MORPHOGENETIC EFFECT OF PHENYLBORIC ACID ON VARIOUS LEAF-SHAPE MUTANTS IN THE TOMATO, DUPLICATING THE EFFECT OF THE LANCEOLATE GENE

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Introduction

In previous reports, MATHAN and JENKINS (1960, 1962) described a leaf-shape mutant in the tomato which they called lanceolate (La). They found the mutant to be caused by a dominant gene in heterozygous condition (La+/La) and to be characterized by a simple, lanceolate leaf instead of the pinnately compound leaf of normal tomato. The homozygote (La/La) shows a further reduction of the leaf. It occurs in several forms: narrow, produces a small simple narrow leaf; modified, forms only one cotyledon and a bud that fails to develop; reduced, is devoid of cotyledons or other foliar structures, growing into a column of tissue up to 5 cm in height and 0.2 cm in diameter.

MATHAN and COLE (1964) have shown that associated with the La+/La leaf-shape there is a twofold increase in the activities of four oxidative enzymes, namely tyrosinase, laccase, peroxidase and catalase. Narrow (La/La) was shown to exhibit a three to fourfold increase, above the level found in normal (La+/La+), of these oxidative enzymes.

HACCIUS and GARRECHT (1963) reported that application of phenylboric acid (PBA) to germinating normal tomato seeds, or the tips of normal tomato plants, transforms the first true leaf that arises after the treatment from a pinnately compound leaf to a simple lanceolate leaf, similar to the lanceolate leaf developed on a plant heterozygous for the La gene.

MATHAN (1965) confirmed this finding and also demonstrated that associated with the change in leaf-shape from normal to phenotypically lanceolate, which resulted from PBA treatment, there was an increase in the activities of the same four oxidative enzymes found to have increased levels in the lanceolate and narrow leaf types conditioned by the gene La. PBA treatment, therefore, duplicated the action of the La gene with respect to both the change in morphology and the associated increase in the activities of certain enzymes.

In the present work the effect of PBA treatment on four other leaf-shape variants is described and compared with that on the La+/La+ and La+/La types. The work was based on the finding (MATHAN, 1956, 1960) that the expression of the gene La was modified in a characteristic fashion when this gene was combined with certain other leaf shape genes. It seemed, therefore, interesting to determine whether the expression of these leaf-shape mutants was affected in a similar manner by PBA, thus lending further support to the idea that the La gene and PBA exert their morphological effects by the same mechanism, probably involving a specific increase in the activity of certain oxidative enzymes.
Four recessive mutants were used: \textit{trifoliolate} (\textit{tt/ff}), \textit{clausa} (\textit{clau/clau}), \textit{potato} (\textit{c/c}), and \textit{entire} (\textit{e/e}). \textit{tt/ff} differs from the normal in that it has a terminal leaflet and only two lateral leaflets instead of the six lateral leaflets characteristic of the normal tomato leaf (Robinson and Rick, 1955). \textit{clau/clau} is similar in general characteristics to normal except that the leaf is highly segmented, having secondary and tertiary leaflets (Stubbs, 1958). \textit{c/c} differs from the normal in that it has a much larger terminal leaflet and only two to four small lateral leaflets (MacArthur, 1934). \textit{e/e} has a leaf similar to \textit{c/c} in general characteristics, but somewhat broader (Butler, 1951).

The former three mutants, together with normal tomato, can be arranged in order of increasing size and number of major and minor lateral leaflets as follows: \textit{La+/La}, \textit{tt/ff}, \textit{La+/La+}, \textit{clau/clau}. \textit{c/c} and \textit{e/e} differ from these four leaf types in that they have a very large terminal leaflet and few and small lateral leaflets.

The first true leaf that arises after germination in all the leaf-shape variants in the tomato is smaller and simpler than the leaves that arise in later stages of development. The work here is confined mainly to the first true leaf.

\section*{Materials and Methods}

Seeds of \textit{La+/La+} plants were obtained from the \textit{La+/La} line, after the latter had been selfed for fifteen generations. Seeds of \textit{tt/ff}, \textit{clau/clau}, \textit{c/c} and \textit{e/e} were obtained from Dr. C.M. Rick of the University of California, Davis. They were selfed in an insect-free greenhouse for five generations.

PBA was applied, at a series of concentrations ranging from 50 to 500 ppm (4 \times 10^{-4} - 4 \times 10^{-3} \, \text{M}) to germinating seeds for 24 hours, either on the first or the second day of germination. Only one application was given in each experiment. The plants were grown and the treatment 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 assay of enzymes was made according to the procedures described previously (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 Fähræus, Tullander and Ljunggren (1958). Guaiacol was used as a substrate to determine peroxidase activity (Maehly and Chance, 1961).

Catalase activity measurements were made using the buoyant effect 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 Esselein, 1959).

\section*{Experimental Results}

\subsection*{Morphology}

\textit{La+/La} — The present observation confirms that made by Mathan (1965) that PBA treatment induces a narrow leaf. In addition it has been observed that, up to 300 ppm, PBA exhibits a concentration effect; the higher the concentration the smaller the leaf that is formed.

\textit{tt/ff} — This mutant is very sensitive to PBA treatment; with 100 ppm given on the first day of germination the first true leaf is a typical lanceolate leaf; at 300 ppm there is a further reduction of the leaf (Fig. 1). As a result of treatment the second leaf in most cases is also of the lanceolate type. In crosses of \textit{La+/La}