

LETTERS TO THE EDITORS

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Immunological Methods in Plant Taxonomy

THE application of immunological analysis to plant taxonomy has been made practicable by the development of gel-diffusion methods, both by the Elek-Ouchterlony technique¹ and by the more recent immuno-electrophoretic technique of Grabar and Williams². By these means the individual proteins in plant extracts may be differentiated and the extracts compared component by component with those from related species. We have applied a modification of these general methods to the investigation of the genus *Solanum*, with particular reference to the interrelationships of certain Mexican species of potato, and the comparison of these with *S. tuberosum*, the domestic potato.

Thin slices of tuber of each species were soaked in sodium hydrosulphite (0.7 per cent solution) for 30 min., rinsed in distilled water and the sap expressed by crushing the slices, supported within folded strips of moist calico, through stainless steel rollers. Antisera were raised in rabbits to crude saps from *S. tuberosum* and *S. ehrenbergii* (a Mexican species), using a combination of Freund's adjuvant technique³ with courses of intravenous injections. Agar containing veronal buffer (pH 8.5) was poured to a depth of 2 mm. on lantern slides in 'Perspex' frames; in the agar, holes were cut with a cork-borer to hold the tuber extracts to be compared, and a trough at a distance of 1 cm. to hold the antiserum. The reagents were allowed to diffuse towards one another and lines of precipitate formed, each line corresponding to an antigen-antibody system. Where lines from different extracts joined end to end they were assumed to belong to identical or cross-reacting proteins. In some cases a much improved separation of the lines was obtained by subjecting the antigen to a short (4-hr.) electrophoresis before adding the antiserum (Fig. 1).

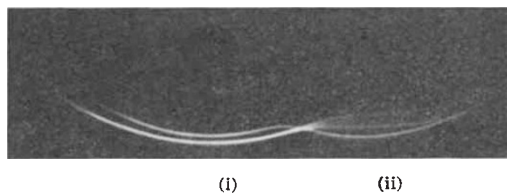


Fig. 1. *S. tuberosum* reacting with homologous antiserum. (i) Without electrophoresis the antigen shows only two lines of precipitate; (ii) with electrophoresis, one of the lines has been split into three, and their relative positions changed.

With both antisera, well-marked cross-reactions occurred between all the forty species examined; but by comparison of the 'line spectra' and by cross-absorptions of the antisera, it was possible to divide the species into well-defined groups.

By using *S. tuberosum* antiserum, the fourteen Mexican species were separated into three divisions (Fig. 2a) and then by the use of the antiserum against *S. ehrenbergii* two of these could be further subdivided, giving a total of five groups (Fig. 2b). Each group, with the exception of one, corresponded to a particular taxonomic series according to the classification proposed by Hawkes⁴; the one exception (Group 4) contained species from two series. The taxonomic series corresponding to the groups mentioned here are as follows: Group 1. Series *Morelliformia*. Group 2: Series *Pinnatisecta*. Group 3: Series *Cardiophylla*. Group 4: Series *Demissa*, and *Longipedicellata*; *S. tuberosum* and other South American species. Group 5: Series *Bulbocastana*.

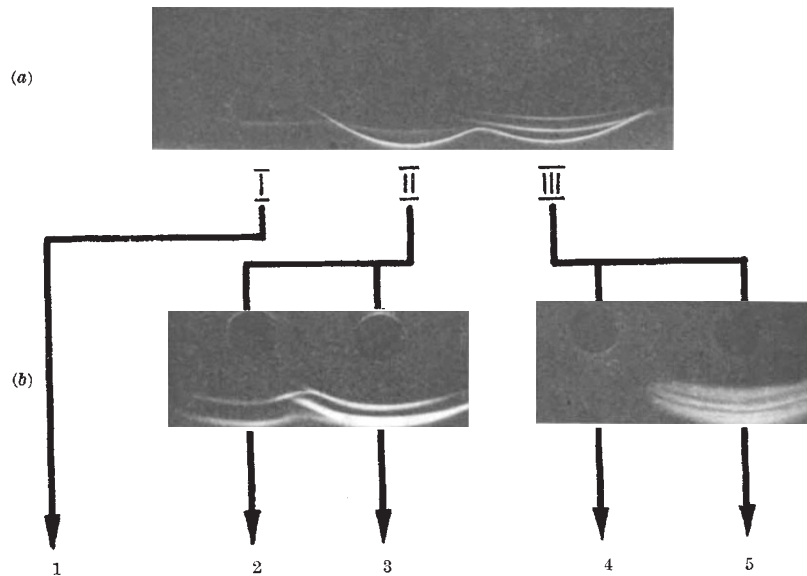


Fig. 2. (a) Separation of the species into three divisions with *S. tuberosum* antiserum. (b) The splitting of group II into two by means of unabsorbed *S. ehrenbergii* antiserum and of group III into two by means of the same antiserum absorbed with *S. tuberosum* extract.

These results will be published in detail elsewhere. The work is being carried out mainly under a grant from the Nuffield Foundation.

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¹ Elek, S. D., M.D. thesis, Univ. London (1948); *Brit. Med. J.*, i, 493 (1948). Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, 25, 186 (1948).

² Grabar, P., and Williams, C. A., *Biochim. Biophys. Acta*, 17, 67 (1955).

³ Freund, J., and Bonanto, M. V., *J. Immunol.*, 48, 325 (1944).

⁴ Hawkes, J. G., in Kappert, H., and Rudolf, W., "Handbuch der Pflanzenzüchtung", 2nd edit., 3, Chap. 1, Potatoes (Paul Parey, Berlin) (in the press).