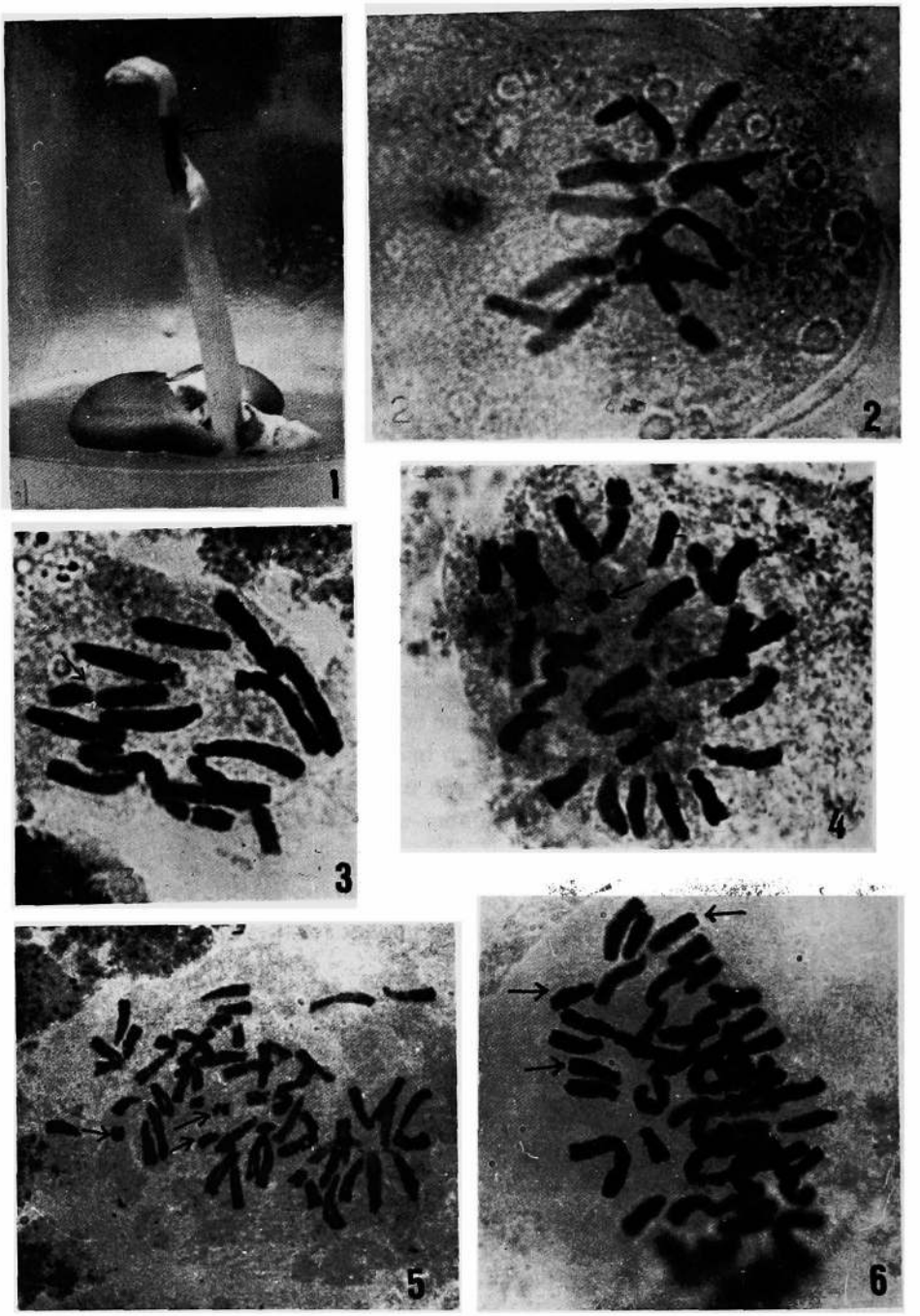
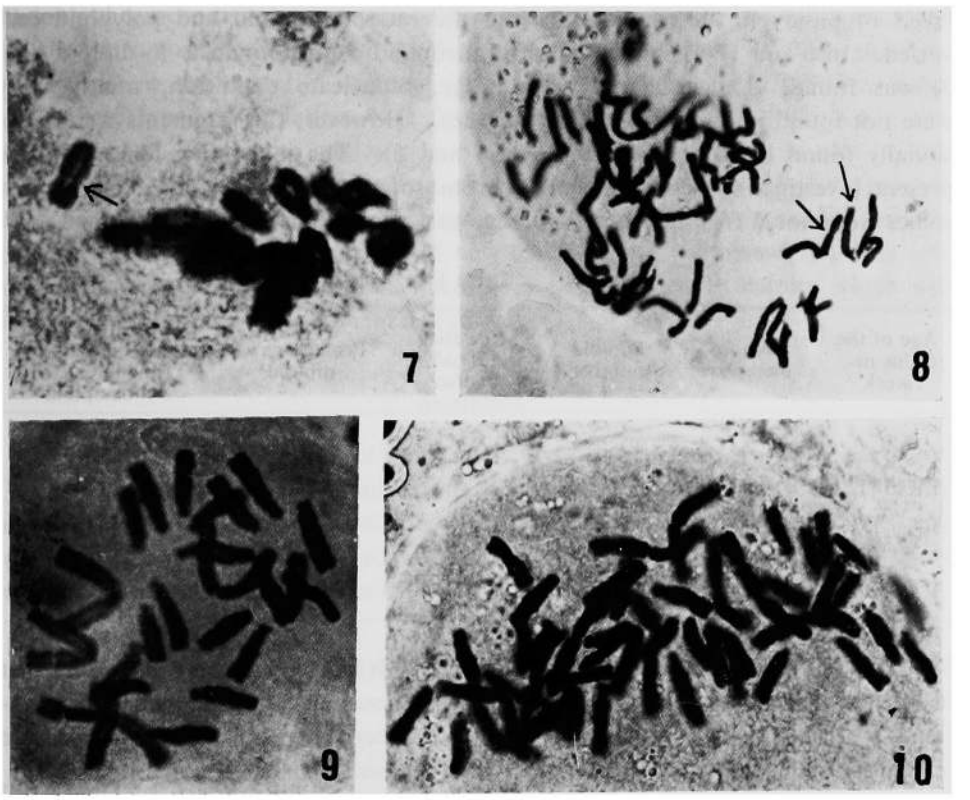


Figs. 1–6. 1, *in vitro* germinated seedling of *Vicia faba* showing "blackening" of epicotyl region. 2, a normal diploid cells with 12 chromosomes from 6 weeks old culture. 3, an aneuploid metaphase plate having 14 chromosomes showing a structurally altered chromosomes. 4, 36 weeks old culture showing one fragment. $2n=>18+1f$. 5, 54 weeks old cutlure showing three fragments. $2n=48+3f$'s. 6, an octaploid cell from 30 weeks old culture showing three altered chromosomes. $2n=48$.

in SH media when it was supplemented with 5 mg/lit nicotinic acid, 5 mg/lit pyridoxin HCl, 10 mg/lit thiamine HCl, 1 gm/lit of mesoinostol, riboflavine .2 mg/lit, calcium pantothenate 1 mg/lit, aminobenzoic acid .02 mg/lit, biotin .01 mg/lit, 24D-.5 mg/lit, KI-.1 mg/lit, In *Vicia faba* one barrier in maintaining cultures is the blackening of tissues. In the field also, the blackening of shoots occur after growth of young seedlings and the plant ultimately dies (Fig. 1). After many trials this has been overcome but the cause is yet to be determined. Proliferation of tissues starts within 10 days of inoculation and is better in dark light at 22–25°C with a



Figs. 7–10. 7, a clumped metaphase plate showing a single structurally altered chromosome from 36 weeks old culture. $2n=>12<18$. 8, an octaploid cell from 54 weeks old culture tissue having two altered chromosomes. $2n=48$. 9, a well-scattered tetraploid metaphase plate from 24 weeks. $2n=24$. 10, an octaploid cell from 24 weeks old cultures. $2n=48$.

maximum humidity of 55%. Friable creamish white tissues were produced within one month of culture. Callus was maintained by subculturing in the same medium at an interval of 6 weeks. The concentration and combination of hormones were changed from 24D-.5 mg/lit and Kinetin .1 mg/lit to 24D-.25 mg/lit, NAA-1 mg/lit and KI-.1 mg/lit for maintaining good growth as well as for reducing the effect of 24D on chromosomes.

For all cytological experimentation cultures were fixed within 7 days after inoculation when growth is in the exponential phase. Cytological studies were