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Kierownik: doc. dr Tadeusz Baszyński

Tadeusz BASZYŃSKI and Hanna STEINBRICH

The Pattern of Tocopherols in Seeds of Lentils (*Lens esculenta* M n c h.) and their Dynamics during Germination

Tokoferole w nasionach soczewicy (*Lens esculenta* M n c h.) i ich dynamika w procesie kiełkowania

Токоферолы в семенах чечевицы (*Lens esculenta* M n c h.) и их динамика в процессе прорастания

INTRODUCTION

Tocopherols are an object of general concern because of their biological activity in the organism as well as their antioxidant properties. The application of paper and thin layer chromatography permitted of closer examination of tocopherols in plants in the last years. In a number of cultivated plants, however, the pattern of tocopherols is not known yet. Similarly the changes of tocopherol content in germinating seeds and their distribution in the particular parts of seeds are known only in several plant species. The knowledge of the dynamics of tocopherols in the germinating seed will supply the data for closer determination of their role in growth and development of plants. The participation of tocopherols in those processes seems to be unquestionable.

Tocopherols in seeds of lentils, which is a food component in many countries, have not been fully examined so far. C h a t t o p a d h y a y and B a n e r j e e (3), Z a k h a r o v a (15), and N a z i r and M a g a r (11) took into account only the total content of tocopherols without considering the participation of individual tocopherols, the biological properties of which are not, as it is known, identical.

In this paper the authors attempted to find the pattern of tocopherols in seeds of lentils and to compare it with that occurring in seeds of other

dicotyledons for the deviations from the characteristic pattern both in mono- and dicotyledons are known (e.g. Lambertsen and coworkers 1967, Green 1958 and others). Moreover, the authors decided to examine the dynamics of vitamine-E-active compounds during germination of those seeds.

MATERIAL AND METHODS

Lentil seeds (*Lens esculenta* Mnch.) harvested in 1968, were sterilized with 0.1% mercuric chloride for 20 min and then washed a few times with sterile water. The sterile seeds were soaked in water for 24 hrs and then allowed to germinate on moist filter paper in Petri dishes, and stored dark in a thermostate at 24°C.

The samples were dissected into their component parts and analyzed on tocopherol content every day during 6 days of germination. Tocopherols were determined by the method of Green and coworkers (5).

Identification of the individual tocopherols was achieved by TLC-chromatography according to Pennock and coworkers (12). For the identification of the γ- and δ-tocopherol dianisidine coupling method (14) was also used. Ultraviolet absorption curves for individual tocopherols in ethanol were given after separation of tocopherols on secondary magnesium phosphate according to Bro-Rasmussen and Hjarde (2). Mono- and dimethyltocols were methylated to α-tocopherol by the method of Eggitt and Norris (4).

The results obtained (5 repetitions) were expressed in relation to seed number.

RESULTS AND DISCUSSION

In lentil seeds three tocopherols were found, which were indentified as α-, γ-, δ-homologues (fig. 1, 2).

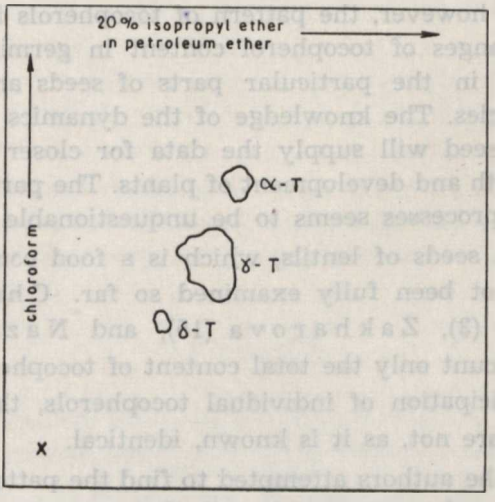

Fig. 1. Two dimensional thin-layer chromatogram of lentil seed tocopherols

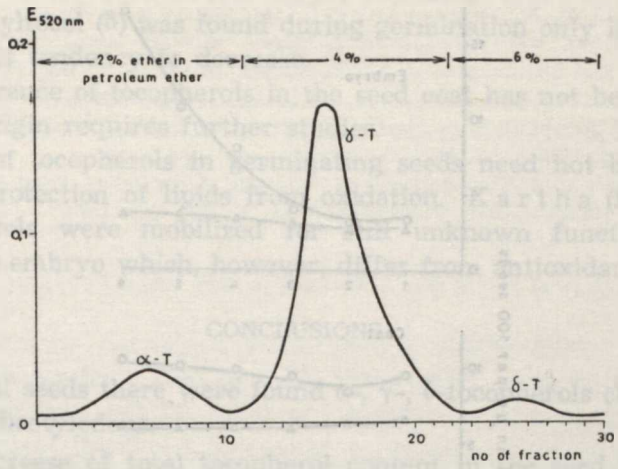

Fig. 2. Fractionation curve for unsaponifiable fraction of lentil oil (by Bro-Rasmussen and Hjarde's method)

The ultraviolet absorption spectrum in ethanol of isolated homologues corresponded with the values characteristic for those tocopherols. The value obtained for the total tocopherol content of whole seeds in 1 g of dry matter was higher than that given earlier by other authors (3, 11, 15, table 1).

Table 1. Tocopherol content in seeds of lentils

Tocopherols, μg/1 g of dry matter				Ratio α : γ : δ
α	γ	δ	total	
10.58	26.82	2.70	40.10	26.4 : 66.9 : 6.7

The pattern and ratio of individual tocopherols was characteristic for seeds of the majority of dicotyledons examined (6). The dominance of γ-tocopherol over the other tocopherols was distinct. After the second day of seed germination a slight decrease of the content of α-tocopherol was observed and then the total tocopherol content increased in terms of germination. This is due to intensive synthesis of α-tocopherol in the embryo (fig. 3). The figures show that, as H a l l and L a i d m a n (8) suppose, the increase of α-tocopherol content is not the only consequence of methylation of the non-α-tocopherols of resting seeds. The possibility of γ-tocopherol methylation found (1) with the use of methionine as the donor of CH_3 groups, as confirmed by T h r e l f a l l and coworkers (13), need not be the only possible mechanism of α-tocopherol synthesis during

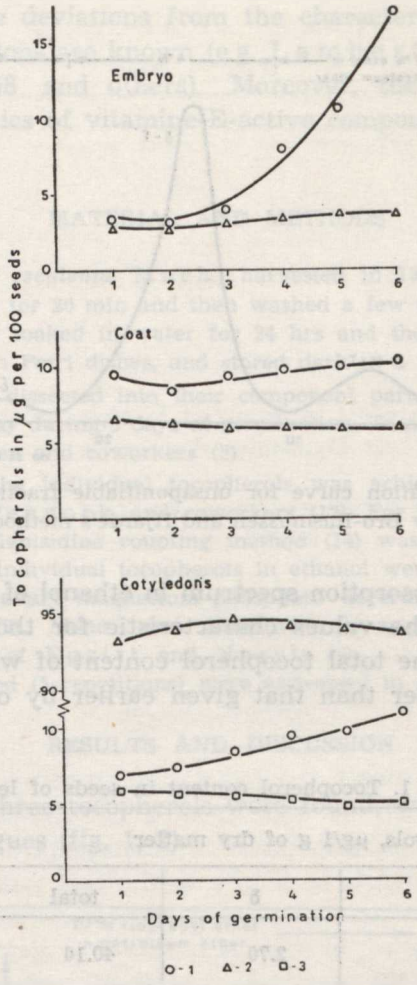

Fig. 3. Tocopherols in the component parts of the germinating seeds of lentils; 1 — α-tocopherol, 2 — γ-tocopherol, 3 — δ-tocopherol

seed germination. The increase of α-tocopherol in the embryo proves that it must be synthesized extra-plastidically, although in the green parts of plants the chloroplasts are a place of its synthesis.

On examining the component parts of seeds it can be stated that in the initial germination period the total of tocopherols increases due to intensive synthesis of α-tocopherol in the embryo.

A slight increase of α-tocopherol observed in cotyledons is connected with simultaneous decrease of γ- and δ-tocopherols. The increase of α-tocopherol in cotyledons is interesting with regard to virtually constant level of tocopherols in the endosperm of monocotyledons (8).

Monomethyltocol (δ) was found during germination only in cotyledons with a distinct tendency to decrease.

The occurrence of tocopherols in the seed coat has not been observed yet and its origin requires further studies.

The role of tocopherols in germinating seeds need not be restricted only to the protection of lipids from oxidation. Kartha (9) suggested that tocopherols were mobilized for still unknown functions during growth of the embryo which, however, differ from antioxidant functions.

CONCLUSIONS

1. In lentil seeds there were found α-, γ-, δ-tocopherols characteristic for seeds of dicotyledons.

2. The increase of total tocopherol content in the seed in terms of germination is mainly connected with intensive synthesis of α-tocopherol in the embryo.

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STRESZCZENIE

Badano występowanie tokoferoli w nasionach soczewicy oraz ich dynamikę w procesie kiełkowania. W tym celu wysterylizowane nasiona poddano kiełkowaniu w ciemności w temp. 24°C i analizowano oddzielnie zarodki, liścienie i łupiny na zawartość tokoferoli metodą Greena i współprac. (5).

Do identyfikacji tokoferoli posłużono się chromatografią ciekowarstwową wg Pennocka i współprac. (12), chromatografią kolumnową wg Bro-Rasmussena i Hjarde (2), reakcją z dwuazo-o-dwuanizydyną oraz widmem absorpcyjnym w UV.

Stwierdzono, nasiona soczewicy zawierają α-, γ-, δ-tokoferole, charakterystyczne dla nasion roślin dwuliściennych. Wzrost ogólnej zawartości tokoferoli w czasie kiełkowania nasion związany jest głównie z intensywną syntezą α-tokoferolu w zarodku.

РЕЗЮМЕ

Исследовали появление токоферола в семенах чечевицы, а также его динамику в процессе прорастания. Для этой цели стерилизованные семена проращивали в темноте при температуре 24°C и определяли отдельно в зародышах, семядолях и семенных оболочках содержание токоферола по методу Грина и соавторов (5).

Для идентификации токоферола применяли тонкослойную хроматографию по методу Пеннока и соавторов (12), колонную хроматографию по методу Бро-Расмуссена и Хйарде (2), реакцию с диазо-о-дианизидином и абсорбционный спектр в UV.

Определили, что семена чечевицы содержат α-, γ-, δ-токоферолы характерные для семян двудольных растений. Увеличение общего содержания токоферолов во время прорастания семян связано, главным образом, с интенсивным синтезом α-токоферолов в зародыше.

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