Journal of Experimental Botany, Vol. 51, No. 349, pp. 1475–1477, August 2000

Journal of Experimental Botany

SHORT COMMUNICATION

The significance of α-amylase under anoxia stress in tolerant rhizomes (*Acorus calamus* L.) and non-tolerant tubers (*Solanum tuberosum* L., var. Désirée)

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Received 22 March 2000; Accepted 4 May 2000

Abstract

Rhizomes of *Acorus calamus* L. were able to maintain a functional α-amylase under anoxia, whereas a steep decrease in the enzyme protein content and activity took place in potato tubers. The stress-induced control in tubers occurred on the translational level. It is suggested that this decrease is one of the key factors with regard to anoxia intolerance.

Key words: α-Amylase, anoxia, starch mobilization, translational control.

Introduction

Potato tubers are considerably more sensitive towards oxygen deprivation than wetland plant rhizomes (Crawford and Braendle, 1996). However, tubers and rhizomes have several characteristics in common. For example, both serve for vegetative propagation and both are rich in starch. The latter is particularly important because long-term anoxia tolerance depends, among other things, on an adapted energy metabolism with adequate ATP supply (Sieber and Braendle, 1991). Thus, a non-interrupted access to fermentable sugars is a prerequisite for survival in oxygen-deprived environments (Barclay and Crawford, 1983).

The aim of this paper is to know whether a restricted starch mobilization is a crucial determinant of the failure of potato tubers to survive waterlogging. Therefore, gene expression, protein synthesis and activity of the α-amylase enzyme, together with sugar levels were measured in extremely tolerant wetland plant rhizomes (*Acorus calamus* L.) and compared to those of potato tubers (*Solanum tuberosum* L. var. Désirée) under anoxia. The Désirée variety was purposely selected because it is noticeably less intolerant than most other potato varieties. Investigations were focused on α-amylase because this enzyme is primarily known to trigger the hydrolytic break up of the amyloplast (Perata *et al*., 1998; Witt and Sauter, 1995).

Materials and methods

Rhizomes (*Acorus calamus* L.) and tubers (*Solanum tuberosum* L. var. Désirée) were cultivated as described previously (Sieber and Braendle, 1991). O_2-free incubations took place in an anaerobic workbench (Model 1029, Forma Scientific, Marietta, Ohio, USA). During incubation (2, 6 and 10 d anoxia), the surface-sterilized material was stored on wetted filter paper in plastic vessels. Afterwards, if not used as fresh material, the still turgescent and healthy organs were peeled to remove the corky periderms, cut and immediately frozen in liquid N_2. The frozen tissue was then homogenized to a fine powder in a ball mill (Mikro-Dismembrator II, Braun, Melsungen, Germany) and stored at $-80\,^{\circ}C$.

For RNA extraction, *A. calamus* powder was treated with hot phenol (Verwoerd *et al*., 1989). After extraction of potato powder by phenol, RNA was selectively precipitated with LiCl. RNA concentrations were determined photometrically and verified by ethidium bromide staining of the agarose/formaldehyde gels. Northern blotting and hybridization were carried out under standard conditions (Sambrook *et al*., 1989). The probe for α-amylase mRNA (Young *et al*., 1994) was randomly labelled.

SDS-PAGE and immunoblotting were adapted from Mitsuhashi and Feller (Mitsuhashi and Feller, 1992). The primary antibody was raised against barley α-amylase. The α-amylase activity of fresh material was measured with the artificial substrate 'blocked *p*-nitrophenyl-maltoheptaoside' (BPNPG7) according to the instructions of the test kit (Alpha Amylase Assay Procedure, Megazyme, Bray, Ireland). Sucrose, glucose and fructose contents of tissue powders were determined with appropriate test kits (Roche Diagnostics, Rotkreuz, Switzerland).

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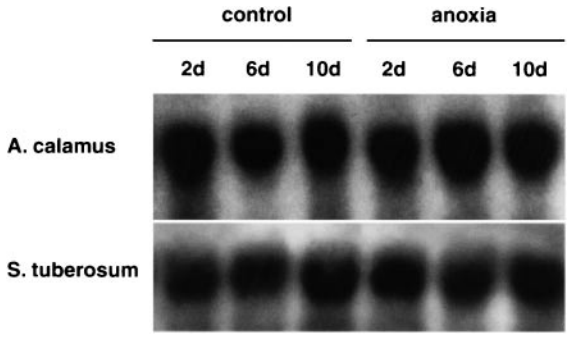

Fig. 1. Northern blot analysis of total RNA (8 µg per lane) isolated from rhizomes of *A. calamus* and potato tubers previously kept for 2, 6 or 10 d under ambient atmosphere (=controls) or anoxia.

Results and discussion

Northern blot analysis of α-amylase RNA showed identical signals in rhizomes under normal air and anoxia treatments (Fig. 1). This was also the case for the Désirée tubers. It is thus obvious that anoxia did not affect the mRNA levels in these two organs.

A translational regulation of the α-amylase synthesis was thus expected since messengers remained present under anoxia (Fig. 1) as well as under other stress conditions (Bailey-Serres, 1999; Crosby and Vayda, 1991). Indeed, clear differences occurred in protein levels as revealed by immunoblotting (Fig. 2). The α-amylase decreased considerably in potato tubers during anoxia treatment. Thus, densitometric analysis showed that the protein level decreased by about 70% in potato tubers after 10 d anoxia whereas it increased by 50% in *A. calamus*.

Moreover, the differences in protein content were mirrored in the *in vitro* enzyme activities (Fig. 3). Whereas the α-amylase activities of *A. calamus* rhizomes were equally increased under both normal air and anoxia, they decreased dramatically in the O_2-deprived potato tubers. The activity increase in *A. calamus* rhizomes reflected probably the higher carbohydrate demand of the starting regeneration processes (Crawford, 1994).

Furthermore, clear differences were also found with respect to free sugar content (Fig. 4). The high amounts

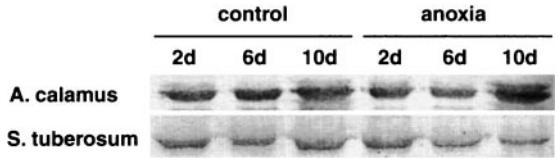

Fig. 2. Western blot analysis of α-amylase under the conditions of Fig. 1. Each lane was loaded with a volume corresponding to 6.5 mg fresh weight (average protein content ±SD was 2.0±0.1 µg protein mg^{-1} fresh weight for potato and 1.2±0.4 µg protein mg^{-1} fresh weight for *A. calamus*, $n=6$). Patterns and concentrations were previously tested by Coomassie blue staining. Densitometric analysis (Desaga, Heidelberg, Germany) showed a 50% increase in *A. calamus* and a 70% decrease in potato after 10 d anoxia.

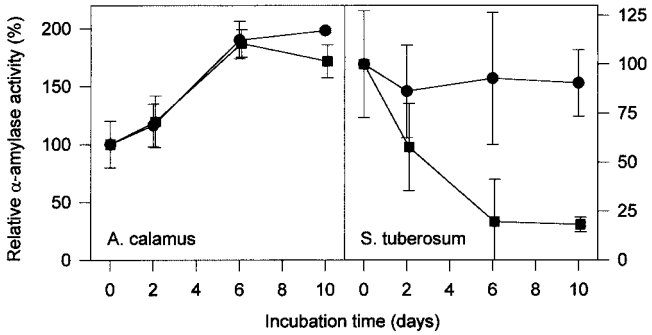

Fig. 3. Changes in the relative α-amylase activities extracted from rhizomes and tubers which were previously incubated under anoxia (■) or ambient atmosphere (controls, ●). The 100% values were 5.45 mUnits g^{-1} fresh weight for *A. calamus* and 38.5 mUnits g^{-1} fresh weight for potato. One activity Unit is defined as the amount of enzyme required, in the presence of excess α-glucosidase and glucoamylase, to release one micromole of *p*-nitrophenol from BPNPG7 in 1 min under the defined assay conditons. Data are means of four experiments ±SD. Note the different scales.

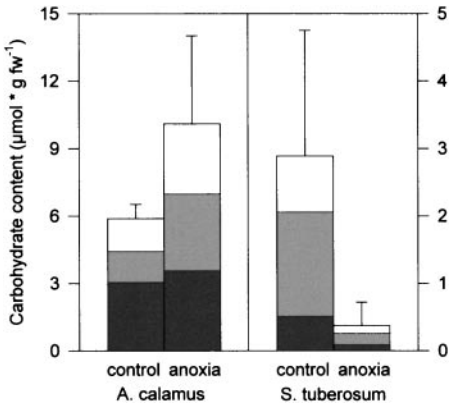

Fig. 4. Sucrose (■), glucose (■) and fructose (□) content of rhizomes and tubers which were kept for 10 d under ambient atmosphere (controls) or anoxia. Data are means of four experiments ±SD. Note the different scales.

of sucrose, glucose and fructose already present in *A. calamus* rhizomes under normal air tended to increase under anoxia. Potato tubers contained generally less free sugars under normal air. A high variability in sugar amounts is not uncommon for this type of tissue (Cone and Wolters, 1990). However, the most striking fact was the almost complete loss of fermentable sugars in anoxic potato tubers.

Similar results have already been reported when comparing seeds of the tolerant rice and of the non-tolerant barley and wheat (Perata *et al.*, 1992, 1996). Transcription of α-amylase under anoxia occurred only in rice, and only rice was able to synthesize a functional enzyme (Perata *et al.*, 1993). These data suggest a similar behaviour for *A. calamus*, although it is not yet known whether the maintenance of the α-amylase mRNA level is due to a well-balanced equilibrium between ongoing mRNA synthesis and degradation. In any case, the α-amylase

level appears to be translationally controlled in potato tubers, in contrast with the transcriptional control exerted by barley and wheat.

Moreover, growth processes (shoot extension) have been demonstrated for wetland plant rhizomes, which are comparable to those occurring during the anoxic germination of rice (coleoptile elongation) (Crawford, 1994). Therefore, it was suggested that the existence of a functional α-amylase is absolutely required to ensure a permanent supply of free sugars for survival and growth under anoxia, and that it is a genetically fixed property of the starchy organs of wetland species. On the other hand, it was concluded that the impaired translation of α-amylase is one of the crucial causes of the intolerance of potato tubers.

Acknowledgements

The cDNA was a gift from Dr TE Young, Riverside, California. The primary α-amylase antibody was kindly provided by Dr P Perata, Pisa, Italy. We also thank Drs S Kopriva, Freiburg im Breisgau, Germany and S Roulin, Berne for methodological advice. This work was supported by the Swiss National Foundation (grant no. 31-53722.98).

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