REVERSING THE MORPHOGENETIC EFFECT OF PHENYLBORIC ACID AND OF THE LANCEOLATE GENE WITH ACTINOMYCIN D IN THE TOMATO

DAVID S. MATHAN

*Division of Biology, California Institute of Technology, Pasadena, and
Department of Genetics, University of California, Berkeley, California¹ 94720*

Received January 18, 1967

THE tomato leaf-shape mutant lanceolate and its normal form have been the subject of intensive comparative studies. The purpose of these investigations is to relate biochemical and physiological differences to morphological changes. These differences may enable us to chart a sequence of events that lead from a mutant gene affecting form so as to result in the changed trait. The merit of these comparative studies is that in such a system a small part of the organism is studied without disturbing the whole. Any information obtained can provide a clue as to how form is determined, and may suggest a mechanism by which differentiation may be accomplished.

The lanceolate mutant has a simple lance-shaped leaf instead of the pinnately compound leaf characteristic of normal tomato. The lanceolate phenotype was observed in individuals of the La^+/La genotype (MATHAN and JENKINS 1960, 1962). MATHAN and COLE (1964) found that the heterozygote when compared with normal (La^+/La^+), exhibits a twofold increase in the level of activity of four oxidative enzymes—tyrosinase, laccase, peroxidase, and catalase. The homozygous lanceolate (La/La) showed a three- to fourfold increase of these oxidative enzymes above the level found in La^+/La^+ .

HACCIUS and GARRECHT (1963) reported that phenylboric acid (PBA) when applied to germinating normal tomato seeds or to the tips of normal tomato plants transforms the first true leaf that is formed after the treatment from a pinnately compound leaf to a simple lanceolate leaf similar to the leaf conditioned by the gene La . MATHAN (1965) confirmed this and showed that treatment with PBA also duplicates the action of the La gene with respect to the level of activity of tyrosinase, laccase, peroxidase and catalase. MATHAN (1965, 1966) has suggested that the primary action of PBA, as well as of the gene La , is to increase the level of activity of the four enzymes. These enzymes in turn influence leaf form via a pathway as yet unknown. The evidence in support of this hypothesis is summarized by MATHAN (1966).

The question arose whether the increase in the level of activity of these enzymes represents newly synthesized enzymes, or an activation of existing enzymes. Furthermore, if the increase in activity represents *de novo* synthesis of

¹ Present address.

the enzymes, would it be possible to prevent the morphological change to lanceolate leaf by inhibiting the formation of these enzymes? The present work attempts to answer these questions. It will be shown that treatment with actinomycin D, when given at the proper time of seedling development and for the right duration, partly reverses the PBA effect on La^+/La^+ seedlings with respect to both the increase in enzyme activity and the change in leaf form. It will also be shown that actinomycin D treatment of La^+/La seedlings overcomes in part the action of the *La* gene.

MATERIALS AND METHODS

Seeds of La^+/La^+ and La^+/La types were obtained from the La^+/La line after the latter had been selfed for 16 generations in an insect-free greenhouse. The progeny of an La^+/La plant segregated in a ratio of 1 La^+/La^+ :2 La^+/La :1 La/La . These different types are easily recognized even after the various treatments. Since the experiments were designed to affect only the first true leaf of the seedling (in some cases also the second), the succeeding leaves developed in accordance with the genetic makeup of the plant.

Phenylboric acid was applied at a concentration of 300 $\mu\text{g}/\text{ml}$ for the first 24 hours of seed germination. About 50 seeds were placed in 5 ml of the PBA solution. They were removed from the solution after 24 hours and placed in distilled water, or were subjected to other treatments. Actinomycin D (a gift of Merck, Sharp, and Dohme) was used at concentrations ranging from 7.5 to 150 $\mu\text{g}/\text{ml}$. It was applied at various times from the first to the fifth day of germination and for the duration of 1 to 5 days.

The seeds were germinated in Petri dishes in the dark, and after treatment the seedlings were planted in a mixture of gravel and vermiculite. The plants were grown and the treatment was conducted under constant environmental conditions in the Earhart and Campbell Plant Research Laboratories. The procedures of treatment and the growing conditions were the same as described by MATHAN (1965).

The homogenate was prepared and the enzymes assayed according to the procedures described by MATHAN and COLE (1964). Colorimetric methods were used to measure the activity of tyrosinase, laccase and peroxidase. A Klett-Summerson photoelectric colorimeter with filter No. 42 was employed. DL-dihydroxyphenylalanine (Dopa) served as substrate for the determination of tyrosinase. Catechol was used as a substrate for the determination of laccase according to the procedure described by FAHRAEUS, TULLANDER and LUNGGREN (1958). Guaiacol was used as a substrate to determine peroxidase activity (MAEHLY and CHANCE 1961). Catalase activity measurements were made using the buoyant effects of the liberated oxygen on paper discs, by recording the time required for the discs to rise to the surface of the reaction solution (3% hydrogen peroxide). Lyophilized beef liver catalase was used as a standard (GAGNON, HUNTING and ESSELEN 1959).

EXPERIMENTAL RESULTS

Leaf Form

Germination in the dark. In previous work (MATHAN and COLE 1964; MATHAN 1965, 1966) seeds of La^+/La^+ and La^+/La were either germinated in a mixture of gravel and vermiculite and placed directly under normal conditions of light and temperature, or were first germinated in distilled water in the dark for the first 24 or 48 hours, and then planted in a mixture of gravel and vermiculite and placed in the light. In the present experiments, owing to the fact that a longer exposure to actinomycin D was required, the seedlings were kept in distilled

water and distilled water containing actinomycin D, and kept in the dark up to 8 days before being planted.

The retention of germinating seeds in distilled water in the dark up to 4 days had little or no effect on the development and form of the first leaf (in some cases it may have caused an elongation of the lamina). Beyond 4 days the effect was to reduce the size and complexity of the leaf the longer the germinating seeds were kept in the dark: La^+/La^+ seedlings developed smaller first leaves and many of them were devoid of leaflets; La^+/La exhibited a reduction in the size of the first leaf.

Table 1 compares sizes of first leaves of La^+/La plants kept in distilled water in the dark for 4 days with those kept for 8 days. Seedlings kept in the dark for 8 days show a much reduced leaf size.

Actinomycin D: When seeds of La^+/La^+ and La^+/La are placed directly in a solution of 50–100 $\mu\text{g/ml}$ actinomycin D for a period exceeding 2 days, they either fail to germinate or show various degrees of retardation in plant and leaf development. If, on the other hand, the seeds are placed in distilled water for the first 2 to 3 days and are then placed in actinomycin D solution for 3 to 4 days, they germinate normally. In addition there is a marked effect on the development and form of the first true leaf. La^+/La^+ plants show variations from the normal: about 10% of the seedlings develop an enlarged terminal leaflet with the complete absence of lateral leaflets, resembling potato leaf conditioned by the recessive gene *c* (MATHAN 1966). The rest of the seedlings show variations from a typical normal leaflet to various degrees of enlarged terminal leaflet and decrease in lateral leaflets (Figures 1, 2). La^+/La seedlings react to actinomycin D treatment by forming an enlarged first leaf. In some cases there is even an initiation of a lobe, which was never observed in typical La^+/La plants (Figure 4). Table 1 gives a comparison in sizes of the first leaf between seedlings kept in distilled water in the dark for 8 days and those kept in distilled water for 3 days and in 100 $\mu\text{g/ml}$ actinomycin D solution for an additional 5 days before being planted in vermiculite and placed in the light. Those treated with actinomycin D show a marked increase in size of the first leaf, especially expressed in the width of the lamina. Longer exposure to actinomycin D causes a reversal of this effect, namely

TABLE 1

Effect of the length of time of retaining germinating seeds in distilled water in the dark, with and without actinomycin D, on the size of the first leaf in La^+/La plants

4 days*			8 days†			8 days + actinomycin D‡		
Lamina length	Lamina width	Petiole length	Lamina length	Lamina width	Petiole length	Lamina length	Lamina width	Petiole length
4.08 ± .84	.93 ± .18	1.90 ± .28	1.93 ± .22	.65 ± .12	1.47 ± .39	2.85 ± .30	1.13 ± .13	2.10 ± .36

Measurements were made of leaves taken from 5-week old plants. Each figure is an average $\pm$ SE of six leaves.

* Germinating seeds were kept in distilled water in the dark for 4 days before planted in a mixture of gravel and vermiculite and placed in the light.

† As in *, except that seeds were kept in distilled water in the dark for 8 days.

‡ Germinating seeds were kept for 3 days in distilled water, and for an additional 5 days in a 100 $\mu\text{g/ml}$ actinomycin D solution in the dark.

FIGURE 1-4:—Effect of treatment with actinomycin D, phenylboric acid, and their combination on the shape and size of the first true leaf in La^+/La^+ and La^+/La plants. Seeds were germinated in distilled water or in the treatment solution for 7 days in the dark, after which they were planted in a mixture of gravel and vermiculite and placed in the light. Leaves were taken from 5-week old plants. FIGURE 1.—Variations in the response to actinomycin D treatment. La^+/La^+ seeds were germinated and kept in distilled water for the first 3 days, and in a 100 $\mu\text{g}/\text{ml}$ actinomycin D solution for an additional 4 days. FIGURE 2.—Effect of various treatment on leaf-shape and size in La^+/La^+ plants. From left to right: (a) control germinated in distilled water for 7 days; (b) actinomycin D as in Figure 1; (c) PBA germinated in 300 $\mu\text{g}/\text{ml}$ PBA solution for the first 24 hours and placed for an additional 6 days in distilled water; (d) PBA and actinomycin D germinated in 300 $\mu\text{g}/\text{ml}$ PBA for the first 24 hours, 2 days in distilled water, remaining 4 days in 100 $\mu\text{g}/\text{ml}$ actinomycin D solution. FIGURE 3.—Variations in the response to PBA plus actinomycin D in La^+/La^+ plants. Treatment as in Figure 2. FIGURE 4.—Effect of actinomycin D treatment on leaf size and shape in La^+/La plants. The control (left) was retained for 7 days in distilled water. The leaf on the right was from a plant treated as in Figure 1. Note initiation of a lobe.

a decrease in leaf size and retardation of leaf development in both La^+/La^+ and La^+/La .

The effect of actinomycin D treatment on La/La seedlings could not be measured or verified since there are variations in form of this class from *reduced* to *narrow* believed to depend on other factors (MATHAN and JENKINS 1962; MATHAN and COLE 1964). However, an increase in the number of *narrow* plants is observed in the actinomycin D treated cultures.

Actinomycin D and PBA: As has been shown previously (MATHAN 1965, 1966), PBA applied to La^+/La^+ seeds for the first 24 hours of germination at a

concentration of 300 $\mu\text{g/ml}$, effectively transforms the first true leaf of all seedlings from a compound leaf to a lanceolate leaf similar to the lanceolate leaf conditioned by the gene *La*. If, however, on the third day of germination following the treatment with PBA, the seedlings are placed in a solution of 50 to 100 $\mu\text{g/ml}$ actinomycin D for a duration of 3 to 4 days, the transformation of the first true leaf to a typical lanceolate leaf does not take place; instead, 25% of the seedlings show a normal first leaf, and the rest range in shape and size from normal to an enlarged lanceolate leaf (Figures 2, 3).

Oxidative Enzymes

Germination in the dark: If dry seeds of La^+/La^+ are assayed for tyrosinase, laccase, peroxidase and catalase, only very slight activity of laccase and catalase can be observed. If the seeds are placed for germination in a Petri dish containing distilled water and are kept in the dark, tyrosinase and peroxidase activity continue to be absent for the first 3 days of germination, while laccase and catalase activities show a gradual but slight increase. From the 4th to 10th days of germination there is a rapid rise in activities of all four oxidative enzymes (Table 2). On the other hand, if the seeds are placed in distilled water for only the first 2 days, then transplanted to vermiculite or sown directly in a mixture of gravel and vermiculite, watered with $\frac{1}{2}$ -strength Hoagland solution and placed under normal conditions of light and temperature, the steep rise in activities of all four oxidative enzymes from the fifth day on does not take place.

Table 2 gives a comparison in enzyme activities between La^+/La^+ seeds germinated in distilled water and kept in the dark and those germinated in a mixture of gravel and vermiculite and kept in the light. The following can be concluded from Table 2: (1) The activities of tyrosinase, laccase, peroxidase and catalase in the dark grown seedlings on the seventh day of germination are much higher than the corresponding light-grown seedlings; (2) the values of enzyme activities reached on the seventh day in the light-grown seedlings are comparable to those reached on the fourth day in the dark-grown seedlings; (3) the activity

TABLE 2

A comparison of enzyme activities between La^+/La^+ seedlings kept in distilled water and grown in the dark and those sown in a mixture of gravel and vermiculite and grown in the light

Activity	Days grown in the dark*										Days grown in the light*			
	0†	1	2	3	4	5	7	8	10		7	8	10	11
Tyrosinase	0	0	0	0	35	60	275	258	340		37	57	50	45
Laccase	3	8	10	15	60	120	160	189	250		43	68	56	42
Peroxidase	0	0	0	0	38	70	75	107	200		47	80	90	127
Catalase	3.7	3.0	7.5	12.1	18.7	30.1	37.5	52.6	61.4		14.7	22.5	15.0	18.0

In this table and in Table 3, results for tyrosinase and peroxidase are given as average increment in colorimetric units per 0.33 g fresh weight, for laccase per 0.066 g fresh weight, for catalase as units per g fresh weight. Roots were excluded from the samples. It should be noted that no differences in water content due to treatment or genotype could be shown. Each figure is the average of three readings.

* Days after the seeds were initially placed for germination.

† Dry seeds.

of the enzymes continues to rise even after the seventh day in the dark-grown seedlings, whereas in the light-grown seedlings the activity stabilizes around the values observed in the seventh day. Peroxidase does not follow the above scheme; it continues to rise in both cultures, but at a higher rate in the dark-grown seedlings.

If one compares the effect of the long retention of seedlings in distilled water in the dark on the activities of the four oxidative enzymes to its effect on morphology, it becomes clear that, associated with the increase in enzyme activities, there is a decrease in size and complexity of leaf in both La^+/La^+ and La^+/La seedlings (Tables 1, 2).

Actinomycin D. When La^+/La^+ seeds are germinated in distilled water in the dark for the first 3 days, then removed from the distilled water and placed in a solution of 50 to 100 $\mu\text{g/ml}$ actinomycin D for an additional 5 days, the activities of tyrosinase, laccase and catalase do not rise to the same level as if they were kept for the entire 8-day period in distilled water (peroxidase activity rises and attains higher levels than the control). On the other hand, if the seeds are kept in distilled water in the dark for 6 days and then transferred to actinomycin D solution for additional 2 days, the activities of all enzymes except catalase climb even beyond the activities attained by the control (Table 3).

Relating the level of activity of the oxidative enzymes in the seedlings treated on the fourth day with actinomycin D to the development of the first leaf, it is clear that the lower level of activity of tyrosinase, laccase and catalase is associated with a larger leaf (Table 3; Figures 1, 2).

Actinomycin D and PBA: MATHAN (1965, 1966) has shown that treatment of La^+/La^+ or La^+/La seeds with PBA in the first 24 hours of germination causes an increase in the level of activity of tyrosinase, laccase, peroxidase and catalase. Associated with this increase there is a reduction in size and complexity of the first true leaf of the seedlings. However, if 2 days after PBA treatment the seedlings are placed in 50–100 $\mu\text{g/ml}$ actinomycin D solution and kept in the dark for additional 3 to 5 days, the level of activity of tyrosinase, laccase and catalase is considerably below that of seedlings treated with PBA alone, and resembles

TABLE 3

The effect of PBA and actinomycin D on the four enzyme activities in La^+/La^+ seedlings

Activity	Control*	PBA†	Act. D‡	Act. D§	PBA + Act. D
Tyrosinase	240	470	140	390	230
Laccase	170	390	100	310	220
Peroxidase	126	316	384	720	426
Catalase	60.2	90.0	52.5	45.0	62.1

All cultures were grown in the dark and the assays were made on 8-day old seedlings.

* Placed in distilled water for 8 days.

† Placed for the first 24 hours in 300 $\mu\text{g/ml}$ PBA, for the remaining 7 days in distilled water.

‡ Placed for the first 3 days in distilled water, for the remaining 5 days in 100 $\mu\text{g/ml}$ actinomycin D solution.

§ Placed for the first 6 days in distilled water, for the remaining 2 days in 100 $\mu\text{g/ml}$ actinomycin D solution.

|| Placed for the first 24 hours in 300 $\mu\text{g/ml}$ PBA solution, for 2 days in distilled water, and for the remaining 5 days in 100 $\mu\text{g/ml}$ actinomycin D.

that of the control. Peroxidase activity continues to rise beyond the level attained by each treatment separately (Table 3).

Actinomycin D reversed the effect of PBA with respect to both the level of activity of tyrosinase, laccase and catalase and the PBA effect on leaf form (Table 3; Figures 2, 3).

DISCUSSION

The results presented show that actinomycin D, when given at a particular stage of seedling development, partially inhibits tyrosinase, laccase and catalase activities. Associated with this inhibition there is an increase in leaf size. Actinomycin D was also found to prevent the increase in enzyme activities, the associated change in leaf form, and the decrease in leaf size due to PBA treatment.

GOLDBERG and RABINOWITZ (1962), BALTIMORE and FRANKLIN (1962), and REICH (1963) have shown that actinomycin D specifically inhibits DNA-dependent RNA polymerase. The inhibition in bacterial and mammalian cells affects all fractions of the RNA, nuclear, ribosomal and soluble RNA. These authors have pointed out that the inhibition of RNA synthesis is not due to an effect on the enzyme protein directly, but is due to the binding of actinomycin D to primer DNA. MITRA and SIRCAR (1966) applied actinomycin D to germinating seeds of wheat at a concentration of 500 $\mu\text{g/ml}$ and reported 70% inhibition of RNA synthesis with an equivalent inhibition of subsequent protein synthesis.

The reduced activity of tyrosinase, laccase and catalase brought about by actinomycin D treatment in the tomato seedlings is of particular interest. Actinomycin D treatment was found to be effective in reducing enzyme activity only when given on the third day of germination, or prior to the steep rise in the activities of these oxidative enzymes. These results indicate that probably the specific RNA's required for the synthesis of tyrosinase, laccase and catalase are not synthesized in appreciable quantities until the fourth day. Treatment with actinomycin D prior to this synthesis effectively prevents RNA formation, which in turn reduces the synthesis of these enzymes. On the other hand, if given after the third day, RNA's for these specific enzymes are already formed in appreciable quantities, and as a result synthesis of these proteins is not affected, as measured by their activities (Table 3).

The finding that actinomycin D treatment prevents the rise in the level of enzyme activity due to PBA treatment, suggests that the PBA effect in increasing tyrosinase, laccase and catalase activities represents induction of newly synthesized enzymes.

In the light of these findings the relationship between the level of activity of these enzymes and leaf form is of particular significance. Actinomycin D prevents the increase in the level of activities of the above enzymes due to PBA effect, and associated with this inhibition of enzyme formation there is a partial reversion to normal leaf. These facts indicate strongly that the level of activity of these enzymes directly affects leaf form in some way as yet unknown. It is realized that actinomycin D inhibition is not specific to these particular enzymes, but the above conclusion is justified by the fact that the increase in the activities of these

enzymes was found to be associated with the lanceolate mutant and the PBA treated plants, while other enzymes such as ATPase, proteinase, α -amylase and cytochrome oxidase did not show such an increase (MATHAN 1965), coupled with the fact that actinomycin D is effective in inhibiting the above enzymes only at a particular stage of seedling development, which coincides with the time prior to the normal rise in these enzymes.

An implication may have been drawn from the foregoing discussion that beginning with germination the seed undergoes certain well defined stages of metabolic activity. It is true that there is a well defined starting point in the seed which shows very little metabolic activity. However, any generalization for a metabolic system involving the whole seedling will be misleading. For convenience the seedling as a whole (except for the roots) has been used in the assay of enzymes, yet there is sufficient evidence to indicate that the activities of phenolases and peroxidases differ in different tissues and regions of the plant (VAN FLEET 1961).

The relationship between the activities of tyrosinase, laccase, peroxidase and catalase and leaf form should be found in the apical meristem where the leaf is determined. We find evidence for such a relationship in the fact that when PBA is applied in later stages of seedling development it affects succeeding leaves. Applied to the tip of a mature plant it affects the leaf that arises after the treatment. On the other hand, if applied to a mature leaf, it does not cause any change in morphology, yet it increases the level of activity of the four oxidative enzymes. This indicates that PBA specifically induces these enzymes in all cells, but only at the tip do their increased activities result in a modified leaf (MATHAN 1965). It is possible that careful application of actinomycin D to the tip of mature plants may likewise affect the leaf that arises after the treatment.

A question that is repeatedly raised in our studies is how to explain the increase in activity of several enzymes in the light of our knowledge of gene action. Lanceolate is believed to be a one-gene mutation and accordingly should be involved in the qualitative determination of one enzyme. How can we explain a quantitative increase in several enzymes due to action of a single gene? The phenomenon of enzyme induction by substrates and related compounds is well known, particularly in microorganisms (HALVORSON 1960). The induction of several enzymes by one metabolite or one compound in bacteria is known as the phenomenon of sequential induction of enzymes (STANIER 1954). Similar to nutritional mutants in *Neurospora* (BEADLE 1960), the *La* gene may be looked upon as rendering an enzyme ineffective in catalysing a reaction converting substance A to substance B. As a result several things can happen—an accumulation of substance A, a deficiency of substance B, or an accumulation of substance A and a deficiency of substance B. In the case of *La*, the accumulation of substance A may cause the induction of several enzymes.

It should be emphasized that the above suggestion is a speculation, resting solely on the fact that PBA added externally to *La*⁺/*La*⁺ plants remarkably duplicates the action of the *La* gene both with respect to enzyme activity and leaf form. Moreover, PBA exhibits a concentration effect similar to the dosage effect exhibited by the *La* gene (MATHAN 1965, 1966). This tends to support an

assumption that PBA is similar in physiological activity to a compound which may be accumulating in the *La* mutant. The fact that Actinomycin D partially reverses the actions of PBA and of the *La* gene also supports the above proposal.

The author wishes to thank DR. LEDYARD STEBBINS and DR. JESSE RABINOWITZ for reading the manuscript.

SUMMARY

Actinomycin D treatment of germinating seeds of normal (La^+/La^+) and lanceolate (La^+/La), causes an increase in the size of the first leaf, when given on the third day of germination for a duration of 3 to 5 days. Associated with the increase in leaf size is a reduction in activities of tyrosinase, laccase and catalase. Actinomycin D reverses the effect of phenylboric acid with respect to both the size and shape of the leaf, and reverses the activities of the three enzymes.

LITERATURE CITED

- BALTIMORE, D., and R. M. FRANKLIN, 1962 The effect of mengo virus infection on the activity of the DNA-dependent RNA polymerase of L-cells. *Proc. Natl. Acad. Sci. U.S.* **48**: 1383-1390.
- BEADLE, G. W., 1960 Physiological aspects of genetics. *Ann. Rev. Physiol.* **22**: 45-74.
- FÄHRÆUS, G., V. TULLANDER, and H. LUNGGREN, 1958 Production of high laccase yields in cultures of fungi. *Physiol. Plantarum* **11**: 631-643.
- GAGNON, M., W. M. HUNTING, and W. B. ESSELEN, 1959 New method for catalase determination. *Ann. Chem.* **31**: 144-146.
- GOLDBERG, I. H., and M. RABINOWITZ, 1962 Inhibition of deoxyribonucleic acid dependent synthesis of ribonucleic acid. *Science* **136**: 315-316.
- HACCIUS, B., and M. GARRECHT, 1963 Durch Phenylborsäure induzierte "Lanzettblättrigkeit" bei *Solanum lycopersicum*. *Naturwiss.* **50**: 133-134.
- HALVORSON, H. O., 1960 The induced synthesis of proteins. *Advan. Enzymol.* **22**: 99-156.
- MAEHLY, A. C., and B. CHANCE, 1961 The assay of catalases and peroxidases. pp. 357-412. *Methods of Biochemical analysis*. Edited by D. GLICK. Interscience, New York.
- MATHAN, D. S., 1965 Phenylboric acid, a chemical agent simulating the effect of the lanceolate gene in the tomato. *Am. J. Botany* **52**: 185-192. — 1966 Morphogenetic effect of phenylboric acid on various leaf-shape mutants in the tomato, duplicating the effect of the lanceolate gene. *Z. Vererb.* **97**: 157-165.
- MATHAN, D. S., and R. D. COLE, 1964 Comparative biochemical study of two allelic forms of a gene affecting leaf-shape in the tomato. *Am. J. Botany* **51**: 560-566.
- MATHAN, D. S., and J. A. JENKINS, 1960 Chemically induced phenocopy of a tomato mutant. *Science* **131**: 36-37. — 1962 A morphogenetic study of lanceolate, a leaf-shape mutant in the tomato. *Am. J. Botany* **49**: 504-514.
- MITRA, S. A. M., and S. M. SIRCAR, 1966 Synthesis of an unstable functional ribonucleic acid in germinating wheat. *Indian J. Biochem.* **3**: 26-29.
- REICH, E., 1963 Biochemistry of actinomycin D. *Cancer Res.* **23**: 1428.
- STANIER, R. Y., 1954 The plasticity of enzymatic patterns in microbial cells. *Symp. Soc. Study Develop. Growth* **13**: 43-67.
- VAN FLEET, D. S., 1961 Histochemistry and function of the endodermis. *Botan. Rev.* **27**: 165-220.