Miscellaneous Note

Kiyonobu TOYODA*: A Complementary Note on the Chlorophylls in Some Spermatophytic Seeds

豊田清修*: 種子中のクロロフィルについての補遺

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In a previous paper¹⁾, the writer reported on the chlorophylls contained in some angiospermous seeds. Chlorophylls were further found in some spermatophytic seeds, which this paper refers to. The seeds investigated are as follows:

1. *Citrus erythrosa* Hort. ex Tanaka and *C. Kinokuni* Hort. ex Tanaka.
2. *Euonymus japonicus* Thunb.

In the fruit of *E. japonicus*, there exist 1-5 seeds which have red arils. The seed has white seed-coat and white albumen, and a green embryo located in the inner part of the seed was used.

3. *Rhaphiolepis umbellata* Makino and *R. umbellata* var. *Martensii* Makino.

The outer layer of the pericarp of *R. umbellata* is dark-purple and the inner layer is green. The seed-coat is brown and covers the light-greenish cotyledons. The pigments in the cotyledons were studied.

4. *Ginkgo biloba* L.

The seed of *Ginkgo* has a soft episperm and a hard endopleura, and the inner part is filled with yellowish-white albumen. A yellowish-green embryo located in the interior of albumen was used.

At first the green pigments were analyzed chromatographically in the same manner described in a previous paper¹⁾. Besides, lest the protochlorophyll should change into chlorophyll *a*²⁾, the experiments were carried out using the seeds after they had been soaked in boiling water for 5-10 minutes. In some cases, after the green parts of the seeds had been exposed to the sunlight for 1-2 hours, the pigments were studied to see the influence of light.

Table 1. The results of paper chromatography on the pigments in some seeds (at 16-25°).

		Rf value and color ^o			
1)	a) <i>Citrus erythrosa</i>	0.38 (YG)	0.80 (Y)		
	b) <i>C. Kinokuni</i>	0.35 (YG)	0.58 (Y)		
	Do. developed after exposing to the sunlight	0.27 (YG)	0.34 (BG)	0.52 (Y)	
2)	<i>Euonymus japonicus</i>	0.18 (YG)	0.21 (BG)	0.69 (Y)	
	Do. developed after soaking in boiling water	0.21 (YG)	0.80 (Y)		
3)	<i>Rhaphiolepis umbellata</i>	0.23 (YG)	0.29 (BG)	0.57 (Y)	0.97 (Y)
	Do. developed after soaking in boiling water	0.23 (G)	0.55 (Y)	0.73 (Y)	0.96 (Y)
	Do. developed after exposing to the sunlight	0.28 (YG)	0.35 (BG)	0.63 (Y)	
4)	<i>Ginkgo biloba</i>	0.52 (G)	0.80 (Y)		
	Do. developed after exposing to the sunlight	0.37 (YG)	0.44 (BG)	0.68 (Y)	0.95 (Y)

^o YG yellowish green; BG bluish green; Y. yellow.

* Fujisawa Higher School attached to Nihon University, Fujisawa, Kanagawa, Japan.

The paper chromatography was carried out using carbon tetrachloride and the results are shown in Table 1.

The chromatogram of the pigments of *R. umbellata* var. *Martensii* was similar to that of *R. umbellata*.

The absorption curves of the green pigments of those seeds dissolved in acetone were taken with the aid of a Beckman spectrophotometer, and the results are shown in Figs. 1 and 2.

From the results of these experiments, following conclusion may be drawn. The embryo of *Citrus erythrosa* has chlorophylls *a* and *b* judging from its absorption curves,

Fig. 1. Absorption curves of the seed pigments dissolved in acetone.

- A) Embryo of *Citrus erythrosa*. — shows the results after soaking in boiling water, --- after exposing to the sunlight.
 B) Embryo of *Euonymus japonica*. — after soaking in boiling water, --- in the dark.
 C) Cotyledon of *Raphiolepis umbellata*. — after soaking in boiling water, --- after exposing to the sunlight.

though it shows only the YG spot on the chromatogram. The embryo of *C. Kinokuni* also has chlorophyll *a* and a small amount of chlorophyll *b*.

The chromatography of the embryo of *Euonymus* was taken in two different ways; in the dark and after soaking in boiling water. A light BG spot which was found on the chromatogram of the untreated seed did not appear after soaking in boiling water. Little difference was, however, seen between the absorption curves of those samples. The embryo of *Euonymus japonicus* seems to contain chlorophylls *a* and *b*.

A light YG spot with a large Rf value was seen on the chromatogram of the pigment of the *Raphiolepis* cotyledons which had been extracted after soaking in boiling water. However, a similar YG spot did not appear for the seed exposed to the sunlight, and the exposure to light seemed to enlarge the area of the BG spot. It is, therefore, inferred that the YG spot having a large Rf value may be protochlorophyll. The illumination may have transformed protochlorophyll into chlorophyll *a*. The cotyledon of *Raphiolepis* seems to contain chlorophylls *a* and *b*, and protochlorophyll (Fig. 1-C).

The embryo of *Ginkgo* seems to have a large amount of carotenoids, protochlorophyll and a small amount of chlorophyll *a*. The results of chromatography indicate that protochlorophyll is changed into chlorophyll *a* by exposure to light.

The seed-coat of *Euonymus* is semitransparent and it may transmit a faint light. While, the seeds of *Raphiolepis* and *Ginkgo* seem to be non-transparent due to thick seed-coat. It is worth to notice that chlorophylls were identical for completely non-transparent samples.

Fig. 2. Absorption curves of the seed pigments dissolved in acetone.
— *Ginkgo biloba*, --- *Citrus Kinokuni*.

References

- 1) Toyoda, K., Bot. Mag. Tokyo **72**: 159 (1959).
- 2) Hejnowicz, Z., Physiolog. Plantarum **11**: 878 (1958).