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BOTANY

THE EFFECT OF HIGH TEMPERATURE ON THE AMINO ACIDS IN PEAS [1]

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INTRODUCTION

The effects of high temperature on the free amino acids of the Alaska variety of *Pisum sativum* (L.) was studied.

Since the stage of development found by Lambert and Linck (1958) to be most severely affected by high temperature (i.e. 90° F. for six hours on five consecutive days beginning five days after full bloom) was close to the period of active protein synthesis as reported by Boswell (1924) and McKee *et al.* (1955), a study of the free amino acid content of the pods, peas, and vegetative parts of the plant was made. It was thought that if some enzyme system involved in amino acid metabolism were labile at this temperature, this study of the qualitative and/or the quantitative distribution of amino acids in the various plant parts might reveal if amino acid metabolism is upset and might suggest which enzyme systems are involved.

MATERIALS AND METHODS

24 seeds of peas were planted one inch deep in each of 20 boxes. 13 days after planting, the plants were thinned to 12 per box, and to 8 per box at full bloom. Prior to treatment all plants were maintained in a controlled environment room at 75° F. during a 12-hour light period and 65° F. during a 12-hour dark period. Five days after blooming, 9 of the boxes were placed in a high temperature cabinet and kept at 90° F. for six hours in the middle of the light period. This treatment was repeated for five consecutive days. At the end of the fifth day of heat treatment, the plants were harvested in the following manner: the pods (at the first bloom node) from 8 plants per box were opened and the peas counted (as normal, small, or vestigial). The normal and small peas from 8 plants were placed in separate tared containers, and the pods and foliage were each placed in separate tared containers. Foliage fraction includes the stem and leaves and is hereafter referred to as "foliage." Fresh weights were obtained and the like material from 3 boxes was composited (making three

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composite samples from the 9 boxes), placed in 70% ethanol (20 ml. alcohol/gram of fresh weight), macerated in a Waring Blendor for one minute, heated to boiling for 7 minutes, and kept for 24 hours at -4° C. The untreated check plants were harvested in exactly the same manner. The material was then filtered through filter paper under suction to remove the alcohol insoluble material. The solutions were then concentrated *in vacuo* at 40° C. to ten ml. and spotted on Whatman No. 1 chromatographic paper. The alcohol insoluble material was dried in a forced air oven at 80° C. for 30 hours, cooled and weighed. This dried, alcohol insoluble material is considered here as dry weight even though the alcohol soluble material is not present. Preliminary experiments were made to determine the proper amount of material to spot to obtain a good separation for each of the subsamples. It was determined that 50 μl of the concentrated extract from one mg. fresh weight was the desired concentration to spot for good separation of amino acids from the foliage while 25 μl from pods or 10 μl from normal or small peas was sufficient. Since different amounts of material were obtained from heat treated and check plants, the amounts of extract spotted were adjusted so that extracts from equal weights of material were compared. This was considered as the most logical comparison to make to determine whether any qualitative changes had taken place during the high temperature treatment. This comparison of extracts from equal amounts of plant material also would show whether any change in the size of the free amino acid pool had taken place. Two dimensional chromatograms were made of the amino acids in the alcohol soluble fraction using phenol-water in the first direction and collidine-lutidine-water in the second direction. Color was developed with ninhydrin.

RESULTS AND DISCUSSION

The fresh and dry weights of the various plant parts and the number of normal peas harvested immediately after a five day treatment (6 hours each day at 90° F. beginning 5 days after full bloom) indicate that yield is reduced as a result of the treatment. Both fresh and dry weight of the pods and normal peas were significantly lower for the heat treated plants when compared by the "t" test (Snedecor, 1950).

Table 1. THE EFFECT OF A SIX HOUR TREATMENT AT 90° F FOR FIVE CONSECUTIVE DAYS ON FRESH AND DRY WEIGHTS OF FOUR PARTS OF THE PEA PLANT [a]

Plant Part	Weight in Grams			
	Fresh Weight [b]		Dry Weight [c]	
	Check	Treated	Check	Treated
Normal Peas	1.6*	0.8*	0.3*	0.1*
Small Peas	0.15	0.19	0.02	0.02
Pods	6.1*	4.3*	1.3*	0.8*
Foliage	13.4	13.0	4.4	3.9

[a] Treatment was begun five days after full bloom. Plants were harvested ten days after full bloom.
[b] Fresh weight averages based on nine replicates of eight plants each.
[c] Dry weight averages based on three composite samples of twenty-four plants each.
* Differences between treated and check plants exceed the 1% level.

On the other hand the fresh and dry weights of the foliage and the small peas of the check plants were not significantly different from the treated plants (see Table 1).

Heat treatment reduced the number of normal peas and ovules and increased the number of vestigial peas. These differences were statistically significant. There was no significant effect on the number of small peas (see Table 2).

Table 2. THE EFFECT OF A SIX HOUR TREATMENT AT 90° F FOR FIVE CONSECUTIVE DAYS ON THE NUMBER OF NORMAL, SMALL, AND VESTIGIAL PEAS, AND THE TOTAL NUMBER OF OVULES FROM EIGHT PLANTS OF THE ALASKA VARIETY OF PEAS [a]

	Check Plants [b]	Treated Plants [b]
Normal Peas	13.6†	7.2†
Small Peas	11.0	11.8
Vestigial Peas	23.9*	26.0*
Ovules	47.4*	45.1*

[a] Treatment was begun five days after full bloom. Plants were harvested ten days after full bloom.
[b] Averages based on nine replicates of eight plants each.
* Differences between treated and check plants exceed the 5% level.
† Differences between treated and check plants exceed the 1% level.

Table 3 contains a listing of the amino acids tentatively identified by Rf and color obtained from the various plant parts of both check and treated plants and an estimate of their relative concentration. Note that the amide asparagine is present in small amounts in the peas and in larger amounts in the pods and foliage, confirming the reports of Hyde (1953, 1954). No differences were noted between the heat-treated and check plants when spotted in the described manner; how-

Table 3. A TENTATIVE IDENTIFICATION OF THE AMINO ACIDS AND AMIDES FROM THE ALCOHOL SOLUBLE FRACTION OF VARIOUS PLANT PARTS OF THE ALASKA VARIETY AS DETERMINED BY PAPER CHROMATOGRAPHY AND AN ESTIMATE OF THEIR RELATIVE CONCENTRATION.

Compound	Normal Peas		Small Peas		Pods		Foliage	
	TRT [a]	CK [b]	TRT	CK	TRT	CK	TRT	CK
Alanine	++++ [c]	++++	++++	++++	++++	++++	++++	++++
Aspartic acid	++	++	++	++	++	++	++	++
δ amino Butyric	++	++	++	++	++	++	++	++
Glutamic acid	+++	+++	+++	+++	+++	+++	+++	+++
Glycine	++	++	++	++	++	++	++	++
Leucines	++	++	++	++	++	++	++	++
Threonine	++	++	++	++	++	++	++	++
Valine	++	++	++	++	++	++	++	++
Asparagine	+	+	++	++	+++	+++	+++	+++
Glutamine	+++	+++	+++	+++	+++	+++	+++	+++

[a] TRT—Plants treated at 90° F for 6 hours on 5 consecutive days beginning 5 days after full bloom.
[b] CK—Plants kept at 75° F during that same period.
[c] Number of plus signs indicate relative concentration.

ever, more extract was spotted, in general, for the treated plants to compensate for the difference in fresh weight of material extracted. If the spotting were done on a per organ basis, differences in quantity but not of quality would have been found.

On the basis of these experiments, high temperature treatment given at this stage of development appears to affect the over-all amino acid metabolism of the pea plant rather than inactivating any one specific enzyme system; however, more work will have to be done to explain the effect of high temperature on peas in physiochemical terms.

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