Different sets of TaCKX genes affect yield-related traits in wheat plants grown in a controlled environment and in field conditions

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Abstract

Background: TaCKX wheat gene family members (GFMs) encode the enzyme cytokinin oxidase/dehydrogenase (CKX), which irreversibly degrades cytokinins. The genes are important regulators of cytokinin content and take part in growth and development, with a major impact on yield-related traits. The goal of this research was to test whether these genes might be differentially expressed in the field compared to laboratory conditions and consequently differently affect plant development and yield.

Results: We compared expression and crosstalk of the TaCKX GFMs and TaNAC2-5A gene in modern varieties grown in a growth chamber (GC) and in the field and looked for differences in their impact on yield-related traits. The TaNAC2-5A gene was included in the research since it was expected to play an important role in co-regulation of these genes. The range of relative expression levels of TaCKX GFMs and TaNAC2-5A gene among tested cultivars was from 5 for TaCKX8 to more than 100 for TaCKX9 in the GC and from 6 for TaCKX8 to 275 for TaCKX10 in the field. The range was similar for four of them in the GC, but was much higher for seven others and TaNAC2-5A in the field. The TaCKX GFMs and TaNAC2-5A form co-expression groups, which differ depending on growth conditions. Consequently, the genes also differently regulate yield-related traits in the GC and in the field. TaNAC2-5A took part in negative regulation of tiller number and CKX activity in seedling roots only in controlled GC conditions. Grain number and grain yield were negatively regulated by TaCKX10 in the GC but positively by TaCKX8 and others in the field. Some of the genes, which were expressed in seedling roots, negatively influenced tiller number and positively regulated seedling root weight, CKX activity in the spikes, thousand grain weight (TGW) as well as formation of semi-empty spikes.

Conclusions: We have documented that: 1) natural variation in expression levels of tested genes in both environments is very high, indicating the possibility of selection of beneficial genotypes for breeding purposes, 2) to create a model of an ideotype for breeding, we need to take into consideration the natural environment.

Keywords: TaCKX, TaNAC, Wheat, Field, Laboratory conditions, Cytokinin, Phytohormone crosstalk, Grain yield

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Background

Wheat is one of the most economically important cereal crops [1]. It can be cultivated in a wide range of environmental conditions and is rich in nutrition components. It provides approximately 20% of protein in the human diet [2]. The large, hexaploid genome, which is composed of three A, B and D diploid genomes, is a rich reservoir of genes determining yield-related traits [3]. However, the increase of wheat yield has remained moderate or even stagnate within the last two decades [4, 5]. This cereal species is much less studied compared to rice and maize [6].

CKX GFMs encode the enzyme cytokinin oxidase/dehydrogenase (CKX), which irreversibly degrades cytokinins [7], and therefore strongly regulates cytokinin content in different organs of plants. Since cytokinins play a diverse role in plant development and affect a number of agriculturally important processes [8, 9], the CKX genes influence yield-related traits. In cereals it was documented by means of silencing of selected CKX in rice [10], in barley [11, 12] and in wheat [13] leading to an increased level of cytokinins, affecting yield components. The phytohormone also regulates changes in gene expression of cytokinin-induced genes to mediate its pleiotropic effect [9, 14].

The number of CKX GFMs varies depending on species. In bread wheat 11 to 14 gene family members have been proposed [15–18]. TaCKX2 underwent gene duplication [15] and the resultant genes were assigned as TaCKX2.1 and TaCKX2.2 based on the Ensembl Plants database [19] and phylogenetic analysis by Ogonowska et al. [16]. The numbering of TaCKX GFMs was recently revised taking advantage of the most up-to-date databases (including IWGSC RefSeq v2.0), their homology with closely related monocot species including barley and diploid genome specificity [20]. Based on these data, there are 13 TaCKX GFMs basically numbered as TaCKX1, TaCKX2.1, TaCKX2.2, TaCKX2.2.1, TaCKX2.2.2, TaCKX2.2.3, TaCKX3 (6), TaCKX4, TaCKX5, TaCKX7, TaCKX8 (11), TaCKX9 (10), TaCKX10 (9) and TaCKX11 (3), and 12 of them are allocated to each of the three chromosomes associated with the A, B and D genomes, giving the number of 35 homologous genes. The exception is TaCKX2.2.2 allocated only to D genome. This new numbering of the TaCKX genes is applied in this publication and the former numbers are shown in brackets.

The CKXs exhibit distinct patterns of organ- and development-specific expression. In our earlier research on HvCKX in barley we found that expression patterns of the genes indicate their role in growth and reproductive development [21]. Therefore, the first step of our investigation of the CKX genes in wheat was focused on expression specificity in different organs and different developmental stages [16]. Based on these results the genes were classified to four groups: i) leaf-specific TaCKX9, TaCKX5, TaCKX4; ii) specific to inflorescence and developing spike TaCKX1 and TaCKX2; iii) seedling root-specific TaCKX10, TaCKX7 and iv) expressed at various levels in all tested organs TaCKX11, TaCKX3, TaCKX8 [16]. The TaCKX GFMs co-operated inside and among organs. The effect of TaCKX1 silencing was further investigated in 7 DAP (days after pollination) spikes [13]. Various levels of TaCKX1 silencing in T1 and T2 generations influenced different modes of co-expression with other TaCKX GFMs and parameters of yield-related traits. Only lines with strongly silenced TaCKX1 and associated with this strong down-regulated TaCKX11 and up-regulated TaCKX2.1 and TaCKX9 were characterized as high yielding. The content of most of the cytokinins in spikes 7 DAP of silenced T2 lines significantly increased, and their interaction with other phytohormones was demonstrated. Each of the tested yield-related traits was regulated by various up- or down-regulated TaCKX GFMs and phytohormones.

Cytokinins regulate gene transcription in target organs and developmental stages through a wide range of transcription factors (TF) [22]. One of the largest groups of plant TFs involved in cytokinin-dependent regulation is the family of NAC (for NAM, ATAF, and CUC) TFs. It has been documented that NACs are involved in the regulation of important agronomic traits [23–26]. Overexpression of nitrate inducible wheat TaNAC2-5A increased root growth, the rate of nitrate uptake under hydroponic conditions and in the field increased grain yield, grain nitrogen (N) concentration, and N harvest index under various N supply levels [23]. This gene was included in our research as an important regulator of cytokinin activity.

Experiments in controlled environments, in contrast to field conditions expose plants to constant irradiance during the day, constant temperature and artificial soil conditions. The growth parameters and the morphology of Arabidopsis plants, grown under artificial light, independent of the source, were significantly different compared with plants grown in natural conditions [27]. Distinct epistatic interactions on flowering time in barley were observed in field and in semi-controlled conditions [28]. Moreover, plant hormones differently regulated grain development in normal and under abiotic stress conditions [29–32]. It was also reported that yield stability genes are differentially expressed in the field compared to laboratory conditions [33].

Transgenic solutions to increase yield in wheat seemed to be very promising, but no transgenic cultivar has yet been approved [5]. On the other hand, natural variation is proposed as a profitable rich source of research objects to understand the responses mediated by jasmonate signalling [34]. We have been testing two alternative strategies of changing TaCKX-dependent cytokinin regulation in order to increase grain yield in wheat. One
is based on the modifications of the *TaCKX* genes’ expression via RNAi-based gene silencing [13]. The second one, presented here, explores the possibility of selection of natural variants differing in expression of *TaCKX* GFMs in wheat genotypes/breeding material. Differences revealed in co-regulation of yield-related traits by these GFMs in different environments, could lead to selection of appropriate genotypes in breeding programmes dedicated to specific environment. Therefore in the first part of the study we examined the range of expression of the *TaCKX* GFMs in 7 DAP spikes and in seedling roots among cultivars and breeding lines in a growth chamber and in the field. We analysed co-expression among themselves and with *TaNAC2-5A* and their influence on yield-related traits in both environments. In the next part of this research we will investigate how expression patterns of selected marker genes are inherited and whether they are consistent with inheritance of yield-related traits.

**Results**

**Expression of *TaNAC2-5A* in different organs of developing wheat plants**

The level of relative expression of *TaNAC2-5A* (NAC2) was highest in leaves, seedling roots and 0 DAP spikes, ranging from 0.015 up to 0.057 (Fig. 1). This wide range was mainly dependent on the large differences of expression of the gene among three tested cultivars. For two of them, the cultivars Ostka and Trappe, the expression levels of the NAC2 in leaves was about three times higher than in Kontesa. Interestingly, opposite data of expression levels in the same cultivars were found in inflorescence, where they were several times lower.

There were no significant differences in expression of NAC2 in 0 DAP spikes among cultivars. Expression levels of NAC2 in 7 DAP and 14 DAP spikes ranged from 0.005 to 0.01. There were some differences between cultivars, but not between the first and the second spike or daily times of sample collection.

**Range of variability of expression of *TaCKX* GFMs and *NAC2* in growth chamber and the field**

Expression levels of *TaCKX* GFMs and *NAC2* were measured in 7 DAP spikes of 34 breeding lines growing in a growth chamber (GC) and in the field. Highest, mean and lowest values of relative expression are presented in Fig. 2. The level of the *CKX1* mean relative expression was higher in 7 DAP spikes from the GC (0.043) compared to the same material from the field (0.026). However the highest (0.126) and the lowest (0.016) values obtained for the same gene in the GC indicated a 7.88-fold difference (quotient of variability), while the values (0.102 and 0.006) in the field showed 18.36-fold difference.

Similar to *CKX1*, mean expression of *CKX2.2.1* and *NAC2* (*TaNAC2-5A*) were higher in the GC (0.0038 and 0.020) compared to the field (0.0025 and 0.015). However for most of the *TaCKX* GFMs (*CKX2.2.2, CKX3, CKX5, CKX8, CKX9, CKX10, CKX11*) the mean values of expression were higher in the field compared to the GC. The range of expression variability between breeding lines in 7 DAP spikes growing in both conditions was similar for *CKX2.2.1, CKX2.2.2, CKX5* and *CKX9*, and showed big differences for *CKX1, CKX3, CKX4, CKX5, CKX10, CKX11* and *NAC2* in the field conditions. The highest variability between expression levels of the genes from the GC compared to the field ranging from 5 to 10 times was observed for *CKX4, CKX10* and *CKX11*. *CKX1* was highly expressed followed by *CKX11, CKX2.2.2* and *CKX9* in both growing conditions. The mean level of NAC2 expression was similar to *CKX11* in the field (0.016 and 0.015 respectively) and two times

![Fig. 1 Specificity of expression of *TaNAC2-5A* gene in developing organs of wheat. ** – significant at p ≤ 0.01; * – significant at p ≤ 0.05; II – second spike](image-url)
higher in the GC (0.020 and 0.008 respectively). Standard deviation of gene expression levels among three biological replicates were higher in spikes from the field compared to the GC.

Co-expression of TaCKX GFMs in growth chamber and the field
Correlation between expression levels of various TaCKX GFMs was different in both growth conditions, GC and the field (Fig. 3, Table S2). Since breeding lines were grown on two different fields of two Plant Breeding Companies Strzelce (S) and Danko (D), correlations are presented for both conditions together (S + D) and separately (S or D). Generally, in field conditions the groups of mutually correlated genes were larger than in GC conditions and co-expression of a few of them was specific to the field (S or D). According to expression specificity, the TaCKX GFMs were divided into four groups. The first one contained the genes specifically expressed in developing spikes; the genes from the second group were expressed in seedling roots; the third were specific in younger plant organs from seedling roots to 0 DAP spikes and the fourth to all organs tested.

expression level of spike specific CKX1 significantly correlated with expression levels of CKX2.2.2 and CKX11 in the GC, but no correlation with others was found in spikes from both fields. Otherwise CKX5 showed specific negative co-expression with CKX1, when spikes were grown on D field. CKX2.2.2, whose expression correlated with CKX1 in the GC, was strongly correlated with CKX3, CKX10 and CKX8 in both fields, and individually with CKX2.2.1 in S field. CKX2.2.1 was co-expressed with CKX5 in both growing conditions. The gene was also co-expressed with CKX3, CKX8 and
CKX10 in both fields and individually with CKX2.2.2 in S field. Expression of root-specific CKX3 from the second group of genes positively correlated with expression of NAC2 and negatively with CKX activity in the GC and with CKX2.2.1, CKX2.2.2, CKX8 and CKX10 in the fields as well as strongly negatively with CKX9 in D field. Another root-specific, CKX10, exclusively correlated with CKX11 in the GC and with CKX2.2.1, CKX2.2.2, and CKX8 in the fields as in the case of CKX3. Moreover, expression level of both, CKX3 and CKX10 showed a mutual correlation in the fields conditions. The CKX5, which was strongly expressed in younger organs, especially in seedling roots, leaves and inflorescences, co-expressed with CKX2.2.1 in both conditions, with CKX9 in GC and with CKX4 in the fields as well as with CKX9 in S field. Expression of the next one from this group, CKX9, which was especially high in leaves, was positively correlated with CKX5 and negatively with NAC2 in GC but positively with CKX4 in both fields, and positively with CKX5 in S field but negatively with CKX3 in D field. No correlation was found between expression of CKX8 and any of the tested genes in GC. However, the CKX8 was positively correlated with CKX2.2.2, CKX3, CKX5 and CKX10 in both fields conditions.

The last group represented genes expressed in all organs. Interestingly, expression of CKX11 correlated with expression of CKX1 and CKX10 in GC. There was no correlation of this gene with others in the field conditions. Conversely, the level of CKX4 expression positively correlated with CKX5 and CKX9 in both fields, but there was no correlation in GC. There was a positive correlation between expression of NAC2 and CKX3 and a negative correlation with CKX9 in GC. No correlation of NAC2 with others was found in the field.

Expression of TaCKX GFMs and NAC2 and CKX activity in 7 DAP spikes differently affect yield-related traits in both growth conditions

In the GC, plant height and TGW were positively correlated with CKX activity in 7 DAP spikes (Fig. 4, Table
S3). However, the plant height and the spike length were negatively correlated with CKX3. Enzyme activity was also positively correlated with number of spikes and number of semi-empty spikes. Moreover, the number of semi-empty spikes is positively correlated with expression of CKX2.2.1 and CKX5, and tiller number is negatively correlated with NAC2. Grain number and grain yield in the same growth conditions are negatively correlated with CKX10. Otherwise, in the fields conditions both grain number and grain yield are negatively
correlated with expression of CKX9, and positively correlated with CKX8, but the grain yield is negatively correlated with CKX11 (spikes from both fields). Additionally grain number is strongly negatively correlated with CKX4 expression in spikes from S field and very strongly positively correlated with CKX10 in case of spikes from D field. Otherwise grain yield is positively regulated by CKX4 and CKX10 in case of D field. The CKX8 expressed in spikes from both fields is not only positively correlated with grain number and grain yield but also with spike length, spike number and tiller number. Both spike number and number of semi-empty spikes are positively correlated with CKX2.2.2 measured in spikes collected from both fields. Individually, spike number is negatively correlated with CKX4 and CKX9 only in case of spikes from S field and tiller number is positively correlated with CKX4 and CKX10 measured in spikes from D and S fields respectively.

Expression of TaCKX GFMs and NAC2 and activity of CKX in seedling roots affect yield-related traits

Expression of CKX1 in seedling roots was positively and strongly correlated with CKX3, CKX5, CKX8 and NAC2 (Fig. 5a, Table S4). There was no correlation between CKX10 and CKX11 and other genes expressed in this organ. CKX3 expressed in seedling roots is the most frequently interacting gene with yield-related traits in the GC (Fig. 5b, Table S4). Its expression positively
correlated with seedling root weight and semi-empty spikes, but negatively with tiller number. Positive correlation of the CKX3 is accompanied by CKX activity in spikes, and in the case of semi-empty spikes, additionally with CKX activity in roots. Moreover, CKX3 and CKX8 are positive regulators of spike CKX activity and, with NAC2, positively correlated with CKX activity measured in seedling roots. Expression of CKX8 is positively correlated with CKX activity in spikes and TGW. Expression of CKX5 is negatively correlated with tiller number.

Discussion

TaNAC2-5A is expressed in different organs of wheat plants

TaNAC2-5A (NAC2) was included into this research on regulation of yield-related traits of wheat by the TaCKX gene family as a possible important regulator of these genes and the corresponding processes. This nitrate-inducible NAC2 encoding gene was reported by He et al. [23] to be an important regulator of root growth and wheat yield. To relate NAC2 expression to our previous research on expression specificity of TaCKX, we performed expression experiments similar to those we did with TaCKX [16]. Relative expression levels of NAC2 in roots, leaves and 14 DAP spikes reported by He et al. [23] were comparable with our results. The NAC2 is also expressed in organs newly tested by us: inflorescence, 0 DAP and 7 DAP spikes, but at a level lower than for 14 DAP spikes. Generally, the gene is expressed in all organs tested. We also demonstrated that, especially in the case of leaves, inflorescence and root expression, levels of NAC2 differed considerably between cultivars.

Large differences in TaCKX GFMs and NAC2 expression levels among breeding lines allow them to be selected for breeding purposes

The differences between the highest and the lowest expression levels of tested TaCKX GFMs and NAC2 in breeding lines were significant and ranged from several to over a hundred times. These differences were much bigger for six of the genes expressed in 7 DAP spikes growing in the field compared to GC. This is consistent with the observation that stress inducible genes were expressed more strongly in field than in laboratory conditions [33]. These differences in expression might be the result of allelic variation among the tested genes, their genetic background and cross-talk with environments. Allelic variation among TaCKX family genes corresponding with yield-related traits was reported by others [35–37]. The data indicate the possibility of using this parameter for selection in the breeding process.

Selected TaCKX GFMs are differentially expressed in field and GC conditions

The similar range of expression variability among breeding lines for CKX2.2.1, CKX2.2.2, CKX8 and CKX9 in the GC and in the field might indicate that expression levels of these genes are independent of growth conditions. The data indicating stable expression being maintained by these genes in changing conditions are in agreement with newly published results of TaCKX1 silencing in the case of both CKX2.2 [13]. There was strongly decreased expression of TaCKX1 gene in silent lines while expression of CKX2.2 remained at the same level. Otherwise, the impact of unstable field growth conditions on expression of CKX1, CKX3, CKX4, CKX5, CKX10, CKX11 and NAC2 was significant. Indeed, TaCKX1 silencing caused strong down-regulation of expression of CKX11 and up-regulation of CKX5 [13]. It may be dependent on the same feedback mechanism between induction of expression of selected CKX genes by cytokinin and their function [38]. It was already documented that constant irradiance during the day in controlled environments and lack of light-day transitions may significantly influence plant phenotype [27]. Consequently, many group of genes determining yield in normal and stress conditions, genes encoding chloroplast located proteins or shade avoidance (including genes involved in hormonal regulation, light and flowering) are differentially expressed in growth chamber versus field conditions [33, 39]. Expression of CKX as well as biosynthetic IPT genes might also be regulated by the levels of available macronutrients, such as nitrate and phosphate [40], and biotic/abiotic stress conditions in the field.

TaCKX GFMs and NAC2 in field or controlled environments form different co-expression groups

The TaCKX GFMs and NAC2 are predominantly co-expressed in the GC and in the field in different groups. Only two of them co-work with each other in both conditions: CKX2.2.1 expressed in developing spikes and CKX5 highly expressed in seedling roots, inflorescences and leaves [16]. This cooperation may indicate their importance in regulation of cytokinin level between roots and spikes during plant growth and development independently of growth conditions. What is remarkable, CKX1 highly expressed in developing spikes and roots, and CKX11 and NAC2 expressed in all organs formed co-expression groups with other genes only in GC conditions. It does not mean that the genes are not susceptible to different nutrition conditions since NAC2 was shown to be inducible by nitrate [23]. However, it is possible that expression of these genes might be independent of main environmental factors as the source of light and light-day transitions as well as changing temperatures and soil moisture. CKX11
highly expressed in all organs, co-worked with CKX1 and CKX10 in the GC conditions but not in the field, although their expression in the field was seven times higher. This strong co-regulation of CKX11 with CKX1 was already observed in our research with silencing the TaCKX1 performed in GC conditions [13]. Conversely CKX4 expressed in all organs and CKX8 expressed in younger organs, which did not show any co-expression with others in GC, were co-regulated by others in the field. Therefore, these genes might take part in regulation of cytokinin content by involving others in more stressful conditions.

Significantly larger were groups of TaCKX genes co-expressed in the unstable, field conditions. CKX8 expressed in younger organs was the most frequently co-working with others, mainly those expressed in developing spikes and roots. However, the gene co-works with CKX5 belonging to the same expression group, which might link coregulation of expression of other genes in both growth conditions. Expression of CKX2.2.1 and 2.2.2 specifically expressed in developing spikes is correlated with the same root-specific CKX3 and CKX10 as well as CKX8 specific to younger organs but only in field conditions. Both CKX2.2 genes correlate with different genes in GC, CKX2.2.1 with CKX5 and CKX2.2.2 with CKX1, which might indicate their different detailed functions (discussed below).

Both root-specific CKX3 and CKX10 are co-regulated with both, spike-specific CKX2.2 and CKX8 specific to all organs, and each other in the field, but in GC these genes specifically co-work with GC-specific NAC2 and CKX11 respectively.

Yield-related traits are regulated by different TaCKX GFM genes and NAC2, depending on the natural or controlled environments

As in the case of co-expression, more CKX genes were involved in regulation of yield-related traits, especially grain number, grain yield, spike number and tiller number in the field conditions than in GC. The most active in the field was CKX8 expressed in all organs co-regulating with CKX9 grain number, with CKX9 and CKX11 grain yield, with CKX11 spike number, with CKX2.2.1 and CKX10 tiller number and as a single spike length. In most cases these traits are positively regulated by CKX8 but negatively by co-expressed TaCKX genes, which indicates their predominant role in maintaining hormonal homeostasis. Interestingly some traits like grain number, grain yield, spike number and tiller number are strongly regulated by CKX4 and CKX10 but this regulation is field dependent. Presumably both genes are strongly dependent on environmental conditions since both research fields differ in type of soil and weather conditions.

The same traits, grain number and grain yield, are regulated in GC negatively by CKX10. None of the genes regulates the same yield-related trait in both growth conditions. Similarly as in the study by Jablonski et al. [13], grain yield and spike number in GC were negatively regulated in 7 DAP spikes by CKX11 which, in cooperation with CKX2.2.1, influenced down-regulation of free base, active trans-zeatin (tZ) and \( \Delta^2 \)-isopentenyl adenine (iT) but up-regulation of benzyladenine (BA). In the same research, spike number in GC-grown, control plants was regulated by CKX2.2.1 as well, which together with CKX1 and CKX5 up-regulated tZ and iP and down-regulated BA. Some of our data do not appear comparable, because in Jablonski et al. [13] only one cultivar with a restricted number of TaCKX genes and yield-related traits was tested. However, it was shown that the genes form different co-expression groups in 7 DAP spikes of TaCKX1 silent and not-silent plants whereby they control the content of various cytokinins and determine final yield-related traits. Different coregulation of both CKX2.2 genes in the GC and the field resulted in their various impact on yield-related traits. CKX2.2.1 together with CKX5 is involved in formation of semi-empty spikes in GC but CKX2.2.2 alone has such involvement in the field. CKX2.2.1 together with CKX8 and CKX10 positively regulates tiller number in the field as well. None exhibits the same role in the GC and in the field. Zhang et al. [35] reported that TaCKX6, renamed by [30] as TaCKX2.2.1-3D, is an orthologue of OsCKX2 regulating rice yield [10]. The cultivar, which has an 18-bp deletion, showed decreased expression of this gene, which was associated with a greater TGW than in other haplotypes. There is a small incompatibility in maximum transcript level, which was reached at 8 DAP in the study by Zhang et al. [35] and was increasing from 0 DAP through 7 DAP and reaching the maximum at 14 DAP in our research (later stages were not tested). This increasing expression in developing spikes up to 14 DAP better explains the possible role of the gene in grain filling, which is an essential factor of greater TGW. Besides this, we did not find any correlation of expression of this gene with TGW, although its expression together with CKX5 positively influenced formation of semi-empty spikes in GC and with two others tiller number in the field. Therefore the joint effect of TaCKX family genes on regulation of yield-related traits depending on growth conditions is important. However, expression of TaCKX2.2.1-3A (originally TaCKX2.4) reduced by RNAi [41] showed a strong correlation with grain number in T3 due to an increased grain number per spike. Similar result is obtained in our research, in which CKX2.2.1 together with CKX5 positively regulates formation of semi-empty spikes in GC, although the same trait was regulated by CKX2.2.2 in the field. Moreover,
function of TaCKX2.2.1-3A is closer to their orthologue OsCKX2 in rice, where is also associated with grain number [10]. According to Li et al. [41], different functions of TaCKX2.2.1-3D and TaCKX2.2.1-3A genes are the result of different locations on chromosomes 3D and 3A respectively. We suppose that these differences might be additionally modulated by possible individual co-operation with other genes within an individual genetic background.

There were two more characterized TaCKX family genes, both demonstrated as determinant of yield-related traits. Isolated by Lu et al. [36], the novel allele TaCKX6a allocated to the TaCKX2.1 gene family [16] and renamed as TaCKX2.1-3D [20] showed, as in our earlier research [13], significant correlations with grain size, weight and grain filling rate in recombinant inbred lines. In the second report one out of two alleles of TaCKX4 found by Chang et al. [37] was positively associated with chlorophyll content and grain yield. The authors did not show any expression data. According to our research, the gene is expressed in all tested organs of developing wheat plant in the GC, showing higher expression in leaves, but their expression was low compared to others [16]. We did not find any correlation of their expression with yield-related traits in the CG. However we found higher expression of this gene in field conditions comparing to the GC as well as their strong correlations with yield-related traits, which was dependent on the individual field conditions.

Selected TaCKX GFM s and NAC2 expressed in seedling roots regulate some yield-related traits

As already reported [16], CKX1 expressed in seedling roots forms a co-expression group with CKX3, CKX5, CKX8 and NAC2 in the same organ. Moreover, the TaCKX GFM s and NAC2 gene expressed in seedling roots formed different co-expression groups with that expressed in developing spikes. It means that the genes also participate in coordinated development of the above ground part of the plant, which is dependent on growth conditions. Such cooperation of TaCKX GFM s is obvious, since they regulate CKX enzyme activity, which inactivates cytokinins, plant hormones known to act opposingly in shoot and root growth [42]. An example is CKX3, highly expressed in seedling roots, which is positively correlated with seedling root weight but negatively correlated with plant height and spike length. NAC2, controlling with CKX3, CKX activity in roots, when expressed in spikes, negatively correlated with tiller number. Moreover, CKX3 with CKX5 expressed in roots negatively regulated tiller number, but CKX3 with CKX activity in roots positively determined formation of semi-empty spikes, and CKX8 expressed in roots positively influenced TGW. Such cooperation of seedling root expressing genes with those expressed in organs from the aerial part of wheat plants and their influence on yield traits was also documented in our earlier research [13, 16].

Generally, overexpression of CKX in roots of other plant species leads to enhanced root growth by reduction of cytokinin levels [43–46]. By contrast, an elevated cytokinin level inhibits root growth but promotes shoot growth in rice [47]. Among the CKX GFM s in rice, Gao et al. [48] indicated a key role of OsCKX4 in the initiation of crown roots by interaction between auxin and cytokinin biosynthesis. Mao et al. [49] underlined the role of NAC transcription factors on rice root development. Overexpressed OsNAC2 increased expression of several IPT, decreased OsCKX4 and OsCKX5 and increased cytokinin level. Therefore OsNAC2 stimulated cytokinin accumulation by promotion of cytokinin biosynthesis and repression of CKX expression. This was affected by the binding of OsNAC2 to the promoter of OsCKX4. In our research, wheat TaCKX4 orthologous to OsCKX4 was not found to correlate with seedling root weight, possibly because of its very low level of expression in roots as well as other organs of wheat plants. However, we showed strong correlations between the CKX4 gene expression and yield-related traits, which was dependent on field conditions. The second orthologue of rice, TaCKX5, together with CKX3, both expressed in roots, was negatively correlated with tiller number, but the same trait was negatively influenced by NAC2 expressed in spike.

**Conclusions**

It was previously indicated that yield stability genes are differentially expressed in the field compared to growth chambers [33]. These differences might mean that many genetically modified plants carrying single, agronomically important genes cannot be applied in practice [5, 50]. Therefore, to indicate which TaCKX family genes might be applicable in breeding programmes, it is important to characterize them in both environments, laboratory conditions and in the field. Indeed, we found large differences in the levels of expression, crosstalk of those genes and correlations with agronomic traits in modern varieties and breeding lines dependent on the environmental conditions. We have documented that to create a model of an ideotype for breeding, we need to take into consideration the field environment. Natural variation in expression levels of tested genes, especially in the field, was very high, indicating the possibility of selection of beneficial genotypes/phenotypes for breeding purposes. Such selection based on variable expression of selected genes, resulting from mutations in the tested genetic background and cross-talk with others, can be carried out among recent breeding material in their field environments. We suppose that combining beneficial genotypes selected in such environments could help breeders to exploit available genetic variation and optimize further yield potential in their
regional soil and climate conditions. Therefore, the next step of our research is to show if and how expression of TaCKX GFMs is inherited in subsequent generations.

**Methods**

**Plant material**

The experimental plant material was collected from 34 breeding lines and cultivars of common wheat (*Triticum aestivum* L.) delivered by two Plant Breeding Companies: Strzelce Ltd., Co. - IHAR-PIB Group (23 breeding lines) and Danko Hodowla Roslin Ltd. (11 breeding lines). Selected genotypes differed in productivity and other interesting breeding features, such as resistance to diseases, plant height, protein content (including gluten), and starch in grain or seed hardness. The plants were grown in two different growth conditions: in a growth chamber and in a field.

Field conditions: The field experiments were conducted on two experimental fields of Strzelce Company (Konczewice) and Danko Company (Choryn). The first is located in the north-central part of Poland (Kujawsko-Pomorski region) and the second in the west-central (Wielkopolska region), about 150 km from each other. Experimental plots were 5 m² (= 5 × 1 m) and 400 grains were sowing per 1 m². White beet and rape were the forecrops respectively. Nitrogen was applied twice at the beginning of spring vegetation and at the beginning of stem elongation in two doses (100–120 kg ha⁻¹ total). The soil was also fertilized by 20–50 kg ha⁻¹ phosphorus and 50–80 kg ha⁻¹ potassium, depending on the field requirements. Humidity and thermal conditions during the experiment are shown in Fig. 6. Water deficiency was supplemented when needed.

Germination of seeds and growth chamber conditions were performed as earlier reported [16]. The 5-days seedlings were replanted into pots with soil after earlier collection of seedling roots, which were cut 3 mm from embryo axis. The tissue samples: 5-day-old seedling roots and the middle parts of first spikes 7 days after pollination (7 DAP) were collected in three biological replicates from the growth chamber and from the field. In both environments spikes with the first 1–3 sticking out anthers from spikelets in the middle part were marked and collected after 7 days. Three biological replicates were selected randomly out of six in the growth chamber (one spike each) and out of whole plots in the field (three spikes each). These in the field were marked the same day and collected 7 days later. All these samples were started to collected at 9:00 am. Additional tissue samples used for testing NAC2 expression were collected from three cultivars of common wheat: Kontesa, Ostka and Trappe according to Ogonowska et al. [16].

**RNA extraction and cDNA synthesis**

Total RNA from 5-day-old seedlings’ roots and 7 DAP spikes was extracted using TRI Reagent (Sigma-Aldrich) according to the manufacturer’s protocol. Purification of RNA and cDNA synthesis was performed as described in Ogonowska et al. [16].

**Quantitative RT-qPCR**

RT-qPCR analysis was performed for 11 target genes: TaCKX1 (JN128583), TaCKX2.2.1 (FJ648070)/TaCKX2.2.2 (GU084177), TaCKX3 (former TaCKX6) (JN128587), TaCKX4 (JN128586), TaCKX5 (Lei et al. 2008), TaCKX8 (former TaCKX11) (JN128592), TaCKX9 (former TaCKX10) (JN128591), TaCKX10 (former TaCKX9) (JN128590), TaCKX11 (former TaCKX3) (JN128585), TaNAC2-5A (AY625683). Sequences of primers designed for the genes tested are shown in Table S1. RT-qPCR reactions were assayed as described in Ogonowska et al. [16]. Each reaction was performed in three biological and three technical replicates. The two standard curves method with
ADP-ribosylation factor (Ref 2) as a normalizer [51] was used for calculation of genes expression. Relative expression for each of the TaCKX family gene was calculated in relation to the control cultivar Ostka set as 1.00.

Analysis of CKX activity
Measurements of CKX enzyme activity were performed in the same samples subjected to TaCKX gene expression analysis according to the procedure developed by Frebort et al. [52] and optimized for wheat tissues. The procedure is described in Zalewski et al. [12]. Concentration of the product was determined at the absorption spectrum ranged from 230 nm to 550 nm. The total protein concentration was estimated based on the bovine serum albumin standard curve according to the Bradford procedure [53].

Measurement of yield-related traits
Morphometric measurement of yield-related traits of selected genotypes was performed. The described traits were: plant height, tiller number, spike number, semi-empty spike number, spike length, grain number, grain yield, TGW and seedling root weight.

Statistical analysis
Statistical analysis was done using Statistica 13 (StatSoft) software. The normality of data distribution was tested using the Shapiro-Wilk test. The significance of changes was analysed using analysis of variance (ANOVA) and post-hoc tests. Correlation coefficients were determined using parametric correlation matrices (Pearson’s test) or a non-parametric correlation (Spearman’s test).

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12870-020-02713-9.

Additional file 1 Table S1. Sequences of primers designed for amplification of the genes. Table S2. Correlations among expression of TaCKX GFM and NAC2 in 7 DAP spikes growing in growth chamber (A) and in the field (B) and in seedling roots (C). 1- non-parametric analysis; bold* – significant correlation at p ≤ 0.05. Table S3. Correlations among expression of TaCKX GFM and NAC2 in 7 DAP spikes from the growth chamber (A) and the field (B) on yield-related traits. 1- non-parametric analysis; bold* – significant correlation at p ≤ 0.05. Table S4. Correlations among expression of TaCKX GFM and NAC2 in seedling roots with seedling root weight and other yield-related traits with. 1- non-parametric analysis; bold* – significant correlation at p ≤ 0.05.

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Authors’ contributions
Conceptualization, A.N-O. and W.O.; methodology, K.S., H.O. and W.O.; software, M.D-B.; validation, W.O.; formal analysis, K.S., M.D-B. and A.N-O.; investigation, K.S., H.O. and R.W.; resources, B.L. and B.Z.; data curation, K.S., H.O., B.L., B.Z. and R.W.; writing—original draft preparation, A.N-O.; writing—review and editing, A.N-O.; visualization, K.S. and A.N-O.; supervision, A.N-O.; project administration, A.N-O.; funding acquisition, A.N-O. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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