BANDING TECHNIQUES AND PLANT CHROMOSOMES

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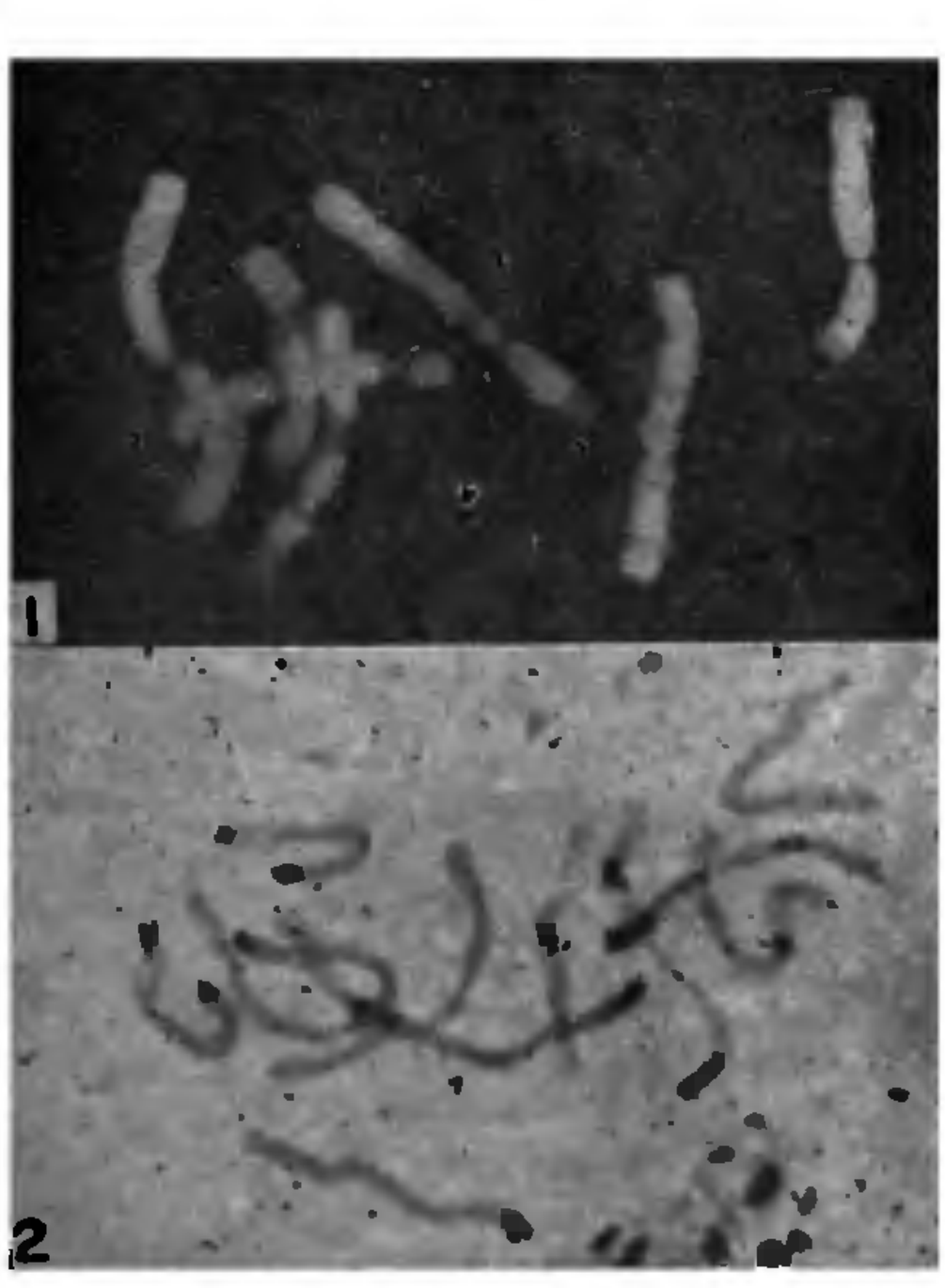
THE introduction of banding techniques in chromosome studies has made two significant contributions, (*i*) individual chromosomes or even part of a chromosome can now be precisely identified, (*ii*) these techniques have opened altogether new avenues of research on structure and molecular organisation of chromosomes. A breakthrough in the development of the technique of banding chromosomes came from the pioneering studies of Caspersson *et al.*[1], that fluorescent dyes such as Quinacrine Mustard (QM) effect selective, discrete fluorescent labelling in both plant and animal chromosomes. The binding specificity of these fluorochromes and intense or reduced fluorescence at localised regions of chromosomes giving a banded appearance is known as fluorescence banding or more specifically Quinacrine banding (Q-banding; Fig. 1). The other banding technique that employs Giemsa stain is the outcome of the studies made by Pardue and Gall[2], who observed denser centromeric regions of chromosomes of mouse complement after *in situ* hybridization with complementary RNA of the mouse satellite DNA and subsequent Giemsa staining. Following this observation, a staining method with Giemsa was developed for the detection of repetitive DNA which is richly localised in the centric regions of the chromosomes[3,4]. The method consists of denaturation of chromosomal DNA of the metaphase preparations by heat or alkali treatment, reannealing it in a suitable buffer, followed by Giemsa staining (Fig. 2). The specific staining of centromeric regions of the chromosomes this way is called as C-banding (staining centromeric type of constitutive heterochromatin). Soon it became apparent that by employing a number of modifications it is possible to obtain differential staining of the constitutive heterochromatin in the chromosome arms (G-banding[5]). Through minor changes in the basic theme of denaturation and incubation prior to staining and use of proteolytic enzymes such as trypsin, many simplified techniques have been developed mostly for mammalian and human metaphase chromosomes[6-17]. This led to the proliferation of nomenclature of the banding techniques, *viz.*, R-banding[12] (reverse Giemsa banding), BSG technique[13] (barium hydroxide/saline/Giemsa), N-banding[16] (nucleolus organiser), cd staining[17] (centromeric dot) and in plants pericentric banding[18] and Hy-banding[10].

Though the initial report on banding with QM was from *Vicia faba*, the subsequent major developments in the methodology of the staining techniques stem from the experiments with the mammalian chromosomes. The ease with which the mammalian metaphase chromosome preparations can be made seems to be the primary reason for this success. Progress in this line of investigation in plant chromosomes is limited. The use of fluorescence for the detection of heterochromatic regions of the chromosomes was attempted in several plants[20-25]. For example, Vosa[20] has shown that there are several types of heterochromatin in plants as defined by allocyclic behaviour and these can be distinguished by their negative or positive sensitivity to cold and by their response to fluorochrome staining. A Giemsa staining technique is outlined by Vosa and Marchi[21] who compared the Giemsa banding with the pattern produced by Quinacrine



FIGS. 1–2. Fig. 1. Somatic chromosomes of *Scilla sibirica* stained with Quinacrine Mustard (Q-banding). The heterochromatic segments correspond to the regions of dull fluorescence (–). Fig. 2. Same, but stained with Giemsa. The deeply stained regions correspond to the regions of reduced fluorescence with Q-banding.

fluorescence in a number of plants with fairly large chromosomes. In all the cases, they observed a close correspondence between the regions darkly stained with Giemsa and those differentiated with Quinacrine. However. the important difference found was that the Giemsa staining does not discriminate between regions with intense and reduced fluorescence with Quinacrine, but stains both in the same way. Schweizer[26] developed a suitable procedure of Giemsa staining for plant chromosomes with a view to study the extent to which Giemsa bands could be correlated with heterochromatic (H)-regions revealed by cold treatment in *Trillium grandiflorum, Scilla sibirica, Vicia faba, Crepis capillaris* and three species of *Fritillaria.* The chromosome segments that stained strongly with Giemsa were shown to be identical with H-regions revealed by cold treatment in all the species studied. In *Crepis capillaris,* he observed, that Giemsa technique to be more sensitive than Quinacrine fluorescence in revealing the longitudinal differentiation of chromosomes. That there is a close correspondence between the regions of the chromosomes that stain darkly with Giemsa or take up bright or dull fluorescence to the heterochromatin was evident in all these investigations. However, this was questioned in the light of findings of G-banding in mammalian chromosomes[6-9] and also in a specific instance in plants[27].

The specific banding pattern produced by these techniques are of immense value in (*i*) identifying the individual chromosomes as has been shown in *Petunia hybrida*[28], rye[29-31] and *Triticale*[32], (*ii*) the detection of structural changes in reconstructed karyotypes of *Vicia faba*[33] and (*iii*) studies in chromosome polymorphism in plant populations as reported in *Scilla sibirica*[22]. There are two illustrious examples to show how these banding techniques could be of specific use in plant cytogenetical investigations. Sarma and Natarajan[29], using a new fluorochrome compound, *bis*-Benzimidazole derivative (Hoechst 33258) characterised the individual chromosomes of rye. The localisation of heterochromatin in the telomeres of the rye complement and its absence at the centromeric regions, as observed by these staining techniques, made possible the identification of chromosome complement of rye in the *Triticale.* Similarly, Natarajan and Sarma (in press) made a complete genome analysis in the hexaploid wheat, *Triticum aestivum* ($2n = 6x = 42$) using Giemsa banding pattern. Of the three genomes of wheat A, B and D, the source of B genome is controversial. The different genomes of hexaploid wheat and its close relatives are composed of chromosomes with median and sub-median centromeres which offer no distinctive features of morphology to identifying them[34]. Giemsa banding of these wheat chromosomes unravelled some morphological details, unobtainable through conventional staining. Some of the chromosomes of the wheat complement showed large, distinct darkly stained regions with Giemsa, located proximal to the centromere. The other chromosomes, however, do not possess such large blocks of centromeric heterochromatin but have heterochromatic regions in the form of bands either sparsely distributed or dispersed at the interstitial and terminal regions of the chromosomes. With such details gathered from the Giemsa banding pattern in wheat, it was possible for them to throw more light on the chromosome characteristics of the B genome. In view of the recent divergence of opinion regarding the validity of *Aegilops speltoides* being the B genome donor[35,36], such a study might help in tracing the B genome donor species.

Giemsa staining technique is also used to specifically stain the telomeres of *Allium cepa* chromosomes, both at metaphase and interphase[37] and in the study of karyotypic differences in some species of *Anemone* and *Hepatica nobilis*[38]. Some modifications in the basic procedure of banding were suggested in plant chromosomes. These include use of acetic-orcein in the place of Giemsa[39], digestion with trypsin prior to Giemsa staining[40]. More recently, Schweizer[41] reported an improved Giemsa C-banding procedure for plant chromosomes. These are some examples where banding techniques were applied in plant chromosome studies. However, in comparison to the literature on this subject in mammalian cytogenetics, they are rather few and limited. The practical difficulties in obtaining cytoplasm free metaphase spreads of plant chromosomes seem to be the main hindrance for the progress. Conventional acid hydrolysis used for this purpose interferes with the production of bands either with Giemsa or fluorochromes. The use of enzymes, snail gut cytase[42] and cellulase[43] for squash preparations of plant chromosomes for fluorescent studies is suggested. Mild hydrolysis with 0·1 N HCl for a few seconds or pretreatment of fixed root tips in 45% acetic acid may also help in softening the tissue. Nevertheless, there is an imperative need for a technique that facilitates chromosome spreads of plant cells on similar lines of mammalian chromosomes.

In spite of a number of investigations on the mechanism of banding in mammalian chromosomes[44-50], what causes the banding is still obscure. While numerous such investigations are still being pursued, the lack of our understanding of the phenomenon of banding at present has not deterred the use of these techniques in chromosome research.

However, a thorough knowledge of the causes of banding production and factors influencing it may not only help in fostering the research on chromosome identification but also elucidate the structure and molecular organization of the chromosomes.

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