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QUANTITATIVE DIFFERENCES IN ALANINE IN RICE PANICLES OF VARIETIES

Resistant and Susceptible to Kernel Smut

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In the course of selecting chromatographic methods for separation and identification of ninhydrin-positive components in rice panicle tissue, it was noted that the compliments of readily extractable amino acids from resistant and susceptible varieties are remarkably similar qualitatively (1). A few amino acids were present in trace amounts in only one variety but the most apparent differences between varieties of different disease reaction were in the levels of alanine, as indicated by size and density of the alanine spot. The concentrations of several other amino acids also varied from variety to variety but not so strikingly as alanine, and the separation of alanine from other spots on the chromatograms was considerably better than some of the other amino acids whose concentration also varied. Also alanine occurs in a relatively high concentration in rice panicle tissues. Therefore alanine was selected as the amino acid with which to begin the selection of a suitable quantitative paper chromatographic method for determination of levels of the various amino acids in resistant and susceptible rice.

This report gives the method selected for color development, elution and colorimetric determination of alanine from paper chromatograms and the differences between alanine content of field-grown rice plants resistant and susceptible to kernel smut.

Materials and Methods: The two varieties of rice used were Bluebonnet 50 and CI 9446, which are resistant and susceptible to kernel smut respectively. The plants were grown in the field at the University of Arkansas, Rice Branch Experiment Station, Stuttgart, Arkansas. Panicles were collected at anthesis, which occurred between 10:00 a. m. and 2:00 p. m. in August, 1961. The panicles were dried, ground, extracted and chromatographed by the procedure selected by Grable, Presley and Templeton (1). Phenol:Water was the solvent for separation in the first direction and n-butanol:acetic acid:water was used for development in the second direction. Color reaction was achieved by dipping the chromatograms in .2 percent ninhydrin in 95 percent ethanol and heating at 65-70°C for 30 minutes in an atmosphere made anaerobic with CO^2 bubbled through ethanol. After removal of the papers they were allowed to dry for one

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hour at room temperature and protected from direct light.

The purplish-blue alanine spots was outlined in pencil and cut out with a razorblade. The removed spots were cut into pieces approximately 1 cm x 1 cm and placed in a test tube. The pieces were washed two times for 1 minute each in five ml aliquots of 50% ethanol. The ethanol was decanted into calibrated colorimeter tubes, made up to 10 ml with 50 percent ethanol and read in the colorimeter at 570 millimicrons. All samples were run in triplicate and a portion of the paper containing no spots was cut out and eluted with ethanol to serve as a blank.

The concentration of alanine was determined by averaging triplicate samples and referring to a standard curve which was prepared from chromatograms of alanine ranging in concentration from 50 to 400 micromoles.

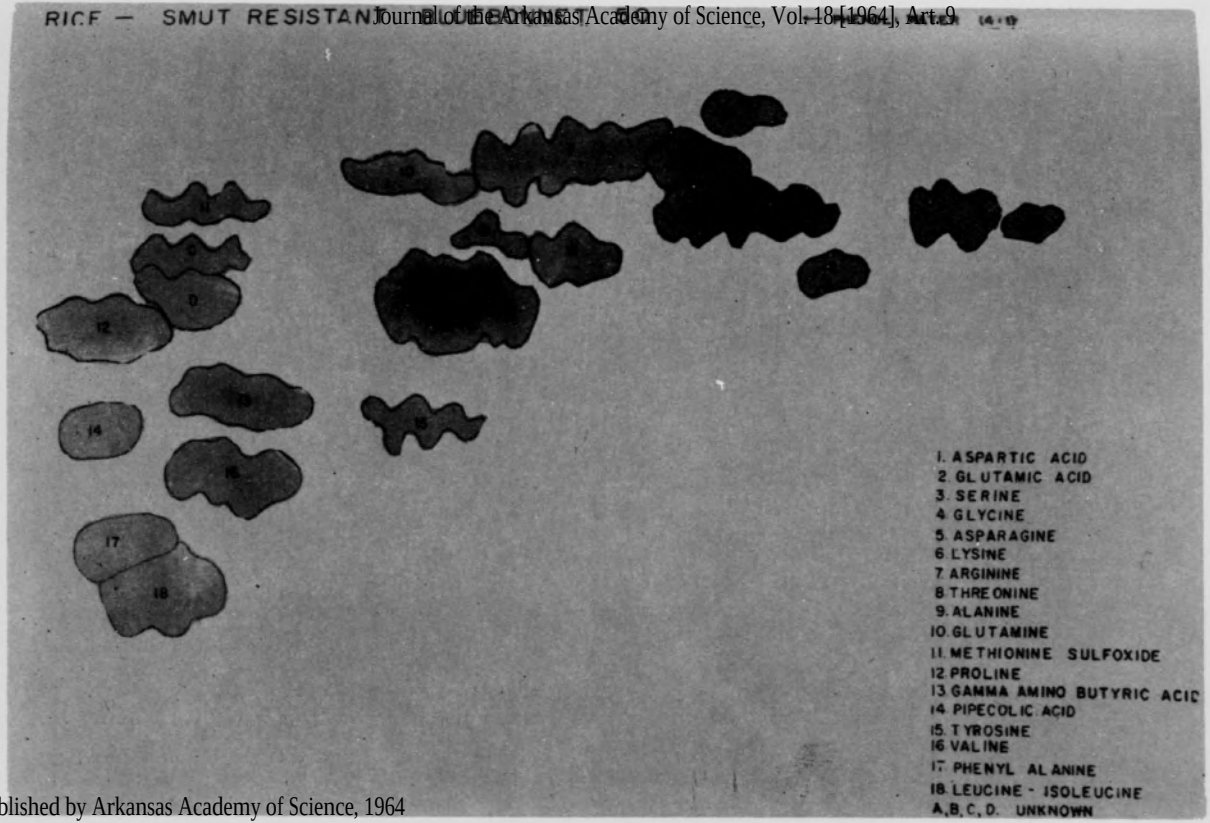
Results:

The chromatograms in Figures 1A and 1B show the relative size and density of the alanine spots from smut resistant and smut susceptible rice panicles. The concentrations of alanine extracted from the two varieties grown at high and low nitrogen fertility levels is in Table 1. The level of alanine in resistant plants was approximately double that in susceptible plants at comparable nitrogen levels. Although there was a consistent increase in alanine in both varieties with increased nitrogen fertilization, this did not prove to be satistically significant. The same results were obtained when data was analyzed with alanine levels expressed as percent of total amino nitrogen.

DISCUSSION, SUMMARY AND CONCLUSION

A suitable colorimetric method was selected for quantitative determination of alanine on paper chromatograms of amino acid mixtures. The method consisted of color development on paper with ninhydrin in an anaerobic atmosphere, elution of the spot from paper with ethanol, and reading absorbance at 570 millimicrons in a colorimeter. The method would be suitable for all amino acids separated on paper, provided that the spots were well separated and account is taken of the fact that color yield per mole for each amino acid is different. Either a standard curve for each amino acid must be made (using 440 millimicron for the yellow proline spot) or adjustment must be made using the experimentally determined color yields as reported by Moore and Stein (2).

The magnitude of the difference between alanine levels in the resistant and susceptible varieties, and the fact that several other of the amino acids in these varieties vary in concentration, suggest that it would be worthwhile to pursue quantitative determination of amino acids in other varieties of plants resistant


RICE — SMUT RESISTANT
1. ASPARTIC ACID
2. GLUTAMIC ACID
3. SERINE
4. GLYCINE
5. ASPARAGINE
6. LYSINE
7. ARGININE
8. THREONINE
9. ALANINE
10. GLUTAMINE
11. METHIONINE SULFOXIDE
12. PROLINE
13. GAMMA AMINO BUTYRIC ACID
14. PIPECOLIC ACID
15. TYROSINE
16. VALINE
17. PHENYL ALANINE
18. LEUCINE - ISOLEUCINE
A, B, C, D. UNKNOWN

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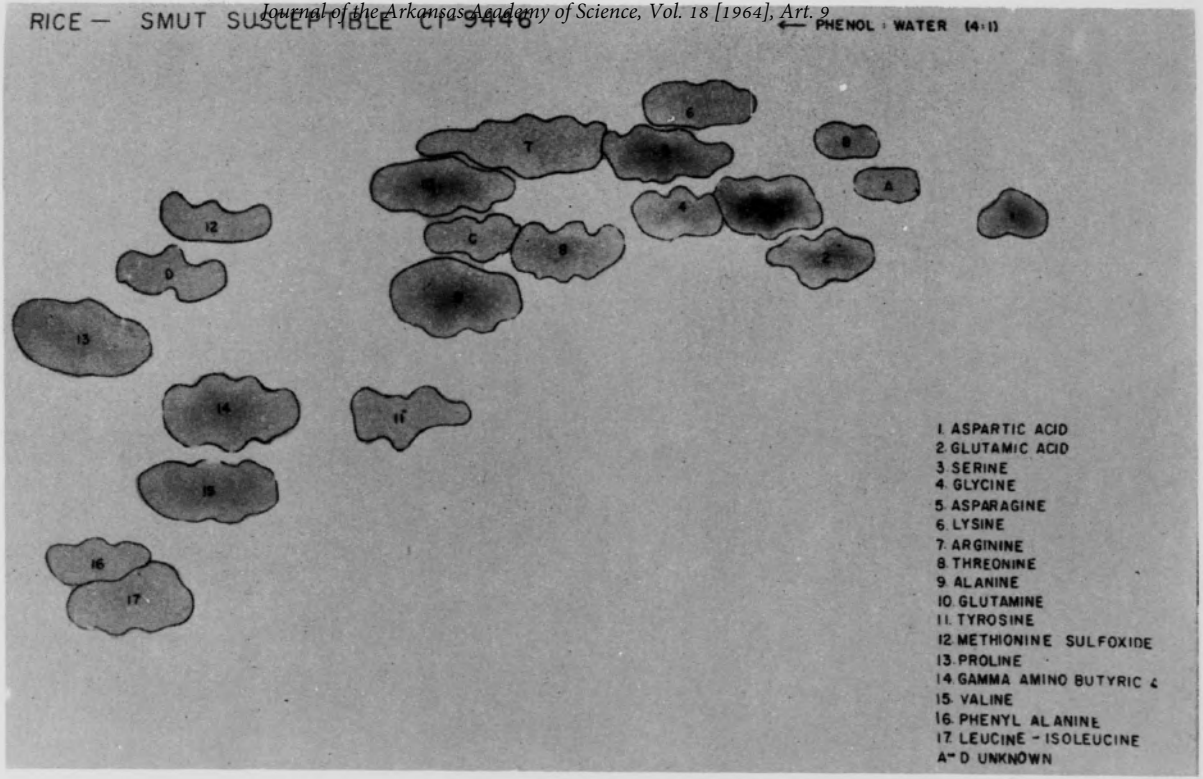
RICE — SMUT SUSCEPTIBLE CI 3446
← PHENOL : WATER (4:1)
1. ASPARTIC ACID
2. GLUTAMIC ACID
3. SERINE
4. GLYCINE
5. ASPARAGINE
6. LYSINE
7. ARGININE
8. THREONINE
9. ALANINE
10. GLUTAMINE
11. TYROSINE
12. METHIONINE SULFOXIDE
13. PROLINE
14. GAMMA AMINO BUTYRIC &
15. VALINE
16. PHENYL ALANINE
17. LEUCINE - ISOLEUCINE
A - D UNKNOWN

and susceptible to this plant pathogen. Since nitrogen fertilization apparently influenced the alanine level and since nitrogen fertilization was reported to influence severity of smut (3), the actual relationship of these factors to disease development must be studied further and under more controlled environmental conditions.

TABLE 1

Alanine in Resistant and Susceptible Rice Panicles Grown at Two Nitrogen Fertility Levels.

		Alanine in Panicles (Millimoles/Gram Dry wt.)				
		Replications				
Variety	Nitrogen level*	A	B	C	D	Ave
Bluebonnet 50 (Res.)	High	6.4	14.3	13.0	8.8	10.6
	Low	8.7	6.6	9.7	8.2	8.3
CI 9446 (Sus.)	High	4.1	6.0	5.3	3.8	4.8
	Low	3.1	4.5	3.0	4.2	3.7

*High—200 lbs. actual Nitrogen per acre.
Low—80 lbs. actual Nitrogen per acre.

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