Studies on the vitamin E content in the course of vernalization of winter wheat

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There exist many facts showing that the presence of vitamin E is of essential importance in the process leading to flower formation (for review — Baszyński 1964; Michniewicz and Kamińska 1967). Vitamin E has induced the flower formation in cold-requiring *Cichorium intybus* (Michniewicz and Kamińska 1964) and winter rye (Bruinsma and Patil 1963). This fact indicates therefore that this vitamin may "substitute" by some way the process of vernalization in these plants.

It is known that during vernalization there occur some special biochemical changes (for review — Kentzer 1959) among others also in the dynamics of vitamins (for review Michniewicz 1961). However we have not as yet any information about the dynamics of vitamin E during this process. It has been therefore decided to examine the changes of tocopherols in the course of vernalization of winter wheat, which up to now is used as the most common object in this kind of experiments.

MATERIAL AND METHODS

For experiments winter wheat var. 'Dańkowska Selekcyjna' was used. The kernels were vernalized in glass pots (10 g per one glass) at a temperature of $2^{\circ}\text{C} \pm 1$ for 60 days, according to Lysenko's method (1950). The water content of grains amounting to 45%, was kept at a constant level during the whole period of vernalization. This kind of cold treatment leads to the earing of the wheat var. 'Dańkowska Selekcyjna' as described in a previous paper (Michniewicz 1965).

The content of vitamin E was defined in 10 g samples of:

- 1) kernels swelled for 24 hours at 23°C (before chilling),
- 2) kernels germinated for 48 hrs (unvernalized grains beeing in the same morphological stage as the vernalized ones), and
- 3) kernels after 3, 5, 10, 20, 40 and 60 days of vernalization.

The determination of vitamin E was carried out according to the method described by the Analytical Methods Committee (1959). The grains were extracted with methanol. The extract was then saponified, shaken with petroleum benzine and evaporated to a small volume. After

purification by column chromatography (Floridin Earth XS) the extract was chromatographed on filter paper Whatman No 4 which was impregnated with zinc amine (two-dimensional, ascending method). As solvent systems: 1.1% acetone in light petroleum ether (first dimension) and methanol (second dimension) were used (Booth and Hobson-Frohock 1961). The spots were marked with pencil under UV light. Readings were taken with UNICAM SP 600 spectrophotometer at a wavelength of 520 m μ and determined with the FeCl₃-dipiridil reagent. Three independent measurements were made for each determination. The content of tocopherols was expressed as μ g/g of fresh weight.

All experiments were repeated 3 times and analysed statistically.

RESULTS AND DISCUSSION

It was found that the grains of winter wheat var. 'Dańkowska Selekcyjna' contain α -, β -, ϵ -, and ζ -tocopherols (Fig. 1). This is in accordance with the results obtained by Green (1958) who experimented

Fig. 1. Two-dimensional paper chromatography of tocopherols from wheat kernels.

with wheat var. 'Atllee'. The presence of the same tocopherols was also shown by Baszyński (1964) in the kernels of barley var. 'Skrzeszowiecki'.

The same as in barley (Baszyński 1964) the content of ζ -tocopherol in wheat kernels was much higher than the content of other tocopherols. Our results did not confirm the data of Green (1958) who stated that the grains of wheat contained more σ - and ϵ -tocopherols than β - and ζ -tocopherols.

During the process of vernalization some changes in the total amount of vitamin E were observed but all the differences were statistically insignificant (Tab. 1, Fig. 2).

Other results were obtained by analysing the particular tocopherols. So, the level of ζ -tocopherol, as compared with the other tocopherols, was significantly higher during the whole period of vernalization,

Table 1

The amounts of vitamin E in the course of vernalization of wheat kernels

Tocopherols	Values in	Days of vernalization							Control**	LSD at p =		
		0*	3	5	10	20	40	60		0,05	0,01	0,001
α	$\mu\text{g/g}$	0.687	0.606	0.461	0.435	1.947	0.512	0.702	0.314	0.102	1.407	1.935
	%***	11.65	9.00	7,04	8.96	26.55	9.43	9.45	4.89			
β	$\mu\text{g/g}$	1.074	1.040	1.073	0.682	1.188	0.753	0.609	1.184	0.960	1.323	1.818
	%	18.21	15.45	16.38	14.05	16.20	13.87	8.20	18.45			
ϵ	$\mu\text{g/g}$	1.383	1.760	1.968	0.860	1.736	0.651	0.891	1.148	1.026	1.413	1.943
	%	23.45	26.14	30.05	17.71	23.67	11.99	11.99	17.88			
ζ	$\mu\text{g/g}$	2.753	3.326	3.047	2.878	2.463	3.513	5.229	3.772	1.170	1.612	2.216
	%	46.69	49.41	46.53	59.21	33.58	64.71	70.36	58.78			
Total	$\mu\text{g/g}$	5.897	6.732	6.549	4.855	7.334	5.429	7.431	6.418	3.004	4.139	5.689

* — The kernels swelled for 24 hrs at 23°C.

** — Sprouting kernels beeing in the same morphological state as the vernalized kernels.

*** — % of total tocopherols.

except of the 20th day of this process. Starting with the 20th day of vernalization the content of ζ -tocopherol markedly increased when compared with its initial value in kernels swelled for 24 hrs at 23°C. The highest amount of this tocopherol was found in fully vernalized

Fig. 2. The dynamics of tocopherols content in the course of vernalization of wheat kernels.

α — kernels swelled for 24 hrs at 23°C. C — unvernallized kernels beeing in the same morphological state as the vernalized kernels. Circle — significant differences in relation to variant O* ($P=0.05$) and variant C ($P=0.01$). Triangle — significant difference at $P=0.001$ in relation to variant O*

kernels. It differed only slightly from that, observed in the unvernializing kernels (germinated for 48 hrs), both samples were being in the same morphological stage. The content of α -tocopherol was significantly increased in relation to both controls (i.e. kernels swelled for 24 and 48 hrs at 23°C) but only after the 20th day of vernalization. Further analyses did not show any significant changes.

Fig. 3. The content of vitamin E in wheat kernels germinated at 23°C and 2°C

All differences significant at $P = 0.05$.

So, the dynamics of vitamin E content during the process of wheat vernalization was quite different from the dynamics of vitamin C. The content of vitamin C gradually increased in the course of vernalization (Michniewicz 1961).

It was revealed that the most intensive biochemical changes occur during the first days of wheat germination (Michniewicz at all 1966). Therefore it was decided to examine the dynamics of vitamin E in the first days of germination — at favourable (23°C) and vernalizing temperature (2°C) and also in dry kernels.

The results of these experiments (Fig. 3) showed an increase in the vitamin E content after 24 hrs of germination at 23°C. Afterwards the level of this vitamin decreased and after 48 hrs it increased again. These observations are therefore in accordance with the data of Leach (see Crocker and Barton 1957) who reported about similar changes

in the intensity of respiration during the first days of wheat germination.

Similar regularity in the dynamics of vitamin E content was also found in kernels germinating at vernalizing temperature. But here the observed changes occurred more slowly, probably because of the inhibitory effect of low temperature on the growth processes. So, the results of these experiments show that the changes of vitamin E content are not specific for the process of vernalization but depend probably on the growth rate of the analyzed plant material.

On the basis of the results presented in this paper, the authors draw the conclusion that similarly to what was shown for auxins (Michniewicz and Kamińska 1966) there is no connection between the dynamics of vitamin E content and the process of vernalization of wheat kernels.

CONCLUSIONS

1. The grains of winter wheat "Dańskowska Selekcynna" contained α -, β -, ϵ -, and ζ -tocopherols. The level of ζ -tocopherol was generally higher than the content of other tocopherols.

2. The changes in the amount of vitamin E occurring during the first days of germination at 23°C corresponded to those in kernels germinated at 2°C. However in vernalizing temperature these changes occur much slower.

3. In the final stages of vernalization the content of ζ -tocopherol markedly increased. The total amount of vitamin E did not change significantly during the process of vernalization.

4. On the basis of the presented experiments it is concluded that there is no connection between the dynamics of vitamin E content and the process of vernalization of wheat kernels.

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Badania nad dynamiką witaminy E w procesie jaryzacji pszenicy ozimej

Streszczenie

Określano zawartość witaminy E w ziarnie pszenicy ozimej 'Dańkowska Selekcja': 1) w ziarnie pieczącym przez 24 godz. w temp. 23°C — bezpośrednio przed chłodzeniem, 2) w nie jaryzowanym ziarnie będącym w tej samej fazie wzrostu w jakiej było ziarno zjaryzowane i 3) w ziarnie po 3, 5, 10, 20, 40 i 60-ciu dniach jaryzacji. Stosowano technikę jaryzacji według metody Łysenki (1950) w temp. 20°C $\pm$ 1.

Wykonano również doświadczenie, w którym porównano zawartość witaminy E w ziarnie kiełkującym w temp. 2°C oraz w temp. 23°C. Zawartość witaminy E oznaczano metodą podaną przez Analytical Methods Committee (1959). Wszystkie doświadczenia powtórzono trzykrotnie, a wyniki poddano analizie statystycznej.

W wyniku przeprowadzonych badań stwierdzono:

1. Ziarna pszenicy zawierały cztery tokoferole: α -, β -, ϵ - i ζ -tokoferol. Poziom ζ -tokoferolu przewyższał zasadniczo wartość pozostałych.
2. Zmiany w zawartości witaminy E jakie zachodziły w pierwszych dniach kiełkowania w temp. 23°C były podobne do tych, jakie obserwowano w temp. 2°C. Zmiany te w temperaturze jaryzującej następowały jednak wolniej.
3. W końcowym etapie procesu jaryzacji wzrastała ilość ζ -tokoferolu, jednakże ogólny poziom witaminy E nie uległ w tym procesie istotnym zmianom.
4. Wyniki niniejszych doświadczeń upoważniają do wniosku, że efekt jaryzacji nie pozostaje w bezpośrednim związku z dynamiką witaminy E w tym procesie.