Proc. Intl. Soc. Citricult. IX Congr. 2000. 691–692.

Potassium-deficient Citrus Rootstocks Accumulate Arginine, Proline, and Putrescine

Yusheng Zheng and Carol J. Lovatt
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124

Abstract. Abstract. **Accumulation of putrescine, proline, or arginine is a well-documented response to potassium deficiency in plants. However, the metabolic mechanism driving this response is unresolved. By quantifying changes in nitrogen metabolism in leaves of two commercially important citrus rootstocks, rough lemon (*Citrus jambhiri* Lush.)and Carrizo citrange [*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.], grown in K-sufficient and K-deficient nutrient solutions for 240 days, it was determined that ammonia accumulated early during K-deficiency stress and was preferentially assimilated into proline. Arginine synthesized de novo was the major compound for storage of excess nitrogen after 210 days of K-deprivation of rough lemon and for the entire period of K-deficiency stress of Carrizo citrange. Putrescine accumulation was delayed until halfway through the stress and was insignificant. Growth of rough lemon, but not Carrizo citrange, was severely limited in the absence K. Rough lemon should be considered a K-dependent rootstock.**

It is well documented that potassium deprivation results in increased leaf concentrations of putrescine (Sarjala and Kaunisto, 1993), arginine (Eppendorfer and Bille, 1996), and proline (Kwon, 1999). The metabolic mechanism driving the accumulation of these compounds is controversial. By quantifying changes in nitrogen metabolism in leaves of two commercially important citrus rootstocks, rough lemon and Carrizo citrange, grown in K-sufficient (+K) and K-deficient (–K) nutrient solutions, we tested the following hypotheses: 1) abiotic stress that restricts shoot growth will cause increased ammonia production early in the stress; 2) arginine de novo biosynthesis will increase to detoxify tissues of excess ammonia; and 3) due to its high N to C ratio, arginine will be the major end product for excess nitrogen (Lovatt, 1990).

Materials and Methods

Plant material. Seven-day-old rough lemon (*Citrus jambhiri* Lush.) and Carrizo citrange [*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] rootstock seedlings were transferred to aerated Shive's nutrient solutions with (+K) or without potassium (–K) and grown for 240 d.

Analytical methods. All analyses were used young fully expanded (YFE) leaves. Ammonia concentration was determined using an Altech Inorganic Nitrogen Analyzer. Arginine and proline in leaf samples were chemically treated and the separate products measured at OD_{500nm} and OD_{520nm}, respectively. Putrescine concentration was quantified by HPLC. Activity of the arginine de novo biosynthetic pathway was assessed by measuring the incorporation of radiolabeled $NaH^{14}CO_3$ into arginine in YFE leaf disks. Activities of arginase and arginine decarboxylase were measured in cell-free extracts of YFE leaves.

Results

Plant growth. Withholding K from rough lemon for up to 240 d severely limited plant growth (Table 1). Compared to +K control plants, average trunk diameter of –K rough lemon plants was 48% less, tree height was 69% less, and leaf area was 48% smaller. Despite leaf chlorosis after 60 d without K, withholding K from Carrizo citrange seedlings for 240 d had no effect on trunk diameter or leaf area; tree height was 18% less than that of the +K control plants. Leaf K concentration averaged 3.3% dry weight for the +K control plants and 0.5% for both rootstocks after 240 d without K.

Changes in ammonia, arginine, proline, and putrescine concentrations. Significant net increases in ammonia and proline concentrations were observed in leaves of rough lemon and Carrizo citrange after only 30 d of K deprivation (Table 2). Net increase in proline concentration was highest after 90 d without K, decreasing over the remainder of the

Table 1. Effect of K deprivation on the growth of rough lemon and Carrizo citrange seedlings.[z]

Treatment duration (days)	K status	Trunk diameter (mm)	Seedling height (cm)	Leaf area (cm^2)
		Rough lemon		
30	+K	1.6	4.5	7.3
	–K	1.6 NS	4.0 *	3.9 ***
120	+K	4.0	27.3	18.0
	–K	2.4 ***	10.7 ***	10.2 **
240	+K	7.4	60.0	44.2
	–K	3.8 ***	18.4 ***	22.8 ***
		Carrizo citrange		
30	+K	1.8	5.0	5.3
	–K	2.0 NS	5.5 NS	5.2 NS
120	+K	2.0	11.6	7.1
	–K	2.1 NS	11.7 NS	7.2 NS
240	+K	3.1	22.0	12.2
	–K	2.9 NS	18.0 **	11.2 NS

[z]Data are the means of three separate experiments. For each parameter measured, paired +K and –K values followed by NS,*,**,*** are nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2. Net increase in leaf concentrations of ammonia, arginine, proline and putrescine for –K rough lemon and Carrizo citrange seedlings determined by subtracting the concentrations observed for +K control plants from those obtained for the –K plants.

Treatment duration (days)	Net increase in nmol•g^{-1} fr wt –K YFE leaves[z]			
	Ammonia	Arginine	Proline	Putrescine
		Rough lemon		
30	361 *	33 NS	4,063 *	14
60	110 **	360 *	6,560 **	23
90	433 *	2,388 ***	26,545 **	52
120	457 *	700 ***	5,433 **	132
150	269 **	2,010 ***	6,768**	308 ***
180	237 *	3,064 ***	12,780 ***	563 ***
210	178 *	4,081 ***	3,258 *	—
240	104 *	6,357 ***	4,144 *	132 ***
		Carrizo citrange		
30	100 **	113 NS	2,456 *	23
60	78 *	689 **	3,661 *	16
90	12 NS	519 **	8,039 **	72
120	339 *	292 *	7,313 ***	63
150	112 **	685 **	5,668 **	37 NS
180	14 NS	341 *	878 NS	59 *
210	135 *	670 ***	1,596 NS	—
240	182 *	1,314 ***	52 NS	354 ***

[z]Net increase = (nmol•g^{-1}fr wt –K leaves) – (nmol•g^{-1} fr wt +K leaves). Data are the means of three separate experiments. Values followed by NS,*,**,*** are nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Values for putrescine not statistically analyzed were the means of two separate experiments that did not differ by more than 20%.

Table 3. Relative distribution of the net increase in total nitrogen in ammonia, arginine, proline and putrescine in rough lemon and Carrizo citrange seedlings during K deprivation.

Treatment duration (days)	Percent distribution of net nitrogen in YFE leaves[z]			
	Ammonia	Arginine	Proline	Putrescine
		Rough lemon		
30	8.0	2.9	88.8	0.3
60	1.3	17.7	80.7	0.3
90	1.2	26.1	72.6	0.2
120	5.2	31.7	61.6	1.5
150	1.8	52.3	44.0	2.0
180	0.9	47.4	49.5	2.2
210	0.9	82.6	16.5	—
240	0.4	85.7	14.0	1.1
		Carrizo citrange		
30	11.4	51.3	32.1	5.2
60	2.3	83.9	12.8	1.0
90	0.4	65.7	29.3	4.6
120	13.5	46.2	33.4	6.8
150	3.1	76.6	18.2	2.1
180	0.7	85.5	6.4	7.4
210	4.5	89.4	6.1	—
240	3.0	85.5	0.1	11.5

[z]Percent distribution for all compounds was based on the average net increase in nmol•g^{-1} fr wt for the –K plants compared with +K plants for three separate experiments × the number of N atoms in each compound. For example, % N in NH_4^+ = (net nmol NH_4^+ × 1N)/(net nmol NH_4^+ × 1N) + (net nmol Arg × 4N) + (net nmol Pro × 1N) + (net nmol Put × 2N) × 100%.

stress period. As proline concentration decreased during K deficiency stress, arginine concentration increased. By the end of the –K stress treatment, the arginine pool accounted for 86% of the net increase in nitrogen for both –K rough lemon and Carrizo citrange (Table 3). In contrast to proline and arginine, the first statistically significant net increase in putrescine concentration was significantly delayed, occurring after 150 and 180 d without K for rough lemon and Carrizo citrange, respectively (Table 2). In addition, concentrations of putrescine that accumulated during K deprivation were insignificant compared to those of proline and arginine. Putrescine contributed little to the storage of excess nitrogen during K deprivation (Table 3).

Arginine de novo biosynthesis. Consistent with the accumulation of arginine during K deprivation, the rate of incorporation of $NaH^{14}CO_3$ into arginine significantly increased over time in leaves of –K rough lemon and Carrizo citrange. At 240 days –K, rates were 2.5-fold and 1.6-fold higher than those of +K rough lemon and Carrizo citrange, respectively.

Arginase and arginine decarboxylase activities. Withholding K had no effect on arginase activity. In leaves of –K rough lemon and Carrizo citrange, arginine decarboxylase activity was significantly accelerated, 3.5-fold and 2-fold, respectively, compared to +K plants but only after 150 d without K, consistent with the delay in putrescine accumulation.

Discussion

Since the growth of rough lemon plants was severely limited during 240 d of K deprivation but the impact of K deprivation on the growth of Carrizo citrange was negligible, rough lemon should be considered a K-dependent rootstock. Rough lemon is known to be P-dependent and dependent on vesicular arbuscular mycorrhizae. Consistent with our hypothesis, ammonia production increased early during K-deficiency stress. A large net increase in leaf proline concentration (μmol·g^{-1} fresh weight) occurred in the leaves of both rootstocks after only 30 d without K, compared to small net increases in arginine and putrescine (nmol·g^{-1} fresh weight) after 60 and 150 d of K deprivation, respectively. These data suggest that ammonia was initially, preferentially assimilated into proline in both rough lemon and Carrizo citrange. Subsequently, during K-deficiency stress of both rootstocks, proline concentrations decreased and arginine concentrations increased. Arginine decarboxylase activity and net putrescine concentration did not increase until more than halfway through the stress period. Putrescine accounted for less than 12% of the net accumulated nitrogen at the end of 240 d without K for both rootstocks. Putrescine, which has been suggested to serve in ammonia detoxification or as the replacement cation for K^+, played an insignificant role during K-deficiency stress of rough lemon and Carrizo citrange rootstocks. During K-deprivation, activity of the arginine de novo biosynthetic pathway increased but arginase activity did not, resulting in arginine accumulation, despite increased arginine decarboxylase activity. Arginine synthesized de novo was the major compound for storage of excess nitrogen after 210 d without K for rough lemon and for the entire period of K-deficiency stress of Carrizo citrange.

Literature Cited

Eppendorfer, W.H. and S.W. Bille. 1996. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower, and potatoes as influenced by nitrogen fertilization and phosphorus and potassium deficiency. J. Sci. Food Agr. 71:449–458.

Kwon, T., P.J.C. Harris, and W.F. Bourne. 1999. Partitioning of Na^+, K^+, proline, and total soluble sugar in relation to the salinity tolerance of *Brassica juncea* and *Brassica napa*. J. Korean Soc. Hort. Sci. 40:425–430.

Lovatt, C.J. 1990. Stress alters ammonia and arginine metabolism, p. 166–179. In: H.E. Flores, R.N. Arteca, and J.C. Shannon (eds.). Polyamines and ethylene: Biochemistry, physiology, and interactions. Amer. Soc. Plant Physiol., Rockville, Md.

Sarjala, T. and S. Kaunisto. 1993. Needle polyamine concentrations and potassium nutrition in Scots pine. Tree Physiol. 13:87–96.