



Full Length Article

Salicylic Acid Alleviates Potassium Deficiency by Promoting Nutrient Absorption and Accumulation in *Zinnia elegans*

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Abstract

Salicylic acid (SA) is involved in improving abiotic stress tolerance. However, the role of exogenous SA on plant potassium (K⁺) deficiency tolerance and mechanisms remain unknown. The present study investigated the effect of exogenous SA (0.75 mM) on growth, nutrient (N, P and K) uptake and accumulation in *Zinnia elegans* seedlings under K⁺ deficiency (4 mM) and K⁺ sufficiency (12 mM) conditions. The results showed that K⁺ deficiency decreased root length and surface area, root uptake ability, nutrient (N, P and K) uptake efficiency and accumulation, which led to reduce plant height and dry weight. Nonetheless, foliar-applied SA increased root length and surface area, which helped to absorb and accumulate more nutrients (N, P and K). Moreover, the improvement of the root uptake ability and uptake efficiency caused by SA may be beneficial to growth. These results indicated that SA could improve K⁺ deficiency stress tolerance in *Z. elegans* by promoting root growth, enhancing root uptake ability and uptake efficiency of N, P and K, which facilitate the better understanding how SA regulates the response of plants to abiotic stress, particularly in horticulture plants. Overall, SA might be considered as an exogenous stimulant to improve K⁺ deficiency tolerance in plants. © 2020 Friends Science Publishers

Keywords: Nutrient absorption and accumulation; Potassium deficiency; Salicylic acid; *Zinnia elegans*

Introduction

Potassium (K⁺), as one of the most important and abundant cations in plants, plays an important role in many fundamental processes (Wang and Wu 2015). The K⁺ is regarded as "resistance element" due to its ability to enhance crop resistance to cold, drought, salt and disease resistance. In most areas of the world, K⁺ deficiency in arable is a major restricting factor for sustainable crop production (Wang and Wu 2015). It has been reported that different crops and genotypes differ in the absorption and utilization of K (Rengel and Damon 2008). Although the K⁺ deficiency tolerance can be improved through genetic manipulation, little progress has been made in the development of K⁺ deficiency tolerant crop cultivars (Zeng *et al.* 2018). K⁺ deficiency not only inhibits the growth of ornamental plants, but also delays the flowering period and decreases the number of flowers (Wang *et al.* 2011; Wei *et al.* 2019).

Zinnia elegans is an annual herbaceous flower and noticeable for its bright and colorful flowers. As one of the four diamonds in herbaceous flowers, its status is next only to marigold. Therefore, it is widely planted as ornamental plants in municipal lands and parks all over

the world (Hemmati and Nikooei 2017). *Z. elegans* had been reported to require a large amount of K, and its deficiency had negative effects on its growth and flowering (Wei *et al.* 2019).

Salicylic acid (SA) has been known as endogenous growth regulator, play a key role in a number of physiological processes (Ahmad *et al.* 2018a). Therefore, SA has been reported to improve the growth and bio-productivity in plants. Some studies have indicated that exogenous SA protect the plants from various abiotic stresses, including chilling, salinity, drought, osmotic, heat, UV and heavy metal toxicity, by altering the resistance-related gene expression (Wang *et al.* 2018; Zheng *et al.* 2018), modulating secondary metabolites pathway (Morris *et al.* 2000), activating proline biosynthesis (La *et al.* 2018), and maintaining ionic balance, photosynthetic activity and reactive oxygen species (ROS) detoxification (Khan *et al.* 2014; Ahmad *et al.* 2018b). SA is also a signaling molecule that induces systemic acquired resistance to pathogen infection (Durrant and Dong 2004). In addition, SA treatment can mitigate the toxic effects of pesticides on *Vigna radiata* seedlings via enhancement of an antioxidative response (Fatma *et al.* 2018). However, scarce information

is available on SA alleviating plant nutrient stress, and whether the nutrient uptake and accumulation were involved in SA-induced enhancement of plant resistance to abiotic stress remains unclear.

Recently, a few studies reported that SA alleviate the salt stress by improving K^+ transport and inhibiting its efflux (Jayakannan *et al.* 2013; Pirasteh-Anosheh *et al.* 2016), and shows that SA may lead to improvement in the tolerance to K^+ deficiency stress.

Therefore, this study analyzed whether the exogenous SA application to the *Z. elegans* under K^+ deficiency conditions affects the growth, root morphology, nutrient uptake and accumulation with the purpose to understand the alleviating effect of SA on plant nutrient stress, the mechanisms involved vital to improve the growth and development of the plants in K^+ deficiency regions.

Materials and Methods

Plant material and growth conditions

The seeds of *Z. elegans* 'Dreamland Pink' were obtained from Takii Seed Co., Ltd. Kyoto, Japan.

According to a preliminary experiment, 0.75 mM of SA was chosen as an optimal concentration for *Z. elegans* seedlings. The healthy seeds were sown in plastic pots (7.0 cm × 7.0 cm × 8.0 cm deep) filled with quartz sand, washed three times with deionized water before used. After emergence (8 d), one seedling per each pot was transplanted and irrigated with 10 mL of nutrient solution [5.0 mM $Ca(NO_3)_2$, 2.0 mM $MgSO_4$, 1.0 mM NaH_2PO_4 , 5.0 mM $NaNO_3$, 0.1 mM EDTA- Na_2 , 0.1 mM $FeSO_4$, 46.0 μM H_3BO_3 , 9.2 μM $MnCl_2$, 0.3 μM $CuSO_4$, 0.8 μM $ZnSO_4$, and 0.4 μM Na_2MoO_4] containing 4 mM K^+ (K^+ deficiency) or 12 mM K^+ (K^+ sufficiency) once a day, and K^+ was supplied by K_2SO_4 (Zhu *et al.* 2018). Plants were cultivated in an artificial climate room with a day/night mean temperature of 25/20°C, a 16-h-day/8-h-night photoperiod, and a photosynthetic photon flux density of 125 $\mu mol m^{-2} s^{-1}$.

After a 30-day pre-treatment (4 mM K^+ or 12 mM K^+) period, at each K^+ level, SA solution (0 mM or 0.75 mM) were prepared according to Li *et al.* (2014) and sprayed onto the adaxial and abaxial surfaces of the leaves once a day for three days. Then, the experimental treatments were designed as follows: 0 mM SA + 4 mM K^+ ; 0.75 mM SA + 4 mM K^+ ; 0 mM SA + 12 mM K^+ ; 0.75 mM SA + 12 mM K^+ . Each treatment was repeated four times with 10 pots per replicate. Plants were sampled after eight days of spraying treatment to assess various growth and physiological parameters.

Plant growth

After harvesting, twelve seedlings from each treatment were measured for plant heights, and then separated into shoots and roots, dried in an oven at 80°C for 48 h until constant dry weight.

Root morphological indexes

In each treatment, 12 plants were selected and their roots were scanned using an EPSON V700 Scanner [EPSON (China) Co., Ltd.], and the length, surface area, volume and average diameter of root were analyzed with WinRHIZO PRO 2012 Root Analysis System (Regent Instruments Inc., Quebec, Canada); (Ding *et al.* 2013).

N, P and K content and accumulation

The dried samples were ground and digested with H_2SO_4 - H_2O_2 , and tissue nitrogen (N) content was determined by the Kjeldahl method, phosphorus (P) colourimetrically using a UV-vis spectrophotometer (Gitari *et al.* 2018), whereas K content using flame spectrophotometer (Liu *et al.* 2017). Tissue N, P and K accumulation was calculated by multiplying nutrient content with respective dry weights.

N, P and K uptake ability, uptake efficiency and use efficiency

Nutrient (N, P and K) uptake ability was defined as the nutrient accumulation per unit of root length ($\mu g cm^{-1}$). Nutrient uptake efficiency was computed as a ratio of nutrient accumulation and nutrient concentration supplied ($g g^{-1}$; Sandaña 2016; Gitari *et al.* 2018), and nutrient use efficiency was calculated as the ratio of dry weight to the plant nutrient accumulation ($g DW g^{-1}$; Shi *et al.* 2009; White *et al.* 2010).

Statistical analysis

Statistical calculations were performed with SPSS 19.0 (SPSS Inc., Chicago, IL, USA; Abbasi *et al.* 2015). Significant differences were assessed using Duncan's multiple range test at $P \leq 0.05$. All data were presented as the mean \pm standard deviation (SD) calculated from at least three replicates.

Results

Plant growth

The K^+ deficiency stress decreased the plant height of *Z. elegans* seedlings (Fig. 1), which increased by the application of SA by 22.65 and 19.30% respectively at 4 mM and 12 mM K^+ level in comparison to water sprayed treatment.

The lowest shoot dry weight was obtained for 0 mM SA + 4 mM K^+ , while the highest for 0.75 mM SA + 12 mM K^+ treatment (Table 1). Relatively similar results were obtained for root and whole plant dry weights. Dry weight of *Z. elegans* seedlings negatively responded to K^+ deficiency, and the application of SA positively compensated for the negative effect of K^+ deficiency. A

positive impact of SA on plant biomass was also observed under K^+ sufficiency condition.

Root morphological indexes

The K^+ deficiency stress produced lower root length and root surface area of *Z. elegans* seedlings compared to K^+ sufficiency, and the application of SA (0.75 mM) significantly increased these indexes at both 4 mM and 12 mM K^+ level (Fig. 2a and 2b). The root volume (Fig. 2c) showed a similar trend for root length and root surface area. However, there was no significant difference in root average diameter among the four treatments (Fig. 2d).

N, P and K content and accumulation

K^+ deficiency led to a significant decrease in N contents of shoot and root while application of SA largely increased these contents by 25% in shoot and 18.18% in root. The adverse effects of K^+ deficiency on N contents were effectively alleviated by SA (Table 2). A positive impact of SA on N content was also observed under K^+ deficiency condition. Similarly, the contents of P and K were strongly decreased in the K^+ -deficient compared to the K^+ -sufficient plants, SA could offset the decrease of P and K contents caused by K^+ deficiency, however, no positive effect on K content were detected under K^+ sufficiency condition.

Although, the accumulations of N, P and K in shoot were higher than in root (Table 3), the patterns response of two parts to the SA and K^+ deficiency were similar, both SA and K significantly increased the accumulations of N, P and K in shoot and root. The highest levels were detected in 0.75 mM SA-treated plants at 12 mM K^+ , while the lowest in plants treated with 0 mM SA + 4 mM K^+ (Table 3). Compared with the application of SA, the increase of K^+ level was more conducive to the accumulation of K^+ .

Nutrient uptake ability

The K^+ deficiency significantly reduced the uptake ability of N, P and K by 51.3, 49.04 and 66.33% respectively, compared with the adequate K^+ (Fig. 3). The response of root nutrient uptake ability to the application of SA was positive under K^+ deficiency condition, but the positive impact of SA was not observed under K^+ sufficiency condition.

Nutrient uptake efficiency

The N, P and K uptake efficiency increased as the K^+ concentration increased. The application of SA largely increased the N, P and K uptake efficiency by 161.11% (N), 127.27% (P) and 151.67% (K) respectively under K^+ deficiency condition (Fig. 4). Under K^+ sufficiency, SA treatment increased the absorption efficiency of N, P and K, but the increases were less than K^+ deficiency.

Table 1: Effects of salicylic acid (SA) on dry weight in *Zinnia elegans* seedlings under potassium (K^+) deficiency and K^+ sufficiency conditions

K^+ concentration (mM)	SA concentration (mM)	Dry weight (g plant ⁻¹)		
		Shoot	Root	Whole plant
4	0	276.67±70.95c	53.33±32.15c	330.00±100.00c
	0.75	586.67±51.32b	80.00±10.00b	666.67±41.63b
12	0	546.67±76.38b	76.67±5.77b	623.33±80.83b
	0.75	770.00±112.69a	123.33±15.28a	893.33±101.16a

Values represent the mean of 12 plants ± SD of three replicates. Values in a vertical column followed by different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test. The same as follows

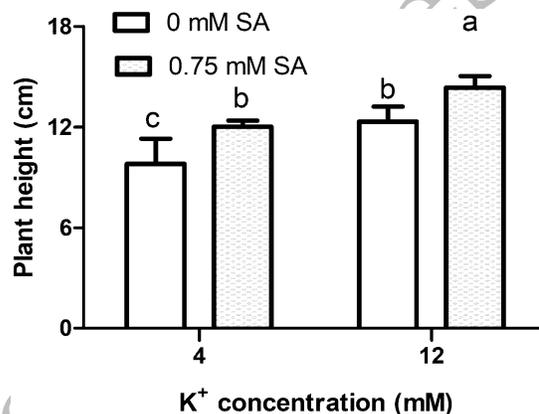


Fig. 1: Effect of salicylic acid (SA, 0.75 mM) on plant height of *Z. elegans* seedlings under Potassium (K^+) deficiency (4 mM) and K^+ sufficiency (12 mM) conditions. Different lowercase letters above the columns in the figure denote significant different ($P \leq 0.05$) according to Duncan's multiple range test

Nutrient use efficiency

As K concentration increased, the N and P use efficiency changed little, but the K use efficiency markedly reduced (Fig. 5). At both K^+ levels, no significant difference in nutrient use efficiency between SA-treated and untreated plants was noted.

Discussion

In most areas of the world, lack of available K in soil and shortage of K resources are prominent problems (Ahanger and Agarwal 2017). Thus, K^+ deficiency has become a limiting factor for crop productivity and quality, especially at the initial stage of planting (Wang and Wu 2015). *Z. elegans*, as an important ornamental plant, has a higher demand for K^+ (Wei *et al.* 2019). According to a preliminary experiment, 12 mM was chosen as an optimal concentration of K^+ , and 4 mM as a low concentration for *Z. elegans* seedlings (Zhu *et al.* 2018). K^+ deficiency (4 mM) significantly reduced the dry weight of *Z. elegans* seedlings compared with K^+ sufficiency (12 mM), (Table 1).

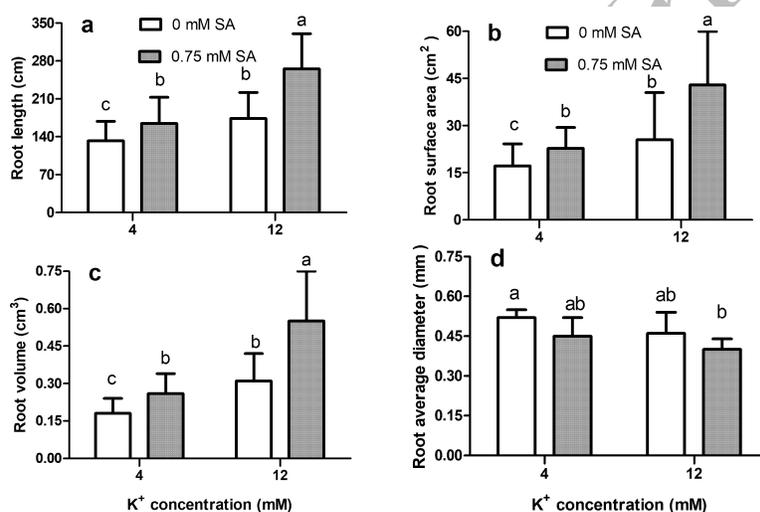
SA plays a critical role in regulating many aspects of plant growth and development, as well as resistance to

Table 2: Effects of salicylic acid (SA) on N, P and K content in *Zinnia elegans* seedlings under potassium (K⁺) deficiency and K⁺ sufficiency conditions

K ⁺ concentration (mM)	SA concentration (mM)	Nutrient content (% DW)					
		N		P		K	
		Shoot	Root	Shoot	Root	Shoot	Root
4	0	2.52 ± 0.03c	2.31 ± 0.03d	0.06 ± 0.01c	0.05 ± 0.01d	3.79 ± 0.05c	4.04 ± 0.43c
	0.75	3.15 ± 0.32b	2.73 ± 0.16c	0.08 ± 0.00b	0.07 ± 0.01c	4.33 ± 0.00b	5.05 ± 0.05b
12	0	3.08 ± 0.08b	3.01 ± 0.00b	0.08 ± 0.00b	0.10 ± 0.01b	7.21 ± 0.05a	5.83 ± 0.00a
	0.75	3.50 ± 0.08a	3.43 ± 0.16a	0.09 ± 0.01a	0.14 ± 0.01a	7.13 ± 0.05a	5.13 ± 0.05b

Table 3: Effects of salicylic acid (SA) on N, P and K accumulation in *Zinnia elegans* seedlings under potassium (K⁺) deficiency and K⁺ sufficiency conditions

K ⁺ concentration (mM)	SA concentration (mM)	Nutrient accumulation (mg plant ⁻¹)					
		N		P		K	
		Shoot	Root	Shoot	Root	Shoot	Root
4	0	2.26 ± 0.74c	0.27 ± 0.01c	0.06 ± 0.02c	0.01 ± 0.00c	3.37 ± 0.96d	0.47 ± 0.07d
	0.75	5.89 ± 0.17b	0.68 ± 0.01b	0.14 ± 0.01b	0.02 ± 0.00b	8.15 ± 0.60c	1.26 ± 0.05c
12	0	5.99 ± 0.76b	0.83 ± 0.02b	0.15 ± 0.02b	0.03 ± 0.00b	13.99 ± 1.51b	1.60 ± 0.03b
	0.75	9.38 ± 1.49a	1.50 ± 0.21a	0.24 ± 0.05a	0.06 ± 0.01a	19.07 ± 2.72a	2.23 ± 0.23a

**Fig. 2:** Effects of salicylic acid (SA, 0.75 mM) on root length (a), root surface area (b), root volume (c) and root average diameter (d) of *Z. elegans* seedlings under Potassium (K⁺) deficiency (4 mM) and K⁺ sufficiency (12 mM) conditions

abiotic stress. The effects of exogenous SA on plants vary according to SA concentration, plant species and environmental conditions. In the present study, foliar spraying of 0.75 mM SA was applied to *Z. elegans* seedlings and results showed that SA not only alleviated the adverse effects of K⁺ deficiency on plant growth and also had positive effects under K⁺ sufficiency condition (Table 1).

The root is the first plant organ to detect nutrient deficiencies, and change of root architecture is the basic factor for plants to respond to nutrient deficiency and adapt to the environment (Song et al. 2018). Physiological, metabolic, and morphological root adaptations to K⁺ deficiency have been reported in many plant species, such as tobacco (Song et al. 2018), sweet potato (Liu et al. 2017), rice (Ma et al. 2012), *Arabidopsis* (Gruber et al. 2013) and so on. The K⁺ deficiency causes the significant decrease in root length and root surface area of seedlings. Similarly, the

root length, root surface area and root volume of the *Z. elegans* seedlings were markedly declined under K⁺ deficiency (Fig. 2). These may be due to the fact that K⁺ deficiency primarily inhibits cell elongation mainly via turgor reduction (Leigh and Jones 1984).

Larger root length and root surface area are conducive to the absorption of nutrients dependent on diffusion. Hence, the application of SA (0.75 mM) caused a significant increase in these indices at both 4 mM and 12 mM K⁺ level (Fig. 2) showing positive role of SA in regulating the response of plants to K⁺ deficiency, and this could be due to SA's ability to stabilize membrane integrity, enhance antioxidant defense system, increase photosynthesis and change gene expression (Jayakannan et al. 2013). The application of SA led to a decrease in the average root diameter, but the difference was not obvious, the result indicated that the

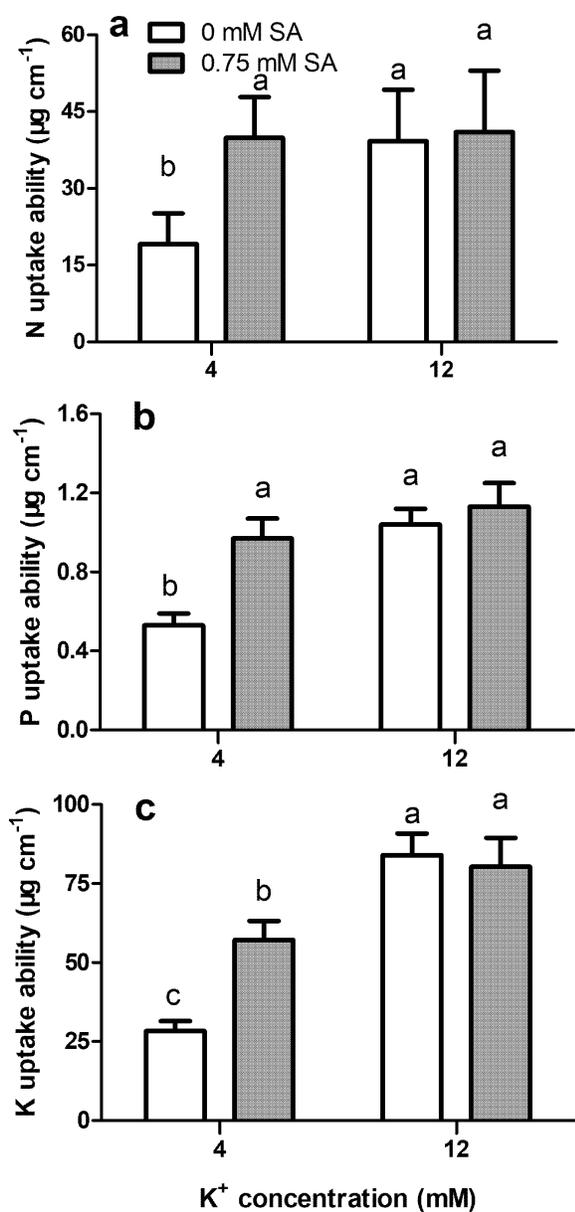


Fig. 3: Effects of salicylic acid (SA, 0.75 mM) on root uptake ability of N (a), P (b) and K (c) of *Z. elegans* seedlings under Potassium (K⁺) deficiency (4 mM) and K⁺ sufficiency (12 mM) conditions

influence of SA on root length and root surface area were higher than on root diameter. The promotion of SA on root growth was beneficial to plant growth, and resulted in an increase in dry weight.

Xu *et al.* (2015) reported that exogenous SA increased the absorption of K, Ca, Mg and Fe in soybean seedlings. Moreover, the effect of SA on the uptake and transport of ions may play a role in promoting salt tolerance in plants (Pirasteh-Anosheh *et al.* 2016). The N, P and K are the three most important and essential elements in plants and results of present study indicated that SA promote nutrient

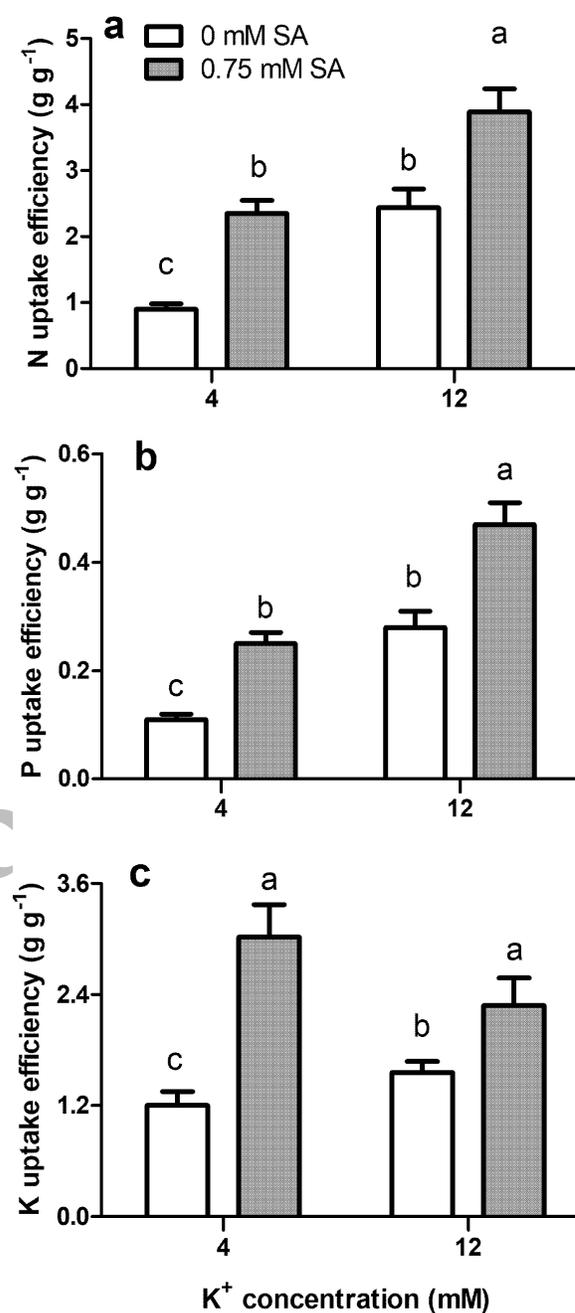


Fig. 4: Effects of salicylic acid (SA, 0.75 mM) on N (a), P (b) and K (c) uptake efficiency of *Z. elegans* seedlings under Potassium (K⁺) deficiency (4 mM) and K⁺ sufficiency (12 mM) conditions

absorption and accumulation, which might be one of the important causes for SA-induced K⁺ deficiency tolerance in *Z. elegans* seedlings. In present study, K⁺ deficiency not only reduced the root traits and also caused a decrease in accumulation of nutrient elements (N, P and K) (Table 3). Furthermore, the effects of exogenous SA on nutrient (N, P and K) accumulation were consistent with these root traits. Thus, it could be concluded that nutrient (N, P and K)

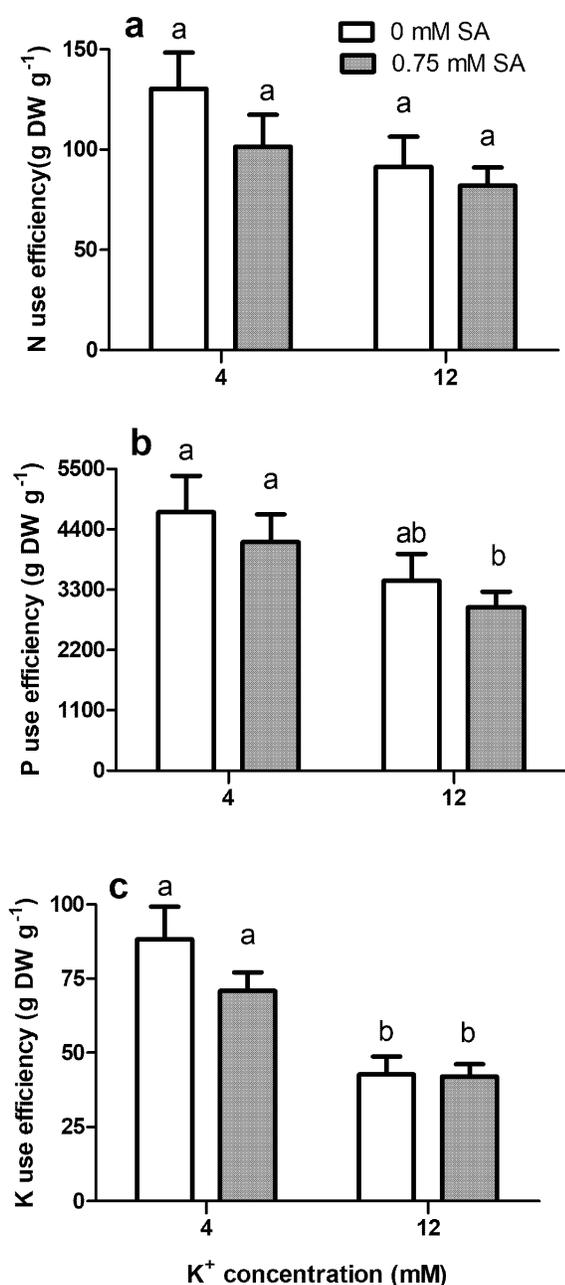


Fig. 5: Effects of salicylic acid (SA, 0.75 mM) on N (a), P (b) and K (c) use efficiency of *Zinnia elegans* seedlings under Potassium (K⁺) deficiency (4 mM) and K⁺ sufficiency (12 mM) conditions

accumulation were positively correlated with two root architecture, traits including root length and root surface area.

Under K⁺ deficiency conditions, SA significantly increased the root uptake ability of N, P and K accumulation. Liu *et al.* (2017) reported that K⁺ deficiency affected the structure of root tip cells and caused metabolic abnormality. SA successfully mitigated salt toxicity in *Caralluma tuberculata calli* by revival of cellular structure

(Rehman *et al.* 2014). Thus, the ability of SA to recover root cell structure damage caused by K⁺ deficiency may be also responsible for enhancing root uptake ability and increasing nutrient accumulation.

Root size is the main factor affecting K uptake efficiency (Chen and Gabelman 1995) and results in present study also showed that both K⁺ sufficiency and SA treated led to a significant increase in N, P and K uptake efficiency (Fig. 4), which could attribute to the larger size of the root system. But with regard to nutrient use efficiency, the positive response to increase of K⁺ level or SA application was not observed, K⁺ sufficiency even reduced the K use efficiency (Fig. 5), that is the ratio of dry weight to the plant K accumulation (g DW g⁻¹; White *et al.* 2010). A similar finding was also observed in Chinese cabbage (Li *et al.* 2015). K uptake efficiency correlated strongly with shoot biomass, but not for K use efficiency (White *et al.* 2010), as confirmed in present study (Fig. 4c; Fig. 5c). Thus, SA could enhance plant resistance by affecting plant uptake and accumulation of nutrients.

Conclusion

Z. elegans seedlings decreased plant height, root length, N, P and K content, accumulation and uptake ability which ultimately reduced dry weight under K⁺ deficiency. On the other hand, exogenous applied SA could efficiently reduced the adverse effects of K⁺ deficiency stress on the growth of *Z. elegans* by promoting root growth, increasing root uptake area, and improving root uptake ability, nutrient (N, P and K) uptake efficiency and nutrient accumulation. Thus, SA could be used to improve the plant growth in the K⁺ deficiency areas.

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