

Table 1 Effect of culture filtrates of *A. porri* on seed germination and seedling vigour of onion and growth of the same fungus

Culture filtrate	Seed germination*		Seedling vigour**		
	Germination (%)	Reduction in germination over control (%)	Weight of seedling (g)	Reduction in weight over control (%)	Average dry weight of fungus (mg)
Cf-1	78.0	18.8	2.7	53.0	6.6
Cf-2	79.5	17.2	2.8	51.8	12.0
Cf-3	85.0	11.5	2.9	49.7	16.0
Control	96.0	—	5.8	—	141.0

* Recorded after 14 days, and based on 400 seeds. ** Recorded after 20 days, and based on 200 seedlings.

there was no appreciable difference in 10 and 14-day-old filtrates with regard to seed germination and seedling vigour.

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MODIFIED C AND QF CHROMOSOME BANDING FOR ARACHIS L CHROMOSOMES

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RECENT cytological techniques for linear differentiation of chromosomes have assisted in genome and chromosome characterization in addition to clarifying the nature of heterochromatin. Certain acridine derivatives such as quinacrine and its mustard have been utilized for this purpose by exploiting their DNA binding specificity and fluorescence¹. Similarly, various methods of denaturation and reannealing of DNA, followed by giemsa staining, have been used for differentiation of heterochromatic regions of chromosomes^{2, 3}. These techniques have been very useful in the animal systems, however there have been some limitations with plant systems because of (a) small size and excessive condensation of chromosomes, (b) small amounts of heterochromatin and (c) technical difficulties in obtaining proper linear differentiation. The present paper compares and illustrates the modifications in the quinacrine method and the new giemsa technique that have provided suitable preparations for the detailed study of *Arachis* chromosomes.

Actively growing root tips were pretreated in a saturated solution of monobromonaphthalene for 3 hr at 5°C and then in 1:1:1 modified Carnoy's fluid II (acetic acid, absolute alcohol and chloroform) for an hour before their final fixation in Carnoy's fluid I (1:3, acetic acid and absolute alcohol). The pretreatment with modified Carnoy's fluid with higher concentra-

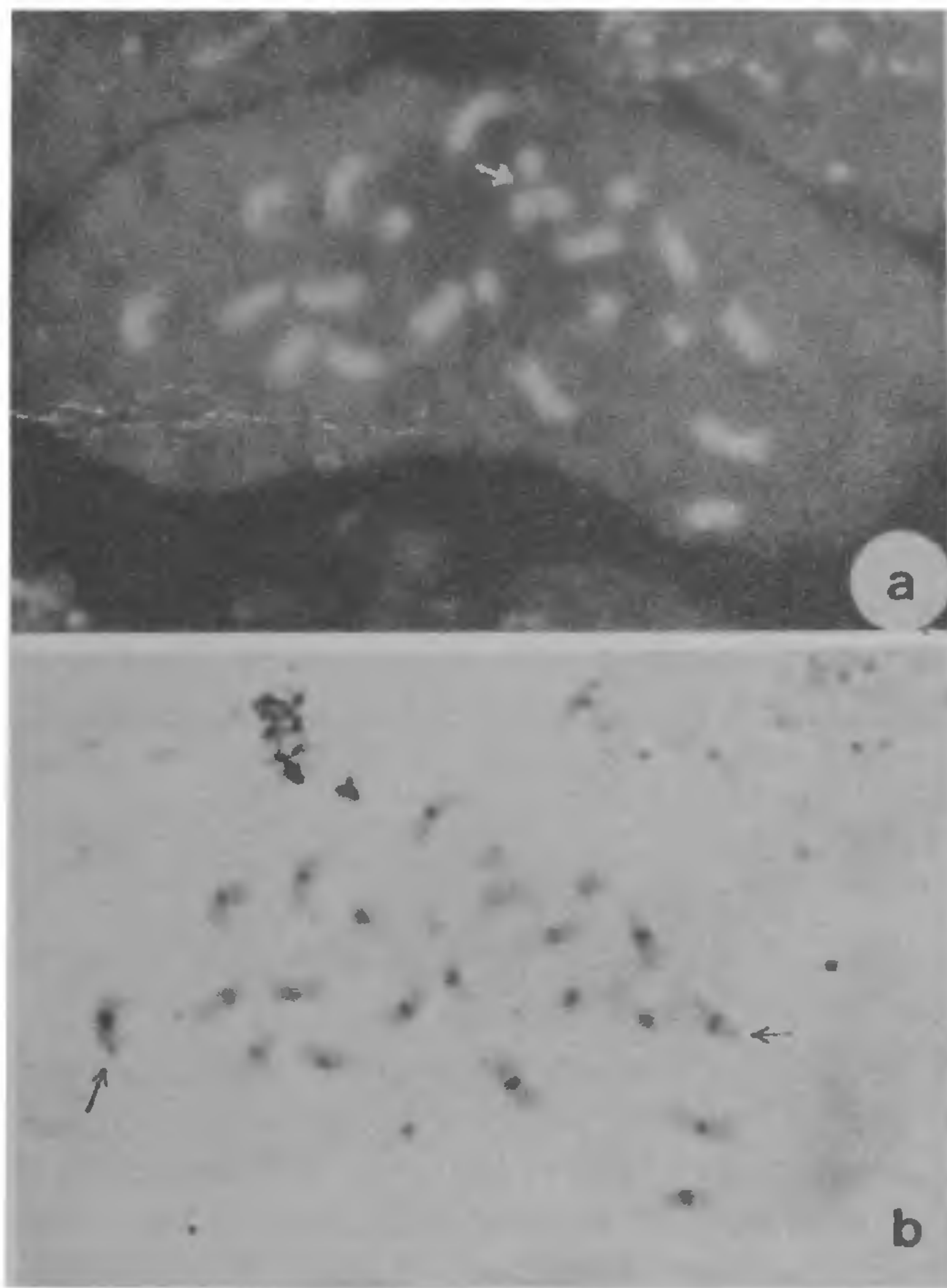
tion of chloroform helps in clearing the cytoplasm by dissolving the excess oil abundant in groundnut.

The fixed root tips of section *Arachis* species were placed in 1N-HCl at 60°C and hydrolyzed between 20 and 30 sec, then squashed in 45% acetic acid. Any debris was removed with fine forceps. This aids proper separation of cells and chemical penetration. A coverslip coated with glycerine-albumin was placed on the material, and delicately tapped and pressed until suitable metaphase plates were observed under phase contrast microscope. Glycerin-albumin helps in sticking of the cells to coverslip and restricts the loss of cells during subsequent treatments and washing. The coverslips were separated either by the dry ice method or by inverting the slide on glass rods immersed in absolute alcohol in a petri-dish. For quinacrine staining the coverslips were dried at room temperature and staining was carried out by immersion of these coverslips for 3 min in recommended 0.5% or even in a solution diluted to 0.01% quinacrine dihydrochloride dissolved either in absolute alcohol, water or phosphate buffer (pH 5.5). The coverslips were mounted in water or in phosphate buffer and observed under fluorescence microscope for a detailed study. All these variations yielded identical staining results (figure 1a).

For the giemsa staining, the dried coverslips were immersed in a saturated solution of barium hydroxide for 5 min at 40°C. In the preparation of barium hydroxide solution, care should be taken that the crystals are washed in running distilled water at least 3 to 4 times. Only a clear solution of barium hydroxide free from scum should be utilized. Masking of coverslip with a scum of barium carbonate inhibits the reaction of the barium hydroxide solution. After careful washing in running distilled water, the coverslips were incubated at 60°C for 20–30 min in 2 × SSC (7.012 g sodium chloride and 3.528 g trisodium citrate dissolved in 200 ml distilled water and adjusted to pH 7). Again after washing in running distilled water the coverslips were stained in 1% solution of giemsa R-66 (Gurr) prepared in phosphate buffer adjusted to pH 6.8. The timing of staining varied but 1 hr treatment has given satisfactory results. The coverslips were again washed in running distilled water, air-dried, mounted in euparal and bands observed (figure 1b).

Other published quinacrine staining methods do not yield satisfactory results for groundnut. Our giemsa method is identical to Vosa's method⁴ with differences in using monobromonaphthalene as pre-treating agent, 2 × SSC concentration and incubation period. The results are illustrated in figure 1a and b.

In all the section *Arachis* species there seems to be a



Figures 1a, b. Mitotic chromosomes of the same cell of *Arachis duranensis* stained with quinacrine for QF bands (a) and subsequently with giemsa for C bands (b). Note knob differentiation in giemsa staining and reduced fluorescence in a pair of chromosomes (arrow) × 1600.

close correspondence between the segments strongly stained with Giemsa and segments differentiated with quinacrine fluorescence. Most of the bands are centromeric suggesting that the heterochromatin is predominantly confined around centromere. There is, however, no differentiation of segments with intense and reduced quinacrine fluorescence except in case of a single pair of chromosomes (figure 1a arrowed). This may either be because of small size of chromosomes that mask such fine differentiation or due to complete lack of G-C rich heterochromatic segments in the chromosomes of these species. Both giemsa and QF banding techniques are closely associated with chromocenters of resting nuclei, which are confined to the centromeric regions.

The important advantage of the present giemsa staining method appears to be that a satellite knob that

is not differentiated by quinacrine technique is differentiated by giemsa as a fine knob at the end of a pair of chromosomes (figure 1b arrowed). Also, the slides with C-bands can be kept permanently for record. On the other hand, the quinacrine technique has the practical applicability for screening of a large amount of material, because of much simpler process of slide preparation.

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NEWS

SEAWEED DRESSING FOR WOUNDS

The healing properties of seaweed have long been known to seafarers, and now a British company has been established to spin the weed into dressings for cuts, post-surgical wounds and external ulcers. The company—Cair Ltd., of Aldershot (Southern England)—claims that the healing properties of its new dressing, called Kaltostat, are quite remarkable.

The "sailor's cure", as it was called in the old days, was put to use again by Major George Blaine, who served with the British Royal Army Medical Corps in World War II. He investigated the use of seaweed, which is rich in calcium alginate, and used it to dress wounds. The practice was never developed.

Biochemical engineer David Tong decided to re-investigate the technology and invented a wet spinning process to convert deep-trawled brown seaweed into sterilisable fibres of pure calcium alginate. The substance works chemically, the calcium combining with a sodium compound in the blood to form a protective gel that keeps the wound moist. The calcium also

encourages fast clotting.

The alginate fibres are absorbed naturally by the body, so that the dressing can be left undisturbed for a long period. If used on a cut, for instance, it need be removed only when the scab is ready to come off.

In cases where frequent dressing changes are needed, the protective gel ensures that the delicate regenerative tissue is not damaged.

Cair has carried out extensive tests with Kaltostat at teaching hospitals in Oxford, Cardiff and London, and has won a product licence from Britain's Department of Health and Social Security.

"It does heal wound very quickly, and does not cause any irritation or allergic reactions," says Mr. Bob Browning, Cair's marketing manager. "We think it ideal for use in casualty departments and are now in negotiations with major pharmaceutical companies to market Kaltostat worldwide." (*Spectrum*, 197, p. 12, 1986; *British Science News*, R. P. Nash, British High Commission, New Delhi 110 028).
