Iowa Science Teachers Journal

Volume 7 | Number 3 Article 8

1970

Identifying and Growing Biochemical Mutants of *Arabidopsis thaliana*

Sujit K. Dhar
Ellsworth College

Identifying and Growing Biochemical Mutants of *Arabidopsis thaliana*

SUJIT K. DHAR
Ellsworth College
Iowa Falls, Iowa

Beadle and Tatum (1945) irradiated bread mold fungus, *Neurospora* spores, with X-ray and isolated biochemical mutants by their inability to grow in minimal medium. They identified the defective pathways by finding a particular amino acid, vitamin, or nucleotide which, being added to the medium, remedied the defect. Biochemical mutants have since been found in other microorganisms such as *E. coli* (Horowitz and Leupold, 1951), phage (Edgar and Lielausis, 1964), and tobacco mosiac virus (Jocklusch, 1966). Since higher plants are diploid, the isolation of mutants is laborious, their large-scale aseptic culture is seldom practicable, and their average life cycle is generally very long. For these reasons, only a few nutritional mutants of higher plants have been reported (Langridge, 1955; Smith and Castle, 1960; Redei, 1962; and Walles, 1963.

The isolation of biochemical mutants in higher plants is important for two reasons: (1) furthering better understanding of the genetic control of metabolic pathways, and (2) providing better tools for physiological genetic studies. The application of chemical techniques to a higher plant like *Arabidopsis thaliana,* which is easy to grow in an aseptic culture (Dhar, 1969), has a short life cycle, and is naturally self-pollinated, may open new approaches to the study of gene function and differentiation.

MATERIALS AND METHODS

Arabidopsis thaliana was used in this investigation. Seeds were soaked in distilled water for 20 hours before X-ray treatment of 20 Kr. Another batch of seeds was soaked in 0.005% streptomycin (dihydrostreptomycin sulfate, Nutritional Biochemical Corporation) for the same amount of time before irradiation. The first generation plants (X_1) were raised in steam-sterilized soil and kept in a controlled environment greenhouse under continuous illumination. For isolation of mutants in the second generation (X_2) bulk progeny, seeds were collected from individual X_1 rows. Minimal medium consisted of mineral elements as described previously (Dhar, 1969). This medium was solidified with 0.5% agar. The seeds were sterilized in 1:1 H_2O_2 and ethyl alcohol for 10 minutes. Fifteen ml. of this medium was dispensed in petri dishes and these were then autoclaved for 10

minutes at 15 lbs. pressure. Three hundred seeds were planted in each petri dish with the help of a platinum loop. In an effort to pick out the nutritional mutants, plants that did not grow beyond the first two-leaf stage in the minimal medium were transferred after two weeks to 18X150 mm. test tubes with 5 mm. of minimal medium plus a nutritional supplement (Table 1). Since there was no way of telling

Table 1

To isolate biochemical mutants, the following supplements were added to the minimal medium

(1) minimal medium plus vitamin solution as in table 3

(2) minimal medium plus amino acid solution as in table 4

(3) minimal medium plus 4 mgm/L of yeast extract

(4) minimal medium plus gibberellic acid 50 mgm/L

(5) minimal medium plus 5-20 mgm/L casein hydrolysate

from the phenotype what the requirement was, it was decided that adding just one nutrient at a time to the minimal medium was rather inefficient. It was decided to make up a more complete medium since the chances of the mutant finding its particular nutrient would be greater. When a mutant grew, all the seeds from this X_2 plant were collected. To identify the specific nutritional requirement, all the seeds from this X_2 plant were then grown in a minimal medium plus one specific nutrient (Table 2). Since the X_2 plants were homozygous recessive for this particular trait, the X_3 and subsequent generations would breed true for this particular trait.

RESULT

Second Generation plants (X_2)

In all the chlorophyll mutants obtained by irradiation after soaking in distilled water, no nutritional requirements for any were found. However, one mutant obtained from the seeds irradiated after soaking in SM completed growth in a medium with the vitamin solution (Table 3). This plant (X2154SM) flowered in three weeks after transfer to the medium plus vitamin solution (Table 3). This plant formed 50 seeds in five pods. (When seeds of this mutant plant were grown in minimal medium plus just one vitamin (Table 4), only those plants that were supplied with nicotinic acid completed their life cycle.

DISCUSSION

Mutant X2154SM has a genetic block in the nicotinic acid synthesis pathway. When this vitamin was added to the plant, it showed normal growth. However, the stem of this plant measured 9 cm. as against 10 cm. in wild-type plants. The difference in measurement seems to be due to the fact that this plant grew only about 0.2 cm. in two weeks in the minimal medium whereas a wild-type plant would grow about 2.0 cm. When this mutant plant was transferred to a medium with the vitamin solution, it showed very rapid growth, and flowered in three weeks. However, seeds from this X_2 mutant plant when grown in a medium with nicotinic acid showed identical growth with wild type plants.

Table 2
Growth of third generation plants. Only one of the following vitamins was added to a minimal medium.

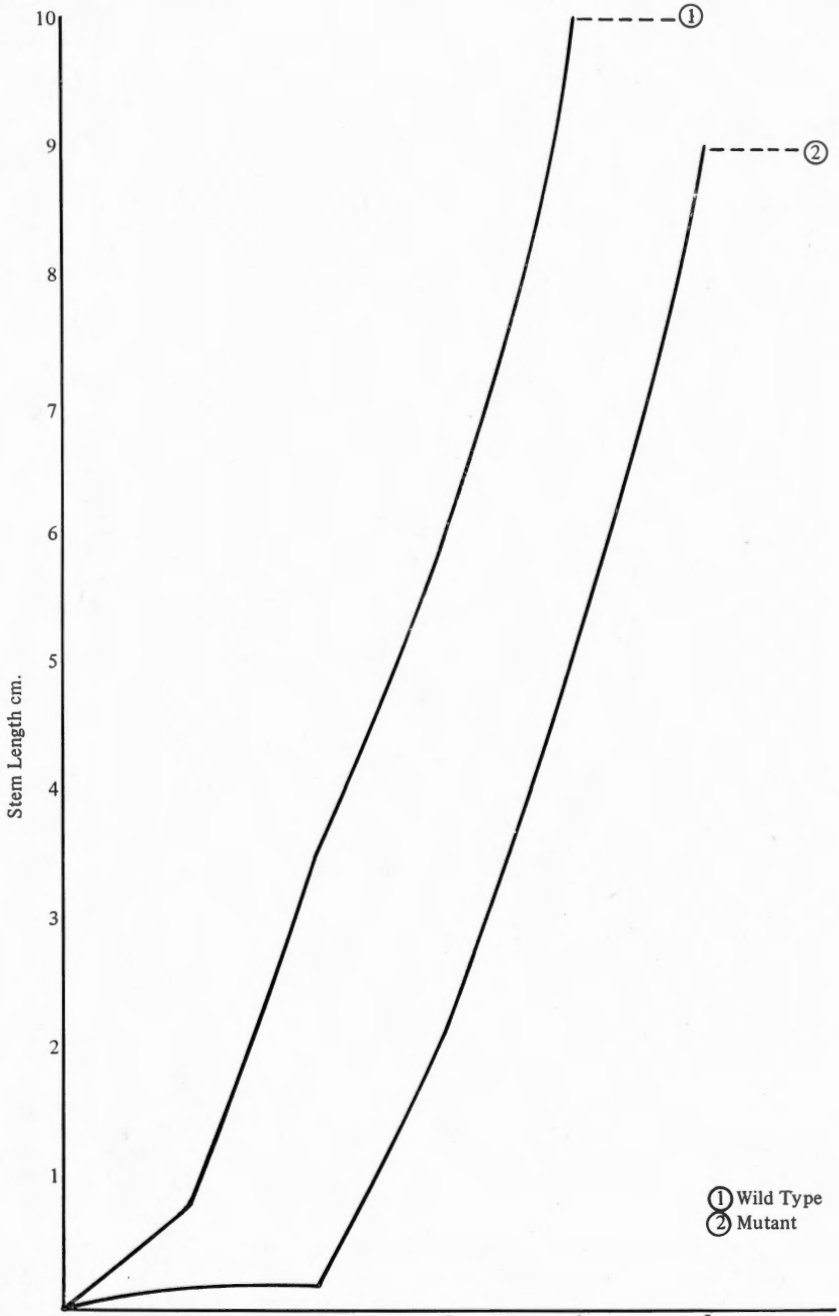
Minimal medium plus	*Amount mgm/L*	*Condition of plant*
nicotinic acid	0.2	plant was green. Stem 10 cm. long. Flowered in 30 days.
thiamine hydrochloride	0.04	chlorotic, first 2 leaves formed, dies in 3 weeks
riboflavin	0.04	chlorotic, first pair of leaves formed, dies in 3 weeks
pantothenic acid	0.04	light green, dies in a week
pyridoxine hydrochloride	0.04	dies in 2 weeks
biotin	0.002	albino plant, dies in a week
folic acid	0.04	chlorotic plant, dies in 3 weeks
inositol	8.0	light green, dies in 2 weeks
ascorbic acid	0.8	chlorotic, first pair of leaves formed, dies in 3 weeks

Table 3
Vitamin Solution

Vitamin	*Amount supplied to give final concentration in mgm/L of each*
thiamine hydrochloride	0.04
riboflavin	0.04
nicotinic acid	0.2
pantothenic acid	0.04
pyridoxine hydrochloride	0.04
biotin	0.002
folic acid	0.04
inositol	8.0
ascorbic acid	0.8

Table 4
Amino Acid Solution

Amino acid	*Amount supplied to give final concentration in mgm/L of each*
glycine	2.0
alanine	1.5
serine	0.5
threonine	1.0
valine	1.0
leucine	1.0
phenylalanine	2.0
tyrosine	2.0
tryptophan	2.0
cystine	2.5
cysteine	2.5
methionine	4.0
histidine	1.5
aspartic acid	4.0
glutamic acid	4.5
arginine	1.0



Growth of Mutant and Wild-Type Plant

Figure 1

Table 5
Growth of third generation plants (X_3)
in medium with nicotinic acid

Type of plant	*Minimal medium plus*	*Days to flower*	*Average stem length, cm.*	*Average root length, cm.*
mutant	0.2 mgm/L nicotinic acid	28	10	3.3
wild type	nothing	28	10	3.3
wild type	0.2 mgm/L nicotinic acid	28	10	3.0

From 250 chlorophyll mutants obtained by irradiating seeds after they were soaked in distilled water, no biochemical mutant was obtained. Out of 151 chlorophyll mutants obtained after the seeds were soaked in SM, only one nutritional mutant which required nicotinic acid to complete its life cycle was obtained. It seems that though SM reduces the percentage of chlorophyll mutants, it gives a different spectrum of mutation. It may be pointed out here that every chlorophyll mutant may not be a biochemical mutant. Many genes control the synthesis of plastid pigments, and also many genes control the development of chloroplast. Since any disturbance in any stage leading to the synthesis of the pigment or plastid will lead to a chlorophyll mutant, it is not surprising that the chlorophyll mutants arise at a rather high rate. However, many of the biochemical mutants will also be chlorophyll mutants, since the pathways are probably in some way connected. Langridge (1955) and Redei's (1962) thiamine mutant in *Arabidopsis*, Walles' (1963) leucine mutant in barley, and the nicotinic acid mutant in this investigation are all chlorophyll mutants also.

LITERATURE CITED

1. Beadle, W., and E. L. Tatum. 1945. *Neurospora.* II. Methods of producing and detecting mutations concerned with nutritional requirements. Am.J.Bot. 32:678-686.

2. Dhar, S. K. 1967. Studies on effects of streptomycin in *Arabidopsis thaliana.* Proc.Iowa Acad.Sc. 74:28-32.

3. _____ 1969. Aseptic culture of *Arabidopsis thaliana:* a flowering plant that can be handled like a microorganism. Iowa Sc.Teach.J. 6(4):14-15.

4. Edgar, R. S., and I. Lielausis. 1964. Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. Genetics 49:649-662.

5. Horowitz, N. H., and U. Leupold. 1951. Some recent studies bearing on the one-gene one-enzyme hypothesis. Cold Springs Harb.Symp. 16:65-74.

6. Jocklusch, H. 1966. Relations between temperature sensitivity, amino acid replacements, and quaternary structure of mutant proteins. Biochem.Biophys.Res.Commun. 24:577-583.

7. Langridge, J. 1955. Biochemical mutations in the crucifer *Arabidopsis thaliana*(L.). Heynh.Nature 176:260-261.

8. Redei, G. P. 1962. Genetic block of "Vitamin thiazole" synthesis in Arabidopsis. Genetics 47:979(Abst.).

9. Smith, D. H., and J. E. Castle. 1960. Production of auxotrophs in a duckweek, *Spirodela polyrhizza.* Plant Physiol.. 35:809-815.

10. Walles, B. 1963. Macromolecular physiology of plastids IV. On amino acid requirements of lethal chlotoplast mutants in barley. Hereditas 50:317-344.