Identification and Analysis of Low Nitrogen Fertilizer Stress-Responsive TCP Transcription Factors in Potato Postprint

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Abstract

TCP transcription factors are a class of plant-specific transcription factors involved in multiple aspects of plant biological processes. To investigate the role of potato TCP transcription factors in response to low nitrogen stress, this study constructed four transcriptomic libraries from potato roots and leaves under nitrogen-deficient (0.05 mmol · L^{-1}) and nitrogen-sufficient (7.5 mmol · L^{-1}) conditions for sequencing, and analyzed the differentially expressed TCP transcription factors. The results showed that: (1) A total of 24 TCP transcription factors were identified across the four transcriptomic libraries, primarily distributed on chromosomes 2, 3, and 6. (2) Domain analysis revealed that all 24 TCP transcription factors possess the typical basic-Helix-Loop-Helix domain. (3) Phylogenetic analysis indicated that potato and Arabidopsis TCP proteins could be clustered together, belonging to 10 subclades. (4) Transcriptome sequencing results demonstrated that under low nitrogen stress, most TCP transcription factors were downregulated, with 3 TCP transcription factors significantly differentially expressed in roots and 5 TCP transcription factors specifically expressed in leaves. (5) Based on GO functional analysis and phylogenetic relationship analysis between potato and Arabidopsis TCP transcription factors, it is speculated that these TCP transcription factors are involved in potato' s response to low nitrogen stress. This study lays a foundation for further investigation of the molecular functions of TCP transcription factors in response to low nitrogen stress in potato and other food crops.

Full Text

Identification and Analysis of TCP Transcription Factors in *Solanum tuberosum* Response to Low Nitrogen Fertilizer Stress

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Abstract

TCP transcription factors are plant-specific transcription factors that participate in multiple aspects of plant biological processes. To investigate the role of potato TCP transcription factors in response to low nitrogen fertilizer stress, this study constructed four transcriptomic libraries from potato roots and leaves under nitrogen-deficient (0.05 mmol · L^{-1}) and nitrogen-sufficient (7.5 mmol · L^{-1}) conditions for sequencing and analyzed differentially expressed TCP transcription factors. The results showed: (1) A total of 24 TCP transcription factors were identified across the four transcriptome libraries, mainly distributed on chromosomes 2, 3, and 6. (2) Domain analysis revealed that all 24 TCP transcription factors possess the typical basic-Helix-Loop-Helix domain. (3) Phylogenetic analysis indicated that potato and Arabidopsis TCP proteins cluster together, belonging to 10 subgroups. (4) Transcriptome sequencing results showed that most TCP transcription factors were suppressed under low nitrogen fertilizer stress, with three TCP transcription factors significantly differentially expressed in roots and five TCP transcription factors specifically expressed in leaves. (5) Based on GO functional analysis and phylogenetic relationships between potato and Arabidopsis TCP transcription factors, it is predicted that these TCP transcription factors participate in potato' s response to low nitrogen fertilizer stress. This study lays a foundation for further research on the molecular functions of TCP transcription factors in response to low nitrogen fertilizer stress in potato and other food crops.

Keywords: *Solanum tuberosum*, TCP transcription factors, nitrogen fertilizer stress, transcriptome sequencing, bioinformatics analysis

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TCP transcription factors are a class of regulatory proteins unique to plants, involved in regulating plant morphogenesis and stress responses during growth (Martín-Trillo & Cubas, 2010; Jean-Michel et al., 2014; Mukhopadhyay & Tyagi, 2015; Challa et al., 2016). For example, the barley gene *BDI1* (Branched and Indeterminate Spikelet 1) encodes a CYC/TB1-type TCP transcription factor that plays a crucial role in barley inflorescence architecture and spikelet development (Shang et al., 2020). Overexpression of the rice gene *OsPCF7* not only increased stem height, root length, and total root number in transgenic rice seedlings, promoted rice tillering and heading, but also increased panicle number and filled grain number per plant (Li et al., 2020). Additionally, research has demonstrated that overexpression of blueberry *VcTCP18* in Arabidopsis significantly reduced seed germination rates, while transgenic plants also exhibited bud dormancy phenomena such as late flowering, fewer rosettes, and fewer main branches (Li et al., 2021). The maple TCP transcription factor ApTCP2 can regulate Arabidopsis leaf morphogenesis via miR319, affect flowering, and positively regulate leaf senescence (Zhu et al., 2022). Studies have also proven that TCP transcription factors participate in plant stress resistance processes; for instance, overexpression of the moso bamboo (*Phyllostachys edulis*) transcription factor *PeTCP10* in rice and Arabidopsis not only improved drought tolerance in transgenic plants but also reduced ABA sensitivity (Liu et al., 2020).

Potato (*Solanum tuberosum*) is the world' s fourth largest food crop, widely cultivated in China, Russia, India, Ukraine, the United States, and other countries. In recent years, with the promotion of potato as a staple food in China, potato cultivation area and yield have increased, playing an increasingly important role in achieving sustainable agricultural development and ensuring food security in China. However, potato growth is consistently limited by nitrogen throughout its entire life cycle, and both excessive and insufficient nitrogen supply can severely affect the yield and quality of commercial potatoes (Maltas et al., 2018). Studies have shown that transcription factors participate in regulating nutrient deficiency stress during plant growth and development. For example, under low nitrogen fertilizer stress, Arabidopsis AtTCP20 can activate key genes in ammonia assimilation after interacting with other proteins, thereby enhancing plant nitrogen assimilation capacity (Guan et al., 2017). Additionally, birch BpTCP3 and banana Ma06_{g15900} also participate in low nitrogen stress responses (Ren, 2019; Kan et al., 2021). With the publication of genome data, research on transcription factors in potato has been reported successively. For instance, potato StMYB44 transcription factor responds to low phosphorus stress, with its expression suppressed under phosphorus deficiency, and can negatively regulate phosphate transport in potato by suppressing the expression of potato tuber phosphokinase 1 (Zhou et al., 2017). Furthermore, Niu et al. (2021) identified 36 MYB transcription factors responsive to low nitrogen fertilizer stress based on transcriptomic methods. Xiao et al. (2018) retrieved 43 TCP transcription factors from the potato genome but did not conduct functional analysis and verification of these transcription factors. Al-

though research has demonstrated that the transcription factor StTCP13 cloned from potato variety 'Favorita' plays a certain role in response to salt stress (Li et al., 2021), whether potato TCP transcription factors participate in nitrogen starvation stress and their functions under insufficient nitrogen supply conditions remain unclear. Therefore, this study, based on transcriptome data from potato under nitrogen-deficient and nitrogen-sufficient conditions, utilized bioinformatics techniques to analyze the basic information and conserved domains of TCP transcription factors, their expression patterns in different tissues and nitrogen application levels, GO functions, and phylogenetic relationships with Arabidopsis TCP transcription factors, laying a foundation for further in-depth research on the role of potato TCP transcription factors in response to nitrogen fertilizer stress.

Materials and Methods

1.1 Experimental Materials

The French fry-type potato variety 'Russet Burbank' was used as material and grown in hydroponic systems with sufficient nitrogen supply (7.5 mmol · L^{-1}) and insufficient supply (low nitrogen stress, 0.05 mmol · L^{-1}) (Sharifi et al., 2007; Li et al., 2010). Plant cultivation methods and growth conditions followed those reported by Xie et al. (2017). After 10 days of cultivation, leaves and roots were collected according to different treatments for transcriptome sequencing library construction [Root control and Leaf control were sequencing libraries from roots and leaves of potato with sufficient nitrogen supply; Root treatment and Leaf treatment were sequencing libraries from roots and leaves of potato with insufficient nitrogen supply]. Plants grown under sufficient nitrogen supply conditions served as the control, with four biological replicates for each treatment, followed by transcriptome sequencing.

1.2 Bioinformatics Analysis of Potato TCP Gene Family Response to Nitrogen Fertilizer Stress

TCP gene sequence information was obtained based on transcriptome data and the potato genome database Phytozome 12.1 (https://phytozome.jgi.doe.gov/pz/portal.html). Online analysis software ProtParam and SOPMA were used to predict the physicochemical properties and secondary structure of TCP proteins. BLAST2GO software was used to analyze functional annotation of TCP transcription factors. Using MEGA 7.0.14 software, potato TCP family protein sequences and Arabidopsis TCP family protein sequences from the database (http://planttfdb.gao-lab.org/family.php?sp=Ath&fam=TCP) were aligned and analyzed, and a phylogenetic tree was constructed using the neighbor-joining method. The MEME Suite 5.4.1 (http://meme-suite.org/) program was used to analyze motifs in potato TCP family proteins, with motif width minimum value set to 6, maximum value to 60, motif number to 6, and other parameters as default values. HemI1.0.3.7 software was used to draw gene expression heatmaps based on the expression levels of potato

TCP transcription factors in the four different sequencing libraries (Deng et al., 2014), and genes with log2(foldchange) greater than 1 or less than -1 and P-value > 0.05 were considered significantly differentially expressed genes for differential analysis of TCP transcription factors obtained from transcriptome data.

Results

2.1 Identification of Potato TCP Transcription Factors

A total of 24 TCP transcription factors were identified from four potato transcriptome sequencing libraries, and relevant information for these genes was obtained using the potato genome database Phytozome as a reference (Table 1). The results showed that these TCP transcription factors mainly originated from chromosome 2 (5 genes), followed by chromosomes 3 and 6, with 4 genes each. Gene lengths ranged from 606 to 2,137 bp, amino acid numbers ranged from 201 to 534, and exon numbers ranged from 1 to 6, with gene PGSC0003DMG400016363 containing the most exons (6).

2.2 Physicochemical Properties of Potato TCP Family Proteins

Physicochemical property analysis showed that the average relative molecular mass of the 24 TCP family proteins was 37,693.70 Da, with the minimum being PGSC0003DMG400021568 encoding a protein with molecular mass of 21,143.87 Da, and the maximum being PGSC0003DMG400024928 encoding a protein with molecular mass of 57,554.24 Da (Table 2). The average isoelectric point (pI) was 7.51, with the minimum being PGSC0003DMG400024254 encoding protein (5.22) and the maximum being PGSC0003DMG400045197 encoding protein (10.08). Eleven TCP family proteins were acidic (pI < 7) and 13 TCP proteins were basic (pI > 7). The average hydrophobicity values of all 24 TCP family proteins were less than 0, with the minimum being PGSC0003DMG400004054 (-1.015) and the maximum being PGSC0003DMG400021568 (-0.283). Secondary structure prediction results showed that all 24 TCP proteins possessed four conformations: alpha helix, beta turn, extended strand, and random coil (Table 2), with average proportions of alpha helix and random coil being 19.20% and 63.34%, respectively, while extended strand and beta turn accounted for 4.09% and 13.38%, respectively. Among these TCP family genes, all 24 TCP proteins showed the largest proportion of random coil, 18 TCP proteins had a higher proportion of alpha helix than extended strand, while 6 TCP proteins (PGSC0003DMG400024632, PGSC0003DMG400004082, PGSC0003DMG400021568, PGSC0003DMG400001362, PGSC0003DMG400026568, and PGSC0003DMG400016363) showed the opposite pattern, with alpha helix proportion less than extended strand proportion.

2.3 Motif Analysis of Potato TCP Transcription Factors

Prediction of structural domains for the obtained potato TCP transcription factors showed that all 24 transcription factors contained Motif 1, which was the longest sequence (60 amino acids) among all motifs (Table 3), indicating that this motif is an indispensable domain in potato TCP transcription factors (Figure 1 [Figure 1: see original paper]). Overall, the TCP transcription factors could be divided into four categories: the first category included 11 genes all containing motifs 1 and 3, with 3 genes also containing motif 5; the second category included 9 genes all containing motifs 1 and 2, among which 3 TCP proteins (PGSC0003DMG400009406, PGSC0003DMG400012396, and PGSC0003DMG400015377) contained motifs 4 and 6; the third category included 3 genes containing only motif 1; the fourth category included only 1 gene (PGSC0003DMG400033078) containing motifs 1, 2, and 3. Further analysis of conserved Motif sequence characteristics revealed that all 24 TCP transcription factors possessed the typical basic-Helix-Loop-Helix (bHLH) domain.

2.4 Protein Evolutionary Analysis of Potato TCP Transcription Factors

To resolve the evolutionary relationships of potato TCP proteins and predict their functions, MEGA 7.0.14 software was used to analyze the evolutionary relationships between potato TCP family proteins and Arabidopsis TCP family proteins (PlantTFDB database) (Figure 2 [Figure 2: see original paper]). The results showed that the 24 potato TCP family proteins clustered together with Arabidopsis TCP proteins and belonged to 10 subgroups, indicating that potato TCP family proteins have relatively high conservation with Arabidopsis TCP proteins. From Figure 2, it can be seen that PGSC0003DMG400000597 and PGSC0003DMG400033078 have close phylogenetic relationships with Arabidopsis AT3G47620.1 (AtTCP14), PGSC0003DMG400015377 with Arabidopsis AT3G15030.1 (AtTCP4), PGSC0003DMG400004054 and PGSC0003DMG400005705, PGSC0003DMG400000823 with AT1G68800.1 (AtTCP12); PGSC0003DMG400004082 and PGSC0003DMG400024632 with Arabidopsis AT5G08070.1 (AtTCP17) and AT5G60970.1 (AtTCP5); PGSC0003DMG400045197, PGSC0003DMG400024254, and PGSC0003DMG400022738 with Arabidopsis AT2G45680.1 (AtTCP9), AT5G51910.2 (AtTCP19), and AT5G51910.1 (AtTCP19); PGSC0003DMG400009406 with Arabidopsis AT2G31070.1 (AtTCP10).

2.5 GO Functional Annotation Analysis of Potato TCP Transcription Factors

GO annotation analysis results showed that the 24 TCP transcription factor sequences were annotated to molecular function (15 sequences), biological process (22 sequences), and cellular component (18 sequences). All TCP sequences were further enriched into 26 functional categories, with DNA binding function (11), nucleus function (17), protein binding function (6), transcription factor activity

(13), transcription regulation (12), leaf morphogenesis (8), cell differentiation (8), positive regulation of development (8), and defense response regulation (6) containing more sequences; while regulation of cell size (4), negative regulation of leaf senescence (4), chloroplast function (3), regulation of secondary shoot formation (3), core promoter proximal region sequence-specific DNA binding (2), core promoter binding (2), abscisic acid response (2), regulation of timing of transition from vegetative to reproductive phase (1), cell proliferation (2), cytokinin response (2), gibberellin response (2), embryo development ending in seed dormancy (2), regulation of seed germination (2), inflorescence development (2), root development (2), circadian rhythm regulation (1), and anatomical structure morphogenesis (1) contained fewer sequences. Biological processes mainly focused on transcription regulation, positive regulation of development, leaf morphogenesis, and cell differentiation; cellular components were mainly enriched in nucleus function; and molecular functions concentrated on DNA binding and transcription factor activity (Figure 3 [Figure 3: see original paper]).

2.6 Expression Analysis of Potato TCP Transcription Factors Under Low Nitrogen Fertilizer Stress and in Different Tissues

Based on potato transcriptome data, expression levels of 24 potato TCP transcription factors in different transcriptome libraries were obtained, and expression heatmaps were drawn (Figure 4 [Figure 4: see original paper]). The results showed that after low nitrogen fertilizer stress treatment, 24 TCP transcription factors were suppressed or induced in leaves but did not reach significant differential expression levels, with only 2 genes PGSC0003DMG400000823 and PGSC0003DMG400022738 showing the largest differential expression levels, upregulated by 1.81-fold and 1.58-fold, respectively. In the two root transcriptome libraries, 19 TCP transcription factors were obtained, including 11 suppressed TCP transcription factors and 8 induced TCP transcription factors, among which 3 TCP transcription factors were significantly differentially expressed (PGSC0003DMG400000597 and PGSC0003DMG400024254 were downregulated, PGSC0003DMG400015377 was upregulated), and 2 TCP transcription factors PGSC0003DMG400022964 and PGSC0003DMG400033078 showed large downregulation.

Additionally, differential expression of TCP transcription factors in different tissues was analyzed. The results showed that 21 TCP transcription factors were significantly differentially expressed in roots and leaves. In addition to 5 TCP transcription factors specifically expressed in leaves, 5 TCP transcription factors showed significantly lower expression in leaves than in roots, while the remaining 11 TCP transcription factors were significantly highly expressed in leaves (2 TCP transcription factors PGSC0003DMG400009406 and PGSC0003DMG400015377 showed the largest differential expression levels, upregulated by 6.40-fold and 8.45-fold, respectively) (Figure 4).

Discussion and Conclusion

Due to the critical regulatory role of TCP transcription factors in plant growth, development, and stress resistance, the TCP transcription factor family has attracted increasing attention from researchers. As China' s fourth largest food crop, potato consistently suffers from nitrogen stress during its growth and development, yet whether TCP transcription factors participate in potato' s response to low nitrogen stress has rarely been reported. In view of this, this study identified 24 potato TCP transcription factor family members based on four transcriptome libraries, analyzed their protein sequence structures, and found that these transcription factors all possess the b-HLH structure. Additionally, significant differences were found in the sequences, isoelectric points, molecular weights, and exon numbers of potato TCP transcription factor family members, with uneven distribution on chromosomes, consistent with previous research results (Xiao et al., 2018). Furthermore, expression patterns of TCP transcription factors under low nitrogen stress in different tissues were analyzed. It was found that TCP transcription factors in leaves did not reach significant differential expression levels, with only 2 genes showing large induction expression, a result different from the response pattern in banana where most genes were upregulated after low nitrogen fertilizer stress (Kan et al., 2021), possibly due to species-specific differences in TCP transcription factor response patterns. In roots, genes PGSC0003DMG400000597 and PGSC0003DMG400024254 were significantly downregulated, while PGSC0003DMG400015377 showed the opposite expression pattern. Thus, potato TCP transcription factors exhibit different expression patterns in response to nitrogen fertilizer stress between leaf and root tissues, and different transcription factor members also show different response patterns to low nitrogen fertilizer stress.

Our research group previously used a recirculating hydroponic system to study physiological-level responses of 20 potato cultivars to low nitrogen fertilizer stress, demonstrating that different potato genotypes have different tolerance and sensitivity to low-level nitrogen supply. Under low nitrogen supply conditions, the height of potato aboveground parts and root length of potatoes (including the 'Russet Burbank'variety) were significantly lower than those of plants grown under sufficient nitrogen supply conditions. Meanwhile, potato biomass accumulation was also severely inhibited under low nitrogen growth conditions (Xie et al., 2017). In this study, transcription factors PGSC0003DMG400000597 and PGSC0003DMG400024254 showed consistent expression trends in roots and leaves under nitrogen stress conditions and were significantly suppressed in roots, while transcription factor PGSC0003DMG400015377 showed the opposite trend, being induced in both roots and leaves. Cluster analysis showed that these three TCP transcription factors have the closest phylogenetic relationships with Arabidopsis transcription factors AT3G47620.1 (AtTCP14), AT2G45680.1 (AtTCP9), and AT3G15030.1 (AtTCP4), respectively, suggesting they may have similar biological functions to Arabidopsis transcription factors AtTCP14, AtTCP4, and AtTCP9. In Arabidopsis, AtTCP14 participates in reg-

ulating the growth potential of embryos during seed germination (Tatematsu et al., 2008) and can also regulate plant morphology by promoting cell division in young internodes together with AtTCP15 (Kieffer et al., 2011). AtTCP4 participates in regulating plant hormone synthesis (such as auxin and brassinosteroid) by binding to cis-acting elements of functional genes, thereby affecting plant growth and development. Moreover, Arabidopsis AtTCP4 is regulated by miR319 and can participate in drought stress response by regulating main root elongation (Schommer et al., 2008). Additionally, AtTCP9 has been reported to participate in the coordinated regulation of isochorismate synthase 1 (ICS1) (Wang et al., 2015) and regulate cell size, leaf senescence, and root development in Arabidopsis (Danisman et al., 2012).

Furthermore, studies have reported that Arabidopsis AtTCP20 can activate the expression of nitrate transporter 1.1 (NRT1.1) to regulate the systemic signaling pathway of lateral root development during nitrate transport, thereby affecting lateral root growth (Guan et al., 2014). In this study, potato TCP transcription factors PGSC0003DMG400020247 and PGSC0003DMG400040232 have the closest phylogenetic relationship with Arabidopsis AT3G27010.1 (AtTCP20). Combined with previous physiological research results on potato response to low nitrogen fertilizer stress, the growth rate of potato aboveground parts and roots under nitrogen-deficient conditions was significantly lower than that under sufficient nitrogen conditions, suggesting that these transcription factors may regulate plant morphogenesis and biomass formation and accumulation by affecting the expression of nitrogen metabolism-related genes under low nitrogen fertilizer stress in potato.

In this study, we also found that 4 transcription factors in potato aboveground tissue (leaves) were suppressed under nitrogen stress treatment, while they were not expressed in potato underground tissue (roots), suggesting they may regulate aboveground part growth by responding to low nitrogen fertilizer stress. Among them, gene PGSC0003DMG400005705 has the closest relationship with Arabidopsis AtTCP12; PGSC0003DMG400004082 and PGSC0003DMG400024632 are closely related to AtTCP17 and AtTCP5; PGSC0003DMG400045197 has close phylogenetic relationships with AtTCP9 and AtTCP19. Studies have shown that AtTCP12 is a transcription factor that prevents axillary bud growth, can delay early axillary bud development, and participates in controlling auxin-induced apical dominance (Aguilar-Martínez et al., 2007). AtTCP17 and AtTCP5 play key roles in regulating aboveground lateral meristem morphogenesis by negatively regulating the expression of boundary-specific genes such as *CUC* genes (Koyama et al., 2007). Additionally, GO functional annotation results show that AtTCP19 participates in plant stress defense regulation, and this gene can also function redundantly with AtTCP20 in controlling leaf senescence (Danisman et al., 2013). Therefore, it is speculated that these potato TCP transcription factors may have similar biological functions to the aforementioned Arabidopsis TCP transcription factors, providing references for further verification of the regulatory roles of these TCP transcription factors in potato response to low nitrogen fertilizer

stress.

In summary, this study identified 24 TCP transcription factors under nitrogen-deficient and nitrogen-sufficient conditions in potato based on transcriptome data, and analyzed their chromosomal locations, TCP domains, phylogenetic trees, conserved motifs, and expression patterns in different tissues under nitrogen stress conditions, aiming to lay a foundation for further research on the functions of potato TCP transcription factors under insufficient nitrogen supply and other stress conditions.

References

AGUILAR-MARTINEZ JA, POZA-CARRION C, CUBAS P, 2007. Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds [J]. *Plant Cell*, 19(2): 458-472.

CHALLA KR, AGGARWAL P, NATH U, 2016. Activation of YUCCA5 by the transcription factor TCP4 integrates developmental and environmental signals to promote hypocotyl elongation in Arabidopsis [J]. *Plant Cell*, 28(9): 2117-2132.

DANISMAN S, WAL F, DHONDT S, et al., 2012. Arabidopsis Class I and Class II TCP transcription factors regulate jasmonic acid metabolism and leaf development antagonistically [J]. *Plant Physiol*, 159(4): 1511-1523.

DANISMAN S, VAN-DIJK AD, BIMBO A, et al., 2013. Analysis of functional redundancies within the Arabidopsis TCP transcription factor family [J]. *J Exp Bot*, 64(18): 5673-5685.

DENG WK, WANG YB, LIU ZX, et al., 2014. HemI: a toolkit for illustrating heatmaps [J]. *PLoS ONE*, 9(11): e111988.

GUAN P, RIPOLLI JJ, WANG R, et al., 2017. Interacting TCP and NLP transcription factors control plant responses to nitrate availability [J]. *Proc Natl Acad Sci USA*, 114(9): 2419-2424.

GUAN P, WANG R, NACRY P, et al., 2014. Nitrate foraging by Arabidopsis roots is mediated by the transcription factor TCP20 through the systemic signaling pathway [J]. *Proc Natl Acad Sci USA*, 111(42): 15267-15272.

JEAN-MICHEL D, MICHAEL W, THOMAS R, et al., 2014. Class I TCP-DELLA interactions in inflorescence shoot Apex determine plant height [J]. *Curr Biol*, 24(16): 1923-1928.

KAN BL, YANG Y, DU PM, et al., 2021. Whole Genome Identification of *Musa acuminata* TCP Family and Its Response Nitrogen Stress [J]. *Mol Plant Breed*, [2021-0506]. https://kns.cnki.net/kcms/detail/46.1068.S.20210506.1613.011.html.

KIEFFER M, MASTER V, WAITES R, et al., 2011. TCP14 and TCP15 affect internode length and leaf shape in Arabidopsis [J]. *Plant J*, 68(1): 147-158.

KOYAMA T, FURUTANI M, TASAKA M, et al., 2007. TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis [J]. *Plant Cell*, 19(2): 473-484.

LI WP, CHEN GL, XIAO GS, et al., 2020. Overexpression of TCP transcription factor OsPCF7 improves agronomic trait in rice [J]. *Mol Breed*, 40(5): 48.

LI YQ, AN S, CHENG QQ, et al., 2021. Analysis of evolution, expression and genetic transformation of TCP transcription factors in Blueberry reveal that VcTCP18 negatively regulates the release of flower bud dormancy [J]. *Front Plant Sci*, 12: 697609.

LI JH, XIE MQ, WAN CY, et al., 2021. Prokaryotic Expression of transcription factor StTCP13in potato and its function of salt stress [J]. *Acta Agric Boreal-Sin*, 36(2):33-39.

LI XQ, SVESHNIKOV D, ZEBARTH BJ, et al., 2010. Detection of nitrogen sufficiency in potato plants using gene expression markers [J]. *Am J Potato Res*, 87(1): 50-59.

LIU HL, GAO YM, WU M, et al., 2020. TCP10, a TCP transcription factor in moso bamboo (*Phyllostachys edulis*), confers drought tolerance to transgenic plants [J]. *Environ Exp Bot*, 172(9): 104002.

MALTAS A, DUPUIS B, SINAJ S, 2018. Yield and quality response of two potato cultivars to nitrogen fertilization [J]. *Potato Res*, 61(5): 97-114.

MARTIN-TRILLO M, CUBAS P, 2010. TCP genes: a family snapshot ten years later [J]. *Trends Plant Sci*, 15(1): 31-39.

MUKHOPADHYAY P, TYAGI AK, 2015. OsTCP19 influences developmental and abiotic stress signaling by modulating ABI4-mediated pathways [J]. *Sci Rep*, 5(1): 9998.

NIU SY, JIANG SH, YI ZF, et al., 2021. Characterization of MYB transcription factors in *Solanum tuberosum* under nitrogen fertilizer stress [J]. *Mol Plant Breed*, 1-21[2021-0617]. http://kns.cnki.net/kcms/detail/46.1068.s.20210617.1124.009.html.

REN L, 2019. Function analysis of *BpTCP3* gene in *Betula platyphylla* [D]. Harbin: Northeast Forestry University.

SHANG Y, YUAN L, DI ZC, et al., 2020. A CYC/TB1 type TCP transcription factor controls spikelet meristem identity in barley (*Hordeum vulgare* L.) [J]. *J Exp Bot*, 71 (22): eraa416.

SHARIFI M, ZEBARTH BJ, COLEMAN W, 2007. Screening for nitrogen-use efficiency in potato with a recirculating hydroponic system [J]. *Comm Soil Sci Plan*, 38(3-4): 359-370.

SCHOMMER C, PALATNIK JF, AGGARWAL P, et al., 2008. Control of jasmonate biosynthesis and senescence by miR319 targets [J]. *Plos Biol*, 6(9):

e230.

TATEMATSU K, NAKABAYASHI K, KAMIYA Y, et al., 2008. Transcription factor AtTCP14 regulates embryonic growth potential during seed germination in *Arabidopsis thaliana* [J]. *Plant J*, 53(1): 42-52.

WANG XY, GAO J, ZHU Z, et al., 2015. TCP transcription factors are critical for the coordinated regulation of isochorismate synthase 1 expression in *Arabidopsis thaliana* [J]. *Plant J*, 82(1): 151-162.

XIAO XO, LIN WQ, FENG XF, et al., 2018. Genome-wide identification and analysis of TCP gene in Potato [J]. *Mol Plant Breed*, 16 (24): 7933-7938.

XIE XF, LI XQ, ZEBARTH BJ, et al., 2018. Rapid screening of potato cultivars tolerant to nitrogen deficiency using a hydroponic system [J]. *Am J Potato Res*, 95: 157-163.

ZHOU XJ, ZHA MR, HUANG J, et al., 2017. StMYB44 negatively regulates phosphate transport by suppressing expression of PHOSPHATE1 in potato [J]. *J Exp Bot*, 68(5): 1265-1281.

ZHU L, LI SS, MA QY, et al., 2022. The *Acer palmatum* TCP transcription factor ApTCP2 controls leaf morphogenesis, accelerates senescence, and affects flowering via miR319 in *Arabidopsis thaliana* [J]. *J Plant Growth Regul*, 41(1): 244–256.

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