A THIAMINE-REQUIRING MUTANT OF THE TOMATO

By J. Langridge* and R. D. Brock*

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Summary

A spontaneous, single-gene mutant of the tomato is shown to be unable to synthesize thiamine. The specific reaction lost is either the methylation of position 2 or the activation of position 5 of the pyrimidine ring.

I. Introduction

Apart from a note (Langridge 1955) on certain growth mutants of *Arabidopsis*, there has been no description in flowering plants of mutants of the *Neurospora* type; i.e. mutants which can be shown to be lethal in the absence of a specific organic compound. This report briefly describes such a mutant in the cultivated tomato, *Lycopersicon esculentum* Mill.

II. Material and Methods

The mutant studied is a spontaneous one occurring in two tomato lines, 11–1–1 and 25–1 (Giles and Hutton 1958), which were being bred for disease resistance. These two lines, now known as Merbein Mid-Season and 25-B had as common parents the varieties South Australian Dwarf and Hawaiian Experiment Station Line No. 4242. The mutant has not been found in any of the parent varieties.

The segregation of mutant plants observed in the selfed progeny of 110 heterozygous plants was 1387 wild-type to 450 mutant plants. Thus the mutant phenotype results from a single gene change (χ^2 for $3:1 = 0{\cdot}24$, $P > 0{\cdot}5$). The mutation is completely recessive to its wild-type allele and neither somatic nor germinal back-mutation has been observed.

Plants homozygous for the mutation may survive for several months under good growing conditions, but they grow extremely slowly. The cotyledons are normal in size, shape, and colour, presumably because of the diffusion of metabolites into the seed from the heterozygous maternal parent. The few leaves formed by the mutant are small and at first pale yellow in colour. Later the leaves may become light green, but they soon lose their chlorophyll and turn white except at the main veins which remain green. After the chlorophyll is lost, the leaves wither and die. Frequently the main shoot apex also dies and short secondary shoots develop. Plants after about four months' growth are only 3–6 in. in height, very chlorotic but still alive. There is some variation in phenotype depending on the time of sowing, but experiments showed that this variation was not correlated with temperature or with light intensity during growth.

* Division of Plant Industry, C.S.I.R.O., Canberra.

Mutant plants were tested for their response to organic substances by spraying them daily with nucleic acid hydrolysate, casein hydrolysate, water-soluble vitamins, and organic acids. The preparation, composition, and concentrations of the supplements are described by Langridge (1958). Three plants were sprayed with each supplement.

III. Results and Discussion

The only effective treatment was the vitamin mixture, which produced a pronounced greening of the tissue after 2 days and a complete disappearance of the deficiency symptoms after 5 days of application. The active vitamin of the mixture was shown to be thiamine by means of the screening test described by

Fig. 1.—Plants homozygous recessive for the gene conferring thiamine requirement; age 106 days. Right, untreated control. Left, plant sprayed three times per week with thiamine at the rate of 2 mg per 100 ml.

Lindegren and Lindegren (1951). After mutant plants, which received three treatments with thiamine (2 mg per 100 ml water) per week, had been grown for about four months, they were almost indistinguishable from the wild-type. The plants were nearly normal in size, normal in chlorophyll formation, flowering, and seed production (Fig. 1); their progeny were all mutant in phenotype.

In all organisms that have been studied, thiamine formation has been shown to occur by the separate synthesis of a substituted pyrimidine and a substituted thiazole ring followed by the linking of these compounds by a methylene bridge. Therefore, mutant plants were sprayed with "pyrimidine" (2-methyl-6-amino-5-aminomethylpyrimidine) and "thiazole" (4-methyl-5-β-hydroxyethylthiazole) both separately

and together to obtain information on the biochemical deficiency. The mutant responded to "pyrimidine" but not to "thiazole" alone, indicating that the genetic block is in the formation of "pyrimidine" and that "thiazole" synthesis and the coupling reaction are unimpaired by the mutation (Fig. 2, *A*). Robbins and Bartley (1937) have shown that isolated roots of the tomato are unable to form thiamine but here the deficiency is in the ability to make the thiazole part (Fig. 2, *B*). Langridge (1958) has described an *Arabidopsis* mutant that is unable to join together the two halves of the thiamine molecule (Fig. 2, *C*).

Since a pyrimidine compound is required by the mutant, tests were made of the plants' ability to synthesize the pyrimidines of the nucleic acids. Deficient plants were sprayed with cytosine (2-hydroxy-6-aminopyrimidine), uracil (2,6-dihydroxypyrimidine), and thymine (5-methyl-2, 6-dihydroxypyrimidine). All these compounds were without visible effect.

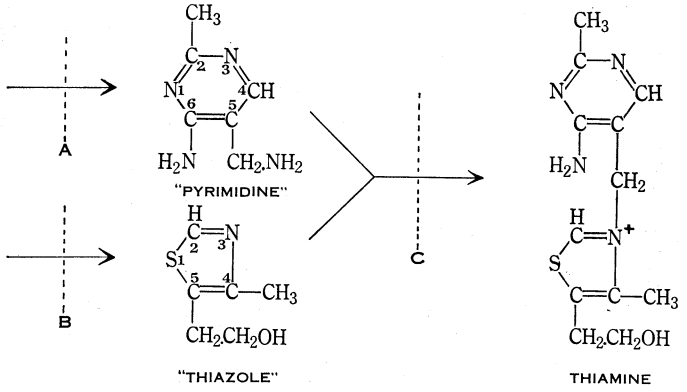

Fig. 2.—Diagram of the structure and synthesis of thiamine. *A*, location of the block in the tomato mutant. *B*, location of the block in isolated roots of the tomato. *C*, location of the block in an *Arabidopsis* mutant.

The various pyrimidines of the nucleic acids have a common precursor, orotic acid (2,4-dihydroxy-6-carboxypyrimidine), which is also considered to be the precursor of the pyrimidine portion of the thiamine molecule. For physiological activity, the pyrimidine ring of thiamine needs an amino group at position 6 (Bergel and Todd 1937), a methyl or ethyl group at position 2 (Huber 1943), and a reactive group at the 5-methyl position for bridge formation with thiazole (Bonner 1938). The absence of response to cytosine and thymine indicates that the mutant is able to perform the 5-methyl and 6-amino substitutions and to remove the hydroxyl group at position 4. Therefore, the chemical deficiency in this mutant must be either in the replacement of a hydroxyl at position 2 by a methyl group, or in the forming of a reactive group at the 5-methyl position.

As a result of the lack of thiamine, there is an almost complete absence of chlorophyll in mutant plants. The chloroplasts of the mutant are normal in size and appearance, so thiamine must be required at some stage in the formation of the chlorophyll molecule itself. However, no chlorophyll was formed by treatment of

deficient plants with δ-amino laevulinic acid, the precursor of the pyrrole ring, or by porphobilinogen, the precursor of the porphyrin structure. Therefore, thiamine deficiency must interfere with chlorophyll synthesis at a stage later than the formation of the pyrrole rings.

IV. References

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