ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIV, nr 1
1975

The influence of soaking injury of seeds on the activity of cytoplasmic NAD⁺-dependent dehydrogenases in the cotyledons of *Pisum sativum* L. and *Phaseolus vulgaris* L.

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(Received: October 17, 1974)

Abstract

In soaking injury of seeds of *Pisum sativum* and *Phaseolus vulgaris*, submerged for 24 hours in aerated or nitrogenated water, distinct changes in NAD^+-dependent aerobic (MDH) and anaerobic (ADH and LDH) dehydrogenases occurred. MDH activity decreased slightly, and the activity of both anaerobic dehydrogenases, especially that of LDH, increased. In both species the increase of LDH was relatively many times higher than of ADH activity. This suggested the use of LDH as an indicator of flooding intolerance and soaking injury of seeds, parallely to ADH in roots.

INTRODUCTION

Leguminous plants have been proved as highly intolerant of experimental flooding, both when growing plant roots are persistently submerged in water (Crawford 1967, 1969; McManmon and Crawford 1971) and when soaking seeds are immersed in water for more than 6 hours (e.g. Kidd and West 1918; Barton 1950; Mazurowa and Schramm 1975). The conditions of flooding result in soaking injury, in which the increase of ADH, but not LDH activity occurs in roots (McManmon and Crawford 1971). Obviously the conditions of experimental flooding are rather far from those in which most investigations on germinating seeds are performed, and a proper equilibrium between several NAD^+-dependent dehydrogenases is established, but not very far from natural conditions in which seeds are repeatedly soaked. Therefore, the problem of soaking injury can not be omitted in study of metabolism of swelling and germinating seeds, and comparison of activity of NAD^+

-dependent dehydrogenases in such conditions seems to be desirable, particularly in confrontation with fairly numerous investigations on seeds growing in so called "normal" conditions, i.e. soaked for 6 hours and then cultivated in moist air or nitrogen.

For assays two species of Leguminous plants, differing distinctly in sensivity for experimental flooding of roots (M c M a n m o n and C r a w f o r d 1971) and on ratio of aerobic (MDH) to anearobic (ADH + LDH) dehydrogenases activity in cotyledons (aer.: anaer., M a z u r o w a and S c h r a m m 1975) were chosen, namely peas (*Pisum sativum* L.) having an extremely high index of sensibility for flooding intolerance (M c M a n m o n and C r a w f o r d 1971) and fairly low ratio aer.: anear. = 1.9. (M a z u r o w a and S c h r a m m 1975), and beans (*Phaseolus vulgaris* L.), not tested for flooding intolerance of roots, but investigated thoroughly in soaking injury of seeds (O r p h a n o s and H e y d e c k e r 1968), and having significantly higher aer.: anaer. ratio = 9.3 (M a z u r o w a and S c h r a m m 1975).

MATERIALS AND METHODS

Chemicals, seeds, cultures, enzyme preparations and assays were as described previously (M a z u r o w a and S c h r a m m 1975). Enzyme assays were performed in crude extracts after 24 hours of culture.

For determination of ethanol produced in seeds in conditions provoking soaking injury, portions of 50 peas, sterilized by 10 min immersion in 0.2% of sublimate and them washed, were submerged in a 200 ml beakers brimmed with distilled water deprived of air by boiling for 30 min. The hermetically closed beakers were kept in thermostat 23 ± 1°C in the dark. Analysis for ethanol, both in the cotyledons and in water, were performed after 3, 6, 12, 24 and 48 hours of culture. Ethanol for determination was distilled from homogenized cotyledons without testa (50 min, about 35 ml of distillate fulfilled to 40 from a pair of cotyledons), and from water in which the seeds were submerged, and estimated after B o n n i c h s e n (1965).

RESULTS

The changes of activity of three NAD^+-dependent dehydrogenases: one of aerobic pathway (MDH) and two of anaerobic pathways (ADH and LDH) in seeds growing in "normal" conditions and after experimental flooding are presented in the Table 1.

The results indicate a distinct difference in activity of dehydrogenases under study regarding experimental flooding of Leguminous seeds. MDH activity in the seeds submerged in water for 24 hours, decreases only sligh-

Table 1

Activity (EU) of NAD$^+$-dependent cytoplasmic dehydrogenases of aerobic (MDH) and anaerobic (ADH+LDH) pathways in the cotyledons of peas (Pisum sativum L.) and beans (Phaseolus vulgaris L.) growing for 24 hours in "normal" conditions (germination on wet filter paper after 6 hors of imbibition) and in conditions of experimental flooding (seeds submerged in water aerated or nitrogenated)

EU unit: removal of 1.0 μmol of NADH·min^{-1}·mg of protein^{+-1}. For details see text

	"Normal" conditions	Seeds submerged in water			
		aerated		nitrogenated	
Pisum sativum					
MDH	0.74	0.74	0.0	0.61	−0.13
ADH	0.36	0.78	+0.42	0.54	+0.18
LDH (EU·10^{-3})	1.30	16.0	+14.7	13.4	+12.1
aer. (MDH)	1.9	0.9		1.1	
anaer. (ADH+LDH)					
LDH(·10^{-2})	0.3	2.0		2.5	
Phaseolus vulgaris					
MDH	2.05	1.88	−0.17	1.78	−0.27
ADH	0.21	0.37	+0.16	0.28	+0.07
LDH(EU·10^{-3})	0.60	10.3	+9.7	6.6	+6.0
aer. (MDH)	9.3	4.7		6.2	
anaer. (ADH+LDH)					
LDH)·10^{-2})	0.3	2.8		2.4	
tabelka str. 199					

tly, if at all. An insignificantly greater decrease occur in nitrogenated water than in aerated. On the contrary, in these conditions activity of both anaerobic dehydrogenases increases. It is noticeable, that in all cases the increase in aerated is higher than in nitrogenated water. In both species the relative increase in LDH activity is significantly higher than the increase in ADH activity; when the highest increase in ADH is about twice (in peas, aerated water), the highest increase in LDH is about 17 times (in beans, aerated water).

DISCUSSION

The phenomenon of aggravation of soaking injury by gas bubbled through the soaking water was observed by Barton (1950) using oxygen, and by Orphanos and Heydecker (1968) using air, while aeration with carbon dioxide (Barton 1950) and soaking of seeds in a weak solution of hydrogen peroxide (Barton and McNab 1956) provoked no injury. Orphanos and Heydecker (1968) suggest the nature of soaking injury of seeds as a deficient oxygen supply to the interior (intercotyledon cavity) of the soaked seeds. The aeration of water with nitrogen provokes deeper aggravation of soaking injury of Leguminous seeds than influenced by air (Table 1). Anyhow, flooding and soaking injury, similarly to anaerobic gas conditions (growing in nitrogen), results always in remarkable changes in dehydrogenases activity both in roots and in seeds of flood-intolerant species. Especially the anaerobic pathway dehydrogenases: ADH and LDH, remarkably increase, although the results of investigations are not always comparable.

Leguminous plant are — as the whole family — typically flood-intolerant, though differences in paticular species are evident (Mazurowa and Schramm 1975); so the changes in several dehydrogenases activity caused by experimental flooding, should be similar. However, some differences between species under study — one highly intolerant (*Pisum sativum*) and the other less intolerant (*Phaseolus vulgaris*) — occur, especially when changes in relative activity of enzymes are compared (Table 1.). In both species in "normal conditions MDH activity is highest, although distinctly higher in beans; so the aer.: anaer. ratio in beans (9.3) is five times greater than in peas (1.9). In flooded pea seeds, especially in aerated water, ADH became the most active enzyme, so that the aer.: anaer. ratio falls below 1. In beans however, the ADH activity reaches, at most, one fifth of MDH activity and the aer.: anear ratio falls slightly below 5.

In comparison with investigations of Crawford (1967) and McManmon and Crawford (1971) on flooding tolerance over roots, some significant differences appear. First — in peas the double growth of ADH activity, although the enzyme becomes the most active of all, is hardly comparable to the pea roots, in which an intensive induced synthesis of ADH occurs and its activity after a certain time is 15 hundred times greater (McManmon and Crawford 1971) and becomes in practice the single acting dehydrogenase. The ability of cotyledons for inductive ADH synthesis is then slight in comparison with roots. Second — although in roots ADH activity is a single index of flooding intolerance (Crawford 1967; McManmon and Crawford 1971), in seeds however, distinctly most significant is LDH, the activity of which rises relatively many times higher than that of ADH, though it always remains markedly lower in absolute value. Then the inductive synthesis of LDH in flooded Leguminous seeds seems to occur similarly to that observed by Sherwin and Simon (1969) in germination under wet conditions, and contraiwise to the roots (McManmon and Crawford 1971).

Practically no changes in MDH activity in cotyledons of both species in conditions under study, in comparison with increase of MDH activity in roots of two flood-intolerant *Senecio* species (Crawford and McManmon 1968), could express either specifity of metabolism of Leguminous family towards other flood-intolerant plants, or specifity of cotyledons towards roots.

Changes in ADH activity, which are considered by McManmon and Crawford (1971) to be an indicator of flooding tolerance in plants, in the initial stage of soaking seem to be strictly connected with ethanol formation (Kollöffel 1970; Leblova and Ehrlichova 1972; Morahashi and Shimokoriyama 1972). With LDH the same has been observed, but only in strictly anaerobic conditions (Sherwin and Simon 1969). However, the K_m values of both enzymes are in peas and beans of the same range (Mazurowa 1973), and the level of lactate accumulating in cotyledons in wet and anaerobic conditions can be even twice higher than that of ethanol, both in peas (Wager 1959, 1961) as in beans (Sherwin and Simon 1969). This could be connected with the insufficiently examined problem of the penetration of both ethanol and lactate from the soaking seeds into the water. In peas soaking in anaerobic conditions, ethanol rises from the 6th hour, to a distinctly higher level in external water than in seeds (Fig. 1). In cotyledons it stabilizes in 12—14 hours on constant level, whereas in water it rises linearly to relative high values. This suggest some special mechanism of exudation of ethonal from seeds. With lactate, however, no similar phenomenon has been observed (Schramm and Piątkowska 1961; Mazurowa and Schramm 1969).

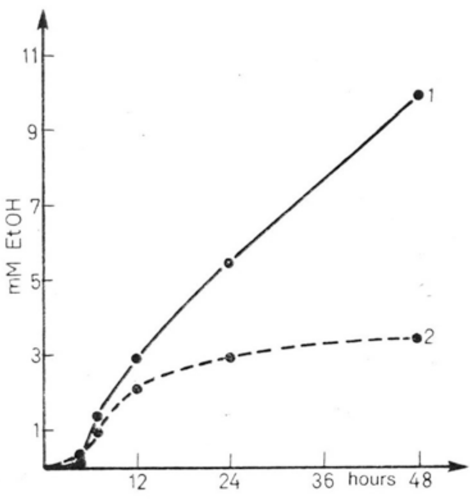

Fig. 1. Production of ethanol by soaking injured peas (*Pisum sativum* L.). Seeds soaking in water deprived of air (for details see methods)

1 — ethanol content in the cotyledons; 2 — ethanol content in submerged water

Slight changes in the activity of the enzymes under study in the period of 12—48 hours in the cotyledons of peas and beans submerged in water (Mazurowa and Schramm 1975), and only gradual decreasing ability of germination of persistently submerged Leguminous seeds (e.g. horse bean — about 20% decrease after 24 hours, less than 50% after 84 hours — Mazurowa and Schramm 1969), together with active exudation of ethanol from the seeds (Fig. 1) indicate that in conditions of flooding of seeds and soaking injury a balance between NAD^+-dependent dehydrogenases is generated, allowing the seed to survive as long as possible in metabolically difficult conditions.

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Wpływ zatrucia wodnego na aktywność dehydrogenaz cytoplazmatycznych NAD+ -zależnych w liścieniach Pisum sativum L. i Phaseolus vulgaris L.

Streszczenie

Nasiona grochu (*Pisum sativum* L.) i fasoli (*Phaseolus vulgaris* L.) moczono przez 24 godziny w wodzie przewietrzanej powietrzem bądź azotem. W obu przypadkach nastąpiło zatrucie wodne (soaking injury), objawiające się silnymi zmianami w aktywności dehydrogenaz cytoplazmatycznych NAD+-zależnych. Aktywność tlenowej dehydrogenazy jabłczanowej (MDH) nieznacznie maleje, natomiast aktywność dehydrogenaz beztlenowych: alkoholowej (ADH) i mleczanowej (LDH) silnie wzrasta. Zmiany te występują silniej u grochu (u którego ADH w nasionach pęczniejących w wodzie przewietrzanej powietrzem staje się najaktywniejszym enzymem), niż u fasoli. Aktywność wszystkich trzech dehydrogenaz jest u obu gatunków nieco niższa w nasionach moczonych w wodzie przewietrzanej azotem niż powietrzem. Względny przyrost aktywności jest u obu gatunków znacznie większy u LDH niż u ADH. Aktywność LDH wydaje się być najlepszym wskaźnikiem stopnia nietoleranpodobnie jak w odniesieniu do systemu korzeniowego roślin wskaźnikiem takim jest ADH.