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**The Dynamics of Tocopherols during Vernalization of Winter Wheat
Kernels Treated with Azide**

Dynamika tokoferoli podczas jaryzacji ziarniaków pszenicy poddanych
działaniu azydu

Динамика токоферолов во время яровизации зерен озимой пшеницы,
находящихся под действием азид натрия

INTRODUCTION

A hypothetical participation of tocopherols in plant development and especially some tests of substituting thermo-induction with a tocopherol in cold-requiring plants, grown in non-inductive conditions (3, 6), encouraged the investigation into the dynamics of tocopherol during vernalization. The hypothesis of Michniewicz and Kamińska about the dependence of the activity of tocopherols in plant reproduction on the control of the level of endogenous gibberellins, not confirmed by these authors (7), promoted the search of elucidating the activity of these compounds. Although vernalization is not likely to directly influence the accumulation of tocopherols in kernels of some varieties of winter wheat, it changes the quantitative tocopherol composition in favour of α -tocopherol (α -T), and affects the level of α -T in seedlings of vernalized kernels in their initial stage of growth (2). The reports of Michniewicz and his coworkers (8) concerning the increase of a homologue different from α -T during vernalization of wheat kernels, do not contradict the fact that during vernalization the per cent composition of particular homologues varies. If these variations are due to low temperature then the "antivernalizing agents" ought to affect the behaviour of tocopherols during vernalization. In the present work sodium azide

served as inhibitor of vernalization; its influence in various vernalization stages of wheat kernels on their generative development was the subject of a detailed study by Kentzer (4).

MATERIAL AND METHODS

Dańkowska Selekcynja, the winter wheat variety, was used to examine the effect of sodium azide (NaN_3) on the dynamics of tocopherols in vernalized kernels. This variety of wheat was used in experiments of Kentzer (4) and it was examined in our laboratory (2). Wheat kernels from Plant Breeding Station at Dańków were harvested in 1967 and required 60 days of vernalization. The kernels for vernalization were germinated at 20°C for 20 hrs. and water was added to reach 40% humidity (5). The material thus prepared was vernalized at $2 \pm 1^\circ\text{C}$ for 65 days. The kernels were treated with an aqueous azide solution at a concentration of 10^{-3}M . This concentration caused a very strong or complete inhibition of the generative development of wheat (4). The inhibitor was used according to Szwedskája and Kruzilin (9) prior and following 5, 10, and 40 days of vernalization. Control kernels were those (a) vernalized without inhibitor and (b) nonvernalized ones, but being in the same morphological stage as the vernalized ones. In each experiment a total of 250 kernels were vernalized. After vernalization the kernels were washed in tap water for 10 hrs and, next, analysed with regard to the content of tocopherols by the method of Green and his co-workers (1). The results expressed as the arithmetic mean of the two separate experimental series (each series was analysed three times) are presented in Figures.

RESULTS

The present examinations confirmed the observations, reported earlier (2), that during vernalization of wheat kernels the content of α -T varied and its ratio to other homologues increased (24.3% of α -T in the initial stage of germination as compared with 47.8% after 65 days of vernalization).

The dynamics of tocopherols is observed to undergo a distinct change in the azide solution. The amount of tocopherols in kernels pretreated with azide decreases with time of vernalization. This decrease is considerable in each stage of vernalization and is possibly caused by the metabolization of tocopherols and inhibition of enzymes which mobilize substrates indispensable for synthesis of tocopherols.

α -T percent in the total of tocopherols is found to be lower than that in the controls vernalized without azide, the per cent decrease being the more considerable the earlier the kernels were treated with the inhibitor. The application of azide in various vernalization stages did not cause changes in the α -T content to such a degree as expected from Kentzer's investigations (4). According to Kentzer azide used prior to vernalization or in its initial stage caused total inhibition of the

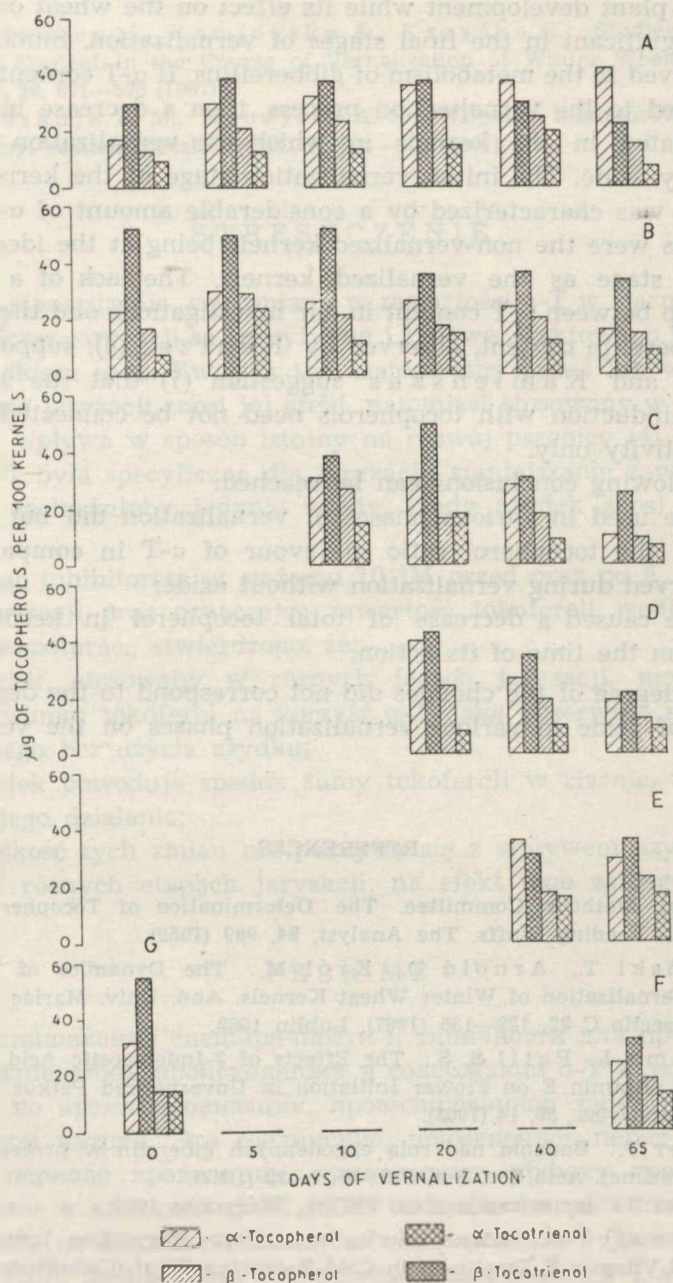


Fig. 1. The dynamics of tocopherols during vernalization of winter wheat kernels treated by azide solution; A — control vernalized without NaN_3 , B — NaN_3 applied before vernalization, C, D, E, F — NaN_3 applied after 5, 10, 20, 40 days of vernalization, G — non-vernalized control

generative plant development while its effect on the wheat development was not significant in the final stages of vernalization. Similar changes were observed in the metabolism of gibberellins. If α -T content in kernels were related to the vernalization process, then a decrease in α -T could be anticipated in the kernels in which the vernalization effect was nullified by azide. The initial vernalization stage of the kernels treated with azide was characterized by a considerable amount of α -tocotrienol similarly as were the non-vernalized kernels being at the identical morphological stage as the vernalized kernels. The lack of a significant relationship between α -T content in our investigations and the variations in the gibberellin content, observed by Kentzer (4), support Michniewicz and Kamieńska's suggestion (7) that the substitution of thermo-induction with tocopherols need not be connected with gibberellin activity only.

The following conclusions can be reached:

1. azide used in various phases of vernalization did not cause any changes in the tocopherol ratio in favour of α -T in comparison with those observed during vernalization without azide;
2. azide caused a decrease of total tocopherol in kernels, which depended on the time of its action;
3. the degree of the changes did not correspond to the degree of the influence of azide in various vernalization phases on the vernalization effect.

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STRESZCZENIE

W celu stwierdzenia, czy zmiany w zawartości α -T w ziarnie pszenicy ozimej podczas jaryzacji są specyficzne i typowe, traktowano jaryzowane ziarno azydkiem sodu. Związek ten zastosowany przed lub w początkowym okresie jaryzacji znosi jej efekt, natomiast stosowany w końcowych fazach nie wpływa w sposób istotny na rozwój pszenicy (4). Gdyby zawartość α -T była specyficzna dla jaryzacji, zmniejszanie zawartości α -T w ziarnie zachodziłoby jedynie wtedy, kiedy azydek znosi efekt tego zabiegu.

Działając inhibitorem w stężeniu $10^{-3}M$ przed oraz po 5, 10, 20 i 40 dniach jaryzacji oraz oznaczając zawartość tokoferoli według metody Greena i współprac., stwierdzono, że:

- 1) azydek, stosowany w różnych fazach jaryzacji, nie powoduje zmiany stosunku tokoferol na korzyść α -T, jaką obserwuje się u ziarna jaryzowanego bez użycia azydku;
- 2) azydek powoduje spadek sumy tokoferoli w ziarnie, uzależniony od czasu jego działania;
- 3) wielkość tych zmian nie pokrywa się z wpływem azydku, stosowanego w różnych etapach jaryzacji, na efekt tego zabiegu.

РЕЗЮМЕ

Для установления специфичности и типичности для процесса яровизации изменений, происходящих в содержании α -Т в зерне озимой пшеницы во время яровизации, яровизированное зерно обрабатывалось азидом натрия. Это соединение, примененное перед или в начальном периоде яровизации, ликвидирует эффект яровизации, а примененное в конечной фазе этого процесса на развитие пшеницы существенно не влияет (4). Если бы содержание α -Т было специфичным для этого процесса, то уменьшение содержания α -Т в зерне происходило бы только тогда, когда азид ликвидирует эффект яровизации.

Воздействуя ингибитором при концентрации $10^{-3} M$ до и после 5, 10, 20 и 40 дней яровизации и определяя по методу Грина и сотр. содержание токоферола, установлено:

- 1) азид, применяемый в разных фазах яровизации, не вызывает изменений отношения токоферолов в пользу α -Т, что наблюдается в зерне, яровизированном без применения азида,
- 2) азид вызывает уменьшение суммы токоферолов в зерне, находящегося в зависимости от времени его действия,
- 3) величина этих изменений не соответствует влиянию азида, примененного в разных этапах яровизации.