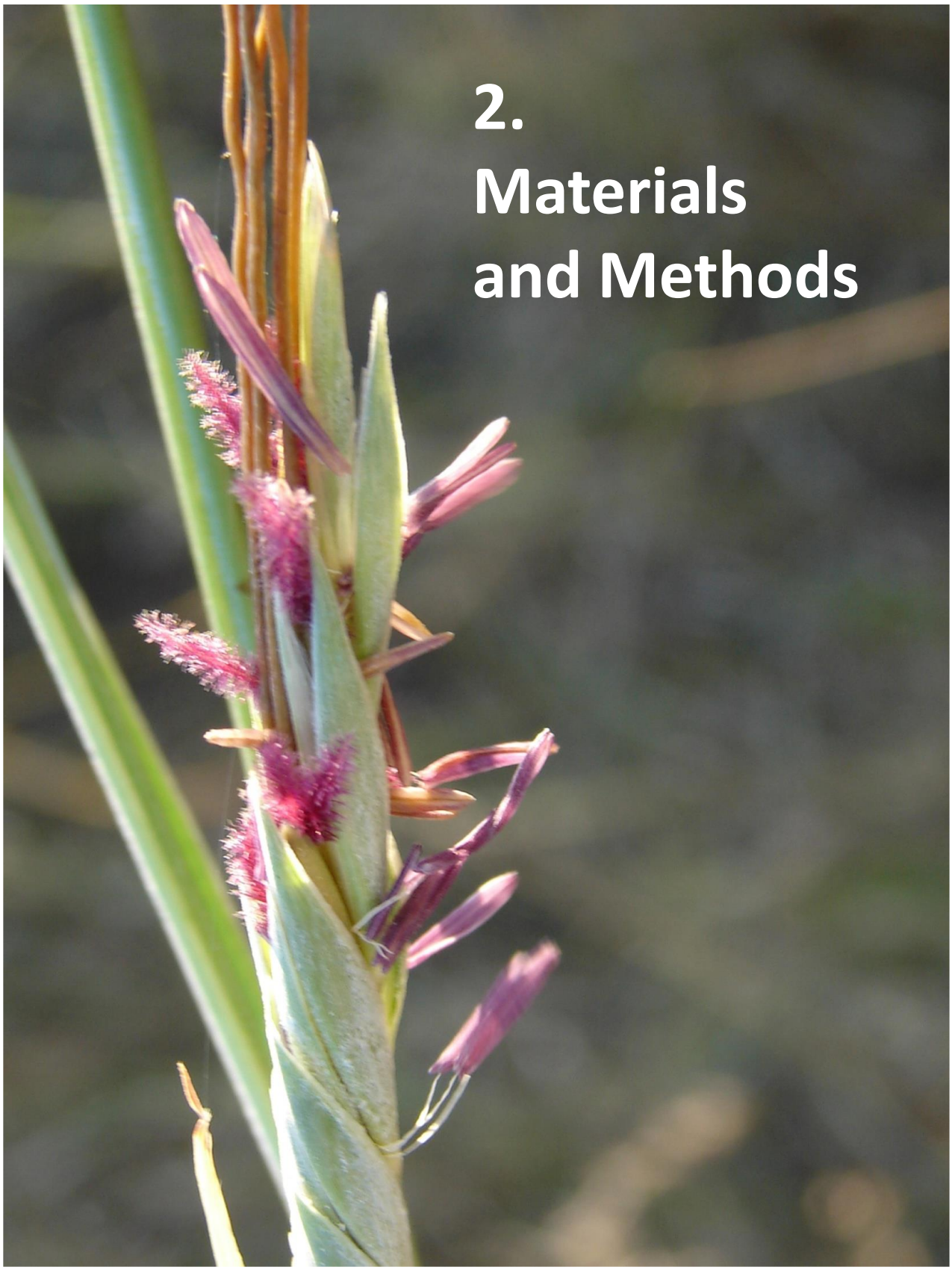

2. Materials and Methods

Leaf and Culm Anatomy:

For anatomical study grass species collected from the field were thoroughly washed and the different parts were separated and fixed in FAA (Formalin: Acetic acid: Alcohol) for further processing.

A leaf in grass can be distinctly differentiated into blade, sheath and ligule. Different parts of the leaf viz. leaf blade, leaf sheath and ligule (the joint of the leaf blade and leaf sheath) were prepared for the study of internal features. About 3-4 mm small pieces of samples were put in FAA for 48 hrs. Fixed samples were then washed 2-3 times with regular water and transferred to 3% commercial hydrofluoric acid for 24 hours or more depending upon the nature of the material for removing silica. Excess amount of hydrofluoric acid was washed in running water for 12 – 18 hours and transferred to 70% alcohol. The washed samples were dehydrated in tertiary butyl alcohol series (TBA series), embedded in paraffin wax (56ºC – 58 ºC). Paraffin blocks were cut and fixed on the wooden blocks and trimmed. 12 - 15 µm sections were obtained on Rotary microtome and slides were prepared for staining. Prepared slides were stained with Saffranin and Fast green and mounted in DPX (Di thalate xylene).

Caryopses Anatomy:

Mature spikelets were collected and stored in polythene bags and brought to the laboratory. Caryopses were carefully separated out from the glumes with the help of dissecting microscope (especially the smaller ones). The collected grass caryopses were immersed in distilled water for 15 – 20 days with few drops of formalin to prevent microbial attack, depending upon the nature of the caryopses for the softening. Soaked caryopses were then fixed in FAA (Formalin: Acetic acid: Alcohol) for 48 hrs., washed 2-3 times with tap water and then dehydrated in tertiary butyl alcohol series (TBA series). Embedding was done with paraffin wax (56ºC – 58 ºC). Wax blocks were cut and fixed on the wooden blocks and trimmed. The trimmed paraffin blocks were exposed and immersed with exposed surface of sample in 50% glycerine for 20 – 25 days to soften the tissue. 12 - 15 µm sections were obtained on Rotary microtome and stained with Toludene blue and mounted in DPX (Di thalate xylene).

Dichotomus Key:

Dichotomous key was prepared as per it described in Volume I.

At the end of the species description of a single genera key to identify them are also provided. A dichotomous key differentiating the 100 species on the basis of leaf anatomical characters has been represented at the end of the discussion.