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INDUCTION OF REDUCTIONAL SEGREGATIONS IN SOMATIC NUCLEI OF *TRAGOPOGON GRACILI* BY CAFFEINE TREATMENTS.

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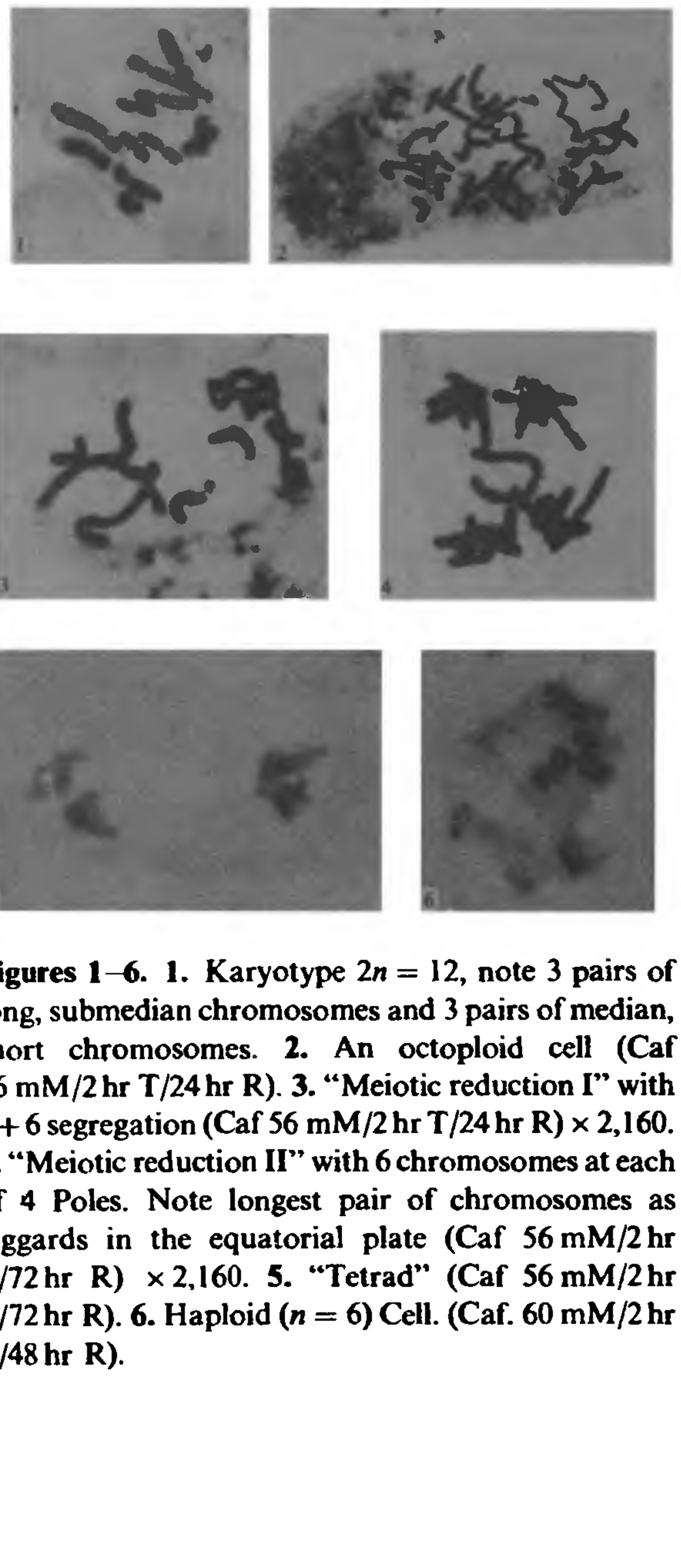
CAFFEINE (1, 3, 7-trimethyl xanthine), an oxypurine, is of particular interest as both a byproduct of human purine metabolism, and is consumed in considerable quantities as a beverage.

Caffeine effect was studied on somatic chromosomes of *Tragopogon gracile* ($2n = 12$ chromosomes) (figure 1) during experimentation of exogenous application of certain cell components on cell systems. As caffeine effects are extensively studied with micro-organisms, plant, animal and human cell systems[1–5] the present communication briefly reports the induction of "meiotic reductions" by high caffeine concentrations in somatic anaphases.

The 3-day old seedlings of *T. gracile* were immersed in neutral caffeine solutions for 1 and 2 hr. The seedlings are then thoroughly washed with distilled



Figures 1–6. **1.** Karyotype $2n = 12$, note 3 pairs of long, submedian chromosomes and 3 pairs of median, short chromosomes. **2.** An octoploid cell (Caf 56 mM/2 hr T/24 hr R). **3.** "Meiotic reduction I" with 6 + 6 segregation (Caf 56 mM/2 hr T/24 hr R) × 2,160. **4.** "Meiotic reduction II" with 6 chromosomes at each of 4 Poles. Note longest pair of chromosomes as laggards in the equatorial plate (Caf 56 mM/2 hr T/72 hr R) × 2,160. **5.** "Tetrad" (Caf 56 mM/2 hr T/72 hr R). **6.** Haploid ($n = 6$) Cell. (Caf. 60 mM/2 hr T/48 hr R).

water, some of them fixed in 1 : 3 acetic ethanol for immediate testing. The rest was put to recovery and fixed at regular intervals upto 96 hr and root-tips were squashed in Feulgen's stain after hydrolysis. Treatment and recovery were carried out at 20 + 2°C along with controls.

The rate of mitosis was not affected appreciably by caffeine. It suppressed cell plate formation resulting in binucleate cells and induced clastogenic effects leading to chromosomal abnormalities. At concentrations higher than 50 mM caffeine, polyploid cells (figure 2) often with fragmented chromosomes and bridges were of predominant occurrence. A significant observation from the same samples was a special type of anaphasic grouping "meiotic reduction I" where homologous chromosomes segregated during mitotic anaphase. Though these groupings were not very frequent they presented a contrast to the high percentage of polyploid cells and could be spotted at once. Only those anaphases (figure 3) showing both qualitative and quantitative haploid complement were identified as "meiotic reductions". The highest percentage of such groupings scored was 13.04 in 50 mM/2 hr T/48 hrR. The frequency of "meiotic reductions" did not bear any relationship to the concentration of caffeine or the amount of recovery allowed after treatment. If this "meiotic reduction" simulates meiosis I, other stages comparable to meiosis II, identified from the same caffeine treated root-tips were "meiotic reduction II" (figure 4) and a "tetrad" (figure 5). Haploid cells (figure 6) probably resultant of cytokinesis after "meiotic reduction I" were observed in some instances. The appearance of all these three stages from samples with 24 hr or more of recovery time suggests their origin after 2 or more cell cycles. Occurrence of reductional groupings and haploid cells was reported by Huskins[6] with sodium nucleate treatment in *Allium* root-tips who referred to it as 'somatic meiosis' without however implying that all other features of meiosis also occur. The induction of "meiotic reductions" with treatments pyrimidine nucleotides, their intermediates and RNA in the same test system and also in *Pterotheca falconeri* was reported[7]. Caffeine, a purine derivative also is capable of inducing a similar effect lends support to the theory that it is the cumulative effect of purine and pyrimidine metabolism.

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REGENERATION OF PLANTS FROM LEAF CALLUS CULTURES OF *SOLANUM TORVUM* SWARTZ

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IN recent years the technique of tissue and organ culture has been effectively used in the mass multiplication of a number of plants of economic importance[1]. However, in a few cases the leaf material has been used as the source of tissue. *Solanum torvum* contains solasonine alkaloid and is currently considered to be a promising species as commercial source of this alkaloid. Vegetative propagation of *S. torvum* has not been possible by cuttings and graftings, limiting the clonal multiplication of high yielding strains. The present investigation was, therefore, undertaken to develop an efficient tissue culture method for plantlet formation from leaf tissues of young plants of *Solanum torvum* Swartz.

Leaves were taken from young plants growing under field conditions in the campus of Banaras Hindu University. They were washed in running tap water for about 30 min and then treated with 1 % solution (v/v) of Cetavlon (a detergent and antiseptic) for 5 min. After rinsing with distilled water several times, the leaves were surface-sterilized with 0.1 % (w/v) mercuric chloride solution for 5 min followed by a thorough washing with double-distilled water. Discs were punched from the leaf lamina with sterile cork borer (5 mm diameter) and single disc was inoculated in each culture vial containing solidified Murashige and Skoog's medium[2] (MS), supplemented with various concentrations and combinations of kinetin, IAA and 2,4-D. Cultures were maintained at 25 ± 2°C with 12 hr illumination at about 4000 lx. For each