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Modified expression of *TaCYP78A5* enhances grain weight with yield potential by accumulating auxin in wheat (*Triticum aestivum* L.)

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Summary

Increasing grain yield has always been the primary goal of crop breeding. *KLUH/CYP78A5* has been shown to affect seed size in several plant species, but the relevant molecular mechanism is still unclear and there are no reports of this gene contributing to yield. Here, we demonstrate that modified expression of *TaCYP78A5* can enhance wheat grain weight and grain yield per plant by accumulating auxin. *TaCYP78A5* is highly expressed in maternal tissues, including ovary and seed coat during wheat development. The constitutive overexpression of *TaCYP78A5* leads to significantly increased seed size and weight but not grain yield per plant due to the strengthening of apical dominance. However, localized overexpression of *TaCYP78A5* in maternal integument enhances grain weight and grain yield per plant by 4.3%–18.8% and 9.6%–14.7%, respectively, in field trials. Transcriptome and hormone metabolome analyses reveal that *TaCYP78A5* participates in auxin synthesis pathway and promotes auxin accumulation and cell wall remodelling in ovary. Phenotype investigation and cytological observation show that localized overexpression of *TaCYP78A5* in ovary results in delayed flowering and prolonged proliferation of maternal integument cells, which promote grain enlargement. Moreover, naturally occurring variations in the promoter of *TaCYP78A5-2A* contribute to thousand-grain weight (TGW) and grain yield per plant of wheat;*TaCYP78A5-2A* haplotype *Ap-Hap*II with higher activity is favourable for improving grain weight and grain yield per plant and has been positively selected in wheat breeding. Then, a functional marker of *TaCYP78A5* haplotype *Ap-Hap*II is developed for marker-assisted selection in wheat grain and yield improvement.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing more than 20% calories and protein for humans. Increasing wheat yield is critical for global food and nutrition security (FAO, http://faostat.fao.org). Wheat yield is composed of the number of panicles per unit area, the number of grains per panicle and grain weight, and among which the grain weight has high heritability and stability, with great potential for improvement (Li *et al*., 2019b). In practice, attempts to enhance grain yield through enlarging grain size/weight have always been impeded by the trade-off between grain weight and grain number. Increasing grain weight without changing grain number has become a major goal of high-yield wheat breeding (Bustos *et al*., 2013). Therefore, increasing grain weight and understanding the mechanism underlying grain size/weight control are pivotal to increase yield of wheat.

Seed is composed of embryo, endosperm and the seed coat from the maternal tissue, which together determine the size and weight of the seed (Shewry *et al*., 2012). It was demonstrated that *KLUH/CYP78A5*, which encodes cytochrome P450 monooxygenase, plays an important role in controlling grain size. In Arabidopsis, *KLUH* increases seed size by non-cell autonomously stimulating maternal integument cell proliferation (Adamski *et al*., 2009). The rice *KLUH* homolog *OsCYP78A13* affects seed size through regulating the balance of resources for cell between embryo and endosperm (Xu *et al*., 2015). In tomato, *SiKLUH* controls fruit weight by increasing cell layer and delaying fruit ripening, as well regulating plant architecture by adjusting the number and the length of branches (Chakrabarti *et al*., 2013). Previous studies in Arabidopsis suggest that *CYP78A5* is involved in the production of downstream mobile signal molecule (Anastasiou *et al*., 2007). Although *KLUH* has been shown to affect seed size in several species, there are no reports of this gene

increasing yield. The molecular mechanism of *KLUH* controlling seed size remains elusive.

Auxin, the first discovered plant growth hormone, plays an important role in plant growth and development, including cell proliferation and expansion at the cytological level, embryogenesis, apical dominance and flowering at the macroscopic level (Pagnussat *et al*., 2009; Sauer *et al*., 2013; Shimizu-Sato *et al*., 2009). Appropriately increasing auxin can increase crop yield (Shao *et al*., 2017). Recent studies showed that increasing the expression of *PLA1/CYP78A1* in maize and *CYP78A9* in rapeseed can increase seed weight and yield by affecting auxin metabolism (Shi *et al*., 2019; Sun *et al*., 2017), but a recent study in Arabidopsis showed that *CYP78A5* mainly affects cytokinin rather than auxin metabolism (Jiang *et al*., 2021).

In this study, we find that *TaCYP78A5* is highly expressed in ovaries and seed coat and locates within the QTLs for grain weight and yield-related traits in wheat. Modified expression of *TaCYP78A5* in maternal integument enhances grain weight and grain yield per plant by 4.3%–18.8% and 9.6%–14.7%, respectively, in field trials. Transcriptome and hormone metabolome analyses reveal that *TaCYP78A5* participates in auxin synthesis pathway and promotes auxin accumulation and cell wall remodelling in ovary. Phenotype investigation and cytological observation show that localized overexpression of *TaCYP78A5* in ovary results in delayed flowering, which prolongs proliferation of maternal integument cells, increases the number of seed coat cell and eventually promotes grain enlargement. Association analysis demonstrates that *TaCYP78A5* haplotype *Ap-Hap*II with higher activity is favourable for increasing grain weight and grain yield per plant and has been positively selected in wheat breeding in China. These findings reveal that *TaCYP78A5* can serve as a valuable gene for improving wheat yield.

Results

TaCYP78A5 is highly expressed in maternal tissues during grain development

Based on the sequence of *KLUH/CYP78A5* in Arabidopsis, we obtained *TaCYP78A5*, a homologous gene of *KLUH/CYP78A5* in wheat. *TaCYP78A5* had three homoeologs (*TaCYP78A5-2A/-2B/-2D*; TraesCS2A01G175700.1, TraesCS2B01G201900.1 and TraesCS2D01G183000.1 respectively) in the wheat genome (Figure S1a). Evolutionary analysis exhibited that *TaCYP78A5* in wheat is most closely related to those in *Thinopyrum* and *Hordeum vulgare* (Figure S1b). Domain analysis showed that TaCYP78A5 is conserved among different species, all containing the typical N-terminal hydrophobic region, oxygen and heme-binding domains (Figure S1b). The subcellular localization of TaCYP78A5 in wheat protoplast showed that it located in the endoplasmic reticulum (Figure 1a). Spatiotemporal expression profile analysis showed that *TaCYP78A5* was ubiquitously expressed in wheat root, stem, leaf and spike, with most abundant in ovary and young seed (Figure 1b). Further investigation of the spatial expression of *TaCYP78A5* in developmental grains showed that it was highly expressed in maternal integument (seed coat) but not in embryo and endosperm (Figure 1c). This indicates that *TaCYP78A5* may be involved in grain development through maternal pathways.

To reveal if *TaCYP78A5* contributes to yield, we integrated the physical location of three homoeologs of *TaCYP78A5* and the known genetic loci of yield component traits on the short arms of chromosome 2A, 2B and 2D in wheat (Table S1) based on the known genetic maps, physical map and wheat genome reference sequence IWGSC Ref v1.0 (IWGSC, 2018). The results showed that *TaCYP78A5-2A* locates within the QTLs associated with grain thickness (GT), grain length (GL) and thousand-grain weight (TGW) in wheat (Figure S2). The above results suggest that *TaCYP78A5* may play an important role in regulating grain weight and yield.

Constitutive overexpression of *TaCYP78A5* enhances grain weight but not grain yield per plant

To verify if *TaCYP78A5* affects grain weight of wheat, we knocked down the expression of *TaCYP78A5* in developing grains of wheat cultivar Shaan 512 that has large-size/heavy weight kernel (with TGW 52 g) by using barley stripe mosaic virus-induced gene silencing (BSMV-VIGS) technique as reported previously (Ma *et al*., 2012). The result showed that the grain size and weight of *TaCYP78A5*-knockdown plants (BSMV:TaCYP78A5) were significantly reduced, compared with those of the control plants (BSMV:00) (Figure S3a–e). We further investigated the cellular characteristics of seed coat, and found that the number of seed coat cells of BSMV:TaCYP78A5 plants was significantly decreased, but the size of the seed coat cell was not altered, compared with those of the control plants (Figure S3f–j). These results suggest that *TaCYP78A5* regulates grain weight by promoting proliferation of seed coat cells.

To obtain increased yield of transgenic wheat and further verify the biological effect of *TaCYP78A5*, we generated transgenic wheat lines constitutively overexpressing *TaCYP78A5-2A* under the control of maize *ubiquitin* promoter (named as UBI lines for simplicity). Nine independent transgenic events were obtained; of which two single-locus transgenic events (UBI-1 and UBI-4) with higher expression levels of *TaCYP78A5* compared to wild-type plants (WT) are shown as representatives of UBI lines (Figure 2). The grain length, width and thickness of the UBI lines increased by 9.3%–10.3%, 9.4%–10.0% and 3.5%–4.0%, respectively, (Figure 2a–c), which resulted in significantly increased grain weight (by 26.9%–30.7%), compared with that of WT (Figure 2d). Further cytological analysis of grains at 15 days after fertilization (DAF) indicated that both the number and the length of seed coat cells of UBI lines were significantly higher than those of WT (Figure 2e,f). Taken together, *TaCYP78A5* has a positive role in increasing grain weight of wheat.

Unfortunately, UBI lines showed apical dominance, the tiller growth was inhibited and hysteresis (Figures 2g–k and S4). Consequently, the grain yield per plant of the UBI lines did not increase (Figure S5), because the grain weight effects at the whole plant level were offset by significantly reduced grain number per tiller spike (Figure 2i).

Localized overexpression of *TaCYP78A5* in maternal integument increases grain weight and grain yield per plant

In view of the fact that constitutive overexpression of *TaCYP78A5* in wheat enhances apical dominance and that *TaCYP78A5* is preferentially expressed in ovary and seed coat, we generated wheat transgenic lines overexpressing *TaCYP78A5* driven by Arabidopsis *INNER NO OUTER* promoter (*pINO*), a maternal integument-specific expression promoter (Villanueva *et al*., 1999), in order to reveal the biological effects of *TaCYP78A5* on grain development and avoid enhancing apical dominance. More than 20 independent transgenic lines (called pINO lines for simplicity) were obtained, and three independent single-locus transgenic

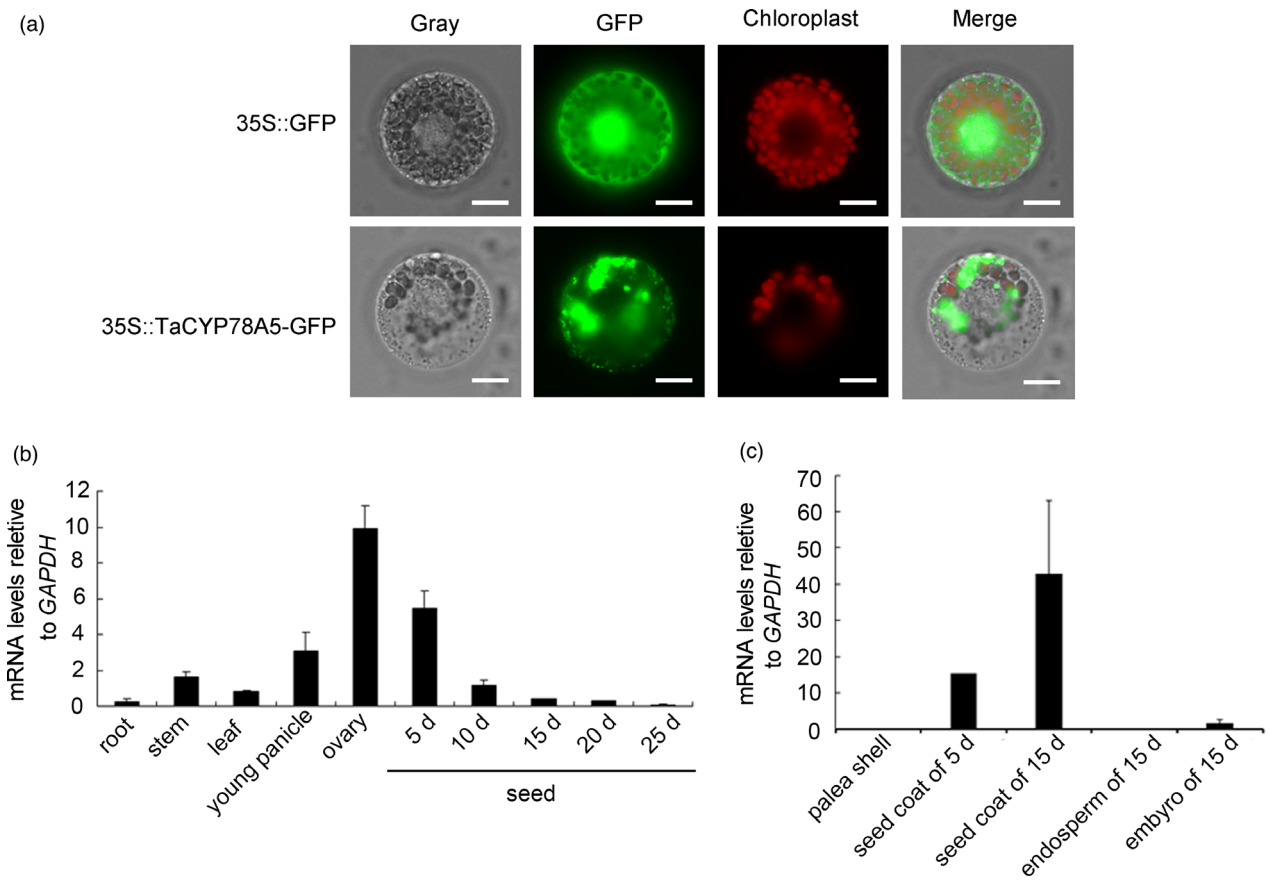


Figure 1 Expression profile and subcellular localization of TaCYP78A5 in wheat. (a) Subcellular localization of TaCYP78A5 in wheat. The vector control (35S::GFP) and fusion protein vector (35S::TaCYP78A5-GFP) were each introduced into wheat protoplasts. GFP was observed with laser scanning confocal microscope. Scale bar = 10 μm. (b, c) Expression profiles of *TaCYP78A5* across developmental cycle of wheat (b) and developmental grains (c). *GADPH* (TraesCS6B01G243700.1) was used as a reference gene. Data are shown as the mean ± SE (n = 3).

lines (pINO-1, pINO-13 and pINO-24) with various transgene expression levels were used for subsequent analysis (Figure 3a). The grain length and thickness of the pINO lines were not significantly different from those of WT, but the grain width was increased by 1.2%–3.4% ($P < 0.01$) (Figure 3b,c), this led to an increase in grain size and grain weight by 7.3%–8.9% and 5.7%–8.6%, respectively, compared with WT (Figure 3d,e). There was no difference in quality traits between the pINO lines and WT (Table S2). We further investigated the numbers of seed coat cells of the pINO lines and WT by cross-cutting the grains 15 DAF (Figure 3f,g). The result showed that the pINO lines had more outer layer cells than WT (Figure 3h); however, the cell lengths of the pINO lines were similar as those of WT (Figure 3i). These indicate that localized overexpression of *TaCYP78A5* in maternal integument causes an increase in the number of seed coat cells, which leads to enlargement in grain size and grain weight.

As we expected, the pINO lines had no obvious apical dominance (Figure 3j). The main spikes and the middle tiller spikes of the pINO lines had similar spike length with those of WT, except the smallest tiller spike (bottom tiller spike) of the pINO lines that had decreased spike length, compared to WT (Figure 3k). There was no difference in the average grain number per spike and grain number per plant between the pINO lines and WT, except for pINO-1 which had significantly increased grain number per plant (Figure 3m). The TGW of the pINO lines was increased by 4.0%–9.5% (Figure 3n).

To further explore the effects of *TaCYP78A5* on grain yield per plant, we investigated the grain yield per plant of the pINO lines for 3 consecutive years, that is, the pINO lines grown in the greenhouse in 2017 and grown in the Transgenic Plant Experimental Station of Northwest A & F University, Yangling (108°4′E, 34°17′N) in natural growth seasons in 2018–2019 and 2019–2020. The results showed that the TGW of the pINO lines significantly increased in all three years (4.9%, 4.3% and 18.8% respectively), compared with those of WT (Figure 4a–c). The grain yield per plant of the pINO lines increased by 11.1% and 14.7% in 2017 and 2018–2019, respectively, and the biomass per plant increased only in 2018–2019 (9.6%), compared with those of WT (Figure 4d–f). There were no differences in other yield-related traits between the pINO lines and WT (Figure S6). Taken together, localized overexpression of *TaCYP78A5* in maternal integument enhances grain size, weight and yield per plant of wheat.

The growth-promoting effect of *TaCYP78A5* on plant organs is limited by the travel distance of a mobile factor

In above study, we noticed that localized overexpression of *TaCYP78A5* in ovaries resulted in a significant increase in

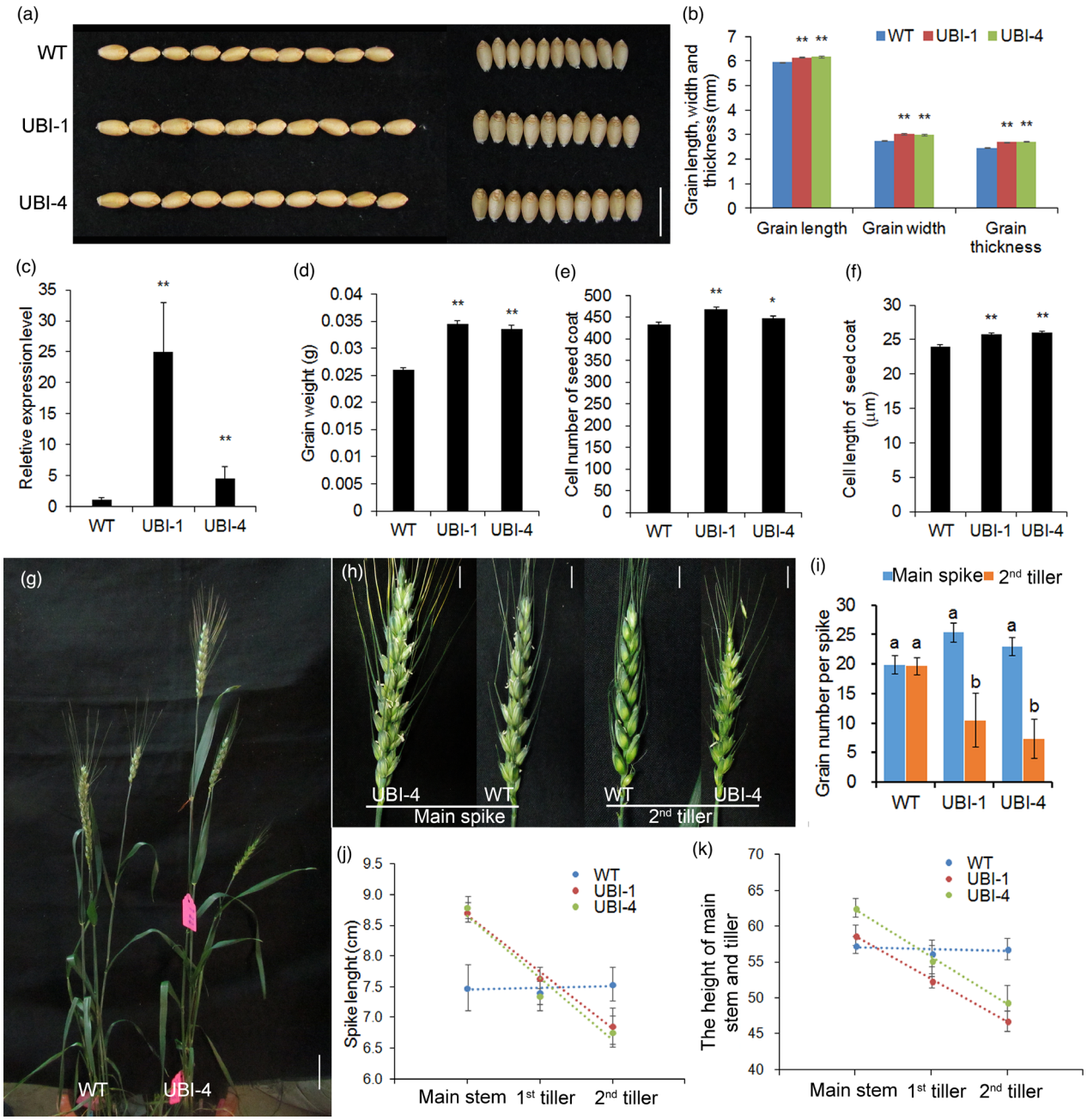


Figure 2 The phenotypes of UBI::TaCYP78A5-transgenic wheat lines (UBI lines) and wild-type wheat plants (WT). (a) The phenotypes of grain length and width of UBI lines and WT. Bar = 1 cm. (b) Grain length, width and thickness of UBI lines and WT ($n \geq 8$). (c) Relative expression level of *TaCYP78A5* in UBI lines and WT, *GADPH* as a reference gene ($n = 3$). (d) Grain weight of UBI lines and WT ($n \geq 8$). (e–f) Cell number (e) and cell length (f) of outer seed coat of wheat grain 15 days after fertilization ($n \geq 8$). (g) The plant architectures of UBI line-4 and WT. Bar = 5 cm. (h) The phenotypes of main spike and the 2nd tiller spike of UBI lines and WT. Bar = 1 cm. (i) The grain number per main spike and per 2nd tiller spike of UBI lines and WT ($n \geq 8$). (j) The spike length of the main stem and the tillers of UBI lines and WT ($n \geq 8$). (k) The height of the main stem and the tillers of UBI lines and WT ($n \geq 8$). Data are shown as the mean ± SE, $*P < 0.05$, $**P < 0.01$ by Student's *t*-test. Different lowercase letters on the bar chart indicate a significant level of difference.

biomass, implying extensive growth-promoting effects of *TaCYP78A5* on other organs. Histochemical observations using β-glucuronidase (GUS) staining showed that the fusion protein TaCYP78A5-GUS only aggregated in the ovaries of the pINO lines (Figure 5a), which resulted in enlarged glumes and lengthen spikes in the pINO lines, compared with those in WT (Figure 5b–i). The flag leaves of the pINO-13 line are also significantly longer than WT (Figure 5j). Cytological observation showed that the cell sizes of the glume outer integument of the pINO lines were similar as those of WT, but the cell numbers of the pINO lines were significantly increased, compared with those of WT (Figure 5d,e). These results suggested that the growth-promoting effect of *TaCYP78A5* may depend on a mobile growth-promoting factor. This is consistent with the previous

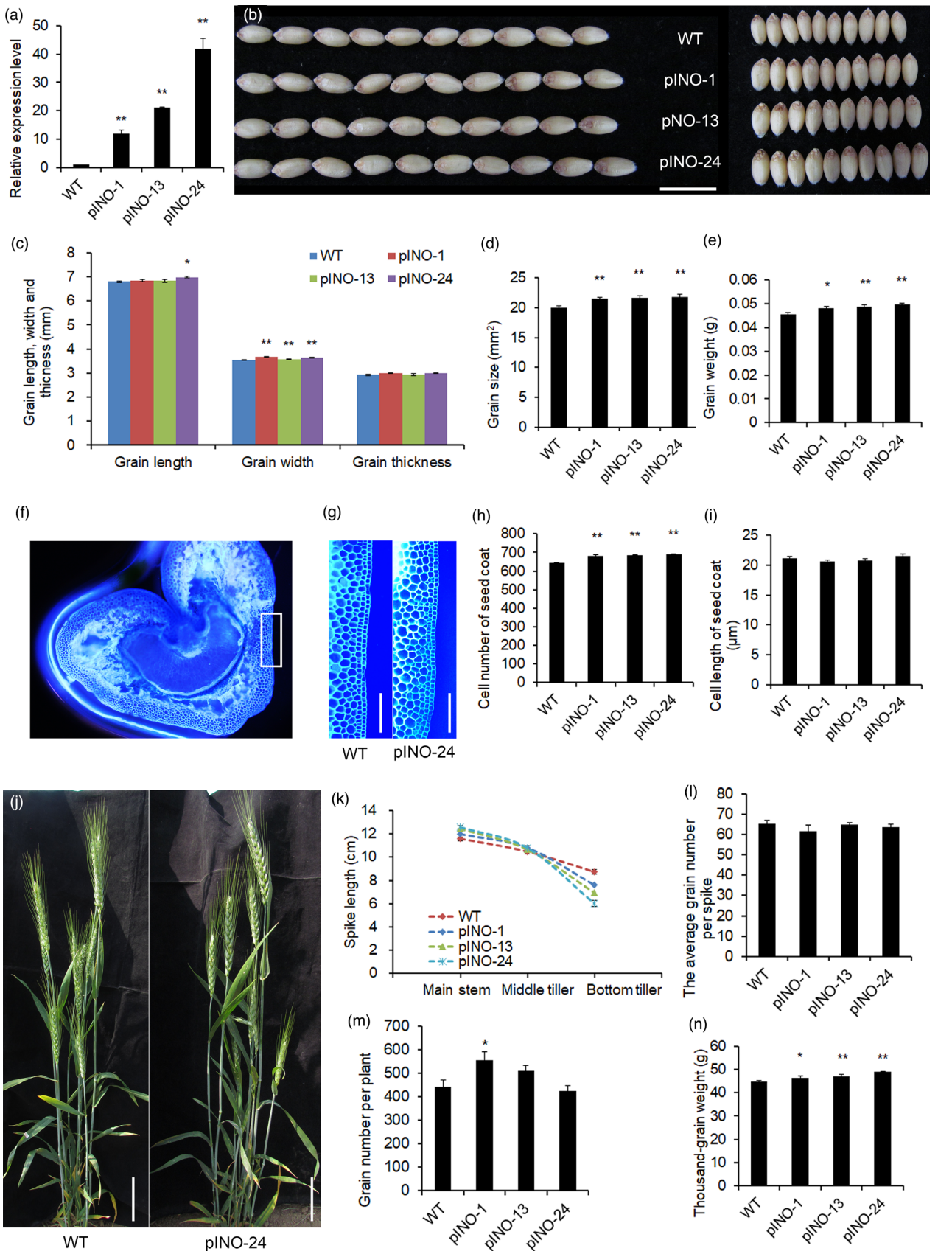


Figure 3 The phenotypes of pINO::TaCYP78A5-transgenic wheat lines (pINO lines) and wild-type plant (WT). (a) Relative expression of *TaCYP78A5* in pINO lines and WT ($n = 3$). (b) Grain phenotypes of pINO lines and WT. Bar = 1 cm. (c) Grain length, width and thickness of pINO lines and WT ($n \geq 10$). (d, e) Grain size (d) and grain weight (e) of pINO lines and WT ($n \geq 10$). (f) A representative cross section of the grain 15 days after fertilization (DAF) stained with Fluorescent Brightener. (g) Enlarged view of the seed coat cells of pINO lines and WT. Bar = 200 μm. (h, i) Cell number (h) and cell length (i) of the outer seed coat of grain 15 DAF ($n \geq 20$). (j) The plant architecture of pINO-24 and WT. (k) The spike length of the main stem and the tillers of pINO line and WT ($n > 10$). (l, m) The average grain number per spike (l) and grain number per plant (m) of pINO lines and WT ($n = 20$). (n) Thousand-grain weight of pINO lines and WT ($n > 10$). Data are shown as the mean ± SE, $*P < 0.05$, $**P < 0.01$ by Student's *t*-test.

inference that *CYP78A5* may promote the growth of reproductive organs through a mobile molecule in Arabidopsis (Adamski *et al*., 2009; Anastasiou *et al*., 2007).

Interestingly, it can be seen that the growth-promoting effects of *TaCYP78A5* on tissues/organs were obviously related to the physical distance where the organ is from the ovary/grain tissues. Glumes and spikes had the closest physical distance to grains, and their enlargement effects were obvious and significant, with an increase of 13.9% and 12.5% respectively ($P = 0.00029$ for glume, $P = 6.62E\text{-}06$ for spike). However, the growth-promoting effects on flag leaf and plant height gradually decreased with increasing distance from the grains (Figure 5f–k). Collectively, overexpression of *TaCYP78A5* only in ovaries can extend the growth-promoting effects beyond the grains of wheat, and this promotion effects may be through a mobile molecule downstream.

TaCYP78A5 promotes grain enlargement through auxin accumulation

To investigate the mechanism underlying *TaCYP78A5* affects grain size and the possible downstream mobile molecule, we conducted transcriptome analysis using the 1-mm size ovaries of

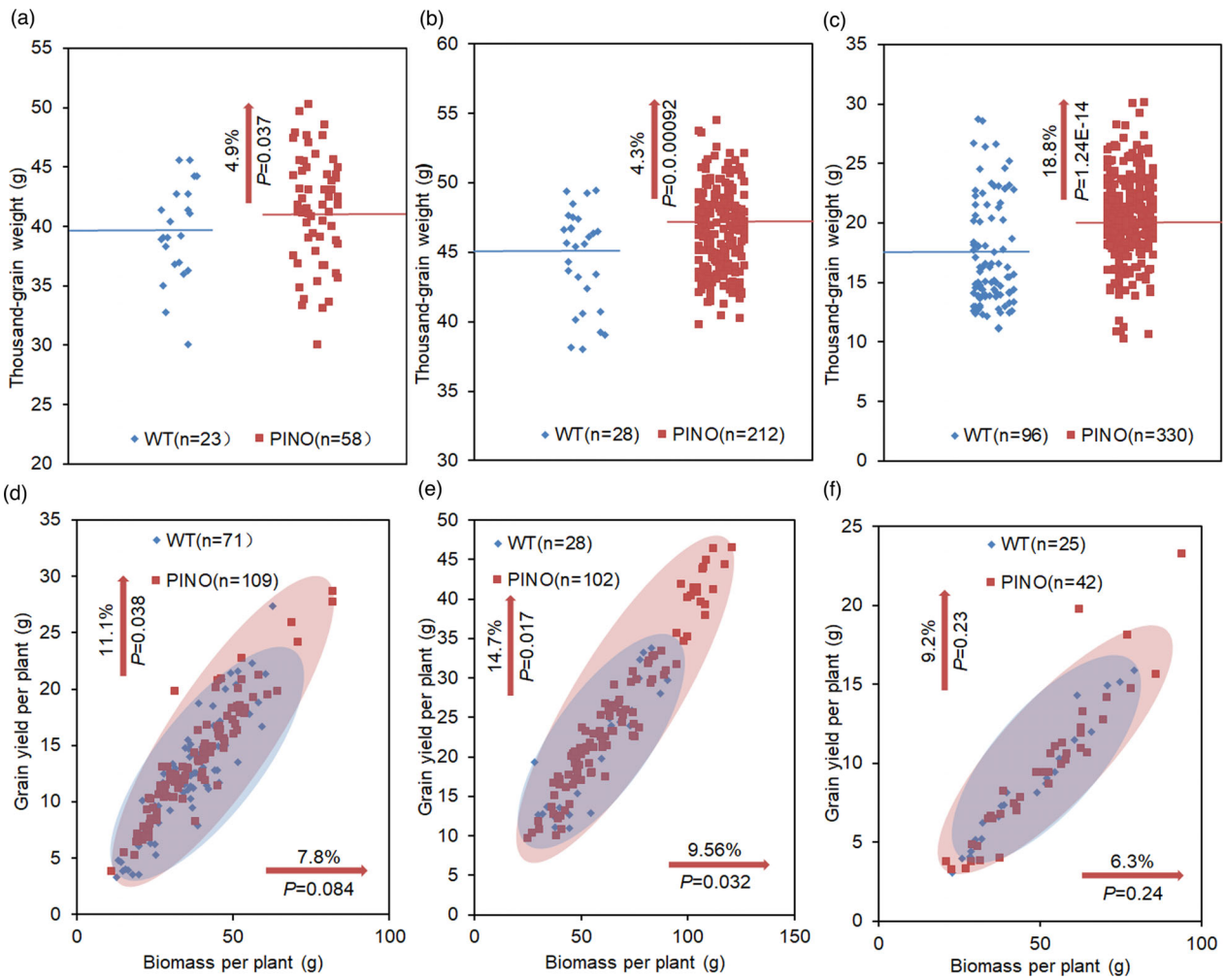


Figure 4 *TaCYP78A5* affects thousand-grain weight and yield of wheat. (a–c) Thousand-grain weight of all transgenic wheat lines overexpressing *TaCYP78A5* in integument under the control of *INO* promoter (pINO lines) and wild-type wheat plant (WT) at green house in 2017 (a) and at field in 2018 (b) and in 2019 (c), the line represents the mean. (d–f) Grain yields per plant and biomass per plant of all pINO lines and WT at green house in 2017 (d) and at field in 2018 (e) and in 2019 (f). The percentages in the vertical of direction in each pane indicate the increase of grain yield per plant, and the percentages in the horizontal direction show the increase of biomass per plant. *P*-values by Student's *t*-test.

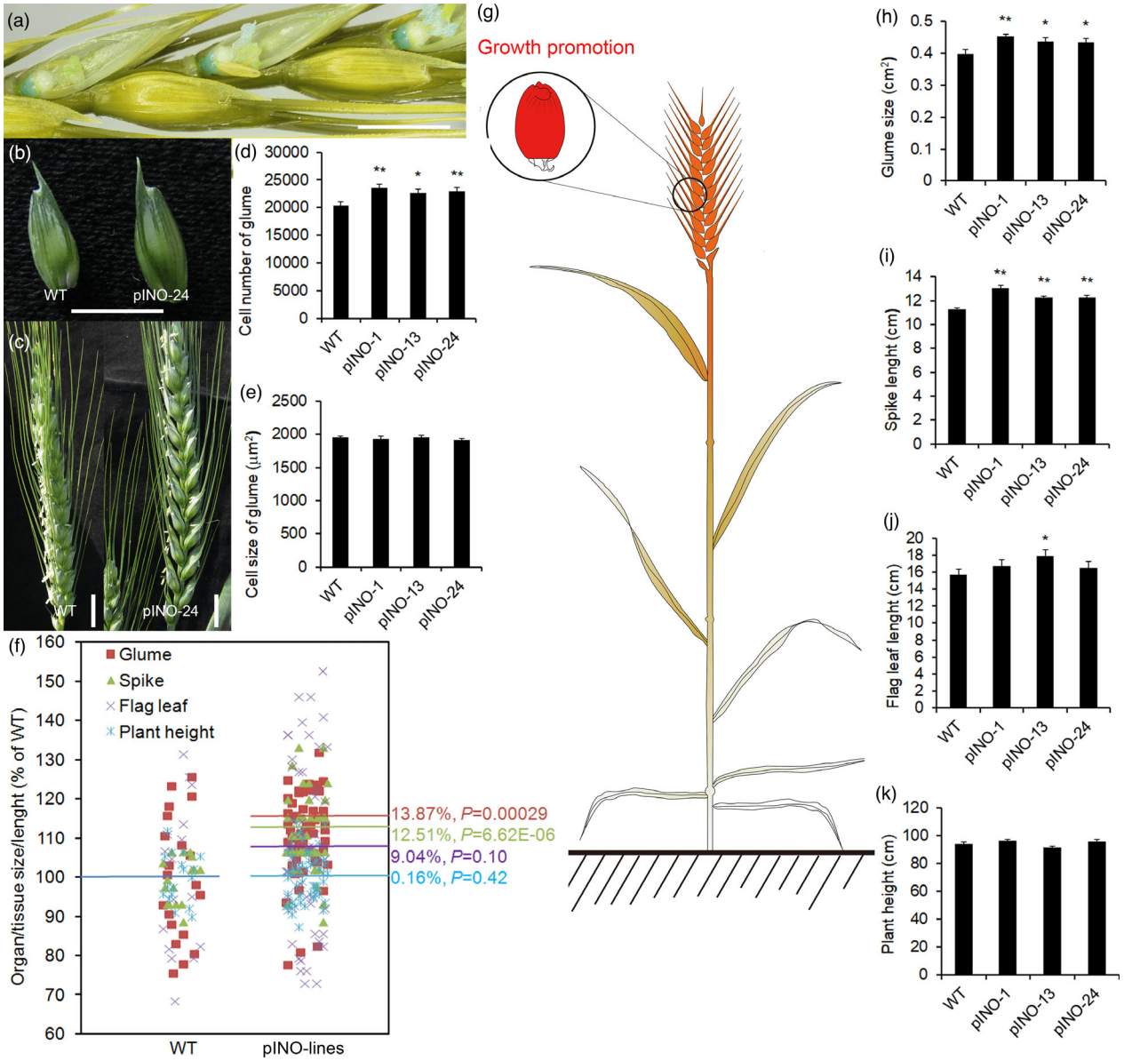


Figure 5 Growth-promoting effects of *TaCYP78A5* overexpressing in ovaries on other tissues/organs of wheat. (a) Histochemical β-glucuronidase (GUS) staining of heading stage spike from transgenic wheat lines overexpressing fusion protein TaCYP78A5-GUS in ovary (named as pINO lines for simplicity). Bar = 1 cm. (b, c) The glumes (b) and the main spike (c) of transgenic line pINO-24 and wild-type wheat plant (WT). Bar = 1 cm. (d, e) Cell number (d) and cell size (e) of the glumes of pINO lines and WT (n = 20). (f) The data of all the glume size, spike length, flag leaf length and plant height of pINO lines and WT, the mean of WT was set to 100%. The horizontal line represents the mean (n > 10). (g) Schematic diagram of the growth-promoting effects of *TaCYP78A5* overexpressing in ovary on other tissues/organs and its distance limitation. (h–k) Glume size (h), spike length (i), flag leaf length (j) and plant height (k) of pINO lines and WT (n > 10). Data are shown as the mean ± SE, *P < 0.05, **P < 0.01 by Student's t-test.

pINO-24 and WT. Consequently, a total of 3328 differentially expressed genes (DEGs) were detected between *pINO*-24 and WT, with 903 up-regulated and 2425 down-regulated (q value <0.05, fold change >2; Table S3-1). Ten DEGs were randomly selected to perform real-time quantitative RT-PCR (qRT-PCR) and the results from qRT-PCR and from RNA-Seq data were highly consistent (Figure S7), suggesting that RNA-Seq data are reliable. To explore the functions, these DEGs involved in Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of the DEGs were performed, and the result showed that the DEGs involved in hormone signal transduction and cell wall metabolic process were significantly enriched (Figures S8 and 6a, Tables S4-1 and S4-2). Further analysis of the hormone signal transduction-related DEGs revealed that 49% (30/61) were involved in auxin signalling (Table S3-2) and that the genes associated with auxin metabolism, transport and response underwent were significantly differentially expressed (Figure 6b, Table S3-3). Considering that the changes in auxin metabolism may affect cell wall process (Pacheco-Villalobos *et al*., 2016), we further analysed the cell wall metabolism-related genes regulated by auxin and found that the genes involved in cell wall remodelling were prominently differentially expressed (Figure 6c,

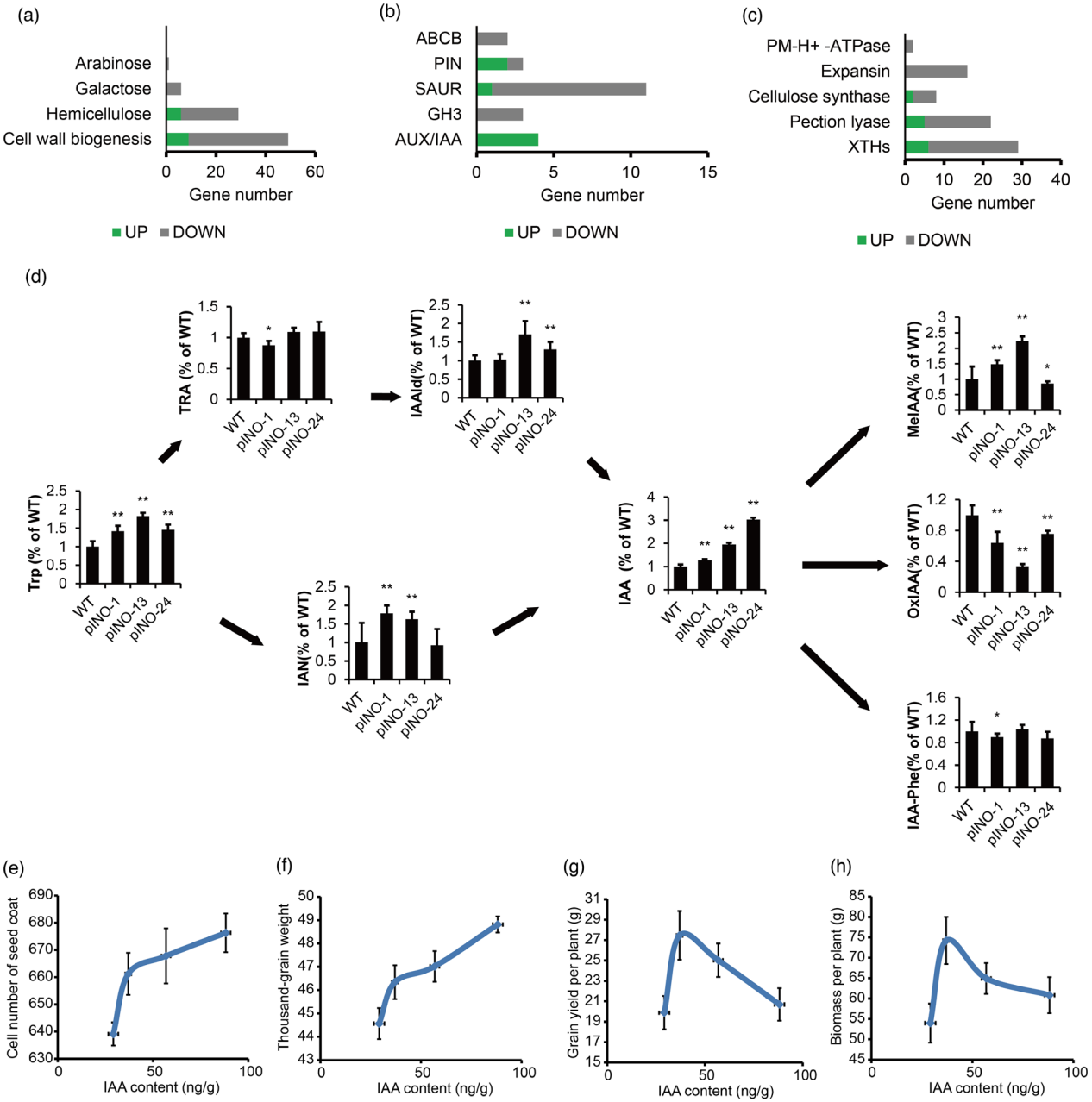


Figure 6 *TaCYP78A5* overexpression affects auxin-related pathways and causes auxin accumulation. WT, wild-type wheat; pINO-1,-13,-24, transgenic wheat lines overexpressing *TaCYP78A5* driven by *INO* promoter. (a–c) Expression changes of genes associated with cell wall metabolism (a), IAA transduction and signalling (b), cell wall metabolism regulated by auxin(c). (d) The relative levels of auxin, auxin precursors and auxin conjugates in pINO lines compared to those of WT. The mean of WT was set to 100%. The contents of these substances were measured by ESI-HPLC-MS/MS, auxin/IAA, indole-3-acetic acid; IAAld, indole-3-acetaldehyde; IAA-Phe, IAA-phenylalanine; IAN, indole-3-acetonitrile; MeIAA, methyl-IAA; oxIAA, 2-oxoindole-3-acetic acid; TRA, tryptamine; Trp, tryptophan. (e–h) The correlation between the auxin concentration and the number of seed coat cells (e), Thousand-grain weight (f), grain yield per plant (g) and biomass per plant (h) in pINO lines. Data are shown as the mean ± SE ($n > 3$), *$P < 0.05$, **$P < 0.01$ by Student's *t*-test.

Table S4-3). This is in line with above conclusion that *TaCYP78A5* overexpression led to an increase in the number of seed coat cells. Interestingly, cell wall expansin-related genes were consistently down-regulated (Figure 6c, Table S4-3), suggesting that cell expansion might be inhibited. Taken together, these results are consistent with above conclusion that *TaCYP78A5* regulates grain size by promoting maternal integument cell proliferation.

As described above, all the auxin transport, auxin response and cell wall metabolism affected by auxin were significantly changed in the pINO line, so we speculated that overexpression of *TaCYP78A5* might lead to changes in auxin metabolism, although a recent study in Arabidopsis showed that *CYP78A5* mainly affects cytokinin rather than auxin metabolism (Jiang *et al.*, 2021). In order to confirm whether *TaCYP78A5* overexpression affects cytokinin or auxin metabolism in transgenic wheat, the

levels of auxin, auxin precursors, auxin conjugates and cytokinin were determined in the ovaries (≥1 mm in length) and the spikes of the pINO lines and WT at heading stage through liquid chromatography–tandem mass spectrometry (LC-MS/MS). Interestingly, the cytokinin levels in the pINO lines were not changed significantly, compared with that in WT, with the exception of pINO-1 (Figure S9). However, the auxin levels of the pINO lines were significantly higher than that of WT and were positively correlated with the expression levels of *TaCYP78A5* (Figures 3a, 6d and S10). Moreover, the levels of auxin biosynthesis precursors, such as tryptophan (TRP), tryptamine (TRA), indole-3-acetaldehyde (IAAld) and auxin conjugates MeIAA in the pINO lines were also higher than those in WT, while the levels of auxin inactivation product (oxIAA) in the pINO lines were much lower than that in WT (Figure 6d). Therefore, we deduced that *TaCYP78A5* might function by affecting the auxin pathway in wheat. Auxin is an essential hormone for plant growth, seed development, cell division and yield. In order to explore the possible influence of *TaCYP78A5* on yield-related traits via auxin, we analysed the correlations between the *TaCYP78A5* activity and the auxin concentration, the number of seed coat cells, TGW, grain yield per plant and biomass per plant of the pINO lines. The results showed that the concentration of auxin in the ovary was positively correlated with the expression levels of *TaCYP78A5* (Figures 3a and 6d). The number of seed coat cell and TGW were continuously increased with the increase of the auxin concentration and the *TaCYP78A5* activity in the pINO lines, while the grain yield and biomass per plant were first increased and then decreased with the increase of the auxin concentration and the *TaCYP78A5* activity in the pINO lines (Figure 6e–h). These results suggest that grain size and TGW increased with the increase of the auxin concentration in the pINO lines, but an optimal auxin concentration existed to maximize grain yield and biomass per plant. This may explain the reason that the UBI lines did not increase grain yield per plant.

In order to further verify that auxin accumulation plays an essential role in enhancing grain weight, we treated wheat (JW1) plants at the booting stage with auxin or auxin synthesis inhibitor 5-methyl-tryptophan (5-MT) every 3 days till the plants at 15 days post flowering, and then measured grain weight after maturity. The results showed that 100 μmol/L of auxin treatment led to increased grain weight, while 50 μmol/L of 5-MT treatment caused reduced grain weight (Figure S11), indicating that auxin accumulation enhances grain weight.

Taken together, transcriptome and hormone metabolome analyses revealed the involvement of *TaCYP78A5* in auxin synthesis pathway and auxin accumulation in the pINO lines to enhance grain weight and grain yield per plant of wheat.

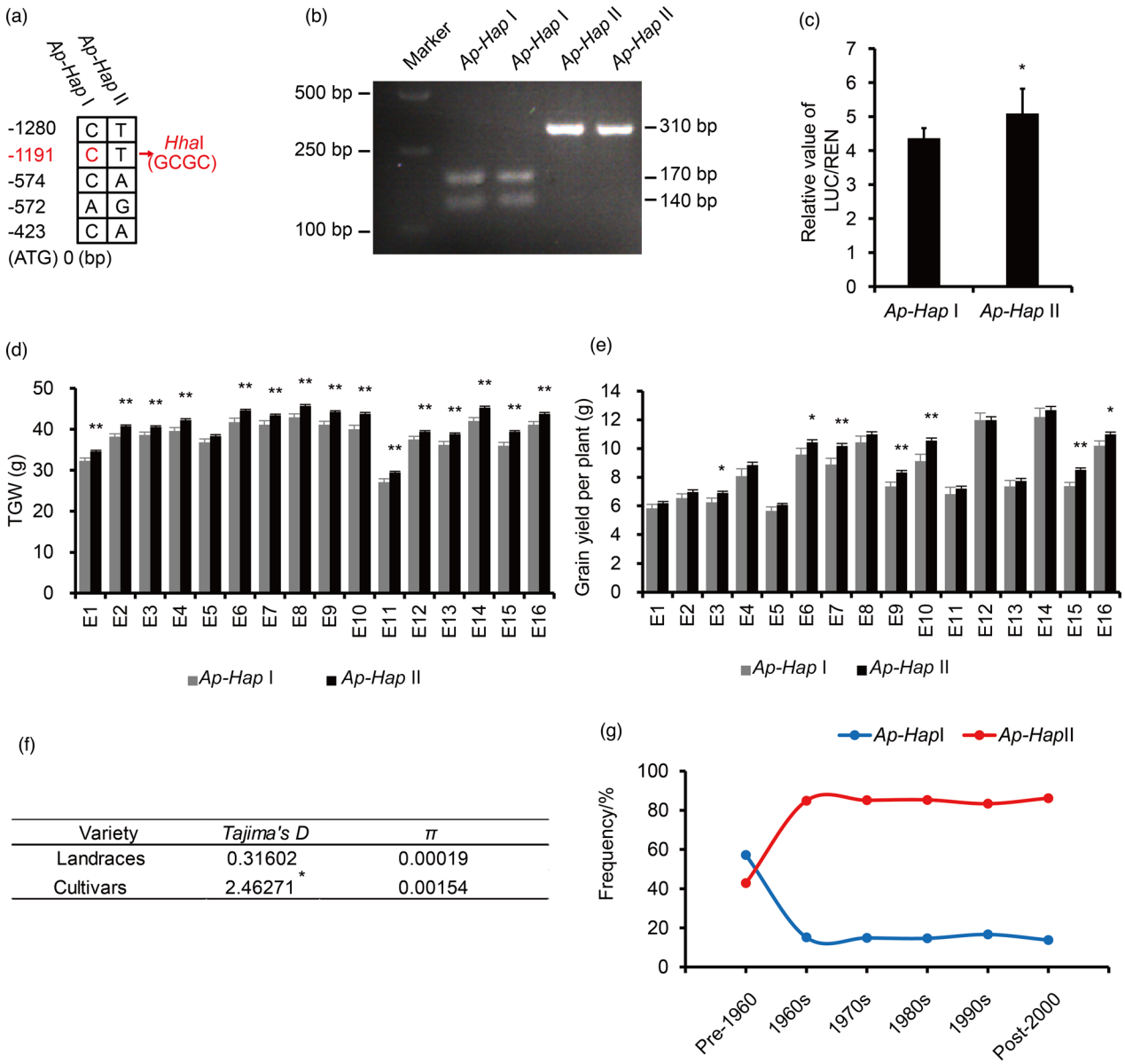
TaCYP78A5 promotes grain enlargement by auxin-mediated prolongation of maternal epidermal cell proliferation

Flowering time and ripening time have important effects on biomass of crops by affecting duration of basic vegetative growth (Andres and Coupland, 2012; Gao *et al*., 2014). In the present study, heading and flowering time of the pINO lines were delayed by 1 and 2–3 days, respectively, compared with those of WT; however, the maturity time of the pINO lines is the same as that of WT (Figure S12a,b). The delayed heading and flowering of the pINO lines may attribute to the increased auxin level, because wheat plants at booting stage treated with exogenous auxin, naphthylacetic acid (NAA), exhibited delayed flowering (Figure S13). This is in line with previous reports that high concentration of auxin can delay flowering and fruit ripening (Dal Santo *et al*., 2020; Zhao *et al*., 2013). Then, we questioned if there is any relationship between auxin-mediated delayed flowering and the enlarged grains due to the increased number of seed coat cells. To answer this question, we selected six time points throughout the period from heading to ripening to observe proliferation of maternal integument/seed coat cells of pINO line-24 and WT, and the results showed that proliferation of maternal integument/seed coat cells mainly occurred during ovary development stage (Figure S12c). A similar phenomenon also appeared in barley (Radchuk *et al*., 2011). Delayed flowering resulted in extending proliferation time of maternal integument cells of the pINO lines, which ultimately led to the increased number of seed coat cells (Figure S12d). Thus, we conclude that *TaCYP78A5* promotes grain enlargement through auxin-mediated delayed flowering, which prolongs proliferation of maternal integument cells and enhances the number of seed coat cell.

Genetic variations in *TaCYP78A5-2A* promoter affect wheat grain weight and the favourable haplotype *Ap-Hap*II has been positively selected in wheat breeding

To uncover the naturally allelic variations of *TaCYP78A5* in wheat, we compared the DNA sequences of the coding regions and the promoters of three homoeologs *of TaCYP78A5* in 30 wheat cultivars with various genetic backgrounds (Table S5). Two haplotypes of *TaCYP78A5-2A* were characterized by five single-nucleotide polymorphisms (SNPs) in the promoter region (named as *TaCYP78A5-Ap* for simplicity), that is, *TaCYP78A5 Ap-Hap*I and *TaCYP78A5 Ap-Hap*II (named as *Ap-Hap*I and *Ap-Hap*II, respectively, for simplicity) (Figure 7a). A cleaved amplified polymorphic sequence (CAPS) marker was developed based on −1191 bp (C/T) in *TaCYP78A5-Ap* to distinguish these two haplotypes (Figure 7b). This CAPS marker was further verified in wheat population with 323 accessions (Table S6). Since the two haplotypes have SNPs in the promoter region of *TaCYP78A5-2A*, we speculated that these SNPs might lead to changes in promoter activity. Therefore, we tested the promoter activity of these two haplotypes, and the results showed that *Ap-Hap*II has higher promoter activity than *Ap-Hap*I (Figure 7c). In order to investigate if the two haplotypes affect wheat yield potential, we conducted association analysis between the two haplotypes and TGW and grain yield per plant of the 323 accessions in 16 environmental sites. The results showed that *Ap-Hap*II had significantly higher TGW and grain yield per plant than *Ap-Hap*I in most environments (Figure 7d,e). These suggested that *Ap-Hap*II with higher promoter activity was a favourable haplotype for TGW and grain yield per plant in wheat.

Breeding selection leaves intense footprints in genomes, showing progressive accumulation of favourable haplotypes (Barrero *et al*., 2011). To examine the evolutionary history of *TaCYP78A5-Ap*, the *Tajima's D* and diversity (π) analysis of *TaCYP78A5-Ap* (1.5 kb of promoter region) were investigated in 43 landraces and 42 cultivars (Table S7). *Tajima's D* of the cultivars showed significant values and was higher than that of the landraces, and the diversity (π) in the cultivars was also higher than that in the landraces, this suggesting that allelic variations of *TaCYP78A5-Ap* were strongly artificially selected during wheat domestication (Figure 7f). To determine whether favourable haplotype *Ap-Hap*II was selected during wheat breeding programs, we evaluated frequency changes of the



Variety	*Tajima's D*	π
Landraces	0.31602	0.00019
Cultivars	2.46271*	0.00154

Figure 7 Sequence variations of *TaCYP78A5-2A* and their associations with grain yield-related traits. (a) Two haplotypes (*Ap-Hap*I and *Ap-Hap*II) based on the sequence variation in the promoter region *of TaCYP78A5-2A*. (b) A cleaved amplified polymorphic sequence (CAPS) marker developed based on −1191 bp (C/T) with restriction endonuclease *Hhal* showed in (a). After enzyme digestion, the *Ap-Hap*I be cleaved into 170 and 140 bp, but *Ap-Hap*II could not be cleaved. (c) The relative activity of *TaCYP78A5* promoters with haplotype *Ap-Hap*I and *Ap-Hap*II. The two haplotypes (*Ap-Hap*I and *Ap-Hap*II) of *TaCYP78A5-2A* were separately cloned into the pGreenII 0800-LUC (firefly luciferase) to generate expression vectors Ap-HapI::LUC and Ap-HapII::LUC, and the two constructs were separately infiltrated into the leaves of 30-day-old *Nicotiana benthamiana*, with the expression of renilla luciferase (REN) as an internal control. The LUC activity and REN activity were measured with GloMax-multi-luminescence reader. The ration of LUC activity to REN activity (LUC/REN) indicates the relative expression level of *TaCYP78A5 Ap-Hap*I or *Ap-Hap*II. Data are shown as the mean ± SE (n = 3). (d, e) Association of *Ap-Hap*I and *Ap-Hap*II with thousand-grain weight (TGW) (d) and yield per plant (e) of 323 wheat accessions at 16 environmental sites. E1–E16 indicate the environments at Shunyi in 2015 under drought stressed (DS), DS + heat stress (HS), well-watered (WW), WW + HS; Shunyi in 2016 under DS, DS + HS, WW, WW + HS; Changping in 2016 under DS, WW; Shunyi in 2017 under DS, DS + HS, WW, WW + HS; Changping in 2017 under DS and WW. (f) The nucleotide diversity (π) and *Tajima's D* of *TaCYP78A5-2A* promoter regions of 43 landraces and 42 cultivars. (g) Frequency changes of the two haplotypes of *TaCYP78A5-2A* over decades in modern cultivars (total of 311 accessions). 14, 33, 47, 34, 60 and 123 accessions were released in pre-1960s, 1960s, 1970s, 1980s, 1990s and post-2000 respectively. *Ap-Hap*I and *Ap-Hap*II indicate two haplotypes of *TaCYP78A5-2A*.

two haplotypes in wheat population with 311 accessions released in different decades in China (Table S6). The results exhibited the frequency of the two haplotypes of *TaCYP78A5-2Ap* in modern cultivars changed over decades. The frequencies of favourable haplotype *Ap-Hap*II sharply increased from 42.9% to 84.8% in the cultivars released from pre-1960 to 1960s, and subsequently remained stable in the decades from 1970s to post-2000, while the frequencies of haplotype *Ap-Hap*I showed

the opposite trend (Figure 7g), this implying that favourable haplotype *Ap-Hap*II was positively selected in wheat breeding in China.

Discussion

Altered expression of *TaCYP78A5* in wheat integument promotes grain weight with yield potential

In plant, seed size is a key factor affecting yield. Larger seeds have greater seed weight and offer the potential to increase yield, but larger seeds usually tend to be accompanied by a decrease in seed number, which counteract the increase in seed yield caused by enlarged seeds (Bustos *et al*., 2013; Foulkes *et al*., 2011; Molero *et al*., 2019). *KLUH/CYP78A5* and its homologous genes have been shown to affect seed/fruit size in Arabidopsis, rice, tomato and other plants (Anastasiou *et al*., 2007; Chakrabarti *et al*., 2013; Nagasawa *et al*., 2013; Zhao *et al*., 2016); but overexpression of *KLUH/CYP78A5* in Arabidopsis did not increase seed yield per plant, because the increase in seed size was offset by the decrease in seed number (Adamski *et al*., 2009). Here, we show that constitutive overexpression of *TaCYP78A5* in wheat leads to enlarged seeds and increased seed weight, but not increased grain yield per plant due to enhanced apical dominance and reduced grain number of tillers (Figure 2g–k). In order to avoid this problem, we generated wheat transgenic lines overexpressing *TaCYP78A5* specifically in integument. Consequently, unlike UBI lines, pINO lines had no obvious apical dominance and normal grain number (Figure 3j–m). Therefore, grain weight and grain yield per plant of the pINO lines were increased significantly compared with those of WT (Figures 3n and 4). The trade-off between grain size and grain number has been reported in wheat, and enhancing grain yield through enlarging grain size had always been impeded by the trade-off between grain weight and grain number (Bustos *et al*., 2013; Foulkes *et al*., 2011; Molero *et al*., 2019). A recent study raised one solution to overcome this problem by ectopic expression of α-expansin in developing seeds, which can lead to grain enlargement but does not reduce the grain number in wheat (Calderini *et al*., 2021). Here, we provide another solution to overcome this problem by localized overexpression of *TaCYP78A5* in wheat integument, which had the potential for grain enlargement by increasing the number of maternal integument /seed coat cells, and ultimately led to the increase in grain size/weight without affecting grain number (Figure 3m,n).

TaCYP78A5 promotes grain weight and grain yield per plant through auxin accumulation

A previous study in Arabidopsis demonstrated that *KLU/CYP78A5* is involved in generating a mobile growth-promoting signal molecule different from known classic hormones (Anastasiou *et al*., 2007). A study in rice indicated that *GE/CYP78A13* does not participate in the biosynthesis of auxin (Xu *et al*., 2015). But studies in maize and rapeseed showed that overexpression of *PLA1/CYP78A1* and *BnaA9.CYP78A9*, both belonging to *CYP78A* family, could affect auxin pathway (Shi *et al*., 2019; Sun *et al*., 2017). More recently, a study in Arabidopsis reported that *KLUH* participates in the cytokinin rather than auxin pathway (Jiang *et al*., 2021). In this study, we find that overexpression of *TaCYP78A5* in integument promotes the growth of organs surrounding, suggesting that *TaCYP78A5* involved in the production of a mobile growth-promoting signalling molecule (Figure 5). Overexpression of *TaCYP78A5* in wheat could enhance apical dominance (Figure 2g–k). Conversely, Arabidopsis *cyp78a5/klu* mutant exhibits reduced apical dominance phenotype (Anastasiou *et al*., 2007), which corresponds to the phenotype of the classic auxin synthesis gene mutant *yucca* in Arabidopsis (Cheng *et al*., 2006). Correspondingly, auxin-synthesis-related gene *MEE45* can promote the cell division of the seed coat, leading to seed enlargement (Li *et al*., 2021), and here overexpression of *TaCYP78A5* in wheat can also promote proliferation of the seed coat cell by prolongation of maternal epidermal cell proliferation, which increases the number of seed coat cell and causes seed enlargement (Figures 2b–i, 3b–h and S12). These findings suggested that *TaCYP78A5* may be involved in auxin-related pathway. The transcriptome and hormone metabolome analyses further confirmed that overexpression of *TaCYP78A5* affects auxin synthesis pathway and causes auxin accumulation in wheat (Figure 6d). However, which step of auxin synthesis pathway *TaCYP78A5* participates in needs further to explore.

Genetic variations of *TaCYP78A5-2A* affect grain yield-related traits and has been selected in wheat domestication and breeding

As one of the most successful crops on the earth, wheat has expanded from the small core area within the Fertile Crescent to all parts of the world in <10 000 years (Lev-Yadun *et al*., 2000; Salamini *et al*., 2002). The genetic diversity of its genome and the convergent adaptation to human selection are one of the important reasons for its evolutionary success (Zhou *et al*., 2020). In the course of evolution, genotypes controlling favourable agronomic traits were preserved. In this study, we found that *TaCYP78A5-2A* locates within QTLs for TGW and yield-related traits by integrating the physical location of *TaCYP78A5* homoeologs with the known QTL maps of group 2 chromosomes (2A, 2B and 2D) in wheat (Figure S2, Table S1), suggesting that *TaCYP78A5-2A* might contribute to grain yield of wheat. Further analysis of naturally genetic variations in *TaCYP78A5-2A* identified two haplotypes, haplotype *Ap-Hap*II exhibiting higher promoter activity than *Ap-Hap*I (Figure 7c). Association analysis between the two haplotypes and the agronomic traits of 323 wheat accessions in 16 environments revealed that haplotype *Ap-Hap*II exhibited significantly higher TGW and grain yield per plant than haplotype *Ap-Hap*I in most environments (Figure 7d,e). This is consistent with the result that overexpression of *TaCYP78A5-2A* leads to an increase in grain size and grain yield per plant (Figure 3). *Tajima's D* and the diversity (π) analysis of *TaCYP78A5-2A* promoter sequences in the 43 landraces and the 42 cultivars showed the genetic variations of *TaCYP78A5-Ap* strongly artificially being selected during wheat domestication and breeding (Figure 7f). Further, the frequency of haplotype *Ap-Hap*II increased rapidly in wheat breeding in China in 1960s and kept stable high level after 1970s (Figure 7g), and this time period is consistent with the time of the wheat green revolution, indicating that favourable haplotype *Ap-Hap*II of *TaCYP78A5-2A* may have been strongly artificially selected during the wheat green revolution in China. Application of marker-assisted selection (MAS) can significantly accelerated wheat breeding (Gupta *et al*., 2010). In this study, a CAPS marker developed to identify *Ap-Hap*I and *Ap-Hap*II (Figure 7b) provides an important functional marker for MAS for improving TGW and grain yield in future wheat breeding.

Materials and methods

Winter wheat cultivar Xiaoyan 6 was used to clone cDNA of *TaCYP78A5* and to analyse its spatiotemporal expression profile. Wheat cultivar Shaan 512 with high thousand-grain weight (52 g) was used to conduct BSMV-VIGS to rapid identification of *TaCYP78A5* function in wheat grain development. The 30 wheat cultivars with various genetic backgrounds were used to detect SNPs of three homoeologs of *TaCYP78A5* (Table S5). The 323 wheat accessions described previously (Li *et al*., 2019a) were used for association analysis (Table S6). Spring wheat accession JW1 was used as a receptor material for wheat transformation.

The growth conditions of the wheat cultivars, wheat accessions and transgenic wheat lines are described in Appendix S1.

Total RNA isolation and cDNA synthesis

Wheat tissue/organ samples kept at −80 were used for RNA isolation. Total RNA was extracted using the Steady Pure Plant RNA Extraction Kit (Accurate Biotechnology, Changsha, China) according to the manufacturer's protocol. The quality of RNA samples was determined by agarose gel electrophoresis and RNA concentration was measured using a Nanodrop 2000 spectrophotometer (ND2000; Thermo Scientific, Wilmington, NC). The cDNA was synthesized using the Evo M-MLVRT Premix (Accurate Biotechnology, Changsha, China).

cDNA cloning and sequence alignment of *TaCYP78A5* in wheat

To clone wheat *TaCYP78A5*, the mRNA sequence of Arabidopsis *KLU*/*CYP78A5* (AT1G13710) was used to blast against wheat genome reference sequences to acquire the putative *TaCYP78A5*. Specific primers were designed based on the putative sequences of *TaCYP78A5*, and the cDNA synthesized from the mixed RNA samples of roots, leaves, young panicles and grains of Xiaoyan 6 was used as the template for *TaCYP78A5* amplification. The primers used are shown in Table S8. The amplified products were constructed into pMD19T vector (Takara, Japan) and more than 10 clones were randomly selected for sequencing. The resulted sequences were compared with IWGSC Ref v 1.0 to obtain full-length cDNA of three homoeologs *of TaCYP78A5* in wheat. DNAMAN8 (www.lynnon.com) was used to compare these sequences. The tree plot of *TaCYP78A5* and its homologues were investigated in Triticeae-Gene Tribe (http://wheat.cau.edu.cn/TGT/) (Chen *et al*., 2020).

Subcellular localization of TaCYP78A5 in wheat

The coding region of *TaCYP78A5-2A* from +1 to +1017 bp that contains the hydrophobic domain and oxygen-binding motif was inserted into 35S::GFP of expression vector p16318 by using restriction endonuclease *Hind*III and *Sal*I site to generate the construct 35S::TaCYP78A5-GFP. The resulted construct and the vector control (35S::GFP) were transferred into wheat protoplast prepared according to previous study (Yoo *et al*., 2007). GFP was observed by inverted fluorescence microscope (DMi8, Leica, Germany). The primers used for developing the constructs are listed in Table S8.

Genetic mapping of *TaCYP78A5* in wheat

The physical locations of three homoeologs of *TaCYP78A5* were localized based on physical maps and wheat genome reference sequence IWGSC Ref v1.0 (IWGSC, 2018). The known genetic loci associated with yield-related traits on the short arms of chromosome 2A, 2B and 2D in wheat (Table S1) were integrated to the physical maps of the short arms of group 2 chromosomes to obtain the genetic maps of *TaCYP78A5* in wheat.

Detection of genetic variations of *TaCYP78A5* in wheat

Single-nucleotide polymorphism (SNP) detection of three homoeologs of *TaCYP78A5* in the 30 wheat cultivar and functional marker development based on the SNPs are described in Appendix S1.

The sequences of *TaCYP78A5-2A* of 43 landraces and 42 cultivars (Table S7) were obtained from GVM database (https://bigd.big.ac.cn/gvm, GVM000082; Zhou *et al*., 2020), and the molecular diversity π values and *Tajima's D* were computed using DnaSP5.0 (Librado and Rozas, 2009).

Association analysis between *TaCYP78A5* haplotypes and agronomic traits of wheat accessions

The genotypic data based on genetic variations *of TaCYP78A5-2A* and phenotypic data of agronomic traits of the 323 wheat accessions at 16 environmental sites (E1–E16) over 3 years were analysed with the TASSEL5.1 software (Bradbury *et al*., 2007). The Details of the 16 environmental sites and agronomic trait measurement of wheat accessions are described in Appendix S1.

Determination of *TaCYP78A5-2A* promoter activity

The promoter activity was detected according to the instruction of Dual-Luciferase Reporter Assay System (Promega), and the procedure is shown in Appendix S1.

BSMV-mediated *TaCYP78A5* gene silencing in wheat

The BSMV-VIGS technique used for knockdown the expression of *TaCYP78A5* in developmental grains of wheat was performed as previously reported (Ma *et al*., 2012). The protocol of BSMV-derived vector construction and BSMV-mediated *TaCYP78A5* gene silencing is described in Appendix S1.

Construction of *TaCYP78A5* overexpression vectors and development of transgenic wheat lines

The protocol for constructing *TaCYP78A5* overexpression vectors UBI::*TaCYP78A5-2A*-GFP/GUS and pINO::TaCYP78A5-GUS is described in Appendix S1. The constructed vectors were, respectively, introduced into *Agrobacterium tumefaciens* strain *EHA105*. The embryogenic calli of the immature embryo 15 days after fertilization (DAF) of wheat line JW1 were used as recipient materials. Wheat transformation was conducted by *A. tumefaciens*-mediated method as described previously (Ishida *et al*., 2015; Zhang *et al*., 2018). The positive transgenic plants were identified by leaf daubing with 0.2% glufosinate (BASTA), and transgenic lines were obtained by continuous self-crossing of single-locus transgenic plants and screening by Basta.

RNA sequencing and data analysis

The RNA samples isolated from the 1-mm size ovaries of transgenic wheat line pINO-24 and WT were used for RNA sequencing. The differentially expressed genes were identified as described in Appendix S1.

All the genes detected were subjected to GO (http://geneontology.org/) to obtain GO annotations, GO and KEGG enrichment were conducted as previously described (Chi *et al*., 2019).

Determination of auxin and cytokinin metabolite levels in wheat

The 1-mm size ovaries of the pINO lines and WT were used to detect auxin levels, and auxin was determined by electrospray ionization–high-performance liquid chromatography–tandem mass spectrometry (ESI-HPLC-MS/MS) method. The spikes of the pINO lines and WT at heading stage were used for testing relative quantification of auxin precursors, auxin conjugates and cytokinin. The protocols of auxin and cytokinin metabolite determination are shown in Appendix S1.

Quantitative real-time reverse transcriptase–polymerase chain reaction (qRT-PCR)

The qRT-PCR was performed using the SYBR Green premix Pro Taq HS qPCR Kit (Accurate Biotechnology) on a CFX96™ Real-time PCR Detection System (Bio-Rad, Hercules, USA), wheat *GAPDH* gene (TraesCS6B01G243700.1) being used as a reference gene. The comparative Ct (threshold cycle) method was used to calculate the relative quantity (Livak and Schmittgen, 2001). All date were obtained from three biological replicates. The primers used are listed in Table S8.

Morphological characterization of transgenic wheat lines

The transgenic wheat lines of T_2 and T_3 generations and wild-type wheat (WT) grown in the Transgenic Plant Experiment Station (108°4′E, 34°17′N) of Northwest A & F University, Yangling, China, were used for assessment of agronomic trait, including plant height, tiller number, spike length, grain number per main spike, thousand-grain weight, grain yield per plant and biomass per plant. At least 15 plants were investigated for each transgenic line. For grain length, width and thickness determination, the grains from the middle spikelets were measured with a digital calliper (Pro'sKit, PD-151).

Cytological characterization of ovary, seed coat and glume cells of wheat

To measure the number and area of outer integument/seed coat cells, the ovaries of the transgenic wheat plants and WT were collected and transected with frozen slicer (POLAR DM; Sakura, Tokyo, Japan), the grains at 15 DAF were rapidly frozen in liquid nitrogen and then cross-cut. The sliced samples were stained with 0.01% solution of Fluorescent Brightener 28 (Sigma, Hongkong, China) for 10 min, and photographed under fluorescent microscope (Olympus, System Microscope BX53) to determine the number and the size of the outermost layer cells of integument/seed coat. At least 10 ovaries or grains from individual plants of each transgenic line or WT were analysed.

Histochemical observations using β-glucuronidase (GUS) staining

Histochemical observations of GUS activity was determined as described previously (Tittarelli *et al.*, 2007). The spikes of the pINO lines and WT at heading stage were incubated in the GUS staining solution (50 mM Phosphate buffer (pH = 7.2), 0.01% Triton X-100, 2 mM $K_3Fe(CN)_6$, 2 mM $K_4[Fe(CN)_6]\cdot 3H_2O$, 10 mM EDTA, 0.2 mM X-Gluc) at 37 °C for 6 h, followed by gradient decolourization, then photographed by stereomicroscope (SMZ25; Nikon, Tokyo, Japan).

Statistical analysis

All data obtained were processed in SPSS, and statistical analysis was conducted by one-way ANOVA or unpaired Student's *t*-test. Significant differences were considered if *P*-values <0.05.

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Conflict of interest

Authors claim that there is no conflict of interest.

Author contributions

H.Z., R.J., M.M. and L.G. conceived and designed the experiments. L.G., M.M., L.W., M.Z., M.L., B.W. and L.L. conducted the experiments. X.L. helped to construct expression vector. L.G. and M.M. analysed the data and wrote the draft. H.Z., R.J. and W.C. revised the manuscript.

Data availability statement

The raw data of the transcriptome in this study were deposited in NCBI's Sequence Read Archive under BioProject number PRJNA718479.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 The sequence characteristics and phylogenetic tree of TaCYP78A5 in wheat.

Figure S2 The genetic loci of yield-related traits on the short arms of chromosome 2A, 2B and 2D in wheat.
Figure S3 *TaCYP78A5* silencing resulted in decreased seed size and seed coat cell number.
Figure S4 The ripening time of UBI::TaCYP78A5-transgenic wheat line (UBI-4) and wild-type plants (WT).
Figure S5 Grain yield per plant of UBI::TaCYP78A5-transgenic wheat lines (UBI-1 and UBI-2) and wild-type plants (WT).
Figure S6 The yield-related traits of transgenic wheat lines and wild-type wheat.
Figure S7 Validation of RNA-seq data by quantitative real-time RT-PCR (qPCR).
Figure S8 KEGG enrichment (a) and GO enrichment (b) of differentially expressed genes (DEGs) between wild-type wheat and transgenic line pINO-24.
Figure S9 Relative levels of cytokinin in the ovaries of wild-type wheat and transgenic lines.
Figure S10 The correlation analysis between the auxin concentration and the relative expression of pINO lines.
Figure S11 Auxin affects grain weight of wheat.
Figure S12 Localized overexpression of *TaCYP78A5* in integument causes delayed flowering and prolongs proliferation of maternal integument/seed coat cells.
Figure S13 Exogenous auxin treatment causes delayed flowering in wheat.
Table S1 The known genetic loci of yield-related traits on the short arms of chromosome 2A, 2B and 2D in wheat
Table S2 The quality traits of pINO lines and WT
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Appendix S1 Supplemental Methods

