BIOLOGIA PLANTARUM 38 (1): 153-157, 1996

BRIEF COMMUNICATION

Polyamines and senescence of maintenance foliage of tea, *Camellia sinensis* L.

R.K. KAKKAR and P.K. NAGAR*

Plant Physiology Laboratory, Biotechnology Division, CSIR Complex,
Palampur - 176 061 (HP), India

Abstract

In leaf discs of maintenance foliage of tea (*Camellia sinensis*) polyamines (PAs) and kinetin retarded chlorophyll (Chl) loss, whereas inhibitors of PA biosynthesis [difluoromethyl arginine, difluoromethyl ornithine, methylglyoxal-bis(guanylhydrazone)] and abscisic acid (ABA) promoted senescence. The contents of RNA and protein were significantly higher in PA and kinetin treated leaf discs as compared to those treated with inhibitors and ABA. The contents of total and reducing saccharides declined with the progressive loss of Chl, and the concentration of starch increased in all the PA treated leaf discs. Free amino acid content also increased under all the treatments, but the increase was comparatively larger in case of inhibitors application.

Additional key words: abscisic acid, chlorophyll, kinetin, putrescine, RNA, spermidine, spermine.

Senescence is a series of degradative programmed processes which ultimately lead to death of the organism or a part thereof. Senescence of leaves is associated with a progressive decrease in certain cellular components (Lalonde and Dhindsa 1990). Recently, considerable attention has been paid to the involvement of polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) in different plant processes (Galston and Kaur-Sawhney 1990, Smith 1990). PAs may function as antisenescence

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Received 27 February 1995, *accepted* 11 August 1995.

Abbreviations: ABA - abscisic acid; CN - control; DFMA - difluoromethyl arginine; DFMO - difluoromethyl ornithine; MGBG - methylglyoxal-bis(guanylhydrazone); Kn - kinetin; PAs - polyamines; Put - putrescine; Spd - spermidine; Spm - spermine.

Acknowledgements: The authors thank Director, CSIR Complex, Palampur for providing necessary facilities and Merrel Dow Research Institute (Cincinnati, OH) for generous gift of DFMO and DFMA.

* To whom correspondence should be sent.

agents (Galston and Kaur-Sawhney 1982) and this property is largely based on (*1*) decrease in PA titer with leaf aging and senescence and specially on (*2*) the ability of exogenous PAs to delay or prevent senescence in excised leaf/leaf segments (Cohen *et al.* 1979, Kaur-Sawhney *et al.* 1982). Tea is a unique perennial crop in which the young developing shoots (two and a bud) are plucked periodically and pruning is a regular practice to sustain the productivity. The permanent leaves retained below the plucking surface (maintenance foliage) are essential for the productivity and survival of a pruned and plucked tea bush. These leaves supply the photosynthates to other parts of the plant which respire and grow (Barua 1989). Besides shading, senescence beyond certain limit reduces the photosynthetic efficiency of maintenance leaves (Manivel 1980). The present study was undertaken to determine the effect of exogenous PAs and their biosynthetic inhibitors along with known senescence promoter (ABA) and retardant (kinetin) on senescence of maintenance foliage of tea and also their effects on certain biochemical constituents.

Pruned and plucked 8 year old bushes of tea [*Camellia sinensis* L. (O) Kuntze] cv. T-78 growing in the Institute's experimental farm, deep skiffed during 1992 and tipped to five leaves (20 cm) above pruning cut were used for the studies. Maintenance foliage (upper) of uniform size (*ca.* 6 × 2.5 cm) was collected in September from each bush and 6 leaf discs (10 mm diameter) were taken from both sides of the midrib. Twenty leaf discs were floated in a Petri dish (9 cm diameter) on 10 cm^3 of test solutions of various PAs (Put, Spd and Spm), of their biosynthetic inhibitors DFMA, DFMO, MGBG, of kinetin and ABA (each at a concentration of 10^{-4} M) and these were incubated for 24 and 48 h in dark at 25 ± 2 °C. The leaf discs (20) floated in double distilled water under the same conditions were used as control.

Chlorophyll was extracted in 80 % chilled acetone from 20 leaf discs (fresh mass 0.5 g) and determined spectrophotometrically at 665 nm. For determination of protein and RNA, samples of 20 leaf discs (about 0.5 g of fresh mass) were homogenized with 10 cm^3 of chilled 0.05 M Tris-HCl buffer (pH 7.5) and centrifuged at 6 000 *g* for 20 min at 5 °C. Supernatant was precipitated by chilled 20 % trichloroacetic acid (1:1 v/v), kept at 5 °C for 2 h, and pelleted by centrifugation as above. The precipitate was dissolved in 0.5 M NaOH. Protein content of an aliquot was determined according to Lowry *et al.* (1951) using casein as standard. In another aliquot, total RNA was estimated by orcinol method (Markham 1955) using RNA as standard.

Starch (McRae 1971), total soluble saccharides (Dubois *et al.* 1956), reducing saccharides (Sumner 1935) and total free amino acids (Lee and Takahashi 1966) were analyzed from treated and untreated (control) leaf discs.

The changes in chl content were used as a senescence index. The decrease in chl content was retarded by PAs and kinetin and accelerated by DFMA, DFMO, MGBG and ABA (Fig. 1A). These effects were more prominent after 48 h: Spm and kinetin were most effective in retarding Chl loss, while MGBG was the most prominent in accelerating this loss. Both the retardation and acceleration of senescence were localized and only the central area of leaf discs remained green or became even more green.

The discs treated with PAs and kinetin showed higher concentrations of RNA,

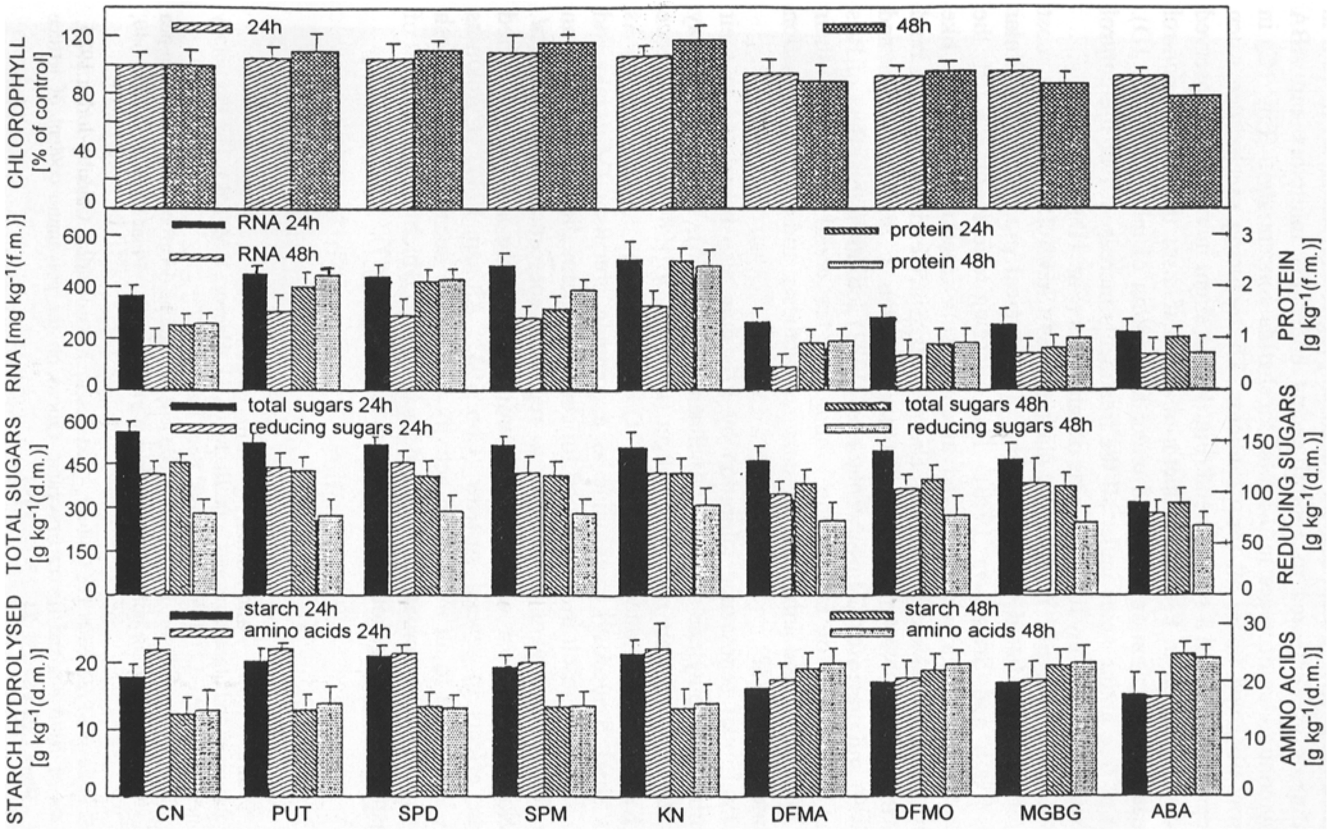

Fig. 1. Changes in contents of chlorophyll (*a*+*b*) (*A*), RNA and protein (*B*), total and reducing saccharides (*C*), and starch and total amino acids (*D*) in leaf discs of maintenance foliage of tea following treatments with various polyamines, their inhibitors, kinetin and ABA (10^{-4} M each). The values are means of three replicates of 20 leaf discs. SE ($n = 3$) for each value are given by vertical bars.

whereas the level declined with inhibitors compared to control during both the periods studied (Fig. 1B). Protein content increased progressively from 24 to 48 h in all the treatments as compared to control except under PA inhibitors and ABA (Fig. 1B). A continuous decrease in the total saccharide concentration (Fig. 1C) in leaf discs was observed under all the treatments. The reducing saccharides when estimated separately showed a similar trend (Fig. 1C). A slight increase was observed in the contents of starch in PAs- and kinetin-treated leaf discs than in the control ones, whereas its concentrations declined under application of inhibitors (Fig. 1D). The content of free amino acids under all the treatments increased over the control although it was comparatively higher in case of inhibitors (Fig. 1D).

PAs can delay senescence in different plant tissues (Smith 1990) and in most cases, this effect is mediated by altering the physiological properties of cellular membranes (Naik and Srivastava 1978). The retarding effects of PA on the maintenance foliage of tea were reflected in changes in reserve metabolites like RNA, protein, starch, total saccharides and amino acids. Since endogenous PAs bind strongly to RNA and DNA and inhibit RNAase (Galston *et al.* 1978, Isola and Franzoni 1989) and protease (Kaur-Sawhney *et al.* 1982) activities, the effect of PAs on retarding senescence of maintenance foliage of tea could be due to their interaction with RNA and inhibition of enzyme synthesis and/or enzyme action (Kaur-Sawhney *et al.* 1982).

The mechanism of senescence inhibition by PAs may also be related to their possible inhibition of ethylene synthesis (Apelbaum *et al.* 1985). This property may account for PAs to act as free radical scavenger (Drolet *et al.* 1986) and senescence retardant (Kaur-Sawhney and Galston 1991). Since both ethylene and PAs biosynthesis share S-adenosyl methionine as a common precursor (Miyazaki and Yang 1987) this could result in competition for common intermediate and provide an explanation for the ability of polyamines to regulate senescence (Roberts *et al.* 1984). Cytokinins are at par with PAs in retarding senescence and PAs exhibited cytokinin-like effects in several systems (Cho 1983, Altman 1989). Cytokinins increase PA levels due to an increase in activity of arginine decarboxylase (Suresh *et al.* 1978) and in RNase activity (Hodge and Sacher 1975) which may be a result of reduced sensitivity to endogenous ethylene (Kao and Yang 1983).

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