INSTITUTUM AGRONOMICUM "DR. PETRU GROZA" CLUJ-NAPOCA (ROMANIA)
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CHEMOTAXONOMICAL RESEARCHES IN HIGHER PLANTS

IX. CAROTENOID PIGMENTS IN SOME SPECIES OF THE GENUS CUCUMIS

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Within the framework of chemotaxonomical investigations in higher plants, we carried on further researches on carotenoid pigments in some yet unstudied species of the genus Cucumis.

The intense chemotaxonomical researches carried out in various countries these last years succeeded in determining some specific and characteristic carotenoid pigments of various groups of lower plants (fungi, bacteria, algae etc. (1-5,14), and also of some heterotrophic higher plants (6-9). Such determinations in autotrophic higher plants, however, are far more difficult as these species though pertaining to the same genus, at times they have quite different carotenoid pigments. Such are, for instance, the species of genera Astragalus, Mimulus, Piracanta, Ranunculus etc. (1,2,4,1o). Nevertheless also in autotrophic higher plants there exist species and even genera having carotenoids which might be considered as specific and characteristic for the whole taxonomic unit. Such is the case e.g. for the plants of the genus Aesculus which possess characteristic pigments at the pollen level: the ketohydroxycarotenoids with a pentacyclic structure of the capsanthin type (12). Alike in all mature fruits of the species of genus Rosa it was found as a characteristic pigment the rubixanthin (1,2,4), while in tomatoe fruits, the lycopene (1,2,4). In the flowers of various varieties of Eschscholtzia californica, the principal and characteristic pigment was eschscholtzxanthin (1,4,12) and in the flowers of Adonis aestivalis, astaxanthin (13).

All the plants of the Cucumis genus are characterized by their flowers and fruits of yellow and yellow-orange colour and by a red colour for the mature fruits of Cucumis citrullus, which points out a high content of carotenoid pigments. Up to the present there was

ascertained the presence of carotenoids in some species of this genus, especially in the mature fruits of Cucumis citrullus (15-17). Still, owing to the fact that the determination of carotenoid pigments was performed some decades ago, when the means for studying carotenoids were not developped yet, it was not possible to identify all the carotenoid pigments actually existing in these species.

The knowledge of carotenoid pigments in the petals and fruits of main species belonging to genus Cucumis cultivated in many countries, is a matter of necessity since they may be utilised rationally in both man and animal food, as sources of carotenoid pigments. We mention that no carotenotaxonomic studies on plants of genus Cucumis have been done so far.

This paper introduces the carotenoid pigments of petals and fruits of Cucumis sativus var. anguria (gherkin), Cucumis melo var. persica and Cucumis citrullus (fodder plant) and, it also points out the carotenoids of carotenotaxonomical importance to the species within this genus.

Material and method

The determination of carotenoid pigments was effected on fresh material (petals and fruits with their peel) harvested and analysed during the period July-September 1976. The plants were cultivated by the Irrigated Farming Department on an experimental field of the Didactic and Experimental Station of the Agronomy Institute of Cluj-Napoca.

The extraction of carotenoid pigments was carried out with a mixture of petroleum ether - acetone - methanol in ratio of 6:3:1. For the separation and purification of carotenoids it was mostly utilized the column chromatography using as adsorbant a mixture of magnesium oxide and fine sand in weight ratio of 2:1. The chromatographical columns were developped with a mixture of petroleum ether - benzene - acetone in ratio of 7:2:1. As to check the purity of pigments and for the mixture chromatograms it was also used the thin layer chromatography, using MgO and Silicagel as adsorbant and a mixture of ethanol - chloroform - petroleum ether in ratio of 2:2:6 as developing solvent. There were also performed chromatograms of mixture of natural carotenoids extracted from various plants.

The saponification of pigments was completed with ethanol solution of potassium hydroxide 10 % for 45 minutes at 40°C.

The identification and determination of carotenoid pigments was carried out by means of the very same methods already shown in previous

papers (10-12).

Results and discussion

The content of carotenoid pigments from plants petals is given in Tab. 1.

Tab. 1

Content of carotenoid pigments from petals of Cucumis sativus var. anguria, C. melo var. persica, C.citrullus expressed in $\mu g/g$ of fresh material

Carotenoid pigments	Cucumis sativus var.anguria (gherkin)	Cucumis melo var. persica	Cucumis citrullus (fodder plant)
Violaxanthin ester	2,34	1.48	0.75
Auroxanthin ester	12.83	15.22	12.58
Flavoxanthin ester	32.46	30.37	27.51
Zeaxanthin ester	15.49	12.75	8.23
Lutein ester	39.28	26.86	23.68
β-Cryptoxanthin ester	8.73	7.40	6.44
β-Carotene	20.16	13.41	14.73
α-Carotene	1.64	1.36	1.57
Total carotenoids	123.93	108.85	95.49

The carotenoid pigments are comprised in the table according to their order of adsorbtion on the adsorbant column. The most strongly retained on the column was violaxanthin ester while the least one was - caroten.

It was assessed from these determinations that the petals had a relatively high content of carotenoid pigments which contributed for the most in determining the colour of petals. In all three above mentionned species, the same carotenoids were identified with quantitative differences only and having no peculiar significance. These differences were mostly assignable to metabolic particularities of plants and to the physiological phase of the harvested material.

Among the carotenoid pigments identified in the pigments prevailed the xanthophylls which were found under the form of esters. Some of the xanthophylls had also epoxide groupings (flavoxanthin ester, auroxanthin ester, violaxanthin ester). From the hydrocarbon

carotenoids only α-and β-carotene were identified.

Among the characteristic carotenoid pigments having a carotenotaxonomical importance for this genus of plants should be mentionned flavoxanthin ester and auroxanthin ester. Both these carotenoids had an α-ionone structure, being mono- (flavoxanthin) and di- furanoids (auroxanthin) of lutein. From a quantitative point of view, the principal pigment was flavoxanthin ester, followed by lutein ester. As the content in carotenoid pigments of yellow colour (α-carotene, lutein ester, flavoxanthin ester, auroxanthin ester, violaxanthin ester) was higher than those with an orange colour (β-carotene, β -cryptoxanthin ester, zeaxanthin ester), the petals of flowers had a yellow colour tinged with slight orange hues.

The content of carotenoid pigments from mature fruits of tested plants is listed in Tab.2.

Tab.2

Content of Carotenoid Pigments from Mature Fruits of Cucumis sativus var. anguria (Gherkin), C. melo var. persica, (Fodder) C. citrullus, expressed in μg/g og fresh material

Carotenoid pigments	Cucumis sativus var.anguria	Cucumis melo var. persica	Cucumis citrullus
Neoxanthin	o.46	-	1.16
Violaxanthin	o.92	1.65	1.46
Flavoxanthin	1.7o	1.28	-
Zeaxanthin	1.96	2.61	2.27
Lutein	3.63	5.72	2.15
Lycopene	-	-	14.34
γ-Carotene	-	-	2.o8
β-Cryptoxanthin	o.85	1.39	1.41
β-Carotene	2.54	3.48	3.78
α-Carotene	-	1.12	o.82
Total carotenoids	12.o6	17.25	29.47

The carotenoid pigments of the fruits of these three plants showed both qualitative and quantitative differences. The fruits had a lower total content of carotenoids versus the petals. Thus, while in the petals the total contenf of carotenoids decreased from Cucumis

sativus var. anguria (gherkin) to fodder (Cucumis citrullus), the ratio was inverse in the fruits.

In the fruits it wasn't possible to assess characteristic carotenoid pigments having a chemotaxonomical importance for the species of genus Cucumis. In fruits, the epoxide carotenoids (neoxanthin, violaxanthin, flavoxanthin) had but a low content, while the xanthophylls were found in a free condition, unesterized. Amongst the common carotenoids which were found in all three plants, should be recalled β -carotene, β -cryptoxanthin, lutein, zeaxanthin and violaxanthin. The principal pigment in Cucumis sativus and C. melo was lutein, while in C. citrullus, lycopene. In the mature fruits of fodder C. citrullus, besides the carotenoid pigments already listed in the table, were also discovered - though in small amounts - some other carotenoids (neolycopene, pro-γ-carotene, neurosporene, phytofluene, phytoene). The red-orange hue mature fruis of fodder C.citrullus was due to the high content of lycopene and also to the presence of β -carotene, β -cryptoxanthin, γ -carotene and zeaxanthin.

From these experiments it can be concluded that the investigated plants have a higher total content of carotenoids in petals than in fruits. From petals, might be considered as characteristic pigments having a chemotaxonomical importance for the genus Cucumis the flavoxanthin ester and auroxanthin ester. There were no qualitative but only quantitative differences between the carotenoid pigments of petals from the searched plants, while, on the contrary, in the carotenoids from fruits, the diferences were both qualitative and quantitative.

Hence, as the petals and fruits of the species of genus Cucumis have a high content of carotenoids, they might be used as sources of carotenoid pigments in the diet of men and ratio of animals.

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DATA CONCERNING PHENOTYPICAL PLASTICITY (SIZE AND SHAPE OF LEAF) IN RANUNCULUS SECT. AURICOMUS

by A. T. Szabó

The section Auricomus Spach of genus Ranunculus L. have throughly been studied from taxonomic and cytological points of view (4, 5, 6, 8, 1o, 12, 14, 15, 17, 2o), but the problem of morphological modifications determined by ecological conditions it's still not entierely agreed upon (1, 2, 8, 9, 13, 11, 16, 17, 18).

While making the monographic review of the genus Ranunculus for the Romanian Flora (12) A. NYÁRÁDY has noticed microsystematically important morphological modifications within the same plants, during the subsequent years. Relying on these observations, transplant experiments have started with individual plants sampled from natural populations and cultivated on experimental plot under natural environmental conditions.

The observations have been done for the following purposes:

(1) the study of general characteristics of phenotypical plasticity with leaves from the plants belonging to genus Ranunculus sect. Auricomus under natural conditions, over a period of four years;

(2) the examination of microtaxonomic consequences of the modifications under surveillance with individual plants.

This paper is dealing only with the first item of general significance in order to demonstrate, that with this plants, adapted mainly to more humid forest and meadow habitats, certain morphological modifications take place during the individual life which - based probably on autoregulatory dependent morphogenesis (19) - may be correlated with certain climatic differences (temperature, soil humidity) in the subsequent years, with the age of plants and with heteroblastic development. Our observations may be put in connection with the temperature dependent leaf morphogenesis and phenotypical plasticity of the amphibious species of Ranunculus (2, 7).