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ICP-MS ANALYSIS OF BREAD WHEAT CARRYING THE *GPC-B1* GENE OF *Triticum turgidum* ssp. *dicoccoides*

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The aim of the research was to analyze effects of the gene *Gpc-B1*, introgressed from wild spelt *Triticum turgidum* ssp. *dicoccoides*, on the presence of biologically important elements in bread winter wheat hybrid families of generations F_4 and F_5. The accumulation of metals in ripe and unripe grains was measured on a mass spectrometer with inductively coupled plasma ICP-MS Agilent 7700x. It was found that the expressing gene significantly increased the content of trace elements Fe, Mn, Zn and Cu in ripe wheat kernels on average by 50–70%, while the increase of secondary nutrients or mesoelements Mg, Ca defined by an average of 20–40%. The enrichment of minerals, confirmed during grain development and ripening provides not only biofortification for the future harvest, but also potentially enhances the resistance of plants to diseases and formation of seedlings with more efficient use of nitrogen.

Key words: *Triticum aestivum*, biofortification, bread wheat, *Gpc-B1*.

The content of trace elements within ISO 3768–2010 in wheat grains is important for nutrition and getting a seed culture with high sowing qualities. There are low levels of Zn, Fe and Mn in soils and crops in all the countries, but it is particularly dangerous in regions of growing crops. Those minerals are the basis of human nutrition. The problem of lack of biologically important trace elements such as Fe and Zn is relevant to more than 2 billion people around the world. Pregnant women and children less than 5 years old suffer shortages most [1–4].

In European countries, grains, especially wheat, provide about 30% of daily calorie intake while in the Central Asia go to an average of 50% [1, 4, 5]. The wheat does not always contain sufficient micronutrients to ensure adequate human diet [6].

Micronutrient content in wheat genetically determined and depends on environmental factors. The level of absorption of trace elements in generative organs of wheat is low. Thus, it is a promising trend of genetic improvement of crops. One of the areas of biofortification of micronutrients is the transfer of genes from wild relatives, including, in particular, as spelt and wild wheat [7, 8]. Gene *Gpc* (grain protein content) within the whole chromosome 6B was transferred from tetraploid hulled wild emmer *Triticum turgidum* L. subsp. *dicoccoides* (Korn. ex Asch. & Graebn.) Thell. in hard tetraploid wheat cultivar Langdon (*Triticum turgidum* L. var. *durum*) [7]. The gene is responsible for accelerated aging of leaves and causes the effect of increasing the content of important trace elements in wheat, including Zn, Mn and Fe. The effect locus was detected in a significant increase compared to the starting line in the grain content of zinc (60 to 47.5 mg/kg), iron (44.2 to 35.9 mg/kg), manganese (53.9 to 40.9 mg/kg) and protein (14.4 to 10.8%) [8–10].

Well known that iron, zinc and manganese are some important elements to ensure the redox homeostasis of living organisms. Iron is available to plants in the forms Fe^{+2} and Fe^{+3}. This element plays an important role in metabolism, is part of enzymes involved in chlorophyll synthesis, a component Citric acid cycle. The lack of iron during plant growing causes intercostal chlorosis and consequently disrupts the synthesis of chlorophyll and photosynthesis activity [11]. Iron is an essential element in the human body and is a part of many substrates and enzymes responsible for transporting oxygen to cells, functioning respiratory chain mitochondrial redox reactions, the functioning of nervous and immune systems. About 60% of iron in the body is in hemoglobin. The lack of this trace element determines, first of all, the development of hypochromic anemia. The recommended daily dose of iron for adults is 10–15 mg [12–14].

Plants often use zinc as the divalent cation Zn^{+2}. It is one of the most important trace elements necessary for the regulation of carbohydrate and a number of enzymes involved in the growth process. It is a cofactor of more than 80 enzymes and acts as a structural component of many proteins [11]. The role of zinc in human life is due mainly to the fact that it is a member of more than 40 important enzymes that catalyze the hydrolysis of peptides, proteins, certain esters and aldehydes. Zinc is involved in carbohydrate metabolism, located in insulin. The presence of zinc only let vitamin A function. This ion required for bone formation. Lack of zinc in the body has pleiotropic effects on mRNA expression of hundreds of genes, altering the biosynthesis of many proteins and zinc transcription factors. The average daily recommended amount of zinc for adults is 12 mg [2, 15, 16].

Plants absorb manganese in the form of divalent cation Mn^{+2}. It performs a structural role in the chloroplast membrane system [17]. The element deficiency leads to intercostal chlorosis of young and old plant tissues [11]. Manganese is a part of the active site of many enzymes involved in blood clotting, regulates conversion of molecular oxygen. Also manganese is involved in the synthesis of vitamins B and affects the synthesis of hemoglobin. The lack of manganese leads to disruption of carbohydrate metabolism, delays the growth of hair and nails, causes dermatitis, abrupt formation of cartilage, osteoporosis. The daily dose of manganese for adults is 2.0–5.0 mg [17].

Given the biological importance of iron, zinc and manganese, the aim of our study was to analyze the wheat hybrid families of generations F_4 and F_5 carrying the gene *Gpc-B1* from *Triticum turgidum* ssp. *dicoccoides* within the substituted chromosome 6B for the content of micro- and macronutrient in grain.

Materials and Methods

To investigate the content of micro- and macroelements the hybrid wheat families of generations F_4 and F_5 marked as 18.2, 50.5, 51.5, 94.4, 98.3, 104.5, 125.5, 158.5 bearing the native gene *Gpc-B1* of *T. turgidum* ssp. *dicoccoides* in Kuyalnyk background (been the second genetic parent) have been selected. The samples of generation F_4 were represented by ripe grains while the samples of generation F_5 were grains in the milk stage of maturation.

The determination of elements in the grain samples was carried out with a mass spectrometer with inductively coupled plasma ICP-MS Agilent 7700x (Agilent Technologies) together with ICP-MS MassHunter WorkStation. The samples (0.400 g) were treated with in nitric acid of ICP-grade in the microwave sample preparation system Milestone Start D. After the cooling, water was added to the samples to the dissolution rate 250×. All solutions were prepared in class 1 water (18 MOm·cm), obtained from the water purification system Scholar-UV Nex Up 1000 (Human Corporation, South Korea). In 2015 Fluka Multielement standard solution 5 for ICP (Switzerland) was used for calibration whereas in 2016 the ICP-MS Complete Standard IV-ICPMS-71A (Inorganic Ventures, USA) was applied which helped identify selenium (Se) too [18, 19]. Settings of the mass spectrometer is given in Table 1.

The selected elements in the argon plasma may produce false peaks. For example, equal by weight CaO^{56} and Fe^{56} or ArN or ArO with other isotopes of iron. ArNH and KO may mask Mn, and Ba^{++} – Zn. All 6 Ca isotopes can interact with O, H and Ar, which leads to inaccuracies in the determination of Cu, Fe, Sc, Se. Determination of selenium prevent most isotopes isobaric overlap Kr or Ge (masses of 74, 76, 78, 80 and 82) or polyatomic interferences mainly on Ar_2 masses 76, 78, 80. Although copper ionizes well in argon plasma (90%), the definition isotope ^{63}Cu is complicated with NaAr interaction and types of P, while ^{65}Cu is covered with SO_2/SO_2H. Besides, copper isotopes interfere with calcium oxide and titanium. Taking in to account the named obstacles the measurement was performed in helium purge mode, which effectively removes the above matrix and element interference. As an internal standard

Table 1. **Setting a mass spectrometer to analyze the Agilent 7700x inorganic elements**

Indicator	Settings	Value
Supply	Power generator, W	1550
Setup argon plasma	Flow of carrier gas, l/min	15.5
	Flow of helium, l/min	0.1
Auto Setup	CeO^+/Ce^+ (%)	1.114
	Ce^+/Ce^+ (%)	1.867
	Sensitivity pulses per second to mg/l (CPS/ppb)	Li (62700), Y (92920), Tl (87080)

the 1 ppb solution of Sc by Inorganic Ventures, USA was used. The statistical analysis of obtained results was performed with ICP-MS MassHunter Software and MS Excel 2014.

Results and Discussion

As a result of ICP-MS detection of a number of elements contents in mature wheat grains generation F_4 8 homozygous hybrid families where parental control served as a cultivar of Kuyalnyk, have the following (Table 2).

Average zinc content among the prototypes of 23.25 mg/kg dry weight, 83% more than the original control (cultivar Kuyalnyk). Maximum accumulation of zinc occurs in the family 94.4 — 33 mg/kg, which is 155% higher than the control cultivar. Minimal accumulation of zinc in the family determined 50.5 — 17 mg/kg of dry matter, but even this level of content significantly exceeded the results of the control options.

The increase in the accumulation of iron in the homozygous hybrid families was slightly higher compared to the levels of accumulation of zinc. The average iron content of test samples is 52.3 mg/kg of dry matter, which, in turn, 61% more than the original line. The maximum iron content was observed in the family 51.5 — 69 mg/kg, minimum — in the family 158.5 — 40 mg/kg.

The average concentration of manganese prototypes of 50.7 mg/kg of dry matter, which is 55% more than the original line. The maximum manganese content was observed in the family 18.2 — 65 mg/kg, minimum — in the family of 158.5 index of 45 mg/kg.

Given the biological importance of other divalent cations detected and copper content as well as magnesium and calcium. This essential elements, high content which determines crop seed quality and nutritional value of wheat flour. Copper content in grain increased in all variants, but did not reach the size limit accumulation of 10.0 mg/kg, which is regulated by law in accordance with ISO 3768–2010. It was also noted the high content of magnesium accumulation in grains and calcium — in most lines. The average concentration of magnesium, compared with the original line was over 46%, calcium — 24%, copper — 64%.

As a result of ICP-MS analysis of the content of Zn, Fe, Mn, Cu, Mg and Ca in all research family is the high content of micronutrients compared to the cultivar of Kuyalnyk. This finding confirms previous studies [9, 10, 19] that *Gpc-B1* gene of *Triticum turgidum* ssp. *dicoccoides* positive effect on increasing micronutrient content in its carrier.

In 2016 was analyzed hybrid generation families F_5. For research took grain maturation phase of the milk. Served as a control parental line (cultivar Kuyalnyk). The task of the research was to determine the degree of accumulation of micro- and macroelement number of hybrid gene carriers family — *Gpc-B1* of *Triticum turgidum* ssp. *dicoccoides* the initial phase of ripening grain. Analysis was performed on 6 hybrid families that they investigated in 2015. The data given in Table 3.

The content of zinc in hybrid families an average of 27.6 mg/kg of dry matter, which is 28% more than in the starting line. The maximum level was observed in the family 98.3 — 33 mg/kg.

The average iron content in samples of 2016 amounted to 28.7 mg/kg of dry matter, while the beans control — 27.0 mg/kg (8% difference). The maximum iron content was observed in the family 51.5 — 32.0 mg/kg, the minimum content 104.5 — 27.0 mg/kg. A small difference can be explained by the

Table 2. **The content of micro and macroelements in physiologically mature grains of wheat crop 2015 (mg/kg dry matter)**

Variant	Microelement				Macroelement	
	Zn	Fe	Mn	Cu	Mg	Ca
Control (cultivar Kuyalnyk)	13a	33a	33a	2.80a	911a	372a
18.2	25c	58e	65d	5.87c	1723d	560d
50.5	17b	46c	47b	4.45b	1257c	399a
51.5	20b	69f	49b	4.09b	1329c	444b
94.4	33d	48c	49b	4.36b	1268c	465c
98.3	21c	57d	52c	4.22b	1351c	403b
104.5	19b	53d	52c	4.37b	1279c	494c
125.5	29d	48c	48b	4.76b	1377c	492c
158.5	22c	40b	45b	4.59b	1023b	434b

Note: the identical letters mark variations that do not differ on the 0.05 significance level.

Table 3. **The content of wheat grain micro- and macroelements in the initial stages of ripening at harvest in 2016 (mg/kg dry matter)**

Variant	Microelement					Macroelement
	Zn	Fe	Mn	Cu	Se	Mg
Control (cultivar Kuyalnyk)	21a	27a	49a	4.2a	0.09a	1665a
18.2	24b	29a	63c	5.0b	0.41c	1682a
51.5	27c	32c	60b	5.0b	0.23b	1631a
94.4	26c	28a	59b	5.2b	0.03a	1681a
98.3	33d	29b	63c	6.0c	0.14a	1853b
104.5	24a	27a	65c	5.8b	0.11a	1648a
125.5	31d	30c	77d	7.2d	0.26b	1960c

fact that the grain is not yet mature and the accumulation of iron in the plant continued until fully ripe grain.

The average concentration of manganese incurred samples was 64.6 mg/kg, which is 31% more than in the starting line Kuyalnyk grade. The maximum level was 125.5 in the family — 77 mg/kg and the minimum — in the family 94.4 — 59 mg/kg of dry matter.

Analyzing the performance of content generation F_4 and F_5 on the content of Zn, Mn, Fe, one can conclude that the gene *Gpc-B1* of *Triticum turgidum* ssp. *dicoccoides* significantly increases the content of trace elements in samples. This effect was observed even in the early stages of ripening grain. Also, we have determined the content of Cu and Mg in hybrid families.

Mean values of copper in hybrid families by 35% higher than the content in the starting line, whereas the magnesium content — 5%. Elevated levels of elements at this early stage indicates that the gene *Gpc-B1* of *Triticum turgidum* ssp. *dicoccoides* multifunctional and not only increases the amount of Fe, Mn, Zn, but Cu, Mg.

One of the important micronutrients for wheat is also selenium. It is a component of over 30 vital biologically active substances of the human body as part of the active centers of antioxidant enzymes involved in the metabolism of nucleic acids, lipids, hormones. Among plant products the main source of selenium in Ukraine is wheat flour, so control of the content of selenium is essential [20, 21].

The average content of selenium in experimental families of 0.207 mg/kg of dry matter, which is 117% more than the father. The maximum level of selenium — family 18.2 — 0.41 mg/kg (4.5 times more than control) in the dry matter.

According to the research in 2015 and 2016 we found that the presence of the gene *Gpc-B1* of *Triticum turgidum* ssp. *dicoccoides* bread winter wheat growth leads to accumulation of important biologically significant nutrition elements — iron, zinc, manganese, copper, selenium, and magnesium (which is correlated with observations of other researchers on a number of cereals [5, 8, 19]). Given the importance of iron, zinc, manganese, copper and selenium in redox homeostasis, we can provide high quality seeds sown grain containing this gene, as well as increased resistance of plants to pathogens destructive diseases — *Fusarium* spp., etc. Enriching copper ions promote the formation of stairs cultures with high levels of nitrogen use efficiency.

Thus, the presence *Gpc-B1* locus of *Triticum turgidum ssp. dicoccoides* and its functioning is important for a bread winter wheat biofortification and further breeding work.

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ICP-MS АНАЛІЗ М'ЯКОЇ ПШЕНИЦІ З ГЕНОМ *GPC-B1* ВІД *Triticum turgidum* ssp. *dicoccoides*

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Метою роботи був аналіз впливу гена *Gpc-B1*, інтродукованого від дикої полби *Triticum turgidum* ssp. *dicoccoides*, на біологічно важливі елементи живлення гібридних сімей пшениці м'якої озимої поколінь F_4 та F_5. Накопичення металів у стиглих та недозрілих зернівках вимірювали на мас-спектрометрі з індуктивно зв'язаною плазмою ICP-MS Agilent 7700x. Встановлено, що експресія цього гена істотно підвищує вміст мікроелементів Fe, Mn, Zn і Cu у стиглих зернівках пшениці м'якої — у середньому на 50–70%, тимчасом як мезоелементів Mg, Ca — у середньому на 20–40%. Збагачення мінералами, що підтверджено під час розвитку та наливу зерна, не лише забезпечує біофортифікацію майбутнього врожаю, але й сприяє підвищенню резистентності рослин до хвороб, формуванню сходів із більш ефективним використанням азоту.

Ключові слова: Triticum aestivum, біофортифікація, м'яка пшениця, *Gpc-B1*.

ICP-MS АНАЛИЗ МЯГКОЙ ПШЕНИЦЫ С ГЕНОМ *GPC-B1* ОТ *Triticum turgidum* ssp. *dicoccoides*

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Целью работы был анализ влияния гена *Gpc-B1*, интродуцированного от дикой полбы *Triticum turgidum* ssp. *dicoccoides*, на биологически важные элементы питания гибридных семей пшеницы мягкой озимой поколений F_4 и F_5. Накопление металлов в зрелых и созревающих зерновках измеряли на масс-спектрометре с индуктивно связанной плазмой ICP-MS Agilent 7700x. Установлено, что экспрессия этого гена значительно повышает содержание микроэлементов Fe, Mn, Zn и Cu в зрелых зерновках пшеницы мягкой — в среднем на 50–70%, в то время как мезоэлементов Mg, Ca — в среднем на 20–40%. Обогащение минералами, подтвержденное при развитии и наливе зерна, не только обеспечивает биофортификацию будущего урожая, но и способствует повышению резистентности растений к болезням, формированию всходов с более эффективным использованием азота.

Ключевые слова: *Triticum aestivum*, биофортификация, мягкая пшеница, *Gpc-B1*.