area) on slides exposed for 7 h. Sporidial counts increased about five-fold (average 106 per mm^2 area) within the subsequent 1 h period.

The trapped sporidia germinated in water drops on the glass slides. Forty to sixty per cent of the sporidia germinated within 4 h of incubation. A germinated sporidium supported germ tubes normally at both of its ends.

The trapped sporidia suspended in water (1×10^5/ml) and used for inoculation of wheat spikes at the boot leaf stage proved infective to all 25 spikes used. On an average an inoculated spike had 50-100% bunted kernels.

The same spike produced repeated crops of sporidia for a week, the duration of the test. Unlike inoculum obtained from cultures grown on PDA, preparations of allantoid sporidia free of filiform ones were obtained.

The detached wheat spike method of inoculum production used in this investigation is inexpensive and precise. It may also be adapted for various *in vivo* investigations on *N. indica*.

23 May 1988; Revised 22 September 1988

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FLOWERING-INDUCED AGEING IN *BUTICA MONOSPERMA*

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DIFFERENTIATION, development and ageing in plants are programmed at the genetic level but the environmental effects also influence these processes. The photosynthetic capacity of mature green leaves is known to decline under induced stress conditions and dark stress is also known to induce ageing in detached leaves[1-2]. The ageing causes loss of chlorophyll, Hill activity, photophosphorylation and CO_2 fixation[3-6].

Flowering and fruit development impose stress on leaves in the flowering/fruiting region. In *Butica monosperma* (*frondosa*), which grows in central India along the western ghats, a clear gradient of ageing can be observed from the apical flowering region to the basal part of the stem. The onset of flowering in February is marked by yellowing of leaves and subsequent vernalization.

We have studied the changes in photosynthetic characteristics of the leaves of *B. monosperma* during flowering under field conditions.

Leaves of *B. monosperma*, collected from a nearby forest, were washed with distilled water, cut into small pieces, and kept overnight at 4°C before processing for isolation of chloroplasts. Five regions on the tree, chosen arbitrarily, were identified and are numbered 1 to 5 (figure 1).

Chloroplasts were isolated according to the method of Karabourniotis *et al*[7]. The leaves from each region were blended separately in ice-cold isolation medium containing 400 mM sucrose, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$, 10 mM KCl, 150 mM NH_4Cl, 2 mg/ml bovine serum albumin (fraction V) and 4 g/l polyvinylpyrrolidone (PVP-10). The homogenate was filtered through four layers of cheesecloth and the filtrate was centrifuged for 1 min at 500 *g*. The pellet was discarded and the supernatant centrifuged for 3 min at 3000 *g*. The pellet containing the chloroplasts was collected and suspended in minimal volume of isolation medium.

The leaves were extracted in cold 80% acetone under dim green light and the amount of chloro-

Figure 1. *B. monosperma* in the month of February under natural forest regime. Leaves attached to the flowering region were yellowed. The arrows mark the five regions from which leaf samples were collected for photosynthetic measurements.

Table 1 *Flowering-induced changes in total chlorophyll and chlorophyll a/b ratio in leaves of B. monosperma*

Region	Distance from flowering region* (feet)	Chlorophyll *a* (mg/g fresh wt)	Chlorophyll *b* (mg/g fresh wt)	Total chlorophyll (mg/g fresh wt)	Chlorophyll *a/b* ratio
1	25	0.54 ± 0.024	0.17 ± 0.032	0.72 ± 0.057	3.05
2	21	0.28 ± 0.057	0.11 ± 0.062	0.39 ± 0.119	2.47
3	17	0.18 ± 0.009	0.09 ± 0.014	0.27 ± 0.023	1.84
4	12.5	0.07 ± 0.004	0.05 ± 0.009	0.12 ± 0.013	1.25
5	7	0.05 ± 0.002	0.03 ± 0.007	0.08 ± 0.009	1.69

*Approximate height of the tree is 30 feet.

phylls in the extract was estimated according to Mackinney[8].

The photoreduction of dichlorophenol indophenol (DCIP) was measured spectrophotometrically according to Mohanty *et al*[9]. The chloroplasts were illuminated with saturating white light and a water filter was placed between the light source and the sample to cut off infrared radiation. The reaction mixture in a final volume of 3 ml contained chloroplasts equivalent to 10 μg/ml chlorophyll, 10 μM DCIP, 10 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$ and 5 mM NH_4Cl. The bleaching of DCIP was measured at 605 nm spectrophotometrically (Shimadzu model UV–VIS 160) and the rate of DCIP reduction was calculated using an extinction coefficient of 21 mM^{-1}.

The translocation of photosynthates from leaf to fruiting body results in ageing of leaves. This is marked by changes in the chlorophyll content of leaves[1,2]. A clear pattern of yellowing was observed from region 1 to region 5 with onset of flowering in *B. monosperma* (figure 1).

A linear decrease in total chlorophyll content was observed from region 1 to region 5 (table 1). The decline in chlorophyll *a* content was higher compared to that in chlorophyll *b* from region 1 to region 5, resulting in a continuous decrease in the chlorophyll *a/b* ratio. The green leaves from region 1 had a chlorophyll *a/b* ratio of 3, which is characteristic of sun plants[10]. The decline in the ratio from region 1 to region 5 indicates disorganization of thylakoid membranes upon onset of flowering.

Chloroplasts isolated from leaves of region 1 gave a red peak at 679.5 nm and had a characteristic blue/red peak ratio of 1.9 (figure 2). Chloroplasts isolated from leaves of region 5 had low absorbance in the red region and the blue/red peak ratio was 3.3. The difference spectrum (green minus yellow) showed peaks at 438, 473 and 679 nm, suggesting disorganization of chlorophyll–protein complexes.

The loss in electron transport efficiency, as measured by DCIP reduction, declined from region 1 to region 5 (table 2). The decrease in DCIP Hill activity can be correlated with decrease in chlorophyll from region 1 to region 5.

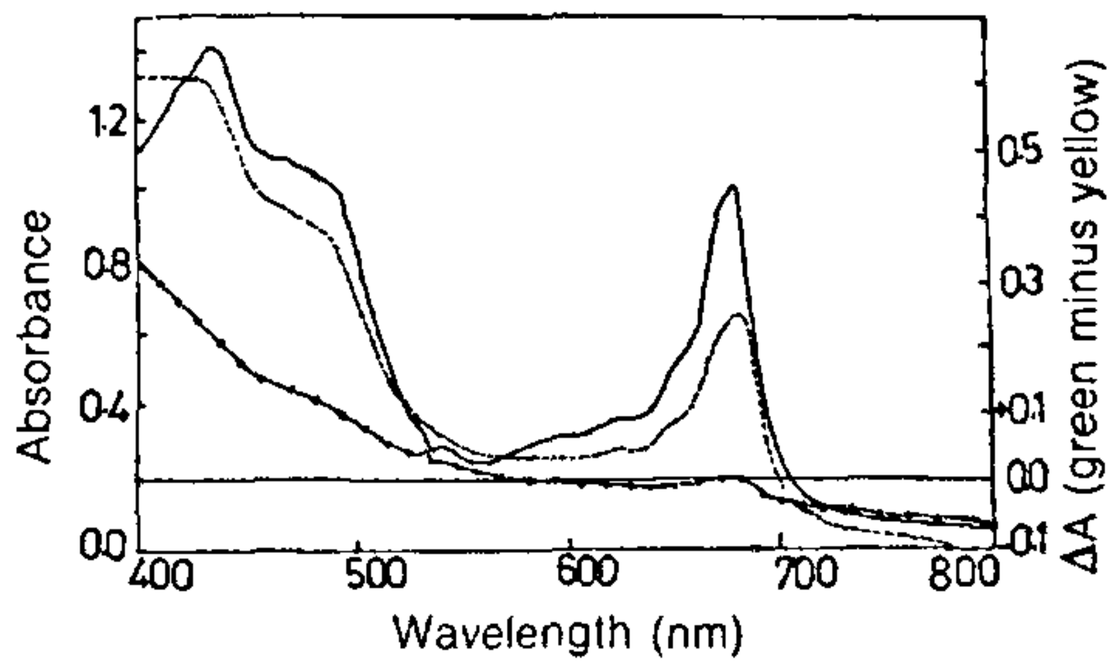

Figure 2. The absorption spectra of chloroplasts isolated from leaves of *B. monosperma* from region 1 (– – – –) and region 5 (–·–·–·–) (yellow leaves). The difference spectrum (green minus yellow, ———) shows peaks at 438, 473 and 679 nm. The absorbance at 546 nm was fixed at 0.25.

Table 2 *Flowering-induced changes in photosystem II activity of chloroplasts isolated from leaves of B. monosperma*

Region	DCIP reduction (% of value for region 1)
1	100*
2	57
3	35
4	21.5
5	15

*79.2 μmoles of DCIP reduced per mg chlorophyll per hour.

All the observations point to the fact that ageing induced by flowering/fruiting causes disorganization of pigment–protein complexes and loss of electron transport capacity in chloroplasts, particularly in leaves close to the region of flowering. In higher crop plants, flowering is not accompanied by ageing but fruit setting marks the onset of ageing. In this respect *B. monosperma* differs from higher crop plants and is an interesting example for studies of ageing in trees under field conditions.

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POLYPLOIDY AND GENE DOSAGE EFFECTS ON PEROXIDASE ACTIVITY IN MULBERRY

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THE effect of increased ploidy in mulberry has been studied only with reference to chromosome affinities, morphological and anatomical comparisons[1,2]. Little information is available on the possible relationship of increased ploidy level and certain bio-chemical parameters[3,4]. A crude enzyme extract when fractionated on a suitable gel medium produces a spectrum of bands which is diagnostic for the species relationship and may also for ploidy differences. The present study deals with the electrophoretic pattern of peroxidase isozymes in mulberry in relation to ploidy level.

S_{30}, S_{36} and S_{41} diploids and their colchicine-induced autotetraploids were utilized for the present study. The peroxidase isozyme separation was carried out with gel electrophoresis apparatus GE-2/4 LS connected with electrophoresis power supply EPS 500/400 of pharmacia fine chemicals, Sweden. The polyacrilamide gel electrophoresis method of Clarke[5] and for peroxidase enzyme staining Scandalios[6] were adopted. For the leaf peroxidase analysis the third to sixth leaf, from the top, youngest leaf expanded were used. Equal amounts of enzyme extract were loaded on the top of each gel. The gels were preserved in 7% acetic acid for photography.

The tetraploids exhibit reduction in length of the shoot, the number of branches per plant, the internodal distance and rooting percentage (table 1). The leaf became slightly larger, thicker, coarser and dark green. Flowering was somewhat delayed and the blooming duration was prolonged in tetraploids. The inflorescences of tetraploids were larger in size and lax with more flowers in comparison to diploids. Among the auto-tetraploids, S_{30} (116.50 cm) showed decreased height in comparison with S_{36} (121.77 cm) and S_{41} (125.70 cm).

The allozymes of diploids and tetraploids of mulberry did not show much differentiation in banding pattern. Among the diploids studied, S_{30} and S_{36} showed one thick and one light band whereas S_{41} showed only one thick big band (figures 1 and 3). Three autotetraploids showed 2 bands, viz. one dark and one light band (figures 2, 4, 6). Among the autotetraploids in S_{36} and S_{41}, the intensity of the first band was less than that of the parent diploids whereas in S_{30} autotetraploid the first band intensity was more than that of its parent diploid (figures 1–6). In S_{41} (4×) a new faint band appeared which was not present in the parent diploid (figures 3 and 6). The R_f values of diploids and tetraploids also differed significantly (table 1).

These observations with three diploids and their auto-tetraploids suggest that the increase in ploidy may not bring any significant numerical changes in the spectrum of bands and in their intensity which may be helpful in identifying the ploidy differences.

Bhatia[7] reported an increase in the number of esterase-1 and alcoholdehydrogenase (ADH) iso-