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RESEARCH ARTICLE

Can fertilization of potato (*Solanum tuberosum* L.) with potassium sulfate cause oxidative stress in the plants?

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Abstract

Background: Potatoes are regarded very prone to chloride toxicity, and the application of sulfate-based potassium fertilizers is therefore recommended. However, in several studies, no significant differences between KCl and K_2SO_4 on tuber yield and starch concentration were found. In addition, plant shoots occasionally showed more pronounced stress symptoms after sulfate compared to chloride treatment.

Aims: This study aimed to identify reasons for enhanced stress occurrence of potato plants after K_2SO_4 instead of KCl fertilization.

Methods: We conducted a pot experiment with the potato cultivar Marabel, which showed to be chloride-resistant in our previous study, and applied 1 g K or 2 g K either as K_2SO_4 or KCl. Plant shoots were analyzed for nutrients, sugars, and antioxidative capacity shortly before start of flowering (intermediate harvest), and tubers were harvested at physiological maturity and their starch concentration and yield were determined.

Results: With sulfate-based K fertilization, the start of flowering and ripening as well as the achievement of physiological maturity occurred earlier than with KCl supply. However, the accelerated development of K_2SO_4-treated plants did not affect shoot fresh and dry mass shortly before flowering and at maturity, showing similar values as in the KCl treatment. With 2 g K as K_2SO_4, the potato plants produced much larger and less than half the number of tubers compared to 2 g K as KCl, resulting in a significantly reduced tuber fresh mass. Tuber starch concentration as well as starch yield per plant were not significantly affected by the kind of K fertilization. In plant shoots shortly before flowering, calcium and magnesium concentrations were significantly decreased, combined with a significant increase in antioxidative capacity after application of 2 g K as K_2SO_4 compared to KCl. Sugar concentrations and contents in potato shoots at flowering were mostly unaffected by the kind of K fertilizer.

Conclusions: Stronger toxicity symptoms and growth inhibition after sulfate compared to chloride application, combined with a significant increase in antioxidative capacity, provide evidence for sulfate-induced oxidative stress. A higher production of organic anions such as succinate may be involved in the stress reaction. This needs to be investigated in more detail in further studies.

KEYWORDS
antioxidative capacity, assimilate availability, calcium signaling, chloride sensitivity, starch concentration, tuberization

1 | INTRODUCTION

Chloride is one of the most common anions in nature. Due to inputs into agroecosystems via irrigation and/or application of chloride-based fertilizers, chloride deficiency of crops is rather unlikely under field conditions (White & Broadley, 2001), whereas chloride toxicity is much more common (Xu et al., 2000). Toxicity occurs in the range of 5–10 mg Cl^- g^{-1} dry matter of leaf tissue for sensitive crops and greater than 40 mg Cl^- g^{-1} dry matter for tolerant ones (Bergmann, 1992; Kopsell & Kopsell, 2015). According to general knowledge, potatoes are supposed to be prone to chloride toxicity, and the application of sulfate-based instead of chloride-based fertilizers (e.g., K_2SO_4 instead of KCl) to potato plants is therefore recommended (e.g., Berger et al., 1961; Panique et al., 1997; Timm & Merkle, 1963). Many of these studies on potato plants rely on data produced decades ago, indicating a need for further investigations (Koch et al., 2020).

However, in studies comparing the effects of sulfate and chloride on potato shoot growth, contrary results were found. Bilski et al. (1988a, 1988b) and Hütsch et al. (2018) tested the effect of salt stress in hydroponics on early growth of different potato cultivars and found a significantly stronger decrease in shoot dry matter after Na_2SO_4 than after NaCl application. However, $CaCl_2$ treatment did not significantly affect shoot growth and no symptoms of chloride toxicity (Heckman, 2007; Kopsell & Kopsell, 2015) were observed, although the chloride concentration reached 65–74 mg Cl^- mg^{-1} dry matter, similar to the NaCl treatment (Hütsch et al., 2018). In contrast, growth reductions were closely related to sodium concentrations; thus, plants suffered sodium toxicity and not chloride toxicity.

Apart from chloride effects on shoot growth, the impact of chloride on tuber yield and tuber quality is of particular importance for farmers and the potato-processing industry. The general parameters to characterize tuber quality are specific gravity, dry matter content, starch concentration, and starch yield. In a pot experiment with two potato cultivars (Désirée and Marabel) grown in a soil substrate under near-natural conditions (day-time natural air and light exposure, night-time in a vegetation hall), no significant effect of the potassium source (K_2SO_4 or KCl) on tuber yield, dry matter content of the tubers, starch concentration, and starch yield occurred at physiological maturity (Hütsch et al., 2018). Moreover, in several field trials, no significant differences between KCl and K_2SO_4 on tuber yields were found (Kumar et al., 2007; McDole, 1978; McDole et al., 1978; Panique et al., 1997; Silva et al., 2018; Westermann et al., 1994), and specific gravity was not adversely affected when KCl was used (Davenport & Bentley, 2001; Silva et al., 2018; Westermann et al., 1994). In a recent field study, using the cultivars Marabel and Laura, no significant effects of K_2SO_4 application compared to KCl were observed on tuber yield, starch concentration, and starch yield (Wilmer et al., 2022). Thus, it is possible to conclude that potatoes can be fertilized with KCl instead of K_2SO_4 without the risk of depression in tuber yield or tuber quality. The statement that potatoes are chloride-sensitive and that chloride has negative effects on yield performance therefore needs reconsideration (Hütsch et al., 2018). In addition, an important positive aspect of chloride application to potato plants was addressed by Koch et al. (2021). The authors concluded that chloride fertilization is a promising tool to improve drought resistance of potatoes, which are very sensitive to water shortage.

In our previous salinity experiment, potato plants treated with 50 mM Na_2SO_4 showed more pronounced stress and toxicity symptoms than plants treated with 100 mM NaCl (Hütsch et al., 2018). The plants were smaller and showed strong necroses at the older leaves. Thus, it is reasonable to assume that the tested potato cultivars are not only characterized by a high tissue tolerance toward chloride but that the sulfate anion has even a detrimental impact on the plants. In studies by Zhao et al. (2020) with *Arabidopsis thaliana*, sulfate salts caused stronger growth inhibition than chloride salts as well as more severe toxicity symptoms in form of necroses on the leaves. The authors proposed the sulfate-induced accumulation of organic anions such as succinate and fumarate, intermediates of the citrate cycle, as reason for the observed reduction in shoot and root dry mass. In particular, the accumulation of succinate can stimulate a reverse electron transport leading to the production of reactive oxygen species (ROS) (Dröse, 2013; Muller et al., 2008) and enhance oxidative stress.

Against the background of these studies, it is worthwhile to investigate if nutrition with K_2SO_4 with its presumed concomitant accumulation of organic anions may induce oxidative stress in potato plants. We conducted a pot experiment with the potato cultivar Marabel and applied 1 g K or 2 g K either as K_2SO_4 or KCl. In order to provide an optimal K supply and to expose the plants to larger amounts of sulfate and chloride, a higher K dosage was chosen than in our previous experiment (Hütsch et al., 2018). Plant shoots were analyzed for nutrients, sugars, and antioxidative capacity shortly before start of flowering (intermediate harvest), and tubers were harvested at physiological maturity and their starch concentration and yield were determined. Our investigation focused on the following hypotheses: potato nutrition with K_2SO_4 instead of KCl (1) increases the antioxidative capacity in the shoot, (2) causes reductions in tuber yield, and (3) leads to a reduction in starch concentrations of the tubers.

2 | MATERIALS AND METHODS

2.1 | Plant cultivation and harvests

The potato (*Solanum tuberosum* L.) cultivar Marabel was chosen, which was registered as a potato cultivar in 1993, and is described as an early table potato with a yellowish color of the tuber flesh (Federal Plant

Variety Office [FPVO], 2022). Marabel was also used in our previous study and was shown to be chloride-resistant (Hütsch et al., 2018). In May 2021, tubers were pre-germinated in a greenhouse for 14 days. On May 17, the germinated tubers were transferred into 14-L Ahr pots, containing a mixture of 7 kg sand and 7 kg of a Luvisol subsoil (loamy sand: 21.2% clay, 34.5% silt, 44.3% sand; pH($CaCl_2$) 5.9, CAL-P: 8.9 mg P kg^{-1}, CAL-K: 58.3 mg K kg^{-1}, DTPA-Fe: 27.1 mg Fe kg^{-1}). Each pot contained one potato plant, which was supplied with the following fertilizers: 2.8 g N as NH_4NO_3, 2.1 g P as $CaHPO_4$, 1.4 g Mg and 1.8 g S as $MgSO_4$, 6.4 mg B as H_3BO_3, 1.6 mg Mo as NH_4-Molybdat, 280 mg Mn as $MnSO_4$, 140 mg Zn as $ZnSO_4$, and 70 mg Cu as $CuSO_4$. Macronutrients were applied in solid form to the air-dry sand/soil mixture, micronutrients in a solution. Different potassium treatments were established with four replicates each. Potassium fertilizer was applied either as KCl or K_2SO_4 at two rates: 1.0 g and 2.0 g K per pot. The whole experimental setup was duplicated in order to enable two harvests: an intermediate harvest during vegetative growth, shortly before start of flowering, and a harvest at maturity of the tubers, resulting in 32 pots in total.

Water content was adjusted to 60% of maximum water-holding capacity during the entire vegetation period by water applications at least twice daily. The pots were set up in a completely randomized design and their position was changed once a week. The pots were placed in the vegetation hall of the experimental station of the Institute for Plant Nutrition in Giessen and moved outside during the day; thus, plants received natural light intensities. In order to prevent infections by *Phytophthora infestans* and *Alternaria solani*, the plants were successively treated either with the fungicide *Terminus* or with Curzate *M WG*. The fungicides were applied in the recommended dosages (provided by the manufacturers) three times each in weekly intervals between 8th June and 16th July.

On June 17, 31 days after planting (DAP) of the tubers and shortly before the start of flowering, the intermediate harvest took place. The aboveground plant material (named "shoot" throughout the paper) of 16 plants was harvested and the shoot fresh mass determined. Subsequently, the material was frozen in liquid N_2 and stored at −80°C until freeze-drying and milling to a fine powder for further analyses. The shoot dry mass was determined after freeze-drying and subsequent oven-drying at 30°C for 3 h to remove residual humidity and facilitate milling of the samples. Although at the intermediate harvest tubers had already been induced, the tuber size was very small, and their contribution to nutrient uptake and metabolization could be neglected.

Fertilization with K_2SO_4 accelerated the maturation of the potato plants. However, at final harvest, which was performed on August 17 (92 DAP), all plants had reached physiological maturity, characterized by the complete dieback of the plant shoots. The shoot fresh and dry mass (105°C drying) were recorded. The tuber yield and the number of tubers per potato plant were determined as well as the individual tuber weight. Prior to freezing at −80°C, the fresh tubers were cut into small pieces. In order to determine tuber dry mass, an aliquot of fresh material was dried at 65°C for 21 h, followed by 105°C for 3 h (Wilmer et al., 2022). Another aliquot was freeze-dried and milled to a fine powder for analysis of tuber starch concentration and calculation of starch yield per plant.

2.2 | Plant analyses

2.2.1 | Chloride and sulfate determination in shoots shortly before start of flowering (31 DAP)

For chloride extraction, 200 mg of dried and finely ground plant material were weighed into 50 mL volumetric flasks, 30 mL bi-distilled water of 60°C were added, and the mixture was kept at 60°C in a shaking water bath for 30 min. After cooling down to room temperature, the flasks were filled up to 50 mL with cold bi-distilled water and shaken thoroughly. The solution was filtered through a folded filter paper (MN 615.25), and the chloride concentration was analyzed potentiometrically in the filtrate using a chloride titrator (Aminco 4-4433 Vintage Chloride Titrator—American Instrument Co.; Steffens & Schubert, 2011). Briefly, two electrically isolated silver electrodes are immersed into the solution to be titrated. A constant, continuous current flows through the pair of electrodes, causing an electrochemical oxidation of the generator anode (silver wire) to silver ions (Ag^+). These ions are released into the solution and combine with Cl^- to silver chloride (AgCl). As long as the rate of produced silver ions is constant, the amount of precipitated chloride is proportional to time. As soon as the chloride has quantitatively reacted with the silver ions, the presence of free silver ions causes a steep increase in voltage. With a timer, located in the chloride titrator, the time between start of titration and start of voltage increase is measured. This time interval and the comparison with standard solutions are used to calculate the chloride concentration in the sample solution.

For sulfate extraction, 200 mg of dried and finely ground (pulverized) shoot material were weighed into 50-mL volumetric flasks, 30 mL bi-distilled water of 100°C were added, and the mixture was kept at 100°C in a shaking water bath for 3 h. After cooling down to room temperature, the flasks were filled up to 50 mL with cold bi-distilled water and shaken thoroughly. The solution was filtered through a folded filter paper (MN 615.25), and the sulfate concentration in the filtrate was analyzed using ion chromatography (Compact IC 761, Metrohm GmbH & Co. KG).

2.2.2 | K, Mg, Ca, P, and N determination in shoots shortly before start of flowering (31 DAP)

For cation and phosphate determination, 500 mg of dried and ground plant material were dry-ashed at 550°C in a muffle furnace. The ash was mixed with 2 mL of bi-distilled water and 2.5 mL of 5 M HNO_3. The mixture was briefly boiled and poured through a filter paper (MN 640 m, 0.2 mm) into a 50-mL volumetric flask, which was filled up with bi-distilled water after cooling down to room temperature. In the filtrate, the concentrations of K^+, Mg^{2+}, and Ca^{2+} were measured using atomic absorption spectrometry (Varian, SpectrAA 220 FS).

P concentrations in the extracts were determined colorimetrically (Steffens & Schubert, 2011).

The Kjeldahl method was used for N analysis (Kjeldahl, 1883). Briefly, the dried and milled plant material was digested with concentrated sulfuric acid, followed by the distillation of the produced NH_3 and the determination of the N amount by titration. In order to include also the nitrate N, the sample was analyzed after reduction of oxidized N by salicylic acid and sodium thiosulfate. The concentration of total N was calculated and is given as mg N g^{-1} dry matter.

The concentrations of nutrients were calculated per g dry mass and are given in the results section. The contents per plant or plant organ were also calculated by multiplying the concentration with the respective dry mass. The content represents the net uptake of a nutrient.

2.2.3 | Sugar determination in shoots shortly before start of flowering (31 DAP)

Sucrose, glucose, and fructose were analyzed in 200 mg of dried and finely ground (pulverized) plant material. Samples were extracted, as described for chloride, with 30 mL bi-distilled water in a shaking water bath at 60°C for 30 min. The extracts were filled up to 50 mL with bi-distilled water, filtered, and stored at −20°C until enzymatic sugar determination was conducted with UV test kits (Roche-Biopharm AG). Each sample was extracted in duplicate prior to sugar analysis.

2.2.4 | Determination of antioxidative capacity of shoots shortly before start of flowering (31 DAP)

For measurement of the antioxidative capacity in potato leaves, the ORAC value (oxidative radical absorption capacity) was determined. The vitamin E derivative trolox was used as reference (unit of measured values: µmol trolox equivalent [TE] g^{-1} dry mass). With the employed method radicals were produced by 2,2′-azobis[2-methylpropionamidin]dihydro-chloride. The radicals oxidize the dye fluorescein and make it visible. Antioxidant compounds in the sample decelerate the oxidation of fluorescein by reaction with the radicals. The concentration of fluorescein was measured photometrically. For the analysis, 50 mg of freeze-dried, milled plant material was homogenized with 12.5 mL of 80% methanol solution in a 25 mL volumetric flask. The samples were extracted in an ultrasonic bath for 30 min. After addition of 12.5 mL of bi-distilled water, the samples were filtered (folded filters, MN 595.5). A calibration curve was prepared with 0, 25, 60, 95, and 130 µM solutions of (±)-6-hydroxy-2,5,7,8-tretramethylchromane-2-carboxylic acid (trolox) in methanol. The results obtained are presented as equivalent of µmol trolox (TE) in 1 g dry leaf mass.

2.2.5 | Starch determination in tubers at the final harvest (92 DAP)

The starch concentration was determined in freeze-dried, milled material of potato tubers from the final harvest. A 250 mg sample was taken and immediately mixed with 5 mL 8 M HCl and 20 mL dimethylsulfoxide in a 100-mL Erlenmeyer flask in order to avoid uncontrolled enzymatic starch hydrolysis. Subsequently, the sample was incubated in a shaking water bath at 60°C for 30 min. After fast cooling down in an ice water bath, the pH value was adjusted between 4 and 5. Then, the sample was transferred into a 100-mL volumetric flask, filled up to the mark, mixed thoroughly, and filtered (folding filters, MN 615.25). For determination of the starch concentration in the filtrate, a UV test kit was used (Roche-Biopharm AG). The concentration of starch in the tuber was calculated after subtraction of free glucose and of the blank value caused by the used chemicals. The starch yield was calculated by multiplying the concentration with the respective tuber dry mass.

2.3 | Statistical analysis

In order to identify significant differences between two independent variables, the two-sided *t*-test (Microsoft Office Excel 2019) was used. Differences between KCl (chloride) and K_2SO_4 (sulfate) treatment were evaluated for each application rate as well as dosage effects (1 g or 2 g K) for each kind of K fertilizer. In the tables and diagrams, means ± standard errors from biological replicates ($n = 4$ per treatment) are given. Statistical significance was referred to a p-value < 0.05 (ns = not significant $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$).

3 | RESULTS

3.1 | Plant shoot growth and development

Until flowering, the potato plants were characterized by homogenous shoot growth with no visible differences between chloride and sulfate nutrition (Figure 1). Shortly before start of flowering (intermediate harvest), the shoot fresh and dry mass were neither significantly affected by the kind of K fertilizer nor by the K dosage (Figure 2A,B).

With transition from the vegetative to the generative growth phase, treatment differences occurred. K sulfate-treated plants of both application rates started to flower earlier with a shorter duration compared to their K chloride counterparts. In addition, plants with 2 g K supply flowered earlier with a shorter duration than those with 1 g K supply. Thus, the transition into the generative phase occurred in the following order: 2 g K as K_2SO_4 → 1 g K as K_2SO_4 → 2 g K as KCl → 1 g K as KCl (Figure 3A–C).

The ripening process, characterized by naturally occurring drying and subsequent dying of the potato shoots, started earlier in K_2SO_4-treated plants of both application rates and proceeded faster compared to their KCl-treated counterparts (Figure 3D–F). In addition, plant shoots with 1 g K application dried faster and were earlier ready for harvest than those with 2 g K. Thus, plants with 2 g K as KCl were characterized by a particularly slow ripening process (Figure 3D–F).

At final harvest (August 17), 4 days after the photos of Figure 3F were taken, the shoots of almost all plants were completely dead, only

FIGURE 1 Potato plants of all treatments, randomized and placed on a carriage at the experimental station; the plants look homogenous without any visible treatment effects; the photo was taken 2 days before the intermediate harvest.

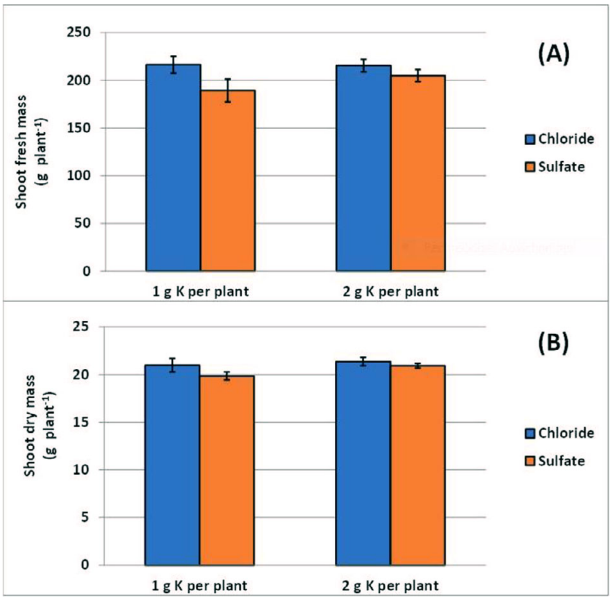

FIGURE 2 Shoot fresh mass (A) and shoot dry mass (B) of the potato cultivar Marabel shortly before start of flowering (intermediate harvest, 31 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); there were no significant differences (*t*-test).

with 2 g K of both kinds of fertilizer (chloride and sulfate) a few leaves still had a greenish color. There were no significant differences in shoot fresh and dry mass between KCl- and K_2SO_4-treated plants (results are not shown).

3.2 | Tuber yield

With application of 1 g K, at final harvest no visible differences in tuber production between chloride and sulfate nutrition occurred. However, with 2 g K as K_2SO_4, the potato plants produced much larger and less than half the number of tubers compared to 2 g K as KCl (Figure 4C), resulting in a significantly reduced tuber fresh mass (Figure 4A). With 1 g K, the tuber dry mass was smaller with K_2SO_4 than KCl treatment (Figure 4B). With 2 g K, the tubers were generally larger than with 1 g K, resulting in significantly enhanced tuber fresh and dry mass (Figure 4A,B).

3.3 | Analyses of potato shoots (intermediate harvest, 31 DAP)

3.3.1 | Nutrient concentrations and contents

The shoot concentrations of the main macronutrients and of chloride are combined in Table 1. There was no significant difference in K concentration between KCl and K_2SO_4 applications, yet the higher dosage caused significant increases for both kinds of K fertilizer. For potato shoots in this growth stage, Bergmann (1992) gave a

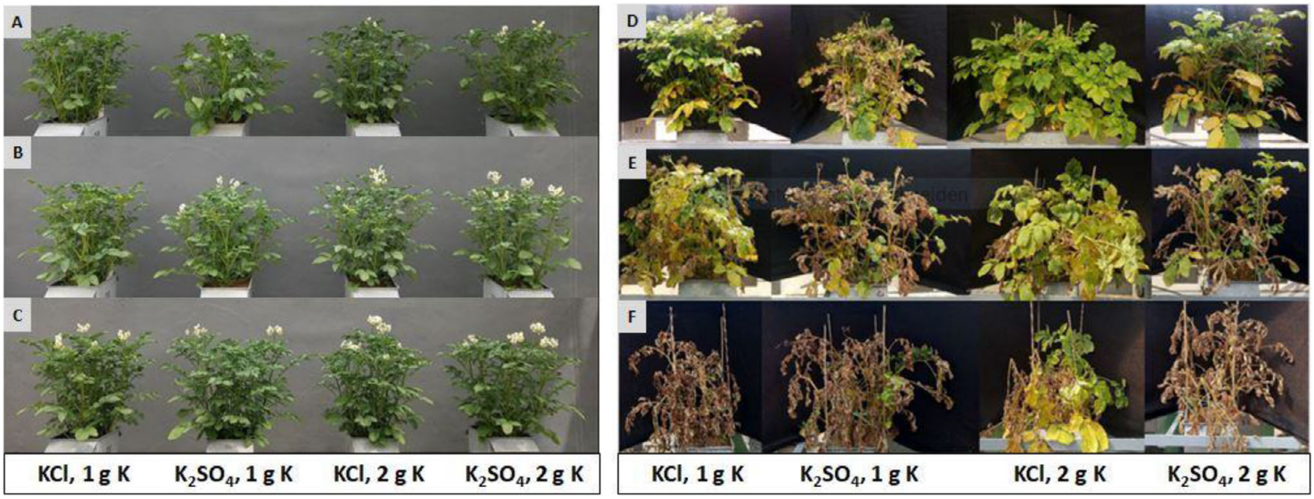

FIGURE 3 Effect of potassium nutrition, applied either as chloride (KCl) or sulfate (K_2SO_4) in two dosages (1 or 2 g K per plant) on the flowering process (left side) of the potato cultivar Marabel, visualized with photos taken on 28 June (A), 1 July (B), and 5 July 2021 (C), and on the ripening process (right side), visualized with photos taken on 5 August (D), 10 August (E), and 13 August 2021 (F).

TABLE 1 Concentrations (mg g^{-1} dry mass) of potassium (K), chloride (Cl), sulfate (SO_4^{2-}), calcium (Ca), magnesium (Mg), phosphorus (P), and nitrogen (N) in shoots of the potato cultivar Marabel shortly before start of flowering (intermediate harvest, 31 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); significant differences are indicated by asterisks.

	Potassium treatment					
	1 g K per plant		2 g K per plant		Effect of K dosage	
Nutrient	KCl	K_2SO_4	KCl	K_2SO_4	KCl	K_2SO_4
K	36.2 ± 1.2	37.4 ± 0.9	46.8 ± 1.4	49.7 ± 0.6	**	***
Cl	9.5 ± 0.5	0.9 ± 0.1***	18.9 ± 0.7	0.9 ± 0.1***	***	ns
SO_4^{2-}	3.4 ± 0.1	4.2 ± 0.1**	3.1 ± 0.2	5.4 ± 0.2**	ns	**
Ca	10.4 ± 0.3	9.4 ± 0.5	10.0 ± 0.3	7.8 ± 0.1***	ns	*
Mg	7.6 ± 0.2	8.4 ± 0.3	7.4 ± 0.3	6.4 ± 0.0*	ns	***
N	48.4 ± 0.7	50.0 ± 1.8	45.4 ± 0.2	48.9 ± 0.8**	**	ns
P	6.2 ± 0.4	5.9 ± 0.2	5.6 ± 0.1	5.7 ± 0.1	ns	ns

*, **, and *** with $p \leq 0.05$, 0.01, and 0.001, respectively; *t*-test; ns = not significant.

critical concentration of 50 mg K g^{-1} DM; thus, the values of the 2 g K dosage are sufficient and they lay slightly below in the 1 g K treatment. The chloride concentration was significantly higher with KCl than K_2SO_4 treatment and was almost exactly doubled with 2 g K compared to 1 g K. The threshold value for chloride toxicity is 5 mg Cl g^{-1} DM (Bergmann, 1992); thus, the Cl concentration of the KCl-treated plant shoots lay well above this value. However, as no symptoms of Cl toxicity were observed, the cultivar Marabel cannot be considered chloride-sensitive. The sulfate concentration was significantly increased with K_2SO_4 supply and its application rate. In the 2 g K treatment, K_2SO_4 caused significant decreases in Ca and Mg concentrations compared to KCl and also compared to 1 g K as K_2SO_4. Treatment with 2 g K as K_2SO_4 resulted in a significant higher N concentration than 2 g K as KCl, which itself showed a significant reduction in N concentration compared to 1 g K as KCl. Neither the kind of K fertilizer nor its dosage affected the P concentration in the potato shoots. According to Bergmann (1992), the plants from all treatments were sufficiently nourished with N, P, Ca, and Mg.

The nutrient contents per potato shoot, calculated by multiplying the concentration with the respective shoot dry mass, are given in Table 2. Effects of KCl versus K_2SO_4 as well as of K dosage on K, chloride, and sulfate contents were similar to the effects observed for their concentrations. It should be pointed out that the increase in K content with 2 g K application was only one-third of the K content of 1 g K, whereas the chloride content with 2 g K as KCl was almost exactly doubled compared to 1 g K as KCl. When KCl is compared to K_2SO_4, the chloride contents were 10 and 20 times increased with 1 g and 2 g KCl applications, respectively. The Ca content was significantly reduced with 2 g K as K_2SO_4 compared to KCl. Mg, N, and P contents were

TABLE 2 Contents (mg shoot^{-1}) of potassium (K), chloride (Cl), sulfate (SO_4^{2-}), calcium (Ca), magnesium (Mg), phosphorus (P), and nitrogen (N) in shoots of the potato cultivar Marabel shortly before start of flowering (intermediate harvest, 31 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); significant differences are indicated by asterisks.

	Potassium treatment					
	1 g K per plant		2 g K per plant		Effect of K dosage	
Nutrient	KCl	K_2SO_4	KCl	K_2SO_4	KCl	K_2SO_4
K	757 ± 20	744 ± 30	1001 ± 50	1040 ± 24	**	***
Cl	198 ± 10	19 ± 2***	405 ± 22	18 ± 2***	***	ns
SO_4^{2-}	70 ± 3	83 ± 2*	66 ± 4	113 ± 6**	ns	**
Ca	218 ± 5	188 ± 12	215 ± 12	163 ± 2**	ns	ns
Mg	159 ± 5	168 ± 9	158 ± 10	134 ± 2	ns	*
N	1015 ± 23	994 ± 52	971 ± 24	1023 ± 24	ns	ns
P	130 ± 4	117 ± 5	119 ± 5	119 ± 3	ns	ns

*, **, and *** with $p \leq 0.05$, 0.01, and 0.001, respectively; *t*-test; ns = not significant.

unaffected by the kind of K fertilizer, and an effect of K dosage was only observed for the Mg content of the K_2SO_4 treatment, resulting in a significantly decreased Mg content with 2 g K compared to 1 g K.

3.3.2 | Sugar concentrations and contents

Sucrose, glucose, and fructose concentrations, determined in potato shoots shortly before start of flowering, were mostly unaffected by the kind of K fertilizer apart from the fructose concentration, which was significantly increased after application of 2 g K as K_2SO_4 (Figure 5A). With the higher K dosage, the sugar concentrations decreased, although the effects were only significant for glucose (chloride and sulfate) and for fructose (chloride). The sucrose and glucose contents per plant shoot were unaffected by kind and dosage of K fertilizer (Figure 5B), only the fructose content increased significantly in the treatment 2 g K as K_2SO_4 compared to KCl, and it decreased between 1 and 2 g K as KCl (Figure 5B). The total content of all three sugars was unaffected by kind and dosage of K fertilizer (results are not shown).

3.3.3 | Antioxidative capacity

With 1 g K supply, no difference in the antioxidative capacity occurred between both kinds of K fertilizer (Figure 5C). However, with 2 g K per plant, the antioxidative capacity of the potato shoot was significantly higher after K_2SO_4 compared to KCl application. No significant effects of K dosage on the antioxidative capacity were observed (Figure 5C).

3.4 | Starch concentration and starch yield of tubers (final harvest, 92 DAP)

The starch concentration in the tuber dry mass as well as the starch yield per plant were not significantly affected by the kind of K fertilization; the K_2SO_4 treatment showed only a tendency to smaller values compared to KCl (Figure 6A,B). Effects of K dosage were only observed for the starch yield with significant higher values in the 2 g K than 1 g K treatment (Figure 6B). The increases in starch yield are exclusively based on the higher tuber dry mass obtained with 2 g K application (Figure 4B).

4 | DISCUSSIONS

4.1 | Potassium sulfate accelerates flowering and ripening of potato plants

With sulfate-based K fertilization, the start of flowering and ripening as well as the achievement of physiological maturity occurred earlier than with KCl supply (Figure 3). Thus, K_2SO_4 accelerated the physiological development, which is in agreement with previous studies (Beringer et al., 1990; Haeder, 1976; Hütsch et al., 2018). However, the advanced development of K_2SO_4-treated plants did not affect shoot fresh and dry mass shortly before flowering and at maturity, showing similar values as in the KCl treatment (Figure 2A,B). This is contradictory to other studies, where K application in chloride form compared to sulfate resulted in increased shoot growth and a higher water uptake, leading to the postulation of a subsequent increase in assimilate competition between tuber and shoot (Koch et al., 2020; Naumann et al., 2020; Naumann & Pawelzik, 2023). In addition, it was considered that chloride-induced higher water uptake of the shoot causes reduced K concentrations with negative impacts on phloem loading and assimilate transport to the tubers (Beringer et al., 1990). In our study, there was no significant difference in shoot concentrations and contents of sucrose, the main transport metabolite in the phloem, between the KCl- and K_2SO_4-treated plants (Figure 5A,B). Thus, at that time of tuber initiation, assimilate availability was similar, and obviously translocation to the root was not restricted with KCl application, as this would have resulted in an accumulation of sucrose in the shoot. These observations at flowering are strengthened by

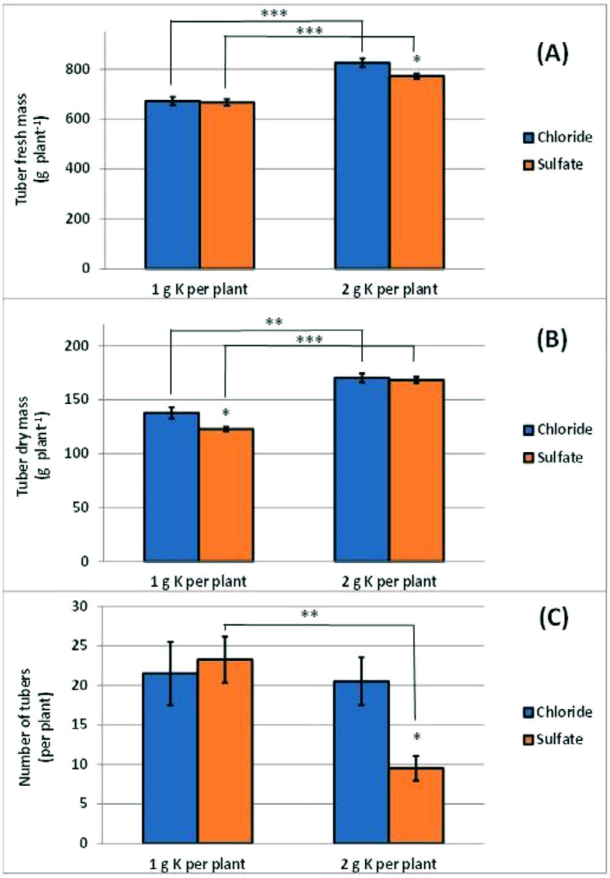

FIGURE 4 Tuber fresh mass (A), tuber dry mass (B), and number of tubers (C) of the potato cultivar Marabel at the final harvest (92 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); significant differences are indicated by asterisks (*, **, and *** with $p \leq 0.05$, 0.01, and 0.001, respectively; *t*-test).

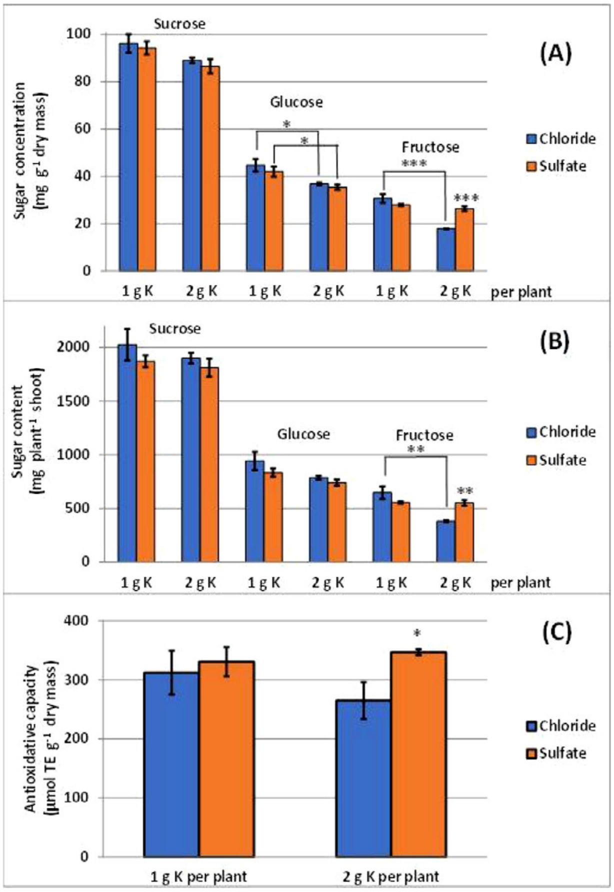

FIGURE 5 Sugar concentration (A), sugar content (B), and antioxidative capacity (C) in shoots of the potato cultivar Marabel shortly before start of flowering (intermediate harvest, 31 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); significant differences are indicated by asterisks (*, **, and *** with $p \leq 0.05$, 0.01, and 0.001, respectively; *t*-test).

the results at physiological maturity, when tuber starch concentration and starch yield were unaffected by the kind of K fertilization (Figure 6A,B).

Differences in start of flowering were also observed between both K application rates with an earlier start in the 2 g K than 1 g K treatment. In this growth stage, with 1 g K the K nutrition was rather suboptimal according to the desired value of 50 mg K g^{-1} DM (Bergmann, 1992), whereas with 2 g K the plants had received sufficient K as this value was achieved (Table 1). Thus, the higher K supply most likely caused faster growth and development of the plants, yet they reached physiological maturity later than those plants with 1 g K addition. Potassium is mainly responsible for establishment of cell turgor and cell extension growth, resulting in a higher proportion of the vacuole in the cells (80%–90% of the cell volume; Rengel et al., 2023). This could have retarded the drying process and thus dieback of the potato shoots during ripening.

4.2 | Potato nutrition with optimal K supply as K_2SO_4 instead of KCl increased the antioxidative capacity in the shoot

The potato plants that had received 2 g K as K_2SO_4 showed a significantly higher antioxidative capacity than those with 2 g K as KCl (Figure 5C). An increase of the antioxidative capacity indicates enhanced oxidative stress of the sulfate-treated plants before start of flowering (intermediate harvest). With 1 g K, no significant difference between both kinds of K fertilizers was observed, probably because the sulfate concentration was not sufficient to achieve a detectable impact on the antioxidative capacity. Plants suffering oxidative stress are mainly exposed to ROS such as hydrogen peroxide (H_2O_2) or the superoxide radical ($O_2^{\bullet-}$), which can attack proteins, lipids, or nucleic acids. This can cause various damages to plant cells, such as loss of cell membrane integrity, impaired functioning of transport proteins located in the membrane, disruption of enzyme activities, and damage to DNA. It is well established that the mitochondrial respiratory

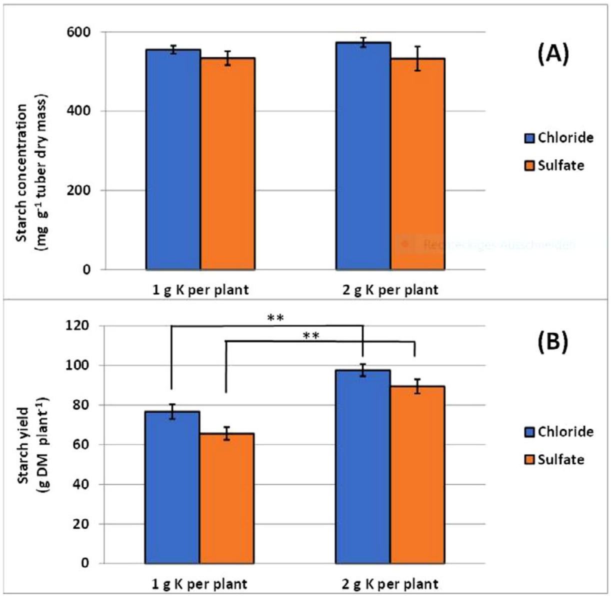

FIGURE 6 Starch concentration in tubers (A) and starch yield (B) of the potato cultivar Marabel at the final harvest (92 days after planting, DAP), as affected by KCl and K_2SO_4, applied in two rates; data are means of 4 replicates ± standard errors (SE); significant differences are indicated by asterisks (*, **, and *** with $p \leq 0.05$, 0.01, and 0.001, respectively; *t*-test).

chain is a major source of ROS (Murphy, 2009). This electron transport chain consists of several complexes containing multiple redox centers that normally enable controlled transfer of electrons to their final acceptor, molecular oxygen, which is reduced by four electrons to water at complex IV (Quinlan et al., 2013). Complex II oxidizes succinate to fumarate and reduces ubiquinone, thereby creating a direct link between the tricarboxylic acid cycle and the respiratory chain (Cecchini, 2003; Dröse, 2013). After sulfate treatment of *A. thaliana*, Zhao et al. (2020) found an accumulation of organic anions such as fumarate and succinate in the leaves. With isolated mitochondria, it has been shown that a high succinate concentration induces reverse electron transfer from complex II into complex I that is associated with a high rate of superoxide production (Dröse, 2013; Muller et al., 2008). Thus, stronger toxicity symptoms and growth inhibition after sulfate compared to chloride application, observed with potato plants in the present study and the experiments of Hütsch et al. (2018) and with *A. thaliana* (Zhao et al., 2020), could probably be due to sulfate-induced oxidative stress originating from a higher production of organic anions such as succinate. This needs to be investigated in more detail in further studies.

4.3 | Potato nutrition with K_2SO_4 instead of KCl caused reductions in tuber yield but not in starch concentration of the tubers

Potato plants with 2 g K as K_2SO_4 produced less tubers than their KCl-treated counterparts, resulting in a significant decrease in tuber fresh mass (Figure 4A,C). Although the tubers of K_2SO_4-treated plants were larger, this size increase could not compensate the reduced number. Obviously, with higher sulfate application, potato tuberization, which represents the morphogenetic transition of underground shoot to tuber (Nookaraju et al., 2012), was impaired. The sulfate anion could either have a direct negative impact on tuber induction or indirectly affect physiological processes in the potato plant. One indirect effect could be related to the Ca^{2+} concentration in the shoots, which was significantly reduced with 2 g K as K_2SO_4 compared to KCl (Table 1), as was also observed by Hütsch et al. (2018). Various studies indicated that Ca^{2+} influences tuberization through Ca^{2+} modulator proteins, calmodulin, Ca^{2+}-dependent protein kinases, and channel proteins. Some of these proteins have shown enhanced expression in stolon tips and developing tubers (Jackson, 1999; Nookaraju et al., 2012). Thus, Ca^{2+} plays an important role in the signal transduction pathway that regulates tuberization in potato. The function of Ca^{2+} as second messenger depends on the large concentration differences between the cytosol with extremely low values (less than 1 µM Ca^{2+}) and the apoplast or the vacuole (McAnish & Pittman, 2009; Pottosin & Schonknecht, 2007). Changes in Ca^{2+} concentration in the cytosol are mediated by transport systems located in the membranes, such as Ca^{2+} ATPase or Ca^{2+} channels. However, controlled Ca^{2+} transport can likely be disturbed due to loss in membrane integrity by ROS, pointing to a contribution of the observed stress reaction in the K_2SO_4 treatment to the impaired tuber induction and reduced tuber yield. The Ca^{2+} uptake of the potato shoots was decreased as well, which could be caused by a restricted synergism of ion uptake. This synergism is less pronounced between the ions Ca^{2+} and SO_4^{2-} than between Ca^{2+} and Cl^-, leading to a lower Ca^{2+} uptake after K_2SO_4 than KCl treatment.

Tuber starch concentration and starch yield were not significantly affected by the kind of applied K fertilizer (Figure 6A,B). Thus, the accelerated development and ripening process of the sulfate-treated plants had no negative impact on overall starch synthesis compared to the chloride treatment. Chloride had no negative effect on starch concentration and yield either, as was already found in previous experiments with the cultivars Marabel and Désirée (Hütsch et al., 2018). For the present study with the cultivar Marabel, it can be concluded that KCl supply showed neither detrimental effects on tuber yield nor on starch concentration and starch yield, whereas K_2SO_4 fertilization can even cause a decrease in tuber yield due to the aforementioned reasons.

5 | CONCLUSIONS AND OUTLOOK

In the present study with the potato cultivar Marabel, it was shown that in the shoots shortly before flowering, oxidative capacity was significantly increased after application of 2 g K as K_2SO_4 compared to KCl, indicating a pronounced impact of oxidative stress on the sulfate-treated plants. Stronger toxicity symptoms and growth inhibition after sulfate compared to chloride application were observed, which are assumed to originate from a higher production of organic anions such as succinate, leading to increased oxidative stress. A disruption of the Ca signal transduction pathway due to loss of membrane integrity by ROS may have contributed to the impaired tuberization and decreased tuber yield observed after K_2SO_4 treatment. This needs to be investigated in more detail in further studies, including various potato cultivars with measurements of ROS and organic anions in the plant shoots at different growth stages.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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