Phytochemistry, 1975, Vol. 14, pp. 57–59. Pergamon Press. Printed in England.

EFFECT OF POTASSIUM NUTRITION ON SOME ENZYMES FROM RIPENING *LYCOPERSICON ESCULENTUM* FRUIT

ROBERT T. BESFORD and GRAEME E. HOBSON

Glasshouse Crops Research Institute, Littlehampton, Sussex BN16 3PU

(*Received* 21 *April* 1974)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato fruit ripening; potassium nutrition; respiration; transaminase; malate dehydrogenase.

Abstract—The maximum respiration rate of tomato fruit during the climacteric period was markedly increased when the plants were grown under potassium-deficient conditions. Whereas potassium deficiency had no effect on cytoplasmic glutamate-oxoloacetate transaminase, there was a significant increase in the activity of this enzyme from mitochondria once the fruit began to change colour. Malate dehydrogenase was reduced in activity by potassium deficiency. It is suggested that the augmented mitochondrial transaminase levels, coupled with reduced malate dehydrogenase activity in low potassium fruit, result in reduced levels of oxaloacetic acid which is a potent inhibitor of Krebs cycle oxidations, thus leading to higher respiration rates for the intact fruit.

INTRODUCTION

In tomato fruit increased potassium (K) nutrition causes an increase in the concentration of organic acids, in particular citric and malic acids [1–3]. The tomato is a climacteric fruit [4–6], so that the preclimacteric respiration minimum is followed by a peak during which the rate rises by 100–250% [7, 8]. Plants supplied with high concentrations of K have a reduced rate of respiration [9], particularly during the climacteric phase. Oxaloacetic acid (OAA) which is also increased [10] by application of K is formed primarily from the oxidation of malate by malate dehydrogenase, has been shown to inhibit malate and succinate oxidation by tomato fruit mitochondria [11], and citrate, malate and succinate oxidation by potato and mung bean mitochondria [12]. The endogenous concentration of OAA could be controlled by the rate of transamination with L-glutamate through the action of glutamate-oxaloacetate transaminase (L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1) [11]. This transaminase could, therefore, influence the rate of turnover of Krebs cycle intermediates. We have examined the effects of K nutrition on fruit respiration, glutamate-oxaloacetate transaminase (GOT) activity and malate dehydrogenase (L-malate: NAD oxidoreductase, E.C. 1.1.1.37) activity during the later stages of fruit development.

RESULTS AND DISCUSSION

The mean peak respiration rate of low K fruit was significantly greater than that of fruit from plants receiving adequate K supplies (Table 1). After the fruit had passed through the climacteric peak, they were individually analysed for their K content. Plots of K concentrations in the fruit (x) against the corresponding maximum climacteric respiration rates (y) were closely correlated and gave a regression line $y = 5{\cdot}72 - 0{\cdot}36\,x$. The gradient, significantly less than zero ($t = 3{\cdot}96$ with $N = 6$, $P \leqslant 0{\cdot}01$), indicates a suppression of the respiration rate with increasing K concentration in the fruit.

As was the case with the apple [13], the climacteric respiration rise in tomato fruit might also involve the oxidation of malate, as its concentration decreases progressively during ripening [14]. In contrast to apple tissue, however, the major pathway for malate oxidation appears to be through the action of malate dehydrogenase rather than by means of the malic enzyme [15]. The activity of the malate dehydrogenase was significantly increased by raising the K level (Table 1). Despite the findings that increased K levels led not only to greater malate dehydrogenase activity but also to higher malate concentrations, the mean climacteric respiration maximum of tomato fruit in these circumstances was diminished.

Table 1. Effect of potassium nutrition on tomato fruit climacteric respiration rate and malate dehydrogenase activity

Source of fruit	Maximum climacteric respiration rate (mg CO_2/kg fresh tissue/hr)	Malate dehydrogenase* Activity per g fresh tissue	Activity per mg protein N
Low potassium nutrition	40·5	1·71	20·03
High potassium nutrition	34·1	4·65	34·27
Significance of the difference between means	$P \leqslant 0{\cdot}05$	$P \leqslant 0{\cdot}01$	$P \leqslant 0{\cdot}01$
LSD between means at $P = 0{\cdot}05$	5·2	1·02	5·03

* The activity is expressed as μmol OAA reduced per min. Tissue at the green–orange ripening stage was used. With the respiration data each value is the mean of four determinations and with the enzyme data the mean of three determinations.

It has previously been suggested that GOT may constitute a mechanism for the control of malate oxidation by restraint on the concentration of OAA [11], a potent inhibitor of the reaction. GOT has been found in both the cytoplasm and mitochondria of tomato pericarp tissue. The values given in Table 2 indicate that there was a progressive decrease in cytoplasmic activity during ripening, but that the activity at any stage of ripeness was not significantly affected by the degree of K nutrition. However, the proportion in the mitochondria from K-deficient and K-sufficient fruit increased up to the green–orange stage of ripeness and then fell again so that red fruit showed less than half the maximum value. Inadequate K nutrition significantly increased the mitochondrial GOT activity at all stages of ripeness once the change in colour of the fruit had begun. This could account for the increase in aspartate at the expense of glutamate, another characteristic of fruit of low K status [14].

In the present work, the climacteric respiration peak for most of the fruit examined coincided with the green–orange/orange–green stage of ripeness, which is approximately the point of maximum mitochondrial GOT activity. The close association between respiration and GOT activity was further demonstrated by the observation that both responded similarly to changes in K nutrition. At the mature green stage, neither was significantly affected by raising the K level, but with green–orange/orange–green fruit both respiration and mitochondrial GOT activity were significantly reduced (Tables 1 and 2).

Table 2. Effect of potassium nutrition on GOT activity during tomato fruit development

Source of enzyme	Enzyme activity*/100 g fresh tissue at the following stages: Large green	Mature green	Green–orange	Green–orange/orange–green	Orange–green	Red
Cytoplasm from						
low potassium fruit	17·18	16·85	15·22	14·68	10·47	9·80
high potassium fruit	21·67	15·27	12·37	12·73	10·20	9·73
Significance of the difference between treatment means	N.S.†	N.S.	N.S.	N.S.	N.S.	N.S.
Mitochondria from						
low potassium fruit	5·10	7·17	8·83	6·25	5·84	3·83
high potassium fruit	4·80	5·57	5·77	3·77	2·72	2·55
Significance of the difference between treatment means	N.S.	N.S.	$P \leqslant 0{\cdot}01$	$P \leqslant 0{\cdot}01$	$P \leqslant 0{\cdot}001$	$P \leqslant 0{\cdot}05$
Specific mitochondrial activity (per mg mitochondrial N) from						
low potassium fruit	1·09	1·54	1·89	1·56	1·46	1·09
high potassium fruit	1·03	1·19	1·24	0·94	0·68	0·73
Significance of the difference between treatment means	N.S.	N.S.	$P \leqslant 0{\cdot}01$	$P \leqslant 0{\cdot}01$	$P \leqslant 0{\cdot}001$	$P \leqslant 0{\cdot}05$

* The activity is expressed as μmol OAA reduced per min. Each value is the mean of three determinations.
† Indicates that the differences are not significant at $P = 0{\cdot}05$.

The climacteric respiration peak occurs in response to the integrated energy requirements of the fruit in order to carry out the various changes in composition and structure that accompany ripening [16, 17]. The time when the major acids begin to fall in concentration [14, 18, 19] is close to the stage in development when fruit enter the climacteric rise in respiration [7, 20], and a connection between these two phenomena may be found in the controlled respiratory oxidations in mitochondria, mediated by the concentration of OAA. From the present results on the effects of K nutrition on malate dehydrogenase, GOT and the extent of climacteric respiration, it is suggested that in normal fruit the level of OAA is sufficient to limit the turnover of the organic acids, in particular malic and citric, thus causing their accumulation. In conditions of K deficiency, the decreased levels of OAA in conjunction with enhanced GOT activity would not then inhibit steps in the Krebs cycle, thus allowing a higher climacteric respiration peak at the appropriate point in development.

EXPERIMENTAL

Tomato fruit cv. Amberley Cross were used for respiration and malate dehydrogenase studies and cv. Potella for transaminase assays. The effect of K on transaminase was similar in both varieties. After germination in peat/sand mixture, the plants were transferred to 23 cm whalehide containers filled with a peat–loam (2:1, v/v) medium supplemented with 0·8 kg NH_4NO_3, 0·4 kg Frit 253A, 2·4 kg $CaCO_3$, 2·4 kg Dolomitic limestone and 1·5 kg superphosphate per m^3, together with either 0·4 or 1·2 kg K_2SO_4 for the "low" or "high" K treatments respectively. A liquid feed containing 1·3 meq K^+ (50 ppm K^+), 5·8 meq NO_3^-, 4·5 meq NH_4^+ and 1·4 meq Mg^{2+}/l. for the low K treatment or 10·2 meq K^+ (400 ppm K^+), 10·2 meq NO_3^-, 1·4 meq Mg^{2+}/l. for the high K feed was given at every watering after flower set on the first truss [21].

The isolation of mitochondria and the method of GOT assay have been described previously [11]. Fruit for respiration studies were picked a few days prior to the mature green stage. CO_2-free air at 18·3° was passed over individual fruit contained in air-tight jars of about 400 ml capacity (flow rate *ca* 3 l./hr). H_2O vapour was removed from the gas stream by a "cold finger" surrounded by a freezing mixture, and the CO_2 concentration was measured by an Infrared Gas Analyser.

For malate dehydrogenase studies, chilled fruit pericarp tissue was macerated with 7 vol. of acetone at −15° for 1 min. The macerate was filtered under vacuum and washed with cold acetone until colourless. Samples were used directly or stored briefly at −15°. Aliquots of the acetone powders were stirred for 20 min with 15 ml 0·1 M Tris-HCl buffer pH 7·8 containing 10 mM 2-mercaptoethanol, the whole kept at 2°, squeezed through muslin and the filtrate centrifuged at 30000 *g* for 10 min at 2°. The supernatant was assayed for malate dehydrogenase activity at 25°. Incubates contained 2·87 ml of 0·1 M Tris-HCl buffer pH 8, 0·10 ml OAA in a similar buffer (2 mg/ml), 0·01 ml Na_2NADH (50 mg/ml) and 20 μl of enzyme preparation. The reaction was initiated by the addition of OAA. The enzyme and buffer concentrations were adjusted to give an initial decrease in A 340 nm of no more than 0·15 units per min. Under these conditions, the rate of reduction of OAA was linear for at least the first 2 min, hence the decrease in A_{340} during the interval 45–105 sec was recorded after initiating the reaction.

The nitrogen content of the disintegrated mitochondrial suspensions was determined by the method of Thompson and Morrison [22] as modified by Biale *et al.* [23]. The protein content of the enzyme extracts was measured after precipitation by 10% TCA and digestion with H_2SO_4 using Se as catalyst [24, 25]. The K in tomato fruit was determined following drying of the tissue at 80°, grinding to pass a 0·5-mm sieve and digestion in H_2SO_4, again with Se as catalyst.

Acknowledgements—We would like to express our thanks to Mr. D. A. Bone and Mrs. J. Finney for their skilled technical help, to Mr. A. R. Brown for results from the AutoAnalyzer, and to Dr. R. Nichols for the use of the Infrared Gas Analyser.

REFERENCES

1. Davies, J. N. (1964) *J. Sci. Food Agr.* **15,** 665.
2. Davies, J. N. and Winsor, G. W. (1967) *J. Sci. Food Agr.* **18,** 459.
3. Carangal, A. R., Jr., Alban, E. K., Varner, J. E. and Burrell, R. C. (1954) *Plant Physiol.* **29,** 355.
4. Gustafson, F. G. (1929) *Plant Physiol.* **4,** 349.
5. Workman, M. and Pratt, H. K. (1957) *Plant Physiol.* **32,** 330.
6. Basham, C. W. (1965) Ph.D. Thesis, University of Maryland.
7. Clendenning, K. A. (1942) *Can. J. Res.* **20C,** 197.
8. De Swardt, G. H. and Rousseau, G. G. (1973) *Planta* **112,** 83.
9. Trudel, M. J. (1969) Ph.D. Thesis, Cornell University.
10. Trudel, M. J. and Ozbun, J. L. (1971) *Naturaliste Can.* **98,** 83.
11. Besford, R. T. and Hobson, G. E. (1973) *Phytochemistry* **12,** 1255.
12. Douce, R. and Bonner, W. D. (1972) *Biochem. Biophys. Res. Commun.* **47,** 619.
13. Hulme, A. C., Jones, J. D. and Wooltorton, L. S. C. (1963) *Proc. Roy. Soc.* **158B,** 514.
14. Davies, J. N. (1966) *J. Sci. Food Agr.* **17,** 396.
15. Macrae, A. R. (1971) *Phytochemistry* **10,** 2343.
16. Hulme, A. C., Rhodes, M. J. C. and Wooltorton, L. S. C. (1967) *Phytochemistry* **6,** 1343.
17. Pratt, H. K. and Goeschl, J. D. (1969) *Ann. Rev. Plant Physiol.* **20,** 541.
18. Stevens, M. A. (1972) *J. Am. Soc. hort. Sci.* **97,** 655.
19. Sakiyama, R. (1966) *J. Jap. Soc. hort. Sci.* **35,** 36.
20. Abdul-Baki, I. I. (1964) Ph.D. Thesis, University of Illinois.
21. Ministry of Agriculture, Fisheries and Food (1962) *Tomatoes.* Bulletin No. 77, H.M.S.O., London.
22. Thompson, J. F. and Morrison, G. R. (1951) *Analyt. Chem.* **23,** 1153.
23. Biale, J. B., Young, R. E., Popper, C. S. and Appleman, W. E. (1957) *Physiol. Plant.* **10,** 48.
24. O'Neill, J. V. and Webb, R. A. (1970) *J. Sci. Food Agr.* **21,** 217.
25. Bould, C., Bradfield, E. G. and Clarke, G. M. (1960) *J. Sci. Food Agr.* **11,** 229.