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Kierownik: doc. dr Tadeusz Baszyński

Tadeusz BASZYŃSKI, Danuta ARNOLD
and Maria KRÓL

The Dynamics of Tocopherols during Vernalization of Winter Wheat Kernels

Dynamika tokoferoli w procesie jaryzacji ziarna pszenicy ozimej

INTRODUCTION

Although many attempts have been made to explain the mechanism of vernalization, the problem has not been elucidated.

Some workers who are in favour of the hormonal theory of plant flowering, according to whom growth regulators may be substituted for thermo-induction, have been obtaining diverse results, dependent on the species of the plant used. Lang's (9) recent report concerning the substitution of thermo-induction for gibberellin has not been confirmed.

In search of factors of hormonal nature, it is necessary to mention the report by Bruinsma and Patil (6) who demonstrated that it was possible to substitute the vernalization of Petkus winter rye for vitamin E for reproduction, if it was grown under non-inductive temperature conditions.

Michniewicz and Kamieńska (10) found also that tocopherol and kinetin were capable of promoting the formation of flowers in cold-requiring, long-day unvernalized *Cichorium intybus* L.

It ought to be mentioned that tocopherols, which according to Sironval and El Tannir-Lomba (13) are supposed to control the flowering of strawberry plants, induced the flowering of long-day plants grown in short day (4, 11).

The recognition of tocopherols in the flowering of plants as those which control only the level of endogenous gibberellin has not been unanimously confirmed (12).

No experiments on the mechanism of tocopherols in the process of vernalization have been reported. Moreover, there is no information on the qualitative and quantitative composition of tocopherols in kernels during vernalization as well as on the level of α-tocopherol at early stages of growth of vernalized and unvernalized plants.

This report is an attempt to partly fill up the gap with regard to several varieties of winter wheat.

MATERIAL AND METHODS

The material for experiments were kernels of four winter wheat varieties:

Dańkowska Biała (classified by Plant Breeding Station at Laski) with unknown period of vernalization.

Dańkowska Selekcyjna (classified by Plant Breeding Station at Danków) which requires 60 days of vernalization.

Leszczyńska Wczesna (classified by Plant Breeding Station at Antoniny) which requires 20 days of vernalization.

Kujawianka Więcławicka (classified by Plant Breeding Station at Więcławice) which requires 20 days of vernalization.

The kernels underwent vernalization for 60 days at 2° (± 1°C) prior to germination at 23°C, for 24 hrs. In the course of vernalization performed at intervals of 6 days this part of vernalized material was examined from which tocopherols had been extracted with ethyl ether in the Soxhlet apparatus. Next, the content of tocopherols was determined by the method of Green and his co-workers (1).

The content of tocopherols was given in μg per unit of dry weight or per 100 kernels.

To estimate the effect of vernalization on α-tocopherol content at early stages of wheat seedlings, kernels were sown after 6, 12, 18, 24, 30, 36, 42 and 48 days of vernalization in garden soil in pots, in the light, at 24°C. Control plants were grown from nonvernalized kernels which were allowed to germinate to the same stage as did vernalized kernels. 5-day-old wheat seedlings were analyzed with regard to α-tocopherol content by the method of Booth (5).

The difference between the α-tocopherol content of seedlings from vernalized kernels and that of the control kernels was given as a percentage (α-tocopherol content in the control seedlings was taken as 100%).

RESULTS

In accordance with the pattern of tocopherols characteristic of cereal kernels, given by Green (7), the kernels of all analyzed varieties of winter wheat were found to contain four tocopherols (α, β, ε, ζ). This pattern of tocopherols remained unchanged both before and during vernalization. The examined wheat varieties differed in tocopherol content and in the ratio of particular isomers (Table 1).

24 hr. germination of kernels resulted in a content decrease of these four tocopherols. This decrease indicates a rapid utilization of tocopherols in the most vigorous phase of growth, i. e. at an early stage of germina-

Table 1. Tocopherols content of winter wheat kernels before vernalization

Variety of wheat	Tocopherols in μg per															Ratio
	g of fresh weight					g of dry weight					10^2 of kernels					
	α	β	ε	ζ	total	α	β	ε	ζ	total	α	β	ε	ζ	total	α : β : ε : ζ
Dańkowska Biała	5.0 ±0.48	3.0 ±0.39	7.3 ±0.10	1.1 ±0.20	16.4	5.4	3.3	7.9	1.2	17.8	21.1	12.9	30.9	4.3	69.2	30,5 : 18,3 : 44,5 : 6,7
Dańkowska Selekcyjna	4.6 ±0.71	2.7 ±0.14	5.1 ±0.80	1.0 ±0.09	13.4	5.1	3.0	5.7	1.1	14.9	15.7	9.2	17.3	3.4	45.6	34.3 : 20.1 : 38.1 : 7.5
Leszczyńska Wczesna	4.6 ±0.40	2.3 ±0.03	5.4 ±0.85	0.9 ±0.17	13.2	5.2	2.6	6.2	1.0	15.0	19.1	10.8	22.4	3.8	56.1	34.8 : 17.4 : 41.0 : 6.8
Kujawianka Więcławicka	4.7 ±0.10	2.7 ±0.16	8.5 ±0.57	1.5 ±0.17	17.4	5.6	3.2	10.2	1.8	20.8	20.9	12.0	37.8	6.7	77.4	27.0 : 15.5 : 48.9 : 8.6

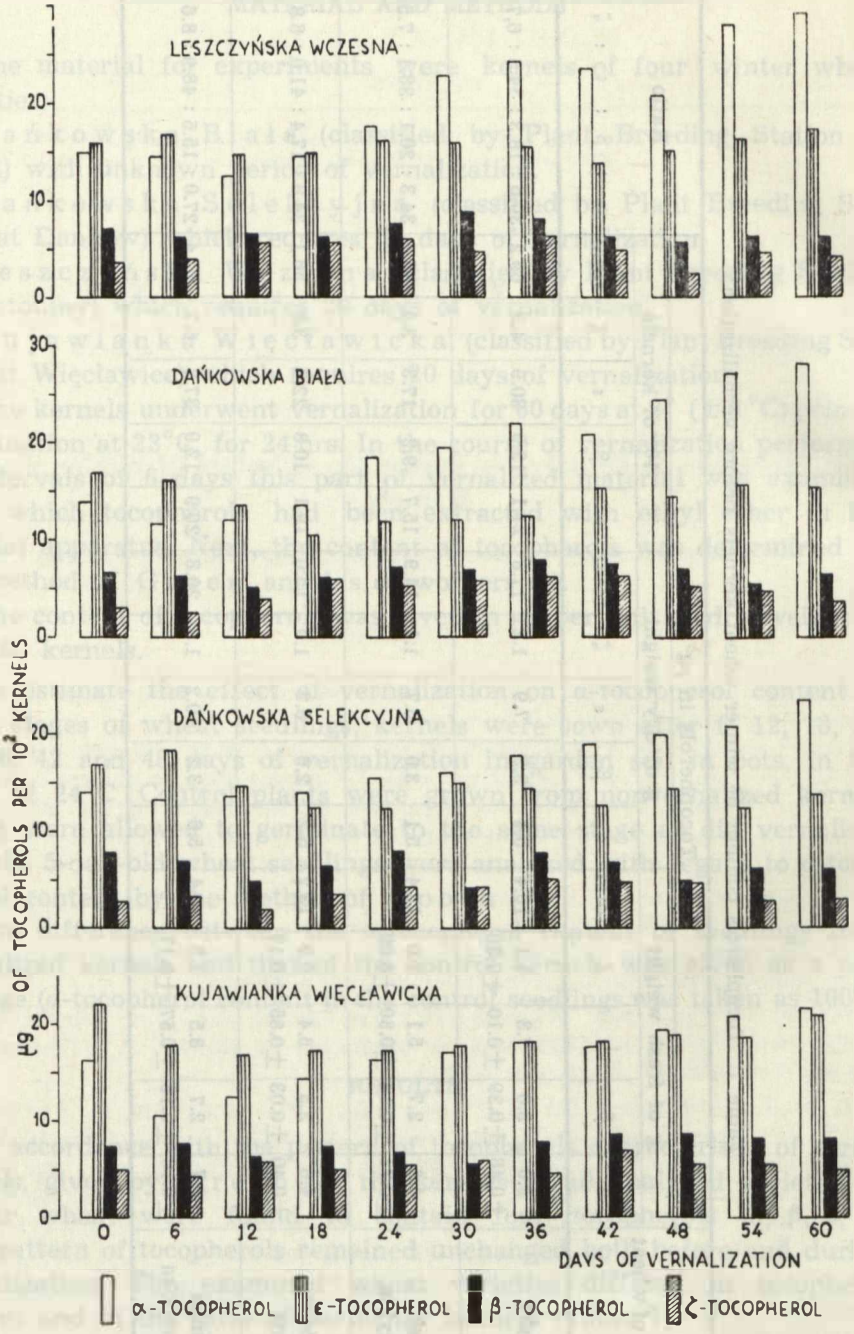

Fig. 1. The dynamics of tocopherols during vernalization of winter wheat kernels

tion. A similar effect or complete disappearance of tocopherols was also found by K a r t h a (8), in a number of seeds of oil plants. As shown in Fig. 1, the dynamics of tocopherols during the vernalization of kernels is characterized by a decrease of the total amount of tocopherols during the first vernalization period and by a gradual increase of their content at later stages.

The gradual increase in the total tocopherol content is caused almost exclusively by α-tocopherol synthesis, the percent participation of which in total tocopherol content was found to have increased significantly. The increase of total tocopherols and the absence of the distinct decrease in β- and ζ-tocopherol content, give basis for the assumption that mono- and dimethyltocols act as precursors of α-tocopherol in the seeds, as suggested in studies concerning the distribution of tocopherols at early stage of germination of pea seeds (2).

It is also difficult to draw some general conclusions from the results obtained with regard to trimethyl compound i. e. ε-tocopherol.

Table 2. The effect of vernalization of kernels on the α-tocopherol content of 5-day-old winter wheat seedlings var. Kujawianka Więcławicka

	Days of vernalisation							
	6	12	18	24	30	36	42	48
% of α-tocopherol content (non-vernalized control plants = 100%)	76.1	81.2	158.2	149.4	138.0	173.8	146.3	134.6

A distinctly higher increase of α-tocopherol level is observed after the vernalization process finished, i.e. after the period of cold-require characteristic of a given variety (especially distinct in Leszczyńska Wczesna, Dańkowska Biała and Dańkowska Selekcyjna). Investigations with Kujawianka Więcławicka variety on the tocopherol content in 5-day-old seedlings from vernalized and nonvernalized kernels showed that the vernalization period longer than 18 days resulted in a higher content of α-tocopherol than that in the controls of the same group age (Table 2). This result is in agreement with a short period of cold-requirement of that variety and it enables a tentative evaluation of the plant development on the basis of tocopherol content. This is also in accordance with some earlier reports according to which the tocopherol content increases with growth of plants at vegetative stage (3).

CONCLUSIONS

1. Tocopherol pattern characteristic of kernels of several winter wheat varietes does not undergo any change during vernalization.

2. Changes in the tocopherol content which occur during vernalization of kernels refer to α-tocopherol.

3. The process of kernel vernalization has an influence on the level of α-tocopherol in the initial growth stage of wheat seedlings.

REFERENCES

1. Analytical Methods Committee. The Determination of Tocopherols in Oils, Foods and Feeding Stuffs. The Analyst, **84**, 999, 1959.
2. Baszyński T.: An Attempt to Explain the Mechanism of the Synthesis of α-Tocopherol in the Seedlings of *Pisum sativum* L. Acta Soc. Bot. Pol., **30**, 307, 1961.
3. Baszyński T.: Investigations on the Synthesis and Function of Tocopherols in Plants. The Synthesis of Tocopherols in the Ontogenesis of Short-day and Long-day Plants. Materiały XXXVI Zjazdu PTB, Lublin, 1964.
4. Baszyński T.: The Effect of Vitamin E on Flower Initiation in *Calendula officinalis* L. Grown in Short Day. Naturwiss., **54**, 13, 1967.
5. Booth V. H.: Determination of Tocopheroll in Plant Tissues. The Analyst, **88**, 1049, 1963.
6. Bruinsma J., Patil S. S.: The Effects of 3-Indoleacetic Acid, Gibberellic Acid and Vitamin E on Flower Initiation in Unvernalized Petkus Winter Rye Plants. Naturwiss., **50**, 14, 1963.
7. Green J.: Tocopherols. In "Enzyme Chemistry of Phenolic Compounds". Pergamon Press, London 1963.
8. Kartha A. R. S.: A Study of the Mobilisation of Free Tocopherols in Germinating Seeds. J. Sci. Fd. Agric., **15**, 11, 1964.
9. Lang A.: Induction of Flower Formation in Biennial *Hyoscyamus* by Treatment with Gibberellin. Naturwiss., **43**, 284, 1956.
10. Michniewicz M., Kamieńska A.: Flower Formation Induced by Kinetin and Vitamin E Treatment in Cold-Requiring Plant (*Cichorium intybus* L.) Grown under Non-inductive Conditions. Naturwiss., **51**, 295, 1964.
11. Michniewicz M., Kamieńska A.: Flower Formation Induced by Kinetin and Vitamin E Treatment in Long-day Plant (*Arabidopsis thaliana*) Grown in Short Day. Naturwiss., **52**, 623, 1965.
12. Michniewicz M., Kamieńska A.: Studies on the Role of Kinetin and Vitamin E in the Flowering of the Cold Requiring Plant (*Cichorium intybus* L.) and the Long-day Plant (*Arabidopsis thaliana* L.,) Grown in Non-inductive Conditions. Acta Soc. Bot. Pol., **36**, 1, 1967.
13. Sironval C., El Tannir-Lomba J.: Vitamin E and Flowering of *Fragaria vesca* L. var. *semperflorens* Duch. Nature, **185**, 4716, 1960.

Dynamika tokoferoli w procesie jaryzacji ziarna pszenicy ozimej

Streszczenie

Badano dynamikę tokoferoli podczas przedsiewnej jaryzacji czterech odmian pszenicy ozimej.

Jaryzację ziarna przeprowadzano w sposób konwencjonalny przez 60 dni. Tokoferole w jaryzowanym ziarnie oznaczano metodą Greena i współprac. w odstępach 6-dniowych.

Ponadto badano zawartość α-tokoferolu (metodą Bootha) w 5-dniowych siewkach pszenicy odmiany Kujawianka Więcławicka, pochodzących z ziarna poddanego jaryzacji. Kontrolę stanowiły siewki otrzymane z ziarna niejaryzowanego, podkiełkowanego do stanu, w jakim znajdowało się ziarno jaryzowane.

Stwierdzono, że:

1. Skład tokoferoli charakterystyczny dla ziarna pszenicy ozimej nie ulega zmianie w procesie jaryzacji.
2. Zmiany w zawartości tokoferoli podczas jaryzacji ziarna odnoszą się głównie do α-tokoferolu.
3. Proces jaryzacji ziarna ma wpływ na poziom α-tokoferolu w początkowym okresie wzrostu siewek pszenicy.

Динамика токоферолов в процессе яровизации зерна озимой пшеницы

Резюме

Исследовалась динамика токоферолов во время предпосевной яровизации четырёх сортов озимой пшеницы.

Яровизация зерна проводилась конвенциональным способом в течение 60 дней. Токоферолы в яровизированном зерне определялись по методу Грина и сотр. каждые 6 дней.

Кроме того, исследовалось содержание α-токоферола (метод Bootha) в пятидневных сеянцах пшеницы сорта Kujawianka Więcławicka, происходящих от яровизированных зерен. Контрольной группой являлись сеянцы, полученные из неяровизированного зерна, пророщенного до состояния, в котором находилось яровизированное зерно.

Установлено:

1. Состав токоферолов, характерный для зерна озимой пшеницы, в процессе яровизации не изменяется.
2. Изменения содержания токоферолов, происходящие во время яровизации зерна, относятся, главным образом, к α-токоферолу.
3. Процесс яровизации зерна влияет на уровень α-токоферола только в начальном периоде роста сеянцев пшеницы.

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