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Lipoquinones of Winter Wheat Grain in Relation to Vernalization

Wpływ jaryzacji na lipochinony ziarna pszenicy ozimej

Влияние яровизации на липохиноны зерен озимой пшеницы

INTRODUCTION

During vernalization of seeds a number of significant changes in the development of plants take place, which either enhance or enable flower differentiation. Vernalization causes an increase in the activity of oxidizing and hydrolytic enzymes (13, 20), which, in consequence, leads to an increase in the number of free saccharides (8) and amino acids (9). A higher ratio of ^{32}P incorporation into the sRNA, rRNA+mRNA and TB-RNA fractions of the leaves of vernalized plants is also observed, as compared with that in the non-vernalized control (6). The chilling effect occurs then on direct change in seed metabolism. Despite the numerous studies on vernalization, we are still far from being able to elucidate the mechanism of the action of low temperatures on plant development.

It appears from the studies of Sironval (quoted by 12) that light effect (photoperiodism), which also influences flower differentiation, exhibits modifications in the ultrastructure of leaves, especially in membrane properties and in general composition. This author proposed a hypohtesis on the role of the plastid apparatus in floral induction.

There is a lack of data on the influence of vernalization on the plastid apparatus of plants. Therefore, the study of the dynamics of some lipids taking part in the formation of plastids in vernalized grains seems to be appropriate. It is known from the present studies that chilling affects the unsaturated fatty acids and the phospholipid and glycolipid components of winter rye (21), and influences the level of α-tocopherol in the initial growth stage of wheat seedlings (5).

The influence of plant growth and age on the level of lipoquinones was studied by many authors; the results, however, are not unambiguous. Barr and Crane (4) found the occurrence of PQA * and PQB * in dry seeds of oats, corn, broad beans and peas. During the greening of etiolated seedlings PQB vanishes and PQC, α-TQ and vitamin K_1 appear. It is believed that PQB does

* Abbreviations: PQA — plastoquinone A; PQB — plastoquinone B; PQC — plastoquinone C; α-TQ — α-tocopherol quinone.

not appear in the green parts of plants, although Barr and Crane (3) found it in small amounts in some plants. PQB synthesis takes place by the end of ontogenesis, in the process of wheat grain formation (14). If, however, we take into consideration the fact that PQB persists in seeds only till their germination in response to light, this compound can be treated as a storage form of plastoquinones, which occurs in seeds.

As it was mentioned earlier, compounds indispensable for further development and growth of plants are mobilized during vernalization. The purpose of this paper was to study the behaviour of lipoquinones under the influence of low temperatures. The role of lipoquinones in the formation of thylakoid membranes and the function of some of them in photosynthetic electron transport entitle us to study their dynamics under the influence of factors accelerating the generative development of plants.

MATERIAL AND METHODS

Dańkowska biała, the winter wheat variety, was used to examine the effect of chilling on the dynamics of lipoquinones. This variety of wheat from the Plant Breeding Station at Dańków required 60 days of vernalization. The grains were surface sterilized with 0.1% mercuric chloride for 20 min, rinsed in glass distilled water and then soaked in the dark at 24°C to reach 40% humidity. The material thus prepared was vernalized at 2±1°C for 60 days according to Lewicki (15). The embryos and endosperm were collected every 5 days and analyzed for lipoquinone content.

Control, non-vernalized grains, taken as a comparison, were in the same morphological stage as the vernalized ones.

Lipoquinones were extracted from the material with acetone and petroleum ether and separated by thin layer chromatography on silica gel GHR and developed with chloroform and isooctane (80:20, v/v) (2). The amounts of benzoquinones were stimated according to Lichtenthaler (16).

Vitamin K_1 was analyzed as described by Lichtenthaler and Tevini (18).

The chlorophyll concentrations were determined by the method of Arnon (1).

The results obtained, being the arithmetical mean of five repetitions, were expressed in relation to grain number.

RESULTS

In this paper the presence of three homologues of the plastoquinone-9: PQA, PQB and PQC was shown in wheat grains, both in the germ and endosperm (Table 1). In the present studies the composition of individual plastoquinones in the wheat grain has not been determined (11, 18).

As seen in Fig. 1, grain germination brings about an increase in the amount of plastoquinones and α-TQ. This is evident because imbibition of water by seeds acts as a triggering mechanism for the biosynthesis of these quinones (23). The increase in lipoquinones (except PQB) develops successively over the entire period of vernalization. This refers above all to the synthesis of PQA, the amount of which after 60 days of vernalization is double the hight of that in the non-vernalized grain. A higher content of benzoquinones results from the more advanced development of the grains subjected to the influence of low temperature, and from their requirement for the development of proplastids and chloroplasts.

The amount of PQB decreases in the course of vernalization. This decrease is also observed in non-vernalized grains, however, at a much slower rate.

Table 1. Lipoquinones content in winter wheat grain

Organ	µg/100 organs			Total
	PQA	PQB	PQC	
Germ	1.2	1.5	0.6	3.3
Endosperm	3.3	2.4	2.0	7.7
Whole grain	4.5	3.9	2.6	11.0

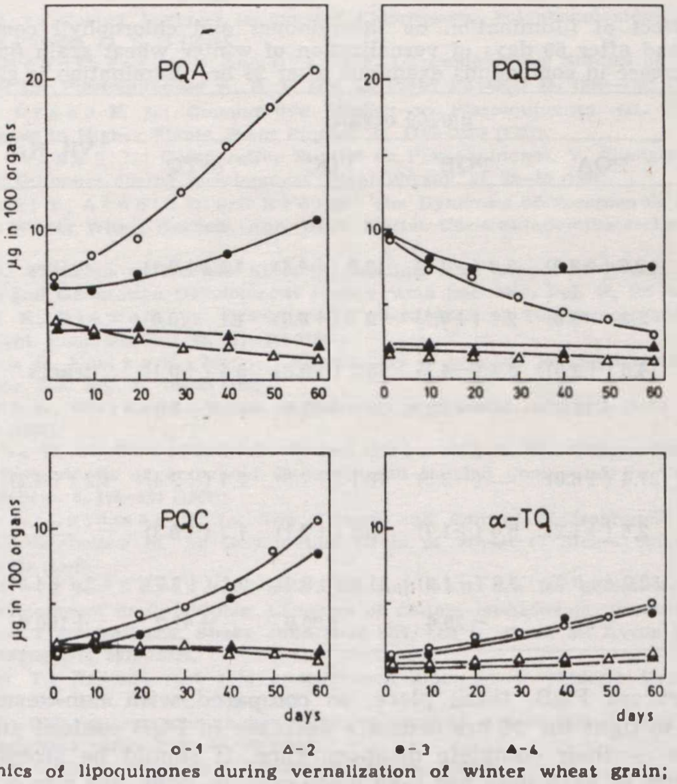

Fig. 1 Dynamics of lipoquinones during vernalization of winter wheat grain; 1 — germ of vernalized grain, 2 — endosperm of vernalized grain, 3 — germ of non-vernalized grain, 4 — endosperm of non-vernalized grain

It is known from numerous studies on the biochemistry of vernalization that chilling increases the activity of hydrolytic enzymes (20). It could thus be assumed that, during vernalization, hydrolysis of PQB (ester) takes place, the result of which is an increase in the amount of PQC (alcohol). Because of lack of quantitative conversions of PQB to PQC, such an assumption is not certain; however, it is very likely.

On the basis of the results of this paper, and the presence of PQB found by Barr and Crane (4) in the seeds and etiolated shoots from a number of plants, as well as the fact that PQB synthesis takes place during naturation of wheat grains (14) we can state that PQB is strictly connected with dormancy and the heterotrophic phase of plant growth. These facts lead us to assume after Barr and Crane, that PQB merely acts as storage from of seeds.

During vernalization the benzoquinones are synthesized de novo in germ. The increase of benzoquinones in germ considerably exceeds the loss of these compounds in the endosperm.

Changes in the level of lipoquinones from vernalized and non-vernalized grains in response to light are in general similar to those observed by other authors (7, 4, 10, 11, 17, 22). The studies of these authors did not concern, however, homologes of plastoquinones and their dynamics in vernalized seeds. In vernalized plants (Table 2) a more intensive synthesis of all the compounds

Table 2. Effect of illumination on lipoquinones and chlorophyll content in non-vernalized and after 60 days of vernalization of winter wheat grain (in paranthesis, increase in compounds examined after 24 hrs illumination is given)

Organs	μg/100 organs				Vit. K_1	Chlorophyll
	PQA	PQB	PQC	α-TQ		
Non-vernalized						
germ	12.6 (+2.0)	3.3 (−4.2)	12.8 (+4.4)	4.5 (+0.1)	traces	25.9 (+25.9)
endosperm	2.2 (0.0)	2.1 (+0.1)	2.7 (+0.9)	1.1 (0.0)	—	—
whole grain	14.8 (+2.0)	5.4 (−4.1)	15.5 (+5.3)	5.6 (+0.1)	traces	25.9 (+25.9)
%	+15.6	−43.2	+52.0	+1.8		+100.0
Vernalized						
germ	27.4 (+6.8)	— (−2.9)	18.1 (+7.5)	7.4 (+2.4)	4.2 (+4.2)	57.4 (+57.4)
endosperm	2.6 (+1.2)	3.0 (+1.7)	3.5 (+2.1)	1.7 (+0.3)	—	—
whole grain	30.0 (+8.0)	3.0 (−1.2)	21.6 (+9.6)	9.1 (+2.7)	4.2 (+4.2)	57.4 (+57.4)
%	+36.4	−28.6	+80.0	+42.2	+100.0	+100.0

analyzed, except PQB, takes place, as compared with non-vernalized ones. Exposition to light for 24 hrs causes a decrease in PQB content and in vernalized plants — their complete disappearance. It should be stressed that the disappearance of PQB does not take place only in light, but also occurs during vernalization. Light only significantly accelerates this process.

Thus the differences in the composition of benzoquinones in vernalized and non-vernalized plants, both in darkness and in light, do not concern qualitative but quantitative changes.

We did not detect vitamin K_1 in etiolated tissues as reported by Lichtenthaler (18), irrspective of their being vernalized or not. After only 24 hrs of illumination we found considerable amounts of vitamin K_1 in vernalized, and only traces of it in non-vernalized grains.

CONCLUSIONS

1. Winter wheat grain contained three benzoquinones: PQA, PQB and PQC, both in germ and endosperm.

2. Moreover, α-TQ synthesis appears during germination and vernalization.

3. Vernalization causes increased accumulation of PQA, PQC, α-TQ and PQB decrease. These changes chiefly concern the germ, and their rate is much higher than that in non-vernalized grain.

4. Changes in the level of the lipoquinones examined, active in synthesis of the plastid structure and photosynthetic electron transport, from vernalized grain, in response to light, are greater that those in non-vernalized ones.

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STRESZCZENIE

Celem pracy było zbadanie dynamiki lipochinonów w procesie jaryzacji pszenicy ozimej odmiany Dańkowska biała. Jaryzację przeprowadzono w sposób konwencjonalny według Lewickiego (15) przez okres 60 dni. Lipochinony oznaczano w odstępach 5-dniowych oddzielnie w zarodku i bielmie metodą Lichtenthalera (16), zaś witaminę K_1 — metodą Lichtenthalera i Teviniego (18). Ponadto oznaczano zawartość lipochinonów i chlorofilu w ziarnie jaryzowanym i niejaryzowanym po 24-godzinnym naświetlaniu. Chlorofil oznaczano według metody Arnona (1). Kontrolę stanowiło ziarno niejaryzowane podkiełkowane do stanu, w jakim znajdowało się ziarno jaryzowane.

Stwierdzono, że:

1. Ziarno pszenicy ozimej zawiera, zarówno w zarodku, jak i bielmie, trzy benzochinony: PQA, PQB i PQC.
2. W czasie kiełkowania i jaryzacji syntetyzowany jest α-TQ.
3. Proces jaryzacji powoduje większe nagromadzenie PQA, PQC i α-TQ w ziarnie oraz spadek zawartości PQB. Zmiany te dotyczą głównie zarodka, a tempo tych zmian jest wyższe aniżeli w ziarnie niejaryzowanym.
4. Zmiany w poziomie lipochinonów, aktywnych w syntezie struktur plastydowych i fotosyntetycznym transporcie elektronów, spowodowane 24-godzinnym naświetlaniem są wyższe w ziarnie jaryzowanym aniżeli w niejaryzowanym.

РЕЗЮМЕ

Авторы изучали динамику липохинонов в процессе яровизации озимой пшеницы (сорт Даньковска Бяла). Яровизация проводилась традиционным способом по Левицкому (15) в течение 60 дней. Липохиноны контролировались через каждые 5 дней в зародыше и эндосперме по методу Лихтенталера (16), а витамины K_1 по методу Лихтенталера и Тевини (18). Кроме того определялось содержание липохинонов и хлорофилла в яровизированном и неяровизированном зерне после суточного облучения. Хлорофилл подсчитывался по методу Арнона (1). Контрольным было неяровизированное зерно на одинаковом этапе прорастания с яровизированным зерном.

Констатировано, что:

1. Зерно озимой пшеницы содержит как в зародыше, так и в эндосперме 3 бензохинона: ПQА, ПQБ, ПQЦ.
2. В период прорастания и яровизации синтезируется α-TQ.
3. Процесс яровизации влечет большое скопление ПQА, ПQЦ и α-TQ в зерне и уменьшение содержания ПQБ. Эти изменения происходят в основном в зародыше, а темп их больше, чем в неяровизированном зерне.
4. Изменения уровня активных липохинонов в синтезе пластидовых структур и фотосинтетическом перемещении электронов, (вызванное суточным облучением) более значительны в яровизированном зерне.