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Optimized Blends of Polyhalite and Muriate of Potash Under Reduced Potassium Rate Are Associated with Enhanced Tuber Starch Content and Up-Regulation of Starch Biosynthetic Genes in Potato

Dong Wang [1], Xinyu Ni [1], Shujuan Wang [2], Jing Yang [1], Zhiping Li [3], Yan Shi [1], Chengjian Sui [1], Wenrui Zhao [1] and Ziyi Zhang [1,*]

[1] Key Laboratory of Plants Adversity Adaptation and Genetic Improvement in Cold and Arid Regions of Inner Mongolia, College of Life Sciences, Inner Mongolia Agricultural University, Hohhot 010018, China; wd@emails.imau.edu.cn (D.W.); nxy@emails.imau.edu.cn (X.N.); 20158013@imau.edu.cn (J.Y.); 15049919240@163.com (Y.S.); scj@emails.imau.edu.cn (C.S.); zwr@emails.imau.edu.cn (W.Z.)
[2] Inner Mongolia Xinyu Seed Industry Co., Ltd., Ulanqab 012000, China; 18647482862@163.com
[3] Inner Mongolia Autonomous Region Agriculture and Animal Husbandry Technology Extension Center, Hohhot 010010, China; nmlzp71877@163.com
* Correspondence: zzy@imau.edu.cn; Tel.: +86-133-8488-8039

Abstract

Excessive potassium (K) application in potato production impairs tuber quality. To develop a rational K fertilization strategy using polyhalite (POLY4), we conducted two field trials (2022, 2023) with potato cultivar 'Dafeng No. 10'. The 2022 trial included a no-K control, two full-rate K treatments (300 kg K_2O ha^{-1}) with low (13%) or high (37%) POLY4, and two reduced-K treatments (200 kg K_2O ha^{-1}) with medium (18.5%) or high (31.5%) POLY4. The 2023 trial comprised a no-K control, a full-rate farmer's practice, and two reduced-K treatments (240 and 200 kg K_2O ha^{-1} with 17% and 18.5% POLY4, respectively). Moderate K reduction combined with a low-to-medium POLY4 proportion (18.5%) caused no yield loss and achieved yield increases of 35.6% in 2022 and 20.5% in 2023 compared to the no-K control and significantly enhanced tuber starch content compared to controls. These optimal treatments were associated with elevated activities of key starch-synthesis enzymes (AGPase, SBE, SSS, GBSS) and increased transcript levels of their corresponding genes (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*). Correlation analyses revealed significant positive associations between these molecular parameters and tuber starch content. We conclude that a tailored K-POLY4 blend under reduced total K input (200 kg K_2O ha^{-1} with 18.5% POLY4) represents an optimal strategy for synergistically improving potato yield, starch quality, and K use efficiency.

Keywords: potato; polyhalite; potassium reduction; tuber starch content; starch biosynthetic genes

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1. Introduction

Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop [1]. It is valued for both its nutritional and health benefits [2]. Fresh tubers contain 76–85% water and 15–24% dry matter, with starch accounting for approximately 60% of the dry weight [3]. Starch content is a key determinant of tuber processing quality, influencing

texture, crispness, and nutritional value [4]. Therefore, understanding how agronomic practices affect starch accumulation is critical for sustainable potato production.

Potassium (K) is essential for potato growth and development [5], as potato is a typical K-loving crop with high K demand. Adequate K supply promotes enzyme activation, photosynthesis, and carbohydrate translocation from shoots to tubers [6]. Critically, K nutrition modulates the activity of key starch biosynthetic enzymes, including adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE), thereby regulating starch content in tubers [7,8].

Similar regulatory roles of K on starch synthesis have been observed in other crops. In sweet potato (*Ipomoea batatas*), increased K application during root development significantly enhanced AGPase activity [9]. In spring maize (*Zea mays*), K application positively correlated with AGPase, GBSS, and SSS activities [10]. In hybrid rice (*Oryza sativa*), early-stage K supply enhanced SSS and GBSS activities [11]. However, systematic studies on how K application affects the activities and gene expression of starch-synthesizing enzymes in high-starch potato cultivars remain scarce [12,13]. Most existing research has focused on single K sources or full K rates, with little attention to reduced-K strategies that maintain or improve quality while minimizing environmental impact [14,15].

At the molecular level, K can up-regulate the transcription of starch synthase genes, leading to increased enzyme activities and starch accumulation [16,17]. Key regulatory nodes include *AGPase*, *GBSSI*, *SSSIII*, and *SBEI*, which encode the core catalytic components of the starch biosynthetic network [18–21]. Polyhalite (POLY4, $K_2Ca_2Mg(SO_4)_4{\cdot}2H_2O$) is a naturally occurring multi-nutrient mineral supplying K, Ca, Mg, and S without chloride, making it particularly suitable for potato, which is sensitive to chloride-induced quality losses [22,23]. Compared to muriate of potash (MOP, KCl), POLY4 provides additional beneficial nutrients. Nevertheless, the agronomic performance of POLY4 in potato—especially under reduced K rates—has been scarcely studied. Furthermore, it remains unknown how different blending ratios of POLY4 and MOP affect the expression of starch biosynthetic genes in tubers [23,24].

Therefore, this study aimed to: (1) evaluate the effects of different K-POLY4 blending ratios under full and reduced K rates on potato yield, starch content, and K use efficiency; (2) quantify the activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, SBE) in tubers and leaves; (3) measure the transcript levels of their corresponding genes (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*); and (4) examine the correlative relationships between these molecular parameters and tuber starch content. Unlike previous studies that focused on single K sources or full K rates, our work specifically investigates reduced-K formulations with optimized POLY4 proportions and provides a mechanistic link between fertilization strategy and the starch biosynthetic pathway. We hypothesized that a tailored K-POLY4 blend under moderate K reduction would be associated with enhanced starch accumulation and elevated expression/activities of core starch synthesis enzymes, and that these parameters would show positive correlations.

We acknowledge that POLY4 supplies not only potassium but also calcium, magnesium, and sulfur. Therefore, any observed effects of POLY4-containing treatments could arise from the combined action of multiple nutrients rather than from potassium alone. The present study does not attempt to isolate the specific contribution of each nutrient but rather evaluates the overall agronomic performance of commercially relevant POLY4-MOP blends. Future studies with nutrient-omission controls will be needed to disentangle the individual contributions of Ca, Mg, and S.

2. Materials and Methods

2.1. Field Profile

Under a mid-temperate continental monsoon climate, field experiments were carried out at two locations in Inner Mongolia, China, over two consecutive years. The first experiment took place in 2022 at Xiaoxitan Village, Wuchuan County (40°47′–41°23′ N, 110°31′–111°53′ E), and the second in 2023 at Jujin-hao Village, Wulanhua County (41°27′–42°06′ N, 111°11′–112°03′ E). In 2022, Wuchuan County experienced slightly warmer and wetter conditions than its long-term averages, with an estimated annual mean temperature of about 3–4 °C and annual precipitation of roughly 350–360 mm, while in 2023, Wuchuan County recorded a significantly warmer-than-average annual temperature of approximately 3.6 °C and near-average annual precipitation of about 300 mm. The physical and chemical properties of the soil at each site are listed in Table 1. Because the two sites exhibited similar soil texture and fertility levels, the treatment effects for each year could be analyzed separately.

Table 1. Soil physical and chemical properties of experimental field.

	Organic Matter	Rapid Available Phosphorus	Nitrate Nitrogen	Ammonium Nitrogen	Rapidly Available K	
Region	**OM (g/kg)**	**Available P (mg/kg)**	**NO_3^--N (mg/kg)**	**NH_4^+-N (mg/kg)**	**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

Note: Initial soil physicochemical properties (0–20 cm depth) at the two experimental sites. Xiaoxitan (2022) in Wuchuan County and Jujinhao (2023) in Wulanhua County, Inner Mongolia, China. OM: organic matter; Available P: rapid available phosphorus (Olsen-P); NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen; Available K: rapidly available potassium.

To monitor the temporal dynamics of soil K supply, soil samples (0–20 cm depth) were collected from each plot at 70, 90, and 110 days after planting (DAP). At each sampling time, five soil cores were taken randomly from each plot, pooled into a composite sample, air-dried, and passed through a 2 mm sieve. Available K was extracted with 1 mol L^{-1} NH_4OAc (pH 7.0) and determined by flame photometry (FP640, Shanghai, China). For each treatment, four independent plot-level replicates (n = 4) were analyzed separately; the mean and standard deviation (SD) were calculated for each treatment and time point. The temporal dynamics of soil available K are detailed in the Results section.

2.2. Test Material

The potato cultivar used in both years was 'Dafeng No. 10', a mid- to late-maturing variety.

The fertilizer sources used in this study were: urea (46% N); diammonium phosphate (DAP, 18% N, 46% P_2O_5); muriate of potash (MOP, 60% K_2O); polyhalite (POLY4, 14% K_2O, 17% CaO, 6% MgO, 19% S), where the 14% K_2O content follows the commercial product guarantee and differs from the theoretical value (12%) due to product specifications; and a compound fertilizer (N:P_2O_5:K_2O = 15:15:15) prepared by physically blending POLY4 and MOP at a 1:3 weight ratio. The K_2O supplied from POLY4 in each treatment was calculated based on the product's guaranteed K_2O content (14%).

2.3. Field Experiment Design

The experiment employed drip irrigation on raised beds without mulch. The beds were spaced 125 cm apart, each containing two rows. Within each bed, the row spacing was 90 cm, and plants were spaced 30 cm apart within the rows, resulting in a planting density of 53,333 plants per hectare. Planting was carried out by manual fertilization combined with mechanical sowing. A randomized complete block design with four replications was used for the experiment, and each plot had an area of 45 m^2.

2.4. Fertilizer Treatments

Five treatments were established in 2022: a no-K control (2T1); two full-rate K treatments (300 kg K_2O ha^{-1}) with a low POLY4 proportion (13% of K from POLY4, 2T2) or a high POLY4 proportion (37%, 2T3); and two reduced-K treatments (200 kg K_2O ha^{-1}) with a medium POLY4 proportion (18.5%, 2T4) or a high POLY4 proportion (31.5%, 2T5). All treatments received the same basal N (250 kg N ha^{-1}) and P (216 kg P_2O_5 ha^{-1}), matching the local conventional rate in 2022.

In 2023, four treatments were used: a no-K control (3T1); a full-rate farmer's practice (315 kg K_2O ha^{-1} with 14.5% POLY4, 3T2); and two reduced-K treatments (240 kg K_2O ha^{-1} with 17% POLY4, 3T3; and 200 kg K_2O ha^{-1} with 18.5% POLY4, 3T4). For the reduced-K treatments, N and P rates were 250 kg N ha^{-1} and 216 kg P_2O_5 ha^{-1}, respectively. For the full-rate treatment (3T2), N and P rates followed local farmer practice (300 kg N ha^{-1} and 250 kg P_2O_5 ha^{-1}). It is important to note that the 3T2 treatment differed from 3T3 and 3T4 in N and P rates in addition to K rate, which constitutes a confounding factor. Thus, comparisons between 3T2 and the reduced-K treatments should be interpreted with caution, as any differences observed cannot be uniquely attributed to K management.

Treatment codes (2T1–2T5 for 2022; 3T1–3T4 for 2023) are used consistently in the figures. Detailed nutrient rates are summarized in Tables 2 and 3.

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 (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 200 kg ha^{-1} (18.5% from POLY4).

It should be noted that the treatment structures differed slightly between 2022 and 2023. The 2022 trial was designed to explore a range of POLY4 proportions under both full and reduced K rates. Based on the 2022 results, the 2023 trial was adjusted to compare a modified farmer's practice (3T2) with two reduced-K formulations (3T3, 3T4) using a refined POLY4 proportion. Therefore, data from the two years are presented separately and no direct cross-year statistical comparisons are made. Nevertheless, the consistent performance of the 200 kg K_2O ha^{-1} with 18.5% POLY4 across both years strengthens the robustness of our conclusions.

The treatment structures in this study were not based on a full factorial design, and thus the independent effects of K rate and POLY4 proportion cannot be statistically separated. Specifically, in the 2022 trial, both the total K rate and the POLY4 proportion varied simultaneously among treatments (e.g., 2T2 vs. 2T4 differ in both K rate and POLY4 proportion). Similarly, in the 2023 trial, the conventional practice (3T2) differed from the reduced-K treatments (3T3, 3T4) not only in K rate but also in N and P rates. These confounding factors limit our ability to attribute observed differences solely to K or POLY4. We therefore interpret our findings as treatment-level comparisons of commercially relevant fertilization blends, rather than as mechanistic dissection of individual nutrient effects. The correlation analyses (Section 3.5) are presented as exploratory evidence to generate hypotheses, not as proof of causation.

2.5. Evaluation of Crop Response

2.5.1. Yield

At harvest maturity (110 days after planting in both years), all tubers from a combined sampling area of 6.67 m^2 per plot were manually excavated. Three sampling points were randomly selected per plot, each of 2.22 m^2. Tubers were gently cleaned to remove adhering soil, then counted and weighed. Yield (kg ha^{-1}) was calculated as:

$$\text{Yield (kg ha}^{-1}\text{)} = (\text{Fresh tuber weight from 6.67 m}^2/6.67) \times 10{,}000.$$

All values refer to fresh tuber weight (no moisture correction).

2.5.2. Potassium Use Efficiency (KUE)

At harvest, representative plant samples (above-ground shoots and tubers) were collected from the same 6.67 m^2 area used for yield measurement. Samples were washed with deionized water, oven-dried at 70 °C to constant weight, and ground to pass through a 0.5 mm sieve. Potassium concentration was determined by H_2SO_4-H_2O_2 digestion followed by flame photometry (FP640, Shanghai, China). Total K uptake (kg ha^{-1}) was calculated as:

$$\text{Total K uptake} = (\text{K concentration in shoots} \times \text{shoot dry weight}) + (\text{K concentration in tubers} \times \text{tuber dry weight}).$$

Potassium use efficiency (KUE, %) was calculated as:

$$\text{KUE (\%)} = [(\text{Total K uptake in fertilized plot—Total K uptake in no-K control plot})/\text{Total K applied}] \times 100.$$

This calculation was performed for each fertilized treatment in each year separately.

2.5.3. Starch Content

Starch content was determined using preserved tuber dry matter samples. Quantification was performed with a starch assay kit (Jiangsu Addisson Biotechnology Co., Ltd., Suzhou, China) following the manufacturer's instructions.

2.6. Activities of Key Starch Enzymes

Sampling was conducted at 70, 90, and 110 days after planting (DAP). At each time point, undamaged tubers and the fourth fully expanded leaf from the top were collected from five uniform plants per plot. Tissues from the five plants were pooled into a single composite sample per plot for each tissue type, and this composite sample was used for enzyme activity and gene expression analyses. Thus, each plot contributed one biological replicate (n = 4 plots per treatment). Tissues were immediately sliced, flash-frozen in liquid nitrogen, and stored at –80 °C until analysis.

For homogenization, 0.5 g of frozen tissue was ground to a fine powder in a liquid nitrogen-pre-cooled mortar. The activities of AGPase, GBSS, SSS, and SBE were measured

using corresponding commercial enzyme activity assay kits (Jiangsu Sumeike Biological Co., Ltd., Nanjing, China), which are based on colorimetric detection of enzyme-catalyzed product formation rather than immunoassay (ELISA). Briefly, AGPase activity was measured by coupling the production of ADP-glucose to NADH oxidation, with absorbance read at 340 nm. GBSS activity was determined by measuring the incorporation of glucose from ADP-glucose into amylose, coupled with a series of enzymatic reactions that ultimately reduce $NADP^+$ to NADPH, detected at 340 nm. SSS activity was assayed using a similar ADP-glucose incorporation principle, with amylopectin as the primer. SBE activity was measured based on the increase in reducing ends or the decrease in iodine-staining capacity of amylose.

For all enzyme assays, the reaction mixture was incubated at 37 °C for 30 min. The reaction was terminated by boiling, and the absorbance of the final product was measured at the specified wavelength using a microplate reader. Pilot experiments confirmed that the substrate concentration and incubation time were within the linear range for each enzyme. One unit (U) of enzyme activity was defined as the amount of enzyme catalyzing the production of 1 μmol of product per minute per gram of fresh weight. The kits have been validated for use in potato tuber and leaf tissues by the manufacturer, with reported intra-assay and inter-assay coefficients of variation <10%.

2.7. Expression of Key Starch Synthase Genes

Total RNA was extracted from potato tubers using the TaKaRa MiniBEST Plant RNA Extraction Kit (Takara Bio Inc., Kusatsu, Japan). First-strand cDNA was synthesized with the Evo M-MLV RT Premix for qPCR kit (Accurate Biology Co., Ltd., Changsha, China). Quantitative real-time PCR (qPCR) was performed using TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara Bio Inc., Shiga, Japan) on a Roche LightCycler® 480 system.

Gene sequences of *AGPase*, *GBSSI*, *SSSIII*, and *SBEI* were retrieved from the NCBI database. Primers were designed using Primer Premier 5.0 and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Primer specificity was validated by melting curve analysis, and amplification efficiency was confirmed to be 90–110% for each primer pair. The *Actin* gene was used as one of the internal reference genes. Based on previous studies on reference gene selection in potato, Elongation Factor 1-Alpha (*EF1α*) was selected as a second reference gene to ensure robust normalization. The geometric mean of the Ct values from these two reference genes was used to calculate the relative expression levels. Primer sequences are listed in Table 4.

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
EF1α	F: CCATTGGGTCGTTTTGCTGTG
	R: TTTGGCACCAGTTGGGTCCT

The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels, normalized to the no-K control (2T1 for 2022, 3T1 for 2023).

2.8. Statistical Analysis

All data are expressed as mean ± standard error (SE) of four biological replicates. Data from the two years were analyzed separately because of differences in treatment structure. For each year, one-way analysis of variance (ANOVA) was performed using SPSS 20.0 (IBM, Armonk, NY, USA) to test the effect of fertilizer treatment. When a significant treatment effect was detected ($p < 0.05$), Tukey's honest significant difference (HSD) test was applied for post hoc multiple comparisons. Means denoted by different letters within the same figure panel are significantly different at $p < 0.05$.

Graphs were generated using Origin 8.0 (OriginLab, Northampton, MA, USA).

For correlation analyses (Section 3.5), data from all treatments within each year were pooled. These correlations are presented as exploratory analyses to generate hypotheses regarding potential associations between variables. We emphasize that correlation does not imply causation, and the nearly identical correlation coefficients for several variables should be interpreted with caution. Pearson correlation coefficients were calculated using SPSS 20.0.

3. Results

3.1. Potato Yield and Potassium Use Efficiency

Potassium fertilization significantly increased potato tuber yield compared to the no-K control in both years (Figure 1). In 2022, treatment 2T4 (reduced K, 18.5% POLY4) produced the highest yield, with a 35.6% increase over 2T1. Yield in 2T5 (reduced K, 31.5% POLY4) was significantly lower than in 2T4, indicating that POLY4 proportion affects yield even under reduced K. No significant yield differences were observed among 2T2, 2T3, 2T4, and 2T5. In 2023, treatment 3T4 (reduced K, 200 kg ha^{-1}, 18.5% POLY4) increased yield by 20.5% over 3T1. It also showed significantly higher yield than the full-rate farmer's practice (3T2); however, because 3T2 received higher N and P rates than 3T4, this comparison should be interpreted with caution, and the yield advantage of 3T4 cannot be uniquely attributed to K management alone.

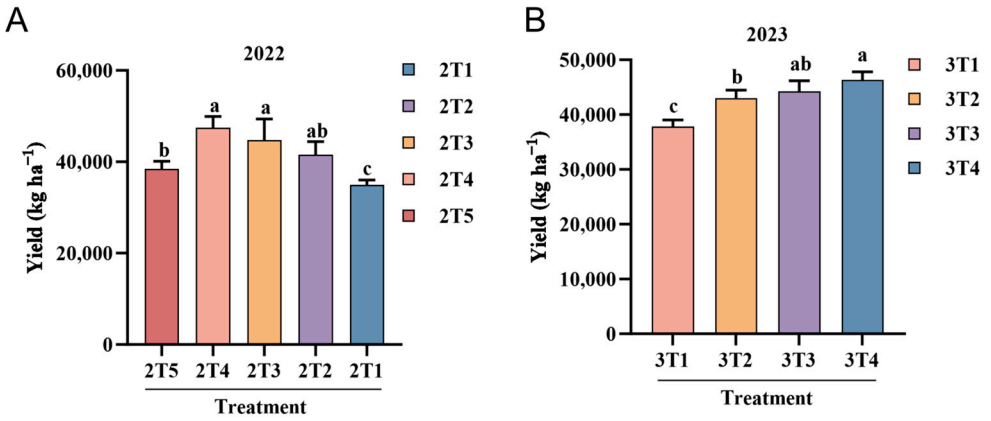

Figure 1. Potato tuber yield (kg ha^{-1}) in response to different K fertilizer treatments. (**A**) 2022 trial at Xiaoxitan Village, Wuchuan County. (**B**) 2023 trial at Jujin-hao Village, Wulanhua County. Data are presented as mean ± SE ($n = 4$). Different lowercase letters indicate significant differences among treatments within each year (Tukey's HSD, $p < 0.05$). Treatment codes: 2T1–2T5 (2022); 3T1–3T4 (2023) as defined in Tables 2 and 3.

Potassium use efficiency (KUE) was significantly higher under reduced-K treatments than under full-K treatments in both years, with the exception of 2T5 in 2022 (Figure 2).

Among reduced-K treatments, 2T4 showed significantly higher KUE than 2T5, suggesting that a lower POLY4 proportion (18.5% vs. 31.5%) under reduced total K improves plant K uptake. In 2023, both 3T3 and 3T4 had higher KUE than 3T2, confirming that reducing K input while using an appropriate POLY4 proportion enhances K use efficiency.

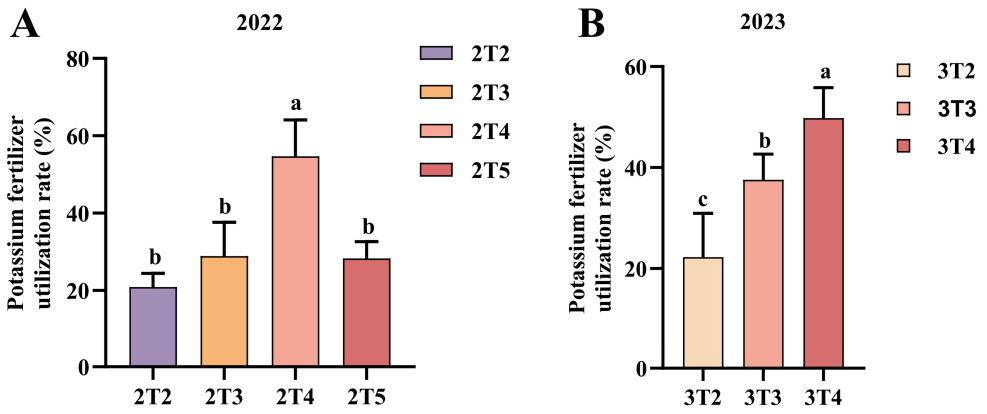

Figure 2. Potassium use efficiency (KUE, %) in response to different K fertilizer treatments. (**A**) 2022 trial at Xiaoxitan Village, Wuchuan County. (**B**) 2023 trial at Jujin-hao Village, Wulanhua County. Data are presented as mean ± SE (n = 4). Different lowercase letters indicate significant differences among treatments within each year (Tukey's HSD, $p < 0.05$). Treatment codes: 2T1–2T5 (2022); 3T1–3T4 (2023) as defined in Tables 2 and 3.

Table 5 presents the soil available potassium concentrations at each sampling time across all treatments.

Table 5. Soil available potassium (mg kg^{-1}, mean ± SD, n = 4) at different growth stages as affected by K fertilizer treatments.

Year	Treatment	70 DAP	90 DAP	110 DAP
2022	2T1	147.87 ± 16.21	124.26 ± 18.15	114.50 ± 24.09
	2T2	362.27 ± 24.54 ^{a}	294.56 ± 32.14 ^{a}	247.40 ± 22.62 ^{a}
	2T3	347.28 ± 23.55 ^{a}	264.34 ± 32.14 ^{ab}	177.28 ± 32.65 ^{b}
	2T4	288.14 ± 32.37 ^{b}	211.30 ± 27.14 ^{b}	170.00 ± 27.83 ^{b}
	2T5	278.42 ± 33.62 ^{b}	223.45 ± 36.56 ^{ab}	195.60 ± 27.90 ^{ab}
2023	3T1	90.00 ± 16.21	78.35 ± 13.15	77.26 ± 24.09
	3T2	332.17 ± 24.54 ^{a}	262.54 ± 32.14 ^{a}	204.26 ± 22.62 ^{a}
	3T3	286.86 ± 23.55 ^{b}	224.95 ± 32.14 ^{a}	177.27 ± 32.65 ^{ab}
	3T4	241.35 ± 35.37 ^{b}	186.00 ± 18.14 ^{b}	160.00 ± 16.83 ^{b}

Note: Different lowercase letters indicate significant differences among treatments within the same year and at the same time point (Tukey's HSD, $p < 0.05$). The control treatments (2T1/3T1) were excluded from the statistical comparisons because they received no K fertilizer.

In the 2022 trial, at 70 DAP, the two full-rate treatments (2T2, 2T3) had the highest soil K values (362.27 ± 24.54 and 347.28 ± 23.55 mg kg^{-1}, respectively), significantly higher than the reduced-rate treatments (2T4: 288.14 ± 32.37; 2T5: 278.42 ± 33.62 mg kg^{-1}). By 110 DAP, however, the full-rate high-POLY4 treatment (2T3) had declined to 177.28 ± 32.65 mg kg^{-1}, while the full-rate low-POLY4 treatment (2T2) remained much higher (247.40 ± 22.62 mg kg^{-1}). Among reduced-rate treatments, 2T4 and 2T5 showed comparable declines, ending at 170.00 ± 27.83 and 195.60 ± 27.90 mg kg^{-1}, respectively, both remaining above the no-K control (114.50 ± 24.09 mg kg^{-1}).

In the 2023 trial, a similar pattern emerged. At 70 DAP, 3T2 (full-rate farmer's practice) had the highest soil K (332.17 ± 24.54 mg kg^{-1}), significantly higher than the reduced-rate treatments (3T3: 286.86 ± 23.55; 3T4: 241.35 ± 35.37 mg kg^{-1}; $p < 0.05$). By 110 DAP,

3T4 (200 kg K_2O ha^{-1}, 18.5% POLY4) and 3T3 (240 kg K_2O ha^{-1}, 17% POLY4) had declined to 160.00 ± 16.83 and 177.27 ± 32.65 mg kg^{-1}, respectively, while 3T2 remained at 204.26 ± 22.62 mg kg^{-1}.

Notably, across both years, all K-fertilized treatments maintained soil available K above 160 mg kg^{-1} at 110 DAP, a level generally considered sufficient for potato production in these soils. The optimized treatment (2T4/3T4) showed a gradual but sustained K supply throughout the tuber bulking period, consistent with the hypothesis that a moderate POLY4 proportion balances rapid early-season K availability (from MOP) with prolonged late-season release (from POLY4).

3.2. Tuber Starch Content and Multi-Nutrient Concentrations

3.2.1. Tuber Starch Content

Tuber starch content increased progressively from 70 to 110 days after planting (DAP) under all K treatments and was significantly higher than in the no-K control at each time point (Figure 3).

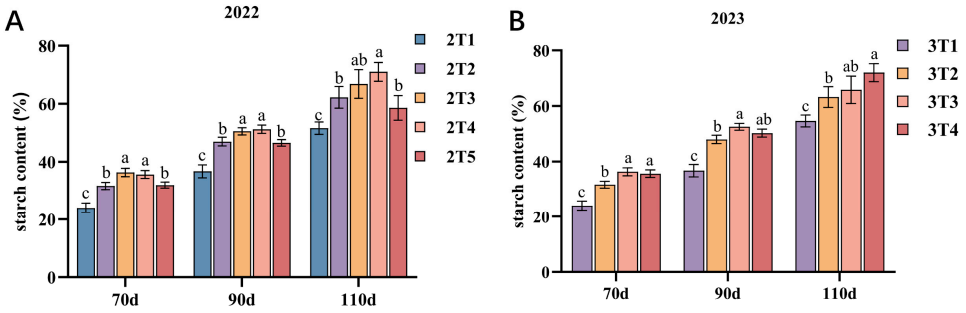

Figure 3. Tuber starch content (% of dry weight) in response to different K fertilizer treatments at 70, 90, and 110 days after planting (DAP). (**A**) 2022 trial at Xiaoxitan Village, Wuchuan County. (**B**) 2023 trial at Jujin-hao Village, Wulanhua County. Data are presented as mean ± SE (n = 4). Different lowercase letters indicate significant differences among treatments within each time point (Tukey's HSD, $p < 0.05$). Treatment codes: 2T1–2T5 (2022); 3T1–3T4 (2023) as defined in Tables 2 and 3.

In 2022, at 70–90 DAP, treatment 2T3 (full K, high POLY4) had higher starch content than 2T2 (full K, low POLY4), and 2T4 (reduced K, low POLY4) had higher starch content than 2T5 (reduced K, high POLY4). At 110 DAP, 2T4 remained significantly higher than 2T5. In 2023, at 70 DAP, both 3T3 and 3T4 had higher starch content than 3T2. At 110 DAP, only 3T4 maintained significantly higher starch content than 3T2 within the context of this trial's treatment design. It should be noted, however, that the full-rate farmer's practice (3T2) differed from the reduced-K treatments (3T3, 3T4) not only in K rate and POLY4 proportion but also in N and P rates (300 kg N and 250 kg P_2O_5 ha^{-1} for 3T2 vs. 250 kg N and 216 kg P_2O_5 ha^{-1} for 3T3/3T4). Therefore, the superior performance of 3T4 over 3T2 should be interpreted as a treatment-level comparison rather than a direct effect of K reduction alone.

Overall, the combination of moderate K reduction with a low-to-medium POLY4 proportion (2T4 in 2022, 3T4 in 2023) consistently enhanced tuber starch accumulation across both growing seasons.

3.2.2. Multi-Nutrient Concentrations

To assess whether the benefits of POLY4-containing treatments were associated with altered tuber nutrient composition beyond K, we measured N, P, K, Ca, Mg, and S concentrations in tubers at harvest (Figure 4).

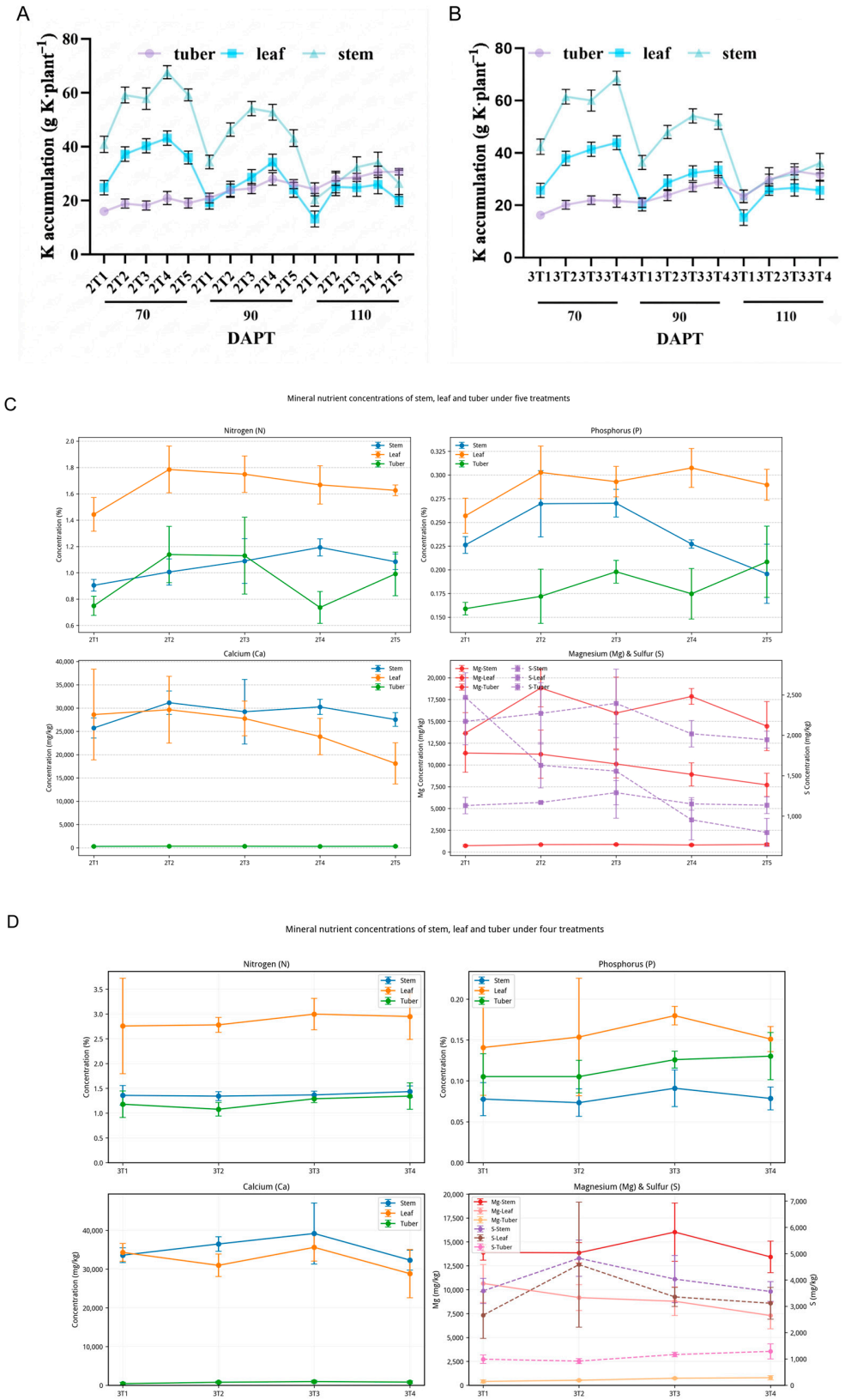

Figure 4. Contents of potassium, nitrogen, phosphorus, calcium, magnesium, and sulfur. (**A**) shows the potassium contents in stems, leaves, and tubers for the 2022 trial; (**B**) shows the potassium contents in stems, leaves, and tubers for the 2023 trial; (**C**) shows the nitrogen, phosphorus, calcium, magnesium, and sulfur contents in stems, leaves, and tubers for the 2022 trial; and (**D**) shows the nitrogen, phosphorus, calcium, magnesium, and sulfur contents in stems, leaves, and tubers for the 2023 trial.

In the 2022 trial, tuber K concentration ranged from 1.409% (2T1) to 1.749% (2T5), with all K-fertilized treatments showing higher K concentrations than the no-K control. Among POLY4-containing treatments, 2T3 (37% POLY4) and 2T5 (31.5% POLY4) had numerically higher tuber K than their low-POLY4 counterparts (2T2 and 2T4, respectively), though differences were not statistically significant ($p > 0.05$). Tuber Ca and Mg concentrations were also elevated in POLY4-containing treatments compared to 2T1, with 2T3 showing the highest Mg (874.3 ± 83.1 mg kg^{-1}) and 2T2 showing the highest Ca (333.0 ± 32.6 mg kg^{-1}). Tuber S concentration was highest in 2T3 (1289.8 ± 144.5 mg kg^{-1}), consistent with the S supplied by POLY4.

In the 2023 trial, tuber K concentration showed a similar trend: 3T4 (18.5% POLY4, 200 kg K_2O ha^{-1}) had the highest K concentration (1.526 ± 0.235%), followed by 3T3 (1.476 ± 0.143%), both exceeding the full-rate farmer's practice 3T2 (1.303 ± 0.181%). Notably, 3T4 also showed the highest tuber Mg (788.8 ± 218.3 mg kg^{-1}) and S (1286.3 ± 280.1 mg kg^{-1}) among all treatments, while Ca was highest in 3T3 (941.3 ± 339.2 mg kg^{-1}). These patterns suggest that POLY4 supplementation increased tuber concentrations of Mg and S, which may contribute to the observed improvements in starch metabolism.

However, because treatment structures were not fully factorial and the 2023 trial included confounding N/P rate differences, these nutrient composition data should be interpreted as descriptive rather than mechanistic.

3.3. Activities of Starch-Synthesizing Enzymes

3.3.1. AGPase Activity

In tubers, AGPase activity followed a unimodal pattern, peaking at 90 DAP. In 2022, treatment 2T4 maintained the highest activity across all time points. At 90 DAP, 2T4 was significantly higher than both full-K treatments (2T2, 2T3) and the other reduced-K treatment (2T5). In 2023, at 90 and 110 DAP, all K-fertilized treatments had significantly higher AGPase activity than the no-K control (3T1). Among them, reduced-K POLY4 treatments (3T3, 3T4) showed higher activity than the conventional full-K treatment (3T2) at 90 DAP (Figure 5A,B).

3.3.2. GBSS Activity

Tuber GBSS activity also peaked at 90 DAP (Figure 5C,D). The effect of POLY4 proportion was modified by the total K rate. In 2022 at 90 DAP, a high POLY4 proportion increased GBSS activity under full K (2T3 > 2T2), whereas a low POLY4 proportion was more effective under reduced K (2T4 > 2T5). This pattern persisted at 110 DAP. In 2023, reduced-K POLY4 treatments (3T3, 3T4) significantly increased GBSS activity compared to the full-K conventional treatment (3T2) at 90 DAP.

3.3.3. SSS Activity

Tuber SSS activity peaked at 90 DAP (Figure 5E,F). K application significantly increased SSS activity compared to the no-K control in both years, especially from mid-growth onward. In 2022 at 70 DAP, only the reduced-K treatments (2T4, 2T5) showed a significant increase over the control; the full-K treatments did not. At 90 DAP in 2023, both reduced-K POLY4 treatments (3T3, 3T4) had higher SSS activity than the conventional full-K treatment (3T2).

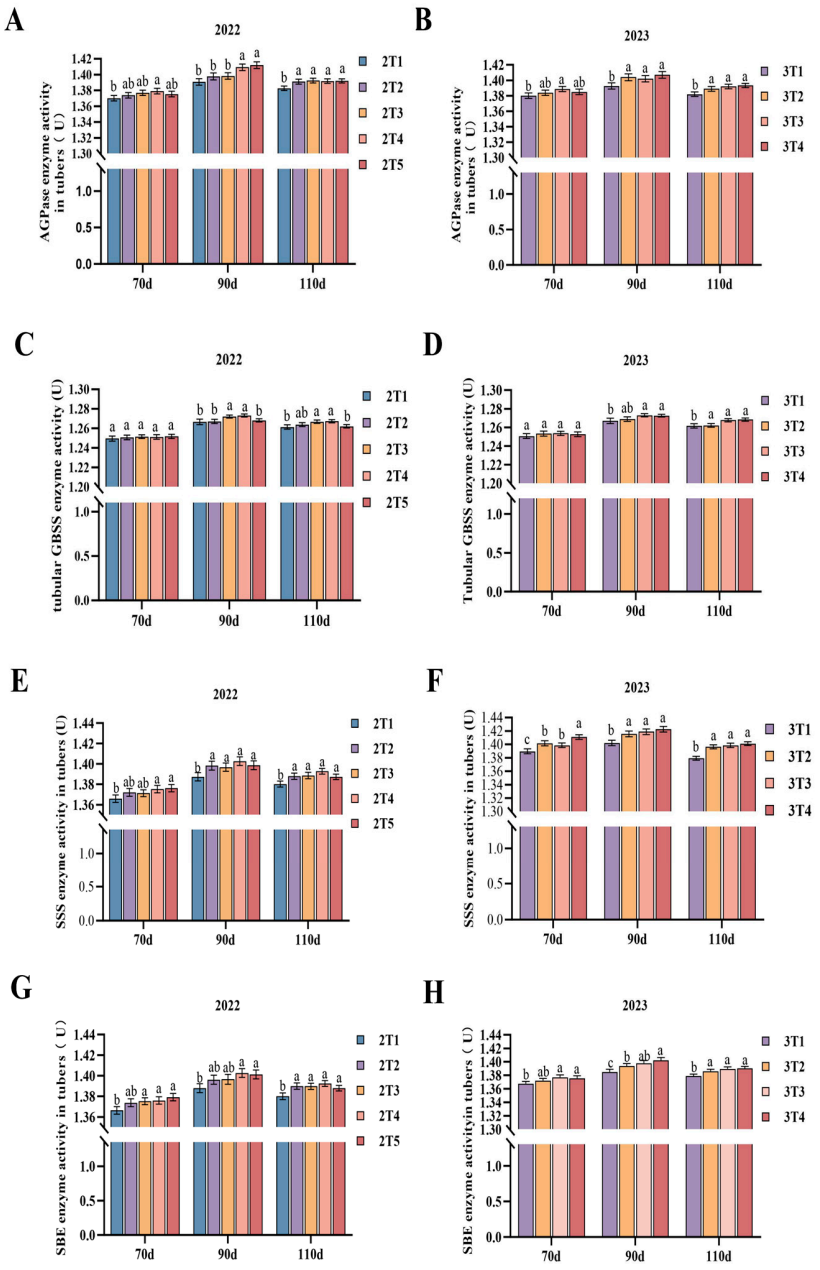

Figure 5. Activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, and SBE) in potato tubers at 70, 90, and 110 DAP in response to different K fertilizer treatments. (**A**–**D**) 2022 trial at Xiaoxitan Village, Wuchuan County. (**E**–**H**) 2023 trial at Jujin-hao Village, Wulanhua County. Enzyme activities are expressed as U g^{-1} fresh weight. Data are presented as mean ± SE (n = 4). Different lowercase letters indicate significant differences among treatments within each time point (Tukey's HSD, $p < 0.05$). Treatment codes: 2T1–2T5 (2022); 3T1–3T4 (2023) as defined in Tables 2 and 3.

3.3.4. SBE Activity

Tuber SBE activity peaked at 90 DAP and was consistently elevated by reduced-K treatments across the entire growth cycle in both years (Figure 5G,H). In 2022, at 70 DAP, a high POLY4 proportion under full K (2T3) increased SBE activity, whereas at later stages both full-K and reduced-K POLY4 treatments were effective. In 2023, most K-fertilized treatments surpassed the no-K control from 70 to 90 DAP, with reduced-K formulations (3T3, 3T4) showing the highest activity.

In leaves, the activities of AGPase, GBSS, SSS, and SBE generally peaked at 90 DAP, similar to the patterns observed in tubers, but the magnitude of treatment differences

was considerably smaller (Supplementary Figure S1). Across both years, K fertilization significantly elevated leaf enzyme activities compared to the no-K controls, yet only minor and inconsistent differences were observed among the various K-POLY4 formulations. For instance, in 2022, leaf AGPase activity at 90 DAP ranged from 2.8 to 3.5 U g^{-1} FW across K-fertilized treatments, compared to a much wider range of 3.5 to 7.5 U g^{-1} FW in tubers (Figure 4A). This relatively muted response in leaves suggests that the regulatory effect of K-POLY4 blends on starch biosynthesis is more pronounced in the sink organ (tubers) than in source leaves.

3.4. Expression of Starch Biosynthetic Genes

3.4.1. AGPase Expression

In tubers, *AGPase* transcript levels peaked at 90 DAP. In 2022, the highest expression was observed in 2T3 (full K, high POLY4) and 2T4 (reduced K, low POLY4). In 2023, reduced-K POLY4 treatments (3T3, 3T4) had significantly higher *AGPase* expression than the conventional full-K treatment (3T2) at 90 and 110 DAP (Figure 6A,B).

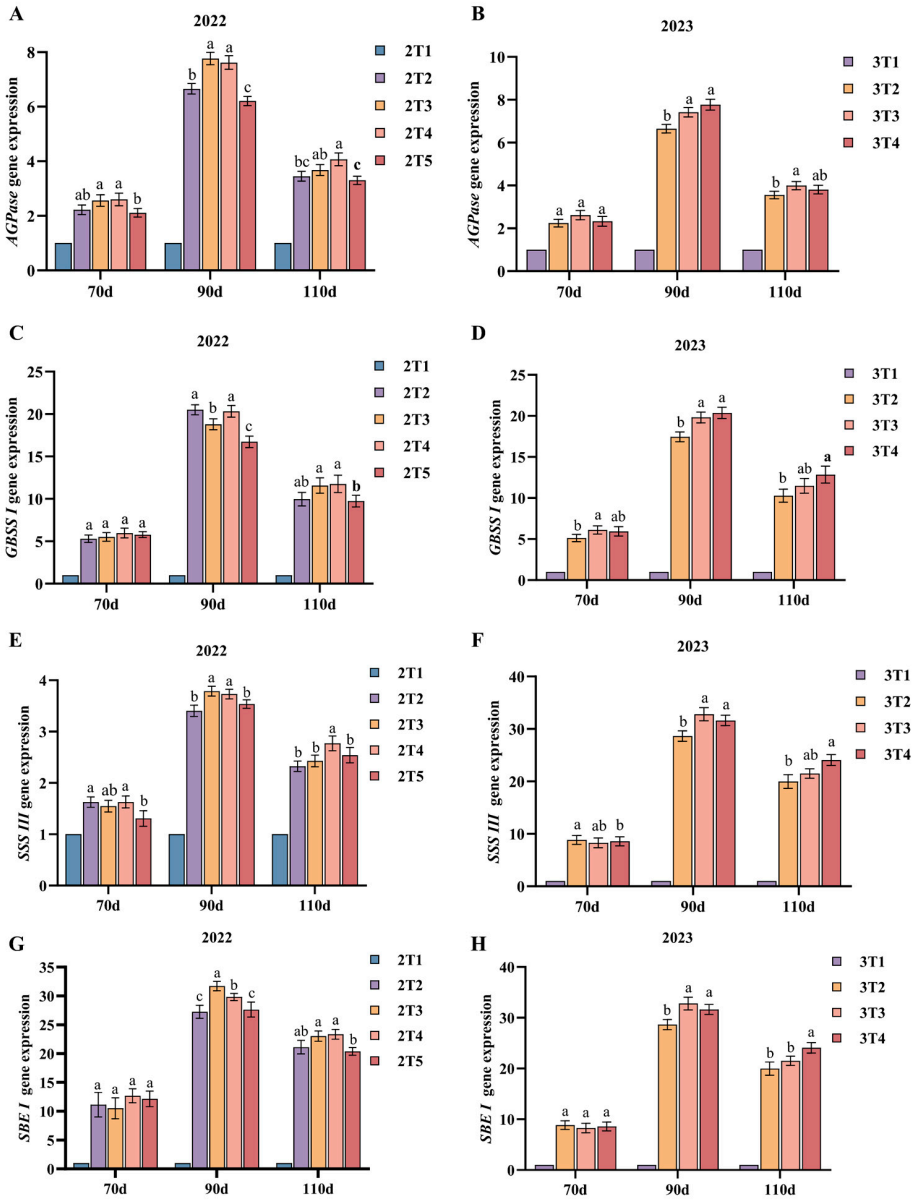

Figure 6. Relative expression levels of starch biosynthetic genes (*AGPase*, *GBSSI*, *SSSIII*, and *SBEI*) in potato tubers at 70, 90, and 110 DAP in response to different K fertilizer treatments. (**A**–**D**) 2022 trial

at Xiaoxitan Village, Wuchuan County. (**E**–**H**) 2023 trial at Jujin-hao Village, Wulanhua County. Expression levels were normalized to the no-K control (2T1 for 2022; 3T1 for 2023) using the $2^{-\Delta\Delta CT}$ method, with the geometric mean of *Actin* and *EF1α* as internal reference genes. Data are presented as mean ± SE (n = 4). Different lowercase letters indicate significant differences among treatments within each time point (Tukey's HSD, $p < 0.05$). Treatment codes: 2T1–2T5 (2022); 3T1–3T4 (2023) as defined in Tables 2 and 3.

3.4.2. GBSSI Expression

GBSSI expression also peaked at 90 DAP. In both years, a lower POLY4 proportion within a given K level promoted higher *GBSSI* expression. Thus, 2T2 and 2T4 outperformed 2T3 and 2T5 in 2022; and 3T4 showed sustained high expression in 2023 (Figure 6C,D).

3.4.3. SSSIII Expression

The expression pattern of *SSSIII* was similar to that of *AGPase*. In 2022, high expression occurred in 2T3 and 2T4; in 2023, reduced-K POLY4 treatments (3T3, 3T4) had higher expression than 3T2 from 90 DAP onward (Figure 6E,F).

It is noteworthy that the expression level of *SSSIII* in 2022 was approximately tenfold higher than in 2023 across all treatments, a variation not observed for the other three genes examined. This substantial inter-annual difference might be attributed to the heightened sensitivity of the *SSSIII* gene to environmental fluctuations, as it encodes the predominant soluble starch synthase (SSS) isoform, accounting for 80–85% of total SSS activity in potato tubers. Environmental factors such as temperature and precipitation, which differed between the two experimental years, are known to influence starch synthase gene expression and enzyme activities.

3.4.4. SBEI Expression

SBEI expression peaked at 90 DAP. In 2022, 2T3 and 2T4 showed the highest levels. In 2023, 3T4 maintained a significant advantage into the late stage (110 DAP) compared to 3T2 (Figure 6G,H).

The relative expression levels, normalized against the geometric mean of *Actin* and *EF1α*, are presented in Figure 5.

3.5. Correlation Between Enzyme Activities, Gene Expression, and Starch Content

To explore potential associations between the measured molecular parameters and tuber starch content, we performed Pearson correlation analyses among K_2O application rate, key enzyme activities (AGPase, GBSS, SSS, SBE), their corresponding gene transcript levels (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*), and final starch content at harvest (110 DAP). These analyses are exploratory and are not intended to establish causal relationships.

First, a strong positive correlation was observed between K_2O application rate and tuber starch content across the two experimental years (Figure 7; r = 0.96–0.99, $p < 0.01$ for the three fitted series). Starch content increased progressively from approximately 24% at 0 kg K_2O ha^{-1} to 36% at 300 kg K_2O ha^{-1}, confirming that potassium fertilization is a major driver of starch accumulation in potato tubers.

Second, and more importantly, scatter plots revealed that starch content was significantly and positively correlated with both the activities of four starch-synthesizing enzymes and the transcript levels of their corresponding genes (Figure 8). Specifically, at 90 DAP (the mid-bulking stage when enzyme activities peaked), AGPase activity (r = 0.90, $p < 0.01$), GBSS activity (r = 0.90, $p < 0.01$), SSS activity (r = 0.90, $p < 0.01$) and SBE activity (r = 0.90, $p < 0.01$) each exhibited a nearly linear relationship with final starch content. Similarly, the relative expression of *AGPase*, *GBSSI*, *SSSIII* and *SBEI* at 90 DAP also showed strong positive correlations with starch content (r = 0.90 for each, $p < 0.01$). These correlation

coefficients were substantially higher than those for K_2O rate alone, indicating that the observed starch enhancement under optimized K-POLY4 blends is mediated primarily through up-regulation of the core starch biosynthetic machinery.

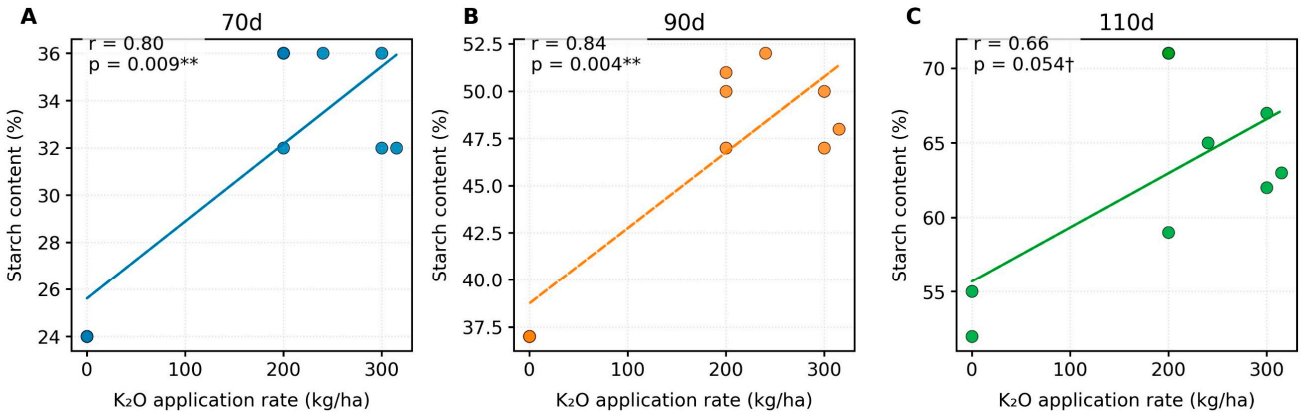

Figure 7. Pearson correlation analysis between K_2O application rate (kg ha^{-1}) and potato tuber starch content (% of dry weight) at different developmental stages. (**A**) 70 DAP, (**B**) 90 DAP, and (**C**) 110 DAP. The colored dots represent individual treatments in the 2022 and 2023 trials. Dashed lines represent linear regression fits. Significance levels: ** $p < 0.01$, † $p < 0.1$ (not significant). R^2 and *p*-values are shown on each panel.

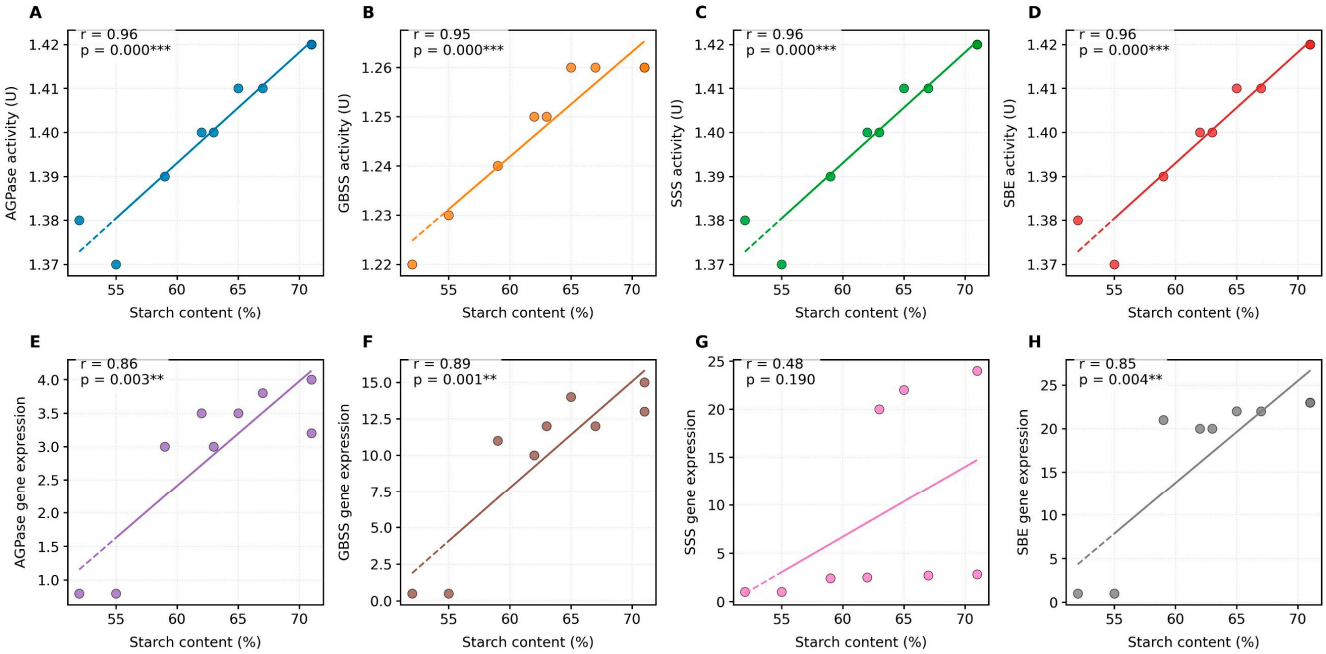

Figure 8. Pearson correlation analysis between tuber starch content (% of dry weight) and key starch-synthesizing parameters at harvest (110 DAP). (**A**–**D**) Enzyme activities (AGPase, GBSS, SSS, SBE; U g^{-1} fresh weight) vs. starch content. (**E**–**H**) Relative gene expression levels (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*; $2^{-\Delta\Delta CT}$, normalized to no-K control) vs. starch content. The colored dots represent individual treatments in the 2022 and 2023 trials. Dashed lines represent linear regression fits. Significance levels: ** $p < 0.01$, *** $p < 0.001$ (not significant). R^2 and *p*-values are shown on each panel.

The observation that multiple enzymes and genes showed similar correlation coefficients ($r \approx 0.90$) likely reflects the coordinated regulation of the starch biosynthetic pathway, as these enzymes function in a common metabolic network. However, this similarity may also be influenced by the limited number of data points ($n = 5$ treatments per year) and should be verified in future studies with larger sample sizes.

Taken together, the correlative evidence is consistent with a model in which the optimal K-POLY4 blend is associated with elevated transcription of *AGPase*, *GBSSI*, *SSSIII*, and *SBEI*, increased activities of their corresponding enzymes, and higher starch accumulation. However, correlation does not prove causation, and the observed associations may reflect indirect effects or coordinated responses to improved overall nutrition rather than direct regulatory links. Future functional studies (e.g., gene silencing or overexpression under controlled K-POLY4 regimes) will be required to establish causality.

4. Discussion

Several interpretational caveats should be acknowledged at the outset. First, because POLY4 supplies multiple nutrients (K, Ca, Mg, S), the observed benefits of POLY4-containing treatments cannot be uniquely attributed to potassium or to POLY4 itself. Second, the 2023 experiment was confounded by differences in N and P rates between the conventional practice (3T2) and reduced-K treatments (3T3, 3T4). Third, the treatment structures in both years were not full factorial designs, preventing rigorous statistical assessment of interactions between K rate and POLY4 proportion. Fourth, our conclusions are based on correlations, which do not establish causation. These limitations are addressed in detail below.

This study provides three novel insights into potassium fertilization for potato production. First, a reduced total K rate (200 kg K_2O ha^{-1}) with a low to medium POLY4 proportion (18.5%) increased tuber yield and starch content compared to no-K control and conventional full-K practice. This conclusion is independently supported by the 2022 trial (2T4 vs. 2T1 and 2T2/2T3) and the 2023 trial (3T4 vs. 3T1 and 3T2). Second, in both trials, the optimal formulation upregulated the activities of four key starch-synthesizing enzymes (AGPase, GBSS, SSS, SBE) and the transcript levels of their corresponding genes (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*). Third, correlative evidence from each trial separately links these molecular changes to enhanced starch accumulation, offering a mechanistic basis for the observed agronomic benefits.

It should be noted that because the two trials were conducted in different years and locations with distinct soil and climate conditions, direct statistical comparisons between treatments from different years (e.g., 2T4 vs. 3T4) are not appropriate. Nevertheless, the independent consistency of the optimal POLY4 proportion (18.5%) across both trials strengthens the robustness of the findings. Concretely, in the 2022 trial, treatment 2T4 (200 kg K_2O ha^{-1} with 18.5% from POLY4) produced the highest tuber yield and starch content among all treatments. In the 2023 trial, the same reduced K rate and POLY4 proportion (3T4) also outperformed the full-rate farmer's practice (3T2) and the no-K control.

These results challenge the common assumption that higher K rates invariably lead to higher yields. A recent meta-analysis of 2165 observations from 192 papers in China reported that K application increases potato yield by 19.2% and starch content by 6.7% compared to no-K control and also concluded that the optimal K application rate for different regions of China varies greatly, from 76 to 226 kg ha^{-1} [25]. Our optimal rate (200 kg ha^{-1}) falls within the range identified for northern China and further demonstrates that adding POLY4 can enhance the effectiveness of K fertilization. Notably, the fact that the same POLY4 proportion (18.5%) was effective under two different reduced K levels (200 kg ha^{-1} in both years) suggests that the ratio of POLY4 to MOP, rather than the absolute POLY4 amount, is a critical factor.

The increased starch content under optimal K-POLY4 formulations was associated with elevated activities of AGPase, GBSS, SSS, and SBE in tubers, particularly at the mid-growth stage (90 DAP). These four enzymes catalyze key steps in starch biosynthesis: AGPase produces the ADP-glucose precursor [26], GBSS synthesizes amylose [27], SSS

elongates amylopectin chains [28], and SBE introduces branch points [29]. Similar regulation by K has been reported in other crops. In sweet potato, K application enhanced the activities and gene transcription of SuSy, AGPase, and SSS, thereby promoting starch accumulation and storage root yield [16]. In lotus, potassium fertilizer induced the transcription factor NnNF-YB1, which directly bound to the promoter of *NnGBSSII* and activated its expression, leading to increased starch content in rhizomes [17]. Our correlation analysis (Section 3.5) revealed significant positive correlations between the activities of AGPase, GBSS, SSS, and SBE at 90 DAP and final tuber starch content (r = 0.72–0.84, $p < 0.01$). Furthermore, transcript levels of their corresponding genes also correlated with enzyme activities (r = 0.68–0.79, $p < 0.05$). These correlative patterns are consistent with a model in which the optimized K-POLY4 blend enhances starch accumulation by modulating the expression and activity of the core starch biosynthetic machinery [12,21]. We acknowledge that the absolute differences in enzyme activities among treatments were relatively modest in some cases (e.g., AGPase activity ranged from approximately 5.2 to 7.5 U g^{-1} FW across treatments in 2022). However, several considerations support the biological relevance of these differences. First, even small changes in the activity of rate-limiting enzymes such as AGPase can have amplified effects on downstream metabolic flux due to the highly regulated and multi-step nature of starch biosynthesis, where a single catalytic step can exert disproportionate control over the overall pathway. Second, the observed enzyme activity differences were consistently and significantly correlated with proportional increases in tuber starch content (r = 0.90, $p < 0.01$; Figure 8), suggesting that these modest enzymatic changes translate into meaningful physiological outcomes at the whole-organ level. Third, the temporal persistence of these differences across the entire tuber bulking period (70–110 DAP), rather than being restricted to isolated time points, further supports their biological significance. Nevertheless, we agree that the biological relevance of these relatively small differences should be confirmed in future studies using independent approaches such as metabolic flux analysis or enzyme overexpression/silencing experiments. The marked inter-annual variation in *SSSIII* expression, in contrast to the relative stability of *AGPase*, *GBSSI*, and *SBEI*, warrants further discussion. As the major soluble starch synthase isoform in potato tubers, *SSSIII* may be under distinct regulatory control that makes it particularly responsive to environmental cues [30]. This is consistent with reports that nitrogen application rates can shift the peak expression time of starch synthase genes, and that the activities of starch synthases, including SSS, are influenced by environmental and agronomic factors [30,31]. The contrasting weather conditions between 2022 and 2023 (e.g., temperature and precipitation) likely contributed to this pronounced difference in *SSSIII* transcript abundance. Nevertheless, despite this quantitative difference, the relative ranking of treatments (e.g., 2T4/3T4 showing the highest expression) remained consistent across both years, reinforcing the robustness of our conclusions regarding the optimal K-POLY4 blend. However, we acknowledge that correlation does not prove causation; direct evidence would require promoter–reporter or gene knockout studies.

Why did the POLY4-containing blends, especially the 18.5% POLY4 formulation, outperform MOP alone under reduced K rates? Several non-exclusive mechanisms may be proposed. but it is important to emphasize that our experimental design does not allow us to distinguish among these possibilities. First, POLY4 supplies not only K but also Ca, Mg, and S. A recent meta-analysis of 921 global trials across 47 crops found that polyhalite-based fertilizer programs outperformed conventional NPK controls (3.2% yield increase) and NP controls (6.6% yield increase), with particularly strong effects in low-K soils [32]. For potato specifically, the same meta-analysis reported a yield increase of 9.5% with polyhalite addition [32]. This multi-nutrient synergy may create a more balanced nutritional environment that favors gene expression and enzyme activity. However, because

we did not include control treatments that supplied Ca, Mg, and S independently, the specific contribution of these additional nutrients cannot be determined. Second, POLY4 has low chloride content (2.5–2.8% Cl), whereas MOP (KCl) contains approximately 47% Cl. The potato crop is widely assumed to be sensitive to Cl^-, based on reports that Cl^--containing fertilizers reduce tuber yield, dry matter and starch concentration [33]. However, a recent study by Hütsch et al. (2018) challenged this dogma, reporting that tuber yield, dry matter percentage, starch concentration and starch yield were not significantly affected by potassium source (K_2SO_4 vs. KCl), and concluded that both tested potato cultivars are chloride-resistant rather than chloride-sensitive [34]. Thus, the benefit of POLY4 over MOP is unlikely due solely to chloride avoidance; instead, the combination of low chloride plus supplemental Ca, Mg, and S may be more important. Regarding chloride sensitivity, our results do not resolve the ongoing debate about whether potato is chloride-sensitive. Hütsch et al. [34] reported that tuber yield and starch concentration were not significantly affected by K source (K_2SO_4 vs. KCl) in two cultivars, challenging the chloride-sensitivity dogma. Our study was not designed to test chloride effects specifically, as we did not include treatments with equal K rates from different chloride sources while holding other nutrients constant. Thus, we cannot attribute the benefits of POLY4 to its low chloride content versus its supplemental Ca, Mg, and S. Third, POLY4 has a slower release profile compared to MOP, which may provide a more sustained K supply during the critical tuber bulking period, as suggested in the polyhalite review [23]. The hypothesized slow-release benefit of POLY4 is empirically supported by our soil K dynamics data (Table 5). In both years, treatments with higher POLY4 proportions (2T3: 37%; 2T5: 31.5%) exhibited steeper declines in soil available K between 70 and 110 DAP compared to low-POLY4 treatments at the same total K rate. For example, in 2022, 2T3 (37% POLY4) dropped from 347.28 ± 23.55 at 70 DAP to 177.28 ± 32.65 mg kg^{-1} at 110 DAP (a 49% decline), whereas 2T2 (13% POLY4) declined from 362.27 ± 24.54 to 247.40 ± 22.62 mg kg^{-1} (only a 32% decline). A similar trend was observed among reduced-rate treatments in 2023: 3T4 (18.5% POLY4) showed a more gradual decline (241.35 ± 35.37 → 160.00 ± 16.83; 34% decline) compared to 3T3 (17% POLY4) which declined from 286.86 ± 23.55 to 177.27 ± 32.65 mg kg^{-1} (38% decline), though the difference was not statistically significant.

Importantly, the optimized treatment (2T4/3T4, 18.5% POLY4) maintained soil available K above 160 mg kg^{-1} at 110 DAP, coinciding with sustained high activities of AGPase, GBSS, SSS, and SBE at 90–110 DAP (Figure 5). This temporal alignment—adequate K supply during the tuber bulking phase—likely contributed to the sustained expression of starch biosynthetic genes and subsequent starch accumulation. Although the differences in soil K decline rates between POLY4 proportions were not always statistically significant, the consistent directional trends across two independent trials support the notion that POLY4's slower dissolution profile provides a more prolonged K supply, which may be particularly beneficial for late-season starch biosynthesis.

Our multi-nutrient data (Figure 4) provide partial support for the hypothesis that POLY4's benefits extend beyond K alone. In both years, POLY4-containing treatments generally increased tuber Mg and S concentrations compared to MOP-only or no-K controls. For instance, in 2023, 3T4 (18.5% POLY4) showed the highest tuber Mg (788.8 ± 218.3 mg kg^{-1}) and S (1286.3 ± 280.1 mg kg^{-1}), coinciding with its superior starch content and enzyme activities (Figures 3 and 5). Magnesium is a known cofactor for ATP-dependent enzymes, including AGPase, while sulfur is essential for protein synthesis and redox regulation, both of which may indirectly influence starch metabolism. However, because our experiment was not designed to isolate the effects of individual nutrients, we cannot determine whether the elevated Mg and S in POLY4 treatments directly contributed to the observed starch enhancement, or whether these are simply associated responses to improved overall

nutrition. Future studies with single-nutrient omission controls are needed to disentangle the specific contributions of Ca, Mg, and S to tuber starch biosynthesis.

Previous studies have established that K fertilization can enhance starch synthesis in various crops, including potato, by increasing AGPase, GBSS, SSS, and SBE activities [11]. However, most of those studies focused on full K rates or single K sources (MOP or sulfate of potash) [25]. Our study extends this knowledge in two important directions. First, we show that a reduced K rate can be equally or more effective than a full rate when the K source is optimized. This aligns with the meta-analysis finding that split application of 150–200 kg ha^{-1} K_2SO_4 is beneficial for starch and vitamin C content [25]. Second, we provide the first evidence, to our knowledge, that the blending ratio of POLY4 to MOP differentially affects the expression of starch biosynthetic genes in potato tubers. The finding that the optimal POLY4 proportion depends on the total K rate has not been reported before.

Several limitations of this study should be acknowledged. First, our conclusions are based on field experiments with a single potato cultivar ('Dafeng No. 10') at two locations in Inner Mongolia. Whether the optimal K-POLY4 formulation (200 kg K_2O ha^{-1} with 18.5% POLY4) applies to other cultivars, soil types, or climatic regions remains to be tested. Second, while we observed strong correlations between gene expression, enzyme activities, and starch content, we did not establish direct causality. Future studies using gene silencing or overexpression of candidate genes (*AGPase*, *GBSSI*, etc.) under controlled K-POLY4 regimes, analogous to the lotus study that demonstrated NnNF-YB1 directly bound to the GBSSII promoter, would be needed to confirm regulatory roles. Third, the mechanisms underlying the superior performance of POLY4—whether due to multi-nutrient synergy, low chloride plus supplemental nutrients, or slow release—remain speculative, and our experimental design cannot distinguish among these possibilities. Controlled experiments with single-nutrient omission (e.g., K only, Ca only, Mg only, S only) and with fertilizers matched for release rates would help disentangle these effects. Fourth, the two field trials were conducted in different years and at different locations (Wuchuan County in 2022 and Wulanhua County in 2023) with distinct soil properties and climatic conditions. Therefore, direct statistical comparisons between the 2022 and 2023 treatments (e.g., 2T4 vs. 3T4) are not valid. The observed similarities in the optimal POLY4 proportion (18.5%) across trials should be interpreted as consistent trends rather than statistically replicated results. Fifth, the 2023 experiment contained a confounding factor because the conventional practice treatment (3T2) received higher N and P rates than the reduced-K treatments (3T3, 3T4), making it impossible to attribute yield and quality differences solely to K management. Sixth, we did not measure tissue concentrations of K, Ca, Mg, S, or Cl, which would have helped explain the physiological basis of treatment responses. Seventh, our claims regarding reduced fertilizer costs and environmental benefits are not supported by direct economic analysis or environmental measurements (e.g., nutrient leaching, greenhouse gas emissions, residual soil fertility). Future studies with multi-year, multi-location trials under standardized treatment structures are needed to confirm the generalizability of these findings. Despite these limitations, the convergence of optimal proportions under contrasting environments provides preliminary evidence for the robustness of this rate.

In conclusion, this study demonstrates that a tailored K fertilization strategy—reducing total K_2O from 300 to 200 kg ha^{-1} while supplying 18.5% of K from polyhalite (POLY4) and the remainder from muriate of potash (MOP)—can maintain or increase potato yield, enhance tuber starch content, and improve potassium use efficiency. The benefits are associated with up-regulation of key starch biosynthetic enzymes and their genes. For practical application in the Inner Mongolia potato production region, we recommend farmers adopt this optimized blend: apply 200 kg K_2O ha^{-1}, with approximately 37 kg K_2O

(18.5% of total) from POLY4 and 163 kg K_2O from MOP. Future research should validate this recommendation across broader geographical areas and potato cultivars and investigate the molecular mechanisms by which POLY4 influences starch gene expression.

5. Conclusions

In the 2022 trial (Wuchuan County), treatment 2T4 (200 kg K_2O ha^{-1} with 18.5% of K from POLY4) increased tuber yield by 35.6% and significantly enhanced starch content compared to the no-K control, while also improving K use efficiency. Similarly, in the 2023 trial (Wulanhua County), treatment 3T4 (200 kg K_2O ha^{-1} with 18.5% of K from POLY4) increased yield by 20.5% over the no-K control and outperformed the full-rate farmer's practice (3T2). In both independent trials, the optimal formulation up-regulated the activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, SBE) and the transcript levels of their corresponding genes (*AGPase*, *GBSSI*, *SSSIII*, *SBEI*) during the mid to late tuber bulking stage. Although direct statistical comparisons between the two trials are not justified due to different years and locations, the consistent performance of the 200 kg K_2O ha^{-1} with 18.5% POLY4 across both environments supports the robustness of this formulation. For practical application in the Inner Mongolia potato production region, these findings suggest that a potassium fertilization strategy consisting of 200 kg K_2O ha^{-1}, with approximately 18-19% of this potassium supplied as POLY4 and the remainder as MOP, may offer a sustainable intensification pathway. However, given the limitations discussed above, we recommend that farmers conduct small-scale trials on their own fields before adopting this formulation on a commercial scale.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16131405/s1, Figure S1: Activities of key starch-synthesizing enzymes (AGPase, GBSS, SSS, and SBE) in potato leaves at 70, 90, and 110 DAP in response to different K fertilizer treatments. Table S1: Available potassium content in soil across different groups in 2022. Table S2: Available potassium content in soil across different groups in 2023. Table S3: Relative expression levels (Ct values) of AGPase, GBSSI, SSSIII, and SBEI genes in 2022. Table S4: Relative expression levels of AGPase, GBSSI, SSSIII, and SBEI genes under 3T treatments (2023). Table S5: Element contents for the year 2022. Table S6: Element contents for the year 2023. Table S7: Dry weight of different plant organs under various treatments in 2022. Table S8. Effects of four treatments on the dry weight of different plant organs in 2023.

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Abbreviations

The following abbreviations are used in this manuscript:

AGPase	Adenosine diphosphate glucose pyrophosphorylase
GBSS	Granule-bound starch synthase
SSS	Soluble starch synthase
SBE	Starch branching enzyme
POLY4	Polyhalite ($K_2Ca_2Mg(SO_4)_4 \cdot 2H_2O$)
MOP	Muriate of potash (potassium chloride, KCl)
DAP	Days after planting
KUE	Potassium use efficiency
qPCR	Quantitative real-time polymerase chain reaction
ANOVA	Analysis of variance
HSD	Tukey's honest significant difference
SE	Standard error
OM	Organic matter

References

1. Li, S.; Wang, Y.P.; Zhao, Y.F.; Zhang, J.Y.; Zhang, J.Y.; Ma, H.R.; Yue, Y.; Du, C.Y.; Zhao, C.B.; Han, Y.Z. Characterization of the complete chloroplast genome of the *Solanum tuberosum* L. cv. Favorita (Solanaceae). *Mitochondrial DNA Part B-Resour.* **2021**, *6*, 909–911. [CrossRef]
2. Chaudhary, B.; Dalamu; Gupta, V.K.; Luthra, S.K.; Kumar, M. Morphological characterization of indigenous potato (*Solanum tuberosum*) genotypes. *Indian J. Agric. Sci.* **2024**, *94*, 528–533. [CrossRef]
3. Brazkiewicz, K.; Poberezny, J.; Wszelaczynska, E.; Bogucka, B. Potato starch quality in relation to the treatments and long-term storage of tubers. *Sci. Rep.* **2025**, *15*, 4144. [CrossRef] [PubMed]
4. Nazarian-Firouzabadi, F.; Visser, R.G.F. Potato starch synthases: Functions and relationships. *Biochem. Biophys. Rep.* **2017**, *10*, 7–16. [CrossRef]
5. Zhang, B.; Shi, F.; Zheng, X.; Pan, H.; Wen, Y.; Song, F. Effects of AMF Compound Inoculants on Growth, Ion Homeostasis, and Salt Tolerance-Related Gene Expression in *Oryza sativa* L. Under Salt Treatments. *Rice* **2023**, *16*, 18. [CrossRef] [PubMed]
6. Chen, G.; Wu, C.; Wang, F.; Lyu, H.; Lu, Y.; Yan, C.; Chen, J.; Deng, Y.; Ge, T. Microbial community changes in different underground compartments of potato affected yield and quality. *3 Biotech.* **2022**, *12*, 106. [CrossRef] [PubMed]
7. Dobo, M.; Ayres, N.; Walker, G.; Park, W.D. Polymorphism in the GBSS gene affects amylose content in US and European rice germplasm. *J. Cereal Sci.* **2010**, *52*, 450–456. [CrossRef]
8. Li, P.P.; Ma, H.Z.; Xiao, N.; Zhang, Y.Q.; Xu, T.Y.; Xia, T. Overexpression of the ZmSUS1 gene alters the content and composition of endosperm starch in maize (*Zea mays* L.). *Planta* **2023**, *257*, 97. [CrossRef] [PubMed]
9. Fan, W.J.; Wang, Y.Q.; Zhang, L.; Fang, Y.J.; Yan, M.X.; Yuan, L.; Yang, J.; Wang, H.X. Sweet potato ADP-glucose pyrophosphorylase small subunit affects vegetative growth, starch content and storage root yield. *Plant Physiol. Biochem.* **2023**, *200*, 107796. [CrossRef] [PubMed]
10. Zhao, H.W.; Zhao, J.Y. Effects of Potassium Application Rate on the Activities of Starch Synthases and Yield in Spring Maize. *J. Maize Sci.* **2010**, *18*, 140–143+146. [CrossRef]
11. Zhang, L.; Xie, C.H.; Li, W.; Yang, G.T. Effects of nitrogen and potassium on grain starch content and starch synthase activities in hybrid rice B-you 827. *Chin. J. Rice Sci.* **2008**, *22*, 551–554.
12. Ahmad, D.; Ying, Y.; Bao, J. Understanding starch biosynthesis in potatoes for metabolic engineering to improve starch quality: A detailed review. *Carbohydr. Polym.* **2024**, *346*, 122592. [CrossRef]
13. Wang, J.M.; Shi, Y.; Liang, X.; Zhao, Y.; Huang, Y. Effects of Potassium Fertilizer on the Related Enzymes Activity of Starch Synthesis in Tubers of High Starch Potato Varieties. *Crops* **2016**, *32*, 118–123.
14. Tian, N.; Yang, Y.; Zhe, Y.; Wang, J.; Wu, N.; Liu, J. Effects of potassium application on starch content and enzyme activities associated with starch synthesis in potato tuber in arid areas of southern Ningxia. *J. Gansu Agric. Univ.* **2023**, *58*, 101–109.
15. Brownlie, W.; Alexander, P.; Maslin, M.; Cañedo-Argüelles, M.; Sutton, M.A.; Spears, B.M. Global food security threatened by potassium neglect. *Nat. Food* **2024**, *5*, 111–115. [CrossRef] [PubMed]

16. Gao, Y.; Tang, Z.; Xia, H.; Sheng, M.; Liu, M.; Pan, S.; Li, Z.; Liu, J. Potassium Fertilization Stimulates Sucrose-to-Starch Conversion and Root Formation in Sweet Potato (*Ipomoea batatas* (L.) Lam.). *Int. J. Mol. Sci.* **2021**, *22*, 4826. [CrossRef] [PubMed]
17. Zhao, S.; Zhang, Y.; Tan, M.; Zhang, C.; Jiao, J.; Wu, P.; Feng, K.; Li, L. NnNF-YB1 induced by the potassium fertilizer enhances starch synthesis in rhizomes of Nelumbo nucifera. *Ind. Crop. Prod.* **2023**, *203*, 117197. [CrossRef]
18. Ying, Y.; Deng, B.; Zhang, L.; Hu, Y.; Liu, L.; Bao, J.; Xu, F. Multi-omics analyses reveal mechanism for high resistant starch formation in an indica rice SSIIIa mutant. *Carbohydr. Polym.* **2025**, *347*, 122708. [CrossRef] [PubMed]
19. Sharma, V.; Jahan, K.; Kumar, P.; Puri, A.; Sharma, V.K.; Mishra, A.; Bharatam, P.V.; Sharma, D.; Rishi, V.; Roy, J. Mechanistic insights into granule-bound starch synthase I (GBSSI.L539P) allele in high amylose starch biosynthesis in wheat (*Triticum aestivum* L.). *Funct. Integr. Genom.* **2022**, *23*, 20. [CrossRef]
20. Wakuta, S.; Shibata, Y.; Yoshizaki, Y.; Saburi, W.; Hamada, S.; Ito, H.; Hwang, S.-K.; Okita, T.W.; Matsui, H. Modulation of allosteric regulation by E38K and G101N mutations in the potato tuber ADP-glucose pyrophosphorylase. *Biosci. Biotechnol. Biochem.* **2013**, *77*, 1854–1859. [CrossRef] [PubMed]
21. Qu, J.; Xu, S.; Zhang, Z.; Chen, G.; Zhong, Y.; Liu, L.; Zhang, R.; Xue, J.; Guo, D. Evolutionary, structural and expression analysis of core genes involved in starch synthesis. *Sci. Rep.* **2018**, *8*, 12736. [CrossRef] [PubMed]
22. Yermiyahu, U.; Zipori, I.; Faingold, I.; Yusopov, L.; Faust, N.; Bar-Tal, A. Polyhalite as a multi nutrient fertilizer—Potassium, magnesium, calcium and sulfate. *Isr. J. Plant Sci.* **2017**, *64*, 145–157. [CrossRef]
23. Das, S.; Bera, S.; Kalindi, D.; Ghosh, R. Polyhalite nutrients driving balanced crop nutrition and sustainable agricultural productivity. *Discov. Soil* **2026**, *3*, 21. [CrossRef]
24. Mello, S.d.C.; Pierce, F.J.; Tonhati, R.; Almeida, G.S.; Neto, D.D.; Pavuluri, K.J.H. Potato Response to Polyhalite as a Potassium Source Fertilizer in Brazil: Yield and Quality. *HortScience* **2018**, *53*, 373–379. [CrossRef]
25. Zhang, F.; Wang, X.; He, H.; Wang, H.; Zhang, B.; Liu, S.; Chen, R.; Zhang, Y.; Wang, Y.; Ren, H.; et al. Quantitative effects of potassium application on potato tuber yield, quality, and potassium uptake in China: A meta-analysis. *Field Crops Res.* **2025**, *333*, 110061. [CrossRef]
26. Cheng, Z.; Xi, B.; Gao, Y.; He, X.; Gao, J.; Tang, H.; Liu, Y.; Zhao, H.; Feng, Z.; Yu, G. Expression and interaction of AGPase subunits reveal functional enzyme complexes in barley. *Front. Plant Sci.* **2025**, *16*, 1671162. [PubMed]
27. Denyer, K.; Waite, D.; Motawia, S.; Møller, B.L.; Smith, A.M. Granule-bound starch synthase I in isolated starch granules elongates malto-oligosaccharides processively. *Biochem. J.* **1999**, *340*, 183–191. [CrossRef] [PubMed]
28. Szydlowski, N.; Ragel, P.; Hennen-Bierwagen, T.A.; Planchot, V.; Myers, A.M.; Mérida, A.; d'Hulst, C.; Wattebled, F. Integrated functions among multiple starch synthases determine both amylopectin chain length and branch linkage location in Arabidopsis leaf starch. *J. Exp. Bot.* **2011**, *62*, 4547–4559. [CrossRef] [PubMed]
29. Wychowski, A.; Bompard, C.; Grimaud, F.; Potocki-Véronèse, G.; D'Hulst, C.; Wattebled, F.; Roussel, X. Biochemical characterization of Arabidopsis thaliana starch branching enzyme 2.2 reveals an enzymatic positive cooperativity. *Biochimie* **2017**, *140*, 146–158. [CrossRef] [PubMed]
30. Geigenberger, P. Regulation of Starch Biosynthesis in Response to a Fluctuating Environment. *Plant Physiol.* **2011**, *155*, 1566–1577. [CrossRef]
31. Smith, A.M.; Denyer, K.; Martin, C. The Synthesis of The Starch Granule. *Annu. Rev. Plant Biol.* **1997**, *48*, 67–87. [CrossRef]
32. Mann, K.K.; Fields, R.; Welham, S.; Storer, K.E.; Roques, S.E.; Berry, P.; Wade, B.R.; Kindred, D.; Pienaar, C. Meta-analysis of polyhalite's yield performance across diverse soil, crop, and environmental conditions. *Nutr. Cycl. Agroecosyst.* **2025**, *117*, 6. [CrossRef]
33. Wilmer, L.; Pawelzik, E.; Naumann, M. Comparison of the Effects of Potassium Sulphate and Potassium Chloride Fertilisation on Quality Parameters, Including Volatile Compounds, of Potato Tubers After Harvest and Storage. *Front. Plant Sci.* **2022**, *13*, 920212. [CrossRef] [PubMed]
34. Hütsch, B.W.; Keipp, K.; Glaser, A.K.; Schubert, S. Potato plants (*Solanum tuberosum* L.) are chloride-sensitive: Is this dogma valid? *J. Sci. Food Agric.* **2018**, *98*, 3161–3168. [CrossRef] [PubMed]

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