

## **BIOFORTIFICATION OF WHEAT FLOUR WITH SELENIUM THROUGH FOLIAR APPLICATION OF WHEAT CROP**

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### **ABSTRACT**

In Egypt, dietary selenium (Se) intake is below recommendation. Foliar fertilization with Se has been reported to increase the Se content in cereals. The main objective of this study was to increase the Se content in the wheat flour using Se foliar fertilization (as biofortification). Two field experiments were selected for the study (Station Farm at Mansoura and Kalabsho-Zayian region, Faculty of Agriculture, Mansoura University) during the winter season of 2010/2011. Sodium selenate were foliar-applied at two different growth stages and different concentrations. A randomized complete block design with four replications was used. Foliar Fertilization of wheat crop with sodium selenite increased the Se content in the whole wheat flour, depending on the level and time of the addition. The maximum Se content ( $22.2 \pm 0.43 \mu\text{g}/100\text{g}$ ) was found in wheat crop after spraying at 50 and 70 days from the agriculture, which is higher than the control sample level ( $1.9 \pm 0.41 \mu\text{g}/100\text{g DM}$ ). There was a relationship between the amount of Se applied to the wheat crop and the carry through/retention of Se in the downstream flour approximately linear indicating efficient incorporation and retention of Se in the wheat crop through to grain harvest and flour production.

**Keywords:** Seelenium, Foliar fertilization, Wheat flour, Biofortification

### **INTRODUCTION**

Micronutrients deficiency are widely distributed (FAO, IFAD& WFP, 2012). The dietary intake of Se, essential micronutrient, is below recommendations of  $55 \mu\text{g}/\text{day}$  (Elmadfa, 2009, Institute of Medicine, 2000). Epidemiological studies have linked insufficient dietary Se intake with a number of health disorders, including oxidative stress-related conditions, reduced fertility and immune function, cardiomyopathy, and an increased risk of cancers (Rayman, 2012; Reid *et al.*, 2008; Zeng & Combs, 2008). The dietary intake of Se is highly depended on foods being consumed. The content of Se in animal and plant-based foods depend on its content in the soil where the animal or plant is grown (Hartikainen, 2005; Uden *et al.*, 2004; Barclay *et al.*, 1995). The Se content in Egyptian foods is relatively low because of the low Se content in the Egyptian soil.

Various approaches by which dietary Se intake could be improved: (1) increasing the consumption of Se-rich foods, (2) biofortification of foods with Se, and (3) using Se dietary supplements. Agronomic biofortification has become cost-effective tool to increase the Se content in various food plants and has been used on many occasions with various products, including broccoli and rice (Finley, 1999).

In Egypt, cereals are the main dietary energy source, providing about 66% of energy (FAO, 2005). Among cereals, wheat (approx. 363 g/capita/day) is frequently consumed as baladi bread with each meal. The increase of Se content in the Egyptian wheat flour could be beneficial to the public health. Foliar application of Se in the form sodium selenate was found to increase the Se content in the wheat crop and the resulted wheat flour. Aim of this study was to produce bio-fortified wheat flour with Se content through Se foliar application to wheat crop.

## **MATERIALS AND METHODS**

### **Materials**

#### **Wheat grains**

The Egyptian wheat cultivar Sakha 93 (resistant to smut, soil salinity and lodging) was obtained from the Wheat Research Section, Agricultural Research Center.

#### **Chemicals**

Selenium as Sodium Biselenite was obtained from Alpha Chemika tm. Nitric acids (HNO<sub>3</sub>), HCL and HClO<sub>4</sub> were obtained from El-Gomhouria Company for Trading Pharmaceutical Chemical & Medical.

### **Methods**

#### **Study design**

Two field experiments were carried out at the Experimental Station Farm in Mansoura and Kalabsho-Zayian region, Faculty of Agriculture, Mansoura University, Egypt during the winter season of 2010/2011 to determine the effect of Se treatments (rates and time of application) as foliar spraying on Se content of wheat flour. A Randomized Complete Block Design of ten treatments with four replications was carried out as follows: Control (without Se addition), spraying with 2.5 g Se/fed after 50, 70 and 50+70 days from sowing; spraying with 5.0 g Se/fed after 50, 70 and 50+70 days from sowing and spraying with 7.5 g Se/fed after 50, 70 and 50+70 days from sowing.

The foliar solution was completed to 200 L/fed and spraying was conducted by hand sprayer until saturation point. The experimental unit area in each location was 3 X 3.5 m occupying an area of 10.5 m<sup>2</sup> (i.e. 1/400 fed). The experiment in Mansoura location was carried out in clay loam soil with medium fertility. While, the experiment in Kalabsho-Zayian location was carried out in a sandy soil with little fertility. Soil samples were taken at random from the experimental field area to determine Se content in the soils.

The experimental field was well prepared through two ploughings, compaction and then divided into the experimental units with dimensions as previously mentioned. The cultivation took place on November 19<sup>th</sup> and 24<sup>th</sup> in Mansoura and Kalabsho-Zayian location, respectively. Wheat grains at the rate of 75 and 90 kg/fed were sown by using broadcasting Afir method in Mansoura and Kalabsho-Zayian locations, respectively. The common agricultural practices such as irrigation, fertilization (NPK), weed and pest control for growing wheat in clayey and sandy soils according to the

recommendations of Ministry of Agriculture and Land Reclamation were followed, except the factors under study.

#### **Wheat milling**

The harvested wheat grain were cleaned from debris and milled in East Delta Flour Mills (Mansoura Flour Mills, Mansoura, Egypt) using tecanor mill to obtain a whole meal flour. Samples from whole meal flour (100 g) were vacuum-packed in polyethylene bags and stored at  $-20\text{ }^{\circ}\text{C}$  until Se quantification. Moisture determinations were carried out on all samples to calculate dry matter by drying in an air oven at  $105\text{ }^{\circ}\text{C}$  for 16 h according to AOAC (2005).

#### **Selenium quantification:**

Sample were digested with 5 ml of a mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  (v/v, 4:1) at  $130\text{ }^{\circ}\text{C}$  for 1 h. After cooling, 5 ml of concentrated HCl was added and heated at  $115\text{ }^{\circ}\text{C}$  for 20 min. Subsequently a clear solution was obtained and made up to 50 ml with distilled water for total Se determination. Se was quantified using atomic absorption spectrophotometer.

#### **Statistical analysis:**

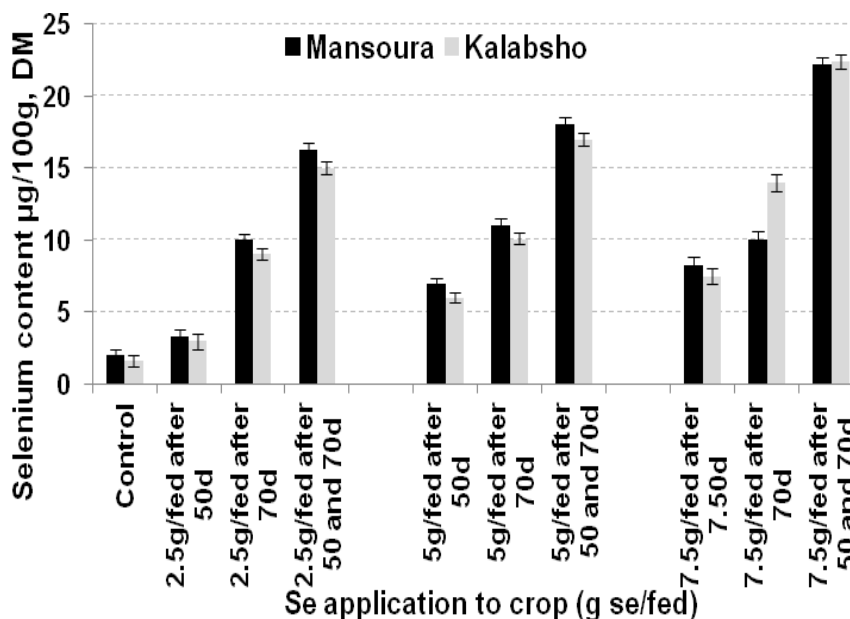
Data were expressed as mean  $\pm$  STD and were statistically analyzed according to the technique of analysis of variance (ANOVA) for randomized complete block design as published by Gomez and Gomez (1984), using MSTAT statistical package (MSTAT-C with MGRAPH version 2.10, Crop and Soil Sciences Department, Michigan State University, USA). Least Significant Difference (LSD) method was used to test the differences between treatment means at 5 % level of probability as described by Snedecor and Cochran (1980).

## **RESULTS AND DISCUSSION**

Se intakes within the Egyptian population are lower than recommended intakes of  $55\text{ }\mu\text{g/day}$ , and therefore increasing the Se levels within the population are required. The results from the current study indicated efficient incorporation and retention of Se in the wheat flour in response to foliar Se application. The findings from this trial and others (Hart *et al.*, 2011; Curtin *et al.*, 2006) reporting increased Se content in wheat flour through foliar fertilization with Se. This approach would in itself probably be adequate to deal with the current shortfall in Se intakes in the Egyptian population.

Wheat is an important source of Se (Broadley *et al.*, 2010), and its content can be increased by application of Se to the growing crop or to the soil (Curtin *et al.*, 2006). The initial Se content in both soils (Mansoura and Kalabsho-Zayian) was similar, 0.087 and 0.031 ppm respectively and therefore no significant difference were found in Se content in wheat grown in both locations after fertilization with Se. Wheat crop showed great ability to accumulate Se (Fig. 1). The highest Se content ( $22.2\pm 0.43\text{ }\mu\text{g}/100\text{g DM}$ ) was found in wheat after spraying with 7.5 g Na-Selenate after 50 and 70 days from agriculture, which is higher than the control sample level ( $1.9\pm 0.41\text{ }\mu\text{g}/100\text{g DM}$ ). The average increase of Se content as result of fertilization

ranged from 0.8 to 12- fold compared to control (Fig. 1). Similar increase was reported by others (Hart *et al.*, 2011; Curtin *et al.*, 2006).



**Figure 1: Selenium content (mean  $\pm$  STD) in whole wheat flour as a function of Se application to the wheat crop cultivated in two different locations (Mansoura and Kalabsho-Zayian, Egypt)**

The increase in Se content (~12 fold) in wheat flour after fertilization of wheat crop with Se depend on the level and time of the addition as no significant differences between the two location of the agriculture (Mansoura and Kalabsho-Zayian) (Tables 1 and Fig. 1) were observed. These results are in agreement with those of Hart *et al.* (2011) who reported that Se content of untreated whole meal flour was 0.035  $\mu\text{g/g}$  and increased to more than 2.2  $\mu\text{g/g}$  in wholemeal flour produced from grain treated with Se (100 g/ha, as selenate).

**Table 1: Selenium content ( $\mu\text{g}/100\text{g}$ , DM) of whole wheat flour a function of Se application to the wheat crop cultivated in two different locations (Mansoura and Kalabsho-Zayian)**

Se application to crop (g se/fed)	Mansoura		Kalabsho-Zayian	
	Se content (DM) $\mu\text{g}/100\text{g} \pm \text{SD}$	Proportion of increase (%)	Se content (DM) $\mu\text{g}/100\text{g} \pm \text{SD}$	Proportion of increase (%)
I. Se application to crop after 50 days from the agriculture				
0	1.9 $\pm$ 0.41		1.6 $\pm$ 0.35	
2.5	3.3 $\pm$ 0.47	73	3 $\pm$ 0.55	85
5	6.8 $\pm$ 0.34	252	5.9 $\pm$ 0.29	266
7.5	8.2 $\pm$ 0.62	327	7.5 $\pm$ 0.55	362
II. Se application to crop after 70 days from the agriculture				
0	1.9 $\pm$ 0.41		1.6 $\pm$ 0.35	
2.5	9.8 $\pm$ 0.42	410	9.1 $\pm$ 0.34	457
5	11.3 $\pm$ 0.49	485	10.4 $\pm$ 0.29	538
7.5	14 $\pm$ .057	400	13.6 $\pm$ 0.62	733
III. Se application to crop after 50 and 70 days from the agriculture				
0	1.9 $\pm$ 0.41		1.6 $\pm$ 0.35	
2.5	16.2 $\pm$ 0.45	746	15.4 $\pm$ 0.41	846
5	18.2 $\pm$ 0.5 $\gamma$	845	17.3 $\pm$ 0.45	963
7.5	22.2 $\pm$ 0.4 $\gamma$	1053	21.2 $\pm$ 0.56	1202
F. test	*		*	

The relationship between the Se concentration applied to the wheat crop and the carry through/retention of Se in the resulted flour was approximately linear (Fig. 2), indicating efficient incorporation of Se in the wheat crop through to grain harvest, flour production. With increasing the amount of sodium selenite applied to the field the Se content was increased linearly ( $R^2 = 0.967$  and  $0.98$  in two different locations) (Fig. 2). This confirmed that the Na-selenite sprayed on the experimental soil is taken up by plants and mainly transformed in cereal grains. These results are in agreement with Hart *et al.* (2011) who reported a linear relationship between Se content of the wheat or flour and the amount of sodium selenate applied to the field up to at least 100 g/ha. Others (Eurola *et al.*, 2004) have also reported that the rate and mode of Se application are likely to have a dominant influence on grain Se levels

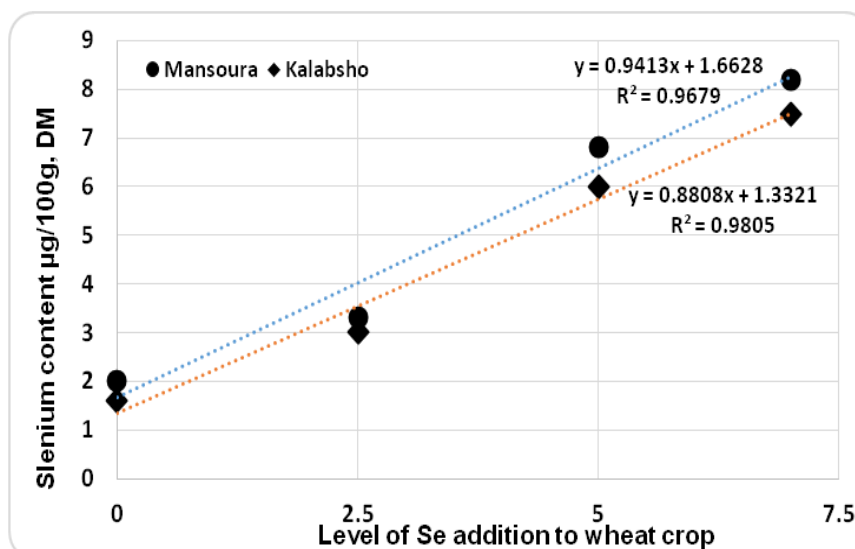


Figure 2: Level of selenium addition to wheat crop in relation to selenium content of flour (µg/100g, DM).

## CONCLUSION

This study shows that Se content in wheat flour increased significantly after foliar spraying with Se. The increase of Se content in wheat flour mainly depend on time and concentration of added Se. Indicating that the quantity of added Se was well retained from the grain to resulted flour. The agronomic biofortification is therefore effective approach to increase the Se content in the wheat flour. This approach could be used as alternative to chemical fortification.

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التدعيم الحيوي لدقيق القمح بالسليينيوم من خلال الرش الورقي لمحصول القمح  
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في مصر تقع كمية السليينيوم المتناول اقل من الموصي بها يوميا. وقد اثبتت الأبحاث ان كمية السليينيوم في الحبوب يمكن زيادتها عن طريق التسميد الورقي. الهدف من هذه الدراسة هو زيادة تركيز السليينيوم في دقيق القمح من خلال التسميد الورقي بالسليينيوم اثناء الزراعة (تدعيم حيوي). أجريت تجربتان حقليةتان بمحطة التجارب والبحوث الزراعية بمنطقتي المنصورة وقلايشو- وزيان - كلية الزراعة - جامعة المنصورة خلال موسم ٢٠١١/٢٠١٠ لتقدير تأثير معاملات الرش الورقي بالسليينيوم (معدلات ووقت إضافة) على محتوى القمح من السليينيوم. أجريت كل من تجربة في تصميم القطاعات العشوائية الكاملة ذو أربعة مكررات في كل من موقعي المنصورة وقلايشو- وزيان. أظهرت النتائج المتحصل عليها أن الرش الورقي بالسليينيوم أدى إلى زيادة كمية السليينيوم في دقيق القمح. وكان أعلى تركيز للسليينيوم (٢٢ ميكروجم/١٠٠ جم مادة جافة) عند رش نباتات القمح مرتين بـ ٧.٥ جم سيلينيوم/فدان بعد ٥٠ و ٧٠ يوما من الزراعة بالمقارنة مع الحبوب الغير معاملة (١.٩ ميكروجم/١٠٠ جم مادة جافة). وكانت هناك علاقة خطية بين كمية السليينيوم المضافة من خلال التسميد الورقي وكمية السليينيوم في دقيق القمح الناتج، مما يدل على كفاءة نبات القمح في الاحتفاظ بالسليينيوم المضاف له من خلال التسميد الورقي وفي دقيق القمح الناتج.