TECHNIQUES OF PLANT CYTOGENETICS

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The second edition of the "Manual of techniques for the use of cytology and cytogenetics laboratories" which has been originally published in 1984 and recently extended of the new techniques elaborated by the INRA working group members for "Cytology and Cytogenetics" of INRA Versailes. Although the authors' attention is preferentially focussed on the chromosomes, the methods of viability testing and observation of reproductive structures of pollen grains and embryo sacs are described as well. In addition a small section of the book has been devoted to the production of interspecific hybrids via in vitro cultivation of fertilized ovaries and embryos. The chapter is logically followed by the part dealing with the methods of obtaining the polyploids as well as with criteria applicable in ploidy level estimation.

Being the cell's structures of paramount genetic importance, the chromosomes are treated in the first two chapters using classic approaches of acetocarmine, Schiff's reagent and DAPI staining as well as the complementary techniques of Giemsa and fluorochrome banding. The details of both these approaches are given for 18 species or the whole families of plants with special reference to chromosome number determination, chromosome morphology description and karyotype construction. Except for the basic principles of chromosome analysis, the differential aspects of the method are also illustrated which respect the diverse nature of such a variety of cells, tissues and organs as are those represented by the protoplasts, mycelium, callus tissue, embryoids, embryos, root and leaf meristems, respectively. As a novel aspect of this part of chromosome study, the application of ∝-bromonaphtalene in the pretreatment step instead of the classical colchicine is worth mentioning.

The six steps which are very common in monitoring the chromosome behaviour during meiosis are detailed with respect to the excision, finding the stage, fixation, storage, mounting and observation, respectively. The observation is not restricted to the meiotic stages only with compact and clearly visible chromosomes, but even the technique is described which allows the study of chromosome pairing during prophase.

In comparison with the conventional methods of karyological analysis, the banding techniques combined with in situ hybridization approach reflect the progress which has been made since 1970 in further discrimination of structural integrity and differential functional activity of individual chromosomes. A very detailed account has in this connection been provided for individual steps of in situ hybridization the resolving power of which has reached even the level of DNA nucleotide sequences. Therefore, it may be looked upon as a new and very efficient tool in chromosome mapping. The potential of both these approaches is illustrated on the examples of cereals, *Allium, Petunia, Hordeum* and *Nicotiana* spp., respectively. The autoradiography and cytophotometry represent the final chapters within the context of the complementary techniques of chromosome and DNA study aiming at precise localization of very low quantities of target substances in tissues, cells or organelles as well as in precise measurement of these substances in relatively large quantities (nuclei). The methods described so far are of universal use, applicable for all plant species.

The reproductive structures of plants are dealt with separately in a chapter devoted to the pollen and embryo sac in which a wide range of agriculturally important species and several fruit tree species are involved. There is mention in the introductory comment on determination of developmental stage of pollen but this aspect of pollen biology has in fact been analyzed only partially, referring to the tetrade level , the stress being laid preferentially on the determination of fertility of mature pollen. Both the vital staining of dormant pollen grains and in vitro germination tests have in this connection been described in detail using Alexander's solution, methylene blue, fuchsin, acetocarmine, fluorescein diacetate and DAPI stains in the former and agar media of different composition in the latter.

The *in situ* germination of pollen, as re-vealed by the aniline or toluidine blue in the styles, is presented in this part of the book as well. Methodologically remarkable aspect of in vitro pollen germination is the analysis of chromosome behaviour during microgametoge-nesis in pollen tubes by means of their contraction with the colchicine pretreatment agent applied directly into the agar medium. In order to allow an easy observation of the whole complexity of embryo sac, only clearing techniques are described including the observation under light field, phase contrast, Nomarski's interferential contrast, and under inter-ferential control, respectively.

The possibilities of overcoming the incompatibility barriers in some interspecific hybrids of *Malus, Prunus* spp., primary triticale, tomato, pepper and egg plants by means of in vitro cultivation of fertilized ovaries and young embryos are treated in the final part of the book. The last chapter on Polyploidization deals with the methods of obtaining of polyploids via colchicine treatment of buds and rooted cuttings as well as by means of utilization of the somaclonal variability phenomenon in cultivated calluses. The efficiency of polyploidization is tested not only by the direct chromosome counting but also by the estimation of such cytological characteristics as are the length of quard cells of the stomata, number of chloroplasts, number of nucleolar chromocentres, number of stomata and size of stomata, pollen grains and foliar lamina, respectively.

As a whole, the book shares all the features which are typical of the manuals covering most of the light microscope techniques currently used in genetic investigation of plant species. Each of its five chapters is introduced by a brief comment on the respective problem followed by an exhaustive description of the corresponding practical steps to cope with it. With a strict orientation on the chromosomes and reproductive structures of plants, the book is expected to address preferentially those who are concerned with basic research of the structures mentioned above.

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