MATERIALS AND METHODS

Anatomy

Anatomical work on the various species was limited to the leaves, shoots and caryopses since it is mainly these organs which afford information of taxonomic value. The material utilized was taken from herbarium sheets, but wherever possible fresh material was used to check on results obtained with the dry material. The dry material was first soaked in a mixture of equal parts of 50 per cent alcohol and pure glycerine for a few days, washed in water, and the silica removed by immersion in 25 per cent hydrofluoric acid for about 1 hour. Sections of the leaf-blade and shoots were cut by hand in 70 per cent alcohol, other methods having proved to be either too time-consuming or not very successful due to the hardness of the material. After sectioning, the material was taken down to water in two stages, 50 per cent, 25 per cent alcohol, and then transferred to full strength commercial " Javel " for a minute or two, which had the effect of bleaching the cell contents and stretching the sections. After wasning thoroughly, the sections were stained by the double-stain technique developed by Evans (1949, p. 191), and made permanent by the usual procedure using Canada Balsam as mounting medium. For determining the distribution of chlorenchyma in the leaf, the bleaching in Javel was omitted, but otherwise the same procedure was followed. Epidermal preparations were made by placing a leaf-blade with its lower epidermis in contact with a glass slide, and scraping away the tissue until only the lower epidermis remained. Prior removal of the upper epidermis, followed by a gentle maceration in " Javel " for about 30 minutes, not only facilitates scraping but results in cleaner preparations. Staining with safranin yielded good results. Caryopses were sectioned by freezing microtome. In order to orientate the grains, a small thin square of agar was placed on the holder and the grain manipulated into the desired position, i.e. with the grain lying on its side and the hilum parallel to the surface of the agar. Thus orientated on, and held in position by the agar, the grain may be frozen and sectioned. By this method reasonably good longitudinal sections of the embryo wree obtained. The sections were double-stained with safranin and haematoxylin (after Evans, 1949, p. 191) dehydrated and permanently mounted in Canada Balsam.

The silica inclusions of leaves of *Aristida* and *Stipagrostis* species were prepared for study by boiling leaves in concentrated sulphuric acid, while adding drops of nitric acid, until all organic matter was dissolved, i.e. until the liquid became quite clear. This liquid was subsequently centrifuged, the acid poured off, and the residue washed in order to remove the acid. Permanent mounts of the silica residue in Euparal were microscopically examined and photographed, under phase contrast.

For all material used in the preparation of drawings the collector's name and number is cited in the relevant text. Except where otherwise stated all specimens used are preserved in the National Herbarium, Pretoria (PRE).

KARYOLOGY

Only root tip material was used for determining the chromosome numbers. This was collected mainly from plants growing under natural conditions. The best results were obtained with root tips gathered on sunny days from vigorously growing plants, between the hours of 10 a.m. and 1.00 p.m., one to two days after soaking rains had fallen. Tips from thick young roots 1–3 cm long were found to be superior to those from older and longer roots. Due to the toughness of even young roots, squashing techniques proved to have no advantages over the standard techniques of embedding in wax and sectioning. Sections were cut at a thickness of 14μ. The best fixative was found to be Randolph's chromo-acetic formalin (CRAF) (Conn & Darrow, 1946, p. 1_2–12). Stockwell's modification of Flemming's Triple Stain gave good results (Stockwell, 1934, p. 121).

Well-spread metaphase plates were drawn with the aid of a Camera Lucida and for each plant investigated several counts were made.

Herbarium specimens of all the individuals examined karyologically are deposited in the National Herbarium, Pretoria (PRE).