*Acta Botanica Neerlandica* **11** (1962) 1–10

# THE DISTRIBUTION OF GLUTAMIC DEHYDROGENASE ACTIVITY AND α-KETOGLUTARATE IN VARIOUS PARTS OF THE TOMATO PLANT

J. VAN DIE

(*Plant Physiological Research Centre, Wageningen*)

(*Received December 21st, 1961*)

## INTRODUCTION

In former studies evidence has been obtained that α-ketoglutarate plays an important role in the synthesis of glutamine and proline in tomato roots, and that glutamic dehydrogenase is probably involved in the initial reaction of the assimilation of supplied ammonium (VAN DIE, 1959, 1960, 1961). On the grounds of these observations and those of other investigators one may assume that the concentrations of α-ketoglutarate and glutamic dehydrogenase in the cells will be of considerable importance for the counteraction of toxic ammonium accumulation in all the tissues which have to deal with relatively large amounts of external supplied—or endogenous—ammonium. Excessive endogenous ammonium formation takes place, for example, after rust infection in leaves of several plant species (ROHRINGER, 1957; SHAW, 1959; WAYGOOD and SMITH, 1959; FARKAS and KIRÁLY, 1961), and under conditions of extreme heat or drought in leaves of sunflower, corn, and water melon (PETINOV and MOLOTKOVSKY, 1957; KURSANOV, 1960).

Although in recent years several studies on ammonium toxicity have appeared (e.g. Goss, 1960; VINES and WEDDING, 1960) little attention has been paid to the distribution of α-ketoglutarate and glutamic dehydrogenase in the various parts of the plant. The aim of the present study was to obtain experimental data about the concentrations of these substances in various healthy tomato tissues.

## MATERIALS AND METHODS

### 1. *Plants*

The plant material used was taken from tomato plants (Ailsa Craig) of about 3 months old, growing in sand culture in the greenhouse.

### 2. *Preparation of the mitochondrial fractions*

Particle fractions were prepared by grinding the plant tissues in a medium composed of 0.4 M sucrose, 0.1 M phosphate buffer of pH 7.4, 0.003 M EDTA, and 0.01 M reduced glutathion. After removing cell wall fragments, plastids, and nuclei by centrifugation

at 1000 × g for 5 minutes, the supernatant was centrifuged at 21,000 × g for 30 minutes in a refrigerated centrifuge. The pellet was resuspended in ice-cold grinding medium and again centrifuged at 21,000 × g for 30 minutes. This washing was once repeated. Although the pellet obtained probably includes various intracellular components other than mitochondria, the metabolic activity of this fraction is generally assumed to be due to the activity of mitochondria. All operations took place at a temperature of 0–2° C.

3. *Standard assays of glutamic dehydrogenase and malic dehydrogenase activity*

Glutamic dehydrogenese activity was determined spectrophotometrically by following the decrease in extinction at 340 mμ upon oxidation of DPNH, at a temperature of 23° C. All measurements were made with a Unicam SP 500 spectrophotometer with cuvettes having a light path of 1.00 cm and a final volume of 3.5 ml. The complete reaction mixture in the cuvette contained: 0.6 micromole DPNH, 50 micromoles $(NH_4)_2$ $SO_4$, 1.5 micromoles NaCN, 30 micromoles nicotinic acid adjusted to pH 7.4, 15 micromoles K-α-ketoglutarate, 250 micromoles of phosphate buffer of pH 7.4, and sufficient enzyme to get a decrease in extinction of about 0.02 to 0.06 per minute. The reaction was started by the addition of the DPNH after which measurements of the change in extinction were made at 3 or 4 minutes intervals, depending on the rate of decrease in extinction, for about 20 minutes.

Malic dehydrogenase activity was also determined spectrophotometrically by following DPNH oxidation at 340 mμ. The complete reaction mixture in the cuvette (3.5 ml) contained 0.6 micromole DPNH, 1.5 micromoles NaCN, 30 micromoles nicotinic acid adjusted to pH 7.4, 5 micromoles of K-oxaloacetate, and 250 micromoles of phosphate buffer of pH 7.4.

A unit of enzyme activity is defined as that amount of enzyme which causes a rate of decrease in extinction at 340 mμ of 0.001 per minute, calculated from the linear portion of the curve relating $E_{340}$ with reaction time.

4. *α-Ketoglutaric acid*

Freshly excised plant tissue was homogenized and several times extracted with 4N sulphuric acid at 0–4° C. One-tenth volume of one percent 2 : 4 dinitrophenylhydrazine in 4N sulphuric acid was added to the combined extracts, followed by one hour incubation at room temperature. The hydrazones were extracted, separated and determined essentially as previously described (Van Die, 1960).

5. *Protein*

The protein contents of mitochondrial extracts were measured by the ultra violet method of Waddell (1956) and Tombs *et al.* (1959). Glutathion, which interferes, was not added to the extracts used for protein determinations. Recrystallized egg albumin (Sigma) was used for the reference curves. 0.05 M phosphate buffer of pH 7.4 was used as solvent and as the blank (Murphy and Kies, 1960).

## RESULTS

### 1. *The time course of the reaction*

The time course of the glutamic dehydrogenase reaction is represented in Fig. 1. In the absence of either α-ketoglutarate or ammonium ions practically no DPNH oxidation takes place. If near the end of the reaction a DPNH-regenerating system consisting of yeast alcohol dehydrogenase and ethanol is added to the complete reaction mixture, all the original absorption at 340 mμ of DPNH is

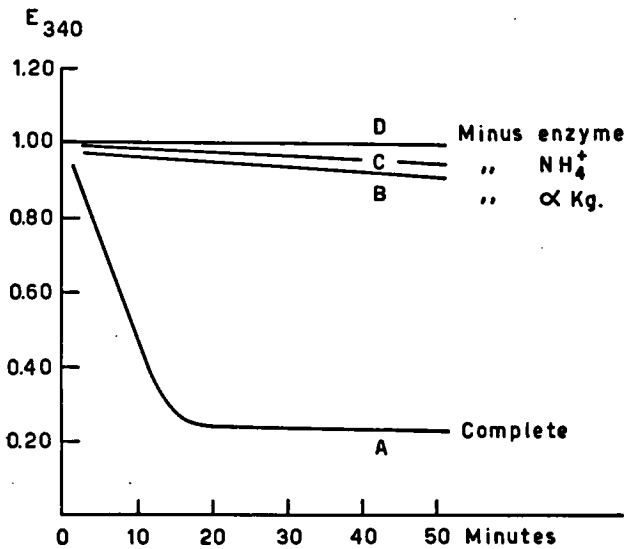


Fig. 1. The rates of DPNH oxidation by an extract of tomato root mitochondria in the presence (curve A) and in the absence (curves B and C, respectively) of α-ketoglutarate and ammonium ions. Curve D represents the same as curve A, except that the enzyme extract was omitted.

recovered. This clearly demonstrates DPN to be one of the products of the enzyme reaction. Paper chromatographic analysis of the cuvette contents when DPNH oxidation was nearly complete revealed the presence of glutamic acid. No other ninhydrin reactive substances were formed.

### 2. *The intracellular localization of glutamic dehydrogenase*

A crude mitochondrial preparation from tomato roots was subjected to two washings with ice-cold 0.4 M sucrose + 0.1 M phosphate buffer of pH 7.4, each washing being followed by 30 minutes centrifuging at 21,000 × g to remove cytoplasmic solutes and soluble components from damaged particles. The extracts were assayed for glutamic dehydrogenase activity and protein content. The particle pellet was resuspended in ice-cold 0.05 M phosphate buffer, frozen in ethanol-solid $CO_2$ and thawed in tap water, after which the

TABLE 1

Protein contents and glutamic dehydrogenase (GDH) activities of extracts from a crude mitochondrial preparation of tomato roots.

| Extract No. | Protein mg | % of total Protein | GDH Units | % of total GDH | Relative specific activity |
|---|---|---|---|---|---|
| W 1 | 2.33 | 36.7 | 216 | 13.5 | 0.37 |
| W 2 | 0.86 | 13.5 | 133 | 8.3 | 0.61 |
| M 1 | 1.72 | 27.1 | 1002 | 62.6 | 2.31 |
| M 2 | 0.74 | 11.6 | 211 | 13.2 | 1.14 |
| M 3 | 0.45 | 7.1 | 35 | 2.1 | 0.29 |
| M 4 | 0.24 | 3.9 | 5 | 0.3 | 0.08 |

W 1 and W 2: extracts obtained respectively from two successive washings of the crude mitochondrial fraction with cold 0.4 M sucrose–0.1 M phosphate buffer of pH 7.4.

M1, M2, M3, M4: extracts obtained after four successive cycles of freezing and thawing of the washed mitochondria in cold 0.05 M phosphate buffer of pH 7.4.

The relative specific activity is the percentage of total GDH-activity divided by the percentage of total protein.

particle-residues were precipitated by centrifuging. A few cycles of freezing and thawing gave almost complete release of glutamic dehydrogenase. Table 1 gives the protein contents and glutamic dehydrogenase activities of the various extracts and shows the mitochondrial origin of the enzyme.

3. *The distribution of glutamic dehydrogenase activity*

Freshly excised tissue parts were homogenized and repeatedly extracted with cold 0.1 M phosphate buffer to which had been added 0.01 M glutathion and 0.003 M EDTA. Each extraction was followed by centrifugation at 21,000 × g. In most cases 3 extractions were sufficient for total extraction of glutamic dehydrogenase activity. Each extract was then separately assayed for enzyme activity. The total amount of such activity, expressed as the number of glutamic dehydrogenase units per gram fresh weight of tissue, was determined for young lateral roots, old roots, secondary xylem and cortex-phloem regions of the roots, and also for stem cortex and pith, and leaves. Except for the secondary xylem, all tissues investigated showed glutamic dehydrogenase activity (Table 2). Of these, the cortex-phloem region of the young roots has an especially high glutamic dehydrogenase activity level, while the other parts of roots and stem show a relatively low glutamic dehydrogenase content.

The explanation for the higher enzyme activity in lateral roots as compared with other plant parts is very probably to be found in the greater concentration of mitochondria in the cells of these tissues rather than in any difference in mitochondrion composition. Assuming that all glutamic dehydrogenase is located in the mitochondria, one can easily calculate from the data in Table 3 that lateral roots contain more mitochondria per unit dry weight than do pith parenchyma cells: Lateral roots contain about 16–20 % dry "mitochondria",

TABLE 2

The distribution of glutamic dehydrogenase activity in various organs and tissues of tomato plants.

| Organ | Tissue | Units GDH per gr. fresh wt. |
|---|---|---|
| Leaves | whole leaves | 52 |
| | whole leaves | 31 |
| Stem | cortex-phloem | 782 |
| | cortex-phloem | 592 |
| | pith | 86 |
| | pith | 98 |
| | pith | 74 |
| Main root | cortex-phloem | 330 |
| | cortex-phloem | 374 |
| | cortex-phloem | 404 |
| | cortex-phloem | 635 |
| | xylem | absent |
| Lateral roots (1st order) | cortex-phloem | 419 |
| | cortex-phloem | 528 |
| | xylem | absent |
| Young roots | whole roots | 2057 |
| | whole roots | 2572 |
| | whole roots | 1852 |

while for pith parenchyma cells this figure is 4–5 %. The so-called "mitochondrial fraction" undoubtedly contains a lot of cell components other than mitochondria, and it is probable that these non-mitochondrial elements contribute in different proportions to the respective mitochondrial fractions derived from root and pith parenchyma. Tables 4 and 5 show that this assumption is probably true: Washed mitochondrial fractions of both plant parts were subjected to a number of extractions—which each followed on one cycle of freezing and thawing. The specific activities of glutamic dehydrogenase and malic dehydrogenase (units per mg protein) in the various

TABLE 3

A comparison of the glutamic dehydrogenase content of dry roots, stem pith parenchyma and their respective mitochondrial fractions.

| Plant part | Units of GDH per mg dry wt. |
|---|---|
| Young roots | 34 |
| Mitochondrial fraction | 169 |
| Young roots | 39 |
| Mitochondrial fraction | 249 |
| Stem pith parenchyma | 1.0 |
| Mitochondrial fraction | 24 |
| Stem pith parenchyma | 1.5 |
| Mitochondrial fraction | 28 |

TABLE 4

The extraction of protein, glutamic dehydrogenase activity (GDH), and malic dehydrogenase activity (MDH) from mitochondrial preparations of pith parenchyma and lateral roots of tomato plants. The relative specific activity is the percentage of total enzyme activity divided by the percentage of total protein.

| Extract no. | Extracted protein in mg | % of total protein | Extracted GDH in units | % of total GDH | Units of GDH per mg protein | Extracted MDH in units | % of total MDH | Units of MDH per mg protein | Relative specific activity GDH | Relative specific activity MDH |
|---|---|---|---|---|---|---|---|---|---|---|
| *pith parenchyma* | | | | | | | | | | |
| 1 | 0.89 | 42.1 | 681 | 54.1 | 765 | 9113 | 47.0 | 10237 | 1.28 | 1.11 |
| 2 | 0.56 | 26.5 | 440 | 34.9 | 785 | 6638 | 34.2 | 11840 | 1.31 | 1.29 |
| 3 | 0.30 | 14.2 | 110 | 8.7 | 366 | 2895 | 14.9 | 9650 | 0.61 | 1.05 |
| 4 | 0.21 | 10.0 | 28 | 2.2 | 133 | 579 | 3.0 | 2757 | 0.22 | 0.30 |
| 5 | 0.15 | 7.1 | — | — | — | 155 | 0.8 | 1033 | — | 0.11 |
| Total . . . | 2.11 | 100 | 1259 | 100 | 642 | 19380 | 100 | 9185 | — | — |
| *lateral roots* | | | | | | | | | | |
| 1 | 1.39 | 57.7 | 1095 | 71.2 | 787 | 13980 | 69.5 | 10057 | 1.25 | 1.23 |
| 2 | 0.63 | 25.7 | 390 | 25.3 | 619 | 4890 | 24.3 | 7762 | 0.98 | 0.94 |
| 3 | 0.28 | 11.4 | 53 | 3.4 | 190 | 1098 | 5.5 | 3902 | 0.30 | 0.48 |
| 4 | 0.15 | 6.1 | — | — | — | 131 | 0.6 | 873 | — | 0.10 |
| Total . . . | 2.45 | 100 | 1538 | 100 | 665 | 20099 | 100 | 8162 | — | — |

TABLE 5

The extraction of protein, glutamic dehydrogenase activity (GDH), and malic dehydrogenase activity from mitochondrial preparations of pith parenchyma and lateral roots of tomato plants.

| Extract no. | Extracted protein in mg | % of total protein | Extracted GDH in units | % of total GDH | Units of GDH per mg protein | Extracted MDH in units | % of total MDH | Units of MDH per mg protein | Relative specific activity GDH | Realtive specific activity MDH |
|---|---|---|---|---|---|---|---|---|---|---|
| *pith parenchyma* | | | | | | | | | | |
| 1 | 1.36 | 56.3 | 369 | 59.6 | 271 | 13200 | 57.6 | 9706 | 1.06 | 1.02 |
| 2 | 0.49 | 20.3 | 220 | 35.6 | 449 | 8400 | 36.6 | 17143 | 1.75 | 1.80 |
| 3 | 0.29 | 12.0 | 29 | 4.6 | 100 | 970 | 4.2 | 3345 | 0.38 | 0.35 |
| 4 | 0.15 | 6.2 | — | — | — | 254 | 1.1 | 1693 | — | 0.18 |
| 5 | 0.12 | 5.0 | — | — | — | 86 | 0.4 | 716 | — | 0.08 |
| Total . . . | 2.41 | 100 | 618 | 100 | 288 | 22910 | 100 | 9506 | — | — |
| *lateral roots* | | | | | | | | | | |
| 1 | 0.75 | 56.0 | 771 | 80.1 | 1028 | 9750 | 79.9 | 13000 | 1.43 | 1.43 |
| 2 | 0.33 | 24.6 | 176 | 18.2 | 533 | 2055 | 16.8 | 6227 | 0.74 | 0.68 |
| 3 | 0.11 | 8.5 | 15 | 1.5 | 131 | 243 | 2.0 | 2132 | 0.18 | 0.23 |
| 4 | 0.06 | 4.7 | — | — | — | 113 | 0.9 | 1765 | — | 0.19 |
| 5 | 0.08 | 5.9 | — | — | — | 69 | 0.5 | 862 | — | 0.08 |
| Total . . . | 1.33 | 100 | 962 | 100 | 808 | 12230 | 100 | 9195 | — | — |

extracts from lateral roots or pith parenchyma do not show much difference. Consequently it seems that the large difference which exist between the glutamic dehydrogenase activity levels of lateral roots and pith parenchyma cells depends on differences in mitochondrion concentrations in the cells of these plant parts.

4. *The α-ketoglutarate content of various plant parts*

In the preceding experiment it was shown that glutamic dehydrogenase is especially active in the young roots, while in the other plant parts its activity is much less. Quantitative determination of the α-ketoglutarate content of various parts of the stem and root system established a more or less comparable situation for this substrate of

TABLE 6

The α-ketoglutarate content of various parts of the root system of a young tomato plant.

| Plant part | α-Ketoglutarate per gr. fresh wt. |
|---|---|
| Stem base | 0.60 μ Mol |
| | 0.52 μ Mol |
| | 0.44 μ Mol |
| Stem/root transition | 0.54 μ Mol |
| | 0.48 μ Mol |
| | 0.82 μ Mol |
| Main root | 0.70 μ Mol |
| | 0.64 μ Mol |
| | 0.69 μ Mol |
| Lateral roots (1st order) | 2.9 μ Mol |
| | 3.0 μ Mol |
| Lateral roots (2nd, 3rd, etc. order) | 6.7 μ Mol |
| | 5.9 μ Mol |

glutamic dehydrogenase (Table 6). In stem-main root axis sections the α-ketoglutarate content was low compared with that found in the lateral roots, which was generally many times higher.

## DISCUSSION

The experimental results demonstrate that an appreciable amount of glutamic dehydrogenase activity is localized in the mitochondria. This is in accordance with the observations of RAUTANEN and TAGER (1955) for *Avena* homogenates, and of ESTERMANN *et al.* (1959) for *Lupinus* cotyledons. The glutamic dehydrogenase activity is easily released from the tomato mitochondria by membrane rupture. The young roots in particular appear to be rich in glutamic dehydrogenase activity, both on the basis of total fresh (Table 2) or dry weight and dry weight of the mitochondrial fraction (Table 3). Thus it appears that young roots contain about 20 to 30 times as much glutamic dehydrogenase activity—on a fresh or dry weight basis—as pith parenchyma. Expressed on a basis of "dry mitochondria", however,

this ratio is about 8 : 1 (Table 3). On a basis of "extractable mitochondrial proteins" there exists hardly any difference between the specific activity of glutamic dehydrogenase derived from roots and that from pith parenchyma (Table 4 and 5). Consequently it seems that the mitochondrion composition with regard to glutamic dehydrogenase, and also malic dehydrogenase, is the same for both plant parts. The lateral roots with their meristematic character possess much more mitochondria than the metabolically less active pith parenchyma cells, and thus have a much higher level of enzyme activity.

The $\alpha$-ketoglutarate determinations show results more or less comparable with those of the glutamic dehydrogenase analyses. The lateral roots especially have a relatively high concentration of $\alpha$-ketoglutarate when compared with, for example, stem sections. The meristematic character of the lateral roots is probably responsible for this phenomenon. Aerobic fermentation is often observed in meristematic plant parts (BETZ, 1960) and will lead to the formation of respiratory intermediates. Apart from that, the accumulation of organic acids in plant cells is a common feature. The accumulation of $\alpha$-ketoglutaric acid, for example, has been observed in *Chlorella* (SYRETT and FOWDEN, 1952) and in yeast (HOLZER, 1958). Probably the organic acids in plant cells are mainly localized in the central vacuoles, and so more or less withdrawn from the pool of rapidly "turned over" respiratory intermediates in the cytoplasm. Animal cells—which lack the central vacuole—normally never accumulate intermediates of the tricarboxylic acid cycle (KREBS and LOWENSTEIN, 1960).

The meristematic character of the root system and its high content of intra-cellular particles makes it a potential synthetic centre. Penetrated ammonium will be rapidly incorporated in the $\alpha$-ketoglutarate skeleton. As the young roots are also the sites at which ion uptake predominantly take place, one may assume that the uptake of ammonium and its assimilation to the amino-acid level are two aspects of the same chemical reaction: the reductive amination of $\alpha$-ketoglutarate in the outer cell layers of the young roots.

Leaf and pith parenchyma cells seem to be insufficiently equiped for a rapid removal of ammonium. Although aerial parts of the plant are normally not exposed to externally supplied ammonium, it may be that the accumulation of glutamine in these plant parts after ammonium uptake by the roots (VAN DIE, 1961), can lead to the formation of endogenous ammonium. According to KURSANOV (1960) dry natural conditions induce the formation of endogenous ammonium in sunflower leaves, which after being translocated to the roots, is again assimilated to amides and amino acids and in this form transported back to the shoots. Experiments of FARKAS and KIRÁLY (1961) demonstrate that a low concentration of $\alpha$-ketoglutarate in wheat leaves may be the limiting factor in glutamic acid formation from endogenous ammonium.

The present results and the data from literature suggest that the harmful effects often observed with ammonium in plant nutrition

may be the result of breakdown of amides in aerial plant parts, which are insufficiently equiped for the detoxication of the endogenous ammonium by reductive amination of α-ketoglutarate.

## SUMMARY

Glutamic dehydrogenase activity proved to be especially high in the cortex-phloem region of the young lateral roots. Extracts of other organs and tissues were much less active. In all tissues examined, glutamic dehydrogenase appeared to be localized in the mitochondrial fractions. The relative amounts of the mitochondria in the cells of the various tissues probably determine the glutamic dehydrogenase content of these tissues.

α-Ketoglutarate concentrations were also highest in the youngest roots and much lower in main root and stem tissues.

It is suggested that the uptake of ammonium from the nutrient solution and its assimilation to glutamic acid are two aspects of the same chemical reaction.

## ACKNOWLEDGEMENTS

The author is much indebted to Miss M. de Bruin and Mr. C. R. Vonk for their assistance with the experimental part, and to Mr. M. M. Martin, MSc., for the correction of the English text.

## REFERENCES

BETZ, A. 1960. Handbuch d. Pflanzenphysiologie (W. Ruhland). Berlin, Göttingen, Heidelberg. Band **12**: 88.

DIE, J. VAN. 1959. Proc. Kon. Ned. Akad. Wetensch. **C 62**: 505.

————. 1960. Proc. Kon. Ned. Akad. Wetensch. **C 63**: 230.

————. 1961. Proc. Kon. Ned. Akad. Wetensch. **C 64**: 375.

————. 1961. Proc. 5th intern. Congr. Biochem. Moscow.

ESTERMANN, E. F., E. E. CONN and A. D. MCLAREN. 1959. Arch. Biochem. Biophys. **85**: 103.

FARKAS. G. L. and Z. KIRÁLY. 1961. Physiol. Plant. **14**: 344.

GOSS, J. A. 1960. Soil Sci. **89**: 296.

HOLZER, H. 1958. Aerobe Gärung und Wachstum. Colloquium d. Deutsch. Ges. f. Physiol. Chem. Heidelberg.

KREBS, H. A. and J. M. LOWENSTEIN. 1960. In D. M. Greenberg: Metabolic pathways. Vol. **1**. New York-London.

KURSANOV, A. L. 1960. The physiology of the whole plant. Occasional publication No. **12**, Wye College (London).

MURPHY, J. B. and M. W. KIES. 1960. Biochim. Biophys. Act. **45**: 382.

PETINOV, N. S. and YU. G. MOLOTKOVSKY. 1957. Fiziol. rast. **4** (3): 225.

RAUTANEN, N. and J. M. TAGER. 1955. Ann. Acad. Sci. Fennicae **2**: 241.

ROHRINGER, R. 1957. Phytopath. Z. **29**: 45.

SHAW, M. 1959. Proc. 4th intern. Congr. Crop Protection. Hamburg.

SYRETT, P. J. and L. FOWDEN. 1952. Physiol. Plant. **5**: 558.

TOMBS, M. P., F. SOUTER and N. F. MACLAGAN. 1959. Biochem. J. **73**: 167.

VINES, H. M. and R. T. WEDDING. 1960. Plant Physiol. **35**: 820.

WADDELL, W. J. 1956. J. Lab. clin. Med. **48**: 311.

WAYGOOD, E. R. and J. E. SMITH. 1959. Proc. 9th intern. Bot. Congr. Montreal.