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BRIEF COMMUNICATION

# Activity of $C_4$ enzymes in $C_3$-type herbaceous plants

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## Abstract

The activity of enzymes characteristic for $C_4$-type photosynthesis was determined in different organs of two herbaceous plants: *Reynoutria japonica* Houtt. and *Helianthus tuberosus* L. The activity of phospho*enol*pyruvate carboxylase (PEPC) was usually higher in the roots, some of the stem tissues and petioles in comparison to the leaf blades. The highest activity of malic enzymes (NAD-ME, NADP-ME) and phospho*enol*pyruvate carboxykinase (PEPCK) was in the petioles and stem tissues of both plants and the lowest in the leaf blades and the pith of *Helianthus tuberosus* L.

*Additional key words*: $C_3$ plants; $C_4$ photosynthesis; conductance; Japanese knotweed; malic enzymes; phospho*enol*pyruvate carboxykinase; phospho*enol*pyruvate carboxylase; stem; topinambur.

The leaves of $C_3$ plants use atmospheric $CO_2$, while in plants where CAM and $C_4$ photosynthesis occurs, there is a mechanism which concentrates atmospheric $CO_2$ in both leaves and stems. In the stems of $C_3$ plants the source of $CO_2$ for photosynthesis is not clear (Pfanz and Aschan 2001, Hibberd and Quick 2002, Berveiller and Damesin 2008). High diffusive resistance of the epidermis or peridermis of the stem (in comparison to leaves) strongly limits the exchange of $CO_2$ between the stem and the external atmosphere. Therefore high concentrations of $CO_2$ are released during dark respiration of the stem tissues (Pilarski 1994, Wittman and Pfanz 2008). The concentration of $CO_2$ in stems can be as much as 300 times higher than in the atmosphere (Teskey *et al.* 2008), while in the CAM plants it can be up to 60 times higher (Lüttge 2004) and just a few times higher in the $C_4$ plants (Furbank and Hatch 1987). This is conducive to photosynthetic reassimilation of the released $CO_2$ that is measured as a difference between efflux of $CO_2$ in the dark and after lighting the stem. It ranges widely from 40 to 110 % (Cernusak and Marshall 2000, Vick and Young 2009). The intensive respiration of the stems also leads to a lower concentration of $O_2$ (Teskey *et al.* 2008) and to the risk of hypoxia (Pfanz *et al.* 2002).

Under such conditions there may be an additional mechanism of building $CO_2$ in malate by PEPC. It is known that PEPC exists in $C_3$ plants in the cytosol of all plant tissues. It has an anapleurotic role of building $CO_2$ in the cycle of citric acid (Andreo *et al.* 1987) that was confirmed in the roots (Höll 1974) and fruits (Blanke and Notton 1991, Muñoz *et al.* 2001). Hibberd and Quick (2002) were the first to notice the additional role of PEPC in the tissues of $C_3$ plants (tobacco) and its participation in a mechanism very similar to $C_4$ photosynthesis. According to their hypothesis, the roots are the source of malate which together with xylem sap is transported and then undergoes decarboxylation in the vascular bundles of stems and leaves where $CO_2$ is reassimilated. A high ability of malate to be decarboxylated by NAD-ME, NADP-ME and PEPCK in the petioles of tobacco (Hibberd and Quick 2002) and in the mid vein of the leaves *Arabidopsis thaliana* (Brown *et al.* 2010) has been shown. Berveiler and Damesin (2008) found that the activity of PEPC and NAD-ME in the bark of the beech, which is a typical $C_3$ plant, is significantly higher than in the leaves.

The aim of this work was to verify the information about the mechanism of $C_4$ photosynthesis in herbaceous $C_3$ plants by defining the activity of the enzymes taking part in the β-carboxylation and decarboxylation of malate. Two species of herbaceous plants that differ from each other in their permeability of the stem's epidermis for gases were used: *Reynoutria japonica* Houtt. (Japanese knotweed) which has a low permeable epidermis

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*Abbreviations*: NAD-ME, NADP-ME – malic enzymes; PEPC – phospho*enol*pyruvate carboxylase; PEPCK – phospho*enol*pyruvate carboxykinase; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase.

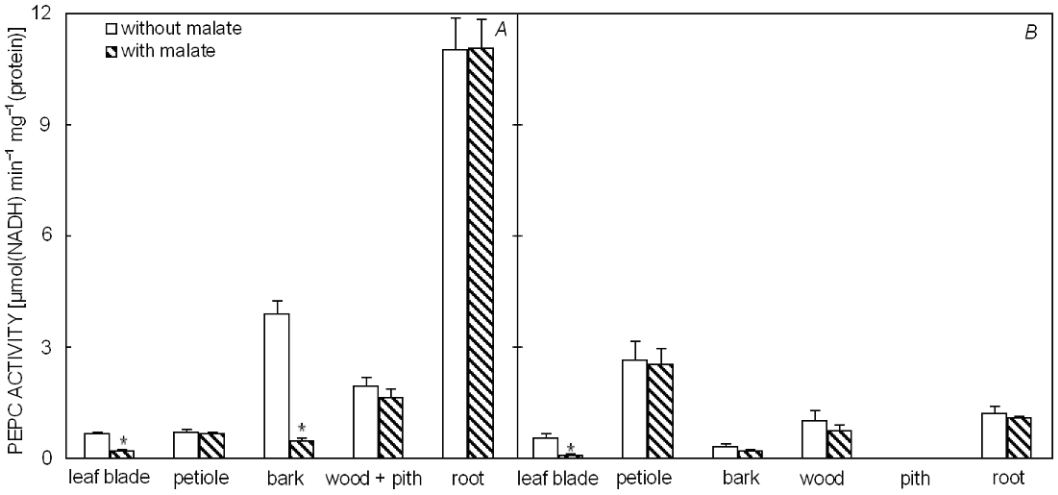


Fig. 1. Phospho*enol*pyruvate carboxylase (PEPC) activity in different parts of (*A*) *Reynoutria japonica* Houtt. (Japanese knotweed) and (*B*) *Helianthus tuberosus* L. (topinambur). Means±SD ($n$ = 5). *T*-tests were performed for pairs of coresponding values (with or without of L-malic acid). *Asterics indicate significant decrease of PEPC activity with presence of 0.2 M L-malic acid at $p<0.001$.

and consequently a high concentration of $CO_2$ inside the stem and *Helianthus tuberosus* L. (topinambur) which has a 5 times higher permeable epidermis and lower concentration of $CO_2$ in its stem (Kocurek 2007).

The determination of PEPC activity was done according to Brulfert *et al.* (1988), while NADP-ME and NAD-ME as well as PEPCK were determined according to Ashton *et al.* (1990). Enzyme activity was marked in the roots, blades, petioles, and stem tissues. In topinambur it was marked in the bark, wood and pith, while in Japanese knotweed – in the bark and wood with pith. In the analyzed tissues the protein content was determined by the Bradford method (1976), and the content of chlorophyll by the Wellburn method (1994).

The plants were grown under field conditions. Experimental samples were collected in July at 9:00 h. The leaves came from the fully developed seventh nodes and fragments of the stem from the seventh internodes.

The activity of PEPC in different organs of Japanese knotweed and topinambur (Fig. 1) ranged from 0.0 to 11.0 µmol(NADH) $min^{-1}$ $mg^{-1}$(protein). As for the Japanese knotweed the lowest activity of PEPC was seen in leaf blades. It was a little bit higher in the petioles and wood with pith. In comparison to the blade, the bark had 6 times and the roots 18 times higher activity. As for topinambur, the highest activity of PEPC was found in the petioles – 5 times higher than in the leaf blade. In wood and roots there was 2 times higher PEPC activity in comparison to the blade. In the bark PEPC activity was 2 times lower than in blades. In the topinambur's pith there was no PEPC activity.

Berveiller and Damesin (2008) found a 13 times higher activity of PEPC in the bark of beech in comparison to the leaves. A high PEPC activity was also observed in the roots (Höll 1973, 1974, Thomas *et al*. 1992, Gao *et al.* 1996, Cramer and Richards 1999). In our case the PEPC isoforms that are indifferent to malate were indicated in the roots of both plants, while the activity of PEPC from Japanese knotweed's bark and leaves of both plants depended on the presence of malate.

In a parallel experiment, xylem sap was obtained from stems of both plants with a pressure chamber (*PMS Instruments*, Corvallis, OR). The determination of L-malate was done according to Möllering (1985). Depending on the time of day and the part of the stem 0.01 to 0.6 mM of malate were found in the xylem sap of the Japanese knotweed's stem, depending on the time of day and the part of the stem. As for topinambur no significant daily fluctuation was seen. Moreover, the content of malate measured at various height of the stem ranged narrow from 0.25 to 0.45 mM. Hibberd *et al.* (1999) recorded a 10-times fall of malate concentration from 0.23 to 0.02 mM in the xylem sap of a tomato depending on the plant height considering the roots as the place of its origin.

A high activity of decarboxylation enzymes was noted in the stem tissues and petioles of Japanese knotweed and topinambur but not in the leaf blades. (Table 1). As for Japanese knotweed, the tissue with the highest activity was the bark. As for malic enzymes, the bark had 5-times higher and for PEPCK 6-times higher activity in comparison to the blade. Topinamburs petioles showed the highest activity of malic enzymes. It was about 2–3 times higher and PEPCK 6 times higher than in the leaf blade. In the bark and wood the NAD-ME and PEPCK activity was, respectively, 1.3–2 and about 5 times higher than in the leaf blade.

Hibberd and Quick (2002) found a similarly high activity of decarboxylating enzymes in the veins of tobacco: NAD-ME activity reached 13, while NADP-ME and PEPCK 9 times higher level than in the leaves. High activities of decarboxylates in mid vein were recorded by

Table. 1. Activity of malic (NADP-ME, NAD-ME) and phospho*enol*pyruvate carboxykinase (PEPCK) enzymes in parts of *Reynoutria japonica* Houtt. (Japanese knotweed) and *Helianthus tuberosus* L. (topinambur). Means ± SD ($n$ = 5).

| Enzyme activity | | NADP-ME [μmol $min^{-1}$ $mg(Chl^{-1})$] | NAD-ME [μmol $min^{-1}$ $mg(Chl^{-1})$] | PEPCK [μmol $min^{-1}$ $mg(Chl^{-1})$] |
|---|---|---|---|---|
| Japanese knotweed | | | | |
| leaf | blade | 0.46 ± 0.01 | 0.52 ± 0.02 | 9.99 ± 0.01 |
| | petiole | 0.98 ± 0.05 | 0.80 ± 0.11 | 17.62 ± 1.07 |
| stem | bark | 2.21 ± 0.14 | 2.50 ± 0.03 | 57.14 ± 4.02 |
| | pith + wood | 0.68 ± 0.07 | 0.76 ± 0.09 | 25.62 ± 3.67 |
| Topinambur | | | | |
| leaf | blade | 0.55 ± 0.04 | 0.61 ± 0.05 | 5.61 ± 0.82 |
| | petiole | 1.08 ± 0.17 | 1.78 ± 0.20 | 35.12 ± 5.53 |
| stem | bark | 0.57 ± 0.06 | 0.79 ± 0.04 | 24.22 ± 6.09 |
| | wood | 0.59 ± 0.08 | 1.29 ± 0.11 | 30.64 ± 3.33 |
| | pith | 0.04 ± 0.01 | 0.05 ± 0.01 | 3.18 ± 0.46 |

Brown *et al.* (2010) in *Arabidopsis thaliana*. In the bark of *Pinus sylvestris* Ivanov *et al*. (2006) recorded about 2-times higher activity of NAD-ME and NADP-ME in comparison to the needles. Hibberd and Quick (2002) also found that the cells of round vessels of celery have not only a high decarboxylase activity but also intensive fluorescence. Strong fluorescence of chlorophyll around vascular bundles is found in many other plant species (Dima *et al.* 2006, Pilarski and Tokarz 2006, Berveiller *et al*. 2007). Moreover experiments on *Arabidopsis thaliana* show that mutants which do not synthesize chlorophyll in the cells surrounding vascular bundle have reduced vigor, height, and harvest of seeds (Janacek *et al.* 2009). Research done by Brown *et al*. (2010) also shows that manipulating the activity of decarboxylases: PEPCK and malic enzymes in mid vein of *A. thaliana* has an impact on a number of processes such as: photosynthesis, synthesis of amino acids, and gluconeogenesis.

A high level of NAD-ME, NADP-ME, and PEPCK activity in stem tissues and the petioles of Japanese knotweed and topinambur indicate that malate from xylem sap of both plants could be decarboxylated and the $CO_2$ realeased is used in photosynthesis by Rubisco. Bervellier *et al*. (2007) located Rubisco in all leaving stem tissues of beech: in xylem, bark, and pith.

High PEPC activity both in the roots and stem tissues of Japanese knotweed and topinambur suggests that malate in xylem sap is loaded not only in the roots (López-Millán *et al.* 2000) but also in the stems and even in the petioles. Especially in topinambur's petioles high activity of PEPC and decarboxylases was noted. We propose that in the same tissues, decarboxylation and carboxylation can occur, depending on conditions favourable for photosynthesis.

Among all stem tissues, bark is in the best light conditions for driving photosynthesis (Pfanz and Ashan 2001). Interestingly that only in Japanese knotweed barks and leaves of both plant PEPC activity is dependent on malate which is known as the PEPC inhibitor (Andreo *et al.* 1987). This may indicate that in the bark of Japanese knotweed, PEPC plays a role in regulation of malate concentration in xylem sap (Martinoia and Rentsch 1994).

The differences in PEPC and decarboxylases activity in Japanese knotweed and topinambur tissues may be explained by the differences in the permeability of the stem epidermis of both plants. The stem of Japanese knotweed has low epidermal conductance to $CO_2$, which results in accumulation of respiring $CO_2$ as well as high PEPC activity in stem tissues. As for topinambur which has a relatively high epidermal conductance, the outer stem tissue – bark, had a lower PEPC activity in comparison to tissues of wood.

Higher $C_4$ enzyme activity in the stem of Japanese knotweed was linked in higher malate oscillation in xylem sap with comparison with the topinambur. Due to the weaker gas exchange even in nonstressed stems there is a low concentration of $O_2$ and hypoxia can occur (Pfanz *et al.* 2002, Kotakis *et al*. 2006). Higher activity of PEPC in the tissues of the stem of Japanese knotweed with low permeable epidermis may be caused by stress condition. Increased activity of PEPC at the conditions of limited stomatal conductance due to salinity (Miteva and Popova 1991, Tsonev *et al.* 1998) and flooding (Yordanova and Popova 2001) was recorded. It is also known that drought- and salt stresses induced PEPC expression in roots (González *et al.* 2003).

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