Table 2 *Increase in water content in lens*

System	% water content	Change in water content (%)
Rat lens incubated in medium	68.66	—
Rat lens incubated in medium +50 mM glucose	79.3	10.7
Rat lens incubated in medium +50 mM glucose+garlic extract	71.4	2.8
Rat lens incubated in medium +50 mM galactose	81.3	12.7
Rat lens incubated in medium +50 mM galactose+garlic extract	73.6	7.0
Rat lens incubated in medium +50 mM xylose	78.2	9.6
Rat lens incubated in medium +50 mM xylose+garlic extract	74.8	6.2

water content, the value being 2.8, 6.2 and 7.0% with glucose, xylose and galactose respectively.

The inhibition in the levels of accumulated polyols in the lens in the presence of garlic suggests its new role like flavonoids. This property of garlic is further strengthened by its ability to inhibit the hydration of incubated lenses in the presence of glucose, galactose and xylose. The inhibitory effect of garlic extract is probably due to its sulphur compounds, which are good acceptors of hydrogen and the biological activity may be due to their reaction with thiol group substances and NADPH[11,12]. The consumption of NADPH will thus retard the conversion of glucose and galactose to their corresponding polyols which is the key factor of polyol pathway[13].

It can thus be suggested that by proper regulation of dose of garlic extract, the formation of diabetic cataract, due to the excessive accumulation of polyols and hydration of the lenses can be effectively inhibited.

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C-BANDING TECHNIQUE FOR KINETOCHORE AND HETEROCHROMATIN DIFFERENTIATION IN *NITELLA MIRABILIS* (DIV. CHAROPHYTA)

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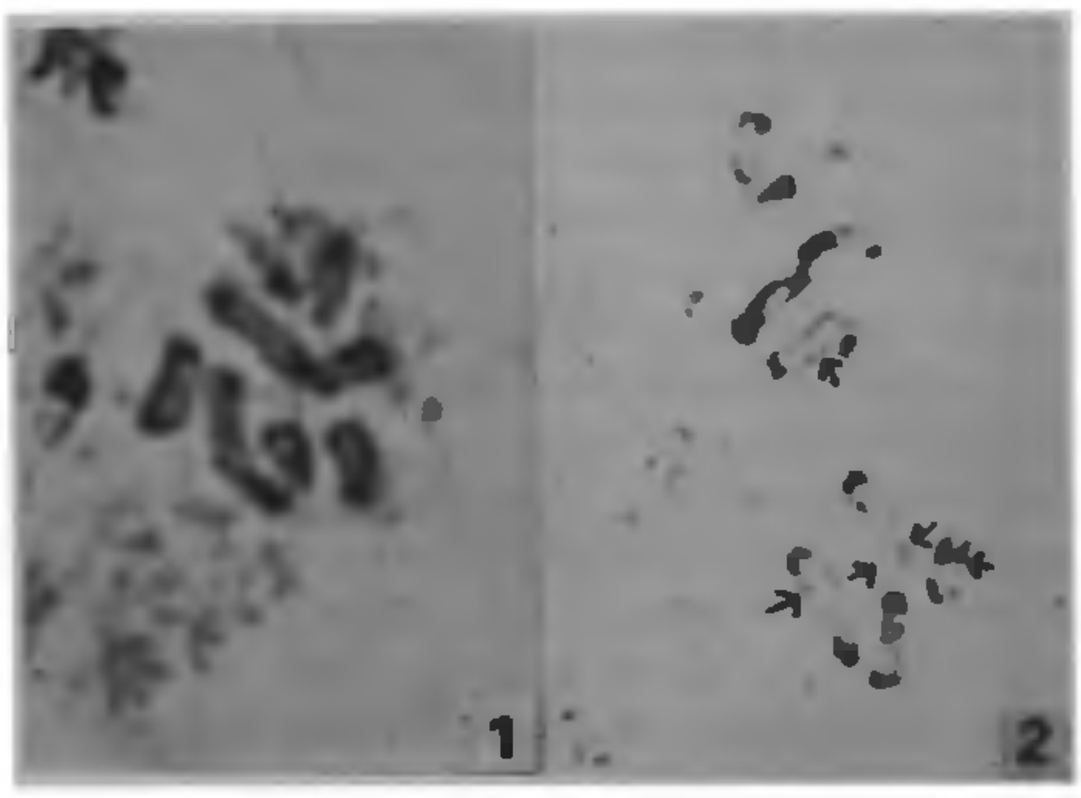
KARYOTYPES, which may be defined as the phenotypic appearance of chromosomes in contrast to their genic content[1,2], have been used extensively for species differentiation in the Charophyta[3-5]. However, the karyotypes studied so far are anaphasic configurations based on assessment of centromere position. The most distinguishing characteristics of a karyotype are the number, position, size and distribution of differentially staining heterochromatic segments[6]. These are being used largely in species differentiation of higher plants. The heterochromatin in Charophyta is probably of the constitutive type and highly localized in the centromeric regions[7]. The C-banding procedure has been found to differentiate the repetitive DNA in centromeric heterochromatin[7]. However, the method also gives bands on the chromosome arms besides the

centromeric bands[7]. In *Chara braunii*, the heterochromatic segments were localized by Pal and Chatterjee[8] using orcein banding. There has been practically no report on the differentiation of the centromere in any algal group by using Giemsa stain[9]. The authors have successfully revealed the constitutive heterochromatin of centromeric regions in *Nitella mirabilis* f. *mirabilis* (Nordst. ex Gr.) em R.D.W. for the first time using Giemsa stain (figure 2). This new technique revealed terminal, intercalary and centromeric bands (figures 1 and 2), indicating that the constitutive heterochromatin in *Nitella mirabilis* is comprised of repetitive DNA sequences in centromeric, terminal and intercalary positions. This technique may be useful for species differentiation of Charophyta because no two species possess exactly the same kind of repetitive DNA[6].

The technique used for C-banding in *N. mirabilis* f. *mirabilis* (Nordst. ex Gr.) em R.D.W. ($n=6$)[3] at metaphase is as follows: (i) Pretreatment of fertile tips in 0.15 ppm triacontanol for 30 min at room temperature. (ii) Fixation in 1:2 acetic alcohol for 24 h and subsequent transfer to 70% alcohol. (iii) Denaturation of repetitive DNA sequences by immersing tips in 0.2 N HCl for differentiating terminal and intercalary bands (30 min) along with centromeric bands (24 h). (iv) Renaturation by incubating tips in 2 × SSC for 10 min at 60°C and subsequent cooling to room temperature. (v) Thorough washing of tips with distilled water 3–5 times. (vi) Staining of tips by Giemsa stain, diluted from stock solution, for 48 h. (Giemsa stock was prepared as follows: One gram of Giemsa powder (IDPL) was dissolved in 66 ml glycerine and the solution was kept at 60°C for 90 min. It was cooled to room temperature and 66 ml methanol was added. For staining, the stock was diluted with distilled water (1:3). Freshly made up stain was used always. Smearing of stained antheridia was done in distilled water. Gentle heating on the flame gives better differentiation. For making permanent slides, coverslips were separated by dry ice method[10] with subsequent drying in an incubator for 15 min at 60°C. The slides and coverslips were mounted in De Pex.



Figures 1 and 2. Metaphase plate of *Nitella mirabilis* ($n=6$) showing (1) terminal and intercalary bands, and (2) centromeric bands (arrows).

The above technique is in accordance with that of Fiskesjo[11] to some extent. The application of barium hydroxide was dropped because it quickly forms barium carbonate scum on the chromosomes, due to which the chromosomes do not take up the stain at all.

The duration of HCl treatment plays an important role in differentiating constitutive heterochromatin in Charophyta, like in other plant groups[13]. This is perhaps due to the strong linkage between non-histone proteins and DNA in the heterochromatin, which is resistant to HCl[12].

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