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THEORETICAL AND APPLIED GENETICS

The Deficiency of Soluble Proteins and Plastid Ribosomal RNA in the Albino Pollen Plantlets of Rice

C.S. Sun, S.C. Wu, C.C. Wang and C.C. Chu
Institute of Botany, Academia Sinica, Peking (China)

Summary. The components of soluble protein and ribosomal RNA in green and albino pollen plantlets of rice were studied by means of polyacrylamide gel electrophoresis. The results were as follows: (1) Soluble protein: the soluble proteins in green pollen plantlets may be fractionated into 15 bands of varying intensities in which the highest content and the most prominent one is band 3 (fraction I protein). Band 3, however, is nearly absent in albino pollen plantlets. (2) Ribosomal RNA (rRNA): rRNA of high molecular weight in green pollen plantlets may be fractionated into 4 bands, i.e. 25S RNA and 18S RNA in cytoplasmic ribosomes, and 23S RNA and 16S RNA in plastid ribosomes. Little or no 23S RNA and 16S RNA, however, is found in albino pollen plantlets. Together with the evidence obtained by other workers, it is suggested that albino forms of pollen plantlets is caused by the impairment of DNA.

Key words: Albino pollen plantlet – Anther culture – Plastid ribosomal RNA – Fraction I protein

Introduction

Some of pollen plantlets obtained from anther culture are albinos, and these have been reported in tobacco (Devreux 1970) and *Datura* (Narayanaswamy and Chandy 1971). This kind of phenomenon, however, is of more frequent occurrence in cereals (Wang et al. 1977). Devreux (1970) suggested that pollen albinos are derived from generative cells on the supposition that they do not contain any plastids. This was soon disproved by later studies (Clapham 1973; Sun et al. 1974). In our studies on the relationship between the occurence of albino plantlets and certain other factors, such as components of culture medium, kinds and levels of hormones, sucrose concentration and cultural conditions (Wang et al. 1977), it was found that only high temperature significantly increased the frequency of albino plantlets and that other factors had no evident influence on it. Electron microscopic observations revealed that proplastids exist in the leaf cells of the albino rice plantlets but owing to the lack of ribosomes they could not develop further into normal chloroplasts with granum lamellae (Sun et al. 1974). All these studies mentioned above provided some data on the albino phenomenon, yet they did not give any clue to the mechanism of their formation. Recently we studied the soluble proteins and rRNA in albino pollen plantlets of rice using polyacrylamide gel electrophoresis. These data obtained may be useful in clarifying the nature of the albino further.

Material and Methods

Culture of Rice Pollen Plantlets

N_6 (Chu et al. 1975) or Miller's basic culture medium (Miller 1963) was used for culturing rice (*Oryza sativa* subsp. *Keng*) anthers. 2 mg/l 2,4-D was supplemented to induce the calli, and 0.2 mg/l Indole-acetic acid (IAA) was supplemented to induce the differentiation of plantlets. The sucrose concentration was 5%. Pollen plantlets were cultured under conditions of illumination of 2,000 lux, 26° C during the day, and in the dark, 18° C at night.

Extraction and Electrophoresis of Soluble Proteins

100 mg of rice albino pollen plantlet and green pollen plantlet leaves were separately collected, shred into pieces and put into glass homogenizers. 1 ml of buffer (0.1 M Tris, 12.5% sucrose, 0.1% cysteine, 1.0% ascorbic acid, pH 8.0) (Rommann et al. 1971) was added to each sample group. These were ground in an ice bath and extracted at 5° C for 4 hr. Homogenates were then centrifuged at 12,000 × g for 15 min (0-4° C). Supernatants were then collected in order to fractionate electrophoretically the soluble proteins.

Davis' (1964) discontinous system was used for polyacrylamide gel electrophoresis. The concentration of the separation gel was

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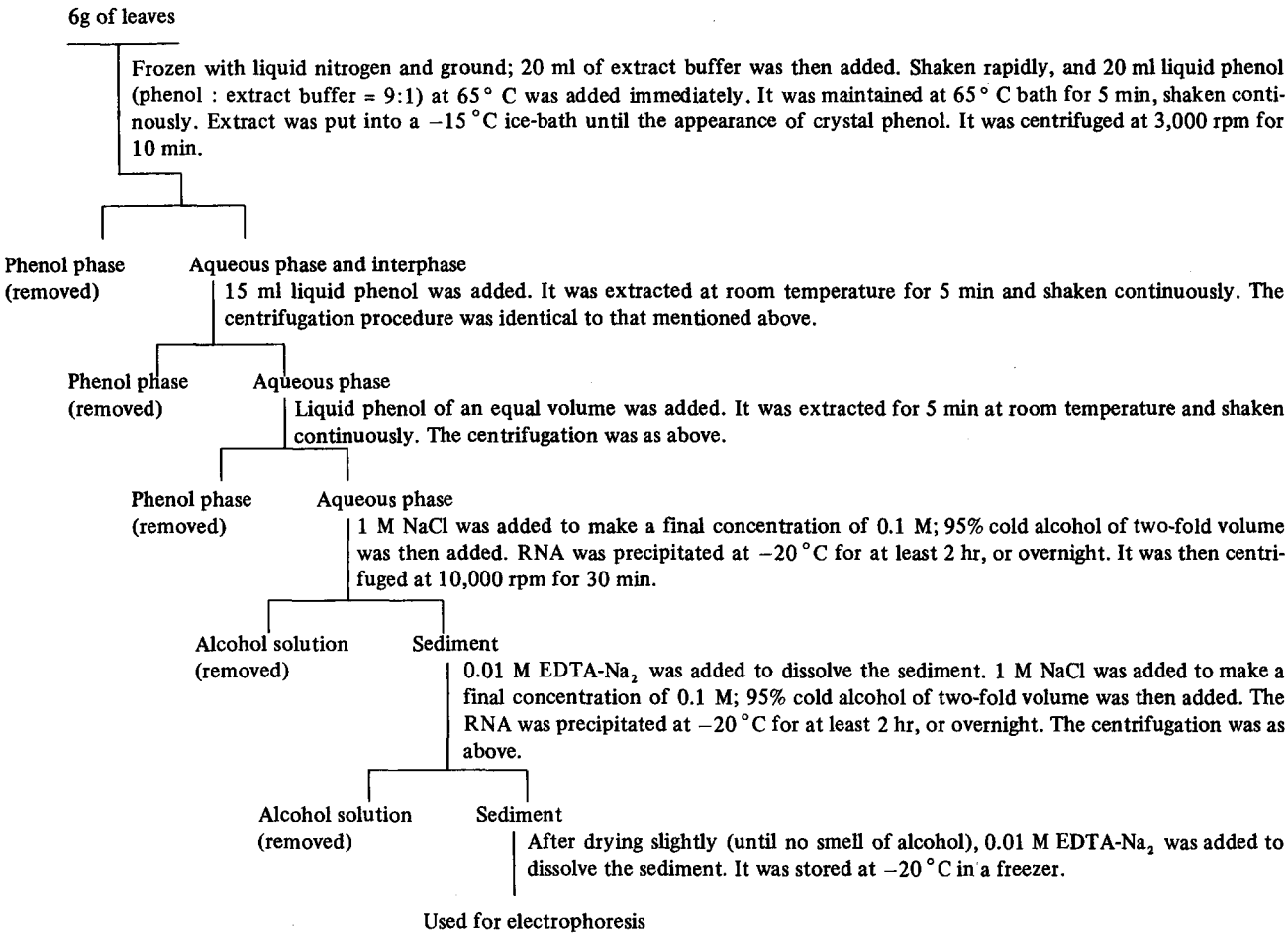
7% (pH 8.9) and that of the spacer gel was 2.5% (pH 6.7). The pH of the electrode buffer was 8.3. A 5 μl sample supplemented with 8% sucrose was added to each tube. Electrophoresis was carried out in an ice-bath. A constant current of 2.5 mA was applied for 2 hr. The gels were then fixed in 12.5% trichloroacetic acid (TCA) for 30 min and subsequently stained in a 0.05% Coomassie blue R-12.5% TCA solution for 1-2 hr. After staining, gel columns were destained in 12.5% TCA and scanned with a densitometer.

Extraction and Electrophoresis of Ribosomal RNA (rRNA)

A somewhat modified hot phenol method (Scherrer 1969) was used to extract the rRNA of albino and green pollen plantlets of rice. The extract buffer consisted of 0.05 M Tris, 0.01 M EDTA-Na_2 and 1% SDS, pH 8.8. The fractionating procedure of rRNA was carried out as follows:



2.5% gels were prepared using Cyanogum 41 (acrylamide : bis-acrylamide = 20 : 1). The concentration of TEMED and ammonium persulfate in the gel was 0.1%, respectively. The buffer contained 0.01 M Tris, 0.02 M sodium acetate and 0.001 M EDTA-Na_2; pH was adjusted to 7.4 using acetic acid. Glass tubes with a length of 95 mm and an inside diameter of 0.5 cm were used for electrophoresis. Each tube was pre-electrophoresed at 5 mA at room temperature for 40 min. The concentration of rRNA in each sample (supplemented with 8% sucrose) was 1-2 mg/ml. 15 μl of sample was added to each tube. Electrophoresis was carried out in an ice-bath for an hr, 5 mA and 25 V for each tube. The gels were then removed from the tubes, stained with 0.1% ethidium bromide (fluorochrome) and subjected to a preliminary examination under U.V. After fixing with 1 M acetic acid for 20 min, staining with 0.2% methylene blue for 1-16 hr and rinsing in distilled water to destain, the destained gels were scanned at 258 nm with a Joyce Loebl 'Chromoscan'.

Results

Soluble Proteins

The electrophoretic patterns of soluble proteins from leaf cells of albino and green pollen plantlets stained with Coomassie blue R are shown in Figure 1. Semi-quantitative measurements of the components of soluble proteins by means of a densitometer are shown in Figure 2. It was shown in these Figures that the soluble proteins of normal green pollen plantlets were able to be fractionated into 15 bands of different intensities. Among these, the most prominent one, with the highest concentration, was band 3, which had a larger molecular weight and comprised the

largest part of the soluble proteins in the cells. It was easily determined as a fraction I protein (Rommann et al. 1971). Band 15, with a smaller molecular weight, and band 10, with an average molecular weight, are the secondary ones. Bands other than these had lower contents and were unimportant. The differences between the components and contents of the soluble proteins in green plantlets and those in albino plantlets was obvious. Among the main proteins in green plantlets, band 3 (fraction I protein) was nearly always absent in albino plantlets. In front of band 4 there was a band 3′ which could be said corresponded to the band 3 of green plantlets but its occurence was rare and it seemed that the molecular weight was different from the band 3 of green plantlets. In addition, band 11, one of the less important proteins was commonly absent in albino plantlets. In Figure 2, the optical density curve shows that apart from a lack of the main protein band 3, the contents of the other two main protein bands, 15 and 10, in the albino plantlets corresponded to those in the green ones. The relative contents of proteins of minor importance, however, were a little higher than those of green ones. The results mentioned above demonstrate that albino pollen plantlets of rice were nearly deficient in the protein synthesis of chloroplasts, i.e. they lost the synthetic capacity for fraction I protein. It is well known that if there is no fraction I protein the dark reaction of photosynthesis cannot be carried out and CO_2 cannot come into the Calvin cycle. The following experiment was done in order to examine fraction I protein in albino plantlets.

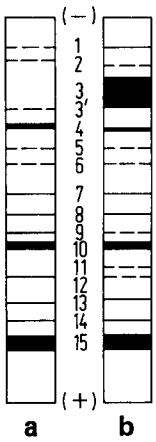


Fig. 1a and b. The patterns of soluble proteins from pollen plantlets of rice after fractionation using polyacrylamide gel electrophoresis and staining using Coomassie blue R. **a** albino plantlets, **b** green plantlets

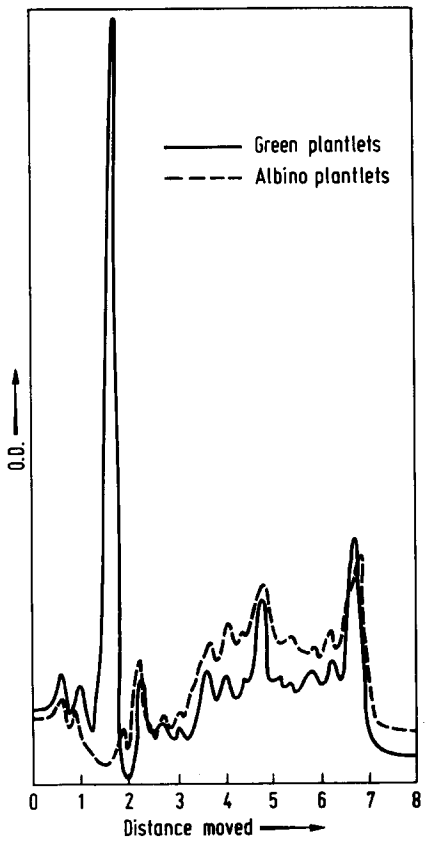


Fig. 2. The optical density curve and migration rate of soluble proteins from pollen plantlets of rice after fractionation using polyacrylamide gel electrophoresis

Albino and green plantlets were incubated together in a glass cabinet full of $^{14}CO_2$. After 2 hrs illumination the plantlets were separately homogenized and then centrifuged. Each supernatant was collected in order to estimate the capacity of CO_2 absorption by means of a liquid scintillation counter. The experimental results are shown in Table 1. These data reveal that albino plantlets almost failed to perform CO_2-fixation. These results are consistent with those obtained with the electrophoresis of the proteins, i.e. fraction I protein was deficient in albino plantlets.

Similar results were obtained from the albino plantlets of wheat (*Triticum vulgare*) and Triticale. It was elucidated that the block in protein synthesis may be the common phenomenon in albino plantlets of cereals.

Table 1. The capacity of $^{14}CO_2$ fixation in albino and green plantlets (pulse/min.)

	Illumination		Dark (control)	
	Green	Albino	Green	Albino
Estimation	15074	681	949	883
Background	118	113	128	100

Ribosomal RNA (rRNA)

After electrophoresis, rRNA of the leaf cells of normal green pollen plantlets could be fractionated into 4 high

molecular weight bands: the 25S and 18S RNA of the cytoplasmic ribosomes, and the 23S and 16S RNA of the plastid ribosomes (Fig. 3b; 4b). The albino pollen plantlets, however, differed greatly from green ones in the rRNA electrophoresis patterns: 25S and 16S RNA of cytoplasmic ribosome were normal whereas 23S and 16S RNA were absent or rare (Fig. 3a; 4a). The deficiency of plastid rRNA was consistent with the electron microscopic observations that no ribosome could be found in the albino plastids (Sun et al. 1974). The capacity of protein synthesis, thereafter, was decreased considerably.

Apart from high molecular weight rRNA, there were other RNA with lower molecular weight in green or albino pollen plantlets. These may be the 5S RNA and 4S RNA.

23S RNA in the plastids was characterized by its unstability. Degradation took place easily during the procedures of fractionation and extraction (Loening and Ingle 1967). Their contents, therefore, were much lower after electrophoresis. Such instability was commonly noted during the experiments.

The case described above, the deficiency of plastid RNA in albino plantlets derived from anther culture, is similar to that in *Pelargonium zonale* albino mutants found by Börner et al. (1973).

Discussion

It is known that the assemblage of the photosynthetic unit of the thylakoid membrane requires the plastid ribosome to participate and that the synthesis of the large subunit in fraction I proteins is carried out on the plastid ribosome (Kung 1977). Therefore, the deficiency of the lamellae structure which exists in normal chloroplasts (Sun et al. 1974) and the fraction I protein in albino rice pollen plantlets have a direct relationship with the deficiency of plastid ribosome and its' RNA.

Chloroplast rRNA is coded by chloroplast DNA. This has been proven by means of DNA-RNA molecular hybridization (Thomas and Tewari 1974) and autoradiography (Gibbs 1967). Therefore, the deficiency of plastid 23S and 16S RNA in albino pollen plantlets suggests that certain impairments may take place in the plastid genome. Such impairments are responsible for the failure of synthesizing the plastid rRNA and subsequently the obstruction of synthesizing the large subunit of fraction I protein and the protein of the chloroplast lamella. Experiments demonstrated, however, that some proteins of plastid ribosome (Bourque and Wildman 1973) and small subunits of fraction I protein (Criddle et al. 1970) are coded by nucleus DNA. The deficiency of plastid ribosome and fraction I protein, therefore, are caused similarly by the impairments of nucleus DNA.

It has been reported that the deficiency of plastid rRNA in the albino variety of some plants is really caused by a variation of plastid DNA (Börner et al. 1972). The variation of nucleus DNA, however, is also responsible for the same deficiency (Sprey 1971, Tôyama 1972).

According to the data cited above and the results in

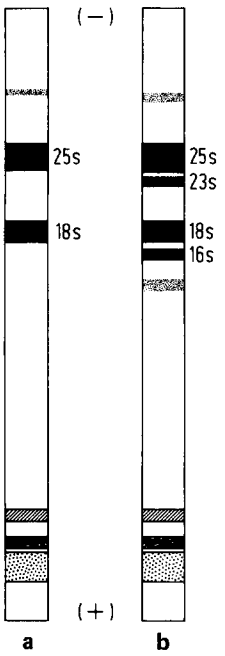


Fig. 3a and b. The patterns of rRNA from pollen plantlets of rice after fractionation using polyacrylamide gel electrophoresis. a albino plantlets, b green plantlets

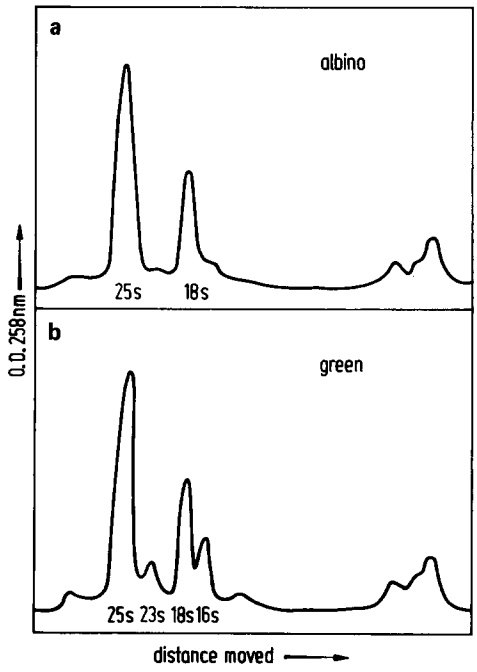


Fig. 4a and b. U.V. scanning patterns of rRNA from pollen plantlets of rice after fractionation using polyacrylamide gel electrophoresis. a albino plantlets, b green plantlets

the present report, it may be suggested that the basic cause of albinos in pollen plantlets is derived from the impaired DNA.

It must be demonstrated further whether the impaired DNA occurs in plastids or nuclei, or in both of them.

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Literature

Börner, Th.; Herrmann, F.; Hagemann, R.: Plastid ribosome deficient mutants of *Pelargonium zonale*. FEBS Letters **37**, 117-119 (1973)

Börner, Th.; Knoth, R.; Herrmann, F.; Hagemann, R.: Structure and function of the genetic information in plastids, V. The absence of ribosomal RNA from the plastids of the plastom-mutant 'Mrs. Parker' of *Pelargonium zonale*. Theor. Appl. Genet. **42**, 3-11 (1972)

Bourque, D.P.; Wildman, S.G.: Evidence that nuclear genes code for several chloroplast ribosomal proteins. Biochem. Biophys. Res. Commun. **50**, 532-537 (1973)

Chu, C.C.; Wang, C.C.; Sun, C.S.; Hsü, C.; Yin, K.C.; Chu, C.Y.; Bi. F.Y.: Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sin. **18**, 659-668 (1975)

Clapham, D.: Haploid *Hordeum* plants from anthers in vitro. Z. Pflanzenzücht. **69**, 142-155 (1973)

Criddle, R.S.; Dau, B.; Kleinkopf, G.E.; Huffaker, R.C.: Differential synthesis of ribulose diphosphate carboxylase subunits. Biochem. Biophys. Res. Commun. **41**, 621-627 (1970)

Davis, B.J.: Disc electrophoresis. II. Method and application to human serum proteins. Ann. N Y Acad. Sci. **121**, 404-427 (1964)

Devreux, M.: New possibilities for the in vitro cultivation of plant cells. Sci. Tech. Rev. Euro. Comm. **9**, 105-110 (1970)

Gibbs, S.P.: Synthesis of chloroplast RNA at the site of chloroplast DNA. Biochem. Biophys. Res. Commun. **28**, 653-657 (1967)

Kung, S.D.: Expression of chloroplast genomes in higher plants. Ann. Rev. Plant Physiol. **28**, 401-437 (1977)

Loening, U.E.; Ingle, J.: Diversity of RNA components in green plant tissues. Nature **215**, 363-367 (1967)

Miller, C.O.: Kinetin and kinetin-like compounds. In: Moderne Methoden der Pflanzenanalyse. Vol. 6. (eds. Linskens, H.F.; Tracey, M.V.), pp. 194-202. Berlin-Heidelberg-New York: Springer 1963

Narayanaswamy, S.; Chandy, L.P.: In vitro induction of haploid, diploid and triploid androgenic embryoids and plantlets in *Datura metel* L. Ann. Bot. **35**, 535-542 (1971)

Rommann, L.M.; Gerloff, E.D.; Moore, R.A. Soluble proteins of alfalfa, white clover, and birdsfoot trefoil. Crop Sci. **11**, 792-795 (1971)

Scherrer, K.: Isolation and sucrose gradient analysis of RNA. In: Fundamental techniques in virology (eds. Habel, K.; Salzman, N.P.), pp. 413-432. London-New York: Academic Press 1969

Sprey, B.: Ribosomal RNA and thylakoid membranes in plastids of chlorophyll-deficient mutants of barley. Z. Pflanzenphysiol. **67**, 223-243 (1971)

Sun, C.S.; Wang, C.C.; Chu, C.C.: The ultrastructure of plastids in the albino pollen-plants of rice. Sci. Sin. **17**, 793-802 (1974)

Thomas, J.R.; Tewari, K.K.: Conservation of 70S ribosomal RNA genes in the chloroplast DNAs of higher plants. Proc. Natl. Acad. Sci. (USA) **71**, 3147-3151 (1974)

Toyama, S.: Electron microscope studies on the morphogenesis of plastids. VI. Plastid development and fine structure in variegated leaves of tomato. Bot. Mag. (Tokyo) **85**, 1-10 (1972)

Wang, C.C.; Sun, C.S.; Chu, C.C.: Effects of culture factors in vitro on the production of albino pollen-plantlets of rice. Acta Bot. Sin. **19**, 190-199 (1977)

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Sun Ching-san
Wu Shih-chun
Wang Ching-chü
Chu Chih-ching
Institute of Botany
Academia Sinica
Peking (China)