Article

The Response of Wheat with Different Allele Statuses of the *Gpc-B1* Gene under Zinc Deficiency

Natalia Kaznina ^{1,*}, Nadezhda Dubovets ², Yuliya Batova ¹, Anna Ignatenko ¹, Olga Orlovskaya ² and Natalia Repkina ¹

¹ Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences, 185910 Petrozavodsk, Russia; batova@krc.karelia.ru (Y.B.); angelina911@ya.ru (A.I.); nrt9@ya.ru (N.R.)

² Institute of Genetics and Cytology of the National Academy of Sciences of Belarus, 220072 Minsk, Belarus; n.i.dubovets@igc.by (N.D.); O.Orlovskaya@igc.by (O.O.)

* Correspondence: kaznina@krc.karelia.ru

Abstract: The aim of this study was to investigate the effect of zinc (Zn) deficiency on the growth and grain yield of wheat with different allele statuses of the *Gpc-B1* gene. For this research, common wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides* (Koern. ex Asch. & Graebn.) Schweinf.), bread wheat (*Triticum aestivum* L. cv. *Festivalnaya*), and two introgressive lines were used. *T. dicoccoides* and introgressive line 15-7-1 carry a functional allele of the *Gpc-B1* gene, while the *T. aestivum* cv. *Festivalnaya* and introgressive line 15-7-2 carry the non-functional *Gpc-B1* allele. Zn deficiency did not affect the shoot height or fresh weight of any of the studied plants. The only exception was *T. dicoccoides*, where a small decrease in shoot height was registered. Additionally, under Zn deficiency *T. dicoccoides* had an increase in flag leaf area, spike length, and dry weight, as well as in grain number and grain yield per spike. The other variants did not experience changes in the above-described parameters under Zn deficiency. Under Zn deficiency, the Zn concentration in the grains was higher in the plants with a functional allele of the *Gpc-B1* gene compared to the plants with a non-functional allele. These results show that wheat with a functional allele of the *Gpc-B1* gene growing under Zn deficiency is capable of grain production with a sufficient Zn concentration without a decrease in yield.

Keywords: wild emmer; wheat; zinc; *Gpc-B1*; growth; grain yield

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1. Introduction

Zinc (Zn) is an essential micronutrient for all organisms, with key catalytic and structural functions [1–3]. Zn is involved in numerous aspects of cellular metabolism [4,5]. Redox-inert metal ions such as Zn are key structural components of a large number of proteins [6]. Among the structural domains of proteins, Zn finger domains have a major physiological relevance [1,7,8]. In addition to this, the role of Zn in membrane integrity and stabilization, in the alleviation of oxidative stress, and as an intracellular second messenger has also been reported [9]. Zn regulates transcription directly through effects on DNA/RNA binding, and through site-specific modifications, regulation of chromatin structure, RNA metabolism, and protein–protein interactions [10]. Zn has a key role in autophagy regulation [11]. As Zn is essential for proliferative cells, its role in the regulation of immune cells is crucial (Zn ions function as chemo-attractants for some immune cells and affect the maturation of dendritic cells and the development and function of T cells, etc.) [12]. Assessments made by the World Health Organization (WHO) concluded that approximately 50% of the world's population has suboptimal zinc nutrition [13]. Disbalances with regard to Zn deficiency as well as Zn excess are linked to a large number of illnesses, particularly immune diseases [14,15]. According to the International Zinc Nutrition Consultative Group, the national prevalence of zinc deficiency is high in South Asia, most of sub-Saharan Africa, and parts of Central and South

America [16]. An estimated 17.3% of people worldwide are at risk of inadequate Zn intake, and Zn deficiency causes a loss of appetite, anemia, growth retardation, hypogonadism, and depressed mental function, etc. [17,18]. Therefore, it is crucial to develop effective measures to mitigate Zn deficiency.

Wheat, rice, and maize are the most important cereal crops across the world. Cereals constitute the largest source of calories in developed and developed countries. Some estimates suggest that wheat alone makes up 20% of the food calories and daily proteins consumed by 4.5 billion people [19]. Global wheat production in 2021 increased to 780 million tons [20].

To mitigate Zn malnutrition in developed countries, supplementation and food fortification are the main strategies. However, these strategies are difficult to put into effect in developing countries, taking into consideration the economic and social conditions. In this case, the increase in Zn concentration in wheat grain and the edible parts of other cereal crops through agronomic intervention or genetic selection is a strategy to mitigate micronutrient malnutrition. Some authors have proposed using some parental species of cultivated wheat (crop wild relatives) such as *Aegilops ventricosa* Tausch (which naturally has high zinc concentrations) for breeding and crossing with cultivated wheat or for direct cultivation as a source of zinc [21,22]. Current agriculture practices can improve the micronutrient content of foods through correcting soil quality, seed quality, and plant breeding (by means of a classical selection process or genetic modification) [23]. Genetic biofortification involving classical breeding, gene discovery, and marker-assisted breeding approaches is a strategy to produce crops with higher micronutrient levels [24]. Previously, it was found that the *Gpc-B1* gene, encoding the NAC (NAM, ATAF, CUC) transcription factor, was involved in the regulation of Zn content in plants [25,26]. The presence of a functional allele of the *Gpc-B1* gene is typical for wild-type tetraploid wheat (*Triticum turgidum* ssp. *dicoccoides* (Koern. ex Asch. & Graebn.) Schweinf.), while most of the created tetraploid and hexaploid varieties of wheat have a non-functional copy of this gene or a deletion of this locus [25,27]. The use of the distant hybridization method makes it possible to breed wheat lines containing a functional allele of this gene. As a result, these lines are characterized by a higher content of proteins and trace elements in grain, including Zn [25,28]. Moreover, the *Gpc-B1* gene is involved in the control of the aging process of plants [26]. The aging of leaves, especially the sub-flag and flag leaves, makes the provision for the remobilization of essential micronutrients from the leaves into the spike. The regulation of this process is crucial for enhancing the supply of Zn into developing grains. There are fragmentary available data on the reaction of wheat with a functional allele of the *Gpc-B1* gene to Zn deficiency. Therefore, the present study was conducted to investigate the effect of Zn deficiency on the growth parameters and grain production of wheat with different *Gpc-B1* allele statuses.

2. Materials and Methods

2.1. Plant Material

Seeds of wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides* (Koern. ex Asch. & Graebn.) Schweinf.), common wheat (*Triticum aestivum* L. cv. *Festivalnaya*) and 2 introgressive lines (15-7-1 and 15-7-2) were received from the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus in Minsk. Introgressive lines were created by crossing of *T. aestivum* cv. *Festivalnaya* and *T. dicoccoides*. Allelic status of the *Gpc-B1* gene was determined using codominant marker *Xuhw89* [29]. Functionally active alleles of the *Gpc-B1* gene were identified in *T. dicoccoides* and introgressive line 15-7-1, whereas *T. aestivum* cv. *Festivalnaya* and line 15-7-2 had non-functional alleles. The non-functional allelic variant is characterized by the presence of a 1bp insertion within the coding region, resulting in a frame shift mutation that introduces a stop codon. The protein calculated for this allele is inactive.

2.2. Growth Conditions

The experiment was conducted in greenhouse conditions during the wheat-growing season at the Agricultural Experiment Station belonging to the Karelian Research Centre (61°45'6" N, 119°25'10.9" E) (WGS84), Petrozavodsk, Republic of Karelia, Russia. Wheat seeds were sown in pots (height 40 cm, diameter 20 cm) containing 5 kg of sand and 12 seeds per pot. Half of the pots were irrigated by Hoagland solution with micronutrient addition including optimal Zn (2 µM) (Zn+, control), and the other pots were irrigated by solution with micronutrients excluding Zn salt addition (Zn−, Zn deficiency). Plants were harvested and analyzed at the seed-filling stage.

2.3. Biometric Measurements

The following morphological parameters and biometric measurements were obtained: main shoot height and shoot fresh weight (FW), flag leaf area, spike length and spike dry weight (DW), number of grains per spike, and grain yield per spike.

Leaf area was calculated as: $S = 2/3ld$, where l is the length and d is the width of the leaf [30].

FW was measured immediately after harvest. Spike and grain DWs were obtained after oven drying at 60 °C until constant mass.

2.4. Chemical Analysis

The Zn concentration in grain was expressed as mg per kg DW and was analyzed by atomic absorption spectroscopy (AA-7000 Shimadzu, Japan) after prior mineralization in a solution of HNO₃ and HCl (9:1 v/v) using Speedwave Digestion (Berghof, Eningen, Germany). Experiments were carried out using the equipment of the Core Facility of the Karelian Research Center of the Russian Academy of Sciences.

2.5. Statistical Analyses

For measurement of each parameter, 20 plants were taken. The data were processed using Excel 2007 (Microsoft, Redmond, WA, USA). Data were analyzed by ANOVA. The Student's t -test was used to compare means. Differences at $p < 0.05$ were considered as statistically significant.

3. Results

3.1. Effect of Zn Deficiency on Plant Growth

Zn deficiency did not affect the shoot height and FW of all variants of wheat plants, with the exception of *T. dicoccoides* which had a decreased shoot height compared to the control (Table 1).

Table 1. The effect of Zn deficiency on shoot height and FW of wheat with different allelic statuses of the *Gpc-B1* gene. Averages followed by different letters within the same parameter indicate statistically significant differences according to the t -test ($p < 0.05$). The data are expressed as means $\pm$ SE ($n = 20$).

Variant	Shoot Height (cm)		Shoot FW (g)	
	Zn+	Zn−	Zn+	Zn−
<i>Triticum dicoccoides</i>	101.5 $\pm$ 1.98 b	95.55 $\pm$ 1.77 c	2.38 $\pm$ 0.17 c	2.60 $\pm$ 0.17 c
<i>Triticum aestivum</i> cv. <i>Festivaalnaya</i>	87.42 $\pm$ 2.58 de	81.85 $\pm$ 2.31 e	5.36 $\pm$ 0.27 ab	5.06 $\pm$ 0.18 b
Line 15-7-1	99.85 $\pm$ 3.78 bc	94.05 $\pm$ 3.59 cd	6.07 $\pm$ 0.46 a	5.87 $\pm$ 0.41 ab
Line 15-7-2	114.26 $\pm$ 3.33 a	114.45 $\pm$ 2.54 a	4.97 $\pm$ 0.27 b	4.84 $\pm$ 0.26 b

At the same time, only in *T. dicoccoides* was the flag leaf area increased under zinc deficiency. The other variants had no differences in this parameter under Zn deficiency or its optimum amount (Figure 1).

Figure 1. The effect of Zn deficiency on the flag leaf area (cm²) of wheat with different allelic statuses of the *Gpc-B1* gene. Averages followed by different letters within the same parameter indicate statistically significant differences according to the *t*-test ($p < 0.05$). The data are expressed as means $\pm$ SE ($n = 20$).

3.2. Effect of Zn Deficiency on Grain Yield Components of Plants

Between all variants, only *T. dicoccoides*, containing a functional allele of the *Gpc-B1* gene, demonstrated an increase in the spike DW under Zn deficiency (Table 2). Zn deficiency did not affect the spike length and DW in the introgressive lines. Moreover, the wheat cv. *Festivalnaya* had no statistical significant differences in the spike length and DW, although a tendency towards a decrease in spike length may be suggested.

Table 2. The effect of Zn deficiency on the spike length and DW of wheat with different allelic statuses of the *Gpc-B1* gene. Averages followed by different letters within the same parameter indicate statistically significant differences according to the *t*-test ($p < 0.05$). The data are expressed as means $\pm$ SE ($n = 20$).

Variant	Spike Length (cm)		Spike DW (g)	
	Zn+	Zn–	Zn+	Zn–
<i>Triticum dicoccoides</i>	4.38 $\pm$ 0.14 c	4.59 $\pm$ 0.13 c	0.45 $\pm$ 0.02 c	0.53 $\pm$ 0.02 b
<i>Triticum aestivum</i> cv. <i>Festivalnaya</i>	7.08 $\pm$ 0.22 b	6.67 $\pm$ 0.11 b	1.01 $\pm$ 0.10 a	1.20 $\pm$ 0.10 a
Line 15-7-1	8.20 $\pm$ 0.32 a	8.41 $\pm$ 0.29 a	1.01 $\pm$ 0.10 a	1.18 $\pm$ 0.11 a
Line 15-7-2	8.21 $\pm$ 0.19 a	7.99 $\pm$ 0.18 a	1.33 $\pm$ 0.08 a	1.42 $\pm$ 0.08 a

The changes in grain number and grain yield per spike also did not depend on the allele status of the *Gpc-B1* gene in wheat under Zn deficiency. Among the studied variants, only *T. dicoccoides* showed an increase in grain number and grain yield per spike under Zn deficiency compared to the control (Table 3).

Table 3. The effect of Zn deficiency on grain number and grain yield per spike in wheat with different allelic statuses of the *Gpc-B1* gene. Averages followed by different letters within the same parameter indicate statistically significant differences according to the *t*-test ($p < 0.05$). The data are expressed as means $\pm$ SE ($n = 20$).

Variant	Grain Number, Pieces per Spike		Grain Yield, g per Spike	
	Zn+	Zn−	Zn+	Zn−
<i>Triticum dicoccoides</i>	12.84 $\pm$ 0.87 d	14.30 $\pm$ 0.65 c	0.29 $\pm$ 0.02 e	0.37 $\pm$ 0.02 d
<i>Triticum aestivum</i> cv. <i>Festivalnaya</i>	20.63 $\pm$ 2.47 b	20.75 $\pm$ 1.92 b	0.68 $\pm$ 0.09 c	0.91 $\pm$ 0.09 abc
Line 15-7-1	21.20 $\pm$ 2.23 b	21.10 $\pm$ 2.08 b	0.76 $\pm$ 0.09 bc	1.01 $\pm$ 0.09 ab
Line 15-7-2	27.79 $\pm$ 1.23 a	26.20 $\pm$ 1.28 a	0.96 $\pm$ 0.06 ab	1.07 $\pm$ 0.06 a

3.3. Effect of Zn Deficiency on Grain Zn Content

Zn deficiency affected the Zn accumulation in the grains differently between wheat with a functional and a non-functional allele of the *Gpc-B1* gene. The plants with a functional allele of the *Gpc-B1* gene (*T. dicoccoides* and line 15-7-1) under Zn deficiency accumulated more Zn in their grains than the control plants (Figure 2). *T. aestivum* cv. *Festivalnaya* with a non-functional allele of the *Gpc-B1* gene under Zn deficiency had a lower Zn concentration its grains compared to the control, whereas line 15-7-2 had no difference in Zn concentration. According the results, the wheat with a functional *Gpc-B1* gene allele had a higher Zn content in its grains than the plants with a non-functional allele of *Gpc-B1* gene under Zn deficiency conditions.

Figure 2. The effect of Zn deficiency on Zn concentration in grains of wheat with different allele statuses of the *Gpc-B1* gene. Averages followed by different letters within the same parameter indicate statistically significant differences according to the *t*-test ($p < 0.05$). The data are expressed as means $\pm$ SE ($n = 3$).

4. Discussion

The *Gpc-B1* gene encodes a NAC (domain present in NAM, ATAF, and CUC genes) transcription factor. NAC proteins are plant-specific transcription factors that have been shown to function in relation to plant development, as well as abiotic and/or biotic stress responses [31–33]. The main motivation for the introgression of the wild-type *Gpc-B1* allele into a modern wheat cultivars is to improve its grain nutritional content [34]. Lines with a functional allele of the *Gpc-B1* gene are capable of accumulating more trace elements

including Zn in grains as compared to lines containing a non-functional allele. This is linked to their high capacity for remobilization of the microelements and their transport from the leaves to the spike during grain filling [35]. However, the response of plants with different functional statuses of the *Gpc-B1* gene allele under Zn deficiency is poorly understood.

Zn deficiency in substrates leads to a decrease in the growth of cereals and their above- and underground biomass [36–38]. Primarily, this is more visible for species (varieties, genotypes) that are less tolerant to Zn deficiency. In our experiments, the height and FW of all of the wheat plants did not differ under the optimal or deficient Zn contents in the substrate. However, *T. dicoccoides* experienced an increase in flag leaf area under Zn deficiency. Considering that the flag leaf is the main donor of assimilates for grain formation in cereals, the increase in its area can result in an enhancement of grain production under micronutrients deficiency.

Zn deficiency in substrates causes a decrease in the Zn concentration in grains and can affect the grain yield. Previously, it was demonstrated that these conditions also resulted in a decrease in spike size, grain quantity in spikes, number of unsterile spikelets, and grain weight. In particular, Zn deficiency led to an almost two-fold decrease in grains per cob in a hybrid variety of maize, namely, Pioneer-32 [39]. Moreover, the maize variety Xun County showed a decrease in the length of its cobs and the weight of 1000 seeds [40]. Zn deficiency caused a decrease in grain weight and quantity per spike in the wheat variety Yumai 49-198 compared to plants under optimal Zn nutrition [41]. These changes could be a consequence of the negative effect of Zn deficiency on the processes of flower formation, pollination, and fertilization, and/or the interruption of physiological, and biochemical processes in mother plants [42–45].

Some researchers demonstrated that plants with different grain protein contents (GPCs) and allele statuses of the *Gpc-B1* gene under sufficient trace element nutrition experienced no differences in grain yield. In particular, high and low GPC inbred lines of hard red spring wheat (ND683/'Bergen', 'Glupro'/'Keene', and 'Glupro'/'Bergen') [46] experienced no differences in yield, although there were differences in the 1000-grain weight [47]. In the same species of wheat, Carter et al. (2012) also demonstrated the absence of a difference in grain yield between isogenies lines with a non-functional or functional allele of the *Gpc-B1* gene [48]. Nadolska-Orczyk et al. (2017) found that, under Zn deficiency, wheat with a non-functional allele of *Gpc-B1* gene had increased grain size but a delayed aging process [49]. The authors supposed that these changes could be a result of a long ripening time. In our experiment, wheat plants with different allele statuses of the *Gpc-B1* gene did not experience a significant decrease in the length and DW of the spike under Zn deficiency. Only *T. dicoccoides* demonstrated an increase in the spike DW under these conditions.

As described above, the low content of Zn in wheat grains is a crucial global problem. The use of the method of distant hybridization with the introduction of a functional allele of the *Gpc-B1* gene from wild wheat aims to eliminate this problem. Particularly, Distelfeld et al. (2007) showed that the recombinant lines containing a functional allele of the *Gpc-B1* gene accumulated more Zn than lines with a non-functional allele of the *Gpc-B1* gene [35]. Winter wheat with the introgressing *Gpc-B1* gene from *T. dicoccoides* has a significantly higher amount of Zn in the grains [28]. Our results demonstrated that under Zn deficiency the wheat plants with a functional *Gpc-B1* gene allele (*T. dicoccoides* and line 15-7-1) accumulated more Zn in their grains than plants with a non-functional allele of the *Gpc-B1* gene. We must then consider that our study takes into consideration only some varieties of cultivated wheat. It would therefore be useful to extend the study to other wheat varieties, preferably ancient ones, to better evaluate the results obtained in the present study.

5. Conclusions

According to our data, the wheat response to Zn deficiency does not depend on the occurrence of a functional allele of the *Gpc-B1* gene. However, the Zn concentration in the

grains was higher in the wheat with a functional allele of the *Gpc-B1* gene in comparison to the wheat carrying a non-functional allele of the *Gpc-B1* gene. We suppose that the realization of the distant hybridization method for the creation of wheat lines with a functional allele of the *Gpc-B1* gene could be a solution for eliminating Zn deficiency in nutrition due to their ability to accumulate higher amounts of Zn in grains without a decrease in yield, even under Zn deficiency in substrates. However, further research is needed to assess the potential for wheat with a functional allele of the *Gpc-B1* gene growing under Zn deficiency. Furthermore, research is still required with the aim of evaluating the capability of other introgressive lines with a functional and non-functional allele of the *Gpc-B1* gene to accumulate the Zn in a higher amount in grains under Zn deficiency in substrate. The mechanisms of these phenomena also need to be studied.

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