bedding in paraffin[6]. 10–12 μm thick sections were cut on a rotary microtome. These were stained with safranin and light green and mounted in DPX.

Leaf segments cultured on media containing benzylaminopurine (BA) (3 to 5 mg/l) + 3-indoleacetic acid (IAA) (1 to 5 mg l) showed prolific adventitious shoot bud differentiation via callus formation after two weeks in culture. Some roots also were formed on a medium with BA (3 mg/l) + IAA (5 mg/l) after the formation of shoot buds in the same culture flask[1]. Direct differentiation of roots from the leaf explants was observed on a medium with kinetin (1 mg/l) + IAA (5 mg l) after 10 to 12 days of culture. Callus also was formed on this medium if cultures were kept for more than 2 weeks.

Prior to rhizogenesis leaf explants showed hypertrophy in the vascular region (figures 1, 2) and differentiation of a large number of tracheid-like elements (figure 2). From the vicinity of this vascular tissue a large number of root primordia differentiated (figure 2). The root primordia originated from the deeper regions of the proliferated leaf explants (figures 2, 3) or callus tissue (figure 4). Lateral roots were also formed from the roots differentiated in cultures.

Prior to shoot bud initiation, differentiation of a large number of tracheid-like cells (figure 5) was observed after one week of culture of the leaf explants. This was followed by the development of islands of meristematic nodules, presumably from single cells situated either deeply (figure 6) or superficially on the callus (figure 7). This meristematic nodule (meristemoid) was composed of smaller cells with dense cytoplasm and large nuclei. These meristemoids developed into shoot meristems after two weeks. The bud primordia consisted of an enormous dome-shaped shoot apex and the two leaf primordia (figure 8). In all the shoot buds, the first leaf primordium had grown considerably by the time the second primordium had differentiated.

Histology of root and shoot development revealed that shoot primordia arose either deep or superficially on the callus, but the root primordia developed always from the deeper cell layers of the proliferated leaf tissue or the callus. Endogenous development of root and shoot has been described in *Convolvulus*[7]. Meristemoids have been traced to deep-seated single cells in *Nicotiana*[8] and *Brassica campestris*[9] callus cultures. Before the appearance of shoot buds, several tracheid-like cells were seen differentiated in the callus tissue. Tracheid differentiation prior to bud formation has also been reported in callus tissue of *Torenia*[10] and in cultured thin layers of *Brassica napus*[11].

16 December 1985

1. Kothari, S. L. and Chandra, N., *Hort. Sci.*, 1984, **19**, 703.
2. Kothari, S. L. and Chandra, N., *J. Plant Physiol.*, 1984, **117**, 105.
3. Kothari, S. L. and Chandra, N., *J. Plant Physiol.*, 1986, **122**, 235.
4. Mohan Ram, H. Y. and Mehta, G., *Plant Sci. Lett.*, 1982, **26**, 227.
5. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.
6. Johansen, D. A., *Plant microtechnique*, 1940, McGraw-Hill, New York.
7. Earle, E. D. and Torrey, J. G., *Am. J. Bot.*, 1965, **52**, 891.
8. Nuti Ronchi, V., *Can. J. Bot.*, 1981, **59**, 1969.
9. Singh, S. and Chandra, N., *Plant. Cell Rep.*, 1984, **3**, 1.
10. Chlyah, H., *Plant Physiol.*, 1973, **54**, 341.
11. Klimaszewska, K. and Keller, W. A., *Plant cell tissue organ culture*, 1985, **4**, 183.

CHANGES IN ENDOGENOUS INDOLE-3-ACETIC ACID LEVELS ACCOMPANYING LEAF AGEING

ABHA ARORA

Department of Botany, Sanatan Dharm College, Muzaffarnagar 251 001, India.

AUXINS have been implicated besides other hormones, in the control of leaf senescence[1,2]. Such views are based mainly on studies with externally applied auxins and other hormones although indole-3-acetic acid (IAA) has been shown to be present in free and bound states in different types of leaves[3–6]. There is a dearth of information on the changes in endogenous hormonal levels accompanying leaf ageing. Hence, studies were carried out on the changes in endogenous free and bound IAA levels accompanying leaf ageing in the young growing, fully expanded mature and senescent stages.

Plants of *Pisum sativum* Bonneville, *Vicia faba*, *Cicer arietinum* G130 and *Phaseolus mungo* S_1 were grown in the experimental plots and samples were taken at three stages, the young growing leaves (1st stage), fully expanded mature leaves (2nd stage) and senescent

leaves (3rd stage). At each stage, the leaves were analyzed for fresh weight, dry weight, total chlorophyll, endogenous free and bound IAA and IAA-oxidase activity. The total chlorophyll was determined according to methods reported earlier[7, 8]. Free and bound IAA was extracted following methods reported earlier[9, 10]. Both free and bound IAA extracts were subjected to ascending/circular paper chromatography in *n*-butanol:acetic acid:water (60:15:25, v/v), IAA spot visualized with Ehrlich reagent against reference spot and quantified with the help of spot area/densitometric standard curves. IAA-oxidase has been extracted and assayed according to Rabin and Klein[11].

In all the plants studied, there is an increase in both fresh weight and dry weight, from young growing leaves to fully expanded mature leaves (table 1). During senescence, both fresh weight, and dry weight decline in *Cicer* and *Phaseolus*. However, in *Pisum* and *Vicia* while there is a decrease in fresh weight during senescence, there is a slight increase in the dry weight. The chlorophyll content increases upto fully expanded mature stage and then declines in the 3rd senescent stage (table 1).

Changes in endogenous free and bound IAA follow varying patterns in different genera (table 1). In *Pisum* both free and bound IAA show parallelism with fresh weight change and such relationships do not seem to exist with changes in dry weight. In *Vicia* free IAA level increases markedly in fully expanded mature leaves followed by a two-fold decline in senescent leaves and this seems to correspond with the fresh weight changes Bound IAA, however, appears to parallel the dry weight growth pattern. In *Cicer* leaves free IAA level increases consistently from young growing stage to the senescent stage. Bound IAA follows the growth pattern, both in terms of fresh weight and dry weight with a two-fold increase in mature leaves and 1.3 times decrease thereafter. Levels of free and bound IAA in *Phaseolus* correspond to the growth pattern, showing a 6-fold rise from 1st to 2nd stage and 3-fold decline in the 3rd stage.

IAA-oxidase activity increases consistently upto 3rd stage in *Pisum* and may be responsible for the decrease in the free IAA level. In *Vicia* also, there is a direct correlation between IAA-oxidase activity and changes in free IAA levels. In *Cicer*, however, changes in IAA oxidase enzyme activity seems to be linked with decrease in the bound IAA level (table 1).

The physiological role of free or bound IAA has been a matter of controversy[12-14]. The present work indicates that, in the first growth phase of the leaf, both free and bound IAA levels show parallel growth and hence in this organ both states may have important regulatory role. In the second phase leading to senescence, there are variations. In *Pisum* and *Phaseolus* both free and bound IAA levels decline and while in *Vicia*, only the free IAA level falls, in *Cicer* there is reduction in bound IAA level. These results besides those with externally applied auxins[1,2] indicate the importance of IAA in the control of senescence, but the relative importance of free and bound IAA may vary with the species[10]. In general, it would seem that the relative importance of free and bound IAA depend on many factors as species, organ and developmental phase of the organ.

Thanks are due to Prof. Y. S. Murty and Prof. D.

Table 1 *Growth pattern, changes in total chlorophyll, free and bound IAA and IAA-oxidase activity in the leaves of different ages.*

Parameter	*Pisum sativum*			*Vicia faba*			*Cicer arietinum*			*Phaseolus mungo*		
Leaf age*	I	II	III	I	II	III	I	II	III	I	II	III
Fresh weight (mg/leaf)	545.70	1162.50	640.00	194.70	673.20	427.10	38.90	88.00	50.00	379.00	2448.00	843.50
Dry weight (mg/leaf)	110.80	212.70	255.50	30.50	106.90	126.50	9.20	22.00	16.40	82.60	473.80	236.90
Total chlorophyll (mg/leaf)	0.64	1.45	0.45	0.22	0.63	0.29	0.06	0.10	0.05	0.11	2.48	0.44
Free IAA (μg/leaf)	Traces	23.77	Traces	Traces	16.53	8.14	0.80	1.53	2.00	12.87	66.90	24.23
Bound IAA (μg/leaf)	30.95	56.18	19.11	Traces	20.33	25.79	0.93	1.93	1.54	9.23	56.21	20.55
IAA-oxidase activity (μg IAA degraded/g FW/hr)	105.04	142.74	171.11	224.30	168.50	207.34	84.66	118.40	436.60	--		--

*(I = young growing leaves; II = fully expanded mature leaves; III = senescent leaves).

Banerji, Botany Department, Meerut University, Meerut, for constant encouragement and helpful suggestions.

16 September 1985; Revised 26 December 1985

1. Osborne, D. J., *Nature* (*London*), 1959, **180**, 1294.
2. Osborne, D. J., *Symp. Soc. Exp. Biol.*, 1967, **21**, 305.
3. Overbeek, I. Van, Vasquez, E. S. DE and Gordon, S. A., *Am. J. Bot.*, 1947, **34**, 266.
4. Vlitos, A. J. and Meudt, W., *Contr. Boyce. Thompson Inst.*, 1953, 171, 197.
5. Linser, H., Mayr, H. and Maschek, F., *Planta*, 1954, **44**, 103.
6. Bulgakov, R. and Chvojka, L., *Biol. Plant.*, 1976, **18**, 78.
7. Arnon, D. J., *Plant Physiol.*, 1949, **56**, 1.
8. Smith, J. H. C. and Benitez, A., In: *Modern methods of plant analysis* (eds) K. Peach and M. V. Tracey, Springer-Verlag, Heidelberg, 1955, **4**, 142.
9. Tamura, S., Nomoto, M. and Nagao, M., In: *Plant growth substances, 7th Int. Conf.*, (ed.) D. J. Carr, Springer-Verlag, Berlin, 1970, p. 127.
10. Bentley, J. A., In: *Encyc. Plant Physiol.*, (ed.) W. Ruhland, Springer-Verlag, Berlin, 1961, **14**, 609.
11. Rabin, R. S. and Klein, R. M., *Arch. Biochem. Biophys.*, 1957, **70**, 11.
12. Gordon, S. A., *Annu. Rev. Plant Physiol.*, 1954, **5**, 341.
13. Fransson, P. and Ingestad, D., *Physiol. Plantarum*, 1955, **81**, 336.
14. Bonner, J. and Foster, R. J., In: The *Chemistry and mode of action of plant growth substances*, (eds) R. L. Wain and F. Wightman, Butterworths, London, 1956, p. 2.

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

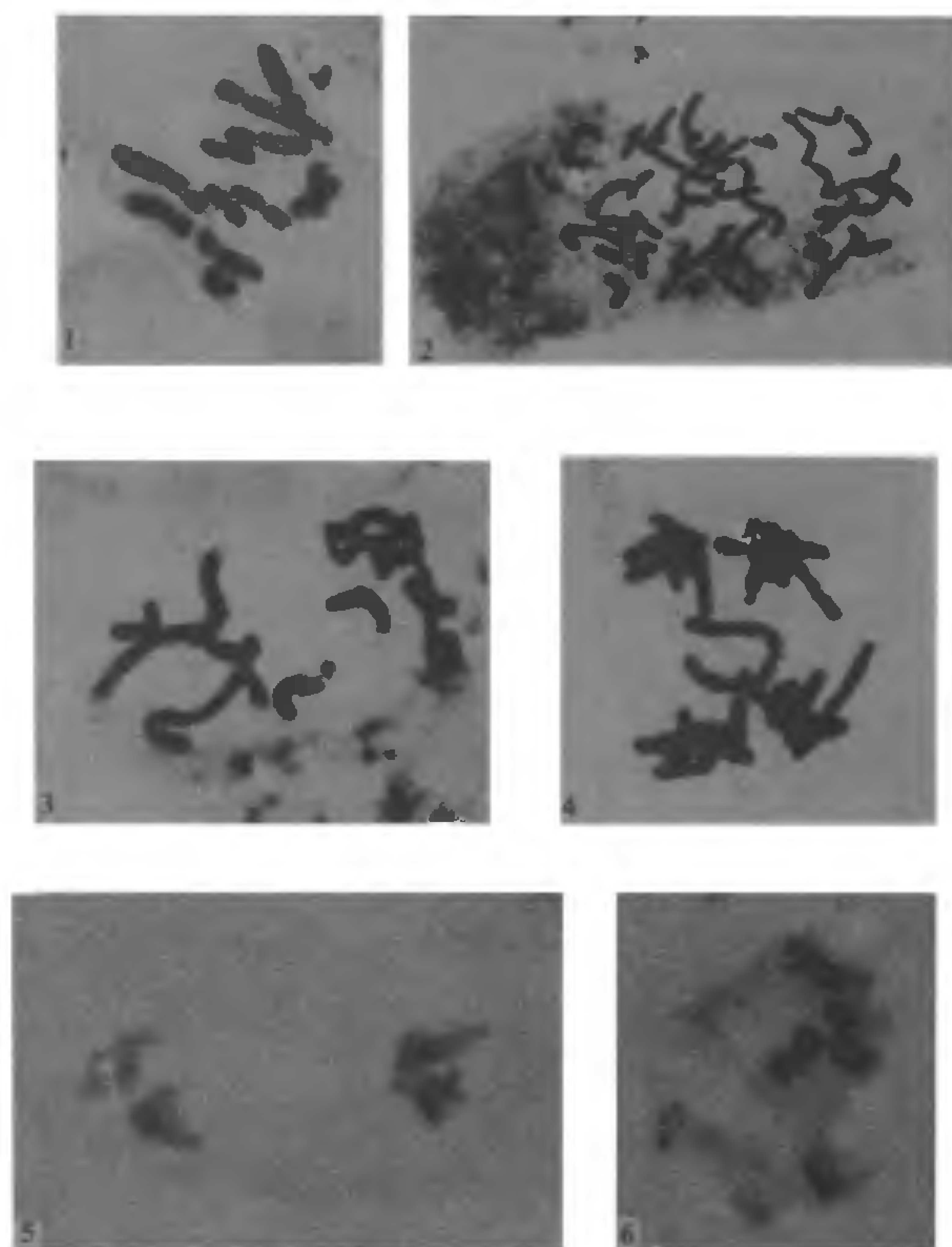
LALITA KUMARI*

Department of Botany, Punjab University, Chandigarh 160014, India.
**Present Address: Documentation Unit, Indian Institute of Chemical Biology, Calcutta 700032, India.*

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).