

Response of potato tubers to hypoxia followed by re-aeration

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Summary

Potato tubers kept under hypoxia (1%) showed improved viability in comparison to anoxia, which was associated with the maintenance of intermediate adenylate energy charge values (A.E.C. = 0.6) and stable adenylate pools at 50% of the initial levels. Re-admission of oxygen to the tuber resulted in an almost full recovery of adenylate energy charge and total adenylates after up to 3 days of hypoxic pretreatment. Tubers exhibited a mixed fermentation. The high lactate, ethanol and acetaldehyde levels proved to be non toxic. Ethanol was degraded to acetaldehyde during re-aeration. Posthypoxic lipid peroxidation was indicated by malondialdehyde and ethane formation. Both products occurred with a temporary delay and in lower amounts compared to post-anoxia. Ethylene release was also considerably smaller. Severe hypoxia and posthypoxia postponed tissue death compared to anoxia. Survival was correlated with an improved energy supply which stabilized membranes.

Introduction

Fleshy plant organs such as potato tubers suffer greatly under the absence of oxygen, as well as during re-aeration after short periods of anoxia, which may cause lethal oxidative stress. Furthermore, potato tubers show an abrupt decline in energy metabolism when subjected to anoxia (Sieber & Brändle, 1991). Potato tubers exhibit a mixed fermentation under anoxia with a considerable predominance of lactate formation followed by pH-decrease in the cytoplasm (Sieber & Brändle, 1991; Pfister-Sieber & Brändle 1994). Cytoplasmic acidosis has been demonstrated to be a major factor in anoxia intolerance (Roberts et al., 1984, 1989; Xia & Roberts, 1994).

In contrast to anoxia, which is rather rare in nature, hypoxia occurs frequently and is usually due to flooding or wetted soils. The question arises as to the differences between anoxia and hypoxia on energy metabolism, on ethanol and lactate formation and on differences occurring during re-oxygenation. With regard to posthypoxic effects, lipid peroxidation could be of intrinsic significance. Potato tubers show the whole range of peroxidation products such as ethane, malondialdehyde and also ethylene after a short period of anoxia. These are indicators of the extreme sensitivity of this tissue to oxidative stress (Pfister-Sieber & Brändle, 1994). Moreover, the rapid conversion of ethanol to acetaldehyde after re-oxygenation may also have toxic effects on membranes (Monk et al., 1987).

Therefore, a knowledge of tuber metabolism under hypoxia and subsequent re-

aeration, in addition to anoxia, could contribute to a more detailed understanding of the extreme sensitivity of this tissue to oxygen shortage. Furthermore, hypoxia and subsequent re-aeration could be more relevant under field conditions than anoxia.

Materials and methods

Seed potatoes (*Solanum tuberosum* cv. Désirée) were cultivated in TrioHum Substrat 2 (Samen Mauser AG, Winterthur, Switzerland) in a growth room (16 h light, 20 °C; 8 h dark, 16 °C). For hypoxic treatments plants were well rinsed, and the tubers were surface sterilized with chloramphenicol (50 µg/ml). The incubation took place in climate boxes (Semadeni, Ostermundigen, Switzerland) in the dark. They were gassed with 1 % O₂ and 99 % N₂. The oxygen concentration was repeatedly controlled at the inlet and outlet with an oxygen analyzer (Toray Type LF 700, Lippke, Neuweid/Rhein, Germany), showing 1.1 % O₂ at the outlet. Following incubation, some of the potato plants were cultivated in soil for the determination of hypoxic survival.

Fermentation products and adenylate energy charge measurements were carried out as described before (Sieber & Brändle, 1991). Acetaldehyde contents in tissue extracts were measured with test kits (Boehringer, Mannheim, Germany). The extracts were prepared according to Sieber & Brändle (1991).

For ethane and ethylene determination, tubers were incubated hypoxically, then transferred into 250 ml gas tight jars with air. After the indicated number of hours, 2.5 ml of air was brought into the jars to compensate the pressure, then the same amount of gas was removed from the headspace with a gastight syringe (Hamilton, Bonaduz, Switzerland) and subsequently analyzed by GC (Sigma 300 dual FID, Perkin Elmer, Norwalk, USA) with a steel column (1.8 m x 1/8", packed with Porapak Q; oven 100 °C, injector 170 °C, detector 190 °C) and a retention time of 14 min. The GC apparatus was calibrated with pure ethane and ethylene.

The ethylene precursor ACC (= 1-aminocyclopropane carboxylic acid) and the bound form M-ACC (= malonyl-ACC) were extracted from deep frozen tuber powder with 80 % ethanol for 16 h at 4 °C according to the method of Bufler et al. (1980). The powder was prepared with the aid of a dismembrator (Braun, Melsungen, Germany) and liquid nitrogen. The homogenate was centrifuged at 10,000 g for 10 min. to remove plant material. Then the supernatant was vacuum dried (Hetovac VR-1, Allerod, Denmark) at 40 °C and redissolved for ACC-determination in 400 µl water. M-ACC was hydrolyzed (Liu et al., 1983) and free ACC determined (Lizada & Yang, 1979).

Malondialdehyde (= thiobarbituric acid reactive material) determinations were done by extracting 200 mg fresh tuber tissue in 2.5 ml water containing 0.12 ml butylhydroxytoluene (0.23 %). Afterwards, 1.2 ml of FeCl₃ (0.29 %) and 2.5 ml of trichloroacetic acid (20 %) containing 0.5 % thiobarbituric acid was added. The probes were well stirred and incubated in a water bath for 45 min. at 95 °C. After cooling on ice, 2 ml of butanol was added, the probes were shaken and centrifuged for 10 min. at 2,500 g (Hunter et al., 1983). The upper phase was measured photometrically at 532 nm and 600 nm. The calculation was done with the difference of both values and the molar

extinction factor of $1.56 \times 10^5 \text{ Mol}^{-1} \text{ cm}^{-1}$ (Heath & Packer, 1965).

For the statistical analysis, the significance of the means was calculated with a t-test for independent variables at the 5% level.

Results

Energy metabolism. The total adenine nucleotide content of normoxic tubers remained constant over a 3 day period at about 50 nMol/g fresh weight (fw, Fig. 1). Under hypoxia, a continuous decrease took place at the beginning of the treatment. Later, the nucleotide level was stabilized at 25 nMol/g fw, equivalent to a decrease of only 50%. After re-aeration, the total adenylate pool showed a large capacity to recover. Under hypoxia, adenylate energy charge (A.E.C.) values behaved similarly to nucleotides. Intermediate values of 0.60 were reached, whereas air controls showed values of about 0.85. Re-aeration of hypoxic tubers was accompanied by an increase of adenylate energy charge, but full recovery was only possible after 24 h of hypoxic treatment (Fig. 2).

Survival. Survival of the green and non-green parts following 3 days of hypoxia was observed for about 60% of the plants. In 20%, only the shoots died while tubers remain turgescient and were able to form new shoots within two weeks. However, death occurred with 20% of the plants. Tubers kept under anoxia survived for about two days and green parts for less than one day (Pfister-Sieber & Brändle, 1994).

Lactate concentrations of tubers. Hypoxic tubers accumulated considerable amounts of lactate especially during the first two hours (Table 1). After 24 h, accumulation slowed down and after 48 h there was no further increase.

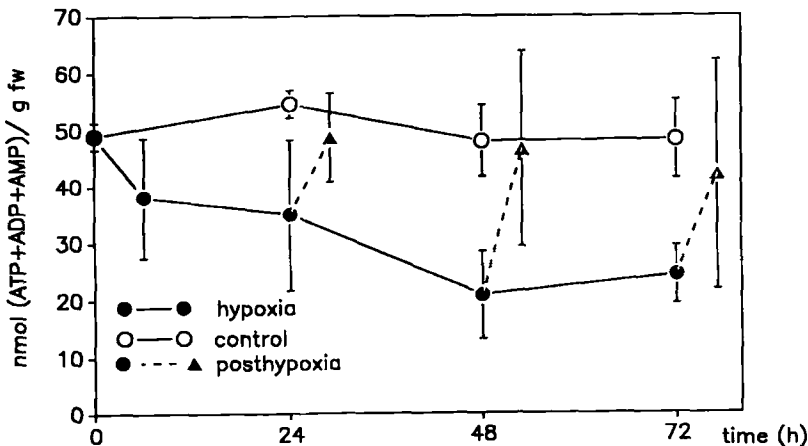


Fig. 1. Total adenine nucleotide content (ATP, ADP, AMP) of potato tubers (*Solanum tuberosum*, cv. Désirée) under hypoxia (1% O₂) and air up to 72 h and after 5 h of re-aeration. Data are means of 5 replicates and bars show standard deviation.

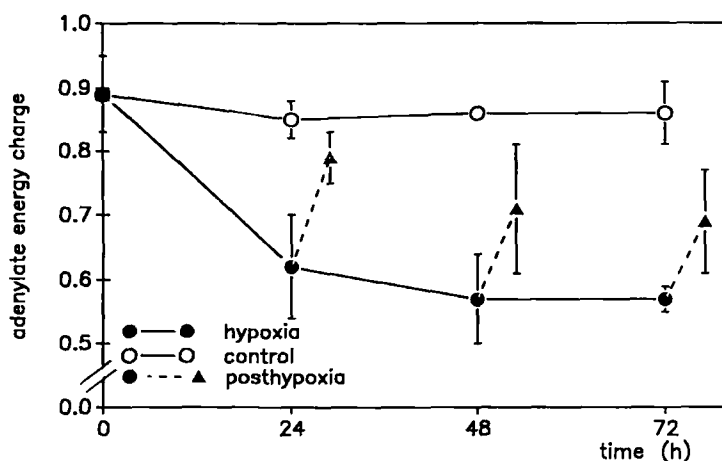


Fig. 2. Adenylate energy charge (A.E.C.) of potato tubers under hypoxia (1% O₂) and air up to 72 h and after 5 h of re-aeration. Data are means of 4 replicates.

Ethanol and acetaldehyde content of tubers. Under hypoxia, ethanol accumulation was very low at the start, but increased continuously and after 72 h it exceeded lactate formation. Re-aeration was followed by a reduction of ethanol content of more than 50% (Fig. 3). Decomposition rates of ethanol per hour were about 0.7–1.2 $\mu\text{Mol/g fw h}$. Hypoxic tubers exhibited relatively high amounts of acetaldehyde, probably originating from ethanol decomposition. Acetaldehyde concentrations showed a linear increase under hypoxia which seemed to be dependent on incubation period. Under air, acetaldehyde concentrations below 0.01 $\mu\text{Mol/g fw}$ were measured. Post-hypoxia increased acetaldehyde tissue concentrations at least 2-fold.

Lipid peroxidation and ethylene release. Re-admission of oxygen resulted in a strong increase of malondialdehyde concentration between day 1 and 2, while previously only very little malondialdehyde was produced (Fig. 4). Ethane showed a similar behaviour: one day of hypoxic pretreatment caused very little change to ethane production during posthypoxia, while an increase took place after 2 and 3 days (Fig. 5).

Table 1. Lactate accumulation of tubers under hypoxia (1%) for up to 72 h. Data are means of 5 replicates \pm standard deviation.

Hypoxic treatment	Lactate concentration ($\mu\text{Mol/g fw}$)
0 h	0.3 ± 0.1
2 h	2.9 ± 0.8
6 h	3.8 ± 1.4
24 h	6.4 ± 0.3
48 h	10.2 ± 1.1
72 h	10.5 ± 0.2

Table 2. ACC (=1-aminocyclopropane carboxylic acid) and M-ACC (= malonyl-ACC) content of tubers under hypoxia and after 5 h of re-aeration. Data are means of 5 replicates \pm standard deviation.

	ACC (nMol/g fw)		M-ACC (nMol/g fw)	
	1.	2.	1.	2.
0 h	0.9 \pm 0.2	0.9 \pm 0.2	2.1 \pm 0.3	2.1 \pm 0.3
24 h	6.2 \pm 1.5	3.4 \pm 1.0	8.3 \pm 1.1	8.1 \pm 0.6
48 h	5.9 \pm 1.1	2.8 \pm 0.9	7.8 \pm 1.3	5.6 \pm 2.7
72 h	8.4 \pm 0.8	4.2 \pm 0.5	8.8 \pm 2.3	5.5 \pm 1.1

1. hypoxia

2. hypoxia + 5 h re-aeration

Tubers kept in air (controls) showed ethane concentrations below 1 pMol/g fw. We conclude that 24 h of hypoxic pretreatment did not induce lipid peroxidation processes in potatoes. Ethylene showed a 40-fold greater release into the headspace than ethane. The large increase was dependent on the pretreatment (Fig. 6) as well as on the length of the posthypoxia period (data not shown). Air controls showed concentrations below 1.5 pMol/g fw. However, all tubers subjected to severe hypoxia showed an increase in the ethylene precursor ACC and its bound form M-ACC (Table 2). After re-aeration, 50% of accumulated ACC was converted into ethylene within 24 h, whereas during the same period no M-ACC decomposition took place. Later, a loss of 30–40% occurred.

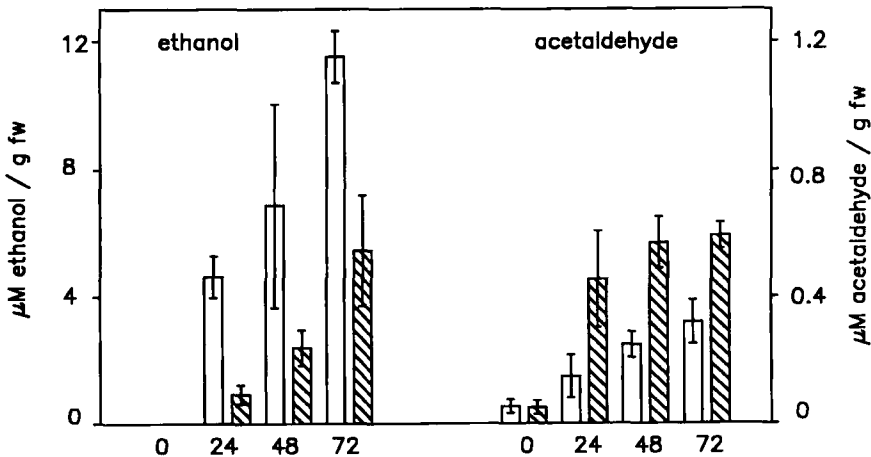


Fig. 3. Ethanol and acetaldehyde content of potato tubers under hypoxia (1% O₂, white bars) up to 72 h and after 5 h of re-aeration (hatched bars). Data are means of 5 replicates.

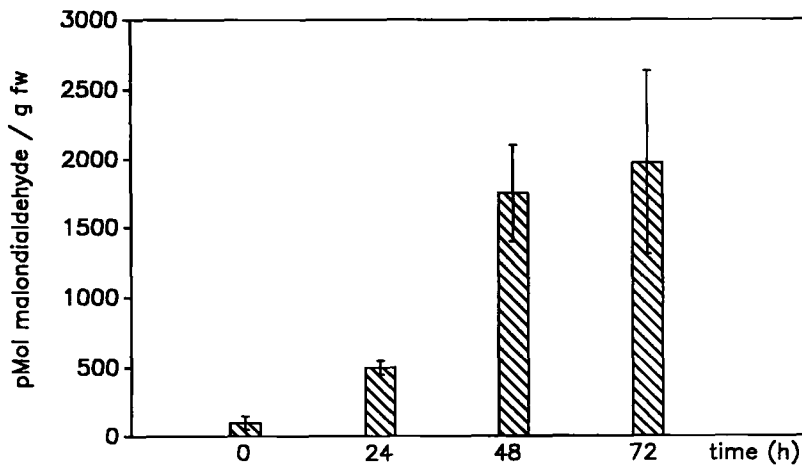


Fig. 4. Increase of malondialdehyde content in tubers after 1, 2 and 3 days of hypoxia (1% O₂) and 5 h of re-aeration. Data are means of 7 replicates.

Discussion

Induction of fermentation pathways is the most widespread response of plants under oxygen deprivation (Muench et al., 1993). The striking difference between anoxic and hypoxic potato tubers lies in the energy metabolism. While anoxic tubers show a complete breakdown of energy metabolism (Sieber & Brändle, 1991), the energy charge of hypoxic tubers with only 1% O₂ in the environment remained constant at

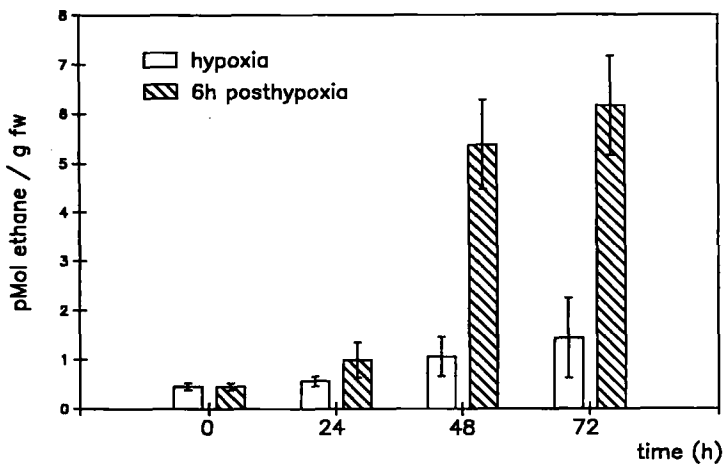


Fig. 5. Ethane release of potato tubers under hypoxia (1% O₂) after 6 h of re-aeration. Data are means of 7 replicates.

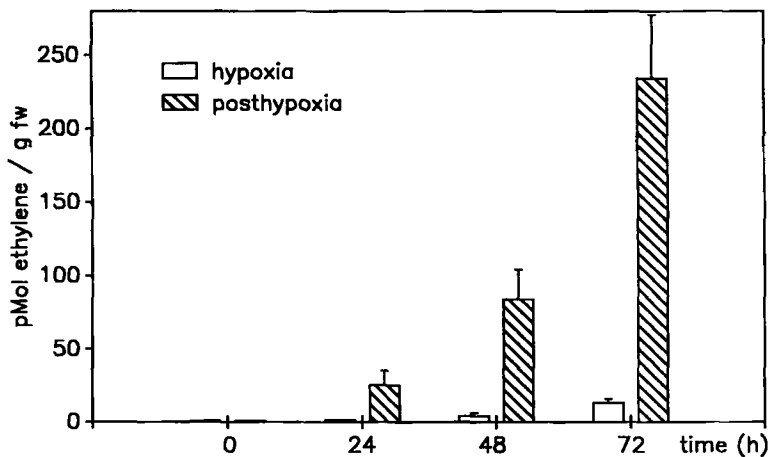


Fig. 6. Ethylene release of potato tubers under hypoxia (1% O₂) and after 6 h of re-aeration. Data are means of 7 replicates.

an intermediate level for at least 3 days of treatment. Furthermore, hypoxic tubers are able to restore energy charge after re-aeration, in contrast to anaerobic tubers (Pfister-Sieber & Brändle, 1994). Energy metabolism is correlated with viability and regeneration of whole plants, since most hypoxic tubers survived 3 days of treatment, while anoxic tubers died earlier (Sieber & Brändle, 1991). A similar response of energy charge in hypoxic tubers to re-aeration has been described for grapes (Tesnière et al., 1994) but potato tubers were more sensitive to oxygen shortage. Moreover, hypoxic tubers exhibited only a 50% loss of the adenylate pool and there was full recovery of nucleotides after re-aeration. Anoxic tubers demonstrate a total dissipation of adenylates, probably as a consequence of metabolic disarray (Sieber & Brändle, 1991).

The fermentative pathway induced under hypoxia has an even higher capacity than under anoxia, mainly for lactate accumulation. Therefore, lactate accumulation seems to be less significant for cytoplasmic acidosis than proton leakage in the tonoplast, which can be hindered by sufficient ATP-availability (Roberts et al., 1984). Consequently, lactate accumulation is characteristic for anoxia-intolerant plants (Roberts et al., 1984, 1985; Hanson & Jacobson, 1984). Ethanol accumulation is widespread and does not allow differentiation between tolerant and non-tolerant species (Muench et al., 1993).

Major problems for tubers under prolonged hypoxia, however, may be the reduced oxygen diffusion and the inability to remove the fermentation end products sufficiently, both resulting from the fleshy nature of the potato. Nevertheless, the higher viability under hypoxia compared to anoxia is probably not due to enhanced fermentation processes but to a residual respiration.

Although posthypoxic tubers accumulate a two-fold higher amount of acetaldehyde than anoxic tubers, there is no immediate effect noticeable. The

restricted toxicity of fermentation products and intermediates is therefore also valid for acetaldehyde.

While anoxic tubers show massive lipid peroxidation after 1 d of pretreatment, hypoxic tubers do not show ethane and ethylene release, and only very small amounts of malondialdehyde upon re-aeration after 24 h. Later, hypoxically pretreated tubers also produce the full range of peroxidation products but to a much lesser extent than under post-anoxia (Pfister-Sieber & Brändle, 1994). The main difference between postanoxia and posthypoxia seems to be a delay of lipid peroxidation in posthypoxic tubers. Nevertheless, both hypoxic and anoxic tubers are more sensitive than rhizomes of the dryland species *Iris germanica* (Henzi & Brändle, 1993). In tolerant species such as *Schoenoplectus lacustris* and *Acorus calamus*, postanoxic peroxidation products appear only after 50–70 days of anoxic pretreatment (Henzi & Brändle, 1993). Furthermore, membrane lipids of these species remain more or less stable under anoxia, while in *Iris germanica* a marked lipid breakdown takes place within 7 days. This happens within 2 days in the even more sensitive potato tubers (Weber, personal communication). Obviously, potato tubers are extremely susceptible to lipid peroxidation processes. One reason could be that their fatty acid composition consists of 75% unsaturated fatty acids (Galliard, 1973).

The ethylene release after 3 days of hypoxic pretreatment may reflect a stress response, rather than the consequence of lipid peroxidation. Compared to postanoxia, posthypoxic ethylene release is significantly smaller (Pfister-Sieber & Brändle, 1994), indicating that post-hypoxic stress is initially less harmful than post-anoxic stress. The source of ethylene is hypoxically accumulated ACC and to a lesser extent M-ACC. This is similar to anoxic tubers where the main source is M-ACC (Pfister-Sieber & Brändle, 1994).

The present data suggest a somewhat lower sensitivity of potato tubers to hypoxia and posthypoxia than to anoxia, which leads to a prolonged survival of 1–2 days. Nevertheless, a prolonged survival of only 1 day in wetted soils might be of considerable benefit for field cultivation of manipulated potatoes with improved ethanolic fermentation.

Acknowledgements

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