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Potassium Fertilizer Formulations Modulate Starch Content and Biosynthetic Gene Expression in Potato Tubers

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Abstract

In Inner Mongolia, a major potato-producing region in China, excessive and unbalanced potassium (K) fertilizer application by farmers in pursuit of higher yields compromises tuber quality and environmental sustainability. To establish a rational K fertilization strategy, two-year field trials were conducted using the medium-late maturing cultivar 'Dafeng No. 10'. The experiments evaluated different K rates and blending ratios with polyhalite (POLY4). Key results show that a moderate K reduction (K_2O 200 kg ha^{-1}) combined with a low POLY4 proportion (18.5%, treatments 2T4 and 3T4) did not cause yield loss but instead achieved the highest yield increases (35.6% in 2022, 22.6% in 2023) and significantly enhanced tuber starch content. This was mechanistically supported by the up-regulation of key starch-synthesis enzymes (AGPase, SBE, SSS, GBSS) and their related genes at transcriptional level under the optimal treatments (particularly 2T3, 2T4, 3T4). The study concludes that a tailored K-POLY4 blend, specifically a reduced K rate with 18.5% POLY4, is an optimal strategy for synergistically improving potato yield, starch quality, and potassium use efficiency, providing a theoretical foundation for sustainable intensification of potato production in China.

Keywords
Potassium fertilization; Potato Yield; Polyhalite; Tuber starch content; Gene expression

Introduction

The potato (*Solanum tuberosum* L.) is an annual herbaceous tuber crop belonging to the genus Solanum in the family Solanaceae, is the fourth most important food crop worldwide (Li et al., 2021), is the most important non -grained food and vegetable crop lauded for its nutritional and health benefits(Chaudhary et al., 2024). Fresh potato tubers typically contain 76%–85% water and 15%–24% dry matter. Starch is the

primary component of the dry matter, accounting for approximately 60% of the total tuber dry weight(Brazkiewicz et al., 2025). Potato starch (PS) ranks third in world production after maize and wheat starches(Reyniers et al., 2020).

Potassium (K) is one of the important mineral elements for potato growth and development(Zhang et al., 2023). As a typical K-loving crop, potato has a relatively large demand for K during its growth and development, and even has the phenomenon of excessive K absorption(Chen et al., 2022). Adequate K can promote plant growth and development in many aspects, such as protein formation, enzyme activation, photosynthesis and stomatal movement of plants(Houmani et al., 2022). K fertilizer can promote the growth of plant roots, enhance the vitality of roots and increase the stem height of potato plants(Xie et al., 2025). The key regulatory nodes in the starch biosynthetic metabolic network are constituted by core catalytic components such as AGPase, GBSS, SSS, and SBE(Dobo et al., 2010; Li et al., 2023). The regulatory role of K nutrition in modulating starch synthase activity has been extensively validated in a variety of crop species. In edible sweet potato, the catalytic efficiency of AGPase was significantly enhanced by increased K application during the root tuber development stage(Fan et al., 2023). Studies on spring maize also demonstrated that, within an appropriate fertilization range, K application rates showed appropriate positive correlations with the enzymatic activities of AGPase, GBSS, and SSS(Zhao, 2010). K application at the early growth stage of hybrid rice ‘B-you 827’ was found to significantly enhance the catalytic efficiency of both SSS and GBSS(Zhang, 2008). As key rate-limiting enzymes in the synthesis of amylose and amylopectin, the activities of GBSS and SSS directly govern the compositional profile of starch(Jiang et al., 2003). Comparative analysis across multiple genotypes further revealed a distinct genotypic specificity in K regulation. Under the treatment of 225 $kg \cdot hm^{-2}$ K_2O, the high-starch maize variety ‘Feiyu 3’ exhibited increases of 42% and 38% in AGPase and SSS activities, respectively, while GBSS activity decreased by 19%. In contrast, the low-starch variety ‘Yuyu 22’ showed a 27% increase in AGPase activity, with reductions of 15% and 21% in SSS and GBSS activities, respectively(Cui, 2011).

Collectively, these findings indicate that K fertilizer can precisely regulate the starch biosynthetic network by modulating the coordinated expression of key starch-synthesizing enzymes, a process dependent on complex genetic backgrounds. Nevertheless, systematic studies on the effects of K application on the activities of starch synthesis-related enzymes in high-starch potato cultivars are still lacking. Further research in this area is required to elucidate the regulatory principles of K nutrition on starch metabolism in potatoes.

As a pivotal nutrient regulator throughout the potato life cycle, K exerts its physiological mechanisms across the entire growth and development process. Studies have shown that K management during the vegetative growth stage can effectively enhance plant vigor, improve stem mechanical stability and stress resilience, optimize the energy conversion efficiency of the photosynthetic system, and accelerate the biosynthesis, translocation, and allocation of carbohydrates. When development enters the tuber initiation and bulking stages, continued K supply further promotes the directional transport of photosynthetic assimilates from the shoot to the underground

storage organs, providing ample substrates for the assembly and accumulation of starch granules in tubers.

The quantitative study by (Amanpreet, 2021)revealed a significantly positive correlation between K ion uptake kinetics in potato tuber tissues and the catalytic efficiency of the starch synthase system. This finding provides molecular-level evidence for the crucial regulatory role of K in potato yield formation. The biosynthesis and accumulation of starch in potato tubers are precisely regulated by a multi-layered physiological and biochemical network. The progression of this biochemical process critically relies on the synergistic catalytic efficiency of a series of key enzymes, which include adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), starch branchingenzyme (SBE), and uridine diphosphate glucose pyrophosphorylase (UGPase) (Bahaji et al., 2014). As a key rate-limiting enzyme in starch biosynthesis, AGPase is widely present in the leaf tissues and storage organs of many plants. In higher plants, it functions as a heterotetramer, describes this structure as consisting of two large subunits (55-60 kDa) and two regulatory small subunits (50-54 kDa). Specifically in potato, purification via gel-filtration chromatography identified a native AGPase with a molecular mass of 206 kDa, comprising two 51 kDa large subunits and two 50 kDa small subunits. The enzyme is expressed in multiple potato organs-including stems, tubers, stolons, leaves, and roots-with the highest abundance observed in tubers and leaves. AGPase catalyzes the synthesis of the starch precursor ADPG from G-1-P and ATP (G-1-P + ATP → ADPG + PPi). Its regulatory large subunits operate through allosteric regulation, promoting activator binding and reducing inhibitor binding to the catalytic small subunits, which directly convert substrates and thereby control starch synthesis rates. Prior work has also established that the genes encoding AGPase subunits in higher plants show marked specificity. The ZmTCP7 transcription factor represses the expression of the AGPase gene *ZmBt2* in maize by binding to its promoter, leading to decreased starch accumulation (Ajayo et al., 2022). While this study focused on maize, the TCP transcription factor family is conserved across plants and may also play a similar regulatory role for AGPase genes in potato.

GBSS is a key enzyme in the starch synthesis pathway and exists in two isoforms: GBSS I and GBSS II. GBSS I is exclusively granule-bound, whereas GBSS II exhibits a dual distribution, being found both in association with starch granules and in the soluble stromal fraction. SDS-PAGE analysis indicates that GBSS has a molecular weight of approximately 60 kDa, while GBSS II shows a significantly larger molecular weight, ranging between 70 and 100 kDa. Although the two isoforms share high sequence homology, GBSS II demonstrates markedly higher catalytic efficiency in starch synthesis(Han et al., 2019). In potato, the expression of the *GBSSI* gene is tissue-specific, primarily localized to tubers where it is responsible for amylose biosynthesis, while the *GBSSII* gene is mainly expressed in vegetative organs such as stems and leaves(Jayarathna et al., 2024). In wheat, the TaABI3-A1 transcription factor exerts a negative regulatory effect on the expression of starch synthesis-related genes at the transcriptional level, ultimately impacting the starch biosynthetic metabolic pathway (Zhao et al., 2024). SSS catalyzes the addition of glucose units from ADP-glucose to the non-reducing ends of adjacent glucan chains via α-1,4-glycosidic linkages, thereby

constructing the branched structure of amylopectin with varying chain lengths. This enzyme system exists in multiple isoforms, with three types—SSS I, SSS II, and SSS III—identified in potato tuber tissues. Studies indicate that the predominant SSS isoform responsible for the major activity varies significantly among different crops. In potato tubers, approximately 85% of the total SSS activity is attributed to SSS III, about 15% to SSS II, while the contribution of SSS I is minimal(Wan et al., 2020; Wang et al., 2023). Starch branching enzymes (SBEs) in higher plants have molecular weights generally between 70 and 114 kDa. Based on phylogenetic analysis of amino acid sequences, SBEs are evolutionarily classified into two major clades: SBE (A) and SBE (B). Representative members of the SBE (A) clade include pea SBEI, maize SBEIIa/b, and rice SBEIII, whereas the SBE (B) clade encompasses enzymes such as maize SBEI, rice SBEI, and pea SBEII(Matsumoto et al., 2012). Initial work by Henríquez et al. (Henriquez et al., 2016) detected two transcripts of the SBEII gene in potato leaf tissues but not in tubers, whereas the *SBEI* gene exhibited the converse expression pattern.
Polyhalite(PH) is a naturally occurring K, calcium, magnesium sulfate mineral with a chemical formula $K_2Ca_2Mg\ (SO_4)4{\cdot}2(H_2O)$ corresponding to 13.0% K, 13.3% Ca, 4.0% Mg, and 21.3% S. Many horticultural crops including potatoes require each of the nutrients in PH, often in significant quantities, and are commonly included in nutrient management programs. Polyhalite has a known potential as a fertilizer (Arunachalam et al., 2025) but has had limited use worldwide because it has not been commercially available. Consequently, literature on the performance of PH fertilizer for agronomic crops is very limited compared with common commercial fertilizer sources used to supply K, Ca, Mg, and S to plants. With the discovery of the largest high-grade known resource of PH in the world, the Zechstein deposit located in the southern North Sea Basin in the United Kingdom (Kemp et al., 2016) , PH fertilizer is becoming available worldwide. Polyhalite fertilizers from the Zechstein deposit have purities approaching 90% and have a guaranteed analysis of 12% of K, 12% Ca, 3.6% Mg, and 19% S. Potatoes make an ideal crop to evaluate PH as a fertilizer. Westermann (Westermann, 2005)reported K uptake in potatoes to exceed N, P, Ca and Mg with an average uptake of 4.2 kg of N, 0.55 kg of P, 6.0 kg of K, 1.6 kg of Ca, 1.1 kg of Mg, and 0.4 kg of S per ton of tuber yield. In addition, chloride or salt content can negatively affect dry matter percentage (Roy et al., 2017) and petiole nitrate contents (James et al., 1994) In potato production, chloride-free K sources are preferred. This is because potatoes are frequently grown on soils of low natural fertility under irrigation regimes aimed at high yield and quality, which necessitates substantial inputs of N, P, and K fertilizers.

Materials and Methods

Field profile

Field experiments were conducted in two locations under a mid-temperate continental monsoon climate: (i) 2022, in Xiaoxitan Village (40°47′~41°23′ N, 110°31′~111°53′ E), Wuchuan County, Inner Mongolia Autonomous Region; and (ii) 2023, in Jujinhao Village (41°27′~42°06′ N, 111°11′~112°03′ E), Wulanhua County, Inner Mongolia Autonomous Region. Physical and chemical properties of the soil in the experimental

sites are shown in Table 1.

Test material

In 2022 and 2023, the potato cultivar used in the experiment was ‘Dafeng 10’, a mid- to late-maturing variety.

Fertilizer sources used for both trials were: urea (46% N); diammonium phosphate (DAP, 18% N, 46% P_2O_5); K chloride (MOP, 60% K_2O), polyhalite (POLY4, 14% K_2O) and compound fertilizer (N: P_2O_5: K_2O= 15:15: 15, POLY4: MOP= 1:3).

Field Experiment Design

A field experiment was conducted using drip irrigation on raised beds without mulch. The beds were spaced 125 cm apart, with double rows per bed. Row spacing within the bed was 90 cm, and plant spacing within the row was 30 cm, resulting in a planting density of 53,333 plants per hectare. Planting was performed using manual fertilization combined with mechanical sowing. The experiment was arranged in a randomized complete block design with four replicates. The area of each plot was 45 m².

Fertilizer Treatments

Five fertilizer treatments were established: a no-K control (T1); two full-rate K treatments (300 kg K_2O ha^{-1}) representing local practice, with a low proportion (T2: 13% supplied by POLY4) and a high proportion (T3: 37% supplied by POLY4) of the K sourced from POLY4; and two reduced-K treatments (200 kg K_2O ha^{-1}), with a medium proportion (T4: 18.5% from POLY4) and a high proportion (T5: 31.5% from POLY4) of POLY4-sourced K. All treatments received the same basal application of nitrogen (250 kg N ha^{-1}) and phosphorus (216 kg P_2O_5 ha^{-1}), which corresponded to the local conventional fertilization rate in 2022. The treatment details are summarized in Table 2

In 2023, with no K fertilizer treatment (3T1) and K fertilizer treatment (K_2O 315 kg ha^{-1}, 14.5% POLY4, 3T2) and K reduction treatment, (K_2O 240 kg ha^{-1}, 17% POLY4, 3T3) and (K_2O 200 kg ha^{-1}, 18.5% POLY4, 3T4), nitrogen (N 250 kg ha^{-1}) and phosphorus (P_2O_5 216 kg ha^{-1}) fertilizer application rates were consistent in K reduction treatment, N (N 300 kg ha^{-1}) and P (P_2O_5 250 kg ha^{-1}) fertilizer application rates in K combination treatment were the customary fertilizer application rates of local farmers.

Evaluation of Crop Response:

Yield

After potato maturation, the tubers were harvested from each plot. Three sampling points were selected per plot, with a combined sampling area of 6.67 m². Each treatment included four replicates for yield measurement. All potato tubers from the root systems within the sampled area were carefully excavated to avoid loss. After removing adhering soil from the tuber surfaces, the number and weight of tubers were recorded separately for yield analysis.

Starch Content

The sta rch content was determined using preserved tuber dry matter samples. A starch assay kit (Jiangsu Addisson Biotechnology Co., Ltd., China) was used for quantification.

Activities of Key Starch Enzymes

Sampling was conducted at 70, 90, and 110 days after planting for enzyme activity assays. Undamaged tubers and the fourth fully expanded leaf from the top of uniform plants were selected, immediately sliced, and flash-frozen in liquid nitrogen. For homogenization, fresh or frozen tissue samples were rapidly transferred to a mortar pre-cooled with liquid nitrogen. Using a pre-cooled pestle, tissues were ground to a fine powder while maintaining the sample in a frozen state by periodically adding liquid nitrogen.

The activities of soluble starch synthase (SSS), adenosine diphosphate glucose pyrophosphorylase (AGPase), starch branching enzyme (SBE), and granule-bound starch synthase (GBSS) were determined. All enzyme activities were measured using corresponding ELISA kits (Jiangsu Sumaike Biological Co., Ltd., China).

Expression of Key Starch Synthase Genes

Total RNA was extracted from potato leaves and tubers using the TaKaRa MiniBEST Plant RNA Extraction Kit (Takara Bio Inc., Japan). First-strand cDNA was then synthesized with the Evo M-MLV RT Premix for qPCR kit (Accurate Biology Co., Ltd., China). To assess the effects of different K fertilizer treatments on the expression levels of key starch synthase genes (including *AGPase*, *GBSSI*, *SSS*, and*SBE*) in potato tubers, quantitative real-time PCR (qPCR) was performed using the TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) kit (Takara Bio Inc., Japan) on a Roche LightCycler ® 480 system. Gene sequences for key starch synthases (e.g., *AGPase*, *GBSSI*, *SSSI*, and *SBEIII*) were retrieved from the NCBI database. Specific primers were designed using Primer Premier 5.0 software and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The *Actin* gene was used as an internal reference, and its primer sequences are listed in Table 4.

Data Analysis

Gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. Data processing and statistical analysis were performed using Microsoft Excel 2016 and SPSS 20.0 software, respectively. Figures were generated with Origin 8.0.

Results and Discussion

Response of Potato Yield to Different K Fertilization

As shown in Figure 1, the results from both the 2022 and 2023 trials demonstrated that K fertilizer application significantly increased potato yield compared to the no-K control. Treatment 2T4 showed the greatest yield improvement, with a 35.6% increase over 2T1. However, yield under 2T5 was significantly lower than that under 2T4,

indicating that the proportion of POLY4 influenced yield under reduced-K conditions. No significant yield differences were observed between treatments 2T2/2T3 and 2T4/2T5. In the 2023 trial, treatment 3T4 increased yield by 20.47% compared to 3T1, and significantly outperformed 3T2, suggesting that under combined MOP and POLY4 application, reduced K rates could enhance yield more effectively than full K rates.

Response of K Use Efficiency to Different K Fertilizer Combinations

K use efficiency (KUE) refers to the proportion of applied K fertilizer that is effectively absorbed and utilized by the crop, serving as a key indicator of fertilization effectiveness. Analysis of Figure 5 reveals that, across both the 2022 and 2023 trials (with the exception of treatment 2T5 in 2022), the reduced-K treatments significantly increased KUE compared to the full-rate K treatments. This indicates that K reduction significantly enhances KUE. Furthermore, KUE in treatment 2T4 was significantly higher than in 2T5, suggesting that a reduced-K treatment with a lower proportion of POLY4 more effectively promotes K uptake by plants (Fig. 2).

Response of Starch Content in Potato Tubers to Different K Fertilization

Throughout the growth and development of potatoes, tuber starch content generally exhibited a progressive increase over time under different K fertilizer treatments. From 70 to 110 days after planting, the starch content in tubers from all fertilizer treatments was significantly higher than that in the no-K control.

In the 2022 trial, during the 70 to 90-day period, treatment 2T3 (full K with a high proportion of POLY4) showed significantly higher starch content than 2T2 (full K with a low proportion of POLY4), while treatment 2T4 (reduced K with a low proportion of POLY4) was also significantly higher than 2T5 (reduced K with a high proportion of POLY4). These results indicate that under full K supply, a higher proportion of POLY4 promoted starch accumulation in tubers during the early-to-mid growth stages, whereas under reduced K supply, a lower proportion of POLY4 was more conducive to starch accumulation in the same period. At 110 days, the starch content in treatment 2T4 remained significantly higher than in 2T5, suggesting that under reduced-K conditions, a lower proportion of POLY4 continued to enhance starch accumulation in the later growth stage. In the 2023 trial, at 70 days, both treatments 3T3 (reduced K with high POLY4) and 3T4 (reduced K with medium POLY4) showed significantly higher starch content than 3T2 (full K with conventional fertilizer), indicating that reduced-K treatments promoted starch accumulation in tubers during the early growth stage compared to the full-K treatment. At 90 days, starch content in 3T3 remained significantly higher than in 3T2, demonstrating its continued advantage in the mid-growth stage. By 110 days, 3T4 exhibited significantly higher starch content than 3T2, suggesting that the specific reduced-K formulation with a medium proportion of POLY4 (3T4) effectively enhanced starch accumulation in the later growth stage (Fig. 3).

Response of Key Starch Synthase Activities to Different K Fertilizer Combinations in Potato

Response in Leaves

During potato growth and development, leaf AGPase activity under different K fertilizer treatments showed a pattern of initial increase followed by decrease. In the 2022 trial, at 70 days, the AGPase activity in all K-applied treatments (2T2–2T5) was significantly higher than that in the no-K control (2T1), with no significant differences observed among the K treatments themselves. This indicates that K application significantly enhances AGPase activity in potato leaves. At 90 days, AGPase activity in treatment 2T3 was significantly higher than in 2T2, and activity in 2T4 was significantly higher than in 2T5. These results indicate that under full K application, a higher proportion of POLY4 enhanced AGPase activity in potato leaves during the mid-growth stage. In the 2023 trial, at 90 days, AGPase activities in both the 3T3 and 3T4 treatments were significantly higher than those in 3T1 and 3T2, indicating that reduced-K applications enhanced leaf AGPase activity during the mid-growth stage. Taken together, these results demonstrate that K fertilization significantly increases AGPase activity in potato leaves (Fig. 4A-B).

As shown in Figure 4C-D, leaf GBSS activity under different K fertilizer treatments exhibited an overall trend of initial increase followed by decrease during potato growth. In the 2022 trial, at 70 days, activity in treatment 2T4 was significantly higher than in 2T1, indicating the most pronounced stimulatory effect of 2T4 on GBSS activity during the early growth stage. At 90 days, all K-applied treatments except 2T5 showed significantly higher activity than 2T1, demonstrating that K application generally enhanced mid-growth GBSS activity. Furthermore, the significantly higher activity in 2T4 compared to 2T5 suggested that under reduced K conditions, a lower proportion of POLY4 was associated with decreased GBSS activity. By 110 days, significantly higher activities were observed in treatments 2T3 and 2T4 compared to 2T1, indicating that both a higher proportion of POLY4 under full K and a lower proportion under reduced K could promote leaf GBSS activity in the late growth stage. Results from the 2023 trial were consistent with those from 2022, confirming the same trend in GBSS activity.

As shown in Figure 4E-F, leaf SSS activity under different K treatments exhibited an overall trend of initial increase followed by decrease. During both the early (70 days) and late (110 days) growth stages, trials in both years demonstrated that K application significantly increased enzyme activity, with no significant differences observed among the various fertilizer combinations. The results from the mid-growth stage (90 days), however, revealed a more complex interaction. In 2022, under conventional K rates, substituting MOP with POLY4 (2T3 vs. 2T2) enhanced SSS activity, whereas under reduced K conditions, the same substitution (2T5 vs. 2T4) decreased it. The 2023 mid-stage data further supported this pattern, showing that treatments with reduced K combined with POLY4 (3T3, 3T4) had significantly higher SSS activity than both the no-K control (3T1) and the conventional K treatment (3T2).

This summary indicates that K application universally increased SBE activity in potato leaves, an effect verified across early, mid, and late growth stages in both experimental years. While the 2023 data showed no differences among K rates—suggesting the amount of K had no significant effect—the 2022 mid-stage data revealed variation

between specific formulation treatments (e.g., 3T3 vs. 3T2, 3T4 vs. 3T5), hinting that the POLY4 ratio may further modulate activity under specific conditions.

Response of Key Starch Synthase Activities in Potato Tubers to Different K Fertilizer Combinations

Tuber AGPase activity followed a unimodal pattern during potato development. Across the entire 2022 growing season, treatment 2T4 maintained the highest activity. Neither POLY4 proportion (within the same K rate) nor K rate itself (when comparing certain treatments) caused significant differences in most cases. Stage-specific analysis in 2022 showed the advantage of reduced-K treatments at 90 days and of all K treatments at 110 days. In 2023, while early-stage results highlighted specific effective treatments (3T2, 3T3), the consistent finding from 90 days onward was that any K application significantly boosted activity compared to the non-fertilized control (Fig.5A-B).

Tuber GBSS activity followed a rise-and-fall trend, with K effects modulated by an interaction between application rate and POLY4 proportion (Fig. 5C-D). In 2022, while early-stage activity (70 d) was unaffected, mid-stage (90 d) activity was higher with high POLY4 under full K but with low POLY4 under reduced K. This pattern persisted at 110 d. The 2023 results reinforced this interaction, showing that reduced-K POLY4 treatments (3T3, 3T4) significantly boosted activity in the mid-late stages compared to the control, whereas early-stage activity remained similar across treatments. Overall, K enhanced GBSS activity mainly after mid-growth, with the optimal POLY4 proportion depending on the K level.

uber SSS activity followed a unimodal pattern during growth (Fig. 5E-F). A consistent finding across both years was that K application significantly increased activity compared to the no-K control, particularly from the mid-growth stage onward. An early-stage nuance was observed in 2022, where only reduced-K treatments (2T4, 2T5) showed a significant boost over the control at 70 days, while other K treatments did not.

Tuber SBE activity was consistently elevated by reduced-K treatments across the entire growth cycle in both years (Figure 5G-H). Beyond this, the effect of full-K application was stage-dependent: in 2022, a high POLY4 proportion (2T3) increased activity early, while conventional full-K treatments (2T2, 2T3) were effective later. The 2023 data broadly supported the positive role of K, as most fertilized treatments surpassed the control from 70 to 90 days. Overall, K application—particularly in reduced-rate formulations—promoted tuber SBE activity throughout development.

Response of Key Starch Synthase Gene Expression in Potato Tubers to Different K Fertilizer Combinations

AGPase gene expression in tubers was co-regulated by K level and POLY4 proportion. In 2022, expression was highest under two optimal formulations: full K with high POLY4 (2T3) and reduced K with low POLY4 (2T4). This interaction was consistent from early to late growth, showing that the effect of POLY4 depended on the K supply level. In 2023, while early expression was unresponsive, reduced-K POLY4 treatments (3T3, 3T4) significantly outperformed conventional K application (3T2) in the mid to late stages. Together, these results indicate that tailored K-POLY4 combinations,

particularly reduced-K formulations, effectively up-regulate tuber *AGPase* expression during key growth phases (Fig. 6A-B).

In potato, GBSS exists as two isoforms, GBSSI and GBSSII. The *GBSSI* gene is predominantly expressed in tubers and is primarily responsible for amylose synthesis(Jayarathna et al., 2024). *GBSSI* expression in tubers peaked at mid-growth (90 days) (Fig. 6C-D). A consistent pattern across both years was that a lower proportion of POLY4 within a given K level (full or reduced) tended to promote higher *GBSSI* expression. This was evident in 2022 where low-POLY4 treatments (2T2, 2T4) outperformed their high-POLY4 counterparts (2T3, 2T5) at peak expression. Additionally, reduced-K treatments with POLY4 (e.g., 3T3, 3T4 in 2023) frequently showed higher expression than the conventional full-K treatment (3T2) from mid to late stages, highlighting the effectiveness of this formulation combination in sustaining elevated expression.

In potato tubers, soluble starch synthase (SSS) exists in three isoforms: SSSI, SSSII, and SSSIII, all of which play critical roles in amylopectin biosynthesis. Among them, the *SSSIII* gene is predominantly expressed in tubers(Marshall et al., 1996). *SSSIII* expression peaked at mid-growth, with its regulation by POLY4 depending on K supply level. In 2022, optimal expression was achieved with high POLY4 under full K but with low POLY4 under reduced K, a pattern evident from early to late stages. In 2023, reduced-K POLY4 treatments consistently promoted higher expression than conventional K application from mid-growth onward. Across both years, reduced-K formulations, especially with appropriate POLY4 proportions, effectively enhanced and sustained *SSSIII* expression during key growth phases (Fig. 6E-F).

Starch branching enzyme (SBE) is one of the key enzymes directly involved in starch biosynthesis. In potato, the *SBEI* gene is exclusively expressed in tubers(Jobling et al., 1999). The expression pattern of the tuber-specific *SBEI* gene mirrored SBE enzyme activity, peaking at mid-growth (90 days) in both years. Early-stage expression was unaffected by K rates. The key regulatory insight emerged at peak and late stages: the optimal POLY4 proportion depended on K supply level. In 2022, high POLY4 was beneficial under full K, while low POLY4 was optimal under reduced K. This was corroborated in 2023, where reduced-K POLY4 treatments consistently surpassed conventional K application, with one formulation (3T4) showing sustained advantage into the late stage (Fig. 6G-H).

Discussion

The starch content serves as a central determinant of potato quality(Hochmuth et al., 2025). Furthermore, beyond being a key quality determinant, starch content is subject to significant modulation by multiple abiotic and managerial factors, ranging from water and temperature to nutrient supply. The application rate of K fertilizer has been identified as a key agronomic factor modulating starch accumulation in potato tubers, primarily through its regulation of key enzymatic activities(Huang et al., 2025). This investigation demonstrated that supplemental K fertilization significantly boosted final starch content compared to the K-deficient control. The effect was dose- and

combination-dependent, becoming prominent only during the active tuber filling period, not in early developmental stages. The superior performance of combination treatments 2T4 and 3T4 over two seasons confirms that synergistic K formulations are key to achieving higher starch content and superior processing quality in potatoes. This aligns with the growing body of evidence, including work by Li et al(Li et al., 2025)., which identifies K management as a critical factor for potato starch content. Starch biosynthesis is coordinately catalyzed by a series of key enzymes, including AGPase, GBSS, SSS, and SBE. These enzymes regulate the structure and formation of starch molecules. Specifically, SBE introduces branch points to increase the number of side chains; GBSS is involved in granule formation, affecting its structure and morphology; and SSS progressively adds glucose units at branch points and chains to form the backbone and branches of starch.

The biosynthesis of starch is a coordinated enzymatic process. AGPase catalyzes the committed step, generating the key precursor ADP-glucose (Yu et al., 2023). This precursor is then utilized by the synthases: Soluble Starch Synthase (SSS) elongates glucan chains by adding glucose units, forming the linear backbone and branches(Hu et al., 2025), while Granule-Bound Starch Synthase (GBSS) is primarily responsible for amylose synthesis within the granule matrix. Subsequently(Jayarathna et al., 2024), Starch Branching Enzyme (SBE) (Ahmad et al., 2025) introduces α-1,6-glycosidic linkages to create branch points, determining the branched architecture of amylopectin. Thus, the concerted actions of these enzymes not only govern the rate of synthesis but also precisely determine the final structure and physicochemical properties of the starch granule. A consistent unimodal trend was observed for the activities of AGPase, GBSS, SSS, and SBE, with a peak at 90 days post-planting across both growing seasons. Notably, differential responses to K regimes were evident: treatments 2T3 and 2T4 (2022) and 3T4 (2023) demonstrated superior and sustained enzyme activity throughout development. This strong temporal and treatment-specific correlation indicates that rational K co-application is an effective agronomic strategy to upregulate the activity of the core starch biosynthetic machinery, directly linking fertilization practice to the enhancement of the starch synthesis pathway. Studies by Gan et al. (Gan, 2017) and Tian et al. (Tian, 2023)have established that the activities of AGPase, SSS, and SBE in potatoes follow a unimodal pattern during development. Similarly, Sui et al. (Sui, 2020) demonstrated that GBSS activity in cereals exhibits the same trend under different K conditions. Extending this pattern to our investigation, we found that under K combination treatments, all four key enzymes (AGPase, GBSS, SSS, SBE) exhibited a consistent unimodal activity curve, aligning with the established physiological model for starch biosynthetic enzymes. The findings of the present study are broadly consistent with (or align with) previous research.

In most crops, the application of different types of K fertilizers affects key genes controlling starch synthesis, up-regulating the expression of some of them. This indicates that these genes play important roles in response to different K fertilizer types and application techniques. The expression dynamics of key starch synthesis genes (*AGPase, GBSSI, SSSIII, SBEI*) in response to different K fertilizer combinations mirrored the unimodal activity patterns of their corresponding enzymes. In the 2022

trial, treatments 2T3 and 2T4 specifically induced a significant up-regulation of the *AGPase* gene at 90 days post-planting. This effect was more pronounced in the 2023 trial, where treatments 3T3 and 3T4 concurrently up-regulated the expression of *AGPase, GBSSI, SBEI,* and *SSSIII* genes at the same developmental stage. These findings demonstrate that optimized K co-application strategies enhance starch accumulation by transcriptionally activating the core biosynthetic machinery during the critical tuber bulking phase.

Conclusion

This study elucidates the mechanisms by which optimized K (K) fertilizer combinations enhance starch accumulation in potato tubers. The principal findings are threefold. First, the activities of key starch biosynthetic enzymes (AGPase, GBSS, SSS, SBE) exhibited a consistent unimodal pattern during tuber development, peaking at approximately 90 days after planting. The co-application of polyhalite enhanced starch accumulation in potato tubers under two specific regimes: a high POLY4 ratio (37% of K supply) and a reduced total K input with a low POLY4 ratio (18.5% of K supply). These treatments induced concomitant increases in the activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, SBE) and the transcript levels of their associated genes. This synergistic enhancement at the transcriptional and translational levels confirms that K fertilization directly modulates the core starch synthesis pathway. Third, the treatment-induced peaks in gene expression and enzyme activity were strongly correlated with final tuber starch content, demonstrating a clear cause-and-effect relationship.

Collectively, our results demonstrate that rational K co-application is an effective agronomic strategy for improving potato quality. It achieves this not merely by providing a nutrient element, but by precisely orchestrating the expression and function of the starch biosynthetic machinery. These findings provide a molecular and physiological basis for recommending targeted K fertilizer formulations to enhance starch yield in potato production.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Dong Wang: Data curation, Writing– review & editing, Visualization, Writing– original draft, Formal Analysis.

Xinyu Ni & Jing Yang: Writing– review & editing, Methodology, Conceptualization.

Zhiping Li & Yan Shi: Software, Formal Analysis, Writing– review & editing.

Chengjian Sui: Supervision, Writing review & editing, Conceptualization.

Wenrui zhao Yuying Sang: Writing– review & editing, Software.

Ziyi Zhang: Project administration, Validation, Writing– review & editing, Funding acquisition, Supervision.

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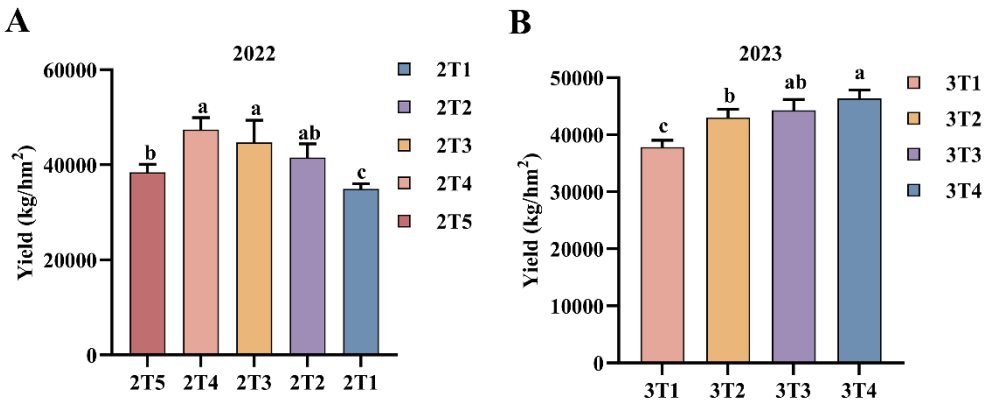


Fig.1

The response of potato yield to different K fertilizer application

The experiments in 2022 **(figure 1A)** and 2023 **(figure 1B)** showed that the potato yield of K fertilizer treatment was significantly higher than that of no K fertilizer treatment, indicating that K fertilizer combined application could significantly increase the potato yield.

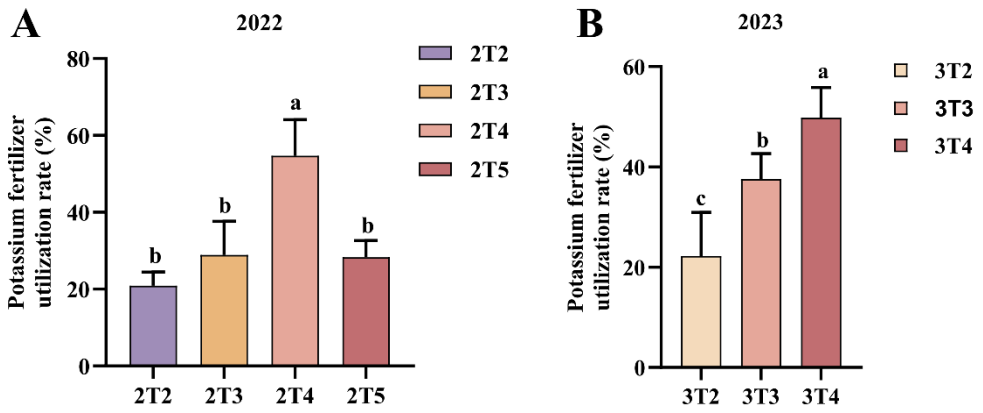


Fig. 2

The response of K fertilizer utilization rate to different K fertilizer applications

2A, Experimental Data of Xiaoxitan Village, Wuchuan County in 2022; **2B,** Experimental data of Jujin Village, Wulanhua County in 2023.

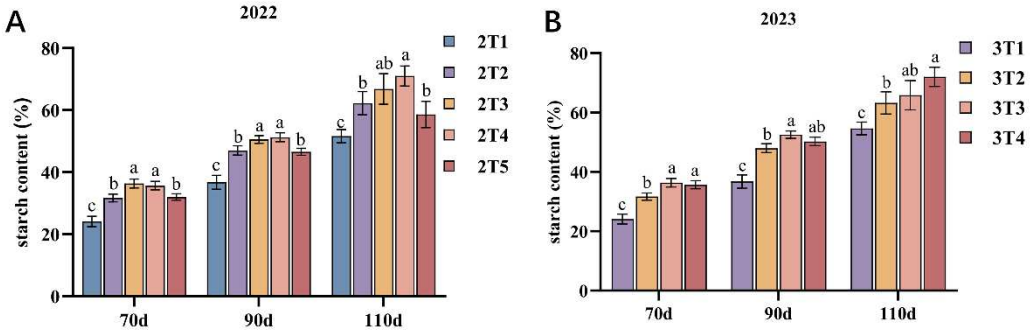


Fig. 3

The response of tuber starch content in potatoes to different K fertilizer applications **A**, illustrates the starch content measured in the 2022 trial, and **B,** displays the corresponding starch content across varying K fertilizer treatments in the 2023 trial.

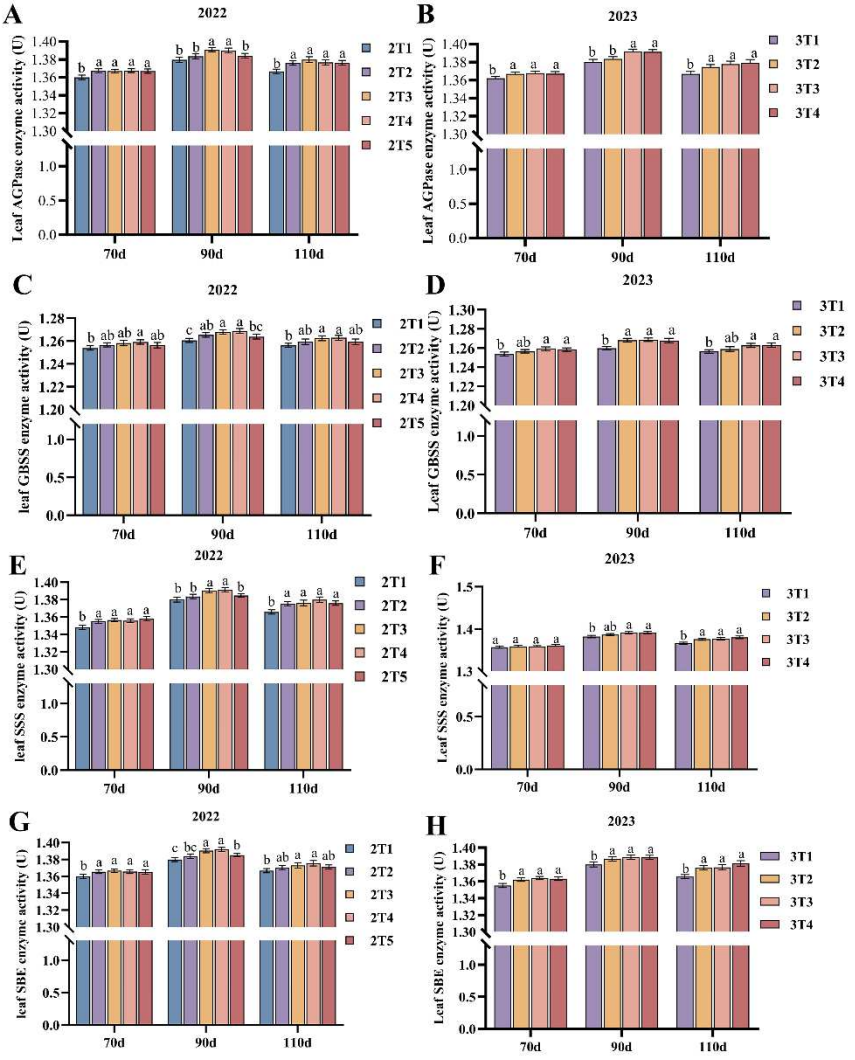


Fig. 4

Response of Key Starch Synthase Activities in Potato Leaves to Different K Fertilizer Combinations

A–H depict the activities of four key starch-synthesizing enzymes—AGPase, GBSS, SSS, and SBE—in leaves under different K fertilizer treatments during the 2022 and 2023 experiments.

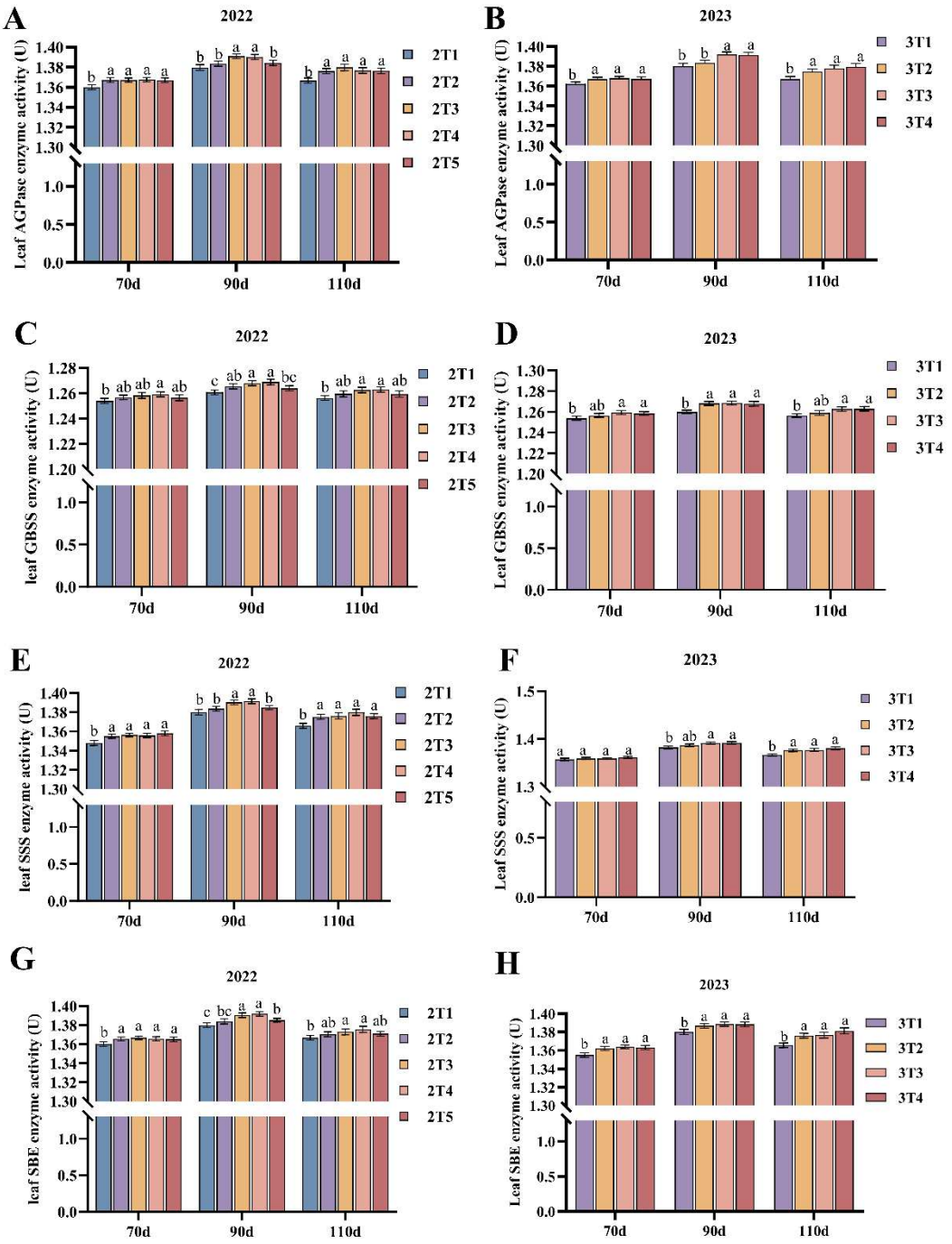


Fig. 5

Response of Key Starch Synthase Activities in Potato Tubers to Different K Fertilizer Combinations

A–H, the activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, and SBE) in tubers under different fertilizer treatments were analyzed at 70, 90, and 110 days after planting. Data were obtained from field experiments conducted in Wuchuan County in 2022 and Wulanhua County in 2023.

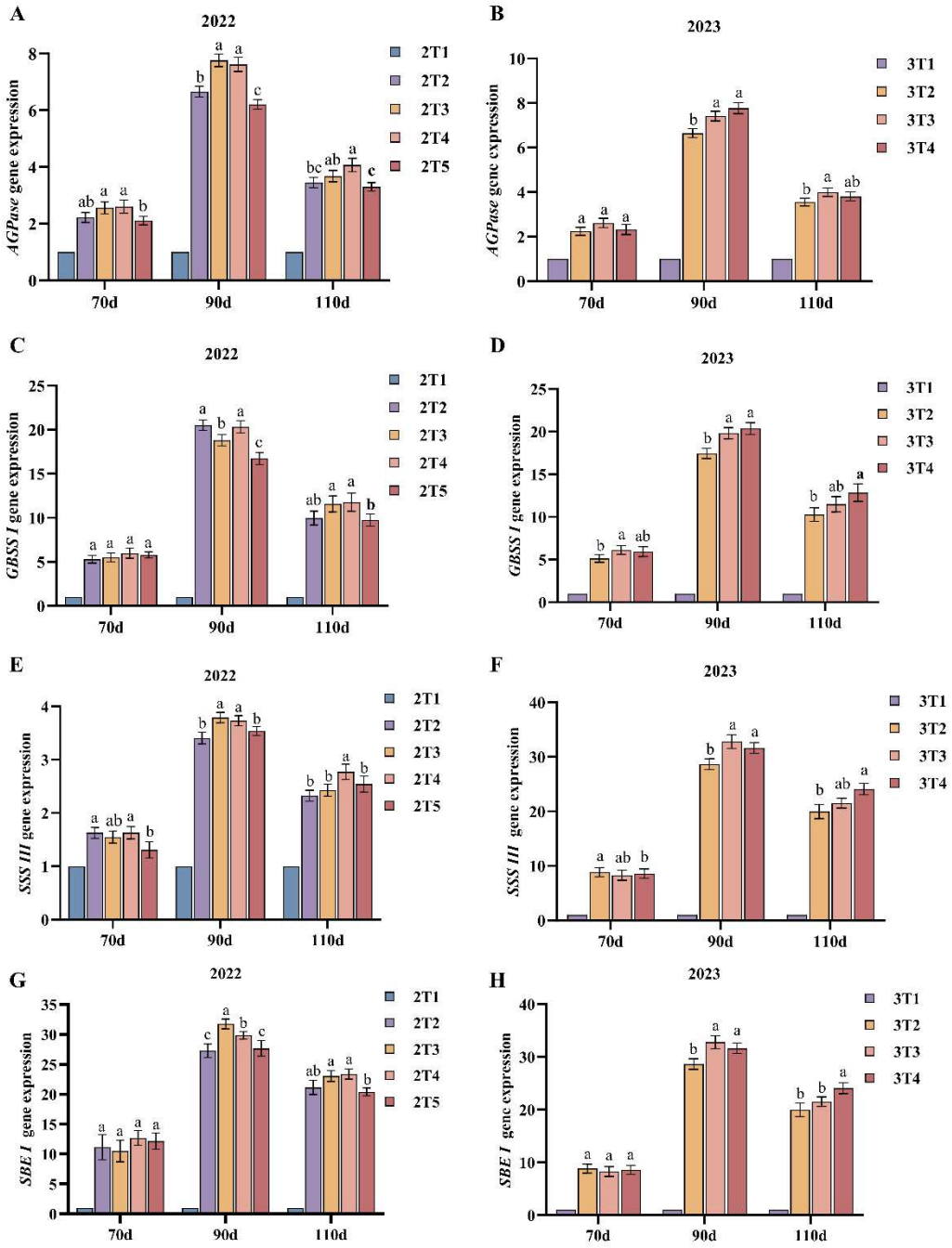


Fig. 6

Expression Levels of Key Starch Synthase Genes at Different Growth Stages

Expression levels of six key genes involved in starch synthesis in potato tubers, **A-B,** *AGPase* gene expression in tubers was co-regulated by K level and POLY4 proportion; **C-D,** *GBSSI* gene is predominantly expressed in tubers; **E-F,** *SSSIII* expression peaked at mid-growth, with its regulation by POLY4 depending on K supply level; **G-H,** the expression pattern of the tuber-specific *SBEI* gene ($p < 0.01$).

Table. 1

Soil physical and chemical properties of experimental field

region	organic matter OM (g/kg)	rapid available phosphorus Available P (mg/kg)	nitrate nitrogen NO_3^--N (mg/kg)	ammonium nitrogen NH_4^+-N (mg/kg)	rapidly available K Available K (mg/kg)	PH
Xiaoxitan	11.1	62.7	10.2	4.1	153.0	7.0
Jujinhao	12.3	63.5	20.1	14.0	90.0	8.2

Table. 2

Potassium fertilizer application rate in 2022 experimental field. (K_2O kg/hm^2)

Treatment	MOP	Compound fertilizer	POLY4	Intertillage fertilizer (Compound fertilizer)	Total K
2T1	0	0	0	0	0
2T2	144	50	0	106	300
2T3	72	50	72	106	300
2T4	52	42	0	106	200
2T5	26	42	26	106	200

Note: In the table, the application rates of nitrogen (N, 250 kg ha^{-1}) and phosphorus (P_2O_5, 216 kg ha^{-1}) fertilizers were identical across all treatments, corresponding to the local conventional fertilization practice, while the K fertilizer treatments varied. 2T1, no K fertilizer was applied. 2T2, K_2O at 300 kg ha^{-1} (13% from POLY4). 2T3, K_2O at 300 kg ha^{-1} (37% from POLY4), 2T4, K_2O at 200 kg ha^{-1} (18.5% from POLY4). 2T5, K_2O at 200 kg ha^{-1} (31.5% from POLY4).

Table. 3

K application rate of each treatment in 2023 experimental field. (K_2O kg ha^{-1})

Treatment	MOP	15-15-15	Intertillage fertilizer 15-15-15	Total K
3T1	0	0	0	0
3T2	132	92	91	315
3T3	79	83	78	240
3T4	52	42	106	200

Note: In the table, the application rates of nitrogen (N, 250 kg ha^{-1}) and phosphorus (as P_2O_5, 216 kg ha^{-1}) fertilizers were identical across all treatments, corresponding to the local conventional fertilization practice, while the K fertilizer treatments varied. 3T1, no K fertilizer was applied. 3T2, K_2O at 315 kg ha^{-1} (14.5% from POLY4), 3T3, K_2O at 240 kg ha^{-1} (17% from POLY4). 3T4, K_2O at 2000 kg ha^{-1} (18.5% from POLY4).

Table. 4

Primers detected by real-time fluorescent PCR

Primer name	Primer sequence(5′-3′)
AGPase	F：*CAGAGTCATCGGCAAAGTGGTG*
	R：*TACTGCCATCGCCTTCAAATC*
GBSSI	F：*TACTCCTTGGACACGCTACAGACT*
	R：*TCAGTGGGACATCATCACGAAC*
SSSIII	F：*CTCAGCAATCAGATCAACACCCT*
	R：*TTGCGTATCGTATAGCGTGC*
SBEI	F：*TTCTTTATGACAGAGGACTCGGGAT*
	R：*CTGGCTTGGACTTTGCTGAGGTT*
Actin	F：*AGGTCCTCTTCCAGCCATCCA*
	R：*CCACTGAGCACAATGTTACCGTAG*