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A Study on the Dormancy of ×*Haynaldoticum sardoum* Seeds *(Poaceae)*

By

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With 3 Figures

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Summary

CAPOCCHI A., GRILLI I. & GALLESCHI L. 1992. A study on the dormancy of ×*Haynaldoticum sardoum* seeds *(Poaceae).* – Phyton (Horn, Austria) 31 (2): 209–216, with 3 figures. – English with German summary.

The possible role of pentose phosphate pathway (PPP) in dormancy release of ×*Haynaldoticum sardoum* Meletti et Onnis seeds was investigated. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities and the C6/C1 ratio in dormant and non dormant embryos from the two natural lines (Culmo Vuoto and Culmo Pieno) were consequently studied. Dry embryos of dormant seeds showed appreciable amounts of both dehydrogenase activities. Furthermore, gibberellic acid incubation of the seeds or their storage in dry conditions (after-ripening), both of which treatments are effective in dormancy breaking, did not produce any serious changes in enzyme activity pattern. Some increase in dehydrogenase activities during late imbibition did not seem correlated with dormancy breaking, which happened earlier. Finally, the C6/C1 ratio of dormant embryos did not change after gibberellic acid treatment, while non dormant embryos showed an increased C6/C1 ratio. Consequently the PPP did not appear to be involved in dormancy breaking of ×*Haynaldoticum sardoum* seeds.

Zusammenfassung

CAPOCCHI A., GRILLI I. & GALLESCHI L. 1992. Eine Studie zur Samenruhe von ×*Haynaldoticum sardoum (Poaceae).* – Phyton (Horn, Austria) 31 (2): 209–216, mit 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Untersucht wurde die mögliche Rolle des Penthosephosphatstoffwechsel (PPP) bei der Auslösung der Samenruhe von ×*Haynaldoticum sardoum*. Dabei wurden die Aktivitäten der Glucose-6-Phosphat-Dehydrogenase und der 6-Phosphogluconat-Dehydrogenase und das C6/C1-Verhältnis in ruhenden und nicht ruhenden Embryonen von den zwei natürlichen Linien (Culmo Vuoto und Culmo Pieno) laufend gemessen. Trockene Embryonen ruhender Samen besaßen merkbare Aktivitäten beider Dehydrogenasen. Darüberhinaus führten Gibberellinsäurebehandlung von Samen oder deren Lagerung bei trockenen Bedingungen (Nachreifung) zu keinen wesentlichen Veränderungen im Verhalten der Enzymaktivitäten. Beide vorher genannten Behandlungen beeinflussen die Beendigung der Samenruhe. Ein geringer Anstieg in den Dehydrogenaseaktivitäten bei fortgeschrittener Wasseraufnahme scheint nicht mit der Beendigung der Samenruhe, welche bereits früher eintritt, im Zusammenhang zu stehen. Das C6/C1-Verhältnis ruhender Embryonen ändert sich nicht nach Behandlung mit Gibberellinsäure während nicht ruhende Embryonen ein gesteigertes C6/C1-Verhältnis aufwiesen. Daraus ist abzuleiten, daß der PPP offensichtlich nicht an der Beendigung der Samenruhe von ×*Haynaldoticum sardoum* Samen beteiligt ist.

Introduction

Embryo growth needs an active metabolism and ATP synthesis during seed germination. Dormant seeds are usually unable to germinate and grow, because of block(s) to germination within the seed itself (BEWLEY & BLACK 1982). The block may disappear slowly from the dry seed (after ripening) or be overcome by some external treatments, such as exposure to high oxygen concentrations or, rather surprisingly, to some respiratory inhibitors, such as potassium cyanide or sodium azide, which reduce oxygen consumption. This evidence induced ROBERTS 1969, 1973 to hypothesize the involvement of pentose phosphate pathway (PPP) in dormancy breaking of some cereal seeds (rice, barley, oat) and consequently to suggest that the breakage of dormancy could be due to a higher activity of PPP which would, on the other hand, have been partly inhibited in dormant seeds. PPP would utilize a cyanide resistent terminal oxidase which competes for oxygen consumption with the conventional respiratory chain and thus the inhibition of this respiratory chain would stimulate the PPP activity with the consequent production of some metabolic intermediates causing dormancy breaking (ROBERTS & SMITH 1977). However, conflicting results on PPP involvement in dormancy release have been published (GOSLING & ROSS 1980, SATOH & ESASHI 1980, FUERST & al. 1983, SWAMY & SANDHYARANI 1986, DE MEILLON & al. 1990).

Previous work on ×*Haynaldoticum sardoum* MELETTI & ONNIS seeds indicated differences between the seeds of the two natural lines, Culmo Pieno (solid stem) and Culmo Vuoto (hollow stem), the former showing a deeper relative dormancy than the latter (ONNIS 1971). ×*Haynaldoticum sardoum* is a hybrid between *Triticum durum* and *Haynaldia villosa* (MELETTI & ONNIS 1975, STEFANI & al. 1987) which grows spontaneously in

Sardinia, Sicily and Southern Italy, where it has the behaviour of a typical weed (MELETTI 1955, 1959).

Therefore, we began a study to gain more detailed information on the causes of the different behaviour, at the dormancy stage, of Culmo Pieno and Culmo Vuoto seeds. Our paper reports on the possible involvement of PPP during dormancy breaking in ×*H. sardoum* seeds and for this purpose glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) activities and C6/C1 ratios in dormant, non dormant and gibberellic acid treated embryos, were determined.

Materials and Methods

Plant material

Seeds of the two lines, harvested during 1989, were utilized in July 1989 (dormant seed) and in February 1990 (non dormant seeds). After sterilization under vacuum (1% NaOCl, v/v, for 20 min) and several washings with sterile water, they were imbibed with distilled water or gibberellic acid (GA_3; 10 µM) in Petri dishes, at 23° C and in the dark for the required times. The percentage of germination was recorded for each imbibition time, and then the seeds were immediately excised and the hand isolated embryos were utilized for enzyme activity or C6/C1 ratio determinations.

Enzyme extraction

Twenty-five embryos were routinely utilized. They were homogenized in a cold mortar with 1 ml of 10 mM sodium phosphate buffer (pH 7) containing 1 mM ethylenediaminetetraacetic acid and 2 mM 2-mercaptoethanol. The homogenate was centrifuged at 60,000 g, 4° C and 30 min and the supernatant utilized as enzymic source.

Each extraction was replicated twice.

Enzyme assay

Glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) activities were measured following the modified method of UPADHYAYA & al. 1981 by utilizing 50 mM tris-HCl buffer (pH 8), 50 µl of 10 mM NADP, 50 µl of 60 mM glucose-6-phosphate or 6-phosphogluconate, as the substrate, and 25 µl of extract. The final volume of the reaction mixture was 1 ml.

Reaction velocities were measured at 340 nm and 26° C by following NADP reduction. The G6PDH activity was calculated according to UPADHYAYA & al. 1981 and both enzyme activities were expresssed as katals (FLORKIN & STOTZ 1973). Each value of enzyme activity was mean of two different extractions and eight determinations. One katal was defined as the amount of enzyme which reduces one mole of NADP per second under standard reaction conditions.

C6/C1 determinations

They were performed as described in FUERST & al. 1983. Twelve embryos from two different Petri dishes were utilized for each imbibition time. Three different determinations were performed for each replica.

Results

1. Seed dormancy and GA_3 effect

Dormant and non dormant seeds of Culmo Vuoto and Culmo Pieno lines showed different germination behaviour (Figs. 1 a, 1 b). Culmo Pieno seeds had a deeper relative dormancy than Culmo Vuoto ones. The dormancy of both seeds could be overcome by a long period (100 or more days) of after ripening (data unshown) or by GA_3 treatment (Figs. 1 a, 1 b). This growth regulator also stimulated the germination energy in Culmo Vuoto line, but the extent of this stimulation was quite different.

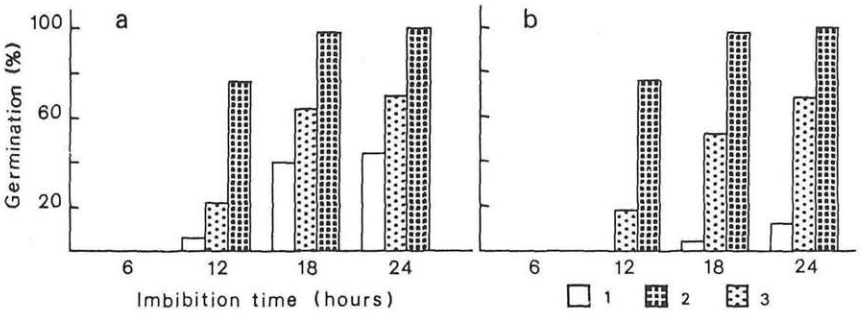

Fig. 1. Germination of ×*Haynaldoticum sardoum* seeds of Culmo Vuoto (a) and Culmo Pieno (b) lines; 1: dormant, 2: non dormant and 3: GA_3 treated dormant seeds.

2. G6PDH and 6PGDH activities

Both dehydrogenase activities were already present in dry embryos, where the levels of 6PGDH were higher than those of 6PGDH (Figs. 2 a, 2 b). After six hours of imbibition both activities increased and subsequently remained nearly constant up to 12 h both in dormant and in non dormant embryos. Finally G6PDH and 6PGDH activitities of dormant Culmo Pieno embryos remained at constant levels while they increased slightly in dormant Culmo Vuoto ones.

After GA_3 treatment of dormant seeds, G6PDH and 6PGDH activities closely followed the pattern observed for water imbibed seeds in Culmo Vuoto line, while slight differences in 6PGDH activity were detected in Culmo Pieno after 24 h of imbibition between the control and the GA_3 treated embryos.

3. C6/C1 determinations

The C6/C1 ratios for embryos excised from dry seeds were similar both for dormant and non dormant embryos and their values were around 0.40.

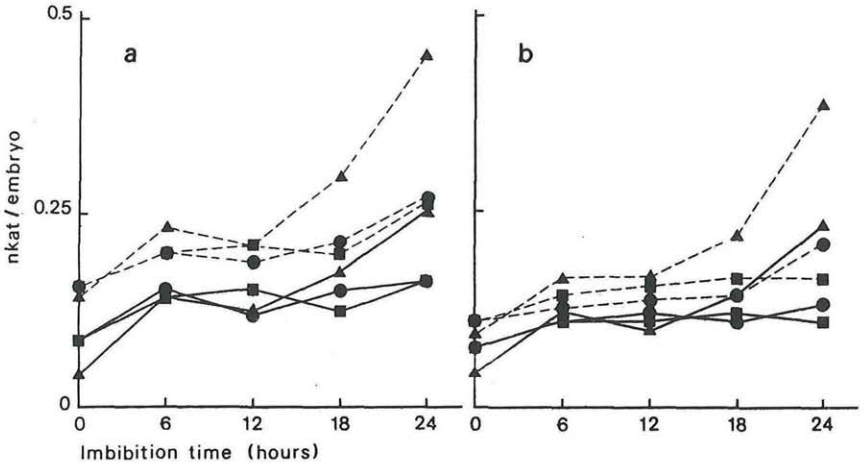

Fig. 2. Changes in G6PDH and 6PGDH activities in dormant (■), non dormant (▲) and GA_3 (●) treated embryos from ×*Haynaldoticum sardoum* seeds. Culmo Vuoto (a), Culmo Pieno (b), G6PDH (–) and 6PGDH (---). The values of the determinations of enzymic activity for the two extractions differed not more than 2%.

This order of magnitude of the C6/C1 ratio for dry embryos indicated the full activity of PPP. After imbibition in water, the ratio was constant up to 12 h in Culmo Vuoto and then increased in non dormant embryos. Culmo Pieno embryos showed an increased C6/C1 ratio by 12 h for non dormant ones, while a slight decrease in the ratio was observed in dormant embryos. Embryos, from GA_3 treated seeds to break dormancy, showed C6/C1 ratios which closely followed those found for the imbibed seeds whether in Culmo Pieno or in Culmo Vuoto.

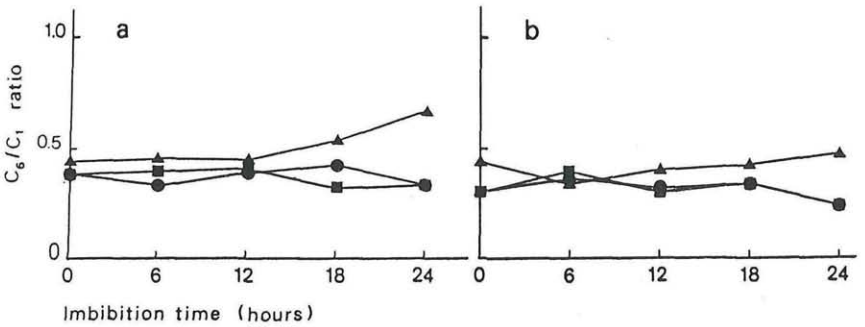

Fig. 3. Activity of the PPP relative to glycolysis-Krebs cycle in dormant (■), non dormant (▲) and GA_3 (●) treated embryos from ×*Haynaldoticum sardoum* seeds. Culmo Vuoto (a), Culmo Pieno (b). The values of the determinations for each replica differed not more than 3%.

Discussion

G6PDH and 6PGDH activities have been demonstrated both in ripening seeds and in dry ones. This has been observed in barley (DUFFUS & ROSIE 1977), castor bean (SIMCOX & al. 1979) and has also been found in ×*H. sardoum* dry embryos. However G6PDH activity, the first enzyme in pentose phosphate pathway (PPP) was lower than in the 6PGDH one – the next enzyme in PPP. This might suggest a mode of modulation of PPP, i. e. it would be the G6PDH limiting activity to regulate the breakdown of glucose in the early stage of germination, as hypothesized for peanuts (SWAMY & al. 1980). Moreover, the levels of both dehydrogenases present during the imbibition could control the overall activity of PPP, which would not operate very well in the presence of low enzyme levels. This would represent one possible mechanism to control the dormancy; many papers have emphasized this point by studying G6PDH and 6PGDH activities in imbibed dormant embryos and in hormone treated ones. SWAMY & al. 1980 showed no difference in both dehydrogenase activities during early germination of dormant and non dormant peanut seeds. Their activities increased in non dormant seeds, while decreased in dormant ones. This was interpreted as a possible metabolic block at the level of PPP and hence a cause of dormancy. Kinetin broke the dormancy and produced an increase in both dehydrogenase activities. Nevertheless, this enzymic pattern cannot be generalized and in fact both dehydrogenase activities in isolated dormant and non dormant embryos of *Avena fatua* remained constant in water or GA_3 imbibed seeds (UPADHYAYA & al. 1981). Thus, the levels of PPP dehydrogenases were not involved in the regulation of seed dormancy.

A similar pattern has been observed in ×*Haynaldoticum sardoum* seeds: dormant and non dormant embryos during water or GA_3 imbibition showed no decrease in dehydrogenase activities and GA_3 treatment failed to modify the above reported activity pattern. A similar increase in dehydrogenase activities during late germination of ×*H. sardoum* was also found in *Avena fatua* embryos, but it was considered to be a post germinative phenomenon and thus unrelated to dormancy breakage (UPADHYAYA & al. 1981). On the other hand, PPP activity could be important during seedling development and this would justify its increase (YAMAMOTO 1963).

Estimations of the relative activities of the glycolytic and PPP in dormant and non dormant ×*H. sardoum* seeds have been experimentally obtained by comparing their ability to utilize glucose-6-^{14}C and glucose-1-^{14}C and by expressing the evolved CO_2 as a C6/C1 ratio. The C6/C1 ratio technique is based on the premise that CO_2 will be released in equimolar quantities from 6-^{14}C glucose and 1-^{14}C glucose when glucose is metabolized via glycolysis-Krebs cycle pathway, while it will only be released from 1-^{14}C glucose when glucose is metabolized via the PPP (BLOOM & STETTEN 1953). Consequently, if the dormancy breaking is associated with

PPP activity, a decrease in the C6/C1 ratio should be expected. The evidence reported here showed an increase in the C6/C1 ratio in non dormant seeds of both ×*H. sardoum* lines, while the ratio decreased slightly in dormant seeds. However GA_3 treatment, effective in dormancy breaking, did not produce any change in C6/C1 ratio values compared to those found in dormant water imbibed seeds. These results confirmed the observations made for *Avena fatua* seeds (FUERST & al. 1983) and suggested a decreased activity of PPP relative to glycolysis-Krebs cycle when dormancy was broken by GA_3 treatment.

In conclusion, in our plant system the G6PDH and 6PGDH activity and C6/C1 deteminations appeared to exclude any role of PPP in dormancy breaking, while they suggested a possible function during late germination. However, it is also clear that not enough plant species have been examined in literature, to draw any conclusions about a general function of PPP in dormancy release.

Further experimental work is in progress to understand the possible causes of dormancy in ×*H. sardoum* seeds.

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