.43^{ns}	50.28^{**}	0.212^{ns}
Shaking (Sh)	1	3.416^{**}	16.38^{**}	0.292^{**}	1.39^{ns}	28.79^{ns}	3.348^{**}
Species (Sp)	1	0.001^{ns}	6.43^{**}	0.916^{**}	2.35^*	100.29^{**}	0.456^*
Pr × Sh	6	0.519^{**}	1.45^{ns}	0.137^{**}	1.83^{**}	3.88^{ns}	0.184^{ns}
Pr × Sp	6	0.506^{**}	1.25^{ns}	0.046^*	3.02^{**}	1.23^{ns}	0.142^{ns}
Sh × Sp	1	11.199^{**}	21.09^{**}	0.001^{ns}	8.36^{**}	0.20^{ns}	0.257^{ns}
Pr × Sh × Sp	6	0.630^{**}	1.33^{ns}	0.042^*	1.20^{ns}	10.66^{ns}	0.134^{ns}
Error	56	0.089	0.88	0.017	0.57	8.02	0.097

**, * = Significant at 1 and 5% confidence levels, respectively; ns = Non-significant

Table 2. ANOVA showing the effect of different priming agents on kernel vitamin concentration in the wheat species differing in kernel hardness.

Source of variation	Df	Ascorbic acid	Riboflavin	Niacin
Priming (Pr)	6	3.109^{**}	1.697^*	2.195^{**}
Shaking (Sh)	1	3.306^{ns}	4.452^*	1.856^{ns}
Species (Sp)	1	2.702^{ns}	4.636^*	1.750^{**}
Pr × Sh	6	1.436^{ns}	2.693^{ns}	6.180^{**}
Pr × Sp	6	9.631^{ns}	2.747^{**}	2.552^{ns}
Sh × Sp	1	2.304^{**}	6.833^{**}	4.683^{ns}
Pr × Sh × Sp	6	2.060^{ns}	1.493^{ns}	4.825^{**}
Error	56	9.075	6.804	1.367

**, * = Significant at 1 and 5% confidence levels, respectively; ns = Non-significant

NO_3^--N contents: Results indicated that soft species, increase over control had been observed in SA, GA and $CaCl_2$ (16, 40 and 13% respectively) with shaking while decrease was noted for HP, AsA and KNO_3 (11, 50 and 35% respectively). Without shaking treatment, all the priming agents brought about decrease in nitrates content (35, 23%, 22, 32, 45 and 27% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively) in comparison with unprimed control. Hard wheat behaves differently than soft one. Without shaking, there was a marked increase in nitrates-N content (123, 99, 123, 79 and123% with HP, SA, GA, AsA and KNO_3 priming respectively). However, only $CaCl_2$ had resulted in decreased nitrate-N contents (14%) than unprimed. With shaking, all treatments had negative impact on nitrate-N accumulation showing a decreasing trend (44, 28, 52, 09, 47 and 55% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively.

PO_4^{3-}-P contents: In case of soft kernel wheat species, all priming treatments with shaking resulted in decreased phosphate-P contents (37, 36, 37, 30, 37 and 35% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively). in comparison with unprimed control. However, GA, AsA and $CaCl_2$ without shaking brought about enhancement in accumulation of phosphate (02, 23 and 13% respectively). HP and SA showed decrease (10 and 30% respectively) while KNO_3 performed similar to that of control. In hard wheat species, all priming treatments with shaking positively influenced in increasing phosphate accumulation (09, 01, 19, 14, 18 and 04% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively). Without shaking, HP, AsA, $CaCl_2$

and KNO_3 brought about an increase (04, 23, 13 and 13% respectively) while SA and GA showed decreasing behavior (04 and 04% respectively) than that of unprimed control.

K^+ contents: In soft wheat species, all priming treatments with shaking resulted in decreased K^+ contents (19, 25, 16, 22, 25 and 02% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively as compared to unprimed control. Similar deceasing trend was also observed without shaking (27, 29, 25, 25, 22 and 15% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively). In hard wheat kernel species, all priming treatments without shaking showed decreased K^+ contents (37, 19, 24, 24, 26 and 25% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively) in comparison with control. With shaking, K^+ contents have been found to decrease (25, 26, 25, 26, 26 and 03% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively).

Ca^{2+} contents: Treatment means showed that in soft wheat species, only HP with shaking were effective in increasing Ca^{2+} contents (02%) as compared to unprimed while all other treatments with and without shaking had been resulted in decreased Ca^{2+} contents (04, 04, 04 and 04% for SA, AsA, $CaCl_2$ and KNO_3 respectively). GA behaved similarly to that of control. Hard wheat species performed differently where SA, GA, AsA and $CaCl_2$ without shaking showed pronounced increase in Ca^{2+} contents (101, 68, 168 and 168% respectively) as compared to control one. HP and KNO_3 behaved similarly to that of control. With shaking, SA, GA and KNO_3 showed increase (68, 67 and 34% respectively) while all other treatments showed a similar pattern in comparison with control.

Mg^{2+} contents: In soft wheat species, all priming agents without shaking have exerted a negative impact on Mg^{2+} accumulation (10, 32, 24, 22 25 and 23% decrease for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively). Likewise, with shaking, decrease in Mg^{2+} contents were observed for all treatments (17, 16, 30, 20 and 20% for HP, SA, AsA, $CaCl_2$ and KNO_3 respectively) except GA which performed similarly to that of unprimed control. Hard wheat specie sowed decrease in response to different priming agents both with shaking (05, 15, 15, 11, 19 and, 16% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively) and without shaking (08, 24, 09, 29, 27 and 21% for HP, SA, GA, AsA, $CaCl_2$ and KNO_3 respectively).
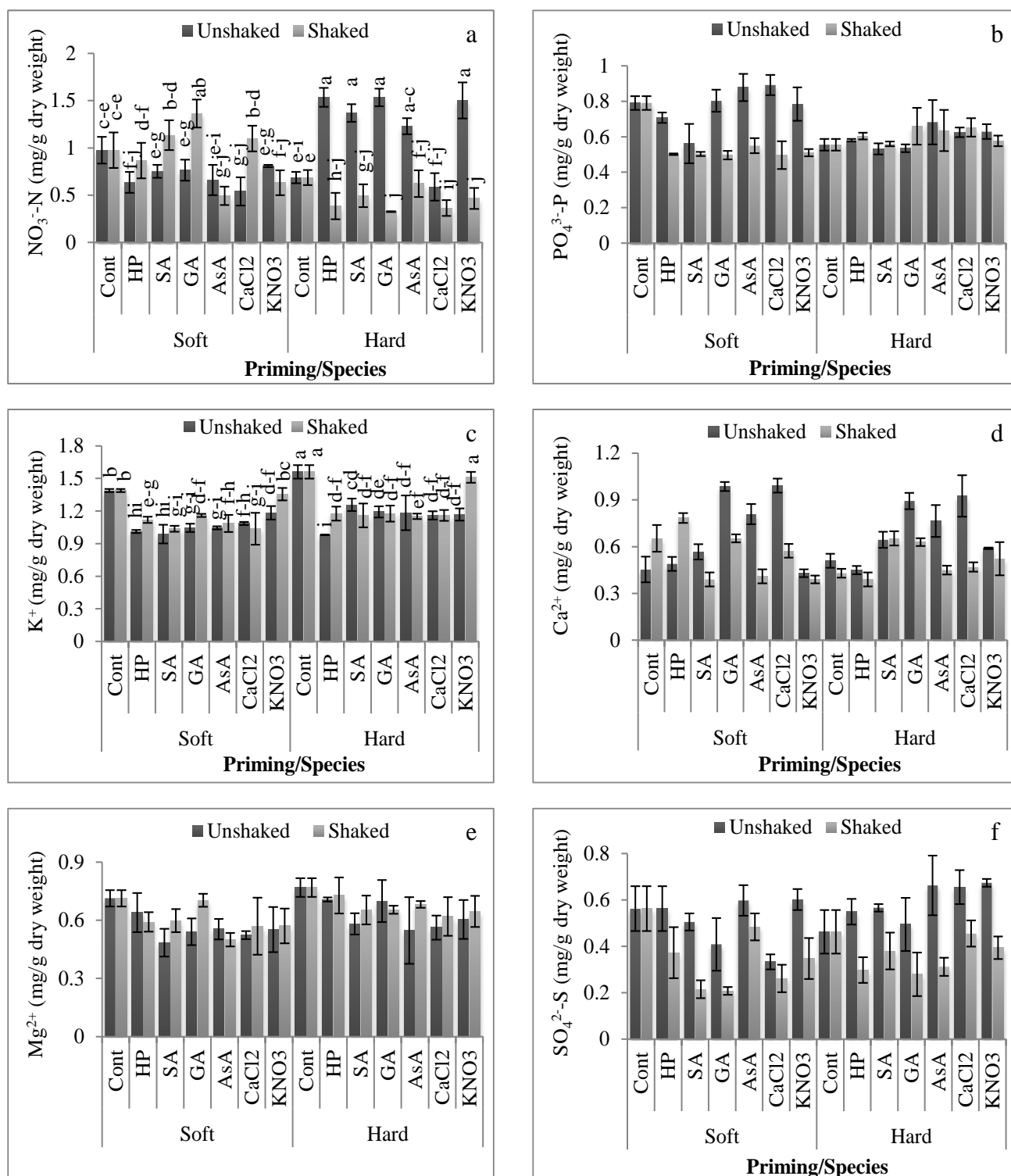
Fig. 1. Changes in the amount of mineral nutrients as affected by different priming agents in the wheat species differing in kernel hardness.

SO$_4^{2-}$-S contents: In soft wheat species, all priming treatments with shaking had been resulted in decreased sulfur contents (34, 62, 63, 14, 54 and 38% for HP, SA, GA, AsA, CaCl$_2$ and KNO$_3$ respectively) while without shaking, SA, GA, AsA and CaCl$_2$ showed a decreasing trend (10, 27, 29 and 41% respectively) than that of control. Only KNO$_3$ brought about an increase in sulfur accumulation (23%) while HP behaved similarly to that of control. In case of hard wheat, an increase had been noted for all priming treatments without shaking (84, 22, 07, 43, 42 and 46% for HP, SA, GA, AsA, CaCl$_2$ and

KNO$_3$ respectively). But with shaking, a decrease (36, 18, 40, 33, 02 and 15% for HP, SA, GA, AsA, CaCl$_2$ and KNO$_3$ respectively) was noted as compared to control.

Ascorbic acid: Increasing trend was noted both with and without shaking in both species. However, more increase was noted with shaking showing 14, 28, 16, 71, 14 and 16% increase over control in HP, SA, GA, AsA, CaCl$_2$ and KNO$_3$ respectively for soft species while Hard species depicted 13, 13, 20, 115, 13 and 01% increase for HP, SA, GA, AsA, CaCl$_2$ and KNO$_3$ respectively.

Riboflavin: It has been clear that all priming treatments with and without shaking had resulted in almost similar enhancement in riboflavin contents in both soft and hard wheat species.

Niacin: In both species, priming with shaking showed more pronounced increase than without shaking depicting (116, 40, 149, 110, 105 and 157% for HP, SA, GA, AsA, CaCl₂ and KNO₃ respectively) in soft species while In case of hard wheat species, (90, 61, 39, 18, 90 and 83% for HP, SA, GA, AsA, CaCl₂ and KNO₃ respectively) was observed.

Fig. 2. Changes in the amounts of kernel vitamins as affected by different priming agents in the wheat species differing in kernel hardness.

Discussion

It has been revealed from present study that different priming treatments performed differently regarding endogenous accumulation of mineral nutrients within the plants. Some induced increased accumulation while some resulted in decreased accumulation of mineral nutrients. In hard wheat species there was a greater accumulation of nitrogen in the form of NO_3^--N when subjected to priming without shaking with SA and AsA. These findings are in accordance with those of Ahmad et al., (2015) for maize. Accumulation of nitrates in soft wheat species and both NO_3^--N and PO_4^{3-}-P in GA and KNO₃ priming treatments was noted in hard wheat species. These results are in accordance with the results reported by Anwar et al., (2020). Both nitrates and phosphates are the source of nitrogen and phosphorus, respectively and are used in the manufacture of proteins which are in greater amounts in hard wheat species than soft wheat species (Holding, 2014) as exogenous application of nitrogen has been found to be associated with increased protein contents in wheat grains (Polisenska et al., 2018). It is likely that the priming induced decline in NO_3^--N may be due to its incorporation in the synthesis of amino acids for use in the embryo growth. Likewise, changes in the PO_4^{3-}-P contents may be attributed to their provision in the ATP synthesis required during embryo activation (Martínez-Ballesta et al., 2020).

Reduction in leachate osmotic potential due to solute leakage has been taken as an essential process shortly after the imbibition and hydration of seed structures (Akhalkatsi & Lösch, 2001). These results showed that although there was a general decline in the kernel K^+, Ca^{2+}, Mg^{2+} and SO_4^{2-}-S contents, shaken condition led to relatively greater contents of these nutrients in the kernel. The possible reason in this regard may be the leakage of these nutrient into the priming media with the softening of seed coat. Their resorption from the germination media could be a strategy for successful seedling establishment once the germination has occurred. This indicated that kernel hardness was partly modulated by priming treatments. Among the treatments, GA, HP, CaCl₂ and AsA were relatively more effective.

Like minerals, vitamins are also considered as nutrients since they concertedly act in performing proper functions of important proteins and enzymes. The availability of vitamins is therefore important for optimal metabolic activities (Chen et al., 2018; Jiang et al., 2021). Enhancement in the endogenous biosynthesis or exogenous supplementation of vitamins is therefore considered as important strategy to promote plant growth (Siva Devika et al., 2021). Different priming agents showed different behavior regarding accumulation of vitamins. There was an overall increase in the accumulation of vitamins over respective controls, although the efficacy of priming treatments was different for different vitamins. This implied that seed priming has tendency to improve the endogenous vitamins accumulation, which presumably has a role in enhancing the seedling vigor during germination.

The behavior of soft and hard wheat species was also different subjected to priming with and without shaking. Priming with shaking exerted more positive influence to accumulate ascorbate. In both soft and hard wheat species, AsA was the most effective in enhancing AsA contents
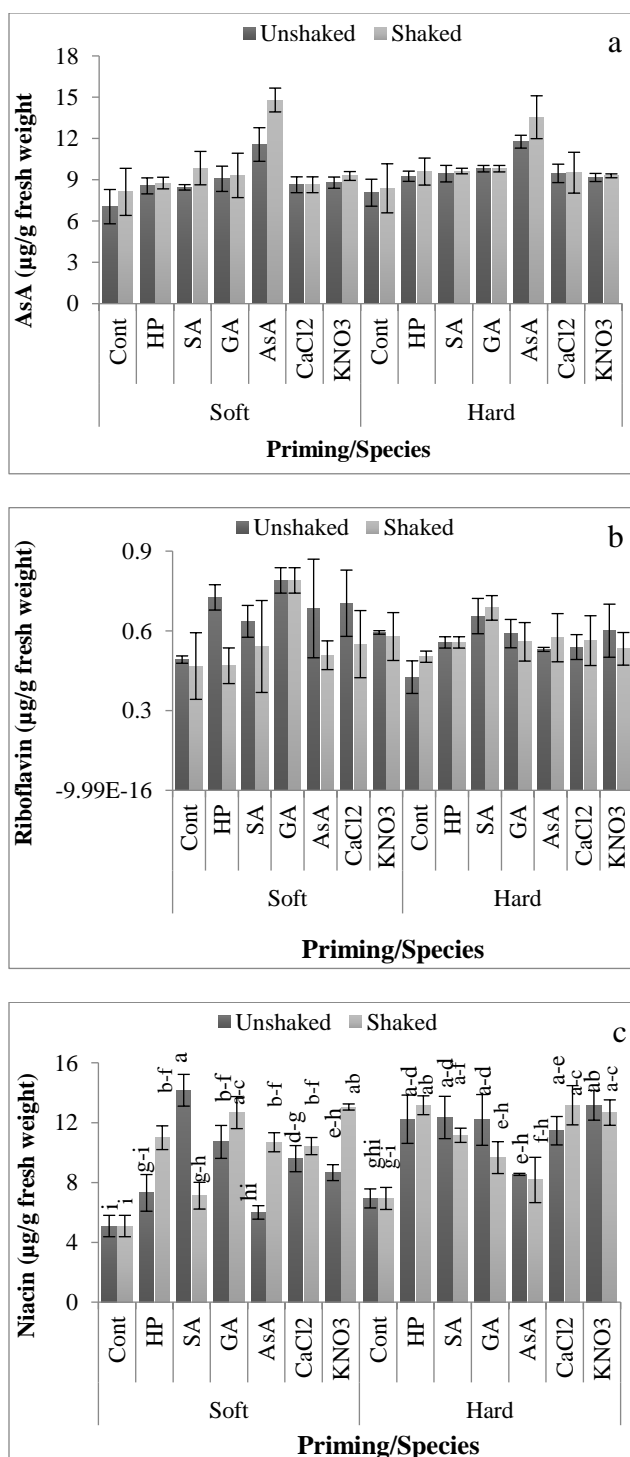
especially under shaken condition. Riboflavin was effectively accumulated with GA in soft kernel wheat while with SA in hard kernel wheat particularly under shaken condition. However, for niacin accumulation, a number of priming treatments including SA, GA and KNO_3 for soft kernel wheat and HP, SA, GA, $CaCl_2$ and KNO_3 proved effective. Increased ascorbate accumulation might be an effective defense mechanism against ROS (Afzal *et al.*, 2006). The accumulation of vitamins has been implicated for the improved germination and seedling growth in wheat (Yasmeen *at al.*, 2013) and maize (Alcantara *et al.*, 2017).

Conclusion

It can be concluded from the findings of present study that different priming agents brought about positive changes regarding endogenous minerals and vitamins contents which might be attributed to the modifications in biochemical activities in endosperm to improve germination and seedling vigour. However, the different behavior of hard and soft grain species might be due to the differences in anatomical characteristics as well as different proportions of chemical constituents within the grain.

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