Phytochemistry, 1970, Vol. 9, pp. 463 to 469. Pergamon Press. Printed in England.

CHEMICAL PREVENTION OF GROWTH REDUCTION CAUSED BY SUPRAOPTIMAL TEMPERATURES IN *ARABIDOPSIS THALIANA**

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(*Received* 4 *February* 1969, *in revised form* 30 *April* 1969)

Abstract—In attempts to prevent the detrimental effects of supraoptimal temperatures, biotin, sucrose, malic acid, and the growth retardant phosfon-S were individually added to the agar suspension on which *Arabidopsis thaliana* plants were grown aseptically under both optimal and supraoptimal temperature regimes. Biotin induced increases over the untreated plants of 54 per cent in fresh weight and 66 per cent in dry weight at supraoptimal temperatures, while the corresponding biotin-induced increases over the untreated plants in fresh and dry weights at optimal temperatures were 38 and 15 per cent, respectively. Addition of 1 per cent sucrose induced 58 per cent increases in fresh and 70 per cent in dry weights of plants grown under supraoptimal temperatures, while under optimal temperature conditions, sucrose actually reduced the fresh weight 12 per cent and had only a slight (2 per cent) promotive effect on dry weights. Phosfon-S increased the fresh and dry weight 30 per cent and 16 per cent respectively, under supraoptimal temperatures and 27 and 9 per cent under the optimal temperatures. Malic acid caused a reduction in both fresh and dry weights under both temperature regimes. However, malic acid treatment was found to increase the number of seed pods, seeds, and the number of secondary roots. The size of seed pods and seeds was greatly increased by malic acid treatment under both temperature conditions.

THE IDEA of "chemical cure of climatic lesions" in higher plants proposed by Bonner[1] has received increasing attention in recent years, probably because of its considerable potential economic importance.

Adenine was one of the first substances which was demonstrated to prevent or reduce an inhibition of growth caused by supraoptimal temperatures.[2] Since then, adenine has been tested by many other workers;[3–6] however, some of these investigators[3,5,6] did not find adenine to be effective in removal of the lesions caused by supraoptimal temperatures. A mixture of B vitamins or mixed ribosides increased the dry weight up to 40 per cent above the untreated plants at supraoptimal temperatures.[7] Sucrose was found to be effective only at temperatures slightly above optimal.[6] Petinov and Molotkovskii[8] reduced the supraoptimal temperature "lesions" in sunflower, corn, pumpkin and oats by spraying the leaves with organic acids. Citric and malic acids were particularly effective in this regard.

* Florida Agricultural Experiment Stations Journal Series No. 3176. This study was a portion of a dissertation presented to the Graduate Council of the University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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TABLE 1. EFFECT OF TEMPERATURE AND ADDED METABOLITES ON NUMBER OF SEED PODS AND SEEDS OF *Arabidopsis thaliana*

Addition to basal medium	Number of seed pods*		Number of seeds*	
	O†	S‡	O†	S‡
None	10·0±1·1	3·5±0·5	127·9±21	44·4±5·1
Biotin	11·5±1·5	10·5±1·3	197·7±18	127·2±17·0
Malic acid	10·2±1·2	6·0±0·7	248·9±38	59·8±7·3
Phosfon-S	10·9±1·7	7·0±0·7	166·4±19	81·2±9·4
1% Sucrose	16·0±2·1	7·6±0·8	235·1±26	105·2±11·1

* Average of two trials, five plants per trial.
† O = Optimal temperatures.
‡ S = Supraoptimal temperatures.

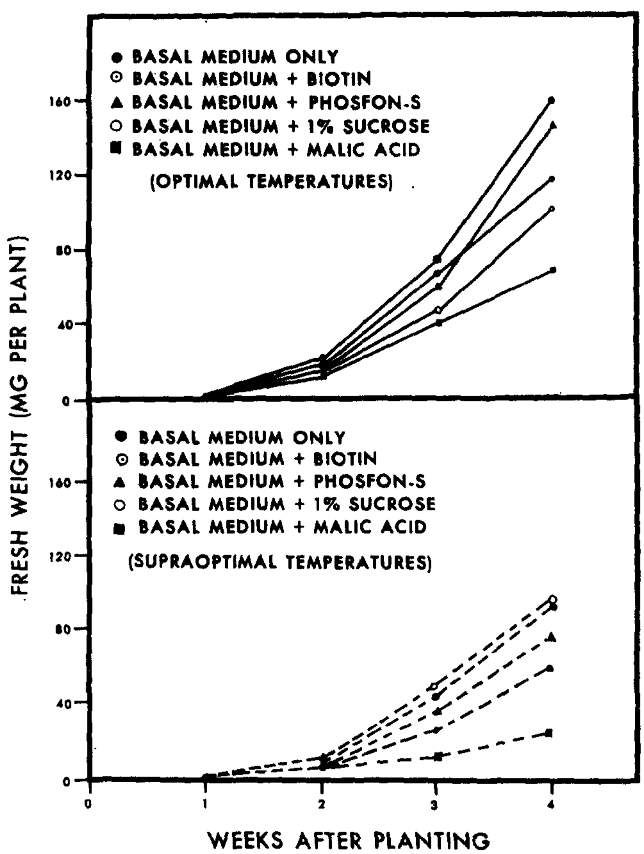

FIG. 1.

In working with *Arabidopsis thaliana*, a very clear-cut case of effective chemotherapy of the inhibition of growth and development at high temperatures was obtained by Langridge and Griffing.[9] Plants were grown with aseptic culture techniques and under controlled environmental conditions. The addition of biotin to the culture medium of two heat-sensitive races prevented the supraoptimal temperature "lesions".

It has been observed that a growth retardant, phosfon-S (2,4-dichlorobenzyltributyl-ammonium chloride), produces resistance to environmental changes in plants. Certain

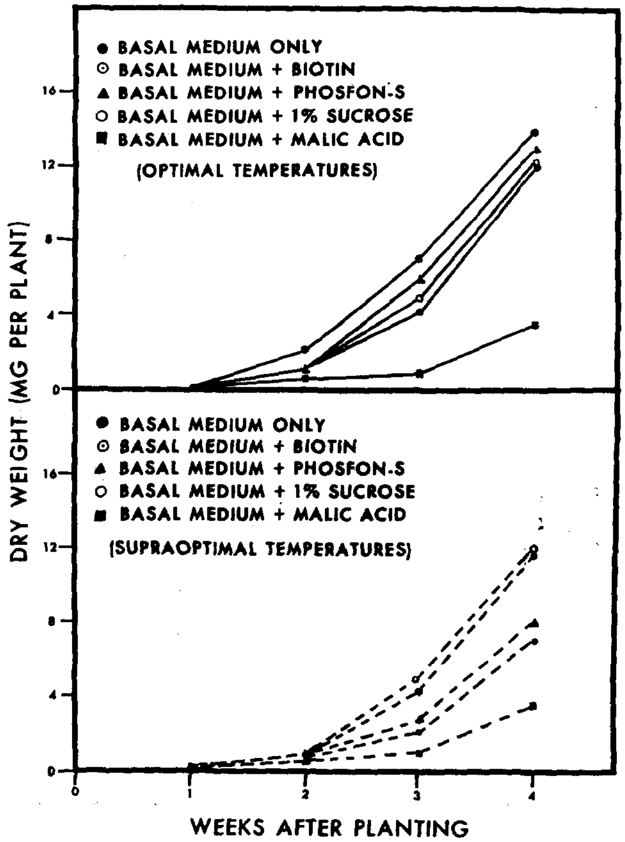

FIG. 2.

plants are more resistant to drought at optimal[10] and supraoptimal temperatures[11] and are also more resistant to frost damage[12] when treated with phosfon-S. The effect of phosfon-S on the resistance of plants to environmental changes encouraged us to test it as well as several other chemicals on the reduction or elimination of the detrimental effects of supraoptimal temperatures on *Arabidopsis*.

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RESULTS AND DISCUSSION

Biotin, sucrose, and phosfon-S partially or completely prevented the detrimental effects of supraoptimal temperatures (Figs. 1–5 and Table 1). Biotin alone prevented the detrimental effects of supraoptimal temperatures at least in so far as growth was concerned. A promotion of growth as a result of biotin treatment was observed in both temperature regimes, but the degree of enhancement of growth compared with the respective untreated controls was much higher at supraoptimal temperatures. Four weeks after planting, the biotin-induced increase

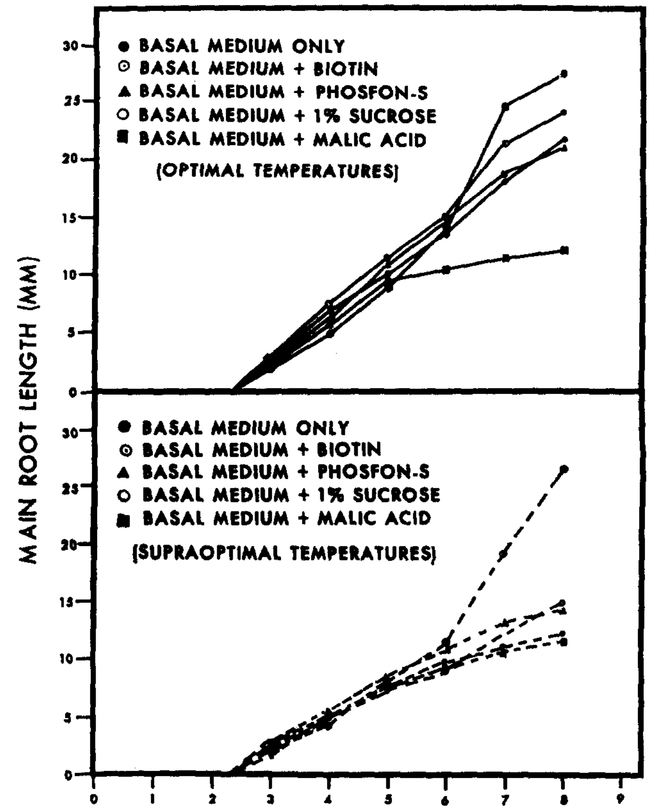

FIG. 3.

in fresh and dry weight over control plants grown under optimal temperatures was 38 and 15 per cent respectively, while under supraoptimal temperatures the corresponding increases compared to controls were 54 and 66 per cent respectively (Figs. 1 and 2). These results are in general agreement with those obtained by Langridge and Griffing[9] for the same plant, although their data were obtained at constant day and night temperatures (25° optimal and 31·5° supraoptimal).

Sucrose was more effective than biotin in preventing injuries caused by supraoptimal temperatures. The addition of 1 per cent sucrose to the medium increased the dry weight only 2 per cent over the controls under optimal temperatures while the increase under supraoptimal temperatures was 70 per cent 4 weeks after planting. Sucrose decreased the fresh weight under optimal temperatures (12 per cent) at the 4-week sampling period, while it increased the fresh weight 58 per cent above that of the controls at supraoptimal temperatures. The decrease in fresh weight at optimal temperatures was probably the result of an osmotic effect of the sucrose medium, which in turn affected the water uptake of the plants. At supraoptimal temperatures, in addition to increasing the fresh and dry weights, sucrose improved most of the other growth characteristics measured (Figs. 3–5 and Table 1).

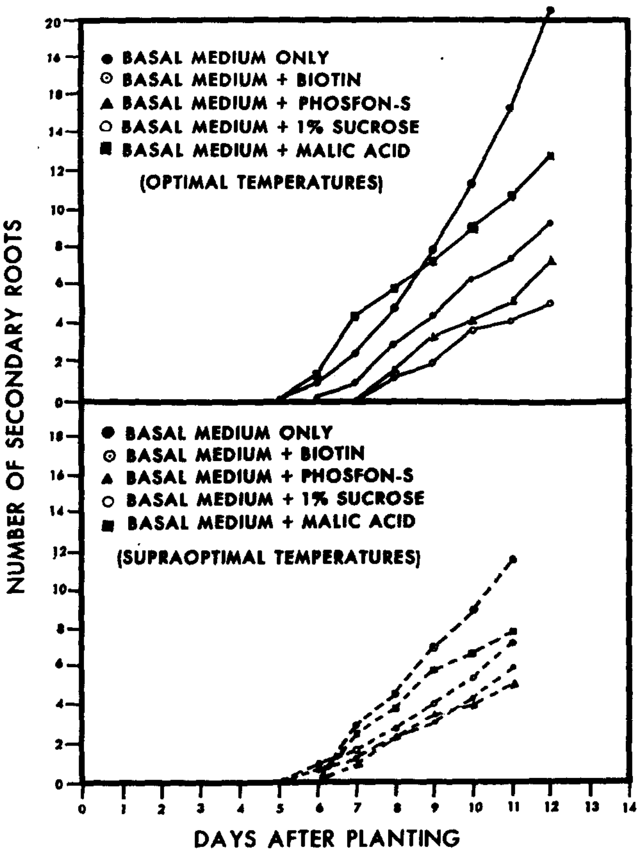

FIG. 4.

Prior to the selection of 1 per cent sucrose medium, an agar suspension containing 10 per cent sucrose, as was suggested by Ketallapper and Bonner,[7] was employed. However, seeds did not germinate at this high concentration of sucrose, presumably because of unfavorable osmotic conditions. To determine the most suitable concentration of sucrose, an experiment with agar media containing 1–5 per cent sucrose was conducted under both temperature regimes. The results obtained showed 1 per cent sucrose to be the most suitable concentration.

A possible mechanism of the protective action of sugars at supraoptimal temperatures has been suggested by Molotkovskii and Zhestkova.[13] They showed that application of

[13] Yu. G. Molotkovskii and I. M. Zhestkova, *Fiziol. Rastenii* 11, 301 (1964).

several sugars, especially sucrose, prevented a drop in the rate of respiration at supraoptimal temperatures. Stabilization of respiration under the influence of sucrose was sufficient to render the plants insensitive to the actions of respiratory inhibitors. They further showed that other sugars and even metabolically inert mannitol had similar effects, but to a lesser degree. In their view, sucrose also prevented the uncoupling of oxidative phosphorylation as shown by the accumulation of inorganic phosphate. Since it had been shown that mitochondria became swollen at supraoptimal temperatures with eventual dissociation of oxidative

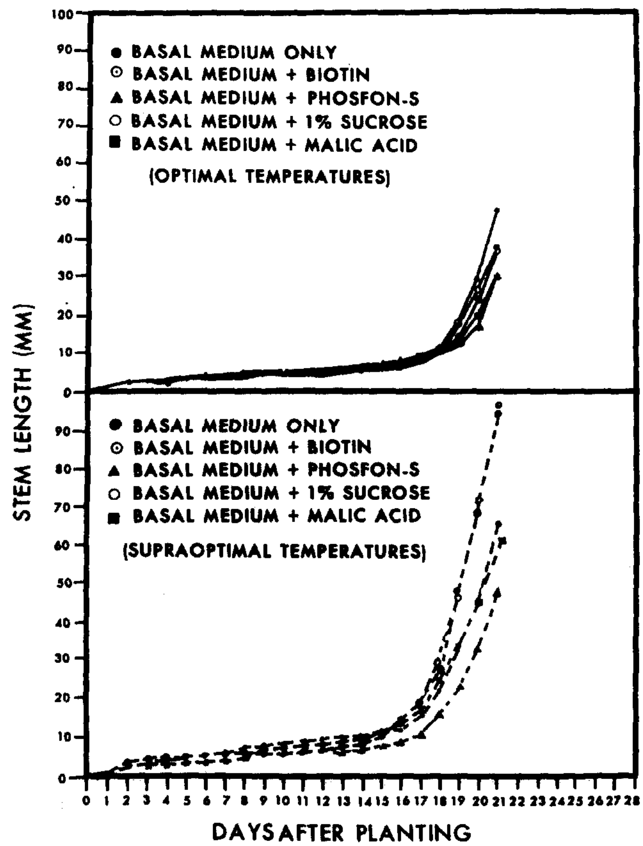

FIG. 5.

phosphorylation and respiration,[14–16] and because the sugars blocked the swelling of the mitochondria,[17] it was suggested[13] that sugars had a "conserving" effect on mitochondria.

Phosfon-S, although not as effective as biotin and sucrose, did produce a 27 per cent increase in the fresh weight under optimal temperatures and a 30 per cent increase above the controls at supraoptimal temperatures 4 weeks after planting. The corresponding increases in dry weight under optimal and supraoptimal temperatures were 9 and 16 per cent respectively (Figs. 1–5 and Table 1).

[14] N. S. PETINOV and YU. G. MOLOTKOVSKII, *Fiziol. Rastenii* 7, 665 (1960).
[15] YU. G. MOLOTKOVSKII, *Izv. AN SSSR, Ser. Biol.* 2, 246 (1961).
[16] YU. G. MOLOTKOVSKII, *Fiziol. Rastenii* 8, 699 (1961).
[17] A. L. LEHNINGER, *J. Biochem.* 49, 553 (1961).

The addition of malate to the medium reduced the fresh and dry weight of plants under both temperature regimes. It was interesting to note a striking effect of malate on the seed pods and seeds. Addition of this metabolite resulted in the production of visibly much larger seed pods and also an increase in both the size and the number of seeds in comparison to the controls at both temperatures (Table 1). Malate failed to prevent any of the other detrimental effects of supraoptimal temperatures on the growth of these plants, except the number of secondary roots. However, the sizes of the secondary roots in this case were smaller than the secondary roots of the plants treated with any other metabolites or those of the control plants (Fig. 4 and Table 1). Our results are largely in disagreement with those of Petinov and Molotkovskii.[8] They demonstrated a reduction in injuries caused by supraoptimal temperatures in sunflower, pumpkin, oats and corn by spraying the leaves of plants with organic acids, mainly citric and malic acids. Although malic acid as employed by Petinov and Molotkovskii was effective on several species, it is possible that this metabolite is not effective on *Arabidopsis thaliana*. In this regard, it has been shown that two species of the same genus or even two ecotypes of the same species may require the addition of different metabolites for prevention or reduction of the injurious effects of supraoptimal temperatures.[9] The supraoptimal temperature used by Petinov and Molotkovskii was extremely high (46°) and was more likely to cause destruction of proteins with a concomitant accumulation of ammonia and an eventual self-toxification of the plant. In their view, the applied organic acids probably formed the organic salts of ammonia or amides thus removing the NH_3 and eliminating one of the detrimental effects of the supraoptimal temperature. Under the temperature regimes selected for our experiments, the accumulation of ammonia in concentrations which could be toxic to the plant seems improbable since Anastasia[18] could not find any detectable amounts of ammonia in pea plants grown under the same temperature regimes. Although the improvement over the controls obtained with sucrose, biotin and phosfon-S were obviously significant, the t-test was applied and showed the results for dry and fresh weights to be highly significant (0·01 level).

EXPERIMENTAL

Plant Material and Growing Conditions

Arabidopsis thaliana plants were grown aseptically on an agar medium in large test-tubes in growth chambers set to provide either supraoptimal temperatures (32° day and 25° night) or optimal temperatures (25° day and 18° night). Light intensity was 33,000+2200 lux; day-length was 16 hr and night was 8 hr (total darkness). All the preparations for plant growth are described earlier.[19]

Chemical Treatments

Sucrose, biotin, phosfon-S (2,4-dichlorobenzyltributylammonium chloride, Virginia-Carolina Chemical Corporation) and malic acid were added to the agar growth medium in an attempt to prevent the detrimental effects of supraoptimal temperatures. A series of concentrations of sucrose (1–5 per cent) in agar suspension in test tubes was prepared, and in preliminary trials were tested for their effect on fresh and dry weight at supraoptimal temperatures. The 1 per cent level was selected as the most effective. Otherwise, standard procedures for handling seeds and seedlings were followed. Since phosfon-S, malic acid and biotin are unstable to heat, they were not mixed with the agar solution in the test tubes before autoclaving the agar. In the case of phosfon-S, the seeds were soaked for 24 hr in a solution containing 20 ppm of the material before they were transferred to the agar medium. After testing different concentrations of phosfon-S (1, 3, 5, 10, 20, 30 and 50 ppm), 20 ppm was selected as the most suitable concentration to retard growth. Stock solutions of biotin and malic acid were sterilized by passing the solutions through membrane filters (pore dia. 0·22 μ). Aliquots containing either 6 μg of biotin or 3·35 mg of malic acid were added to the sterile test-tubes just before solidification of the autoclaved and still warm agar suspension. The pH of the stock solution of malic acid was adjusted to 6·0 by addition of concentrated KOH before sterilization.

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