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Selenium effect on wheat grain yield and quality applied in different growth stages

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Abstract: Small field plot experiments were carried out at the testing station of the Central Control and Testing Institute in Agriculture in Veľký Meder (Slovakia) in the experimental years 2014/2015, 2015/2016 and 2016/2017. Selenium salts in the form of sodium selenite and sodium selenate were applied in growth phases: end of tillering (BBCH 29) and flag leaf ligule and collar visible (BBCH 39). The effect of experimental years 2014/2015, 2015/2016 and 2016/2017 on the yield of wheat grain was not statistically significant within the observed variants. The achieved mean yields were in the range from 10.06 ± 0.81 to 11.07 ± 0.29 t/ha in 2014/2015, from 9.82 ± 0.54 to 10.32 ± 0.10 t/ha in 2015/2016 and from 11.23 ± 0.76 to 11.64 ± 0.51 t/ha in 2016/2017. Selenate in comparison with selenite influenced the selenium accumulation in wheat grains more positively. However, a significant difference was recorded in variants with selenite application in the flag leaf growth phase in comparison with the end of tillering phase. The influence on the content of macroelements P, K, Ca and microelements Cu and Fe was observed in sodium selenite only; its application decreased the element content in comparison with the control variant. Statistically significantly higher values of fiber and fat were achieved after application of selenium in the flag leaf growth stage in comparison with the end of tillering.

Keywords: *Triticum aestivum* L.; nutrition; biofortification; dietary deficiency; antioxidant

Selenium (Se) belongs to the group of essential elements needed for normal growth and function of living organisms. Besides that, selenium has antioxidant properties and protects the organisms against negative effects of free radicals and carcinogenic factors. The importance of selenium is in its participation in physiological functions in organisms (Kieliszek and Blažejak 2013). The selenium content in human organism and its impact on human health are often discussed. The lower level of selenium in human organism can lead to a risk of cardiovascular diseases, cancer and other problems caused by free radicals (Rayman 2000). One of the possible ways to increase Se in human body seems the Se supplement into the food chain; some authors suggested biofortification of different arable crops including wheat as the most worldwide cultivated crop (Lahermo et al. 1998, Hlušek et al. 2005, Galinha et al. 2015). Due to the extremely narrow line between dietary deficiency of Se and its toxicity to humans (Wang et

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Table 1. Summary of individual agrotechnical operations

Operation	2014/2015	2015/2016	2016/2017
Sowing	04/10/2014	10/10/2015	17/10/2016
Harvest	12/07/2015	09/07/2016	15/07/2017
1st fertilisation at BBCH 29 (end of tillering)	19/03/2015	17/03/2016	23/03/2017
2nd fertilisation at BBCH 39 (flag leaf ligule and collar visible)	07/05/2015	05/05/2016	08/05/2017

al. 2019), it is necessary to regulate or determine the safe content of Se in crops. By comparing breeding crops, genetic engineering and Se-enriched crops, biofortified crops are considered the optimal choice to increase Se intake in many countries with selenium deficiency (Zhou et al. 2018).

Due to the various physiological characteristics of crops at different growth phases, its uptake and utilisation also vary with different growth stages (Deng et al. 2017, Li et al. 2018). Previous studies have suggested that selenium application into the soil at the emergence stage was less effective in increasing the selenium content in grain than foliar application of selenite during flowering, because the interaction between selenite and soil subsequently reduced the bioavailability of selenium in soil with the application date (Curtin et al. 2006). A significant increase of selenium content in brown rice was recorded especially at selenium application in in the growth phase of development of grains in comparison with the end of rooting (Deng et al. 2017).

This study expects a significant effect of two foliar Se applications in two different growth stages to achieve yield and improve selected qualitative parameters (macro- and microelements, ash, starch and fat) in wheat grains.

MATERIAL AND METHODS

Field small plot trials were established at the testing station of the Central Control and Testing Institute in Agriculture in Veľký Meder (Slovakia) at the experimental seasons 2014/2015, 2015/2016 and 2016/2017. The sown crop was wheat (*Triticum aestivum* L.) cv. Tacitus. The soil of the trial field is characterised as Haplic Chernozem clay soil. The block method was used for trial establishment with the plot size of 10 m^2 (8 × 1.25 m) in four replications. The experimental area is characterised by warm, windy, mildly humid climate with annual mean temperature 9.6 °C and annual mean precipitations 545 mm. The altitude is 112 m a.s.l., groundwater level is in the depth of 1.5 m. The aim of small plot experiments was to observe the effect of two different forms of selenium salts in two different growth phases on wheat grain yield and content of macro- and microelements in wheat grain. Solutions of sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) were used for fertilisation. Work schedule is described in Table 1. The soil sampling of experimental field for elemental composition analysis was performed before the selenium application; the results are shown in Table 2. Selenium fertilisation was realised by hand sprayer brand STIHL (Andreas Stihl AG & Co KG, Waiblingen, Germany). The application dosage was 400 L/ha. The scheme of the treated variants is given in Table 3.

The harvest was carried out by a small plot harvester in full maturity (BBCH 91 growth stage). The impact of treated variants on selenium and selected macro- and microelements content in wheat grains was observed. Analysis of soil and specifications of wheat grains (content of macro-, microelements, ash, starch, dietary fiber and fat) were determined by standard methods. The samples were mineralised by a microwave

Table 2. Agrochemical characteristics of soil (to the depth of 30 cm) before selenium application

Soil analyses	2014/2015	2015/2016	2016/2017
pH_{KCl}	7.39	7.21	7.35
N_{in} (mg/kg)	13.6	14.7	12.8
$P_{Mehlich\ 3}$ (mg/kg)	53.8	41.5	50.5
$K_{Mehlich\ 3}$ (mg/kg)	275.0	312.2	278.0
$Mg_{Mehlich\ 3}$ (mg/kg)	619.2	587.5	642.5
Se-total content (HF + HNO_3 + HCl) (mg/kg)	0.22	0.23	0.22

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Table 3. Scheme of individual fertilisation variants

Control	variant without selenium foliar application
Se^{4+} 20 ET	application of 20 g Se/ha in the form of an aqueous solution of sodium selenite in BBCH 29–30 (end of tillering)
Se^{4+} 20 FL	application of 20 g Se/ha in the form of an aqueous solution of sodium selenite in BBCH 39 (flag leaf ligule and collar visible)
Se^{6+} 20 ET	application of 20 g Se/ha in the form of an aqueous solution of sodium selenate in BBCH 29–30 (end of tillering)
Se^{6+} 20 FL	application of 20 g Se/ha in the form of an aqueous solution of sodium selenate in BBCH 39 (flag leaf ligule and collar visible)

decomposition method during the increased pressure with the used reagents (hydrogen peroxide and concentrated nitric acid) in the following conditions: max. power: 800; power: 100%; ramp time: 20 min; temperature: 170 °C; hold 15 min. The achieved mixture was poured into a volumetric bank and deionised water was added to the capacity of 0.025 L. The content of Se in wheat grains was determined by the ICP-MS method (inductively coupled plasma mass spectrometry). Equipment model: ICP-MS Agilent 7900 (Agilent Technologies, Tokyo, Japan).

Statistical analysis. The achieved experimental results were statistically evaluated by the standard methods using the Statgraphics plus 5.1 statistical software (Rockville, Maryland, USA). A multifactor ANOVA model was used for the individual treatment comparison at $P = 0.05$, with separation of the means by the *LSD* (least significant difference) multiple-range test.

RESULTS

The statistically non-significant effect of selenium foliar application on the yield of wheat grains was described within evaluation of individual treated variants during the cultivation years 2014/2015, 2015/2016 and 2016/2017. Statistically significantly higher yield 11.07 ± 0.29 t/ha in years 2014/2015 was recorded at variants treated by sodium selenate in the flag leaf growth stage in comparison with variants treated by sodium selenite and control (Table 4). The achieved mean yields were in the range from 10.06 ± 0.81 to 11.07 ± 0.29 t/ha in years 2014/2015, from 9.82 ± 0.54 to 10.32 ± 0.10 t/ha in years 2015/2016 and from 11.23 ± 0.76 to 11.64 ± 0.51 t/ha in years 2016/2017. The different weather conditions of individual experimental seasons did not influence statistically significantly the yield amount within the monitored variants. The weather conditions of individual cultivation years are given at Figure 1.

The application impact of different selenium salt forms in different growth stages was demonstrated by statistical evaluation of selenium content in wheat grains (Table 5). Significantly the highest mean content of selenium in wheat grains as average of three years (0.203 ± 0.053 mg Se/kg) was recorded in variant treated by sodium selenate in the flag leaf

Table 4. Influence of selenium foliar application on the wheat grain yield

Dose (g Se/ha)	Grain yield (t/ha)			
	2014/2015	2015/2016	2016/2017	mean of years
0	10.91 ± 0.41^{ab}	10.09 ± 0.10^{a}	11.36 ± 0.66^{a}	10.79 ± 0.68^{a}
Se^{4+} 20 ET	10.06 ± 0.81^{a}	10.15 ± 0.22^{a}	11.39 ± 0.65^{a}	10.53 ± 0.83^{a}
Se^{4+} 20 FL	10.14 ± 0.34^{a}	9.90 ± 0.83^{a}	11.23 ± 0.76^{a}	10.42 ± 0.84^{a}
Se^{6+} 20 ET	10.19 ± 0.21^{ab}	10.32 ± 0.10^{a}	11.59 ± 0.53^{a}	10.70 ± 0.73^{a}
Se^{6+} 20 FL	11.07 ± 0.29^{b}	9.82 ± 0.54^{a}	11.64 ± 0.51^{a}	10.84 ± 0.90^{a}
$LSD_{0.05}$	0.91	0.60	1.14	0.50

The values in the columns with different letters are significantly different from each other at $P < 0.05$. Se^{4+} 20 ET – selenite form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{4+} 20 FL – selenite form of selenium, dose 20 g/ha in the flag leaf growth stage; Se^{6+} 20 ET – selenate form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{6+} 20 FL – selenate form of selenium, dose 20 g/ha in the flag leaf growth stage; *LSD* – least significant difference

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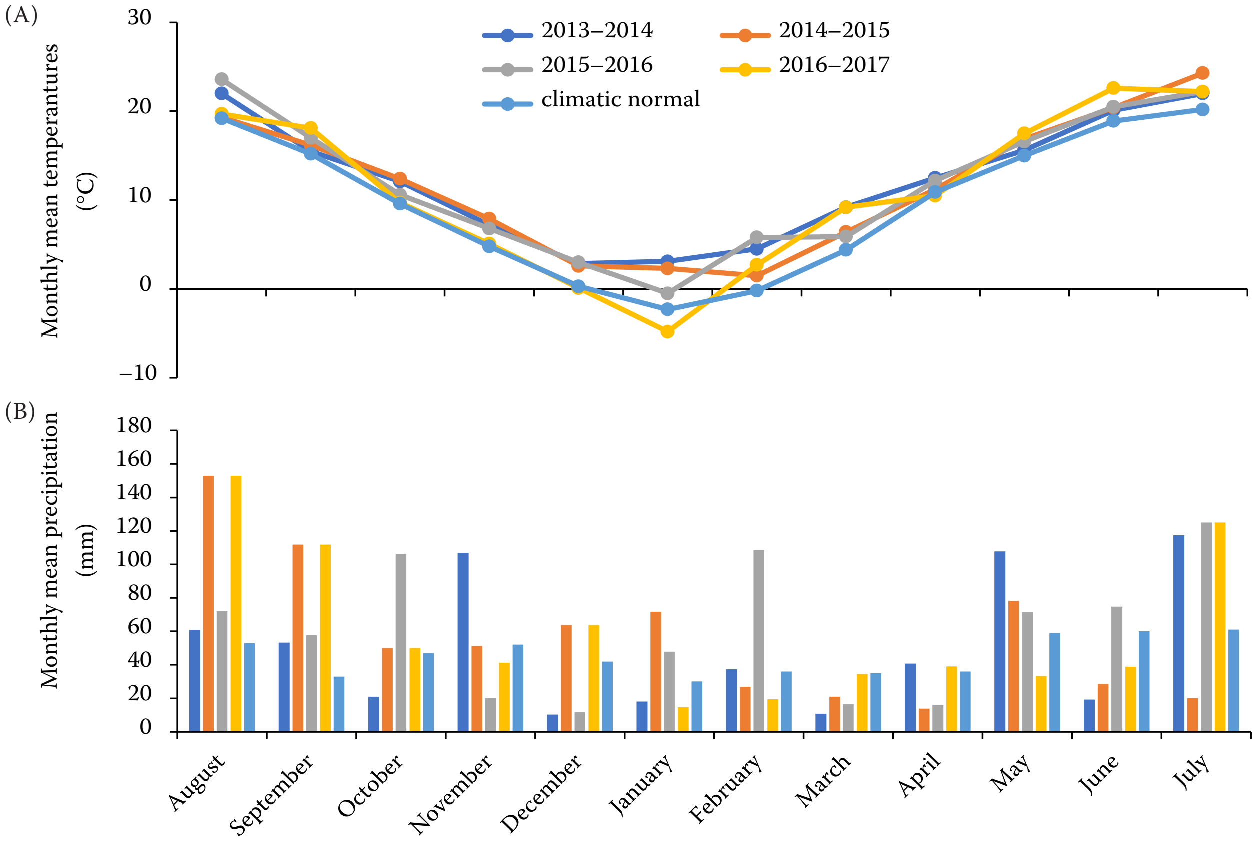

Figure 1. (A) Monthly mean temperatures and (B) monthly mean precipitations compared with climatic normal in experimental seasons 2014–2017

phenological phase. Achieved mean contents were in the range 0.043 ± 0.011 to 0.220 ± 0.082 mg/kg in the season 2014/2015, from 0.055 ± 0.004 to 0.210 ± 0.044 mg/kg in 2015/2016 and from 0.030 ± 0.010 mg/kg to 0.190 ± 0.052 mg/kg in 2016/2017. Statistically significant differences were detected in all treated variants in individual growth stages.

Table 6 shows the differences of selenium application effect in different growth stages on the macroelement content in wheat grains. A non-significant selenium effect was found by analysing the nitrogen content in wheat grains. The evaluation shows that the highest values of P content 2.78 ± 0.27 g/kg, K 4.53 ± 1.73 g/kg, Ca 0.30 ± 0.15 g/kg were recorded at the non-treated

Table 5. Effect of selenium foliar application on the selenium content in wheat grains

Dose (g Se/ha)	Selenium content (mg/kg)			
	2014/2015	2015/2016	2016/2017	mean of years
0	0.043 ± 0.011[a]	0.055 ± 0.004[a]	0.030 ± 0.010[a]	0.043 ± 0.013[a]
Se^{4+} 20 ET	0.046 ± 0.007[a]	0.082 ± 0.012[a]	0.06 ± 0.020[a]	0.063 ± 0.019[a]
Se^{4+} 20 FL	0.190 ± 0.030[b]	0.092 ± 0.026[a]	0.120 ± 0.028[b]	0.134 ± 0.049[b]
Se^{6+} 20 ET	0.200 ± 0.036[b]	0.140 ± 0.025[b]	0.190 ± 0.052[c]	0.177 ± 0.044[c]
Se^{6+} 20 FL	0.220 ± 0.082[b]	0.210 ± 0.044[b]	0.180 ± 0.035[c]	0.203 ± 0.053[c]
$LSD_{0.05}$	0.077	0.047	0.059	0.037

The values in the columns with different letters are significantly different from each other at $P < 0.05$. Se^{4+} 20 ET – selenite form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{4+} 20 FL – selenite form of selenium, dose 20 g/ha in the flag leaf growth stage; Se^{6+} 20 ET – selenate form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{6+} 20 FL – selenate form of selenium, dose 20 g/ha in the flag leaf growth stage; *LSD* – least significant difference

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Table 6. Effect of selenium foliar application of on the macroelement content in wheat grains

Dose (g Se/ha)	Nutrient content (g/kg), mean of years 2014/2015, 2015/2016, 2016/2017					
	N	P	K	Ca	Mg	S
0	21.8 ± 1.3[a]	2.78 ± 0.27[c]	4.53 ± 1.73[b]	0.30 ± 0.15[c]	1.55 ± 0.17[a]	1.22 ± 0.57[b]
Se^{4+} 20 ET	22.2 ± 1.5[a]	2.42 ± 0.34[a]	4.20 ± 2.08[ab]	0.24 ± 0.09[a]	1.52 ± 0.17[a]	1.22 ± 0.60[b]
Se^{4+} 20 FL	21.9 ± 1.5[a]	2.47 ± 0.17[ab]	4.10 ± 1.8[a]	0.28 ± 0.14[bc]	1.75 ± 0.12[b]	1.31 ± 0.53[c]
Se^{6+} 20 ET	21.9 ± 1.3[a]	2.61 ± 0.26[bc]	4.16 ± 1.75[ab]	0.27 ± 0.11[ab]	1.57 ± 0.13[a]	1.21 ± 0.58[ab]
Se^{6+} 20 FL	22.3 ± 2.9[a]	2.41 ± 0.27[a]	4.31 ± 1.73[ab]	0.24 ± 0.08[a]	1.55 ± 0.12[a]	1.13 ± 0.44[a]
$LSD_{0.05}$	0.64	0.17	0.41	0.03	0.08	0.08

The values in the columns with different letters are significantly different from each other at $P < 0.05$. Se^{4+} 20 ET – selenite form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{4+} 20 FL – selenite form of selenium, dose 20 g/ha in the flag leaf growth stage; Se^{6+} 20 ET – selenate form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{6+} 20 FL – selenate form of selenium, dose 20 g/ha in the flag leaf growth stage; *LSD* – least significant difference

control variant. The significantly highest contents of Mg 1.75 ± 0.12 g/kg and S 1.31 ± 0.53 g/kg were achieved at variant with the Se^{4+} application in the flag leaf growth phase. The impact of different selenium salt forms on accumulation of microelements is described in Table 7. The significantly highest values of Cu 6.16 ± 1.88 mg/kg and Fe 88.3 ± 30.8 mg/kg were found in control variants. The significantly highest content of Mn 34.2 ± 5.5 mg/kg and Zn 54.8 ± 8.4 mg/kg was recorded after the Se^{4+} application in the end of tillering stage. From the results obtained by statistical evaluation of the application effect of different selenium forms in different growth stages on the quality of wheat grains is clear that the significantly highest values of fat content 2.29 ± 0.13% and fiber 4.17 ± 0.86% were recorded in the flag leaf growth stage by application of selenate and selenite, respectively. Statistically non-significant differences were found by the evaluation of ash and starch content (Table 8).

DISCUSSION

The yield formation of wheat grain within the selenium application variants is not always influenced by the cultivation season. Ducsay and Ložek (2016) in their study described the important effect of season on wheat grain yield, which can be concluded from their two-year experiment results. Due to different physiological characteristics of crops, selenate has higher biological availability in comparison with selenite. The studies of Rodrigo et al. (2013) and Ekanayake et al. (2015) describe the recovery efficiency of Se in barley and lentil grains that was four times higher at selenate foliar application than at selenite foliar application. Other experiments with potatoes point to the fact that the later selenium foliar application (in tuber enlargement phase) resulted in the increased total concentration of Se in tuber (Zhang et al. 2019). Relatively few studies are aimed at the Se effect on

Table 7. Effect of selenium foliar application on the microelement content in wheat grains

Dose (g Se/ha)	Nutrient content (mg/kg), mean of years 2014/2015, 2015/2016, 2016/2017			
	Cu	Fe	Mn	Zn
0	6.16 ± 1.88[a]	88.3 ± 30.8[b]	33.7 ± 3.3[ab]	49.8 ± 9.6[a]
Se^{4+} 20 ET	5.66 ± 0.81[ab]	85.9 ± 25.5[b]	34.2 ± 5.5[b]	54.8 ± 8.4[b]
Se^{4+} 20 FL	5.11 ± 1.06[b]	74.9 ± 14.2[a]	31.7 ± 4.5[a]	47.4 ± 6.3[a]
Se^{6+} 20 ET	5.35 ± 1.11[b]	84.8 ± 24.5[b]	32.1 ± 3.9[a]	47.8 ± 3.4[a]
Se^{6+} 20 FL	5.44 ± 0.95[b]	78.7 ± 29.3[ab]	31.7 ± 3.5[a]	50.7 ± 7.1[ab]
$LSD_{0.05}$	0.65	9.8	2.1	4.8

The values in the columns with different letters are significantly different from each other at $P < 0.05$. Se^{4+} 20 ET – selenite form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{4+} 20 FL – selenite form of selenium, dose 20 g/ha in the flag leaf growth stage; Se^{6+} 20 ET – selenate form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{6+} 20 FL – selenate form of selenium, dose 20 g/ha in the flag leaf growth stage; *LSD* – least significant difference

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Table 8. Effect of selenium foliar application on ash, starch, fat and fiber content in wheat grains

Dose (g Se/ha)	Content (%), mean of years 2014/2015, 2015/2016, 2016/2017			
	ash	starch	fat	fiber
0	3.74 ± 2.33[a]	56.6 ± 1.3[a]	2.02 ± 0.65[ab]	3.71 ± 0.60[a]
Se^{4+} 20 ET	4.01 ± 2.26[a]	57.3 ± 1.4[a]	2.25 ± 0.23[bc]	3.73 ± 0.86[a]
Se^{4+} 20 FL	3.90 ± 2.80[a]	56.3 ± 1.9[a]	1.95 ± 0.38[a]	4.17 ± 0.86[b]
Se^{6+} 20 ET	3.71 ± 2.41[a]	57.1 ± 2.6[a]	1.91 ± 0.17[a]	3.66 ± 0.50[a]
Se^{6+} 20 FL	3.84 ± 2.17[a]	57.3 ± 2.0[a]	2.29 ± 0.13[c]	3.66 ± 0.49[a]
$LSD_{0.05}$	0.33	1.6	0.24	0.56

The values in the columns with different letters are significantly different from each other at $P < 0.05$. Se^{4+} 20 ET – selenite form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{4+} 20 FL – selenite form of selenium, dose 20 g/ha in the flag leaf growth stage; Se^{6+} 20 ET – selenate form of selenium, dose 20 g/ha in the end of tillering growth stage; Se^{6+} 20 FL – selenate form of selenium, dose 20 g/ha in the flag leaf growth stage; *LSD* – least significant difference

the accumulation of macro- and microelements important for the proper growth and development of plants (Tobiasz et al. 2014). Similar results were described in the study of Tobiasz et al. (2014), who found higher sulphur content in the flag leaf growth phase in comparison with the three leaves and grain stages. Zeidan et al. (2010) also noted low values of Cu and Fe after selenium application. Zembala et al. (2010) observed the same decrease in Fe concentration after Se fertilisation of rape (*Brassica napus* L.) and wheat, while Wang et al. (2013) found out non-significant effect of selenium application on the maize grain content. These results point to the fact that the content of elements in wheat grain can be influenced by various factors. The literature provides minimum information about the effect of different selenium forms applied at different growth stages on the quality of wheat grain. For this reason, the results are compared with the results of Havrlentová et al. (2013 who evaluated oat quality, i.e. the content of beta glucan in oat grains. Statistically significant differences were noted in two variants out of five in peeled oat. A statistically significant increase of polyphenols after the foliar application of selenium was found at the evaluation of other crops and their qualitative parameters (Andrejiová et al. 2019).

The presented results of three-year means point to a non-significant effect of the cultivation years 2014/2015, 2015/2016 and 2016/2017 within the monitored variants. Sodium selenate has shown as a more effective selenium form in comparison with sodium selenite in selenium accumulation to wheat grains. A significant influence of sodium selenite application was found in the flag leaf growth stage in comparison with the end of tillering phase. The impact on the content of macroelements (P, K, Ca) and microelements (Cu and Fe) was observed only at sodium selenite; its application decreased the element content in comparison with variants without selenium application. Statistically significantly higher values of fiber and fat were achieved at selenium application in flag leaf growth stage.

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