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Full Length Article

Selenium Nutrition for Yield Enhancement and Grain Biofortification of Wheat through Different Application Methods

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Abstract

Selenium (Se) is an essential nutrient for humans and it enters in the food chain by plant uptake from the agricultural soils. However, about 15% of world's population is Se deficient due to its deficiencies in soil causing deficiency in human nutrition. This study was carried out to evaluate the influence of Se supplementation through various methods on two bread wheat cultivars (Shafaq-2006 and Lasani-2008) under field conditions. Selenium was applied through seed priming (0.125 and 1.25 m*M* Se solution), seed coating (0.5 and 1.0 g Se kg^{-1} seed), foliar spray (50 and 100 g Se ha^{-1}) and soil application (50 and 100 g Se ha^{-1}) using Na_2SeO_4 as source. Results show that Se application by either method significantly improved the growth, grain yield and grain Se concentration compared to no Se application. Among the application methods, soil fertilization at 100 g ha^{-1} and foliar spray at 50 g ha^{-1} displayed greater leaf area index, crop growth rate, grains per spike, thousand grain weight, biological yield, grain yield and harvest index. Performance of cultivar Lasani-2008 remained better than Shafaq-2006 in both quantitative and qualitative terms. Maximum grain Se enrichment was recorded in Lasani-2008 with foliar spray of 100 g Se ha^{-1}. Seed priming with 0.126 m*M* Se solution proved more economical and showed highest net return (Rs. 73176) followed by foliar spray at 50 g ha^{-1} (Rs 71385). Present study suggests that Se application by either method could improve growth, yield and grain Se concentration in bread wheat.

Keywords: Wheat; Selenium; Seed priming; Seed coating; Foliar application; Soil application

Introduction

Selenium (Se), a metalloid, is an essential element for human beings (Combs, 2001). It is an integral constituent of more than 30 seleno-enzymes involved in various metabolic activities (Rayman, 2002). Selenium acts as an antioxidant, antiviral and anticancer, and helps in imparting resistance against diseases associated with oxidative cell damage like hepatitis, and AIDS (Lyons *et al*., 2003). About 15% of the world population is suffering from Se deficiency (Grusak and Chakmak, 2005; Thacker *et al*., 2006). In Pakistan, very low levels of blood Se have been noted in the rural masses (Farrakh *et al*., 2011). It is alarming to note that intake of diet having low Se may cause about 40 health disorders and diseases (Clark *et al.*, 1998).

Intake of foods having low Se content is the primary reason for Se deficiency in human populations (Gibson, 2006; Graham *et al*., 2007). Issue of low Se in plant-based food may be addressed through agronomic bio-fortification techniques (Broadley *et al*., 2010; Fairweather-Tait *et al*., 2011; Chilimba *et al*., 2012). The prime purpose of agronomic biofortification is to produce micronutrient dense, safe and nutritious food. Such approaches are effective owing to their better adaptability and high benefit to cost ratios (Mayer *et al.*, 2008). Reports indicated that cereals accumulate more bioavailable Se than other sources (Bugel *et al.*, 2002). Bread wheat (*Triticum aestivum* L.) is the principal dietary source around the globe (Kroon and Williamson, 1999) and is known as an efficient Se accumulator than other cereals (Golubkina and Alfthan, 1999). Beside this, wheat can also be grown successfully on high Se containing soils (seleneferous soils) (Ducsay *et al.*, 2009). However, wheat grains are quite low in Se because of low Se in the soil rhizosphere (i.e., 0.05 mg kg^{-1} (McNeal and Balistrieri, 1989). This demands Se fertilization to maintain the optimum Se level in soil for plant uptake (Ahmad *et al.*, 2009; Khan *et al.*, 2010). Rate and supplementation methodologies for Se application are extremely vital for the enhancement of Se concentration in wheat grains (Natasha *et al.*, 2018).

Soil and foliage applications are considered the most reliable ways to supply the micronutrient. However, risk of

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leaf burn, high cost of application equipment, lack of skills required for its application and damage of standing crop prevent wider adoptability of foliar application of fertilizers (Farooq *et al.,* 2012). In case of soil application, plants utilize only a little quantity of the total applied micronutrient while the major portion becomes inaccessible due to leaching or fixation (Mikkelsen *et al.,* 1989). Seed treatments are gaining attention for supply of micronutrients because of less nutrient loss and low cost. Less quantity of nutrients is required for seed treatment, so it may be economical than other methods (Farooq *et al.,* 2012). Seed treatment with micronutrients may be done either by coating of micronutrient on the surface of seed or by soaking of seeds in nutrient solution for specific time at specific concentration named as osmopriming (Farooq *et al.,* 2009). Various studies have exhibited positive impact of seed treatment (seed priming and/or coating) with different micronutrients on seed germination, seedling growth, yield and concentration of micronutrients in grains. Recently, Imran *et al*. (2017) reported that seed priming of maize with 4 m*M* solution increased 3-fold zinc concentration in endosperm and 50 folds in testa compared to hydro-primed seeds. They also observed higher early seedling growth and Zn translocation towards shoot in priming treatments than hydro-priming. Iqbal *et al.* (2017) observed that seed priming with 0.05 *M* boron solution of borax improved grain yield and grain B concentration up to 27% compared to control. Wheat seeds primed in Se solution (5 mg Se L^{-1}) for different time intervals were found effective in promoting chlorophylls, anthocyanin, proline, antioxidant activities and plant growth under cold stress (Akladious, 2012). Chen and Sung (2001) studied the impact of different treatments of seed priming with sodium selenite (1, 2, 5 and 10 mg L^{-1} for 48 h) on the emergence of bitter gourd and reported that seed priming with solution at 2 mg L^{-1} of sodium selenite exerted more positive effects on the germination of bitter gourd by scavenging the production of free radical and peroxides. Rehman and Farooq (2016) reported that seed coating with $ZnSO_4$ and $ZnCl_2$ improved growth, productivity and zinc concentration in grain of wheat. Cartes *et al.* (2005) found that pelleting of ryegrass seed with selenium increased antioxidant ability and Se contents in shoot.

Although seed treatment emerged as a promising technique in boosting seedling emergence, vigor and yield, the potential and performance of seed treatment for grain enrichment needs further exploration. To the best of our knowledge, different Se application methods (including seed priming, seed coating, foliar spray and soil application) have never been compared for their influence on growth, yield and grain enrichment in wheat under field conditions. Therefore, this study was conducted to explore the potential of various Se fertilization strategies including seed priming, seed coating, soil application and foliar spray on growth, yield and Se grain concentration of bread wheat.

Materials and Methods

Experimental Design

This study was carried out at Agronomic Research Farm, University of Agriculture, Faisalabad (31.25°N, 73.06°E and 183 msl) during 2011–2012 and 2012–2013. Seeds of two bread wheat cultivars (Shafaq-2006 and Lasani-2008) were obtained from the Wheat Research Institute, Faisalabad, Pakistan. The experiment was laid out in factorial arrangement under randomized complete block design with a net plot size of 2.7 m × 6 m having three replications.

Crop Husbandry

Wheat was planted on November 23, 2011 and 2012 with a hand drill in 22.5 cm spaced rows using 100 kg ha^{-1} seed rate. Before sowing, composite soil samples were taken from different locations of the experimental field to a depth of 30 cm. The samples were air dried, ground and sieved through 2 mm strainer before analysis. The physico-chemical analysis of experimental soil is presented in Table 1. Nitrogen and phosphorus were applied as urea and di-ammonium phosphate (DAP) at 100 kg N and 90 kg P_2O_5 ha^{-1}, respectively. Whole of P and 1/3rd of N were applied at seed bed preparation, whereas remaining N was applied in two equal installments at first and second irrigations. Crop was irrigated 20 days after sowing (DAS) and succeeding irrigations were applied keeping in view the crop water requirements. Selective herbicide Atlantis 306-WG (30 g kg^{-1} mesosulfuron-methyl + 6 g kg^{-1} iodosulfuron-methyl-sodium) was sprayed 25 DAS to ensure weed free field. Experimental plots were harvested manually on 15th and 20th April, 2012 and 2013, respectively and threshed to record the yield and yield components.

Experimental Treatments

The study comprised of four Se application methods including seed priming, seed coating, foliar application and soil application. Selenium solution was prepared by dissolving Na_2SeO_4 in distilled water and applied through osmopriming (0.125 and 1.25 m*M* Se), seed coating (0.5 and 1.0 g Se kg^{-1}), foliage spray (50 and 100 g ha^{-1}) and soil addition (50 and 100 g ha^{-1}), respectively. Four control treatments including untreated (dry seed), hydro priming (HP), seed coating with Arabic gum (SC AG) and water foliar spray were also included. Sprayer was calibrated before foliar spray and equal amount of water was used for all treatments i.e., 250L/ha. Selenium was applied on the soil at the time of sowing while foliar application of Se was performed in two splits, at jointing (45 days after sowing) and booting (60 days after sowing).

For seed priming, seeds of both wheat cultivars were soaked in aerated respective Se solutions (0.125 m*M* and 1.25 m*M*) and distilled water (for hydro priming treatment) for 12 h by maintaining 1:5 (w/v) seed solution ratio.

Aquarium pump was used to ensure aerated conditions during priming. Seeds were surface washed thrice with distilled water after priming treatment and allowed to dry back under forced air at 27ºC ± 3 under shade closer to original weight.

For seed coating, at first, seeds were coated with inert adhesive material (Arabic gum). Consequently, seeds treated with Arabic gum were coated with a fine layer of Se (0.5 and 1 g Se kg^{-1} seed), respectively.

Observations and Data Collection

Allometric Parameters

Leaf area index (LAI): Leaf area index was calculated following Watson (1952). A randomly selected area of 0.5 × 0.5 m^2 was harvested at ground level with an interval of 15 days started at 45 days after sowing up to 120 days after sowing. Each plant sample was divided into green leaves and stem. At first, fresh weight of detached green leaves was recorded. Leaf area of 5 g leaf sample was noted with leaf area meter (JVC TK-5310).

Crop growth rate (g m^{-2} day^{-1}): The above harvested plant samples was dried in an oven at 70˚C till constant weight and recorded total dry weight. Crop growth rate was calculated after an interval of 15 days using formula of Hunt, (1978).

Morphological and Yield Parameters of Wheat

Number of productive tillers were counted at two different sites from each plot and averaged. From each plot, ten spikes were harvested manually, threshed and the grains were counted. Two samples of 1000 grain were obtained from each experimental unit by using electric counter and weighed to compute average 1000 grain weight. Wheat was manually harvested, tied into bundles and kept in field for sun drying for a week in respective plots. Balance was used to monitor the biological yield from each treatment which was later transformed to tones per hectare (t ha^{-1}). After threshing, grain yield was recorded with weighing balance in kilogram and transformed to tons per hectare (t ha^{-1}). Harvest index (%) of each experimental plot was computed using formula of Beadle (1987).

Grain Se Analysis

Dried ground (2 mm sieve) grain samples were taken in a digestion tube and gently poured 10 mL of concentrated HNO_3 in small portions. Then 10 mL of $HClO_4$ was added in digestion tubes with gentle swirling and incubated overnight. Again, 5 mL nitric acid (concentrated) along the walls of digestion tubes was added and the tubes were placed in digestion block and again heated. The digestion tubes were detached from the block, allowed to cool and diluted to 10 mL volume with 6 *M* HCl to reduce oxidation of Se^{+6} to Se^{+4} and shifted the digest to a 50-mL flask for Se analysis (Gupta, 1998). The filtrate was analyzed for Se contents by ICP-OES (Optima 2100 DV Perkin-Elmer).

Statistical Analysis

Analysis of variance for all the studied parameters were performed using computer aided software Statistix 8.1 (Analytical software, Statistics; Tallahassee, FL, USA, 1985–2003). For comparison of treatment means, least significance difference (LSD) was applied at 5% probability level (Steel *et al.*, 1997). Orthogonal contrasts analysis was performed to compare Se application methods for Se grain enrichment.

Results

Data show that wheat growth, yield contributing parameters, biological and economic yield and Se enrichment in grains of both wheat cultivars were significantly affected by all the Se supplementation methods at $P \leq 0.05$. Interaction between methods of Se application and wheat cultivars was mostly non-significant for various parameters except grain Se concentration. It is evident from our results that Se supplementation by either method or rate showed beneficial effects in promoting growth and yield as well as Se accumulation in wheat grains.

Crop Growth

Selenium application significantly improved the LAI and CGR in both tested wheat cultivars (Fig. 1 and 2). Overall, Lasani-2008 exhibited higher LAI than Shafaq-2006. Highest LAI was observed in Se priming treatments (Fig. 1). A gradual rise in crop growth rate (CGR), up to third harvest, was observed and after that a declining trend was noted. Highest CGR was recorded in treatments where Se was applied through soil application at 100 g ha^{-1} than other Se treatments (Fig. 2).

Grain Yield and Related Traits

Selenium application methods significantly affected the productive tillers and number of grains per spike. Wheat cultivars also varied for productive tillers and number of grains per spike; however, interaction of Se application methods and wheat cultivars was non-significant (Table 2). Cultivar Lasani-2008 had more productive tillers and grains per spike than the cultivar Shafaq-2006 during both growing seasons. Soil and foliar application of Se improved the productive tillers while other methods did not differ for the productive tillers significantly (Table 2). Results also show that Se application methods improved the number of grains per spike during first year of study while during second year of study, seed priming with 0.125 m*M* Se, foliar spray at 50 g Se ha^{-1} and soil application at 100 g ha^{-1} improved the number of grains per spike; while rest of application methods did not differ significantly with control treatments (Table 2). Maximum productive tillers and number of grains per spike were noted from soil Se application at 100 g ha^{-1} (Table 2).

Table 1: Physico-chemical analysis of soil of experimental site

Characteristics	Unit	Value
Texture		Sandy loam
pH		8.1
EC_e	dSm^{-1}	0.29
Organic matter	%	0.65
Nitrogen (N)	%	0.049
Phosphorus (P)	ppm	8.1
Potassium (K)	ppm	110
Total Se concentration	ppm	0.058

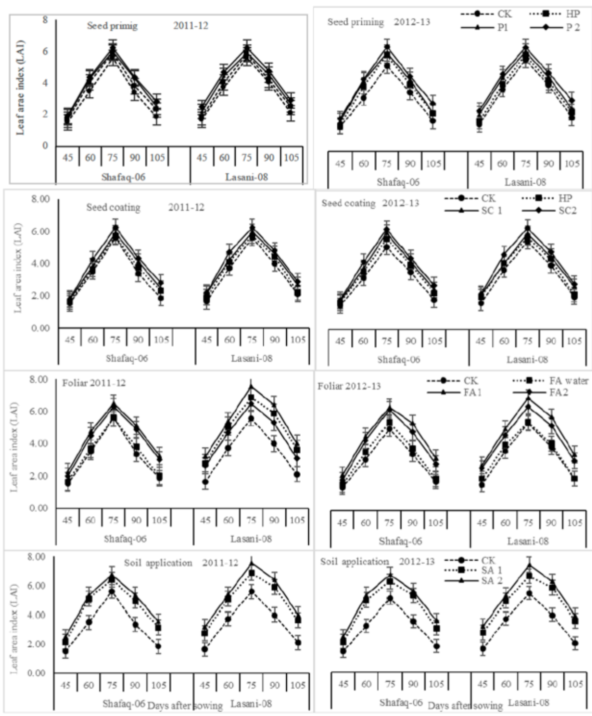

Fig. 1: Leaf area index (LAI) of bread wheat as influenced by Se application methods

Where CK = Control without any treatment, HP = Hydro priming, SP_1 = Seed priming with Se at the rate of 0.125 m*M*, SP_2 = Seed priming with Se at the rate of 1.25 m*M*, SC AG = Seed coating with Arabic gum only, SC_1 = Seed coating with Se at the rate of 0.5 g kg^{-1}, SC_2 = Seed coating with Se at the rate of 1 g kg^{-1}, FA water = foliar spray with water only, FA_1 = Foliar application of Se at the rate of 50 g ha^{-1}, FA_2= Foliar application of Se at the rate of 100 g ha^{-1}, SA_1 = Soil application of Se at the rate of 50 g ha^{-1}, SA_2 = Soil application of Se at the rate of 100 g ha^{-1}

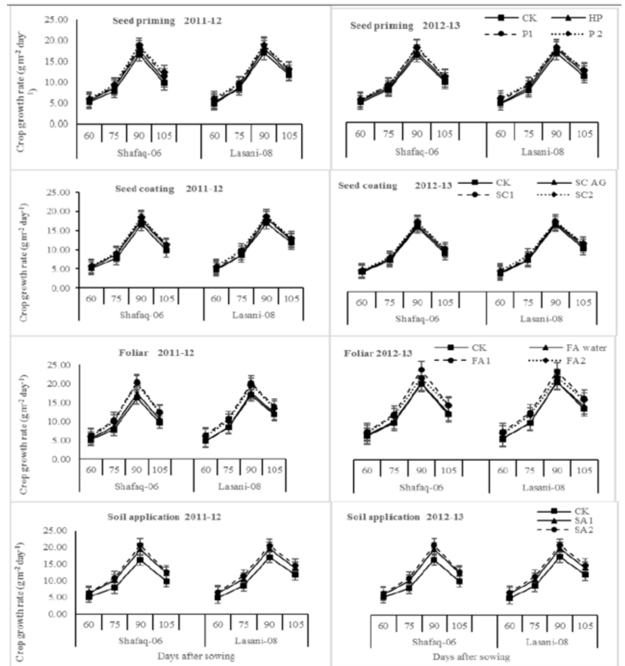

Fig. 2: Crop growth rate (CGR) of bread wheat as influenced by Se application methods

Where CK = Control without any treatment, HP = Hydro priming, SP_1 = Seed priming with Se at the rate of 0.125 m*M*, SP_2 = Seed priming with Se at the rate of 1.25 m*M*, SC AG = Seed coating with Arabic gum only, SC_1 = Seed coating with Se at the rate of 0.5 g kg^{-1}, SC_2 = Seed coating with Se at the rate of 1 g kg^{-1}, FA water = foliar spray with water only, FA_1 = Foliar application of Se at the rate of 50 g ha^{-1}, FA_2 = Foliar application of Se at the rate of 100 g ha^{-1}, SA_1 = Soil application of Se at the rate of 50 g ha^{-1}, SA_2 = Soil application of Se at the rate of 100 g ha^{-1}

Selenium application methods significantly affected 1000 grain weight and biological yield. Highest 1000 grain weight was observed in treatment from soil application of Se at 100 g ha^{-1} during 2011–2012, which was statistically similar with all the treatments where Se was applied irrespective of methods during 2011–2012. However, during 2012–2013, soil application of Se at both rates, foliage feeding with 50 g Se ha^{-1}, and seed priming with 1.25 m*M* Se solution exhibited higher 1000 grain weight than other treatments (Table 3). Application of Se also enhanced the biological yield of wheat irrespective of application methods. Highest biological yield was recorded with soil applied Se at the rate of 100 g ha^{-1} during both years of experimentation. Lasani-2008 showed higher biological yield than Shafaq-2006 during both years (Table 3).

Selenium application methods significantly affected the grain yield of wheat. Wheat cultivars also differed for grain yield. Cultivar Lasani-2008 had higher yield than Shafaq-2006 during both growing seasons (Table 4). Highest grain yield was observed in Se treated soil at 100 g Se ha^{-1}, which was statistically at par with soil applied Se at the rate of 50 g ha^{-1}, foliar application at 50 g Se ha^{-1} and seed priming at 1.25 m*M* Se during both growing seasons (Table 4). Harvest index of wheat was affected significantly by Se application methods during both growing seasons. However, there was no significant difference between wheat cultivars. Interaction of selenium application methods and wheat cultivars was non-significant. During first growing season (2011–2012), a highest harvest index was observed in soil applied Se treatment at 100 g Se ha^{-1} while during 2012–13, foliar nourishment at 50 g ha^{-1} produced the maximum harvest index (Table 4).

Economic analysis of wheat as affected by Se supplementation through various application methods and wheat cultivars on two-year average basis for Shafaq-

Table 2: Influence of selenium application methods on productive tillers and number of grains per spike of bread wheat

SAM	Productive tillers (m^{-2})						Number of grains per spike					
	2011–2012			2012–2013			2011–2012			2012–2013		
	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean
UT	300	332	316 D	288	336	312 D	33.55	35.50	34.53 CDE	33.45	35.14	34.30 E
HP	312	348	330 CD	300	344	322 CD	33.93	36.83	35.96 DE	33.73	36.06	34.90 E
SP_1	352	372	362 A-D	336	360	348 A-D	34.57	37.70	36.13 BC	34.58	37.87	36.26 CDE
SP_2	360	384	372 A-D	348	376	362 A-D	36.22	38.77	37.49 AB	36.17	41.23	38.7 BC
SC AG	316	344	330 CD	304	328	316 CD	33.58	35.22	34.41 DE	33.72	35.55	34.63 E
SC_1	348	360	354 BCD	332	356	344 BCD	34.73	36.60	35.67 B-E	34.83	36.48	35.66 DE
SC_2	356	368	362 A-D	340	368	354 A-D	35.68	37.00	36.42 BC	35.87	37.12	36.49 CDE
WFS	320	348	334 CD	308	340	324 CD	33.23	35.20	34.22 D	33.45	34.91	34.18 E
FA_1	384	436	398 ABC	376	420	398 AB	38.43	39.45	38.94 A	38.18	40.33	39.26 AB
FA_2	372	412	392 ABC	356	400	378 ABC	34.90	37.67	36.28 BCD	34.67	37.65	36.16 DE
SA_1	376	420	410 AB	364	416	390 AB	36.52	38.35	37.43 AB	36.30	39.03	37.98 BCD
SA_2	392	456	424 A	388	436	412 A	38.60	40.00	39.30 A	38.43	44.37	42.25 A
Mean (WC)	349 B	381.67 A		336.67 B	373.33 A		35.33 B	37.35 A		35.28 B	37.98 A	
LSD ($p \leq 0.05$)	SAM = 0.086, WC = 0.35			SAM = 0.92; WC = 0.38			SAM = 1.903, WC = 0.777			SAM = 2.536, WC = 1.035		

Where UT = Untreated seed, HP = Hydro priming, SP_1 = Seed priming with Se at 0.125 m*M*, SP_2 = Seed priming with Se at 1.25 m*M*, SC AG = Seed coating with Arabic gum, SC_1 = Seed coating with Se at 0.5 g kg^{-1}, SC_2 = Seed coating with Se at 1 g kg^{-1}, WFS = Water foliar spray, FA_1 = Foliar application of Se at 50 g ha^{-1}, FA_2 = Foliar application of Se at 100 g ha^{-1}, SA_1 = Soil application of Se at 50 g ha^{-1}, SA_2= Soil application of Se at 100 g ha^{-1}, LSD= Least significant difference, SAM=Se application methods, WC= wheat cultivars, SH-06 = Shafaq-2006, LS-08 = Lasani-2008. Figures sharing the same letters for a parameter do not differ significantly at $p \leq 0.05$

Table 3: Influence of selenium application methods on 1000-grain weight (g) and biological yield (t ha^{-1}) of bread wheat

SAM	1000-grain weight (g)						Biological yield (t ha^{-1})					
	2011–2012			2012–2013			2011–2012			20112–2013		
	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean
UT	42.45	34.27	37.62 D	40.67	34.10	37.38 D	12.43	12.83	12.63 Cd	11.78	12.43	12.11 D
HP	43.09	34.23	38.66 BCD	41.37	34.30	37.84 CD	12.87	12.87	12.87 Bcd	12.00	12.80	12.40 D
SP_1	44.40	35.47	39.93 A-D	43.23	35.53	39.38 BCD	12.87	13.77	13.32 A-D	12.67	13.77	13.22 BCD
SP_2	44.83	35.93	40.38 ABC	44.57	37.10	40.83 AB	13.83	14.10	13.97 Ab	13.32	14.05	13.68 ABC
SC AG	42.00	33.83	37.92 CD	40.63	34.22	37.43 D	12.22	13.12	12.67 Cd	11.62	12.58	12.100 D
SC_1	44.17	35.50	39.83 A-D	42.77	35.31	39.04 BCD	12.48	13.50	12.99 Bcd	12.48	13.20	12.84 CD
SC_2	44.50	35.78	40.14 A-D	43.77	35.65	39.71 BCD	13.13	14.10	13.62 Abc	12.69	13.67	13.18 BCD
WFS	42.20	34.03	38.12 CD	40.40	34.03	37.22 D	12.33	12.27	12.30 D	11.92	12.42	12.17 D
FA_1	45.15	36.70	40.93 AB	45.15	38.00	41.57 AB	13.97	14.52	14.24 A	13.80	14.62	14.21 AB
FA_2	44.98	36.40	40.69 AB	43.80	35.34	39.57 BCD	13.17	13.93	13.55 Abc	13.13	13.32	13.23 BCD
SA_1	45.57	37.23	41.00 A	44.38	36.32	40.35 ABC	13.50	14.23	13.87 Ab	13.67	13.72	13.69 ABC
SA_2	46.20	37.95	42.08 A	46.13	39.17	42.65 A	14.03	14.77	14.40 A	14.20	14.97	14.58 A
Mean (WC)	44.13 A	35.61 B		43.07 A	35.76 B		13.07 B	13.67 A		12.77 B	13.46 A	
LSD ($p \leq 0.05$)	SAM = 2.49; WC= 1.02			SAM = 2.76; WC= 1.13			SAM = 1.130; WC = 0.461			SAM = 1.22; WC = 0.499		

Where UT = Untreated seed, HP = Hydro priming, SP_1 = Seed priming with Se at 0.125 m*M*, SP_2 = Seed priming with Se at 1.25 m*M*, SC AG = Seed coating with Arabic gum, SC_1 = Seed coating with Se at 0.5 g kg^{-1}, SC_2 = Seed coating with Se at 1 g kg^{-1}, WFS = Water foliar spray, FA_1 = Foliar application of Se at 50 g ha^{-1}, FA_2 = Foliar application of Se at 100 g ha^{-1}, SA_1 = Soil application of Se at 50 g ha^{-1}, SA_2 = Soil application of Se at 100 g ha^{-1}, LSD = Least significant difference, SAM= Se application methods, WC= wheat cultivars, SH-06 = Shafaq-2006, LS-08 = Lasani-2008. Figures sharing the same letters for a parameter do not differ significantly at $p \leq 0.05$

2006 and Lasani-2008 respectively presented in Table 7. Results of the two-year average economic analysis of Shafaq-2006 revealed that seeds primed with 0.125 m*M* Se solution resulted in highest net return (Rs. 73176) while foliar spray of 50 g Se ha^{-1} was ranked second (Rs. 71385). Selenium supplementation through seed priming at 1.25 m*M* and foliar spray at 50 g ha^{-1} proved equally better (Rs. 66900, 66059). However, seed coating with Se at 1 g kg^{-1} and Se foliar spray at 100 g ha^{-1} proved less economical due to lower net benefits than other treatments.

Selenium Concentration in Wheat Grain (μg g^{-1})

Selenium supplementation methods significantly affected the Se accumulation in wheat grains during both years of study. Significant difference was noticed between wheat cultivars (Table 5). Cultivar Lasani-2008 had higher accumulation of Se in grains than the cultivar Shafaq-2006. Foliar application of Se at 100 g ha^{-1} was the most effective among the Se supplementation treatments and showed maximum Se enrichment in grains in wheat cultivar Lasani-2008 that was statistically higher as compared to Shafaq-2006 during both years of study (Table 5). Orthogonal contrasts analysis showed that grain Se concentration was significantly influenced by all the Se supplementation methods compared to control. It is also evident from the contrast table that Se supplementation methods differed significantly among each other except seed priming and seed coating (Table 6).

Table 4: Influence of selenium application methods on grain yield (t ha^{-1}) and harvest index (%) of bread wheat

SAM	Grain yield (t ha^{-1})						Harvest index (%)					
	2011–2012			2012–2013			2011–2012			20112–2013		
	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean	SH-06	LS-08	Mean
UT	4.01	4.23	4.12 EF	3.73	4.01	3.87 F	32.25	32.99	32.62 D	31.68	32.28	31.96 DEF
HP	4.25	4.28	4.27 DEF	3.82	4.17	3.99 DEF	33.05	33.29	33.17 AB	31.81	32.55	32.21 C-F
SP$_1$	4.27	4.64	4.45 B-F	4.17	4.55	4.36 CDE	33.16	33.66	33.41 BCD	32.89	33.08	33.45 A-D
SP$_2$	4.65	4.80	4.73 ABC	4.47	4.67	4.57 ABC	33.56	34.02	33.79 ABC	33.54	33.21	33.18 A-E
SC AG	4.05	4.29	4.17 EF	3.68	4.08	3.88 EF	33.06	32.71	32.89 CD	31.71	32.40	31.24 F
SC$_1$	4.14	4.50	4.32 C-F	4.07	4.35	4.21 C-F	33.16	33.32	33.24 BCD	32.58	32.98	33.35 A-E
SC$_2$	4.37	4.68	4.52 B-E	4.26	4.52	4.39 BCD	33.25	33.17	33.21 BCD	33.54	33.10	33.77 ABC
WFS	4.08	3.99	4.03 F	3.76	4.07	3.91 DEF	33.10	32.52	32.81 CD	31.52	32.75	31.61 EF
FA$_1$	4.75	4.94	4.85 AB	4.67	5.03	4.85 AB	34.01	34.02	34.01 AB	33.82	34.40	34.91 A
FA$_2$	4.38	4.65	4.52 B-E	4.35	4.42	4.39 BCD	33.20	33.39	33.29 BCD	33.12	33.18	32.95 B-F
SA$_1$	4.51	4.81	4.66 A-D	4.58	4.65	4.62 ABC	33.37	33.80	33.59 A-D	33.54	33.90	33.72 A-D
SA$_2$	4.80	5.16	4.98 A	4.83	5.20	5.01 A	34.17	34.95	34.56 A	34.04	34.74	34.42 AB
Mean (WC)	4.35 B	4.58 A		4.20 B	4.48 A		33.28	33.49		32.82	33.21	
LSD ($p \leq 0.05$)	SAM = 0.445, WC = 0.181			SAM =0.481, WC = 0.196			SAM = 1.002			SAM= 1.807		

Where UT = Untreated seed, HP = Hydro priming, SP$_1$ = Seed priming with Se at 0.125 m*M*, SP$_2$ = Seed priming with Se at 1.25 m*M*, SC AG = Seed coating with Arabic gum, SC$_1$ = Seed coating with Se at 0.5 g kg^{-1}, SC$_2$ = Seed coating with Se at 1 g kg^{-1}, WFS = Water foliar spray, FA$_1$ = Foliar application of Se at 50 g ha^{-1}, FA$_2$ = Foliar application of Se at 100 g ha^{-1}, SA$_1$ = Soil application of Se at 50 g ha^{-1}, SA$_2$ = Soil application of Se at 100 g ha^{-1}, LSD = Least significant difference, SAM= Se application methods, WC= wheat cultivars, SH-06 = Shafaq-2006, LS-08 = Lasani-2008. Figures sharing the same letters for a parameter do not differ significantly at $p \leq 0.05$

Table 5: Influence of selenium application methods on grain Se concentration (μg g^{-1}) of bread wheat

SAM	2011–2012			2012–2013		
	SH-06	LS-08	Mean (Se)	SH-06	LS-08	Mean (Se)
UT	0.04 m	0.04 m	0.04 H	0.04 l	0.04 l	0.04 G
HP	0.03 m	0.03 m	0.03 H	0.02 l	0.03 l	0.03 H
SP$_1$	1.92 j	2.31 hi	2.11 F	1.82 j	2.63 h	2.22 E
SP$_2$	2.56 h	3.06 g	2.81 E	2.88 h	3.47 g	3.18 E
SC AG	0.04 m	0.04 m	0.04 H	0.04 l	003 l	0.03 G
SC$_1$	1.14l	1.53 k	1.34 G	1.18 k	1.59 j	1.39 G
SC$_2$	2.09 ij	2.01 ij	2.05 F	2.21 i	2.26 h	2.24 F
WFS	0.03 m	0.04 m	0.04 H	0.03 l	0.04 l	0.04 H
FA$_1$	5.04 d	5.28 d	5.16 C	5.24 d	5.53 d	5.39 C
FA$_2$	6.90 b	7.70 a	730 A	7.18 b	8.25 a	7.72 A
SA$_1$	3.80 f	4.37 e	4.08 D	4.21 f	4.78 e	4.50 C
SA$_2$	5.83 c	6.11 c	5.97 B	6.31 c	6.30 c	6.30 B
Mean (WC)	2.45 B	2.71 A		2.60 B	2.91 A	
LSD ($p \leq 0.05$)	SAM = 0.242; WC = 0.0991; Interaction = 0.3435			SAM = 0.2302; WC = 0.0940; Interaction = 0.3256		

Where UT = Untreated seed, HP = Hydro priming, SP$_1$ = Seed priming with Se at 0.125 m*M*, SP$_2$ = Seed priming with Se at 1.25 m*M*, SC AG = Seed coating with Arabic gum, SC$_1$ = Seed coating with Se at 0.5 g kg^{-1}, SC$_2$ = Seed coating with Se at 1 g kg^{-1}, WFS = Water foliar spray, FA$_1$ = Foliar application of Se at 50 g ha^{-1}, FA$_2$ = Foliar application of Se at 100 g ha^{-1}, SA$_1$ = Soil application of Se at 50 g ha^{-1}, SA$_2$ = Soil application of Se at 100 g ha^{-1}, LSD = Least significant difference, SAM= Se application methods, WC= wheat cultivars, SH-06 = Shafaq-2006, LS-08 = Lasani-2008. Figures sharing the same letters for a parameter do not differ significantly at $p \leq 0.05$

Table 6: Contrast analysis for grain Se concentration (μg g^{-1}) of bread wheat as influenced by Se supplementation through different application methods

	2011–2012 2012–2013		2012–2013 2012–2013	
Contrasts	SH-06	LS-08	SH-06	LS-08
Ck *VS*. All	0.04 VS 2.67**	0.036 VS 2.952**	0.04 VS 2.83**	0.03 VS 3.17 **
SP *VS*. SC	1.50 VS 1.09 ns	1.798 VS 1.191 ns	1.57 VS 1.14 ns	2.04 VS 1.29 ns
SP *VS*. FA	1.5 VS 3.58 **	1.798 VS 4.039**	1.57 VS 3.81 **	2.04 VS 4.36 **
SP *VS*. SA	1.5 VS 5.43 **	1.798 VS 5.693**	1.57 VS 5.78**	2.04 VS 5.91**
SC *VS*. FA	1.09 VS 3.58**	1.191 VS 4.039**	1.14 VS 3.81**	1.29 VS 4.36**
SC *VS*. SA	1.09 VS 5.43**	1.191 VS 5.693**	1.14 VS 5.78**	1.29 VS 5.91**
FA *VS*. SA	3.58 VS 5.43**	4.039 VS 5.693**	3.81 VS 5.78**	4.36 VS 5.91**

Where CK = Control; SP = Seed priming; SC = Seed coating; FA = Foliar application; SA = Soil application; LS-08= Lasani-2008; SH-06 = Shafaq-2006

Discussion

This study evaluated the impact of different Se application methods including seed priming, seed coating, foliar and soil application on growth, yield and grain bio-fortification of wheat. Our results show that Se fertilization by either methods improved leaf area index, crop growth rate, yield attributes and grain yield. Stimulatory effects of Se on

Table 7: Economic analysis of wheat as influenced by Se supplementation using different application methods (two-year average)

SAM	Grain Yield ($t\ ha^{-1}$)	Straw Yield ($t\ ha^{-1}$)	Adjusted Grain Yield ($t\ ha^{-1}$)	Adjusted Straw Yield ($t\ ha^{-1}$)	Income Grain Yield ($t\ ha^{-1}$)	Income Straw Yield ($t\ ha^{-1}$)	Gross Income	Total cost	Net Benefit
UT	3.87	8.24	3.48	7.41	113198	27793	140991	82883	62255
HP	4.03	8.40	3.63	7.56	117975	28350	146325	83773	65707
SP_1	4.22	8.55	3.80	7.70	123338	28856	152194	85600	73176
SP_2	4.56	9.02	4.10	8.12	133331	30431	163763	99970	66900
SC AG	3.87	8.05	3.48	7.25	113100	27169	140269	83467	62460
SC_1	4.10	8.38	3.69	7.54	120023	28283	148305	99357	54594
SC_2	4.31	8.60	3.88	7.74	126116	29025	155141	115103	45415
WFS	3.92	8.21	3.53	7.38	114660	27692	142352	83322	60819
FA_1	4.71	9.17	4.24	8.26	137768	30960	168728	102061	71385
FA_2	4.37	8.78	3.93	7.91	127725	29644	157369	115400	44951
SA_1	4.55	9.04	4.09	8.13	132952	30503	163455	100446	66059
SA_2	4.82	9.30	4.34	8.37	140888	31388	172275	116505	61768
Remarks			10% less than actual	10% less than actual	RS 32500/t	RS 32500/t			

Where UT = Untreated seed, HP = Hydro priming, SP_1 = Seed priming with Se at 0.125 m*M*, SP_2 = Seed priming with Se at 1.25 m*M*, SC AG = Seed coating with Arabic gum, SC_1 = Seed coating with Se at 0.5 g kg^{-1}, SC_2 = Seed coating with Se at 1 g kg^{-1}, WFS = Water foliar spray, FA_1 = Foliar application of Se at 50 g ha^{-1}, FA_2 = Foliar application of Se at 100 g ha^{-1}, SA_1 = Soil application of Se at 50 g ha^{-1}, SA_2 = Soil application of Se at 100 g ha^{-1}, SAM= Se application methods

growth of various crops have been reported including tea leaves (Hu *et al.,* 2003), lettuce (Xue *et al*., 2001; Rios *et al*., 2009), ryegrass (Hartikainen *et al*., 2004) and potato (Turakainen *et al*., 2004). Various investigations revealed that Se supplementation enhanced grain yield of wheat (Nawaz *et al.,* 2015), maize (Chilimba *et al.,* 2012), lentil (Thavarajah *et al.,* 2015), soybean (Yang *et al*., 2003) and rice (Zhang *et al.,* 2014). Recently, Jiang *et al.* (2015) reported that application of optimum dose of Na_2SeO_3 (6 mg kg^{-1}) in tobacco accelerated the growth of plants by stimulating photosynthesis, stomatal conductance, carboxylation and rubisco contents. Though, Se is not considered essential nutrient for plants, it exerts numerous beneficial effects in maintaining plant growth especially under various stresses like drought, light, radiation, salinity and heavy metals (Kong *et al*., 2005; Lyons *et al*., 2009; Cartes *et al*., 2011; Xu *et al.,* 2017; Zhou *et al*., 2017). Selenium application improves antioxidant system in plants which resultantly stimulate plant growth and increase grain yield by scavenging radicals and better translocation of metabolites during photosynthesis (Lyons *et al.,* 2009; Pilon-Smits and Quinn, 2010). It has been observed that plant experiences various types of stresses during growth period under field conditions. Higher growth and yield in our study because of Se application might be because of above mentioned beneficial impacts of Se on plants.

Our results also reveal that Se contents in wheat grains were significantly influenced by Se application methods and maximum Se accumulation was recorded with foliar application of Se at 100 g ha^{-1} followed by soil application at the rate of 100 g ha^{-1} compared to other Se supplementation methods. Our results are in line with the findings of Thavarajah *et al.* (2015) who reported that Se applied on the plant foliage substantially boosted lentil biological and grain yield, and seed Se enrichment. The greater accumulation of Se in grain by foliar application might be due to better transport of Se through vascular system when applied to leaves (Boldrin *et al*., 2013; Nawaz *et al*., 2015).

Pre-seeding treatments (seed coating and seed priming) with Se also proved viable option for wheat grain enrichment, though comparatively less effective than soil and foliar application but substantially significant than control. Cartes *et al.* (2005) also reported enhanced Se uptake by ryegrass seedlings as a result of pelleting of seed with selenium. Ožbolt *et al.* (2008) found higher Se concentration in leaves, stem and seeds of buckwheat as a result of seed soaking in selenium solution. Higher uptake of Se due to seed treatment might be due to closer association of Se with seeds, which allows early uptake and faster translocation of Se from roots to shoots. Although, various studies have reported enhanced uptake of different micronutrients by seed treatment including seed coating and seed priming (Cartes *et al.*, 2005; Farooq *et al.*, 2009; Rehman and Farooq 2016; Imran *et al.,* 2017; Iqbal *et al.*, 2017), mechanisms (molecular and physiological) behind excessive uptake is unknown and need elucidation.

Soils in the world are Se deficient and contain low Se content (average 0.05 mg kg^{-1}) and Se fertilization strategies have been found effective in boosting the yield and nutritional quality of the wheat grains on low Se soils. This shows that Se fertilization at suitable rate is important for obtaining higher yield and better grain Se contents. Application of Se at optimised rates proved safe and did not show any signs of toxicity on wheat growth and can be safely used for Se biofortification on broader scale as evidenced in Finland to raise the Se content in the soil derived food to combat Se malnutrition (Wang *et al*., 1998).

Nonetheless, our findings suggest that Se supplementation by either method improved both yield and nutritional status of wheat grain. The results of our study will encourage the farmers to adopt our Se supplementation strategies and rates without any fear of crop failure and at the same time assist the stake holders to formulate and launch a policy to counteract the Se malnutrition. Frequent availability of Se fertilizer and better market rate for the Se biofortified wheat may be a positive step for ensuring secure food to keep the world free form sufferings of Se malnutrition.

Conclusion

Application of Se by either method, at all rates, was effective in improving the performance of wheat, and grain Se enrichment. However, soil application at 100 g ha^{-1} was better in improving the performance of wheat, and foliar spray at 100 g Se ha^{-1} in Se grain enrichment. Further studies are required to explore the mechanism of Se-induced growth promotion and grain enrichment.

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