

Contrasting potassium responses of growth, magnesium status and steviol glycoside traits in *Stevia rebaudiana*

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Research Article

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Abstract

Background: Potassium (K) is essential for stevia production, but increasing K beyond sufficiency may not simultaneously improve root growth, harvestable biomass, magnesium (Mg) nutrition, and steviol glycoside production. This study examined whether these traits share a common response across a broad K gradient in *Stevia rebaudiana*.

Results: Plants of ‘Tiannong 5’ were grown at five nutrient-solution K concentrations of 1, 4, 8, 16, and 24 mmol L⁻¹. Root architecture, biomass, leaf and root K–Mg status, and 13 steviol glycosides were quantified, and treatment comparisons were integrated with restricted dose–response models and bootstrap-derived response ranges. Intermediate K supported root expansion and whole-plant biomass. Root length and whole-plant dry weight had model-estimated peaks at 4.69 and 5.38 mmol L⁻¹ K, with 90% high-response ranges of 2.88–7.65 and 3.17–9.16 mmol L⁻¹, respectively. Leaf dry weight differed among treatments but showed no supported continuous optimum. Leaf Mg concentration declined from 1.769 to 0.896 mg g⁻¹ as K increased, accompanied by lower leaf Mg content and a higher leaf K/Mg molar ratio. Neither total steviol glycoside concentration nor content per plant showed a supported K breakpoint. Their observed means at K24 were 9.05% and 20.54% lower than those at K16, respectively. In contrast, the rebaudioside A/stevioside ratio increased with K supply, indicating divergent responses of glycoside composition and total productivity. The candidate K16–K24 interval corresponded to fitted leaf Mg concentrations of approximately 0.9–1.1 mg g⁻¹, but this treatment-level association does not represent a diagnostic Mg threshold.

Conclusions: Root expansion, biomass accumulation, Mg status, glycoside productivity, and glycoside composition showed distinct K-response patterns. Once K supply exceeded the intermediate range supporting root and whole-plant growth, further K provided diminishing production returns, intensified leaf K–Mg imbalance, and failed to sustain total steviol glycoside productivity. These findings provide a rationale for independently testing coordinated K–Mg nutrition as a strategy to sustain stevia leaf and glycoside production.

Highlights

- Intermediate K supported root expansion and whole-plant biomass.
- Increasing K lowered leaf Mg status and raised the leaf K/Mg molar ratio.
- TSG productivity showed no sustained gain while RA/STV increased with K supply.

1 Introduction

Stevia rebaudiana is an economically important leaf crop and a major plant-derived source of low- and no-calorie sweeteners. Its leaves accumulate a structurally diverse group of steviol glycosides, among which stevioside (STV) and rebaudioside A (RA) are prominent components^[1]. Crop value therefore depends on more than glycoside concentration. Harvestable leaf biomass determines the amount of raw

material, glycoside concentration determines product density, and their product determines per-plant glycoside output. Glycoside composition adds another dimension because the relative abundance of individual compounds may change independently of total output. Crop environment and nutrition can alter biomass and specialized-metabolite accumulation in different directions^[2,3]. Fertilizer form can also shift plant nutrient status and agronomic performance^[4]. A production-oriented assessment should therefore integrate leaf yield, total steviol glycoside (TSG) productivity and composition.

A central challenge in stevia production is to sustain harvestable leaf biomass and glycoside output while maintaining a desirable component profile. A high glycoside concentration does not necessarily produce a high yield per plant when leaf biomass is restricted, whereas greater leaf mass may raise total output even when concentration changes little. Organo-mineral and compost-based inputs have produced nonparallel responses among growth, nutrient uptake and steviol glycosides^[5,6]. Microbial inoculation and foliar nutrition have likewise affected growth, nutrient acquisition or product formation^[7,8]. Potassium (K) is often emphasized as a quality-related macronutrient because of its roles in plant carbon economy and product formation. However, this emphasis can encourage the assumption that further K input will continue to improve both yield and quality. That assumption becomes uncertain once the marginal growth response has declined or additional K disrupts the balance with other nutrients.

Adequate K is indispensable for plant growth and carbon allocation, but its benefit is expected to diminish once crop demand is met. K contributes to osmotic regulation, stomatal function, enzyme activation, photosynthesis and assimilate transport^[9]. Earlier stevia studies linked K supply with physiological performance^[10,11], while K deficiency restricted leaf sugar metabolism and steviol glycoside synthesis^[12]. Foliar nano-K has also been evaluated, although that treatment cannot be equated with root-zone K supply^[13]. Evidence that low K is harmful does not establish that increasing K beyond sufficiency will continue to enlarge roots, raise harvestable leaf weight or increase glycoside yield. Tomato studies similarly show that K-dependent changes in growth, nutrient acquisition, yield and quality do not follow a single response pattern^[14-16]. The relevant question is therefore where the beneficial K response ends and additional supply begins to produce diminishing returns.

A potential cost of excessive K supply is disruption of magnesium (Mg) nutrition through K–Mg antagonism. Mg supports chlorophyll, photosynthetic carbon assimilation, ATP-dependent metabolism and assimilate export from leaves^[9,17]. Imbalanced K and Mg nutrition has altered growth or carbon allocation in tomato, sugarcane and potato^[18-20]. The interaction is not uniform across crops: K-induced suppression of Mg uptake was limited in oat, whereas high K intensified Mg-deficiency stress in rice^[21,22]. Grapevine responses also depend on K/Mg supply, cultivar and tissue^[23-25]. Maize studies show that fertilizer balance and transporter adjustment both contribute to the response^[26-28], while sugarcane data further link cation interactions with crop performance^[29]. Nutrient effects reported in basil, tomato and citrus reinforce the importance of species- and organ-specific interpretation^[30-32]. Yet a complete K gradient linking growth, leaf Mg status, TSG productivity and glycoside composition has

not been resolved in stevia. Together, this evidence makes leaf Mg status a necessary counterpart to growth and product traits when evaluating increasing K supply.

We therefore examined root architecture, leaf and whole-plant biomass, leaf and root K–Mg status, TSG concentration and per-plant content, and glycoside composition across five K supply levels in *S. rebaudiana*. We hypothesized that intermediate K would alleviate low-K constraints on root expansion and biomass accumulation, whereas further K supply would progressively lower leaf Mg status and provide diminishing returns in steviol glycoside productivity. We also assessed whether TSG productivity and RA/STV composition exhibited concordant or divergent responses along the K gradient. By integrating treatment comparisons with dose–response analysis, this study aimed to determine whether the benefits of increasing K were sustained across growth, Mg nutrition, and glycoside traits, thereby providing a scientific basis for avoiding excessive K application and balancing leaf yield, mineral nutrition, and glycoside productivity in stevia production.

2 Materials and methods

2.1 Plant material and seedling establishment

The plant material used in this study was *Stevia rebaudiana* ‘Tiannong 5’, a newly developed stevia cultivar. Uniform cuttings approximately 5–6 cm long, each bearing two pairs of leaves and an apical bud, were collected from healthy stock plants. Cuttings were inserted to approximately half their length into 72-cell trays filled with nutrient soil, vermiculite and perlite (2:1:1, v/v/v) and allowed to root for approximately 15 d. Uniform rooted cuttings were transplanted individually into 1-L pots containing the same substrate. Plants were acclimated for 7 d with half-strength Hoagland solution before the K treatments began. During pre-culture, each pot received 100 mL nutrient solution every 2 d.

2.2 Experimental design and K treatments

A single-factor pot experiment was conducted with five nutrient-solution K concentrations: 1, 4, 8, 16 and 24 mmol L⁻¹, designated K1, K4, K8, K16 and K24, respectively. K was supplied as K₂SO₄, while Mg was maintained at 1 mmol L⁻¹ in all treatments. Each treatment comprised 20 pots, and one pot containing one plant was the biological experimental unit. The experiment followed a completely randomized design.

The basal solution contained 2.5 mmol L⁻¹ Ca(NO₃)₂·4H₂O, 0.5 mmol L⁻¹ NaH₂PO₄·2H₂O and 1 mmol L⁻¹ MgSO₄·7H₂O, together with 40 µmol L⁻¹ FeSO₄·7H₂O, 92 µmol L⁻¹ H₃BO₃, 18.2 µmol L⁻¹ MnCl₂·4H₂O, 1.6 µmol L⁻¹ ZnSO₄·7H₂O, 0.6 µmol L⁻¹ CuSO₄·5H₂O and 1 µmol L⁻¹ (NH₄)₆Mo₇O₂₄·4H₂O. Solution pH was adjusted to 6.50 ± 0.05 with 1 mol L⁻¹ HCl or NaOH.

During the K treatment period, plants were maintained in a controlled-environment chamber at 25/15 °C (day/night) under a 16 h light/8 h dark photoperiod, with a photosynthetic photon flux density of 100

$\mu\text{mol m}^{-2} \text{ s}^{-1}$ and 80% relative humidity. Each pot was supplied with 100 mL of nutrient solution every 2 d until the budding stage, when the plants were harvested.

2.3 Sampling and growth measurements

At the budding stage, relative chlorophyll content was measured on middle, fully expanded leaves from 16 living plants per treatment using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan). Eight plants per treatment were then harvested destructively for biomass, leaf-area and root measurements, as well as mineral and glycoside analyses. Among the eight destructively harvested plants per treatment, four plants were used for mineral analysis and the other four for steviol glycoside analysis. Each mineral or glycoside concentration was paired with the corresponding dry-weight record from the same plant.

All leaves from each harvested plant were scanned individually with an Epson Expression 12000XL flatbed scanner (Seiko Epson Corp., Suwa, Nagano, Japan) at 300 dpi in 16-bit grayscale mode. Total leaf area was quantified using ImageJ version 1.53e. Roots were washed and scanned separately, and total root length, surface area, volume, tip number and mean diameter were quantified using WinRHIZO software (Regent Instruments Inc., Quebec, QC, Canada).

Each harvested plant was separated into leaves, stems and roots. Tissues were dried in a forced-air oven at 60 °C to constant mass and weighed. Whole-plant dry weight was calculated as the sum of leaf, stem and root dry weight. Specific root length was calculated as total root length divided by matched root dry weight, and the root-to-shoot ratio used combined leaf and stem dry weight as the shoot denominator.

2.4 Tissue K and Mg determination

Dried leaf and root tissues were ground and digested with $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$. K concentration was determined by flame photometry (Model 410, Sherwood Scientific Ltd., Cambridge, UK) and Mg concentration by atomic absorption spectrophotometry (AAnalyst 300, PerkinElmer, Waltham, MA, USA). Concentrations were expressed on a dry-weight basis (mg g^{-1}). Tissue K and Mg contents per plant were calculated by multiplying concentration by the matched leaf or root dry weight. The molar K/Mg ratio was calculated as $(\text{K concentration}/39.10)/(\text{Mg concentration}/24.31)$.

2.5 Steviol glycoside determination

Dried leaves were ground and passed through a 60-mesh sieve. Two aliquots of approximately 1.0–1.1 g were each extracted with 100 mL purified water at 80 ± 2 °C for 1 h. Evaporative loss was replaced gravimetrically, and the extracts were filtered through a 0.45- μm membrane. Separation was performed using an Agilent 1260 HPLC system with UV detection and a C18 column (250 $\times$ 4.6 mm, 5 μm) at 40 °C. The mobile phase was 10 mmol L^{-1} sodium phosphate buffer (pH 2.6) and acetonitrile, programmed at 74:26 (v/v) from 0–13 min, 67:33 from 15–40 min and 74:26 from 42–45 min. Flow rate, detection wavelength and injection volume were 0.8 mL min^{-1} , 210 nm and 10 μL , respectively. Twelve glycosides were assigned using a mixed standard; SX was retained as a source-defined chromatographic

component with unconfirmed identity. The 13 measured components were quantified with RA- or RD-based calibration and relative response factors. TSG concentration was their sum; per-plant TSG content equalled TSG concentration multiplied by matched leaf dry weight; and RA/STV was calculated from component concentrations.

2.6 Statistical analysis

Analyses were performed in R version 4.6.1. Plant-level observations were retained, and treatment summaries are reported as means with 95% confidence intervals. Welch's one-way test compared the five K treatments. Six designated primary endpoints were leaf dry weight, whole-plant dry weight, total root length, leaf molar K/Mg, TSG content per plant and RA/STV; their omnibus P values were adjusted jointly by Holm's method. Secondary endpoints were adjusted within measurement families using the Benjamini–Hochberg false-discovery rate. Three biological contrasts compared K1 with the mean of K4–K24, the mean of K4 and K8 with the mean of K16 and K24, and K24 with the mean of K4–K16. Contrast P values were Holm-adjusted within each endpoint. The planned contrasts are reported in Supplementary Table S1. For the 13-component screen, BH-FDR adjustment was applied separately to concentration, per-plant content and relative composition (Supplementary Fig. S1).

Dose responses were screened using linear and quadratic models of centred $\ln(K)$. A quadratic model was retained only when its quadratic term had $P < 0.05$ and its corrected Akaike information criterion was at least 2 units lower than that of the linear model. A peak and the K range retaining at least 90% of the fitted gain above K1 were reported only for a supported concave response. Within-treatment bootstrap resampling quantified uncertainty in fitted response positions. For leaf Mg, fitted K positions associated with 5%, 10%, 15% and 20% declines from fitted K1 were treated as operational intersections. The observed K16-to-K24 TSG change was summarized without imposing a breakpoint, and the fitted leaf Mg values at K16 and K24 were aligned with this interval to integrate response positions across nutritional and productivity domains. Model equations, resampling procedures and downturn criteria are described in Supplementary Methods S1–S2; model comparisons, diagnostics and sensitivity analyses are shown in Supplementary Figs. S3–S5.

3 Results

3.1 Intermediate K increased biomass accumulation, whereas K24 reduced whole-plant dry weight

Whole-plant appearance at harvest is shown in Fig. 1a. Whole-plant dry weight differed significantly among K treatments, increasing from $2.741 \text{ g plant}^{-1}$ at K1 to $3.138 \text{ g plant}^{-1}$ at K16 before decreasing to $2.648 \text{ g plant}^{-1}$ at K24 (Holm-adjusted $P = 0.007$; Fig. 1d). The planned high-K contrast showed that whole-plant dry weight at K24 was $0.421 \text{ g plant}^{-1}$ lower than the mean of K4–K16, with a 95% CI of -0.711 to -0.131 and a Holm-adjusted P of 0.016 (Supplementary Table S1).

Leaf dry weight also differed significantly among treatments (Holm-adjusted $P = 0.011$; Fig. 1b). It increased from $1.144 \text{ g plant}^{-1}$ at K1 to an observed maximum of $1.354 \text{ g plant}^{-1}$ at K16 and then

declined to $1.162 \text{ g plant}^{-1}$ at K24. The mean across K4–K24 exceeded K1 by $0.105 \text{ g plant}^{-1}$, supporting a low-K limitation (95% CI, 0.047–0.163; Holm-adjusted $P = 0.003$). However, the K24-versus-K4–K16 contrast for leaf dry weight was not significant after correction ($P = 0.078$; Supplementary Table S1).

Root dry weight and total leaf area also differed significantly among treatments, with their lowest or near-lowest means at K24. SPAD had its highest observed mean at K16, whereas root-to-shoot ratio did not differ significantly (Fig. 1c, e–g). No significant continuous K response was detected for leaf dry weight (Fig. 5b). In contrast, whole-plant dry weight followed a supported concave response, with a model-estimated peak at $5.38 \text{ mmol L}^{-1} \text{ K}$ and a 90% high-response range of $3.17\text{--}9.16 \text{ mmol L}^{-1}$ (Fig. 5c; Supplementary Table S2).

3.2 Root-system expansion showed a low-to-intermediate K response window

Root length, surface area, volume, and tip number all differed significantly among K treatments, with the greatest root-system expansion occurring at K4–K8 (Fig. 2a–d). Total root length increased from 667 cm at K1 to 1443 and 1364 cm at K4 and K8, respectively, before declining to 792 cm at K16 and 696 cm at K24. The overall treatment effect on root length was significant (Holm-adjusted $P < 0.001$), and the planned contrast showed that mean root length at K4–K8 exceeded that at K16–K24 by 659 cm (95% CI, 490–829; Holm-adjusted $P < 0.001$; Supplementary Table S1).

Root surface area, volume, and tip number showed comparable low-to-intermediate-K responses, whereas average root diameter and specific root length exhibited distinct but significant treatment patterns (Fig. 2b–f). The supported concave model for root length yielded a model-estimated peak at $4.69 \text{ mmol L}^{-1} \text{ K}$ and a 90% high-response range of $2.88\text{--}7.65 \text{ mmol L}^{-1}$ (Fig. 5a; Supplementary Table S2). This range was numerically narrower than, but overlapped with, the corresponding whole-plant dry-weight range.

3.3 Increasing K reduced leaf Mg status and shifted leaf K–Mg stoichiometry

Leaf Mg concentration decreased significantly from 1.769 mg g^{-1} at K1 to 0.896 mg g^{-1} at K24 (BH-FDR $q = 0.007$; Fig. 3b). Leaf Mg content per plant also declined significantly (Fig. 3c), whereas the leaf K/Mg molar ratio increased across the K gradient (Holm-adjusted $P = 0.002$; Fig. 3d). At K24, the leaf K/Mg ratio exceeded the K4–K16 mean by 28.17 units (95% CI, 19.96–36.39; Holm-adjusted $P < 0.001$; Supplementary Table S1). Leaf K concentration increased numerically but its overall treatment effect did not pass BH-FDR adjustment ($q = 0.053$; Fig. 3a).

Leaf Mg concentration showed a significant negative response to $\ln(K)$. The fitted K levels corresponding to 10% and 20% reductions from fitted K1 were 1.95 mmol L^{-1} and 3.81 mmol L^{-1} , respectively (Figs. 5d and 6a). Their bootstrap intervals were $1.76\text{--}2.74$ and $3.09\text{--}7.38 \text{ mmol L}^{-1}$.

Root Mg concentration showed no categorical treatment effect after BH-FDR adjustment ($q = 0.323$), although a secondary $\ln(K)$ model gave a negative slope ($P = 0.033$; Fig. 3e). Root K/Mg also showed no

significant treatment effect ($q = 0.323$; Fig. 3f). Of the additional endpoints, leaf K content differed among treatments ($q = 0.007$; Supplementary Fig. S2a), whereas root K concentration, root K content and root Mg content did not ($q = 0.323, 0.079$ and 0.323 , respectively; Supplementary Fig. S2b–d).

3.4 Steviol glycoside productivity and composition responded differently to K

Neither TSG concentration nor TSG content per plant differed significantly among the five K treatments, and neither endpoint showed a supported continuous K response (Figs. 4a–b and 5e). Although the five-treatment omnibus test was nonsignificant, the low-K contrast descriptively indicated that mean content across K4–K24 exceeded K1 by $30.0 \text{ mg plant}^{-1}$ (95% CI, 9.09–50.92; Holm-adjusted $P = 0.026$; Supplementary Tables S1–S2).

At the high-K end of the gradient, the observed TSG concentration at K24 was 9.05% lower than that at K16, while per-plant content was 20.54% lower. These numerical differences did not establish a statistically supported breakpoint or identify K16 as an optimum (Fig. 6b). The sample-matched decomposition showed that the higher mean TSG content at K16 reflected concurrent numerical contributions from leaf dry weight and TSG concentration (Fig. 4e).

RA/STV increased significantly with $\ln(K)$ ($P = 0.003$), although its categorical omnibus test was nonsignificant ($P = 0.302$; Figs. 4c and 5f; Supplementary Tables S1–S2). Thus, the ordered response in glycoside composition was not accompanied by a significant overall treatment effect on TSG productivity. The RA and STV shares showed numerical changes but no significant categorical effects after BH-FDR adjustment (Fig. 4d). Among the 13 measured components, RUB was the only component that remained significant after BH-FDR adjustment in the concentration, per-plant content, and relative-composition families (Supplementary Fig. S1a–d).

3.5 Integrated analysis revealed distinct K-response positions across functional domains

Supported intermediate-K response ranges were identified for total root length and whole-plant dry weight, whereas leaf dry weight and TSG content per plant had no supported continuous optima (Fig. 5a–c, e; Supplementary Table S2). The fitted 10% and 20% declines in leaf Mg occurred at lower K supplies than the sampled K16–K24 interval in which the TSG means decreased (Fig. 6c). Restricted model comparisons and standardized focal coefficients are shown in Supplementary Fig. S3a–b; residual-versus-fitted and normal Q–Q plots for the same six endpoints are provided in Supplementary Fig. S4a–b.

In bootstrap resampling, K24 was lower than K16 in 78.4% of samples for TSG concentration and 87.6% for TSG content per plant. Strict internal-downturn model support occurred in only 7.4% and 11.8% of resamples, respectively; thus, the K16-to-K24 comparison supports a lack of sustained glycoside gain at the highest K supply rather than a discrete breakpoint (Supplementary Fig. S5a). Increasing the gain-retention criterion from 90% to 95% narrowed both the root-length and whole-plant dry-weight response windows (Supplementary Fig. S5b).

Fitted leaf Mg concentration declined from 1.075 mg g⁻¹ at K16 to 0.964 mg g⁻¹ at K24. The corresponding fitted leaf-Mg interval therefore spanned approximately 0.96–1.08 mg g⁻¹, with an outer bootstrap envelope of 0.884–1.135 mg g⁻¹ (Fig. 6d).

4 Discussion

Potassium is essential for stevia growth and glycoside formation, but the present results show that its benefits were strongly dose dependent. Intermediate K supported root expansion and whole-plant biomass, whereas further K supply progressively reduced leaf Mg status and did not sustain gains in TSG productivity. At the same time, RA/STV increased, indicating that glycoside composition and total glycoside output responded differently. The present results therefore describe a transition from K limitation at low supply to diminishing growth returns and increasing K–Mg imbalance at high supply. The central nutritional question is not simply whether K is beneficial, but how far its supply can be increased before additional input ceases to support production and begins to disturb cation balance.

4.1 Intermediate K supports growth whereas further K provides diminishing returns

Potassium promotes plant growth through osmotic regulation, stomatal function, enzyme activation and assimilate transport, but biomass responses are expected to plateau once plant demand is met^[9]. In this experiment, K4–K8 promoted total root length, surface area, volume and tip number, indicating greater root-system exploration and branching under intermediate K (Fig. 2a–d). The comparatively small root system at K1 is consistent with low-K restriction, while the decline at K16–K24 shows that further K did not maintain the same root response. Earlier stevia studies related K nutrition to physiological and biochemical performance^[10,11], and K deficiency constrained leaf sugar metabolism and glycoside synthesis^[12]. The present study extends this evidence by showing that several dimensions of root-system size responded together across a broad K gradient. Mean diameter and specific root length followed different patterns, however, so K changed root architecture rather than enlarging every trait uniformly. Nutrient effects on root morphology and joint cation acquisition are also strongly context dependent^[33,34]. Thus, the root response is best interpreted as an intermediate-K expansion window rather than a uniform stimulation of all root traits.

Whole-plant dry weight showed a similar, although broader, intermediate-K response. Its fitted high-response range overlapped the corresponding range for root length, identifying a common K region that supported both acquisition capacity and accumulated biomass (Fig. 5a, c; Supplementary Table S2). These overlapping ranges should not be interpreted as two formally distinct optima or as universal fertilizer recommendations. At K24, root expansion and whole-plant dry weight were lower than at intermediate supply. Imbalanced K and Mg nutrition can alter photoassimilate allocation and biomass formation in potato and sugarcane^[19,20]. Maize studies likewise show that biomass and yield depend on the balance between the two nutrients^[35,36]. Long-term maize and alfalfa experiments further indicate

that plant density, interacting nutrients and harvest context can modify the production response^[37,38]. Together, these studies support the importance of balanced K and Mg nutrition for sustaining carbon allocation and biomass formation. The supported conclusion is therefore narrower: the growth benefit of relieving low-K limitation was largely realized before the highest K treatments.

For stevia production, the response of the harvested leaf is more important than the response of total plant weight. Leaf dry weight differed among treatments and was relatively high across K4–K16, but it did not show a supported continuous peak. Consequently, the fitted whole-plant peak cannot be used as a leaf-yield optimum. Nevertheless, K24 produced no additional leaf-weight benefit, and the numerical decrease in leaf dry weight contributed to the lower per-plant TSG output at the highest K level (Figs. 1 and 4). Nutritional and ecological treatments in stevia have likewise altered biomass and specialized metabolites in different directions^[2,5], and fertilizer form can modify nutrient status and agronomic performance^[4]. Evidence from other crops also shows that fertilizer efficiency and production responses depend on crop demand and environmental context^[39,40]. Thus, adequate K relieved low-K limitation, but increasing K beyond the intermediate range produced diminishing returns in root development, leaf yield and total biomass.

4.2 Increasing K lowers leaf Mg status and intensifies K–Mg imbalance

The agronomic benefit of K depends not only on K availability itself but also on its balance with other cations, particularly Mg. Mg supports chlorophyll structure, photosynthetic carbon assimilation, ATP-dependent reactions and assimilate transport, functions that are especially important in a crop harvested for leaf biomass and leaf-derived glycosides^[9,17]. As K supply increased, leaf Mg concentration and per-plant Mg content declined together, while the molar K/Mg ratio rose sharply (Fig. 3b–d). The parallel decreases in concentration and content argue against simple growth dilution as the sole explanation because the measured Mg pool in the harvested leaves also became smaller. Root Mg responses were weaker and more variable, making the leaf the more responsive tissue indicator in this experiment.

The leaf response agrees with evidence that high K can lower Mg status when cation supply becomes unbalanced. High K/Mg ratios have restricted Mg acquisition or growth in tomato, and K–Mg imbalance has affected metabolism and biomass formation in sugarcane^[18,19]. The magnitude and physiological site of the interaction remain crop specific. In oat, K-induced suppression of Mg uptake and translocation was limited, while maize transporter responses illustrate active adjustment to high K^[21,28]. Tomato and oat studies also show that responses can differ among organs or nutrient pools^[41,42]. Together with prior crop evidence, the declining leaf Mg pool and rising K/Mg ratio show that continued K supply progressively shifted leaf cation balance away from Mg. The leaf therefore emerged as the more responsive tissue indicator under this K gradient.

These patterns suggest a progressive shift from K limitation at low supply to a greater leaf K/Mg imbalance at high supply. At low K, the benefit of relieving K limitation was expressed in root expansion

and biomass formation, even though leaf Mg had already begun to decline along the fitted gradient. At K24, the marginal growth benefit of K had disappeared while leaf Mg status deteriorated further and the leaf K/Mg ratio continued to increase. This sequence explains why more K was not equivalent to more production and defines the nutritional transition central to this study. Related studies in rice and grapevine also show that high K or altered K/Mg ratios can affect tissue nutrition and stress responses, although their thresholds cannot be transferred to stevia^[22,23]. The implication for nutrient management is that K sufficiency should be judged together with Mg status rather than from K supply alone.

The mapped leaf-Mg range of approximately 0.9–1.1 mg g⁻¹ provides a practical target for testing whether Mg resupply under high K can restore Mg status and recover leaf or glycoside productivity (Fig. 6d). This range is a validation target rather than an established deficiency threshold. A factorial K × Mg experiment should quantify leaf Mg, leaf dry weight and glycosides across controlled nutrient combinations while accounting for accompanying sulfate and electrical conductivity. Mg-resupply studies in apple and tomato support the value of directly manipulating both nutrients, but they cannot predict the stevia response^[43,44]. Such a design would determine whether the high-K response is reversible through Mg supply and would separate K–Mg interaction from the direct effects of K concentration.

4.3 Additional K shifts glycoside composition without sustaining TSG productivity

Potassium is often regarded as a quality-related nutrient because it supports photosynthesis, carbohydrate transport and secondary metabolism. Greater K supply, however, does not necessarily translate into higher product yield or uniformly improved quality. In the present study, K1 had comparatively low per-plant TSG output, consistent with evidence that severe K deficiency restricts sugar metabolism and glycoside synthesis in stevia^[12]. Above this low-K condition, neither TSG concentration nor per-plant content increased consistently across the full gradient. K16 had the highest observed mean, whereas the lower observed means at K24 indicate no evidence of sustained glycoside gain at the highest K supply (Figs. 4 and 6). The evidence therefore supports diminishing glycoside returns at high K rather than an additional quality benefit from further K input.

Per-plant glycoside output integrates two components that can respond differently: harvestable leaf weight and glycoside concentration. Their sample-matched decomposition showed that the higher mean TSG content at K16 reflected contributions from both components (Fig. 4e). At K24, lower leaf dry weight and lower TSG concentration likewise contributed numerically to the decrease in content. This identity is useful for production interpretation because a concentration value alone can obscure the amount harvested from each plant. Nitrogen supply has produced nonparallel growth and glycoside responses in stevia^[45], as have organo-mineral inputs^[5]. Compost-based and microbial treatments provide further examples of growth and product formation responding differently^[6,7]. The current result therefore argues against treating K as a uniformly yield-enhancing input after low-K limitation has been relieved.

RA/STV increased with K even though total glycoside productivity showed no sustained gain (Figs. 4a–c and 5e–f). This divergence demonstrates that composition and output are different response domains. A larger RA/STV ratio may arise from a change in RA, STV or both, and it cannot by itself establish higher sweetness, better sensory quality or a superior commercial grade. RUB was the only individual component with a treatment effect that remained significant across the concentration, per-plant content and relative-composition families (Supplementary Fig. S1). Other component-level patterns should therefore remain exploratory. The genetic and enzymatic basis of glycoside biosynthesis is complex^[1]. In the present study, the rise in RA/STV is best interpreted as a compositional response that occurred without a corresponding sustained gain in total glycoside output.

At high K, declining leaf Mg concentration and content, a rising leaf K/Mg ratio and the absence of a sustained TSG gain defined a common shift away from the productive intermediate-K range (Figs. 1, 3, 5 and 6). This coordinated response shows that K remained essential but became progressively less beneficial once supply exceeded the range supporting root expansion and biomass formation. The implication is not that K ceased to be a quality-related nutrient, but that its contribution depended on dose and cation balance. Overall, increasing K beyond the range supporting root expansion and biomass shifted leaf Mg status and glycoside composition more clearly than it enhanced total glycoside output.

5 Conclusion

Potassium produced distinct, dose-dependent responses in stevia growth, mineral nutrition and glycoside traits. Intermediate supply supported root expansion and whole-plant biomass, whereas leaf dry weight showed treatment differences without a supported continuous optimum. Further K input provided no supported additional gain in harvestable leaf biomass or TSG productivity. Instead, leaf Mg concentration and content declined progressively and the leaf K/Mg molar ratio increased, marking a shift toward K–Mg imbalance. RA/STV rose along the K gradient, but this compositional response was separate from total glycoside output and should not be interpreted as uniformly improved product quality. The K16–K24 comparison defines a candidate high-K interval in which mean TSG output was lower while fitted leaf Mg declined. These findings provide a quantitative rationale for independently testing coordinated K–Mg supply to sustain stevia leaf yield and glycoside output.

Abbreviations

DA, dulcoside A; K, potassium; Mg, magnesium; RA, rebaudioside A; RB, rebaudioside B; RC, rebaudioside C; RD, rebaudioside D; RE, rebaudioside E; RF, rebaudioside F; RM, rebaudioside M; RN, rebaudioside N; RO, rebaudioside O; RUB, rubusoside; SPAD, relative chlorophyll content; STV, stevioside; TSG, total steviol glycosides.

Declarations

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CRedit authorship contribution statement

Huanhuan Chen: Conceptualization, Validation, Formal analysis, Writing – original draft. **Jiawei Liu:** Conceptualization, Investigation. **Sihong Ye:** Supervision, Writing – review & editing; **Jia Shen:** Supervision, Writing – review & editing. **Chaoqiang Jiang:** Supervision, Writing – review & editing.

Data availability statement

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

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

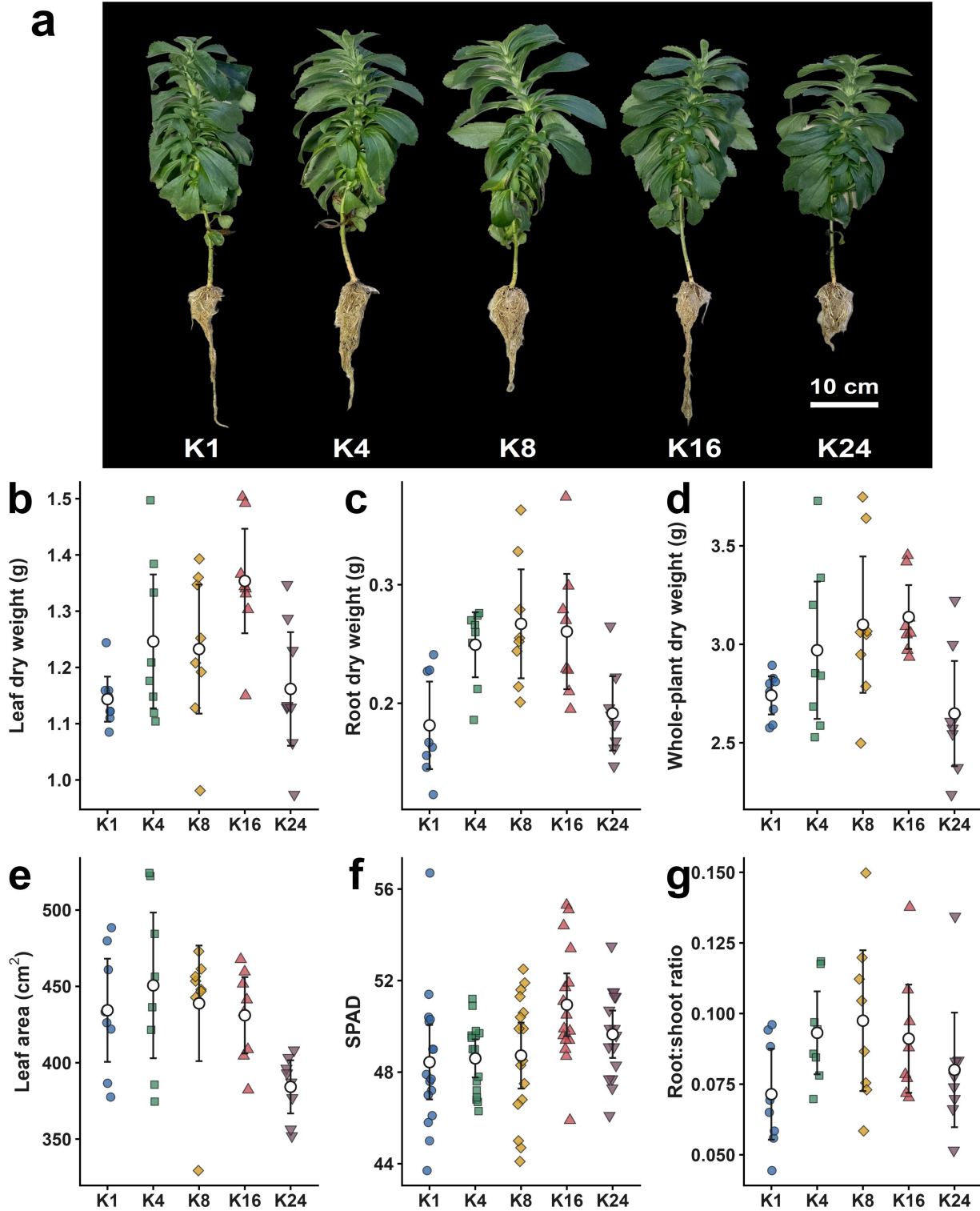


Figure 1

Whole-plant appearance, biomass, and leaf traits under K supply levels. (a) Photographs of one plant from each treatment at harvest (scale bar, 10 cm); (b) leaf dry weight; (c) root dry weight; (d) whole-plant dry weight; (e) total leaf area; (f) SPAD value; and (g) root-to-shoot dry-weight ratio. In panels b–g, coloured symbols represent individual biological replicates, and open circles with vertical error bars denote treatment means and 95% confidence intervals. Biomass and leaf-area traits were measured in

eight plants per treatment, whereas SPAD was measured in sixteen plants per treatment. K1, K4, K8, K16, and K24 correspond to 1, 4, 8, 16, and 24 mmol L⁻¹ K, respectively. These treatment abbreviations and plotting conventions apply to Figs. 2–5 unless otherwise stated.

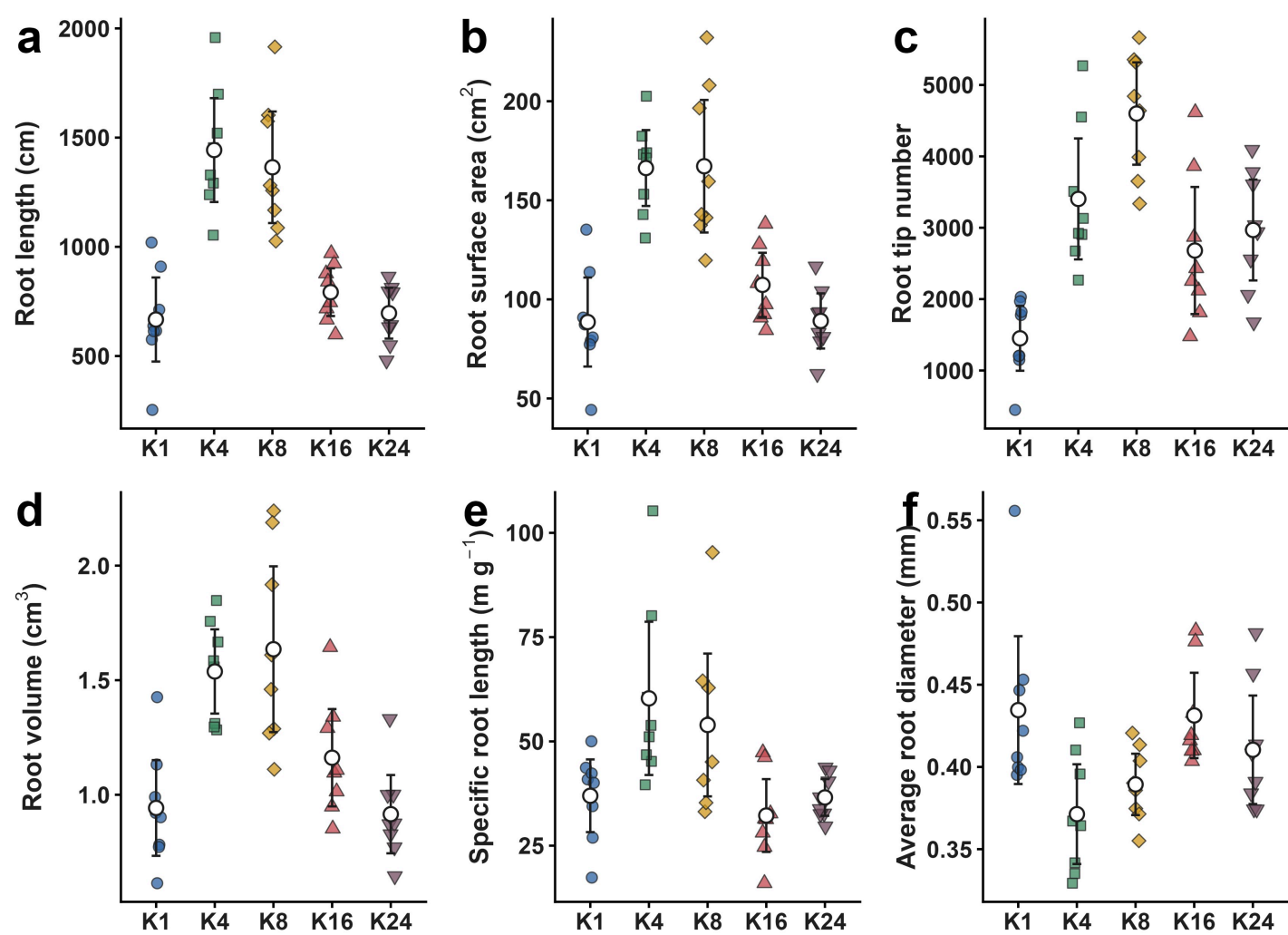


Figure 2

Root-system architecture across the K gradient. Panels show (a) total root length, (b) root surface area, (c) root tip number, (d) root volume, (e) specific root length, and (f) average root diameter. Eight independent plants were analysed per treatment.

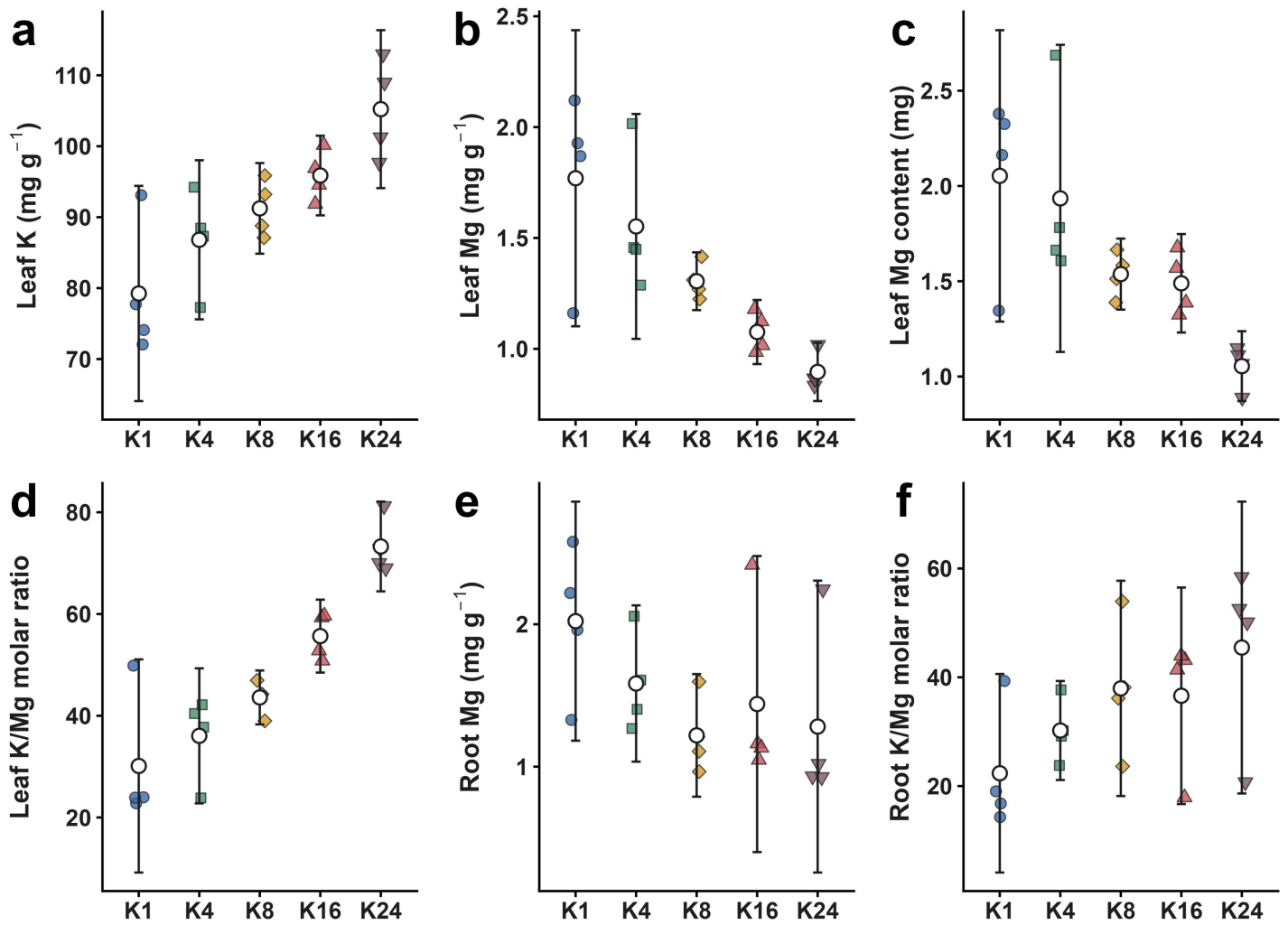


Figure 3

Leaf and root K–Mg status across the K gradient. Panels show (a) leaf K concentration, (b) leaf Mg concentration, (c) leaf Mg content per plant, (d) leaf K/Mg molar ratio, (e) root Mg concentration, and (f) root K/Mg molar ratio. Four independent plants were analysed per treatment. For root Mg concentration (panel e), the family-adjusted categorical test was nonsignificant (BH-FDR $q = 0.323$), although a secondary linear model against centred $\ln(K)$ gave a negative slope ($P = 0.033$).

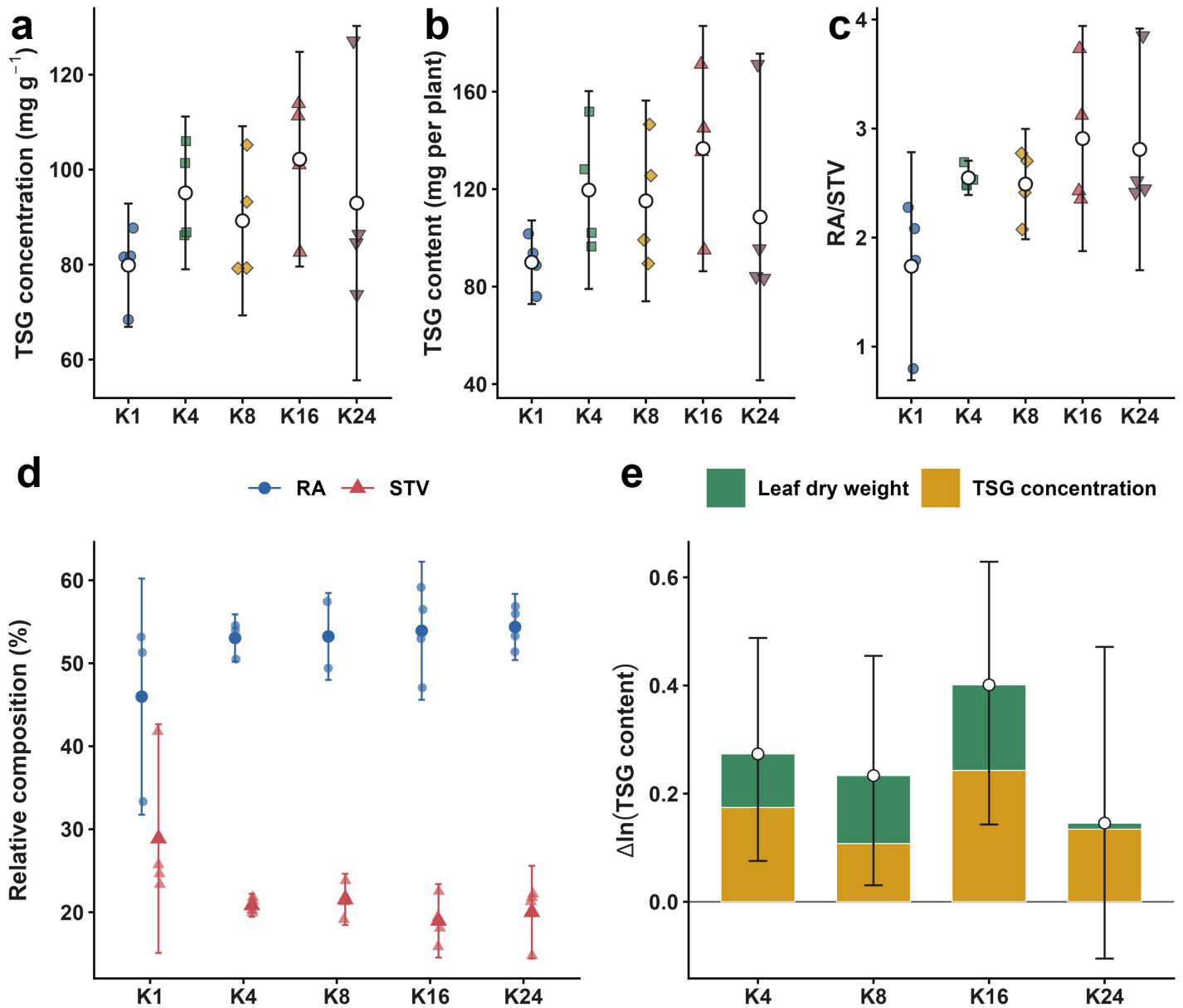


Figure 4

Steviol glycoside productivity and composition across the K gradient. Panels show (a) total steviol glycoside (TSG) concentration, (b) sample-matched TSG content per plant, (c) the rebaudioside A-to-stevioside ratio (RA/STV), (d) the relative shares of RA and STV within the sum of the 13 measured components, and (e) the sample-matched algebraic decomposition of the treatment change in $\ln(\text{TSG content per plant})$, relative to K1, into leaf dry-weight and TSG-concentration components. Four independent plants were analysed per treatment.

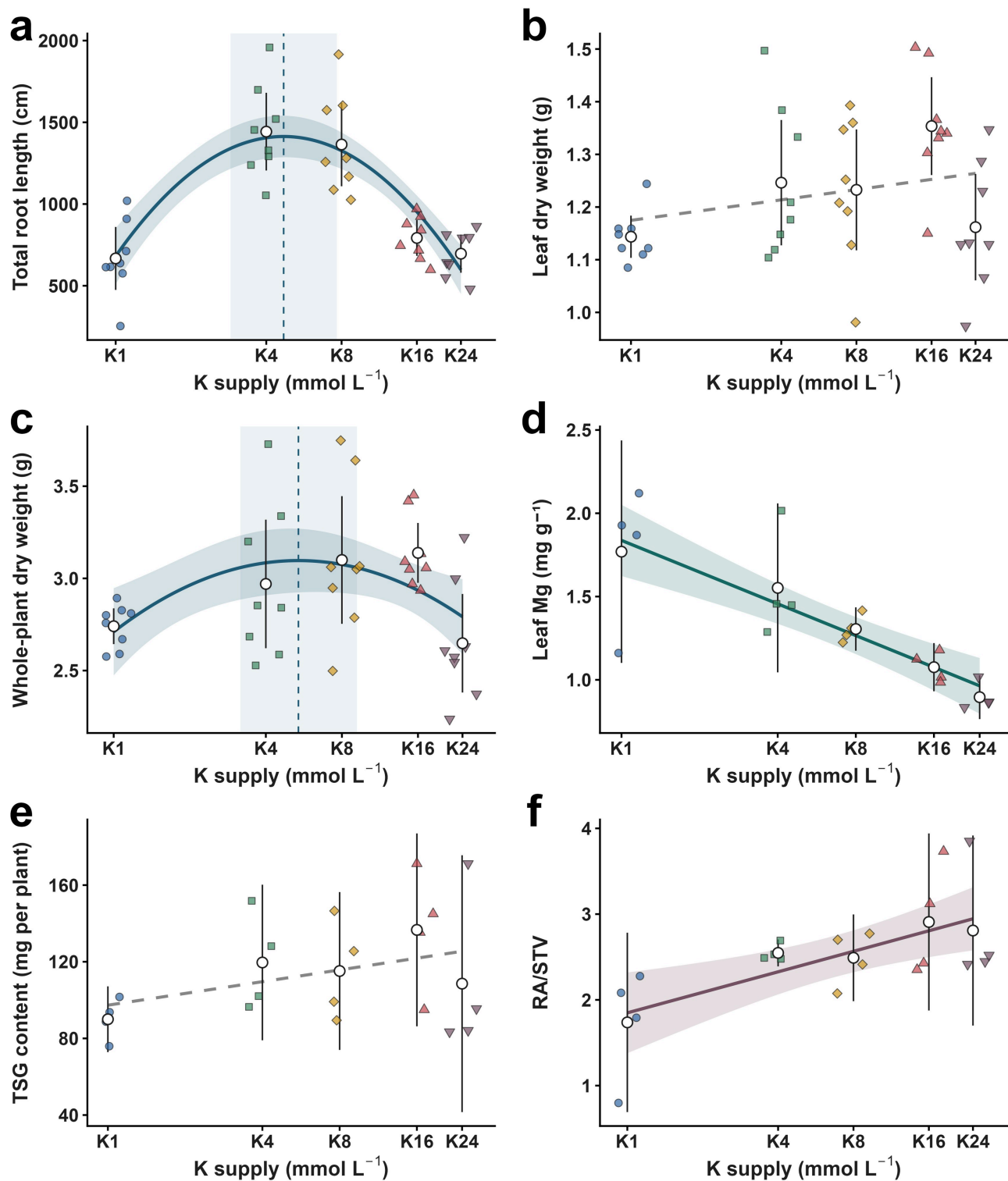


Figure 5

Dose-response relationships for six focal endpoints across the K gradient. Panels show (a) total root length, (b) leaf dry weight, (c) whole-plant dry weight, (d) leaf Mg concentration, (e) TSG content per plant, and (f) RA/STV. Panels a–c used eight plants per treatment, whereas panels d–f used four plants per treatment. Restricted linear or quadratic models were fitted as functions of centred $\ln(K)$, with the measured K levels shown on the horizontal axes. Solid curves and their surrounding ribbons denote

statistically supported models and model-based 95% confidence intervals. Dashed curves denote selected but unsupported models and are included for descriptive purposes only; no peak or response range was inferred from these fits. The translucent vertical bands in panels a and c indicate the operational 90% high-response ranges for total root length and whole-plant dry weight, respectively.

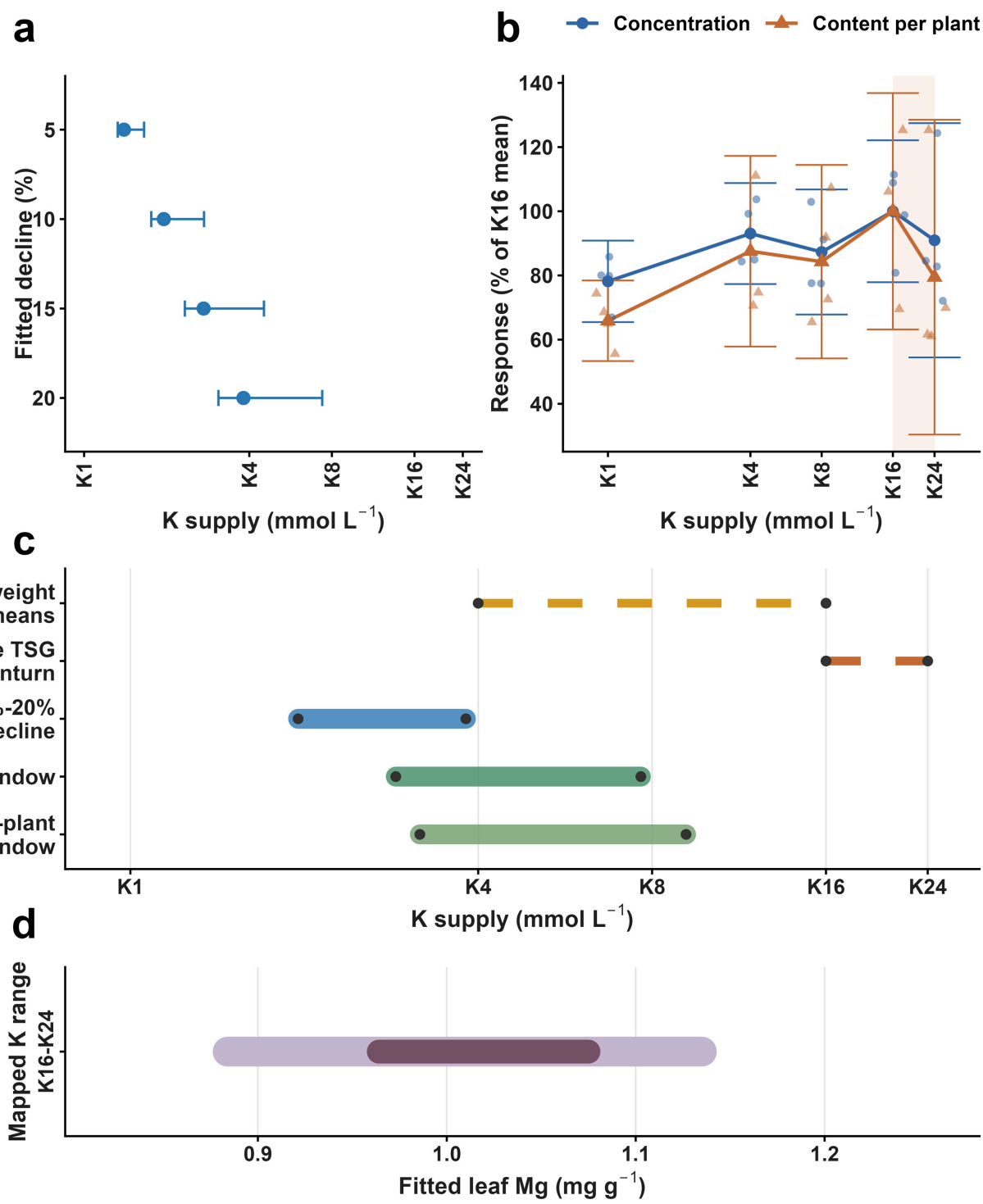


Figure 6

Exploratory treatment-level alignment of leaf Mg decline with the candidate high-K TSG response. (a) Model-derived K positions associated with 5%, 10%, 15%, and 20% declines in fitted leaf Mg concentration relative to fitted K1. Points and horizontal error bars denote estimated positions and 95% bootstrap intervals. (b) TSG concentration and TSG content per plant expressed relative to their respective K16 means (K16 = 100%). The K24 means were 9.05% lower for TSG concentration and 20.54% lower for TSG content per plant than the corresponding K16 means. (c) Treatment-level comparison of the fitted 10%–20% leaf-Mg decline interval, the 90% high-response ranges for total root length and whole-plant dry weight, the K4–K16 interval for leaf dry weight, and the K16–K24 TSG interval. (d) Fitted leaf Mg concentrations corresponding to K16–K24. The darker band denotes the central fitted interval (approximately 0.96–1.08 mg g⁻¹), and the lighter band denotes the outer bootstrap envelope (0.884–1.135 mg g⁻¹).

Supplementary Files

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