

Sufficient potassium supply enhances tolerance of potato plants to PEG-induced osmotic stress

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ABSTRACT

Potassium (K) plays a crucial role in reducing the severity of drought stress. Potatoes are considered drought sensitive crops due to their shallow root system. In this study, potato cultivars 'Milva' and 'Agria' grown under -K (75 μ M) and +K (1000 μ M) supply in a hydroponic system were examined under greenhouse conditions. Half of the plants for each cultivar and K supply were treated for two weeks with polyethylene glycol (PEG) to induce osmotic stress at 67 days after planting (dap). For recovery, plants grew two more weeks without PEG. Plant height, biomass, and water consumption were measured weekly. Leaflets were analysed before, during, and after PEG addition to subsequently determine parameters, for example, total free amino acids and metabolites. Additionally, the K allocation in plant parts was determined. K supply and PEG positively affected biomass production, and the production of side shoots was enhanced with -K and PEG. The water consumption increased with PEG addition in Milva, which was not observed in Agria. K and sugars accumulated in side shoots, showing cultivar and treatments specific reactions. Individual amino acids, including isoleucine, leucine, valine, and alanine, accumulated under low K supply and PEG-induced osmotic stress, and functional characterization could provide information on the response to drought stress. However, K remained an important variable, providing functions of other osmolytes to maintain plant metabolism. The results revealed morphophysiological and biochemical indications for adaption mechanisms against osmotic stress and may help to identify drought stress tolerant cultivars.

Introduction

Climate change influences crop production worldwide due to longer and more unpredictable periods of drought stress (Calzadilla et al., 2013). Drought stress occurs because of reduced water availability in the soil and enhanced water loss through evapotranspiration processes caused by atmospheric conditions (Jaleel et al., 2009). It is one of the most severe factors affecting plant growth and yield in agricultural crops (Bündig et al., 2016). During periods of stress, plants are influenced at different scales ranging from phenological to morphological and molecular levels (Kaur and Asthir, 2017). In such cases, overall plant growth is reduced, where shoots are more inhibited in growth compared to roots (Anithakumari et al., 2012). Moreover, photosynthetic processes are affected by impaired assimilate transport of sugars and amino acids to the plant parts where they are needed (Obidiegwu, 2015), leading to an accumulation of these osmotically active solutes in plant

tissues (Batista-Silva et al., 2019; Dorneles et al., 2021). An increase in organic or inorganic solutes lowers the osmotic potential, improving cell hydration to maintain several metabolic processes under drought stress conditions (Sanders and Arndt, 2012). However, the severity of drought stress depends on the intensity and duration of the stress event and which plant species are affected (Pinheiro and Chaves, 2011). Potatoes are known to be sensitive to drought stress and water deficit due to their poorly developed root system (King et al., 2020). According to Hijmans (2003), the global yield potential of potatoes could decrease by 18–32% between 2040 and 2069 due to drought stress. However, drought stress effects differ in developmental stages during plant growth (Stark et al., 2013; Aliche et al., 2018). Tuber initiation and bulking are the most sensitive stages, while plants at maturity level are more tolerant to drought stress (Hirut et al., 2017). During tuber formation, optimal temperatures between 15–20 °C and soil moisture above 65% favour tuber initiation while higher temperatures and lower soil moisture can

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reduce tuber formation and yield (Thornton, 2020). Consequently, regulating plants' water supply is particularly important, which can be improved especially by an adequate supply of potassium (K) (Kanai et al., 2007; Tavakol et al., 2018). K is the most abundant inorganic cation in plants (Marschner and Rengel, 2012) and is well described as an osmotic substance for maintaining cell turgor during drought stress (Oosterhuis et al., 2014). It regulates stomatal opening and closing, and therefore, water loss through transpiration (Zörb et al., 2014). In addition, sufficient K supply positively affects phloem loading and consequently assimilate transport, e.g., sugars, from source to sink (Mokrani et al., 2018). For most crops, tissue concentrations of K ranging from 5 to 40 mg g⁻¹ DM are considered adequate (White and Karley, 2010). According to Sharma and Arora (1989), the critical K content in leaves of potatoes varies between 3.69 and 5.15%, which is necessary to achieve 95–100% of the yield maximum and refers to the fourth leaf from the top. However, this range depends on the leaves harvested and the maturity of the plants.

The availability of K in plant cells strongly influences a wide range of enzymes for primary metabolism which are related to protein and carbohydrate synthesis (Marschner and Rengel, 2012). Furthermore, K is important for plant growth and improves crop productivity by increasing yield (Zeleeuw et al., 2016). However, the functions of K in osmotic regulation can be replaced by organic acids, amino acids or sugars, whose concentrations increase under K deficiency (White and Karley, 2010). Nevertheless, such production of compatible solutes is more energy-intensive compared to the accumulation of K in plant tissues (Shabala and Shabala, 2011). Adaptation mechanisms to low K supply include a wide set of mechanisms, e.g., the reduction of plant growth for maintaining cell functions due to adequate tissue K concentrations or the redistribution of K to developing plant part (White, 2013). However, even under low K supply, plants can survive because of specialised high-affinity K transporters which were first identified and described for plants in the model plant *Arabidopsis* (Hyun et al., 2014). Maintenance of cellular K homeostasis is the most important function of the high affinity K⁺/K⁺ uptake/K⁺ transporter (HAK/KUP/KT) family (Han et al., 2016), and they are triggered under low K supply (Ashley et al., 2006). Several transporters are described in *Arabidopsis*, but only limited information is available for potato plants. In particular, the expression level of these genes under drought stress or K supply is still scarce and needs to be further explored. The positive effect of K on plants exposed to drought stress affects many metabolic processes in plant development. It has been shown on different plant species that K addition increased biomass partitioning to roots, and enhances leaf membrane stability (Anokye et al., 2021). Moreover, K regulates the photo-assimilation and translocation processes of carbohydrates together with related enzyme activities (Zahoor et al., 2017). In addition, K increases the abscisic acid (ABA) concentration in the leaves, thus reducing the transpiration of plants (Tavakol et al., 2018).

Measuring the effects of drought stress on plants with differing fertilisation regimes on a field scale is challenging. For this reason, controlled (e.g., hydroponic) systems offer a suitable approach to exclude external factors and provide detailed insights into physiological and metabolic processes within a plant. To simulate drought stress, in hydroponic systems, polyethylene glycol (PEG) can be used to induce osmotic stress conditions in plants by promoting water deficiency and imitating soil drying (Dorneles et al., 2021; Emmerich and Hardegee, 1990; Joshi et al., 2011).

The hypothesis that sufficient K supply to the plant under drought stress conditions induced by PEG mitigates the adverse effects by triggering various adaptation mechanisms on several plant levels such as on phenological, morphological and molecular level was the basis for this study. The specific objectives were (I) to investigate the effects of osmotic stress, induced by PEG, on biomass production, water consumption, mineral and sugar allocation in potato plants under low and sufficient K supply, (II) to evaluate changes in metabolites and gene expression levels in leaflets before, during, and after drought stress

simulated by PEG-induced osmotic stress under low and sufficient K supply, and (III) to determine indicators of adaptation strategies by assessed parameters. The investigations were carried out using a hydroponic experiment with PEG 6000 to induce osmotic stress, with the two cultivars, Agria as tolerant and Milva as susceptible to drought stress. Leaf and whole plant analyses were performed to obtain insights into the stress responses of the two cultivars and to gain further information on adaptation mechanisms.

Materials and methods

Experimental setup

Medium-early potato (*Solanum tuberosum* L.) cultivars Milva and Agria (Europlant Pflanzenzucht GmbH, Lüneburg, Germany) were selected according to their drought stress tolerance (further characterisation of the cultivars in Supplementary Table S1). Agria is specified as a tolerant cultivar (Banik et al., 2016; Ahmadi et al., 2017; Alhoshan et al., 2019), whereas Milva is described to be more sensitive to drought stress (Sprenger et al., 2018; Alhoshan et al., 2019). At first, seedlings of potato tubers were grown in 3 L pots filled with quartz sand at a sufficient K supply (600 ppm K₂SO₄, later grown in +K) and low K supply (30 ppm K₂SO₄, later grown in -K). All other nutrients were added in adequate amounts (Supplementary Table S2). At 28 days after planting (dap), the plants were transferred to 5.5 L pots filled with nutrient solutions (Supplementary Table S3), according to Koch et al. (2020). After transferring the plants to the hydroponic system, the nutrient concentration was gradually (20, 40, 60, 100%) increased every 3 days until the desired nutrient level was reached at 35 dap. The nutrient solution was constantly aerated. In addition, K was applied at low levels (75 µm K₂SO₄; designated as '-K') and sufficient levels (1000 µm K₂SO₄; designated '+K'). Twenty biological replicates of each cultivar were grown, using 10 plants for each of the two different K supply regimes. Nutrient solutions were changed twice a week until 53 dap. During the last weeks of the experiment (53-81 dap), nutrient solutions were changed once a week. At 60 dap, osmotic stress was induced by adding 8% (w/v) polyethylene glycol (PEG) 6000 to half of the pots for each cultivar and K supply to simulate drought stress (designated as '+K+PEG' and '-K+PEG', respectively). The osmotic potential of the nutrient solutions with and without PEG was calculated according to Bündig et al. (2017) from measured values determined by using an osmometer (Osmomat 3000 freezing point osmometer, Gonotec® GmbH, Berlin). For nutrient solutions without PEG, an osmotic potential of -0.013 MPa was calculated, while the solutions with PEG revealed a potential of -0.16 MPa. PEG was added over a period of two weeks (60-74 dap) during the regular change of nutrient solution. From 74 to 89 dap, nutrient solutions were changed without the addition of PEG (recovery phase) for both cultivars and K supplies (Fig. 1). The plants grew for a total of 89 days in a greenhouse with 12 h light and 12 h darkness at an average temperature of 20.6 ± 7.5 °C in a completely randomised design.

Phenotypic observation, biomass development and water consumption

Phenotypic changes were recorded during 67 dap by taking photographs of the whole plant. The number of internodes and leaves were counted, and root length was measured using a measuring scale at 34, 41, and 48 dap before inducing osmotic stress with PEG. Plant height and biomass (shoot and roots) were determined weekly after being transplanted to the hydroponic system (29 dap) and each time the nutrient solution was changed (between 39 and 88 dap). Additionally, water consumption was recorded on the same days by weighing the pots directly after and before changing the nutrient solution.

Leaflet sampling and harvest

The youngest fully-developed leaflets were sampled at three time

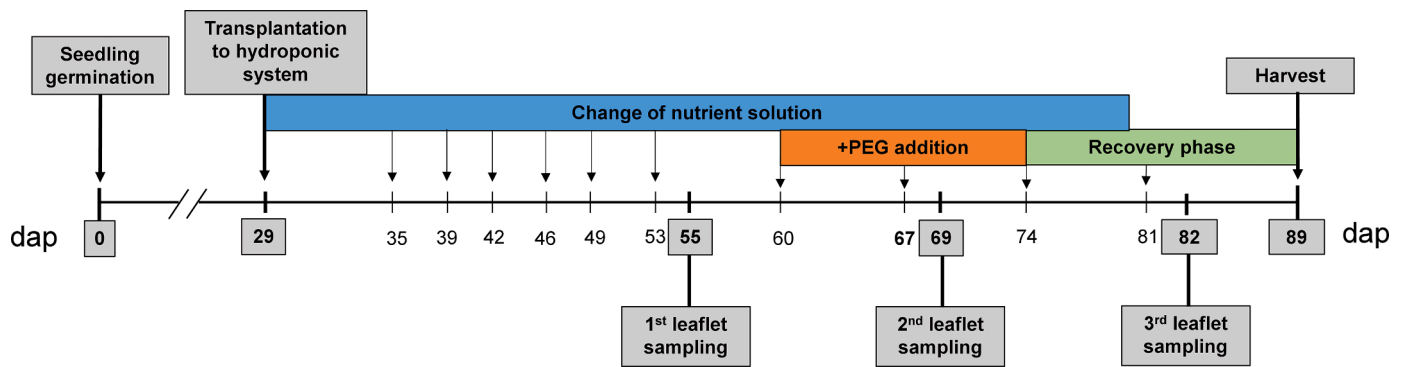


Fig. 1. Timeline of experiment with sampling dates.

points—before (55 dap), during (69 dap), and after (82 dap, recovery phase) PEG-induced osmotic stress (Fig. 1). The youngest fully-developed leaflets were leaves from the upper part of the plant, usually the 3rd or 4th fully-expanded leaf from the top, with ca. 5g FM. An average of 15g FM was removed from each plant for sampling during the experiment, which did not have a strong effect on plant physiology. The harvested leaflet samples were frozen with liquid nitrogen immediately after harvest. Four or five leaves of these leaflets were separated and ground with liquid nitrogen. Chlorophyll, total free amino acids, mineral content, metabolites, and gene expression analysis were determined on leaflets, as illustrated in Fig. 2.

At final harvest (89 dap), the biomass of leaves (only from the main stem), stems (only the main stem), side shoots (side stems with associated leaves), roots, stolons, and tubers were weighed separately to determine the fresh matter (FM). Harvested plant parts were first stored at -20 °C until they were freeze-dried (EPSILON 2-40, Christ, Germany). After freeze drying, samples were weighed again for dry matter (DM) determination, ground into fine powder and stored in dry conditions at room temperature until further measurement. The freeze-dried material of all plant parts was used to determine the mineral and sugar content (Fig. 2).

Potassium, chlorophyll, and total free amino acid analysis in leaflets

Ethanol extraction of chlorophyll was performed on 20 mg fresh leaflet material according to Koch et al. (2019). Chlorophyll a and b were calculated according to the formulae published by Arsovski et al. (2018). Ethanol extraction of free amino acids was performed as described in Chea et al. (2021) on the same leaflet material used for chlorophyll extraction. For amino acid measurement, the ethanolic extract was used and analysed according to Chea et al. (2021).

Metabolite analysis in Milva leaflets

A set of metabolites was determined on fresh Milva leaflets during PEG-induced osmotic stress (69 dap), with the expectation that the more susceptible cultivar would have a stronger metabolite profile response. Immediately after harvest, samples were frozen in liquid nitrogen and stored at -80 °C until further processing. Extraction and analytics were conducted at Lifespin GmbH (Regensburg, Germany). The samples were freeze-dried overnight and ground with a ball mill. Of each sample, an aliquot of 50–100 mg was weighed and extracted for 20 min at 85 °C and 40 min at room temperature after adding 1,500 µL of aqueous phosphate buffer (1 M, pH 6.8, 5% D₂O, 0.01% NaN₃). After centrifugation for 10 min at 10,600 × g, 630 µL of the supernatant was mixed with 70 µL of

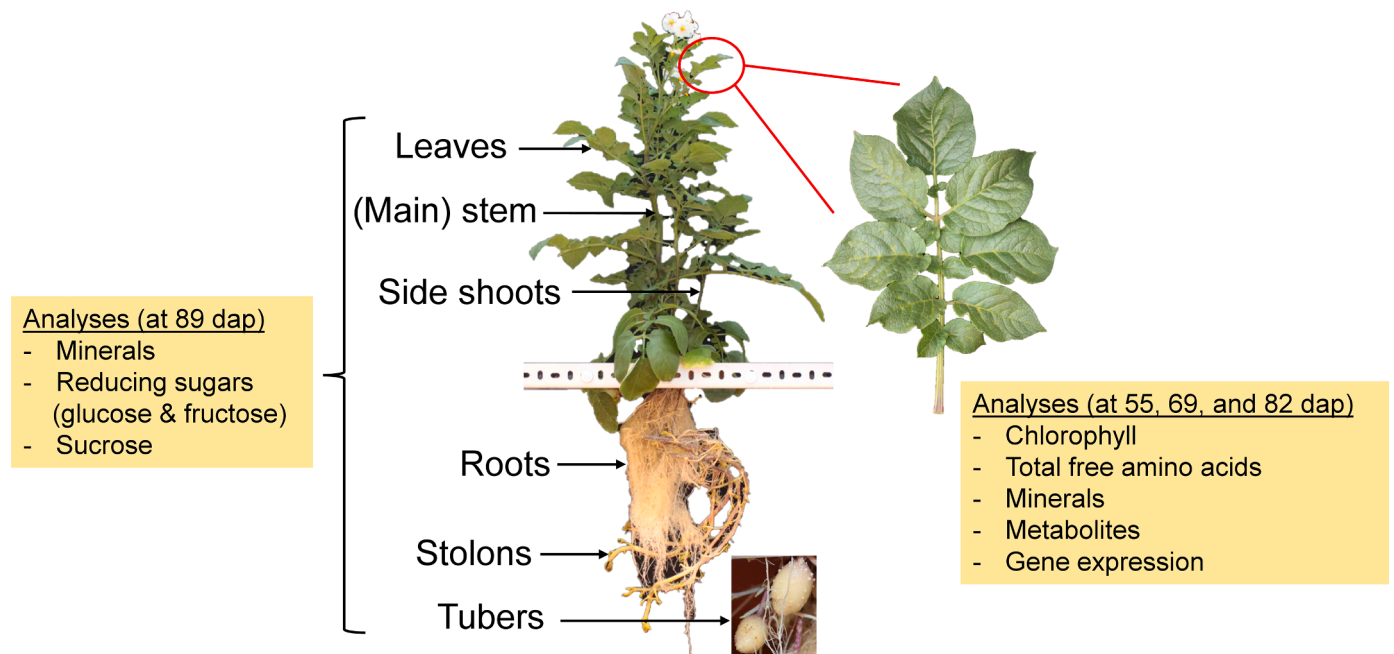


Fig. 2. Schematic illustration of the analyses performed on leaflets and other plant parts.

nuclear magnetic resonance (NMR) additive solution, including trimethylsilyl propionic acid (TSP) as the internal standard. The solution (600 μ L) was filled into NMR tubes (5 mm) and analysed by NMR-spectroscopy using a Bruker AVANCE III HD 600 MHz spectrometer (1D 1H noesygppr, NS=32, T=298K). The resulting spectra were analysed using lispin profiler V1.2 software. This included quality checks by intra- and inter-serial comparison of absolute integrals of internal standard signals, deconvolution of NMR signals, metabolite identification by comparison with lispin databases, and subsequent quantification. The metabolites were quantified against the internal standard TSP (0.0 ppm, 9 protons). Metabolite data were expressed as the fold changes between different K supplies and PEG treatments at 69 dap.

Expression analysis of K transporter StPOTs in leaflets

RNA extraction was performed according to Koch et al. (2019) using the innuPREP Plant RNA Kit (Analytic Jena AG, Jena, Germany). Using the iScript™ cDNA Synthesis Kit (BioRad, California, USA), RNA was transcribed into cDNA according to the manufacturer's instructions. Further, the cDNA was prepared for analysis by RT-PCR using the SsoAdvanced Universal SYBR Green Supermix (BioRad, California, USA). For measurement, the CFX96 Touch Deep Well Real-Time PCR Detection System (BioRad, California, USA) and CFX Manager™ software (BioRad, California, USA) were used. The primer combinations used for RT-PCR are shown in Supplementary Table S4. The house-keeping gene (*StUbiquitin*) was used, as reported by Koch et al. (2020), for normalization the reference at 55 dap was set to 1.0 to show differences compared to the other treatments.

Mineral analysis in leaflets and on plant parts

Mineral analysis was performed according to a method described by Koch et al. (2019) with 100 mg freeze-dried material for all harvested plant parts at 89 dap and the youngest fully-expanded leaflets sampled at 55, 69, and 82 dap. The determination of minerals was performed by using inductively coupled plasma atomic emission spectroscopy (ICP-OES iCap6000Duo, Thermo Fisher Scientific, Waltham, USA). The K content was calculated based on the measured K concentration and individual dry matter of each plant part. The K distribution was further calculated based on the individual dry matter of each plant part and K content.

Soluble sugar analysis on plant parts

Reducing sugars (fructose and glucose) and sucrose were determined in all freeze-dried plant parts at 89 dap. For extraction, 50 mg were weighed into 2 mL reaction tubes and 1 mL of distilled water was added. The samples were vortexed and shaken horizontally for 3 h. For protein precipitation, 50 μ L Carrez I (0.25 M $K_4(Fe(CN)_6) \cdot 3H_2O$) and 50 μ L Carrez II (0.09 M $ZnSO_4 \cdot 7H_2O$) were added to each sample and vortexed. The mixture was centrifuged at $10,600 \times g$ (Eppendorf centrifuge 5804 R; Eppendorf AG, Germany) for 20 min at room temperature. The supernatant was transferred into a new 2 mL reaction tube. Subsequently, samples were filtered (13 mm syringe filter w/ 0.2 μ m PTFE membrane; VWR International, USA) and centrifuged at $10,600 \times g$ again to obtain a transparent solution which was measured by High Performance Liquid Chromatography (LC-2000 Plus, Jasco, Pfungstadt, Germany) according to Koch et al. (2019). The sugar content was calculated based on the measured concentration and individual dry matter biomass of each plant part.

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 25 (IBM statistics, Armond, NY, United States). Data were checked for a normal distribution and

homoscedasticity before performing analysis of variances (ANOVA) to detect differences between the mean values of the treatments, followed by Tukey's post hoc test. All statistical tests were performed at a significance level of $p \leq 0.05$, if not described differently.

Results

Phenotype, plant height, biomass development, and water consumption

The phenotype was influenced by K supply, as shown by the example of cultivar Milva in Fig. 3 (the phenotype of cultivar Agria is shown in Fig. S1). The +K plants produced more biomass and showed no K deficiency symptoms (Fig. 3A). Even after the addition of PEG, the plants remained vigorous and only a few chlorosis and necrosis were observed on the oldest leaves (Fig. 3B). In comparison, the -K plants produced less biomass and both necrosis and chlorosis were observed on the oldest leaves (Fig. 3C). Under PEG addition, this effect was further enhanced, so that the oldest leaves were almost completely necrotic (Fig. 3D).

Plant height and biomass were significantly affected by K supply with higher rates for +K plants compared to -K plants in both cultivars (Fig. 4A–D). The differences between K supplies were higher in Agria starting from the beginning of the measuring period. With PEG addition, plant height and biomass increased for both K supplies compared to plants without PEG addition for 'Agria' and 'Milva'.

However, the effect was not significant. In -K plants, water consumption was significantly reduced compared to +K plants in both cultivars (Fig. 4E, F); but, differences between the K levels were again greater in Agria. The addition of PEG decreased water consumption in Agria at both K levels but increased again after 74 dap (Fig. 4F). In contrast, water consumption in Milva initially increased due to PEG addition in -K and +K plants (67 dap), and a delayed decrease in water consumption was observed at 74 dap (Fig. 4E). However, PEG-induced osmotic stress did not significantly affect water consumption. The number of leaves and internodes was higher in +K plants than in -K plants for both cultivars. Differences were only significant in Agria, which also produced more leaves compared to Milva (Supplementary Table S5).

Potassium, chlorophyll and total free amino acids in leaflets of Agria and Milva

Leaflet samples were taken before PEG addition (55 dap), during PEG-induced osmotic stress (69 dap), and in the recovery phase (82 dap). Higher K concentrations were measured due to increasing K supply in leaflets for both cultivars but with higher concentrations in Milva (Table 1). The addition of PEG did not affect the K concentration in -K and +K plants of both cultivars.

The total chlorophyll (Chl a + b) concentration was higher in -K plants in comparison to +K plants in Milva and Agria at all three growth stages. However, the K supply did not significantly affect the chlorophyll concentrations. The PEG supply also showed no significant influence, but a higher chlorophyll content was found in Agria than in Milva (Table 1). Compared to -K plants, in +K plants, concentration of free amino acids was increased two-fold in Milva and three-fold in Agria. With PEG addition, the concentration of free amino acids was reduced in -K plants compared to +K plants. Further reductions were recorded in the recovery phase for all treatments but to a greater extent for -K+PEG in comparison to -K. In summary, free amino acids were significantly influenced by PEG-induced osmotic stress and K supply but not by cultivar (Table 1).

Metabolites in Milva leaflets

A total of 42 different metabolites, such as organic acids, amino acids, and sugars, in all treatments were measured in Milva leaves during osmotic stress (69 dap). All values were represented as fold

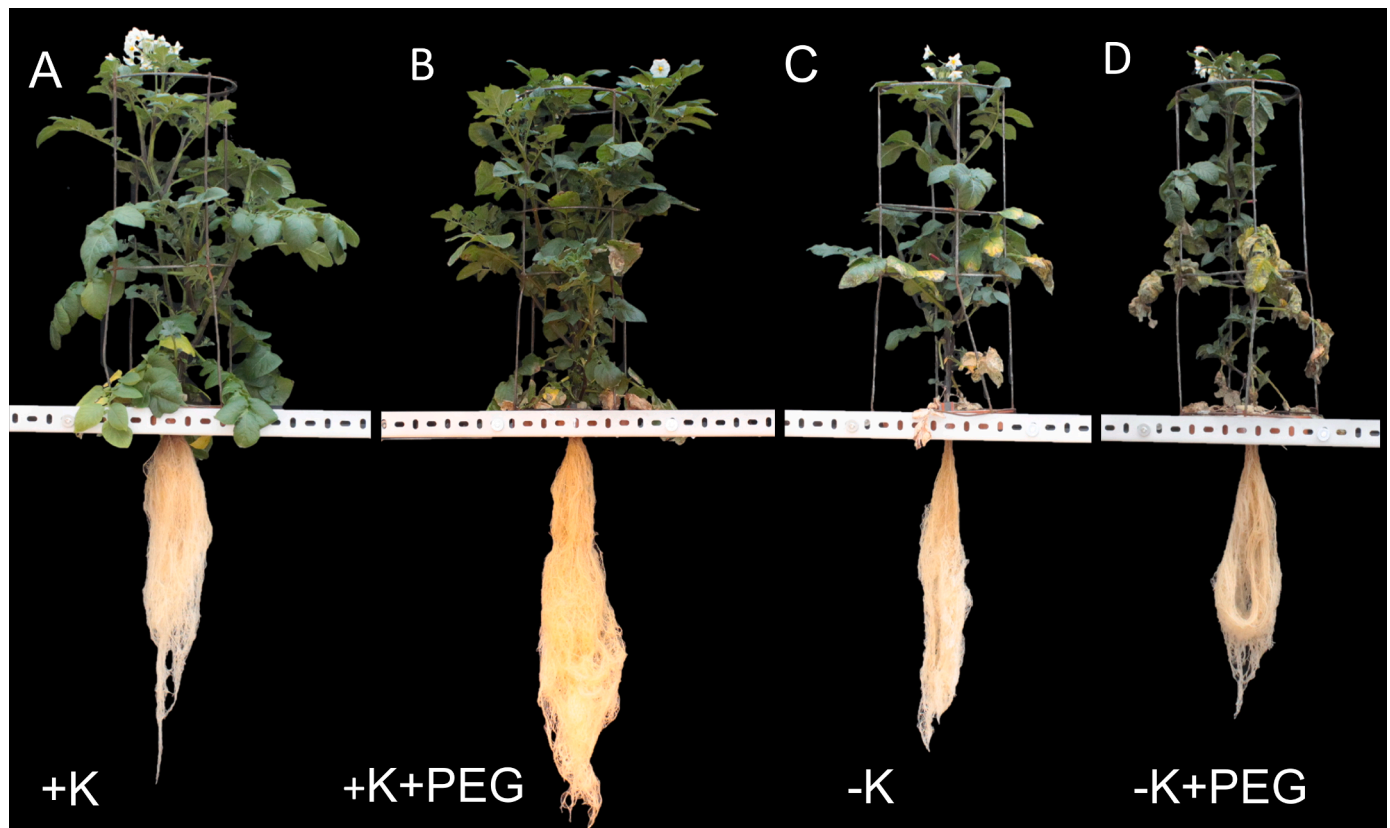


Fig. 3. Phenotype of +K (A), +K+PEG (B), -K (C), and -K+PEG (D) plants of cultivar Milva on 67 days after planting (dap).

changes on 69 dap to evaluate the effect of K or PEG-induced osmotic stress (-K/+K; -K+PEG/+K+PEG; +K+PEG/K; and -K+PEG/-K; Fig. 5). Raw data can be obtained from Supplementary Table S6.

In the group of organic acids, -K plants showed an increase for intermediates of the tricarboxylic acid cycle (TCA) of 9.8- and 4.3-fold in α -ketoglutarate and succinate, respectively, compared to +K plants. For the same metabolites, -K+PEG plants showed a reduction of 80 and 70%, respectively, compared to -K plants. The concentration of citrate increased 1.8-fold in -K+PEG-treated plants compared to +K+PEG plants (Fig. 5). However, a reduction of 40 and 50% in PEG-treated plants at both K levels was observed. Single amino acids such as lysine, leucine, and proline accumulated two to three-fold higher in leaves of -K+PEG plants compared to +K+PEG plants. Proline, especially, showed a 5.2-fold and 3.6-fold increase in -K+PEG-treated plants compared to those treated with +K+PEG and -K, respectively. However, neither K supply nor PEG-induced osmotic stress affected the concentration of monosaccharides fructose and glucose (Fig. 5). Only sucrose increased by 1.7-fold in -K plants compared to +K plants and 2.8-fold in +K+PEG plants compared to +K plants. However, in -K+PEG plants fructose and glucose were reduced 40 and 60% compared to +K+PEG and -K plants, respectively.

Gene expression

The relative expression of three K transporters—*StPOT2*, *StPOT4*, and *StPOT12*—were examined in leaflet samples before (55 dap), during (69 dap), and after PEG-induced osmotic stress (82 dap). Transporters were analysed in leaves, since first deficiency symptoms were observed in the above-ground biomass. Furthermore, expression of these transporters might give additional information about K distribution processes within leaflets. In Milva, expression of all three *StPOTs* was two-fold higher in the -K plants before PEG induction (55 dap) compared to the +K plants. Neither *STPOT2* nor *StPOT4* showed a significant response to

the addition of PEG at both K levels (Table 2). However, *StPOT4* was upregulated in the +K and +K+PEG plants but downregulated in -K and -K+PEG plants at 69 dap (Table 2). In contrast, *StPOT12* was downregulated at 69 dap for all treatments and increased again in the recovery phase, mainly in -K and -K+PEG plants (Table 2). In contrast to Milva, the expression of all three *StPOTs* was significantly lower in the -K-treated Agria plants at 55 dap. In Milva, a reduction in expression was observed for all treatments during PEG induction, especially the expression of *StPOT12* at 69 dap (Table 2).

Biomass, K and sugar content, and their distribution in plant parts at final harvest

More biomass was produced in +K compared to -K plants of both cultivars, but differences were only significant in Agria. In Milva, +K+PEG and -K+PEG plants produced 13 and 43% more biomass, respectively, than plants of the same K supply without PEG (Fig. 6A). Side shoots made up the largest proportion of the biomass, followed by leaves. In comparison to the +K-treated plants, the -K plants produced fewer stems, whereas the root biomass developed equally. A similar distribution was observed in Agria, although the +K+PEG plants did not produce more biomass compared to +K plants. The +K plants of Milva accumulated up to three-fold higher (40 mg DM) K on the whole plant level compared to -K plants (12 mg DM; Fig. 6B). A higher K content was found in +K plants compared to +K+PEG Milva plants, although the biomass was higher in +K+PEG plants.

The highest K content was measured in side shoots and leaves and thus correspond to the biomass and K distribution of individual plant parts (Fig. 6B, C). Compared to +K plants, less K was translocated into the leaves and stems of -K plants, reflecting the biomass and K distribution for both cultivars. However, the K content in Agria was almost equal between +K and +K+PEG plants, although the biomass of +K+PEG plants was 20 g lower based on DM. The results for stolons and

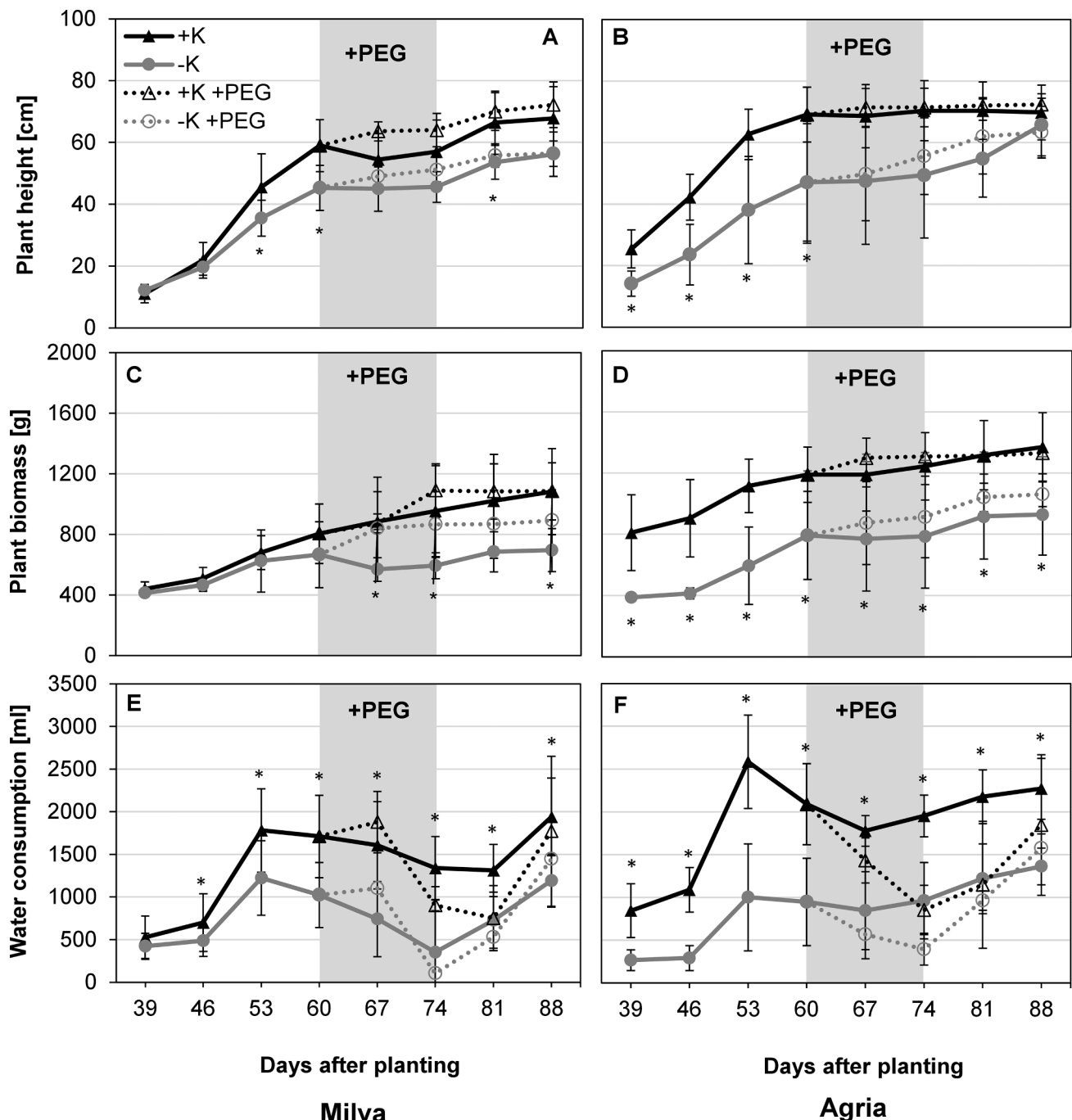


Fig. 4. Effect of potassium (K) supply and simulated drought stress by polyethylene glycol (PEG)-induced osmotic stress on plant height (A, B), plant biomass (C, D), and water consumption (E, F) of 'Milva' and 'Agria' at 39 to 88 dap. Mean \pm standard deviation (SD), $n = 10$ until 60 dap; $n = 5$ from 60 until 88 dap. * = significant differences between +K and -K for each investigated growth stage analysed by Tukey's post hoc test at $p \leq 0.05$.

tubers are shown in Supplementary Table S7.

Reducing sugars accumulated more in -K plants compared to +K plants of both cultivars (Fig. 7A). However, almost twice the content of reducing sugars was detected in +K and +K+PEG plants of Milva than in Agria. Within plant parts, reducing sugars were accumulated mostly in side shoots and leaves at both K levels and in both cultivars. PEG-induced osmotic stress did not affect the accumulation of reducing sugars in -K and +K plants. In contrast to reducing sugars, the sucrose content was not affected by K treatments. However, the sucrose content was two to three-fold lower than the reducing sugar content and was highest in side shoots and leaves (Fig. 7B). In Milva more sucrose was measured in +PEG treated plants at both K supplies, while the opposite

was observed in Agria. The results for stolons and tubers are shown in Supplementary Table S8.

Discussion

Effect of K supply and drought stress simulated by PEG-induced osmotic stress on phenotype, biomass, water consumption, and the K and sugar distribution on the whole plant level

For adaptations to changing environmental conditions, it is important to understand the morphophysiological and metabolic processes of plants to provide specific stress-mitigation strategies. In this study,

Table 1

Influence of K supply and drought stress simulated by PEG-induced osmotic stress on concentrations of K, chlorophyll a + b, and free amino acids (55 dap), during (69 dap), and after osmotic stress induced by PEG (82 dap, in the recovery phase) in the youngest fully-developed leaves.

Cultivar [cv]	K supply [K]	K [mg g ⁻¹ DM]			Chl a+b [mg g ⁻¹ FM]			Free amino acids [μmol g ⁻¹ FM]		
		dap	55	69	82	55	69	82	55	69
Milva	+K	36.98 ±8.63 ^a	20.31 ±4.41 ^a	31.80 ±7.68 ^a	0.55 ±0.08 ^a	0.52 ±0.12 ^a	0.81 ±0.07 ^a	23.71 ±4.35 ^a	19.71 ±3.28 ^b	8.89 ±3.60 ^b
	-K	12.69 ±2.11 ^b	7.56 ±2.67 ^b	8.92 ±4.59 ^b	0.60 ±0.22 ^a	0.58 ±0.14 ^a	0.69 ±0.22 ^a	44.27 ±6.17 ^b	55.94 ±19.12 ^a	49.99 ±30.61 ^a
	+K +PEG	-	21.70 ±2.32 ^a	22.49 ±2.47 ^a	-	0.48 ±0.15 ^a	0.84 ±0.11 ^a	-	20.04 ±6.50 ^b	14.75 ±10 ^b
	-K +PEG	-	9.92 ±2.80 ^b	8.56 ±1.55 ^b	-	0.76 ±0.28 ^a	0.79 ±0.16 ^a	-	42.74 ±9.62 ^a	22.32 ±4.42 ^{ab}
	+K	27.30 ±4.13 ^a	20.77 ±3.91 ^{ab}	23.42 ±3.89 ^b	0.60 ±0.21 ^a	0.88 ±0.59 ^a	0.76 ±0.17 ^a	15. ±8.00 ^b	15.93 ±3.45 ^b	13.99 ±5.49 ^b
	-K	12.03 ±2.12 ^b	12.32 ±4.66 ^b	8.83 ±2.13 ^b	0.85 ±0.22 ^a	0.99 ±0.18 ^a	0.83 ±0.07 ^a	46.27 ±5.13 ^a	60.49 ±31.91 ^a	60.38 ±22.17 ^a
Agria	+K +PEG	-	31.82 ±9.21 ^a	62.48 ±19.14 ^a	-	0.75 ±0.21 ^a	0.98 ±0.18 ^a	-	25.88 ±10.07 ^b	11.72 ±4.11 ^b
	-K +PEG	-	21.38 ±14.50 ^{ab}	10.03 ±5.01 ^b	-	0.78 ±0.20 ^a	0.81 ±0.05 ^a	-	37.84 ±4.40 ^{ab}	29.80 ±8.82 ^b
	Significance	K	***	***	***	n.s.	n.s.	***	n.s.	***
	cv	*	*	*	n.s.	**	n.s.	n.s.	n.s.	
	PEG	*	*	*	n.s.	n.s.	n.s.	n.s.	**	
	PEGx cv	*	***	*	n.s.	n.s.	n.s.	n.s.	n.s.	
	Kx cv	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	
	KxPEG	*	*	*	n.s.	n.s.	n.s.	n.s.	***	
	KxPEGx cv	*	***	*	n.s.	n.s.	n.s.	n.s.	n.s.	

Mean ± SD (n = 3–5). Lowercase letters = differences between K supply treatment within cultivar and growth stage. Levels of significances were tested via analysis of variances (ANOVA) with *, **, and *** for p ≤ 0.05, 0.01 and 0.001. n.s. = not significant. Statistical influence of PEG analysed within 69 and 82 dap by Tukey's post hoc test at p ≤ 0.05

osmotic stress was induced by adding PEG—with an osmotic pressure in the nutrient solution of -0.16 MPa—to simulate drought stress under hydroponic conditions. Two different K rates were applied to investigate the effect of K on plants tolerance to osmotic stress. Phenotypic observations revealed more biomass and almost no osmotic stress symptoms due to +K fertilisation. In comparison -K plants showed typical chloroses and necrosis on older leaves, which were even more severe with PEG addition. PEG symptoms on older leaves were also described by Büssis et al. (1998), which might be related to a water deficit.

Furthermore, the results of this study showed that K supply has a wide range of effects on plant physiological parameters. Weekly determined plant height and biomass decreased under -K conditions compared to +K for both cultivars. This is in accordance with a study on potatoes (Koch et al., 2019) and other plant species, including tomato (Kanai et al., 2007) and wheat (Thornburg et al., 2020). A reduction in plant growth is known to be a physiological adaption to insufficient K supply for maintaining the tissue K concentration sufficient for several cell functions (White and Karley, 2010). During PEG-induced osmotic stress, plants were still able to grow under both K treatments, which was also observed by Büssis et al. (1998). However, in contrast to their study where PEG-treated plants were inhibited in growth, in our experiment, +PEG plants showed higher growth rates within the osmotic stress period. An increase in growth due to the influence of PEG has been rarely described (Khalid et al., 2010), and several studies show contrasting results (Pei et al., 2010; Barra et al., 2013), primarily when investigated under *in vitro* conditions. According to Ahmad et al. (2020), who investigated the *in vitro* growth processes of *Stevia rebaudiana*, it was presumed that water deficiency induced by PEG at a critical level led to the manipulation of plant physiology and biochemistry. Changes in the cellular environment can result in stress stimuli affecting cellular receptors and further triggering signal cascades involved in physiological, and therefore growth processes. Another explanation by Khalid et al. (2010) refers to an increased carbohydrate and mineral content including K, N, and P under PEG addition, which enhanced the growth parameters of *Pelargonium odoratissimum* (L.). This could also explain the results of our study, as increased concentrations of carbohydrates were

found in the +PEG treated plants for both cultivars and K levels, except for sucrose content in Agria.

Similar to plant height and biomass, K deficiency negatively affected water consumption, as root growth is frequently reduced under deficient K supply (Koch et al., 2019). For Milva, water consumption increased in the first week after PEG addition in +K+PEG and -K+PEG plants compared to plants without PEG. This was not observed for Agria, where water consumption decreased in PEG-treated plants in both K supplies when osmotic stress was induced. In a study by Dorneles et al. (2021), the stress responses of potato plants exposed to water deficit under osmotic and matric induction were investigated. They observed a more negative osmotic potential in plants than in nutrient solutions containing PEG, suggesting that plants osmotic potential may promote an osmotic force and thus water uptake under PEG conditions. This could explain the briefly higher water uptake rates of Milva immediately after PEG addition for both K supplies, indicating cultivar-specific responses to osmotic stress. Maintaining water consumption and general growth processes, despite stress situations, could therefore reflect a possible tolerance mechanism to osmotic and drought stress in Milva.

At final harvest, the total biomass was lowest in -K-treated plants than +K plants for both cultivars. Sufficient K supply enhances photosynthetic processes, leading to increased leaf area expansion, which results in elevated biomass production (Tavakol et al., 2018) Milva produced more biomass during PEG-induced osmotic stress for both K treatments, whereas +PEG Agria plants produced a higher biomass only under -K, probably related to increased carbohydrate and mineral content, enhancing growth parameters (Khalid et al., 2010). Overall, the biomass of side shoots was higher in -K plants compared to +K plants, in which the proportion of leaves was greater. Presumably, the plants under K deficit attempt to maintain the photosynthetic capacity by developing more side shoots to increase leaf biomass. Schittenhelm et al. (2006) showed that potatoes can produce a large above-ground biomass as a strategy against soil water deficit. Accordingly, the increased biomass of side shoots in the -K+PEG plants could be the result of a similar mechanism to maintain biomass production under osmotic stress. This may as well be an adaptation to K deficiency, such that -K

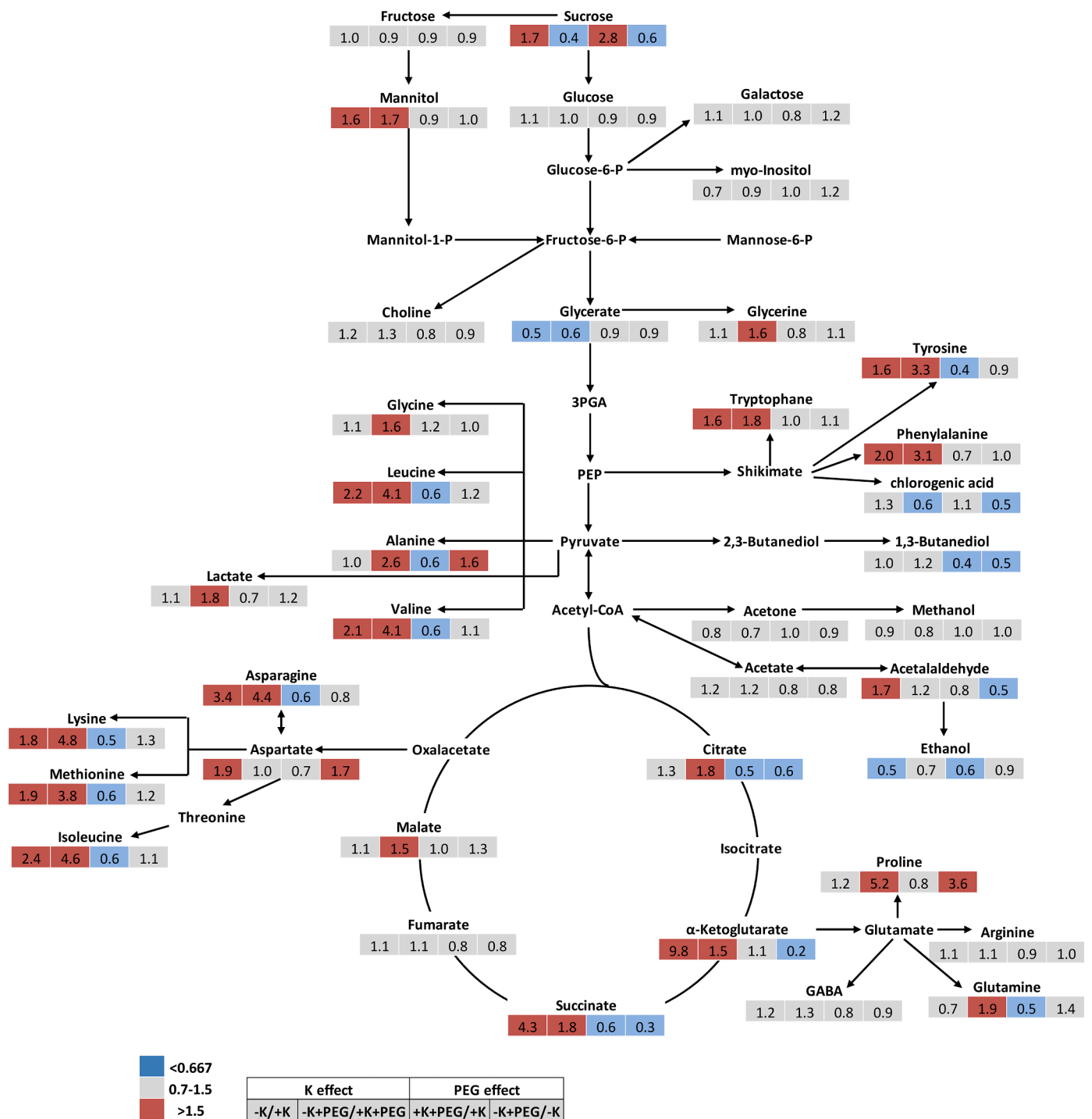


Fig. 5. Effect of K deficiency and PEG-induced osmotic stress on metabolites in leaves of potato plants cultivar Milva. The relative ratios were calculated by the division of metabolite concentration in leaves of -K/+K, -K+PEG/+K+PEG, +K+PEG/+K and -K+PEG/-K during PEG stress at 69 dap. (n = 4). Red and light blue present the significant increase (>1.5 = 1.5-fold increase) and decrease (<0.667 = 1.5-fold decrease) (p ≤ 0.05), respectively. 3PGA = 3-phosphoglycerate; PEP = Phosphoenolpyruvate; Acetyl-CoA = Acetyl Coenzyme A; TCA = tricarboxylic acid; GABA = γ-aminobutyrate. Arrows with one direction show synthesis of a metabolite and arrows with double direction show reversible reactions.

plants increased the leaf area by producing additional shoots, thus maximising the potential for additional photosynthetic activity. However, the cultivars used in this study could be divided according to their shoot morphology, which is based on genotypic characteristics. Cultivar Agria, used in our study, belongs to the stem type (according to the International Union for the Protection of new Varieties in Plants, UPOV), supporting the results of stem biomass for +K plants, which was higher than that in Milva. In contrast, Milva belongs to the intermediate type

and equally forms both stems and leaves. Therefore, the increased biomass production of side shoots could be an adaptation to the prevailing conditions. Another reason for the high production of side shoots could be due to the experimental arrangement, as under greenhouse conditions light was available from all sides and the plants had enough space to spread out, which is usually not the case in the field due to narrow plant spacing.

Reducing sugars accumulated more in -K plants than in +K plants.

Table 2

Relative expression of K transporter *StPOT2*, *StPOT4* and *StPOT12* before (55 dap), during (69 dap), and after (82 dap) drought stress simulated by PEG-induced osmotic stress for plants treated with -K and +K in Agria and Milva.

Cultivar [cv]	K supply [K]	<i>StPOT2</i>								
		[rel. expression]			[rel. expression]			[rel. expression]		
		dap	55	69	82	55	69	82	55	69
Milva	+K	1.43	1.71	2.43	1.16	3.18	2.89	1.63	0.46	5.19
	-K	±1.04 ^b	±1.41 ^a	±0.68 ^a	±0.61 ^b	±0.47 ^a	±1.17 ^a	±1.20 ^b	±0.30 ^a	±3.77 ^a
	+K +PEG	3.59	1.40	4.89	3.14	1.80	5.15	4.80	1.18	8.10
	-K +PEG	±1.54 ^a	±0.55 ^a	±3.26 ^a	±1.26 ^a	±0.44 ^a	±4.51 ^a	±2.01 ^a	±0.77 ^a	±7.12 ^a
		-	1.45	1.76	-	2.34	2.00	-	1.73	1.83
Agria	+K	1.23	0.28	1.27	1.35	0.51	1.84	1.64	0.15	2.42
	-K	±0.68 ^a	±0.15 ^a	±1.64 ^a	±0.96 ^a	±0.31 ^a	±2.28 ^a	±1.09 ^a	±0.13 ^a	±3.01 ^a
	+K +PEG	1.01	0.35	2.05	0.63	0.61	2.83	0.74	0.06	3.40
	-K +PEG	±0.44 ^a	±0.01 ^a	±1.07 ^a	±0.32 ^a	±0.11 ^a	±2.18 ^a	±0.50 ^a	±0.01 ^a	±2.62 ^a
		-	0.28	1.50	-	0.56	1.53	-	0.09	1.21
Significance	K		***			n.s.		**		
	cv		***			*		**		
	PEG		n.s.			n.s.		n.s.		
	PEGx cv		n.s.			n.s.		n.s.		
	Kx cv		*			n.s.		n.s.		
	Kx PEG		n.s.			n.s.		n.s.		
	Kx PEGx cv		n.s.			n.s.		n.s.		

Mean ± SD ($n = 4$). Lowercase letters = differences between K supply treatment within cultivar and dap. Levels of significance were tested via ANOVA with *, **, and *** for $p < 0.05$, 0.01 and 0.001 , respectively. n.s. = not significant. Statistical influence of PEG analysed within 69 and 82 dap by Tukey's post hoc test at $p < 0.05$

Since K is involved in phloem loading (Tränkner et al., 2018), impaired sucrose transport from source leaves to sink organs is a result of K deficiency (Zörb et al., 2014). Compared to +K plants, reducing sugars accumulated more in the roots of -K plants. This is in accordance with Sung et al. (2015), who found high concentrations of sugars in the roots of tomatoes grown under K deficient conditions. However, compared to the concentration of sugar in the roots, more reducing sugars were accumulated in the stems of the -K plants, which, in turn, could indicate an inhibited sugar transportation. Overall, the influence of PEG-induced osmotic stress also tended to affect the sugar concentration. Thus, +K+PEG and -K+PEG plants produced more sugars compared to plants without PEG addition. However, +K+PEG-treated Agria plants were an exception, suggesting that Agria is less susceptible to osmotic stress and may also be less susceptible to drought stress under optimal K supply. This also confirms the classification from different studies, where Agria was considered a more tolerant cultivar to drought stress under pot and field conditions (Banik et al., 2016; Ahmadi et al., 2017; Alhoshan et al., 2019). However, other studies classified Agria as sensitive to drought stress in a pot experiment (Demirel et al., 2020) or as susceptible to water deficit under field conditions (Hassanpanah, 2010). Thus, there is no clear classification for Agria. In contrast, Milva was described as sensitive to drought stress (Sprenger et al., 2018; Alhoshan et al., 2019) in pot experiments under greenhouse and under field conditions, which could not be confirmed in our study. The literature review showed that due to very different experimental conditions, an exact classification and a respective comparison is not easily possible. However, our investigation has shown that Milva and Agria can adapt to PEG-induced osmotic stress under hydroponic conditions. These strategies could also be used when adapting to drought stress under field conditions. Comparing matrix and osmotic stress, Dorneles et al. (2021) found that stress responses to both forms of stress were similar. Accordingly, the results from the hydroponic system may be applied to field conditions.

Changes in leaflet parameters due to K supply and drought stress simulated by PEG-induced osmotic stress at different growth stages

Leaf samples at three different growth stages during the experiment

provided detailed information on the physiological and physiochemical processes under altered conditions induced by sufficient and deficient K supply, and by PEG-induced osmotic stress. The K content in the leaf was positively influenced by the K supply, so that three-fold more K was detected in the leaves of +K plants compared to -K plants. Similar results were found by Koch et al. (2019). Nevertheless, the K content, even in leaves of +K plants, was below the range of 5–40 mg K (g^{-1} DM) indicating an adequate supply of K according to White and Karley (2010). However, since the percentage of K in the DM decreased significantly during growth, this value may not be suitable for predicting K deficiency. Thus, a clear indication of an optimal K concentration should be defined for each growth stage and crop species (Johnson and Goulding, 1990).

Chlorophyll, being an important pigment to absorb light energy (Kayser and Aversch, 2015), was not affected by K supply. In studies by Armengaud et al. (2009) on *Arabidopsis* and by Veazie et al. (2020) on *Brassica rapa*, the chlorophyll content was not affected by K deficit. Presumably, K was directed into the older leaves or side shoots to maintain chlorophyll production by activating specific enzymes. However, several studies revealed a decreased chlorophyll content in leaves of plants under low K supply (Lu et al., 2016), which was demonstrated by the decreased photosynthetic efficiency. Similarly, the effect of PEG-induced osmotic stress did not affect the chlorophyll content. However, both chlorophyll a and b content decrease because chlorophyll synthesis is inhibited by water deficit (Anithakumari et al., 2012; Kaur and Asthir, 2017), which was obviously not severe in our study. Generally, the chlorophyll content varies strongly in potato leaves, depending on the cultivar, time of drought stress induction (van der Mescht, 2010) and leaf development (Büssis et al., 1998). The decrease in chlorophyll content was caused by reactive oxygen species that are formed as a response to drought stress damaging chloroplasts (Ashraf and Harris, 2013). This was not demonstrated in our study, perhaps because of the short osmotic stress period. It is also likely that the stress situation occurred at a time when the plants were already fully mature, and therefore less susceptible to abiotic stress.

To protect cellular structures and maintain important physiological processes, plants can accumulate low-molecular-weight compounds,

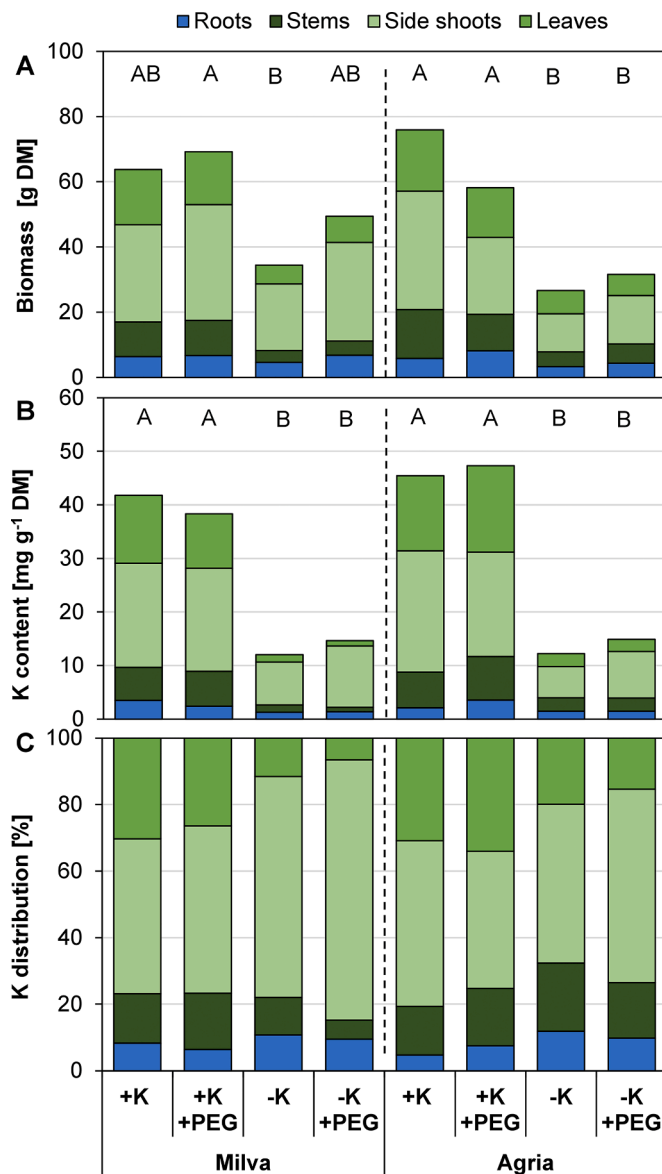


Fig. 6. Biomass (A), K content (B), and K distribution (C) in plant compartments of Milva and Agria influenced by K supply and drought stress simulated by PEG-induced osmotic stress. Sum of mean values of different plant parts ($n = 5$). Capital letters = significant difference for total biomass and K content within one cultivar analysed by Tukey's post hoc test at $p \leq 0.05$.

which are involved in osmotic adjustment during stress conditions (Rawat et al., 2016). These include amino acids, measured as free amino acids in leaflets in this study, before, during, and after PEG-induced osmotic stress. In both cultivars at each sampling day, K deficiency increased the free amino acid concentration. This effect might be related to the substitution of K by organic solutes. An increased concentration of organic solutes, such as amino acids, lowers the osmotic potential of plant cells, improving cell hydration and therefore cell turgor to maintain physiological and metabolic processes, which enhance plant growth under stress conditions (Sanders and Arndt, 2012). Interestingly, the free amino acid content of leaves during PEG induction was not affected by PEG addition in +K plants but reduced in -K+PEG plants compared to -K plants in our study. A sufficient K supply enhanced amino acid accumulation during PEG-induced osmotic stress compared to that at a low K supply. However, the K contents in the leaves increased during the same period. In accordance with Shabala and Shabala (2011), under K deficiency and PEG-induced osmotic stress, K ions were distributed to

developing tissues and accumulated in favour of osmotic compounds since production of these compounds is metabolically more expensive for plants.

Effect of K supply and drought stress simulated by PEG-induced osmotic stress on the metabolite profile of Milva leaflets

To characterise the metabolic responses of the plant during stress mitigation under different K supplies, metabolite profiling was used to investigate changes in the concentrations of primary and secondary metabolites. In the metabolomic pathway for Milva, single amino acids showed a higher concentration in -K plants compared to +K plants, which was also shown by higher concentrations of free amino acids in -K leaflets. This increase was even higher when comparing -K+PEG to +K+PEG plants. In some plant species, amino acids are known to increase during drought stress or K deficiency, which was already explained in the previous section.

Proline increased under PEG-induced osmotic stress in -K+PEG plants compared to +K+PEG (5.2-fold) and -K (3.6-fold) plants. Proline concentrations are frequently considered a metabolic stress indicator for several stress factors and its accumulation is often associated with tolerance mechanisms against abiotic stress (Szabados and Savouré, 2010). Many studies have confirmed that proline accumulates in plant leaves for osmotic adjustment (Schafleitner et al., 2007; Liu et al., 2019). In this regard, K deficiency alone did not increase the concentration of proline in the sample in our study, which indicates the importance of the proline accumulation under K deficiency when at the same time leaves are exposed to osmotic stress. Furthermore, the amino acids isoleucine, leucine, and valine accumulated, which has been shown in *Arabidopsis thaliana* plants exposed to drought stress and was caused by abscisic-acid regulated protein degradation (Huang and Jander, 2017). These amino acids belong to branched chain amino acids formed by pyruvate or aspartate. They are involved in abiotic stress response and act as signaling molecules further regulating gene expression (Nambara et al., 1998). The increase in concentration of these branched chain amino acids might therefore reflect a response mechanism that is triggered under K deficiency with more pronounced effects when K deficient leaves are exposed to osmotic stress. Another branched chain amino acid is alanine, which is also formed by pyruvate and its accumulation was reported to occur under PEG-stress (Batista-Silva et al., 2019). Furthermore, Lea et al. (2007) could show that asparagine and lysine accumulate in plant tissues under different stress conditions, revealing stress responses due to K deficiency and PEG, which was also shown in our study. However, the concentrations of amino acids vary strongly due to the stress effect (K supply or PEG), since individual amino acids react differently to various factors. Therefore, it is difficult to compare studies determining amino acids because of the differences in growth and environmental conditions (Ammann and Rubio, 2012). Responses of the mentioned free amino acids in potato leaves might provide information related to secondary metabolites of interest.

Accumulations were also observed due to -K for some organic acids involved in the TCA cycle, including α -ketoglutarate and succinate, whereas PEG led to a reduction in both K treatments. In rapeseed, Zhu et al. (2020) showed an increase in α -ketoglutarate and succinate under low K and drought stress conditions. Contrasting results were found by Das et al. (2017) in soybeans, where α -ketoglutarate and succinate decreased. The generally low accumulation of organic compounds and higher contents of TCA intermediates could indicate enhanced respiratory rates and energy metabolism (Warth et al., 2015). However, this supports the presumption that K accumulation as an osmotic adaptation against drought stress, is significantly more energy efficient than the formation of organic solutes (Shabala and Shabala, 2011). K is transported into young leaves via the phloem during stress to fulfill the osmotic function of other solutes. This could explain the lower sugar content in the youngest fully-developed leaves and the increased sucrose content in the -K+PEG plants on the whole plant level, at least for

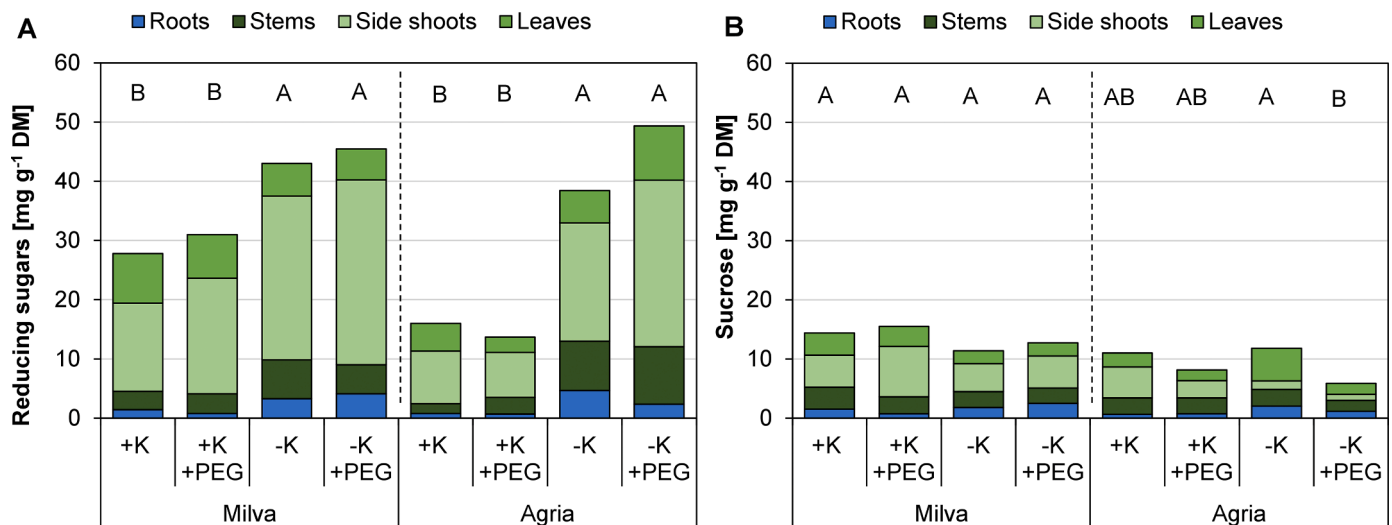


Fig. 7. Influence of K supply and drought stress simulated by PEG-induced osmotic stress on reducing sugars (A) and sucrose (B) in roots, stems, side shoots and leaves at final harvest (89 dap). Sum of mean values of different plant parts ($n = 5$). Capital letters = significant difference concerning total concentration of reducing sugars or sucrose within one cultivar analysed by Tukey's post hoc test at $p \leq 0.05$.

cultivar Milva. However, differences in metabolite profiles vary strongly depending on the type of stress, duration, severity, and cultivar.

Influence of K supply and drought stress simulated by PEG-induced osmotic stress on the expression of K transporters in leaflets

For potatoes, there is less information on K transporters and how they respond to nutrient deficiencies and abiotic stress. The analysed K transporter genes (*StPOTs*) belong to the HAK/KUP/KT family, which have different functions in K uptake and transport (Osakabe et al., 2013). In addition, they play a role in stress tolerance and osmotic potential regulation (Li et al., 2018). The expression of related genes is often triggered by a low K supply, enhancing the overall K uptake (Ashley et al., 2006). The genes of this family are expressed in different plant parts, e.g., in the leaves, roots, or stems, which indicates a connection with the primary uptake of K from the soil, as well as with cellular K homeostasis (Hyun et al., 2014). The transporters studied—*StPOT2*, *StPOT4*, and *StPOT12*—correspond to homologous genes of *Arabidopsis thaliana*, where they are referred to as *AtKUP2*, *AtKUP4*, and *AtKUP12*, respectively. They belong to different clusters in which the various transporters can be subdivided according to their sequence homology (Ragel et al., 2019). *AtKUP2* and *AtKUP4* are assigned to cluster II and described as low-affinity K transporters involved in cell expansion (Rigas et al., 2001). The transporters of these clusters have many homologous transporters from other plant species and are described in more detail. However, *AtKUP12* is assigned to cluster III and has rarely been studied (Véry et al., 2014). Our results showed different expressions of the three transporters depending on K supply, PEG-induced osmotic stress, and cultivar. *StPOT2* and *StPOT4* were more highly expressed in Milva and showed greater expression rates in -K plants at 55 dap. *StPOT* expression in Agria decreased at 69 dap. The expression rates of all K transporters increased again for all treatments and both cultivars during the recovery phase. Due to the fact, that the plants were already mature and had almost completed their growth cycle, the demand for K in young tissues was no longer as high as at the beginning of plant growth, which could explain the low expression levels of all the three *StPOTs* in Agria. Thus, Milva was not yet fully matured and was still taking up K. Accordingly, *StPOT2* and *StPOT4* were still active in Milva since both are related to growth processes. Compared to Agria, higher expression rates of the three *StPOTs* in Milva could indicate differences in the involved genes, which may vary genotypically.

StPOT2 in potato and the homolog gene *AtKUP2* in *Arabidopsis*, as well as *AtKUP6* and *AtKUP8*, play a role in lateral root development by enhancing abscisic acid and responding to osmotic stress. In contrast, *AtKUP4* is involved in growth processes by participating in auxin transport, which in turn contributes to root hair development (Rajappa et al., 2020). These two transporters are also known to increase their expression more in roots than in leaves, which may explain the lower expression rates in our study. Increases in the three *StPOTs* at 82 dap could be related to the increased water uptake of plants in the recovery phase, because more K was available, and therefore, the expression of the three *StPOTs* increased again. Our results showed that *StPOT12* reacted similarly to *StPOT2* and *StPOT4*, but showed higher expression rates in -K Milva plants at 55 dap and up to eight-fold higher expression at 82 dap. Therefore, protein synthesis for *StPOT12* may be enhanced, participating more in K uptake compared to proteins of *StPOT2* and *StPOT4*. Zhang et al. (2021) also showed a connection between *AtKUP12* and increasing K uptake in addition to improving the salt tolerance of *Arabidopsis*. Therefore, *StPOT12* is also involved in abiotic stress. However, the detailed physiological functions remain unclear, and further investigations are needed.

Conclusions

The present study investigated the influence of adequate and deficient K supply and drought stress simulated by PEG-induced osmotic stress on potato plants. A sufficient K supply enhanced biomass production and water consumption in this study and had the greatest influence on most parameters. However, PEG-treated plants produced more biomass irrespective of the K supply with almost constant water uptake, especially for Milva and developed more side shoots under -K and +PEG conditions for both cultivars. The production of additional side shoots enhanced photosynthetic activity and could be interpreted as an adaptation mechanism that both cultivars employed. Both proved to be widely tolerant to osmotic stress under hydroponic conditions. Osmotic stress and K deficiency further increased the content of individual amino acids, indicating the functional characterisations of amino acid responses to osmotic and nutrient stress. However, the production of osmotic substances, such as amino acids, is energetically more expensive for plants; therefore, it is better to use K for osmotic adjustment. K remains an important variable, fulfilling the functions of other osmolytes to maintain plant metabolism. The results from this study could provide indications for adaption mechanisms during osmotic stress, which can

also be transferred to drought related stress situations. However, in general, it is still necessary to investigate the functional characterisation of amino acids and regulation of K transporters, especially in relation to different tissue types and developmental stages in potato plants. Plant responses to induced stress conditions were cultivar dependent and should be further verified with other cultivars. Consequently, specific cultivar management offers the opportunity to prevent negative environmental influences, such as abiotic stress, on plants.

CRedit authorship contribution statement

Lisanne Wilmer: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Merle Tränkner:** Conceptualization, Supervision, Resources, Writing – review & editing. **Elke Pawelzik:** Conceptualization, Supervision, Resources, Writing – review & editing. **Marcel Naumann:** Conceptualization, Supervision, Writing – review & editing.

Declaration of 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2022.100102.

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