SHORT COMMUNICATIONS

Plant & Cell Physiol., 8, 749-751 (1967)

BIOCHEMICAL STUDIES ON ETHYL METHANE SULFONATE-INDUCED CHLORENA MUTANT OF *TRITICUM DICOCCUM* VAR. KHAPLI

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(Received February 21, 1967)

SWAMINATHAN et al. (*1*) reported the occurrence of EMS (ethyl methane sulfonate)-induced chlorophyll deficient mutant of *Triticum dicoccum* var. Khapli. In view of the reports in literature (*2-4*) attributing the changes in morphological and physiological charactersitics to the lack of one of the essential elements in the mutant, and describing the reversal, in some cases, caused by various external treatments, a number of experiments were performed in our laboratory, using the chlorena mutants. These are: (A) analysis of various elements in the leaves, viz. calcium, potassium, magnesium, phosphorus and iron, (B) foliar sprays of urea, copper, zinc, manganese, iron, boron and molybdenum, (C) placing the seedlings under long and short day conditions, and (D) placing the seedlings at different temperatures.

It was observed that there was no difference in concentrations of the elements analysed, except for phosphorus. In 30 day-old seedlings, its value is about 0.3% (on dry weight basis) while in normal plants values of 0.18% were observed. External treatments described above did not result in any recovery of the chlorophyll content of the leaves and the plants remained yellow in appearance.

In this communication, results of experiments on determinations of chlorophyll, carotenoids and total nitrogen in the leaves and various fractions of phosphorus in the chloroplasts of mutant and normal plants are reported.

Normal plants and M_2 generation of chlorena mutant (*5*) were raised in the field during 1965-66 and leaf samples of each were collected at 30 and 75 day stage. 30 Grams of leaves were cut into small pieces and homogenized in a Waring blendor with 100 ml portions of 0.3 M sodium chloride solution containing 0.02 M Tris buffer, pH 8.3 and 0.01 M sodium ascorbate. The homogenate was passed through muslin cloth and centrifuged at $40 \times g$ to remove the cell wall material. The superenatant was centrifuged at $1500 \times g$

for 10 min. The residue was washed twice with the sodium chloride solution and centrifuged (*6*).

Various fractions of phosphorus in the chloroplast pellet were obtained by the method of SMILLIE and KROTKOV (*7*): *Methanol-P fraction*-extracted three times with 15 ml each of cold methanol containing 0.05 M formic acid. The fine suspension in each case is allowed to stand for a few minutes before centrfugation. *Acid-P fraction*—The residue thus obtained was extracted with 10 ml of 5 per cent trichloroacetic acid at 4°. Centifuged. *Lipid-P fraction*—The residue, after the isolation of the above fraction, was extracted with 15 ml lots of (i) 95 per cent ethanol (ii) ethanol: ether (1:1) and (iii) ether. For each extraction, the solvent was kept boiling for 20 sec. *Nucleic acid-P fraction*—The insoluble residue, after the above extraction, was dried, powdered and then extracted with 5 per cent perchloric acid at 90° for 15 min. centrifuged.

Phosphorus was estimated by following the method of KING (*8*). Extraction and estimation of chlorophylls and carotenoids were made according to the technique adopted by FRANK and KENNY (9). Total nitrogen was determined by KJELDAHL's method (*10*).

The contents of chlorophyll and carotenoids in the mutant were lower than in the normal plants. In the mutant, the ratio of the two pigments

TABLE I

Chlorophyll and carotenoid contents in normal and chlorena mutant plants (Triticum dicoccum *var. Khapli*)

Age (days)	Strains	Chlorophyll OD/mg, Fr. Wt. 660 mμ	Carotenoids OD/mg, Fr. Wt. 440 mμ	Chlorophyll/ Carotenoids OD/OD
		(10^{-4})	(10^{-4})	
30	Normal	14.5	44.5	1:3.0
	Mutant	3.2	17.8	1:5.6
75	Normal	12.5	38.7	1:3.1
	Mutant	5.0	35.3	1:7.1

TABLE II

Analysis of chloroplast fractions of normal and mutant plants (Triticum dicoccum *var. Khapli*)

Age (days)	Strains	Dry wt. (%)	Chloroplast material						
			Dry wt. μg	Total N μg	Alcohol-P μg	Acid-P μg	Lipid-P μg	Nucleic acid-P μg	Total P μg
30	Normal	16.3	431	244.2	6.93	7.30	2.83	6.27	23.33
	Mutant	12.5	315	191.5	3.50	15.50	4.70	5.88	29.58
75	Normal	20.1	628	261.5	8.02	11.44	2.89	8.66	31.01
	Mutant	19.2	443	206.3	4.88	14.89	6.97	8.22	34.96

varied depending on the age of the plant. As compared with the normal chlorophyll: carotenoids ratio (OD/OD) of 1:3, the mutant showed ratios of 1:5.6 and 1:7.1 in 30 and 75 day-old seedlings, respectively (Table I). The dry weight (%) of the leaves in the mutant was less than that in the normal plant. The difference, however, diminished with the growth of the plants (see Table II), reflecting the recovery in chlorophyll content and photosynthetic activity in the older seedlings (Table I).

The results of analysis of various fractions of chloroplast material are summarized in Table II. A marked difference was observed only in acid soluble P, the mutant chloroplast showing two times as high a content of this fraction of P as compared with that in normal ones. Also in this case, the difference diminished with age of the plants.

More detailed characterization of the non-colored material in the mutant and normal chloroplasts of *Triticum dicoccum* needs further investigation of the individual components of the plastids.

The authors are very grateful to Dr. M. S. SWAMINATHAN, Director, Indian Agricultural Research Institute, New Delhi, for kindly providing the material, for his encouragement in this work and for his valuable advice in preparation of the manuscript.

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