Pyruvate Kinase Activity as an Indicator of the Level of K^+, Mg^{2+}, and Ca^{2+} in Leaves and Fruits of the Cucumber: The Role of Potassium Fertilization

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Little is known about the effects of K^+ fertilization on pyruvate kinase (PK) activities in cucumber (*Cucumis sativus* L. cv. Brunex) grown in the greenhouse on calcareous soils. Here, the effect of K rates on the concentrations of K^+, Mg^{2+}, and Ca^{2+} and on the PK activity as a possible indicator of the levels of these cations in the leaves and fruits of cucumber plants has been studied. The treatments consisted of applications of three rates of K in the form of K_2SO_4 (K1 = 0.075 mg mL^{-1}, K2 = 0.15 mg mL^{-1}, and K3 = 0.30 mg mL^{-1}). In general, K^+ application in calcareous soils proved beneficial. The highest application of K^+ (K3) to the culture medium reduced, in both the leaves and fruits, the foliar concentrations of Mg^{2+} and Ca^{2+}. These results are reflected by the lowest basal PK activities and the highest differences between the basal PK activity and activities stimulated by these two cations. The opposite effect resulted with K2, with maximal basal PK activity and minimal differences between this activity and activities stimulated by the cations, indicating a better balance in this treatment between the different cations under the experimental conditions. Finally, this trend might partly account for the highest commercial yield in plants treated with K2.

Keywords: *Cucumis sativus; leaves; fruits; cations; pyruvate kinase; bioindicator; yield*

INTRODUCTION

Pyruvate kinase (PK), and thus glycolysis, is essential to the growth and development of plants. Detailed studies on the interactions between carbon and nitrogen metabolism have revealed that the N assimilation rate in this system is critically dependent on the regulation of PK (Lin et al., 1989; Schuller et al., 1990; Vanlerberghe et al., 1990). Work on the kinetics of this enzyme has revealed that its activation depends on the levels of PEP and ADP, in addition to the presence of cations, principally Mg^{2+} (Podestá and Plaxton, 1991, 1992; Day and Copeland, 1993).

The elucidation of the metabolic regulation of glycolysis in plants requires an study of the key control enzymes, principally cytosolic PK (EC 2.7.1.40) (Plaxton et al., 1993; Huppe and Turpin, 1994; Podestá and Plaxton, 1994a,b). PK activity is a potentially good physiological indicator of the cation levels (K^+, Mg^{2+}, and Ca^{2+}) present in an organ studied (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996).

Cations such as K^+, Mg^{2+}, and Ca^{2+} are important major nutrients for higher plants. Like other nutrients, these cations display individual functions as well as mutual interactions. The absorption of a given ion may be influenced by the presence in the medium of another ion or ions. In high concentrations, an ion or ions such as K^+ in the external solution are taken up at high rates, and this may lead to excessive accumulation in the tissue. Excessive uptake of K^+ or other ions may inhibit the uptake of the other mineral nutrients into the root and their transport to the shoot, thereby leading eventually to a deficiency in the tissue (Cramer et al., 1991).

High levels of K^+ decreased Mg^{2+} and Ca^{2+} uptake and translocation from root to shoot (Claasen and Wilcox, 1974; Ohno and Grunes, 1985; Song and Fujiyama, 1996). Magnesium fertilization decreased leaf Ca^{2+} and K^+ concentrations (Huang et al. 1990; Reinboot and Blevins, 1994), and Ca fertilization decreased leaf Mg^{2+} concentration (Miyasaka and Grunes, 1990; Fenn et al., 1994; Song and Fujiyama, 1996).

Many agricultural soils in Spain and elsewhere are calcareous and may be relatively rich in K^+, Mg^{2+}, and Ca^{2+}, but the relative balance of these nutrients may be inadequate for optimal production. Generally, nutritional imbalances have serious consequences for growth and yield. Deficiencies in K^+ and Mg^{2+} result in an accumulation of photosynthates in the leaf, diminishing their distribution to dependent organs such as fruits (Cakmak et al., 1994a,b; Marschner et al., 1996). In addition, deficiency in these cations causes an accumulation of toxic oxide species and afterward foliar chlorosis (Cakmak, 1994). Moreover, Ca^{2+} deficiency results in the appearance of tipburn in leaves and blossom ends as well as blossom rot (Sonneveld and Mook, 1983).

In view of the excessive use and accumulation of inorganic salts in intensive agriculture, for example, greenhouse cultivation, the control and prevention of these nutritional imbalances are essential. Our work focuses on determining to what degree the PK activity serves as a reliable bioindicator or reflects the adequate levels of K^+, Mg^{2+}, and Ca^{2+}, both in the leaves and in the fruits of cucumber. In this effort, we applied differ-

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ent rates of K to cucumber plants to evaluate the interaction between different cations and the behavior of PK.

MATERIALS AND METHODS

Crop. Cucumbers (*Cucumis sativus* L. cv. Brunex) were seeded in cell flats (cell size = 90 cm^3) filled with a peat-lite mixture and placed on benches under the greenhouse conditions described below for a period of 8 weeks; seedlings were then transplanted (October 1996) and grown under controlled conditions in an experimental greenhouse at Centro de Investigación y Desarrollo Hortícola, El Ejido, Almería, Spain. The experiment was conducted from 1996 to 1997. Local climate is semiarid, and the land is intensively used for agriculture. A loamy-sand soil was used, which had the following characteristics: sand (37.3%), silt (48.6%), and clay (10.1%); $CaCO_3$ equivalent (26.8%); $CaCO_3$ active (14.4%); total N (3.5 g kg^{-1}); total organic C (36.1 g kg^{-1}); PO (890 mg kg^{-1}); K^+ (5.3 g kg^{-1}); pH (H_2O, 8.5; KCl, 8.01); electrical conductivity (EC = 4.6 dS m^{-1}). The relative humidity was 60−80%, and the mean temperature was 24 ± 4 °C with extremes of 15 and 30 °C in the greenhouse. The experimental design was a factorial arrangement in a randomized complete block with three treatments. Container-grown cucumbers were transplanted into two rows 100 cm apart and drip irrigated. Each treatment had four replicates in four individual plots of 4 m × 2 m wide (16 plots). Each plot contained eight plants. The irrigation water had the following properties: pH, 8.05; EC = 2.03 dS m^{-1}; Cl^- = 484 mg L^{-1}; Na^+ = 306 mg L^{-1}; K^+ = 10.2 mg L^{-1}; HCO = 279 mg L^{-1}. The quantity of water used for irrigation at the end of cultivation was 130 L m^{-2}, distributed in 21 irrigations. Irrigation was controlled by a computerized system that regulated the amount of water according to the temperature and humidity inside the greenhouse.

Treatments consisted of applying K in the following manner: K as K_2SO_4 (K1 = 0.075 mg mL^{-1}, K2 = 0.15 mg mL^{-1}, and K3 = 0.30 mg mL^{-1}); N as NH_4NO_3 (0.20 mg mL^{-1}); P as H_3PO_4 (0.10 mg mL^{-1}); calcium (0.085 mg mL^{-1}) and magnesium (0.025 mg mL^{-1}) as sulfates. Fertilization−irrigation was complemented with the following micronutrients: Fe = 0.5 mg L^{-1}; B = 0.1 mg L^{-1}; Mn = 0.1 mg L^{-1}; Zn = 0.075 mg L^{-1}; Cu = 0.075 mg L^{-1}; and Mo = 0.05 mg L^{-1}. Both the micro- and macronutrients were applied at a steady concentration over the entire cultivation cycle. The pH values of the solution oscillated between 5 and 6; Fe was applied as FeEDDHA, B was applied as H_3BO_3, and the remaining micronutrients were applied as sulfates.

Plant Sampling. Leaf samples were taken only from plants with fully expanded leaves of similar size. Leaves and fruits were removed from a zone at about two-thirds of the total height of the plant, when the fruits reached maturity (95 days after transplanting; January 1997). Leaves and fruits were rinsed three times in distilled water after disinfecting with nonionic detergent at 1% (Wolf, 1982) and then blotted on filter paper. At each sampling, fresh matter (leaf and fruit pulp) was used for the PK assay; a subsample was dried in a forced-air oven at 70 °C for 24 h, ground in a Wiley mill, and then placed in plastic bags for the further analyses.

Plant Analysis. *Assay of Activity of Basal PK.* The activity of the different forms of PK was determined according to the method of Bar-Akiva et al. (1976) as modified by Podestá and Plaxton (1991). A total of 0.5 g of fresh samples was ground (0−4 °C) in 50 mM Tris-HCl buffer (pH 7.5), 50% glycerol (v/v), and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 3000*g* for 5 min at 0 °C to eliminate tissue and cell remains. The supernatant solution was centrifuged again at 24000*g* for 15 min, providing a clear solution with soluble cytosol proteins, mainly PK. To 100 μL of the supernantant solution from the second centrifugation was added 500 μL of 50 mM Tris-HCl buffer (pH 7.4), together with 0.25 mM sodium molybdate, 25 mM PEP, 5 mM ADP, and 200 μL of H_2O. The mixture was incubated at 37 °C for 10 min, and the reaction was stopped by adding 500 μL of 2,4-dinitrophenylhydrazine in 2 N HCl and 500 μL 2 N NaOH to avoid possible absorbance changes from altering the pH of the reaction mixture. After centrifugation for 5 min at 3000*g*, the absorbance at 510 nm was measured against a standard curve of pyruvate. Triplicate assays were performed for each extract. Enzymatic activity was expressed as micromoles of pyruvate formed per gram of fresh weight (fw) per hour.

Assay of PK stimulated in the presence of K^+, Ca^{2+}, and Mg^{2+} followed the same method as for basal PK, with the difference of adding to the extract either 0.1 mL of K^+ in the form of KCl (50 mM), 0.1 mL of Ca^{2+} in the form of $CaCl_2$ (50 mM), or 0.1 mL of Mg^{2+} in the form of $MgSO_4$ (50 mM). The processes of incubation, centrifugation, and activity measurement were the same as described for basal PK.

Cation Determination. Dry matter was digested with 96% H_2SO_4 in the presence of hydrogen peroxide (H_2O_2). Total potassium (K^+) was determined according to the flame photometer method (Lachica et al., 1973), whereas total calcium (Ca^{2+}) and total magnesium (Mg^{2+}) were analyzed by atomic absorption spectrophotometry (Hocking and Pate, 1977). All cations were expressed as milligrams per gram of dry weight (dw).

Yield. Plant yield was expressed as the mean of fruit weight. Cucumbers collected from each plant were weighed at sampling. Commercial yield (kilograms of fresh weight per plant) represents fruits with acceptable color and size.

Statistical Analysis. Analysis of variance was used to assess the significance of treatment means. Differences between treatment means were compared using the LSD at the 0.05 probability level. The mean of separation according to Duncan's multiple-range test was represented with letters as shown in the tables and figures. Levels of significance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and ns (not significant).

Table 1. Concentrations (Milligrams per Gram of Dry Weight) of K^+, Mg^{2+}, and Ca^{2+} in Leaves and Fruits in Response to K Fertilization[a]

K^+ rate (mg mL^{-1})	K^+		Mg^{2+}		Ca^{2+}	
	leaf	fruit	leaf	fruit	leaf	fruit
0.075	14.9c	5.64c	10.5a	2.97a	37.1a	2.01a
0.15	26.3b	8.26b	11.9a	3.22a	37.8a	2.12a
0.30	37.2c	13.3a	7.03b	1.85b	32.8b	1.04b

[a] Values within a column followed by the same letter are not different ($P > 0.05$).

RESULTS AND DISCUSSION

Ions at high concentrations in the culture medium are absorbed and transported rapidly to the aerial part of the plant, leading to a potentially excessive ion accumulation in those tissues (Cramer et al., 1991). With cucumber plants, these processes were depressed with K^+ in the leaves (Table 1). Foliar concentrations of K^+ increased as K fertilization increased ($P < 0.001$), registering the highest concentrations in the plants treated with K3 and the lowest with K1. The uptake and subsequent distribution of a given cation can inhibit or diminish both absorption and translocation of other cations toward the aerial part (Cramer et al., 1991). Here, as in other studies (Claasen and Wilcox, 1974; Ohno and Grunes, 1985; Hasegawa et al., 1995; Song and Fujiyama, 1996), high levels of K^+ lowered the foliar concentrations of Mg^{2+} and Ca^{2+} (Table 1). The foliar concentrations of Mg^{2+} ($P < 0.01$) and Ca^{2+} ($P < 0.01$) were highest with the K1 and K2 treatments and lowest with K3, indicating the antagonistic effect between K^+ and divalent cations when K^+ is applied at the K3 rate. It is notable that this antagonism was stronger between K^+ and Mg^{2+}, because the reduction of the latter was more significant (Table 1).

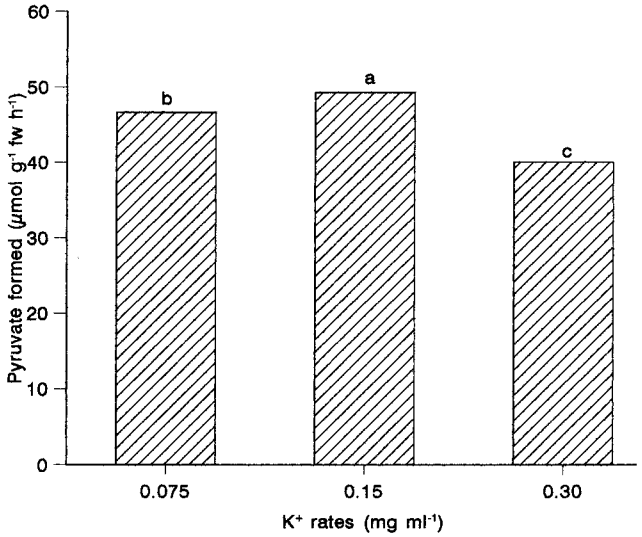

Figure 1. Influence of K fertilization on the basal PK activity in cucumber leaves. Bars topped by the same letter are not different ($P > 0.05$).

Kinetics of the activation of PK depend on the presence of the cations, principally Mg^{2+} (Podestá and Plaxton, 1991, 1992; Day and Copeland, 1993). Therefore, different works consider PK activity to be a possible physiological indicator of favorable cation levels or balance in the organ studied (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996). The basal PK activity in leaves (Figure 1) was highest with the treatment K2 and 23% greater than that of the K3 treatment ($P < 0.001$). The relationship between the basal PK activity and the foliar concentration of the different cations was closer with Mg^{2+}, reflecting the dependence of this enzyme on the presence of Mg^{2+} (basal PK activity with K^+, $r = -0.54$ ns; basal PK activity with Mg^{2+}, $r = 0.85^{**}$; basal PK activity with Ca^{2+}, $r = 0.79^{*}$).

Table 2 presents the PK activities stimulated with K^+, Mg^{2+}, and Ca^{2+}, as well as increases or decreases in these activities with respect to the basal PK activity. The PK activity stimulated with K^+, with the treatment K1, reached its highest activity ($P < 0.001$) as well as the greatest increase (27%) with respect to the basal PK activity in the same treatment, possibly reflecting deficient levels in K^+ at K1 (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996) due to the elevated Mg^{2+} and Ca^{2+} concentrations both in the soil and in the plant. The contrary trend occurred at K3, possibly indicating excess K^+, a condition that could have inhibited the PK activity either due to the direct action of the K^+ on this enzyme or due to the antagonism between K^+ and Mg^{2+}. Finally, in treatment K2, the PK activity stimulated with K^+ showed a minimal change (4%) with respect to the basal activity, indicating appropriate conditions of this cation (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996).

The trend of PK stimulated by Mg^{2+} (Table 2) was quite similar to the PK activity stimulated by K^+ (Table 2). The only striking feature is that the greatest activity ($P < 0.01$) and greatest increase (45%) were registered at K3, due to the lesser Mg^{2+} concentrations in this treatment. Finally, the PK activity stimulated with Ca^{2+} (Table 2) declined in treatments K1 and K2 ($P > 0.05$) compared with the basal activity of this treatments, reflecting appropriate or excessive amounts of Ca^{2+} (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996). The PK activity due to excess Ca^{2+} declined, probably because of the formation of Ca−PEP and Ca−ADP complexes, which impeded substrate use by the enzyme (Podestá and Plaxton, 1992). On the contrary, in treatment K3 there was a minimum increase of the PK activity stimulated by Ca^{2+} (6%) with respect to basal values, suggesting that the foliar concentrations of Ca^{2+} were inadequate.

For fruit growth, the mobility of inorganic nutrients and of organic compounds through the phloem is fundamental (Foyer and Galtier, 1996; Ho, 1996). With increasing rates of K^+ the concentration of K^+ in the fruit (Table 1) reached its highest concentration at K3 and its lowest at K1 ($P < 0.001$). The trends recorded for Mg^{2+} ($P < 0.01$) and for Ca^{2+} ($P < 0.01$) in the fruits were similar to that for the leaves, given that the highest concentrations were registered at K2 and the lowest at K3, also showing the antagonism, within the fruit, between K^+ and the other cations. Finally, the predominant cation in the fruits was K^+, due principally to its greater mobility through the phloem (Marschner et al., 1996), and therefore excessive K^+ application to the culture medium together with the fact of the lesser mobility through the phloem of Mg^{2+} and Ca^{2+} (Marschner et al., 1996) could aggravate the antagonistic effect between these cations in organs such as fruit.

The basal PK activity in fruit (Figure 2) followed a trend similar to that of the leaves. The greatest activity occurred at K2 and the lowest at K3, a decrease of 35% ($P < 0.01$). Again, the relationship between the concentration of the cations and the basal PK activity was closer for Mg^{2+} (basal PK activity with K^+, $r = -0.50$ ns; basal PK activity with Mg^{2+}, $r = 0.91^{**}$; basal PK activity with Ca^{2+}, $r = 0.81^{*}$).

The PK activity also proved to be a good indicator of the levels of cations in fruits. The PK activity stimulated by K^+ was greater at K1 and lesser at K3 than that at K2 ($P < 0.01$). On the other hand, the highest increase (32%) in the PK activity stimulated by K^+ in K1 with

Table 2. PK Activity (Micromoles per Gram of Fresh Weight per Hour) in Leaf and Fruit Extracts Treated with 50 mM K^+, Mg^{2+}, or Ca^{2+} [a]

K^+ rate (mg mL^{-1})	$PK_{(K^+)}$		$PK_{(Mg^{2+})}$		$PK_{(Ca^{2+})}$	
	leaf	fruit	leaf	fruit	leaf	fruit
0.075	59.2a (+27%)	9.75a (+32%)	50.2b (+8%)	8.22b (+12%)	41.6a (−11%)	9.46b (+29%)
0.15	51.1b (+4%)	10.4a (+9%)	51.6b (+5%)	10.3a (+7%)	40.3a (−18%)	11.5a (+20%)
0.30	33.9c (−15%)	5.04b (−19%)	58.2a (+45%)	10.9a (+76%)	42.5a (+6%)	9.36 (+51%)

[a] Values within a column followed by the same letter are not different ($P > 0.05$). Values in parentheses represent increases (+%) or decreases (−%) in the stimulated versus basal PK activities. PK_{K^+}, PK activity stimulated with K^+; $PK_{Mg^{2+}}$, PK activity stimulated with Mg^{2+}; $PK_{Ca^{2+}}$, PK activity stimulated with Ca^{2+}.

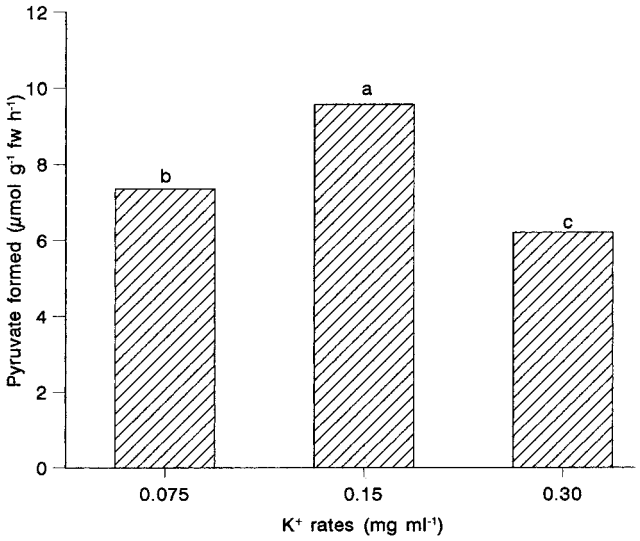

Figure 2. Influence of K fertilization on the basal PK activity in cucumber fruits. Bars topped by the same letter are not different ($P > 0.05$).

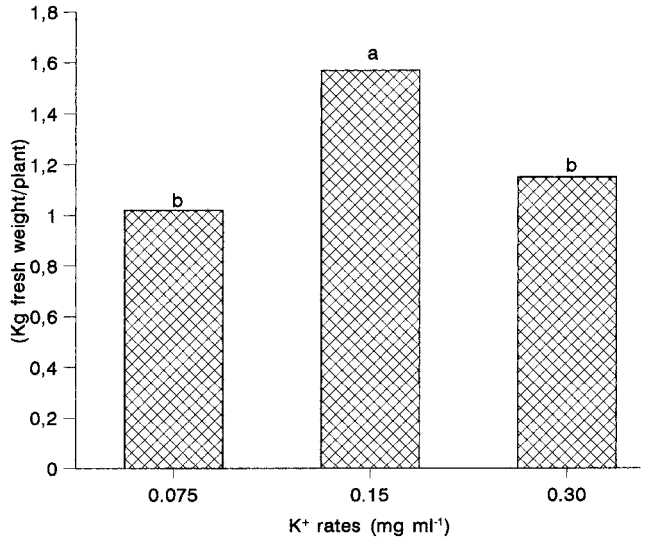

Figure 3. Influence of K fertilization on commercial yield in cucumber plants. Bars topped by the same letter are not different ($P > 0.05$).

respect to its basal PK activity indicates a possible deficiency of this nutrient in the fruit under K1 fertilization (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996). On the contrary, the decrease (−19%) registered at K3 suggests the possibility of excess K^+ in this treatment. Finally, the trend of PK stimulated with K^+ in K2, with a rise of only 9% with respect to the basal activity of this treatment, could represent adequate conditions of K^+ in the fruit (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996).

Meanwhile, the trends of the PK activities stimulated with Mg^{2+} and Ca^{2+} (Table 2) were similar. It is noteworthy that, although the PK activities were highest at K2 ($P < 0.01$), the increases with respect to the basal PK activity appeared at K3, indicating the possible deficit of Mg^{2+} and Ca^{2+} in the fruits of the K3 treatment. The most appropriate concentrations of Mg^{2+} and Ca^{2+} were approached in the K2 treatment, given that the increases in the PK activities stimulated with these cations were lower (Bar-Akiva et al., 1976; Lavon et al., 1988; Pulgar et al., 1996). It is also striking that, as opposed to the leaves, in the fruits the PK activities stimulated by Ca^{2+} (Table 2) were greater in all treatments than the corresponding basal activities. This behavior reflects a possible Ca^{2+} deficiency in the fruit, caused both by the low mobility of this ion through the phloem (Marschner et al., 1996) and by the antagonistic effect with regard to K^+.

Finally, the highest commercial yield values were reached at K2 ($P < 0.01$), with an increase of 36% over the lowest with K1 (Figure 3).

Our results lead to the following conclusions: (1) With the essential role of PK in plant growth taken into account (Plaxton et al., 1993; Huppe and Turpin, 1994; Podestá and Plaxton, 1994a,b), the highest basal PK activities were recorded both in leaves and in fruits in the K2 treatment. In addition, the relationship is well-known between the PK activity and N metabolism (Lin et al., 1989; Schuller et al., 1990; Vanlerberghe et al., 1990), the latter being fundamental to commercial yield (Mattson et al., 1990; López-Cantarero et al., 1997; Ruiz and Romero, 1998). In our experiment (data not shown) the K2 treatment resulted in the greatest foliar assimilation of N. (2) The results of the PK activities stimulated with K^+, Mg^{2+}, and Ca^{2+}, both in leaves and in fruits, indicate that in treatments K1 and K3 the plants displayed greater nutritional imbalance with regard to cation level, a situation that would generally carry grave consequences for growth and yield. On the other hand, in plants treated with K3, a possible antagonism results from the uptake of the NH applied, which would diminish N assimilation. This might explain both the fact that foliar assimilation of N in this treatment was lowest (data not show) and therefore the reduction in commercial yield, as reported in other works (López-Cantarero et al., 1997; Ruiz and Romero, 1998).

In conclusion, the highest application of K^+ (K3) to the culture medium reduced, in both the leaves and fruits, the foliar concentrations of Mg^{2+} and Ca^{2+}. These results are reflected by the lowest basal PK activities as well as by the highest differences between the basal PK activity and activities stimulated by these two cations. The contrary trend resulted at T2, with the highest basal PK activity and lowest differences between this activity and activities stimulated by the cations, indicating a better balance in this treatment between the different cations. Finally, this trend might partly account for the maximum commercial yield in plants treated with K2.

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