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Seed Priming Influence on Growth, Yield, and Grain Biochemical Composition of Two Wheat Cultivars

M. Khaing[1], V. U. Ultra, Jr.[2*], and S. Chul Lee[1*]

ABSTRACT

The influence of seed priming on the growth, amino and fatty acids contents of two wheat (*Triticum aestivum* L.) cvs. Keumkang and Backjung were evaluated through pot experiments in greenhouse and at field conditions. Four priming treatments involving three soaking media: 2.5% potassium Chloride (KCl), 1% potassium Sulfate (K_2SO_4), distilled water (H_2O) and unprimed (dry seeds) as the control were laid out in a randomized complete block design with four replications for each experimental condition. Under greenhouse condition, 1% K_2SO_4 priming enhanced growth and yield of wheat, while 2.5% KCl reduced the dry matter yield in Keumkang, but not in Backjung. In field condition, 1% K_2SO_4 improved the growth of both cultivars and increased the yield of Backjung, while 2.5% KCl in Keumkang had the lowest yield. Overall, this study showed that seed priming with 2.5% KCl and 1% K_2SO_4 triggered specific changes in the Amino Acids (AA) and Fatty Acids (FA) compositions in grain and had carry-over effects on the plant's metabolic adjustments, which were specific to the cultivar and the growing environmental conditions. The compositional changes in AA and FA induced by seed priming have a profound impact on grain and flour quality of wheat.

Keywords: Amino acid profile, Fatty acid profile, Potassium chloride, Potassium sulfate, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum* spp.) is among the big three-cereal crops, with over 750 million tons being harvested annually (FAO, 2017). To increase wheat productivity over limited arable lands, different strategies are being employed by farmers and agricultural scientists involving a combination of genetic improvements, integrated nutrient, pest, moisture and environmental stress management, and application of effective agronomic and farming systems practices (van Rees *et al*., 2014). Establishing a good crop stand is also considered an effective method of improving yield, especially in areas with sub-optimal and unpredictable weather and climatic conditions prevailing in most production areas (Nawaz *et al*., 2016). In South Asia where rice-wheat cropping system is common, a general observation of low wheat yield is attributed to wheat sowing delay because of delayed rice harvest, short period of winter seasons, less developed irrigation facilities and poor crop stands due to lack of optimal moisture (Meena *et al*., 2014). In an arid and semi-arid environment, undependable irrigation supply and erratic weather conditions brought environmental stress resulting in poor crop establishment (Webber *et al*., 2017). For optimal crop stand, pre-sowing

[1] School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu, South Korea.
[2] Department of Earth and Environmental Science, Faculty of Science, Botswana International University of Science and Technology, Palapye, Botswana.
[*]Corresponding authors; e-mail: vuultra@gmail.com; leesc@knu.ac.edu

treatments of seed are performed to enhance speed and uniformity of germination (Hussian *et al*., 2014). Seeds are soaked in water or in an osmotic solution that allows them to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. Aside from improved germination, this treatment enhanced rapid and uniform emergence of seedlings with high seedling vigor and better yields in different crops (Paparella *et al*., 2015). It has also been established that seed priming, which induces several biochemical changes in the seeds, is required to start the germination process such as breaking of dormancy, hydrolysis or mobilization of inhibitors, imbibition and enzyme activation (Zheng, 2016). Common priming techniques include osmopriming [soaking seed in solutions of sugars, PolyEthylene Glycol (PEG), glycerol, sorbitol or fertilizers such as urea], halopriming (soaking seeds in salt solutions), and hydropriming (soaking of seed in water) before sowing (Singh *et al*., 2015). The effectiveness of seed priming depends on the plant species, the type and concentration of priming solution, the priming duration, and temperature and storage conditions (Jisha *et al*., 2013). Singh *et al*. (2015) presented a comprehensive review of the different seed priming techniques in field crops showing that, aside from improved germination, seed priming also reduced seedling emergence time, improved stand establishment, and improved yield for many field crops under a wide range of environmental conditions. It was regarded as the best solution to germination-related problems, especially when crops are grown under unfavorable conditions.

Because seed priming is an effective technology to enhance rapid and uniform emergence and to achieve high vigor leading to better stand establishment and yield, it is hypothesized that seed priming will have a carryover effect on the overall physiology sustained until reproductive and maturity stage. There is a high possibility that the wheat grain quality will be influenced by changes in the fatty and amino acids content. Although García del Moral *et al*. (2007) showed that grain protein content and amino acid composition of wheat fluctuate largely with genotype and environmental characteristics such as nitrogen fertilization rate, nitrogen-application time, residual soil nitrogen, soil moisture availability, and temperature during grain-filling, there is no evidence so far about the influence of seed priming on the biochemistry of the grain of wheat. Therefore, this study was conducted to determine the carryover effect of seed priming with 2.5% KCl and 1% K_2SO_4 on two cultivars of wheat based on the differences on growth and yield of plants and the amino and fatty acids on the whole grain produced by plants grown under two distinct environmental conditions.

MATERIALS AND METHODS

Plant Materials and Priming Treatments

A pot experiment was conducted in greenhouse and at the field located at the Central Experiment Station, Kyungpook National University, Daegu, South Korea to evaluate the effects of seed priming agents on growth, yield and biochemistry of harvested grain of wheat. Two wheat cvs. Keumkang and Backjung were tested with four priming treatments including the control. Keumkang is more heat-tolerant cultivar than Backjung (Truong *et al*., 2017). The priming solutions were 2.5% potassium Chloride (KCl), 1% potassium Sulfate (K_2SO_4), and distilled water (H_2O). These were evaluated against the performance of plants from dry seeds as a control. The 2.5% KCl and 1% K_2SO_4 priming solution was selected based on preliminary germination test of wheat involving several concentrations of KCl and K_2SO_4 at different soaking duration (data not shown). Pure seeds of Keumkang and Backjung were washed with distilled water before soaking on priming solutions. Seeds of the control were blot-dried and placed under a laminar flow hood to air dry, while those for the 2.5% KCl, 1% K_2SO_4, and water priming were fully immersed in priming solutions and stored in the dark at 24°C for 24 hours, then, the seeds were rinsed thoroughly with distilled water and blot-dried prior to sowing.

Pot Experiments

The experiment was laid-out following a two-factor completely randomized design with 4 replications in green house and at field condition. A total of 64 pots (30×30 cm) were filled with 10 kg of sandy loam soil and were fertilized at the rate of 14-12-9 kg ha^{-1} N-P-K in the form of urea, TSP (Triple Super Phosphate), and MP (Muriate of Potash), respectively. The whole quantity of TSP and MP, and 1/2 of urea were basal dressed at the time of pot preparation. The remaining urea was top dressed by two equal splits at 50 and 120 days after sowing. Six seed were sown in each pot and then were thinned into two plants per pot one week after sowing. Thirty-two pots for each environmental condition were randomly arranged inside the greenhouse and in the field for the whole duration of the experiments. The average monthly temperatures from November 2016 to March 2017 under greenhouse and field conditions are presented in Table 1. The greenhouse condition had higher maximum and minimum temperature than the field condition. The mean difference between the maximum and the minimum temperatures was higher in the greenhouse (20.16 to 35.26°C) compared to that in the field condition (8.30 to 13.67°C). The required cultural and other management practices for winter wheat were employed throughout the duration of the experiment. Imidacloprid was applied at a dosage of 5 g 10 L^{-1} to control the aphids.

Growth and Yield

At ripening stage (215 days after sowing), plants were harvested and their heights (cm), number of tillers, aboveground dry matter yield (g $plant^{-1}$), panicle length (cm), number of panicles per plant, total number of grain, total weight of grain (g per pot), one hundred seed weight (g) and Harvest Index (HI) were determined for all the plant samples. Plant dry weight was determined after oven drying at 70℃ for three consecutive days, while the weight of grain was determined at 14% moisture content. The harvest index was calculated as the total weight of grain divided by the aboveground dry matter yield.

Biochemical Analysis of Grain

Grains were milled into flour and analyzed for total Amino Acids (AA) and Fatty Acids (FA) contents. The total AA contents were analyzed after being hydrolyzed with 6N HCl for 24 hours at 110°C (AOAC, 2000, Procedure 4.1.11, Alternative 3). The fifteen AA were analyzed using an AA Analyzer (Hitachi L-8900, Tokyo, Japan). For FAs, the wheat flour samples were

Table 1. Monthly maximum, minimum and mean difference temperatures in and outside greenhouse during growing period of wheat.

	Greenhouse			Outside		
Month	Max temp (°C)	Min temp (°C)	Mean diff (°C)	Max temp (°C)	Min temp (°C)	Mean diff (°C)
November 2016	29.85	9.69	20.16	15.88	7.58	8.30
December 2016	27.19	2.32	24.87	10.71	0.71	10.00
January 2017	25.13	-2.61	27.74	7.03	-5.45	12.48
February 2017	32.14	-0.62	32.76	8.83	-3.00	11.83
March 2017	39.23	3.97	35.26	15.9	2.23	13.67

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analyzed based on the method described by Gerits *et al*. (2013). Briefly, FA from flour was extracted with an ASE 200 (Dionex, Amsterdam, The Netherlands), using Water Saturated Butan-1-ol (WSB). Lipid separation and detection were conducted with a modular High-Performance Liquid Chromatography (HPLC) system (Shimadzu, Kyoto, Japan). All analyses were conducted in duplicate for each sample and were repeated if the results differed by more than 5%.

Statistical Analysis

The data of growth and yield parameters and the concentration of different amino and fatty acids were subjected to two-way analysis of variance (data of main effects were not shown) followed by Tukey's Test for treatment mean comparison for each experimental condition. The statistical analysis was performed using SPSS 12 software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

RESULTS

Greenhouse Experiment

Significant interaction effect of cultivars and priming treatments were observed on all growth and yield parameters determined in wheat grown under greenhouse conditions (Table 2). Specifically, seed priming using 1% K_2SO_4 improved the growth of both cultivars as indicated by taller plants and higher dry matter yield. Backjung had relatively higher number of tillers compared to others regardless of the seed priming treatments. Priming with 2.5% KCl, 1% K_2SO_4 and water resulted in longer panicle length in Keumkang compared to the control while in Backjung, the panicle length did not vary significantly between treatments. In contrast, Backjung treated with 2.5% KCl had the highest number of seeds per plant and total grain yield compared to other treatments. The weights of 100 seeds among the different priming treatments in Keumkang were not significantly different with each other, but in Backjung, 1% K_2SO_4 and H_2O priming resulted in lighter weight compared to the seed produced from plants treated with 2.5% KCl, and the control (unprimed). The harvest index of wheat in Backjung treated with 2.5% KCl was the highest among the treatment combinations.

Significant interaction effects between cultivars and priming treatments were observed on most of the amino acids in the grain of wheat (Table 3). Among the different AA, only serine, glycine and cysteine contents did not vary significantly due to cultivar and priming treatment; all other AA showed significant interaction effects (Table 3). The aspartic acid, threonine, glutamic acid, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and proline in Keumkang significantly increased due to 1% K_2SO_4 priming compared to other priming treatments. On the other hand, Backjung primed with 2.5% KCl had the lowest contents of threonine, methionine, isoleucine, leucine, tyrosine and phenylalanine compared to other treatment combinations including the control. Water priming resulted in AA contents comparable with the control in Keumkang. However, in Backjung water priming resulted to higher AA contents compared to 2.5% KCl, 1% K_2SO_4, and the control. Among the different AA, proline had the highest amount across different cultivars and priming treatments. Overall, the total AA was significantly increased by seed priming with 1% K_2SO_4 in cv. Keumkang, while seed priming with 2.5% KCl in Backjung had the lowest total AA among the different treatment combinations under greenhouse conditions.

Unlike the AA, the Fatty Acid (FA) contents only showed significant interaction effect due to cultivar and priming treatment on the hexadecanoic, oleic, and linoleic acids contents in grains (Table 4). The total FA, total saturated FAs, and total unsaturated FAs only showed main effects

Table 2. Growth and yield performance of two wheat cultivars as affected by different seed priming solutions grown under greenhouse conditions with summary of the P-values of two- way analysis of variance.[a]

Treatment Combinations Cultivars	Priming	Plant height (cm)	Number of tillers (no $plant^{-1}$)	Dry matter yield (g $plant^{-1}$)	Panicle length (cm)	No of seeds/Plant	Grain yield (g $plant^{-1}$)	100 Seeds weight (g)	Harvest index
Keumkang	2.5% KCl	93.8 bc	49.3 ab	137.4 ab	13.2 a	978 c	38.6 b	3.9 a	0.28 b
	1% K_2SO_4	98.8 ab	42.0 bc	148.5 a	14.1 a	945 c	37.9 b	4.0 a	0.26 b
	H_2O	90.0 c	41.8 bc	140.9 ab	13.5 a	916 d	38.7 b	4.3 a	0.28 b
	Unprimed	92.5 bc	49.5 ab	119.7 b	12.4 b	919 d	36.6 c	4.0 a	0.31 a
Backjung	2.5% KCl	94.3 bc	52.5 a	129.1 ab	12.3 b	1284 a	44.1 a	3.5 b	0.35 a
	1% K_2SO_4	104.2 a	51.5 a	146.7 a	13.0 ab	1250 a	40.4 a	3.2 c	0.27 b
	H_2O	101.8 a	50.8 ab	145.5 a	13.1 ab	1050 b	32.6 c	3.1 c	0.23 c
	Unprimed	91.8 b c	53.2 a	137.2 ab	12.5 b	964 c	35.4 c	3.8 ab	0.26 b
P value	Cultivar	ns	0.046*	ns	ns	ns	ns	0.028*	ns
	Priming	0.013*	0.018*	0.027*	0.039*	0.016*	0.035*	0.037*	0.015*
	Cultivar× Priming	0.016*	0.024*	0.008*	0.043*	0.028*	0.042*	0.045*	0.042*

[a] Means within the same column followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance. * Significant at 5%, ns: Not significant

Table 3. Amino acid contents (mg g^{-1}) of grains of wheat cv Keumkang and Backjung grown under greenhouse conditions as affected by seed priming with summary of the P-values of two way analysis of variance.[a]

	Cultivars×Seed priming								P values		
	Keumkang				Backjung						
Parameters	2.5% KCl	1% K_2SO_4	H_2O	Unprimed	2.5% KCl	1% K_2SO_4	H_2O	Unprimed	Cultivar	Priming	Cultivar×Priming
Aspartic acid	0.78 b	1.23 a	0.78 b	0.89 b	0.62 b	0.68 b	1.03 ab	0.74 b	ns	0.038*	0.045*
Threonine	0.20 b	0.33 a	0.19 b	0.23 b	0.15 c	0.16 c	0.26 ab	0.19 b	ns	0.042*	0.048*
Serine	0.16 a	0.18 a	0.13 a	0.16 a	0.14 a	0.14 a	0.17 a	0.14 a	ns	ns	ns
Glutamic acid	1.16 b	2.56 a	1.14 b	1.35 b	0.67 c	0.83 c	1.32 b	0.96 c	ns	0.028*	0.033*
Glycine	0.06 a	0.07 a	0.05 a	0.06 a	0.06 a	0.06 a	0.07 a	0.05 a	ns	ns	ns
Alanine	2.58 b	3.69 a	2.58 b	2.73 b	1.96 b	2.23 b	3.07 ab	2.25 b	ns	0.047*	0.043*
Cystine	0.66 a	0.84 a	0.66 a	0.68 a	0.53 a	0.60 a	0.73 a	0.58 a	ns	ns	ns
Valine	2.49 b	3.57 a	2.67 b	2.78 b	1.88 b	2.22 b	3.04 ab	2.29 b	ns	0.032*	0.026*
Methionine	0.22 b	0.36 a	0.27 b	0.25 b	0.04 d	0.15 c	0.21 b	0.16 c	0.043*	0.024*	0.018*
Isoleucine	2.02 b	3.09 a	2.21 b	2.24 b	1.41 c	1.69 c	2.41 b	1.8 bc	ns	0.028*	0.046*
Leucine	2.32 bc	4.01 a	2.54 bc	2.66 bc	1.46 c	1.86 c	2.97 b	2.04 c	ns	0.016*	0.037*
Tyrosine	0.44 b	0.89 a	0.45 b	0.45 b	0.25 c	0.32 bc	0.60 ab	0.34 bc	ns	0.019*	0.027*
Phenylalanine	1.19 b	2.30 a	1.32 b	1.39 b	0.69 c	0.89 bc	1.51 b	1.03 bc	ns	0.024*	0.036*
Lysine	0.04 bc	0.16 a	0.04 bc	0.06 b	0.01 c	0.03 bc	0.07 b	0.04 bc	ns	0.016*	0.027*
Histidine	0.30 b	0.62 a	0.32 b	0.38 b	0.20 b	0.23 b	0.44 b	0.28 b	ns	0.042*	0.046*
Arginine	1.5 b	2.35 a	1.55 b	1.66 b	1.16 b	1.32 b	2.02 ab	1.38 b	ns	0.032*	0.042*
Proline	8.73 b	11.16 a	9.51 b	8.71 b	7.23 c	7.17 c	9.3 b	7.65 c	ns	0.047*	0.049*
Total AA	24.86 b	37.42 a	26.43 b	26.69 b	18.47 c	20.59 c	29.2 ab	21.94 c	ns	0.036*	0043*

[a] Means within the same row followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance. * Significant at 5%, ns: Not significant.

due to cultivar (see P-values), while the ratio of saturated/unsaturated showed significant variation due to the interaction effect of cultivar and seed priming treatment (Table 4). The hexadecanoic acid contents of grains in Backjung for all treatments and those in Keumkang primed with 2.5% KCl were significantly higher compared with those grains of unprimed and 1% K_2SO_4 primed Keumkang while grains from H_2O primed Keumkang had higher hexadecenoic acid content comparable to those primed with 2.5% KCl but was not significantly different from other treatments. Seed priming with 1% K_2SO_4 resulted in lowest hexadecenoic acid in Keumkang. Similarly, grains of Backjung had significantly higher contents of linoleic, α-linoleic acid, total FA, the total saturated and unsaturated FA compared to those in Keumkang regardless of the priming treatments. In Keumkang, priming with H_2O and the control (unprimed) had lowest linoleic and α-linoleic acid, total FA, the total saturated and unsaturated FA as compared to other treatment combinations.

Field Condition Experiments

Under field condition, significant interaction effect of cultivars and priming treatments were observed in dry matter yield, number of seeds per plant, grain yield, weight of 100 grains and harvest index (Table 5). Main cultivar effect was observed on panicle length only (Data not shown). Specifically, seed priming using 2.5% KCl reduced the dry matter yield in Keumkang but not in Backjung, while seed priming with 1% K_2SO_4 improved the growth of both cultivars as indicated by the higher dry matter yield. Seed priming with H_2O in Backjung resulted in lower dry matter yield comparable to the control. Keumkang had longer panicle length than Backjung regardless of the priming treatments. In terms of yield parameters, priming with 1% K_2SO_4 in Backjung had the highest increase in the number of seeds per plant, grain yield and harvest index, while seed priming with 2.5% KCl in Keumkang led to lowest number of seeds, total weight of grain and harvest index as compared to other treatments. The weight of 100 seeds was generally higher in Keumkang compared to Backjung regardless of treatment, except those produced by plants primed with 2.5% KCl. Priming with H_2O resulted in inferior yield quality parameters values compared to other treatments in Keumkang but not in Backjung.

Significant interaction effects between cultivar and priming treatments were observed in majority of the amino acids and the total AA in the grain of wheat grown under field conditions (Table 6). Among the different AA, only alanine, cysteine and valine contents did not vary significantly due to the interaction effect of cultivar and priming treatments; all other AA showed significant interaction effects (Table 6). The aspartic acid, threonine, serine, glutamic acid, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and proline in Keumkang significantly increased due to 2.5% KCl priming compared to other priming treatments. On the other hand, Backjung primed with 1% K_2SO_4 had the lowest contents of aspartic acid, threonine, serine, glutamic acid, glycine, methionine, isoleucine, leucine, tyrosine and phenylalanine compared to other treatment combinations including the control. Among the different AA, glutamic acids had the highest amount across different cultivar and priming treatments. Overall, the total AA was significantly increased by seed priming with 2.5% KCl in Keumkang while 1% K_2SO_4 in Backjung had the lowest total AA among the different treatment combinations under field condition. Like the greenhouse conditions, water priming led to AA contents comparable with the control in Keumkang, which was lower than those from the 2.5% KCl and 1% K_2SO_4 treatments. In Backjung, the total AA content was not affected by different priming agents.

Under field conditions, the Fatty Acid (FA) contents only showed significant interaction effect due to cultivar and priming treatment on the hexadecanoic, gondoic, oleic, and α-linoleic acids of grain (Table 7). The linoleic acid, the total FA, the total of saturated FA,

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Table 4. Fatty acid contents of grains (mg g^{-1}) in wheat cv Keumkang and Backjung grown under greenhouse conditions as affected by seed priming with summary of the P-values of two way analysis of variance.[A]

		Cultivars×Seed priming								P-values		
		Keumkang				Backjung						
Fatty acids	Symbol	2.5%KCl	1%K_2SO_4	H_2O	Unprimed	2.5%KCl	1%K_2SO_4	H_2O	Unprimed	Cultivar	Priming	Cultivar×Priming
Tetradecanoic acid	C14:0	0.03 a	0.03 a	0.03 a	0.03 a	0.03 a	0.03 a	0.03 a	0.03 a	ns	ns	Ns
Pentadecylic acid	C15:0	0.96 a	0.97 a	0.96 a	1.00 a	0.96 a	0.97 a	0.94 a	0.93 a	ns	ns	ns
Hexadecanoic acid	C16:0	4.20 a	3.93 b	3.83 ab	3.79 b	4.40 a	4.34 a	4.40 a	4.38 a	0.032*	0.046*	0.038*
Octadecanoic acid	C180	0.24 a	0.28 a	0.25 a	0.25 a	0.23 a	0.25 a	0.27 a	0.26 a	ns	ns	ns
Arachidic acid	C20:0	0.03 a	0.04 a	0.03 a	0.03 a	0.03 a	0.03 a	0.04 a	0.04 a	ns	ns	ns
Eicosanoic acid	C22:0	0.05 a	0.06 a	0.05 a	0.05 a	0.05 a	0.05 a	0.06 a	0.06 a	ns	ns	ns
Tricosanoic acid	C23:0	0.01 a	0.01 a	0.01 a	0.01 a	0.02 a	0.02 a	0.02 a	0.02 a	ns	ns	ns
Tetracosanic acid	C24:0	0.06 a	0.07 a	0.06 a	0.06 a	0.06 a	0.06 a	0.06 a	0.07 a	ns	ns	ns
Palmitoleic acid	C16:1	0.03 a	0.03 a	0.03 a	0.05 a	0.04 a	0.04 a	0.04 a	0.04 a	ns	ns	ns
Gondoic acid	C20:1	0.12 a	0.10 a	0.10 a	0.10 a	0.13 a	0.14 a	0.13 a	0.14 a	ns	ns	ns
Nervonic acid	C24:1	0.02 a	0.01 a	0.01 a	0.01 a	0.02 a	0.02 a	0.02 a	0.02 a	ns	ns	ns
Oleic acid	C18:1ω9	2.37 b	2.38 b	2.11 c	2.16 c	2.41 b	2.71 a	2.7 a	2.72 a	0.026*	0.038*	0.045*
Linoleic acid	C18:2ω6	11.82 b	11.30 b	10.78 c	10.82 c	12.64 a	12.67 a	12.56 a	12.93 a	0.046*	0.032*	0.048*
α-Linoleic acid	C18:3ω3	0.68 b	0.60 b	0.61 b	0.58 b	0.87 a	0.90 a	0.93 a	0.88 a	0.034*	ns	ns
Erucic acid	C22:1ω9	0.01 a	0.01 a	0.01 a	0.01 a	0.02 a	0.02 a	0.02 a	0.02 a	ns	ns	ns
Total	20.64 b	19.81 b	18.89 b	18.95 b	21.91 a	22.25 a	22.22 a	22.55 a	0.042*	ns	ns	
Saturated	5.58 b	5.39 b	5.22 b	5.22 b	5.78 a	5.75 a	5.82 a	5.79 a	0.038*	ns	ns	
Unsaturated	14.9 b	14.3 b	13.52 b	13.58 b	15.96 a	16.32 a	16.23 a	16.57 a	0.046*	ns	ns	
Ratio(Saturated-Unsaturated)	0.374 ab	0.377 a	0.386 a	0.384 a	0.362 b	0.352 b	0.359 b	0.349 b	0.034*	ns	0.046*	

[a] Means within the same row followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance. * Significant at 5%, ns: Not significant.

Table 5. Growth and yield performance of two wheat cultivars as affected by different seed priming solutions grown under field conditions with summary of the P-values of two-way analysis of variance.[a]

	Treatment Combinations		Plant height (cm)	Number of tillers (no $plant^{-1}$)	Dry matter yield (g $plant^{-1}$)	Panicle length (cm)	No of seeds/Plant	Grain yield (g $plant^{-1}$)	100 Seeds weight (g)	Harvest index
	Cultivars	Priming								
Keumkang	2.5% KCl		73.5 a	27.8 a	79.8 b	13.4 a	619 d	26.2 b	4.2 a	0.32 c
	1% K_2SO_4		75.2 a	30.2 a	91.3 a	13.3 a	809 c	34.7 ab	4.4 a	0.37 b
	H_2O		75.2 a	35.2 a	93.7 a	13.2 a	985 b	35.1 ab	3.6 b	0.37 b
	Unprimed		73.0 a	31.8 a	88.8 a	13.4 a	812 c	33.4 ab	4.1 a	0.38 b
Backjung	2.5% KCl		74.5 a	30.0 a	87.3 a	11.9 b	983 b	40.4 a	4.2 a	0.46 a
	1% K_2SO_4		75.5 a	31.2 a	89.8 a	11.7 b	1055 a	40.4 a	3.8 b	0.45 a
	H_2O		74.8 a	30.0 a	85.6 b	11.8 b	1037 a	37.7 ab	3.7 b	0.44 a
	Unprimed		73.5 a	33.8 a	82.0 b	11.5 b	1129 a	39.9 a	3.6 b	0.49 a
P value	Cultivar		ns	ns	ns	0.028*	ns	0.013*	ns	0.016*
	Priming		ns	ns	0.042*	Ns	0.026*	0.018*	0.014*	ns
	Cultivar× Priming		ns	ns	0.034*	Ns	0.031*	0.003*	0.008*	0.043*

[a] Means within the same row followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance. * Significant at 5%, ns: Not significant.

Table 6. Amino acids (mg g^{-1}) content of grains of wheat cvs. Keumkang and Backjung grown under field conditions as affected by seed priming with summary of the P-values of two way analysis of variance.

Amino acids	Cultivars×Seed priming								P-values		
	Keumkang				Backjung						
	2.5% KCl	1% K_2SO_4	H_2O	Unprimed	2.5% KCl	1% K_2SO_4	H_2O	Unprimed	Cultivar	Priming	Cultivar×Priming
Aspartic acid	3.66 a	3.51 a	3.16 b	3.13 b	3.17 b	2.74 c	3.4 ab	3.01 b	Ns	0.023*	0.042*
Threonine	1.8 a	1.66 b	1.31 c	1.42 bc	1.27 c	1.05 d	1.35 c	1.22 c	Ns	0.032*	0.036*
Serine	1.82 a	1.64 b	1.17 c	1.26 c	1.05 c	0.88 c	1.1 c	1.1 c	0.036*	0.024*	0.034*
Glutamic acid	27.19 a	23.24 b	19.23 c	21.36 b	17.14 c	13.53 d	16.83 c	15.24 c	0.038*	0.018*	0.022*
Glycine	1.74 a	1.55 a	0.86 b	0.98 b	0.94 b	0.6 c	1.03 b	0.69 c	Ns	0.046*	0.034*
Alanine	4.35 a	4.12 a	3.83 a	4.06 a	3.86 a	3.49 a	4.03 a	3.86 a	Ns	ns	Ns
Cystine	0.78 a	0.68 a	0.61 a	0.65 a	0.60 a	0.54 a	0.61 a	0.60 a	Ns	ns	Ns
Valine	5.00 a	4.60 a	4.37 a	4.61 a	4.30 a	3.86 a	4.37 a	4.24 a	Ns	ns	Ns
Methionine	1.26 a	1.11 a	1.02 a	1.07 a	0.95 b	0.82 ab	0.92 b	0.90 b	0.043*	0.032*	0.023*
Isoleucine	4.44 a	3.98 b	3.83 b	4.10 b	3.53 c	3.16 d	3.53 c	3.53 c	0.046*	0.028*	0.036*
Leucine	7.93 a	7.13 b	6.7 b	7.22 b	6.34 c	5.59 c	6.35 c	6.22 c	0.032*	0.024*	0.034*
Tyrosine	2.21 a	1.94 a	1.77 a	1.88 a	1.78 a	1.48 b	1.70 a	1.62 a	Ns	0.028*	0.032*
Phenylalanine	5.44 a	4.78 ab	4.44 b	4.84 ab	3.90 c	3.42 c	3.91 c	3.83 c	0.043*	0.044*	0.046*
Lysine	1.88 a	1.84 a	1.39 b	1.41 b	1.44 b	1.17 c	1.59 b	1.26 c	Ns	0.032*	0.022*
Histidine	2.24 a	2.04 ab	1.75 b	1.88 b	1.71 b	1.43 c	1.75 b	1.57 c	0.023*	0.038*	0.039*
Arginine	3.89 a	3.75 a	3.30 b	3.43 b	3.57 b	3.08 c	3.74 a	3.28 b	Ns	0.022*	0.033*
Proline	12.79 a	10.99 b	10.77 b	11.88 ab	9.69 bc	8.78 c	9.35 bc	9.86 bc	0.019*	0.037*	0.029*
Total	88.41 a	78.55 b	69.51 c	75.18 b	65.24 cd	55.61 d	65.56 cd	62.03 cd	0.026*	0.046*	0048*

[a] Means within the same row followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance. . * Significant AT WHAT LEVEL?? ns: Not significant.

Table 7. Grain content of fatty acids (mg g^{-1}) in wheat cvs. Keumkang and Backjung grown under field conditions as affected by seed priming with summary of the P-values of two-way analysis of variance.[a]

Fatty acids	Symbol	Cultivars × Seed priming								P-values		
		Keumkang				Backjung				Cultivar	Priming	Cultivar×Priming
		2.5% KCl	1% K_2SO_4	H_2O	Unprimed	2.5% KCl	1% K_2SO_4	H_2O	Unprimed			
Tetradecanoic acid	C14:0	0.02 a	0.02 a	0.02 a	0.02 a	0.02 a	0.03 a	0.02 a	0.03 a	ns	ns	ns
Pentadecylic acid	C15:0	0.99 a	0.94 a	0.94 a	0.93 a	0.95 a	0.96 a	0.96 a	0.94 a	ns	ns	ns
Hexadecanoic acid	C16:0	3.50 c	3.64 c	3.83 b	3.57 c	4.03 ab	4.42 a	3.96 b	4.16 ab	0.042*	ns	0.032*
Octadecanoic acid	C180	0.16 a	0.17 a	0.17 a	0.16 a	0.15 a	0.16 a	0.14 a	0.15 a	ns	ns	ns
Arachidic acid	C20:0	0.02 a	0.03 a	0.02 a	0.03 a	0.02 a	0.02 a	0.02 a	0.02 a	ns	ns	ns
Eicosanoic acid	C22:0	0.04 a	0.04 a	0.04 a	0.04 a	0.04 a	0.05 a	0.04 a	0.05 a	ns	ns	ns
Tricosanoic acid	C23:0	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a	0.01 a	ns	ns	ns
Tetracosanic acid	C24:0	0.04 a	0.04 a	0.04 a	0.04 a	0.04 a	0.04 a	0.03 a	0.04 a	ns	ns	ns
Palmitoleic acid	C16:1	0.03 a	0.03 a	0.03 a	0.03 a	0.03 a	0.04 a	0.03 a	0.04 a	ns	ns	ns
Gondoic acid	C20:1	0.08 b	0.09 ab	0.11 a	0.10 a	0.11 a	0.11 a	0.10 a	0.10 a	0.043*	0.033*	0.044*
Nervonic acid	C24:1	0.01 a	0.01 a	0.02 a	0.01 a	0.02 a	0.02 a	0.02 a	0.02 a	ns	ns	ns
Oleic acid	C18:1ω9	1.61 c	1.71 b	1.77 b	1.70 b	1.82 a	1.96 a	1.73 b	1.76 b	ns	0.046*	0.048*
Linoleic acid	C18:2ω6	9.05 c	9.34 c	9.98 b	9.6 bc	10.34 b	11.3 a	10.12 b	10.49 b	0.036*	ns	ns
α-Linoleic acid	C18:3ω3	0.48 c	0.50 c	0.60 b	0.53 c	0.69 b	0.78 a	0.66 b	0.70 ab	0.042*	0.028*	0.046*
Erucic acid	C22:1ω9	0.01 a	0.01 a	0.02 a	0.01 a	0.02 a	0.02 a	0.02 a	0.01 a	ns	ns	ns
Total		16.06 c	16.59 c	17.59 b	16.79 c	18.28 b	19.93 a	17.87 b	18.51 ab	.022*	ns	ns
Saturated fatty acids		4.78 c	4.89 c	5.07 c	4.80 c	5.26 b	5.69 a	5.18 b	5.4 ab	0.043*	ns	ns
Unsaturated fatty acids		11.16 d	11.57 c	12.39 c	11.85 c	12.89 b	14.08 a	12.55 b	12.98 b	0.039*	ns	ns
Ratio (Saturated/Unsaturated)		0.43 a	0.42 a	0.41 b	0.41 b	0.41 b	0.40 b	0.41 b	0.42 b	ns	ns	0.04*

[a] Means within the same row followed by the same letter(s) are not significantly different from each other based on Tukey's Test at 5% level of significance* Significant AT WHAT LEVEL?? ns: Not significant.

and the total unsaturated FA only showed main effects due to cultivar (data not shown), while the ratio of saturated/unsaturated FAs was significantly affected by the interaction effect of cultivar and seed priming (Table 7). Significantly higher contents of hexadecanoic acid were observed in Backjung primed with 2.5% KCl and 1%K_2SO_4. Seed priming with 1% K_2SO_4 in Backjung significantly increased the Linoleic, α-linoleic acid, total FA, the total saturated and unsaturated FA compared to other treatment combinations. On the other hand, priming with 2.5% KCl in Keumkang resulted in lowest hexadecanoic, gondoic, linoleic and α-linoleic acid, total FA, and the total saturated and unsaturated FA in grain as compared to other treatment combinations. The ratio of the total saturated to unsaturated FA were significantly higher in Keumkang with 2.5% KCl and 1% K_2SO_4 priming treatments as compared to other treatment combinations.

DISCUSSION

Seed priming is widely used to invigorate seed in order to reduce emergence time, accomplish uniform emergence, and give better crop stand (Ashraf and Foolad, 2005; Ibrahim, 2016; ur Rehman *et al*., 2015; Zheng *et al*., 2016) by improving the seed's potential to rapidly imbibe and revive the seed metabolism for germination. Additional benefits of seed priming include increasing the tolerance of plants to adverse environmental conditions (Singh *et al*., 2015). In this study, the influence of seed priming using 2.5% KCl, 1% K_2SO_4 and water resulted in different growth, yield, and metabolic response based on the AA and FA profiles of grain of two wheat cultivars grown under two distinct environmental conditions. It is established that environmental condition dominantly influenced plant growth, yield and the biochemical properties of plants; hence, this study primarily examined the response of the two cultivars of wheat (Keumkang and Backjung) on different priming solutions independently under the greenhouse and field conditions.

Influence of Priming on Growth and Yield

In the greenhouse environment, seed priming with 1% K_2SO_4 consistently improved the growth of Keumkang and Backjung much better than 2.5% KCl and water. Under field condition, contrasting and inconsistent effects of seed priming with 2.5% KCl and 1% K_2SO_4 were observed on several growth and yield parameters of Keumkang and Backjung. While 1% K_2SO_4 increased the dry matter and grain yield in Backjung, 2.5% KCl decreased the dry matter and grain yield of Keumkang. Although several reports had established that seed priming of wheat using KCl and water improved germination, growth, and yield of wheat, results showed the higher potential of K_2SO_4 as seed priming agent in improving growth and yield (Islam *et al*., 2015; Ashraf *et al*., 2018; Mustafa *et al*., 2017). This result corroborates with that of Hussain *et al*. (2013) which showed that seed soaked in 0.5 to 1% solution of potassium sulfate (K_2SO_4) significantly increased plant height, yield attributes and grain yield in wheat. Aliloo (2015) also found out that fertilization of wheat with K_2SO_4 improved seedling vigor and enhanced seedling performance. Results of this study suggest that seed priming with 2.5% KCl or 1% K_2SO_4 have a carryover effect on the growth and yield of wheat based on enhanced growth and development of plants from vegetative stage until the reproductive stage. However, the response of plants is specific on the genetic make-up as shown by the different responses of the two cultivars on a priming agent. Further, this study showed that the carry-over effect on growth and yield due to seed priming varied under different environmental conditions. For example, seed priming with 2.5% KCl increased the yield of Backjung and

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Keumkang under greenhouse condition but decreased the dry matter and grain yield of Keumkang under field conditions. Indeed, 1% K_2SO_4 improved the growth of both cultivars in greenhouse condition, but not for Keumkang under field condition. Moreover, even if there was no consistent effect of these priming agents on the growth and yield, with specific testing of most appropriate priming agent in each cultivar or plants, beneficial carry-over effect on growth of plants was observed.

Biochemical Properties of Wheat Grain

In wheat, grain protein content and amino acid composition are the most important characteristics in determining its nutritional value. Unlike the previous studies on the influence of different seed priming agents (H2O, $CaCl_2$ and others) on the biochemical properties of germinating seeds (Mouradi *et al*., 2016; Kubal *et al*., 2015; Seyyedi *et al*., 2015), results of this study showed a distinct modification of the AA and FA profiles in grain harvested from plants that were treated with 2.5% KCl and 1% K_2SO_4 under different environmental conditions (Tables 3, 4, 6, and 7). Under greenhouse conditions, seed priming of Keumkang with 1% K_2SO_4 increased the total amino acids in the grain, especially the aspartic acid, threonine, glutamic acid, alanine, valine, methionine, isoleucine, leucine, tyrocine, phenylalanine, lysine, histidine, arginine, and proline. On the other hand, seed priming with 2.5% KCl and 1% K_2SO_4 decreased the total AA in the grain of Backjung, especially the amount of threonine, methionine isoleucine, leucine, tyrosine, phenylalanine and proline. Similarly, under field conditions, specific effects of seed priming treatments included the increase of the total AA in Keumkang due to 2.5% KCl priming. Seed priming with 1% K_2SO_4 in Backjung produced the lowest AA in grain. These results indicate that seed priming with 2.5% KCl and 1%K_2SO_4 has a carry-over effect on the quality of amino acids and hence grain protein. In previous studies, the influence of K^+ in the priming media resulted in increase in the AA and protein synthesis (Mengel, 2016; Hojjati and Maleki, 1972). Similarly, sulfur status in relation to nitrogen levels in spring wheat changes the amino acid composition of the whole grain, especially the amount of cysteine, threonine, leucine, isoleucine, lysine and methionine (Granvogl *et al*., 2007). Based on these results, it appeared that the potassium and sulfur in the priming media increased the synthesis of AA, but its effect was more expressed in Keumkang than in Backjung. The potassium and sulfur in, respectively, KCl and K_2SO_4 is responsible for the alteration of the AA profiles in the grains of wheat as compared to water priming. These imply that, depending on the cultivar, different seed priming agent can modify the AA profile of grain, which will have a profound and consequential effect on the nutritional and rheological properties or bread-making potentialities of the flours. For instance, increase in glutamic acid and proline in the grain of Keumkang due to 1% K_2SO_4 priming under greenhouse condition and due to 2.5% KCl priming under field condition will increase the gluten contents of the flour and will affect the workability and elasticity of the dough during bread production (Gallagher *et al*., 2004). Gluten contains hundreds of protein components, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups (Wrigley *et al*., 2006). Follow up studies on the impact of AA alteration specific to a cultivar and due to different priming agents on the rheological properties is warranted.

Lipids are present only in a small quantity in cereals, but they have a significant effect on the quality and the texture of food because of their ability to associate with proteins due to their amphipathic nature with starch, forming inclusion complexes (Šramková *et al*., 2009). The degree of influence of seed priming on FA is not evident as compared to the changes on AA, but there exists a distinct modification on the

FA components due to seed priming on different cultivars of wheat. The higher amounts of the total FA contents, saturated FAs, and the unsaturated FAs in the grain of Backjung compared to Keumkang indicate that the amount of FA in the grain of wheat is highly dependent on the genetic material of plant, regardless of the priming treatments (Tables 4 and 7). However, under greenhouse conditions, seed priming with 2.5% KCl increased the hexadecanoic acid, oleic acid, linoleic acid and α-linoleic acid in Keumkang, but no effect was observed in Backjung. Seed priming using 2.5% KCl and 1% K_2SO_4 did not trigger significant changes in FAs contents in Backjung, except in methionine and threonine. On the other hand, under field conditions, the priming effects were expressed as an increase in the hexadecanoic, gondoic, oleic, and α-linoleic acids of grain of Backjung primed with 1% K_2SO_4 and a reduction of the same FAs in Keumkang primed with 2.5% KCl and 1% K_2SO_4. These results indicate that seed priming triggered a carry-over effect on FA composition in grain, which is highly specific of the plant type or cultivar. It appears that Keumkang is more sensitive to seed priming than Backjung in terms of modification of FAs in grain. Further investigation is necessary to elucidate the molecular basis of the differences of cultivar response to seed priming.

In addition, seed priming with 2.5% KCl and 1% K_2SO_4 promoted unsaturation of FA in Keumkang grown in the greenhouse and in Backjung in the field, respectively. Some studies show that the extent of unsaturation of fatty acids is correlated with the potential of photosynthetic machinery to tolerate stress by alleviating the damage to PSI and PSII and improving the healing of injury due to abiotic stress (Allakhverdiev *et al*., 2001; Sui *et al*., 2010). However, this result contrasts with the well-established findings that high temperature decreases lipid unsaturation, while low temperature promotes it in order to maintain optimal membrane fluidity and membrane stability as temperature changes (Upchurch, 2008). This discrepancy could be attributed to a wider range of day-night temperature, rather than the increase in temperature per se. This would also indicate that the effect of different priming agent on FA profiles is dependent on the growing condition and on the cultivar of wheat.

Overall, this study showed that seed priming with 2.5% KCl, 1% K_2SO_4 and water influenced the growth, yield, and the AA and FA profiles in the grain of Keumkang and Backjung and the magnitude of change is the product of the plant's interaction with growing environmental conditions and genetic make-up of the plants. It was also found out that seed priming with 2.5% KCl and 1% K_2SO_4 triggered specific changes in the AA and FA composition in grain, as carry-over effects on the plant's metabolic adjustments that started from alteration of biochemical processes during its germination until maturity. These carry-over effects are specific to the cultivar and the growing environmental conditions. These relative compositional changes in amino acids and fatty acids induced by seed priming will have a profound impact on grain quality and this necessitates future investigation. Amino acids and lipids are present only to a small extent in cereals, but they have a significant effect on the quality and the texture of foods (Sramkova *et al*., 2009).

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اثر آماده سازی بذر روی رشد، عملکرد، و ترکیب بیوشیمیایی دانه دو کولتیوار گندم

م. کهاینگ، و. ی. اولترا جر، و س. چول لی

چکیده

اثر آماده سازی(priming) بذر روی رشد، و محتوای آمینو اسید ها و اسیدهای چرب دو کولتیوار گندم (*Triticum aestivum* L.) به نام های Keumkang و Backjung با استفاده از یک آزمایش گلدانی در گلخانه و آزمون مزرعه ای ارزیابی شد. در هر یک از شرایط آزمایش، چهار تیمار آماده سازی شامل خیساندن در سه محیط کلرید پتاسیم ۲/۵٪ ، سولفات پتاسیم ۱٪، و آب مقطر، و تیمار شاهد بدون آماده سازی بذر در یک آزمایش با طرح بلوک های کامل تصادفی در چهار تکرار اعمال شد. در شرایط گلخانه، تیمار سولفات پتاسیم ۱٪ منجر به افزایش رشد و عملکرد گندم شد در حالیکه کلرید پتاسیم ۲/۵٪ عملکرد ماده خشک را در کولتیوار Keumkang کاهش داد و لی نه در کولتیوار Backjung. در شرایط مزرعه، سولفات پتاسیم ۱٪رشد هر دو کولتیوار را بهبود بخشید و عملکرد Backjung را افزایش داد در حالیکه کلرید پتاسیم ۲/۵٪ کمترین عملکرد را داشت. به طور کلی این آزمایش نشان داد که آماده سازی با کلرید پتاسیم ۲/۵٪ وسولفات پتاسیم ۱٪ منجر به تغییرات مشخصی در ترکیب اسیدها آمینه(AA) و اسیدهای چرب (FA) در دانه شد و اثر ادامه دار روی تنظیمات متابولیک داشت که ویژه هر کولتیوار و شرایط محیطی محل رشد بود. تغییرات در ترکیب AA و FA ناشی از آماده سازی بذر روی کیفیت دانه و آرد گندم تاثیر عمده ای میگذارد.

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