## Iron Alum as a Mordant in Cytology of Uredinales

The cytology of fungi has remained relatively unexplored, as the vegetative nuclei are too small to define with precision details of nuclear structure and divisional figures. Information on these is primarily based on the study of meiotic division of the diploid nucleus during germination of the resting spore (zygote cell). The chromosomes often show a weakly staining reaction with the usual aceto-carmine methods and seldom stain deep enough for analytical study of configurations of the chromatin material. Addition of a ferric salt hardly improves the stain density. Thus, choice of suitable stains and staining techniques to bring out critical details has been an important factor, as the stain reactions have often been incomplete, uncertain or erratic. Use of iron alum as a mordant is well known in the advanced and elaborate procedures for staining the nuclei in the higher plant cells. However, not infrequently these have been unsatisfactory and inadequate for critical cytological studies in fungi and other microorganisms. Interpolation of iron alum solution of a suitable strength for staining the nuclei in *Chlorophyceae-Conjugales*[1] and *Volvocales*[2] and in *Rhodophyceae*[3] has been successfully employed in the aceto-carmine squash technique. A similar procedure briefly described here proved very satisfactory in the cytological studies of the rust fungi Uredinales as well.

The teliospores were stuck onto the slides by alternately wetting and drying the smears and the slides inverted over wet cotton towelling for moisture condensation as described by THIRUMALACHAR et al.[4,5]. The spore germination time varied with different rust species depending on the dormancy period. Germinating teliospores at a desired stage were fixed in situ in modified Carnoy's fixing fluid (6 rectified alcohol: 4 glacial acetic acid: 4 chloroform) for 1 h and transferred directly to 70% alcohol. The slides bearing the spores were brought down to distilled water through 4 descending alcohol concentrations with 5 min in each grade and washed thoroughly with 2–3 changes in distilled water. Immersion of the smears in 0.5% aqueous Taka-diastase (Parke Davis (India) Ltd., Bombay) for 10 min helped clarify the cytoplasm[6]. A dip in distilled water removed the enzyme residue over the smears and they were upgraded back to 70% alcohol through the same alcohol series. The slides may be retained at this stage for any length of time desired without impairing the stainability of the nuclei. The slides were directly transferred to the mordant 0.5% aqueous iron alum for 2–5 min. Concentration of the mordant and time for mordanting would vary with the fungal species. They were thoroughly washed in distilled water. Care was taken not to leave any traces of the mordant on the smears which crystallized the stain or stained the nuclei dark, masking structural details. The spore mounts were flooded with 0.5% carmine in 45% glacial acetic acid and covered as usual. Immediate observation facilitated analysis of nuclear details as the stain darkened gradually with lapse of time. The technique yielded excellent results with a variety of other fungi as well.

*Zusammenfassung*. Es wird ein Beizverfahren beschrieben, das sich bei der Färbung von Kernteilungsfiguren von keimenden Rotpilzsporen bewährt hat.

U. P. SINGH and M. S. PAVGI

*College of Agriculture, Banaras Hindu University, Varanasi (India), March 6, 1966.*

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## Table of Replaceable Amino Acids in Proteins

In looking for general laws governing the development of primary protein structure[1], some amino acids in peptide chains were found to be frequently replaceable[2]. The regularities observed were expressed in the form of a chart of amino acid interchanges[3] (summarized in [4] and [5]). On this basis it was possible, as early as 1962, to construct 8 variants of codons[6] of the first two bases in triplets, including their sequence. Variant No. 6 (8) (Table I, ref. [6]) corresponds to the presently accepted codons, with the exception of that for methionine.

At present it is possible to make exact the previously published tables of amino acid interchanges[3,5,6] and to extend them on the basis of existing knowledge of the nucleotide sequence in a degenerated code[7,8]. A practical form in this connection is that of a chessboard, as shown in the Table. It is constructed on the assumption that in codon triplets with a sequence[7,8] only a single base will be interchanged for another. The Table differs from the spatial design of VERSTEEG and VLIEGENTHART[9] not only in the discrepancies in a number of interchanges, since more recent codons are being used here, but also in the ease of application. The chessboard form makes it possible to find readily all the interchanges of a given

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