ROLE OF CALCIUM AND PHYTOCHROME IN RETARDATION OF LEAF SENESCENCE IN SORGHUM BICOLOR

Dissertation Submitted to
Jawaharlal Nehru University in
partial fulfilment for the Degree of

MASTER OF PHILOSOPHY

1986

S. SREEDHARA

**SCHOOL OF LIFE SCIENCES
JAWAHARLAL NEHRU UNIVERSITY
NEW DELHI-110067
INDIA**

CONTENTS

PREFACE

The research work embodied in this dissertation has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full for any other degree or diploma of any university.
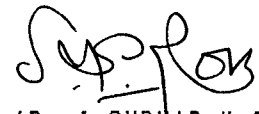
(S.SREEDHARA)

candidate

(Prof. G.S. SINGHAL)

Dean

(Prof.SUDHIR K.SOPORY)

supervisor

School of Life Sciences

Jawaharlal Nehru University

New Delhi - 110 067, India

ACKNOWLEDGEMENTS

(S. SREEDHARA)

INTRODUCTION

Senescence is the terminal phase of ageing characteristically showing decreasing viability and increasing vulnerability. There are two major views as to the physiological nature of senescence. One, going back many years, is that senescence is the consequence of degenerative changes in the biochemical machinery, changes which can be slowed or accelerated but not really reversed (Wear and Tear Theory). The other view is that senescence is the result of increased activity in specific biochemical processes, within the framework of the molecular-biological programme (Genetic Programming Theory).

In animals, senescence occurs gradually over a considerable part of their life span, but senescence in plants, although it can come gradually, can also be induced very rapidly. Thus, the life span i.e., the lapse of time required for senescence is not always a characteristic factor in plants. It can vary both inter and intra-specifically and with the environmental conditions.

Thus, the time at which the onset of senescence occurs and the rate at which it progresses can be modified by many factors. In addition to genetic programming, such factors involve phytohormones, other chemical regulators and several physico-chemical factors of dynamic environment.

The present study aims to characterize the effect of light in delaying senescence in excised leaves of _Sorghum bicolor_ and to check the involvement of calcium in this process.

PREVIOUS WORK

Senescence in plants is considered to be under atleast four different types of control. (1) Genetic; (2) Hormonal; (3) Chemical and (4) Environmental. In this review, I will briefly consider the first three types and give in some detail the effect of environmental factors especially the light.

Genetic Control

Genetic control of senescence is common in many plants. One of the most promising approaches to the more detailed analysis of genetic basis of senescence is the use of mutants, such as the form of _Festuca pratensis_ which is unable to breakdown chlorophyll as the leaves senesce (Thomas and Stoddart, 1975). The non-yellowing characteristic is due to a single nuclear gene (NY); it appears to be confined in its effects to aspects of metabolism which are closely concerned with chlorophyll catabolism since other general features of senescence such as loss of RNA and protein, changing enzyme activities and patterns of isozymes remain unaltered

in the mutant (Pearson et al., 1978; Thomas, 1977;
Thomas and Stoddart, 1975, 1977). Electron micro-
graphs of chloroplasts of the NY mutant show the
thylakoid membranes becoming released from the
general arrangement but not undergoing the usual
pattern of degradation, suggesting that the muta-
tion may involve some aspect of lipid degradation
so that the chlorophyll is not released to sites at
which it is normally degraded.

In the fungus, Podospora there are two genes
which delay senescence. Esser and Keller (1976) have
found that when both are present in the double mutant,
senescence is postponed indefinitely. Postponement
of flowering from the first growing season (annuals)
to the second (biennials) is due to a single recess-
ive gene in sugar beet and in henbane (Hyoscyamus
niger) or   several genes in wheat (Sinnott, 1960;
Halloran and Boydell, 1967). Since monocrapy has
probably evolved independently several times, different
mechanisms may be employed by different species.

Another feature of the genetic control of senescence is that interactions must presumably occur between the nucleus, chloroplasts and mitochondria in order to coordinate events. Yoshida (1961) provides an intriguing example which suggests the involvement of the nucleus in regulating chloroplast senescence in _Elodea densa._ When leaves were plasmolysed in $CaCl_2$ solution, protoplasts of some of the mesophyll cells separated into two halves, one with and one without a nucleus. When leaves were subsequently cultured, the chloroplasts of the enucleate portion of the protoplast remained green and accumulated starch for atleast five days, whereas chloroplasts in the protoplasts containing nuclei disintegrated and were broken down over the same period. Thus, there are several reports supporting the view of genetic regulation of senescence (Koll et al., 1984; Pierce et al., 1984; Osiewacza and Esser, 1984). Although, the precise mechanism of induction of senescence is not known at present, the process of senescence is known to be under genetic control (Stoddart and Thomas, 1982).

Hormonal Control

All well characterized phytohormones have been implicated some way or the other in the regulation of foliar senescence (Debata and Murthy, 1981; Kumar and Khan, 1984; Ray et al., 1983; Mondal and Choudhuri, 1984b; Ray and Choudhuri, 1984).

The first observations were of the effects of cytokinins in delaying senescence in excised leaves of Xanthium (Richmond and Lang, 1957) and the effects of several cytokinins in delaying senescence have now been described for many species, both for excised leaves and for leaves attached to the plant (Richmond and Lang, 1957; Ray et al., 1983). In leaves of Prunus species, IAA delayed senescence (Osborne and Hallaway, 1964; Osborne, 1967). Gibberellins were shown to delay the senescence of excised leaves of Taraxacum officinale but the cytokinins were without any effect (Whyte and Luckwill, 1966; Fletcher, 1975).

In Tropaeolum majus and Rumex species, cytokinins or gibberellins,over similar range of concentration were

equally effective in delaying senescence (Beevers, 1968).
The case of _Tropaeolum_ is particularly interesting
because it was found that the GA content of the leaves
decreased during senescence, thus providing circumstan-
cial evidence that the effects of particular hormones
may be related to their rates of turnover (Elkenawy,
1984), which will affect the rate at which they fall
below the threshold necessary to sustain metabolism
(Woolhouse, 1967).

Abscisic acid accelarated senescence in leaves
of many species (Beevers, 1968; Paranjothy and Wareing,
1971; Ray _et al.,_ 1983). Colohoun and Hillman
(1972) failed to find any correlation between ABA content
and the stage of senescence in leaves of _Phaseolus_
vulgaris, though it is not of course to be expected
that many such correlation between total content of the
hormone and degree of senescence should exist of nece-
ssity; it could, for example, trigger the function in
the early stages of senescence and thereafter changing
in amount in a manner unrelated to the progress of the
process of senescence.

Excised leaves of oat, incubated in darkness, showed a five-fold increase in the ABA content which may be responsible for promoting rapid senescence (Gepstein and Thimann, 1980). Mozer (1980) has clearly shown that both GA and ABA added to barley aleurones, which are known to share many attributes of a senescing tissue, induce the appearance of unique mRNAs which are translatable *in vitro*. It was found that GA treatment altered *in vivo* protein synthesis by stimulating one set of proteins, the hydrolases, exclusively, whereas ABA induced the synthesis of several new proteins, whose functions are as yet unknown and prevents synthesis of α-amylase. Addition of both GA and ABA together to aleurones, to which no hormones were added, showed that the GA-induced mRNAs were inhibited. Thus, it was concluded that ABA is acting to block the translation of GA-induced messages *in vivo*. It is still not clear whether this effect of ABA on translation is mediated via the ABA induced proteins (Woolhouse, 1982). It would be of particular interest to know whether ABA is operating in a similar manner in senescing leaves.

Ethylene has also been implicated to have a role in senescence (Kao and Yang, 1983) though, according to some ethylene has no role in affecting the rate of senescence in some species (Thimann, 1978). It has been observed during senescence of excised leaves of tobacco that there was a rapid rise in ethylene production prior to the final rapid phase of senescence (Aharoni et al., 1979a).

Auxin treatment of many plant tissues leads to an increased production of ethylene (Abeles, 1973), which has been shown to result from increased activity of 1-amino cyclopropane 1-carboxylic acid (ACC) synthase, the enzyme which catalyses the conversion of 3-adenosyl-methionine (SAM) to ACC (Adams and Yang, 1979). The inhibitors of ACC synthase, AVG and CO^{2+}, which inhibits the conversion of ACC to ethylene, delay the senescence of excised leaves, and Ag^+ ions and high CO_2 concentrations, which inhibit ethylene action, also give rise to similar effects (Aharoni and Lieberman, 1979; Aharoni et al., 1979b).

examination of interactions between ethylene and other phytohormones (Lieberman, 1979) in the specific context of leaf senescence. In essence all these observations of changes which occur in the endogenous patterns of hormones in relation to factors influencing the initiation and acceleration of leaf senescence (Chin and Beevers, 1970; Aharoni and Richmond, 1978; Nooden, 1980; Thomas and Stoddart, 1980) are evidences of the implication of the hormonal balance in the complex system of ordered senescence.

There are also evidences for one or more special senescence promoting hormone(s) operating in whole plants (Nooden and Leopold, 1978). Some direct evidences for a senescence hormone, or atleast a transmissible factor have been uncovered in peas. Here, one genetic line, called G_2, has the senescence of its apex delayed by short days (Marx, 1968). When another, more normally senescing, cultivar is grafted as scion on to the G_2 stock, its senescence also becomes sensitive to short days (Proebsting et al., 1977). The nature of this factor is not yet known.

Chemical Control

There are several chemicals known to either promote or retard senescence. Several metal cations (Jana and Choudhuri, 1984; Hemantaranjan and Garg, 1984), polyamines and guanidines (Srivastava et al., 1983; Cheng and Kao, 1983), inorganic nutrient deficiency (Malek and Cossins, 1983), terpenoid analogues (Grossmann and Jung, 1984), dipyridyls (Cheng and Kao, 1984a), metal chelators (Cheng and Kao, 1984b), imidazole derivatives (Dogra and Tiwary, 1983), serine (Satler and Thimann, 1983a) and transcription and translation inhibitors (Pjon, 1984) have been implicated to have either retarding or accelerating effects on the process of leaf senescence. Several evidences indicate that Ca^{2+} can be regarded as having antisenescent properties (Halevy and Mayak, 1981; Ferguson, 1983; Ferguson et al., 1983; Ferguson, 1984). Calcium is thought to act through calmodulin, a calcium binding regulatory protein (Leshem et al., 1984). Calcium and cytokinins have been known to interact in delaying senescence in corn leaves (Poovaiah and Leopold, 1973a).

Calcium status of the cell would depend on the
activity of Ca^{2+}-ATPase, the activity of which
is promoted by activated calmodulin (Dieter and
Marme, 1980). Calcium-calmodulin mediated protein
phosphorylation has been recently demonstrated in
corn coleoptiles (Veluthambi and Poovaiah, 1984).
Calcium has also been observed to interact with
membranes, conferring structural and functional
integrity to them (Poovaiah and Leopold, 1976;
Paliyath et al., 1982). Senescence in post climac-
teric apples has been found to be retarded by the
application of calcium (Poovaiah and Shekhar, 1978).

Environmental Control

The environmental factors include primarily
light, temperature and the access of water. The
present discussion is restricted to the effect of
light only.

Effect of Light - Light plays a very crucial
role in photosynthetic green plants, particularly in
relation to its quality, duration and intensity. In
addition to its role in photosynthesis, it also decis-
ively controls plant development and morphogenesis

(Mohr and Shropshire, 1983). Light absorption by various photoreceptors, transmission of the signal, and the final responses in the greening process are well investigated (Kasemir, 1983). However, very little is known about the mechanism of light action in controlling leaf senescence although it has been known either to stimulate, or retard the process.

Although photostimulation of leaf senescence has been reported in certain cases, the action of light in controlling leaf senescence is normally attributed to its delaying effect on senescence. The precise role of light in retarding the process is confined to two views : whether the action is photosynthetic or photomorphogenic (Biswal and Biswal, 1984).

Role of phytochrome - The non-photosynthetic action of light was reported by Haber et al. (1969). They did extensive experiments on the effect of spectral zones of visible light on the senescence response, and reported that the role of light in retarding plastid senescence is photomorphogenic. However, they did not demonstrate the precise nature

of photoreceptors involved in the process. Later
De Greef et al. (1971) working on the light retard-
ing effect of senescence in Marchantia, revealed that
phytochrome is the photoreceptor involved. This view
has been supported by many papers that have reported
that phytochrome is the major photoreceptor involved
in light-mediated senescence retardation (Biswal et al.,
1983a). Steinitz et al. (1980) who have investigated
the effect of light in retarding senescence of the
flower stalk, also clearly suggest that the effect of
light on retardation of chlorophyll degradation is
totally mediated by the phytochrome system. Phytochrome
is shown to regulate plastid degradation during barley
leaf senescence as reported by Biswal and Sharma (1976)
and Pfeiffer and Kleudgan (1980). The latter authors
have shown that phytochrome not only retards chloro-
phyll loss but also the loss of carotenoids, plasto-
quinones and variable fluorescence of chloroplasts.
Phytochrome as the photoreceptor in controlling leaf
senescence of cucumber and tomato has been reported
by Tucker (1981). In these two systems, pulses of red
light retarded senescence approximately to the same
extent as that of continuous white light and the effect

of red pulse was nullified with a brief irradiation
of far-red light after red light treatment, suggest-
ing again phytochrome involvement during leaf
senescence. The effective control of phytochrome in
retarding chlorophyll loss and disintegration of
plastid ultrastructure has been demonstrated by
Biswal et al. (1982).

Biswal et al. (1983a) confirmed that phyto-
chrome is involved in light mediated retardation
of leaf senescence. They irradiated the leaves
with a single far-red light pulse, which was photo-
synthetically ineffective. If the white light period
was terminated by this far-red light pulse, before
the transfer of light grown seedlings to darkness,
there was a significant effect on the rate of
senescence.

At this juncture, it is difficult to propose any
molecular mechanism of phytochrome action in delaying
leaf senescence. However, at the biochemical level several
possibilities have been suggested to explain the mode of
phytochrome action in relation to retardation of leaf
senescence.

Light-retarding action on leaf senescence is stimulated in the laboratory by physiological modifications of the leaves, particularly by the treatment of leaves with exogenous ions and specific growth regulators in dark. It is thus logical to assume that light might be retarding senescence through its control over these factors in nature. In that case, its action on senescence is rather indirect and through production of secondary messengers, a concept which has been thought to operate during photomorphogenesis.

Light-induced changes in the level of phytohormones in relation to senescence - Although, the involvement of light in hormone production is well established (Wareing and Thompson, 1976; Rohwer and Schierle, 1982), the precise relationship between light and phytohormones in leaf development is not known. It was assumed that light could regulate the level of some specific plant hormones with a consequent effect on leaf senescence. In this context, Gepstein and Thimann (1981) showed that white light induced retardation of oat leaf senescence was mediated

through the light inhibition of ethylene accumulation. Wareing and Thompson (1976) have reported a significant reduction in the level of endogenous ethylene with red light pulses, which on the other hand, stimulate the level of cytokinins. It is, therefore, possible that the effect of red light pulses could be stimulated by the exogenous application of hormones, like gibberellic acid and cytokinins. However, the interaction between phytochrome and exogenous hormones in controlling plant development seems to be complex in nature (Kasemir and Mohr, 1982). The effects of these two factors are shown either to be independent or additive. As present, however, there is no strong evidence that phytohormones could substitute for light in photomorphogenesis.

Light controlled polyamine level in relation to senescence - Polyamines, namely, putrescine, cadaverine, spermidine, spermine and their precursors, L-arginine and L-lysine are known potent inhibitors of leaf senescence (Galston, 1983; Muhitch et al., 1983). Interestingly, light which retards senescence in excised leaves is also reported to increase the level of polyamines (Kaur-Sawhney et al., 1982; Altman, 1982). Exogenous

polyamine addition to the excised leaf segments of a
number of plant species retards senescence (Shih et al.,
1982; Altman, 1982; Kaur-Sawhney et al., 1982; Galston,
1983). Polyamine induced senescence retardation
through light may be interpreted in terms of their
inhibitory action on the production of ethylene, a
potent promotor of senescence (Evan-Chen et al., 1982;
Apelbaum et al., 1982). The mechanism of regulation of
polyamine levels by light and its ultimate effect on
retardation of senescence is not yet clear. Dai and
Galston (1981) and Goren et al. (1982) have attributed
light action through phytochrome in controlling poly-
amine levels in pea plants.

Light mediated stomatal opening in relation to
senescence - Senescence retarding action through the
opening of stomata as controlled by light has been
attributed by some authors (Thimann and Satter, 1949;
Morison and Jarvis, 1983; Nejidat et al., 1983).
Gepstein and Thimann (1981) demonstrated that the
closure of stomata in dark results in an increase in
abscisic acid level that accelerates the process of
leaf senescence. However, the concept of light mediated
stomatal opening and its consequent retarding effect on

leaf senescence has had a set back by the recent reports
which do not suggest any direct correlation between the
status of stomata and senescence regulation (Wardle and
Short, 1983; Satler and Thimann, 1983b; Biswal et al.,
1983c).

Light induced proton secretion in relation to
senescence - Light has recently been shown to
regulate senescence through secretion of protons from
the leaf cells (Gepstein, 1982). It has been suggested
that the proton pump in membranes eliminates H^+ from
cytoplasm in the presence of light. This may prevent
acidosis, consequently delaying the process of senes-
cence by decreasing the activity of acid hydrolases.

Role of Free Radicals in Senescence

The free radical theory as an alternative
explanation of. the mechanism of senescence process
has recently attracted increasing attention. Particu-
larly for animal and humans, the theory was developed
that aging occurs as a result of particular predominant
accumulation of deleterious free radical reactions
(Herman, 1981). As accumulation of free radicals
was also claimed to occur in aging leaves by Leshem

cytokinin was attributed to its ability to act as a
free-radical scavenger (Leshem et al., 1981).

Free radicals participate, briefly in the form
of activated O_2 species such as superoxide (O_2^-) or
H_2O_2, in several electron-transfer reactions of normal
cell metabolism and are usually controlled by the
appropriate protective mechanisms such as superoxide
dismutase, catalase and peroxidases (Fridovich, 1976;
Elstner, 1982). It has been postulated (Leshem, 1981)
that activated oxygen may leak away from faulty defense
mechanisms, and in addition, give rise to other, even
more deleterious oxygen radicals, such as the hydroxyl
radical (OH$^{\cdot}$), singlet oxygen (O_2), or lipid hydro-
peroxides, that readily react with nucleic acids,
proteins and lipids (Fridovich, 1976; Gardner, 1979;
Elstner, 1982) and may initiate senescence.

$TH- 7111$

During the last few years, some aspects of activa-
ted oxygen metabolism, such as the behaviour of lipoxy-
genase (Grossmann and Leshem, 1978; Leshem, 1981; Leshem
et al. 1981), superoxide dismutase and lipid peroxidation
(Dhindsa et al., 1981, 1982) or peroxide levels (Parida
et al., 1978; Mondal and Choudhuri, 1984b) have been
studied during the senescence of leaves in different plant
species.

MATERIALS AND METHODS

Seed Material, Germination and Seedling Growth

Seeds of _Sorghum_ _bicolor_ var. Pusa Chari-6
were obtained from Cummings Laboratory, Indian
Agricultural Research Institute, New Delhi and
were stored at 26 ± 1°C in dessicators.

The seeds of uniform size were selected,
washed thoroughly with double distilled water
and soaked for about 12 h in darkness. Then
seeds were plated for germination on two layers
of wet germination paper in petri dishes and were
maintained in dark in a BOD incubator at 26 ± 1°C.
Seedlings were watered once every 24 h with distilled
water. At 48 h the ungerminated seeds or seedlings
of abnormal size were removed. Handling of the
plant material and such other manipulations were
done under a green safe light.

Light Treatments and Light Sources

The excised leaves of uniform size of 12 day
old seedlings, grown initially for 6 days in dark

and then in continuous white light for 6 subsequent
days, were given red light or far-red light treat-
ments for 10 min every 24 h and were again incubated
in darkness for a specified period. This period
between the time of preirradiation and the end of
subsequent dark period is referred to as 'Dark
Incubation Period' (DIP). All the light treatments
were carried out at 26 $\pm$ 1°C, the DIP in all experi-
ments was of 24 h duration. The characteristics of
light sources were according to Sharma et al. (1976)
and Rao et al. (1983) with some modifications as
stated below.

Green safe light -   The green safe light was
obtained through 8-10 layers of green cellophane
paper with emission maximum at 520-525 nm from 40 watt
cool white fluorescent tube light. The intensity of
green light at the leaf level was never more than
1 μW cm^{-2}.

Red light -   Red light was obtained from two
100 watt tungste lamps wrapped with two layers of
red cellophane paper with emission maximum at 650 nm.
The intensity of the red light at the height of
excised leaves was 500 μW cm^{-2}.

Far-red light - Far-red light was obtained from one 300 watt reflector lamp (Westinghouse, U.S.A.), filtered through 8 cm layer of continuously flowing tap water and a CBS 750 filter (Carolina Biological Supply Company, North Carolina, U.S.A.), with emission maximum at 750 nm. The intensity of far-red light at the height of leaves was 140 μW cm^{-2}.

White light - White light of varying intensities at the seedling level was obtained from 9 cool white fluorescent tubes with no emission in far-red region. The intensity of white light at the plant height was 2000 lux.

Determination of Total Chlorophyll Content

Extraction of chlorophyll - Excised primary leaves (about 50-100 mg fresh wt.) were ground using mortar and pestle for 5 min in 80% acetone to make the final volume to 5 ml. The homogenate was centrifuged at 26,000 x g for 10 to 15 min at 4°C. The pellet was re-extracted with 80% acetone for the complete extraction of chlorophylls.

Chlorophyll estimation - The absorbance of the

extracts was recorded at 645 nm and at 663 nm in a

Shimadzu visible recording spectrophotometer, Model

UV-240 or UV-260. The total chlorophyll content was

then estimated according to Arnon (1949) as given

below.

$$\text{mg chlorophylls/g fresh wt.} = 20.2 \ (A_{645}) + 8.02 \ (A_{663})$$
$$X \ \frac{V}{1000 \times W}$$

where, V = volume in ml of the complete extraction
medium and

W = fresh wt. in g.

Total chlorophylls are expressed as μg/g fresh weight.

Determination of Total Carotenoid Content

The method of Liaaen-Jensen and Jensen (1971) was

followed to determine total carotenoid content. The

total carotenoids were extracted in 80% acetone in a

manner essentially similar to that of chlorophylls.

Since only carotenoids show maximum absorption at

475 nm the absorbance at this wavelength was recorded

(Virgin, 1966). By using the following formula the

total amount of carotenoids was determined:-

$$\text{mg carotenoids/g fresh wt.} = \frac{A_{475} \times V}{W} \times \frac{10}{2500}$$

where, V = total dilution of the sample in ml, and

W = fresh wt. in g.

Estimation of Superoxide Dismutase Activity

Superoxide dismutase catalyses the following reaction :

$$2O_2^- + 2H^+ \xrightarrow{\quad SOD \quad} H_2O_2 + O_2$$
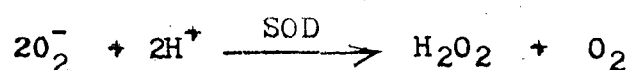
Superoxide dismutase activity was assayed according to the method of Thomas et al. (1976). The assay as well as extraction mixture contained the following: 0.5M carbonate buffer (pH 10.2), 10^{-4}M EDTA, 2.5 x 10^{-5}M nitroblue tetrazolium, 10^{-4}M xanthine, 25 μl of xanthine oxidase (an appropriate amount of xanthine oxidase which can result in a ΔO.D. of 0.60 per 5 min was used and 0.05 ml of enzyme extract in a final volume of 3 ml was added. The reduction of nitroblue tetrazolium was measured by observing change in the absorbance against reagent blank at 560 nm using Shimadzu UV-260 spectrophotometer. The superoxide dismutase content was estimated by comparison with the commertially available erythrocyte superoxide dismutase. A 50% inhibition

of tetrazolium reduction was obtained by 0.862 ug of
the standard superoxide dismutase used. The uninhibited
reduction of nitroblue tetrazolium in the absence of
superoxide dismutase is shown in Table I.

An amount of xanthine oxidase was chosen such
that it gave an activity of $\triangle$O.D. 0.012/min.

Estimation of Fluorescence for Lipid Peroxidation

The crude extract was prepared with the $\approx$ . .
extraction/assay buffer used for superoxide dismutase
assay and the fluorescence was measured in a Shimadzu
Spectroflurometer as reported (Roy et al., 1983).

TABLE 1 : Standard xanthine oxidase activity was recorded as change in the absorbance in the absence of superoxide dismutase.

Time (Minutes)	Absorbance (at 560 nm)
5	0.060
10	0.115
15	0.149
20	0.183
25	0.207
30	0.228

To find out the possible mechanism of the
regulation of foliar senescence the role of phyto-
chrome, and possible secondary messengers has been
studied in the present investigation.

Photoreversibility of the Retardation of Pigment Breakdown

As shown in Table 2 and 3 when 12 day old
(6 days dark + 6 days continuous white light) excised
leaves, floated on water for 6 days were irradiated
with red light (10 min per day), a 57% increase in
chlorophyll level and 20% increase in carotenoid level
over dark control was observed. An immediate exposure
of 10 min far-red light reversed the red light effect.
This photoreversibility of protection/degradation of
pigment molecules indicated the involvement of phyto-
chrome in the retardation of pigment breakdown. The
effect observed with continuous white light was more
pronounced than that observed with red light. The
continuous white light increased the levels of chloro-
phyll by 82% over dark control. But in case of
carotenoid levels, red light was more effective than
continuous white light, as continuous white light

TABLE 2

Role of phytochrome in chlorophyll breakdown during leaf senescence. From plants grown for 12 days (6 Dark + 6 D light), leaves were excised and floated on distilled water. Percent of pre-senescent level is calculated with respect to leaves from 12 days old plants, before they were excised. At 12 day the value was 2.218 mg/gm fresh wt^{-1}.

Treatment	Chlorophyll mg g fr. wt.$^{-1}$	Percent	Percent of pre-senescent
Dark	0.606	100	27
Red 10 min	0.950	157	43
Far-red 10 min	0.645	106	29
Red 10 min + Far-red 10 min	0.644	106	29
Red 10 min + Far-red 10 min + Red 10 min	0.820	135	37
Continuous white light	1.102	182	50

TABLE 3

Role of phytochrome in carotenoid breakdown during leaf
senescence. Experimental details as in Table 2. The
value of pre-senescent leaves was 0.509 mg gm fr. wt.$^{-1}$.

Treatment	Carotenoids mg gm fr. wt.$^{-1}$	Percent	Percent of pre-senescent leaves
Dark	0.258	100	51
Red 10 min	0.310	120	61
Far-red 10 min	0.285	111	56
Red 10 min + Far-red 10 min	0.272	105	53
Red 10 min + Far-red 10 min + Red 10 min	0.298	116	59
Continuous white light	0.295	114	58

showed an increase of only 14% over dark control.
The data given in Table 2 and 3 indicates that
the levels of pigments during senescence are
determined by the quality of final irradiation as
it is observed in other phytochrome-mediated
processes. The effects are also clear when chloro-
phyll level is compared with pre-senescent level.

To trace the mechanism of action of phytochrome
in leaf senescence the role of several possible secon-
dary messengers was worked out. Such possible candidat__
to act as secondary messengers, studied were polyamines,
calcium and cytokinin. Serotonin which is known to
regulate cytosolic Ca^{++} concentrations through inositol
metabolism in animals was also tested for its effect
on foliar senescence. Since the effect of phytochrome
was better noticed for chlorophyll in further experi-
ments only chlorophyll was taken as a parameter of
assay of senescence.

Effect of Polyamines

Polyamines like spermine, spermidine and
putrescine were found to retard leaf senescence. There
was an increase in the retardation effect of polyamines

when their concentration was increased from 0.1 mM to 10 mM. At a concentration of 10 mM, spermine, spermidine and putrescine showed an increase in chlorophyll amount by 200%, 210% and 290% respectively over the dark control as shown in Table 4. This effect was more than in leaves kept in continuous white light.

Effect of Calcium and Benzylaminopurine

The effect of calcium was tested at different concentrations and BAP, only at the 10^{-5}M concentration. With an increase in Ca^{2+} concentration the chlorophyll level retained was more and an optimal of 92% increase was obtained at 10 mM concentration. Even when the chlorophyll content was compared to the chlorophyll level of pre-senescent leaves (i.e. at 12th day) a similar trend was seen (Table 5). With benzylaminopurine (BAP), as expected, the increase was 223% and 87% of chlorophyll was retained. With Ca^{2+} (at 10 mM) the values were comparable to white light controls but with BAP, the values obtained were much higher.

Effect of Serotonin in Regulation of Leaf Senescence

Serotonin, a neurotransmitter which is known to regulate cytosolic calcium concentration through

TABLE 4

Effect of different concentrations of polyamines on senescence retardation

Treatment	Chlorophyll mg gm fresh wt.$^{-1}$	Percent
Dark	0.534	100
+ Spermine 0.1 mM	0.887	166
0.5 mM	0.913	171
1.0 mM	1.251	234
10.0 mM	1.600	300
+ Spermidine 0.1 mM	0.767	144
0.5 mM	1.484	278
1.0 mM	1.801	337
10 mM	1.654	310
+ Putrescine 0.1 mM	0.869	163
0.5 mM	1.514	284
1.0 mM	1.846	346
10.0 mM	2.083	390
Continuous white light	1.160	217

TABLE 5

Effect of calcium and cytokinin on senescence retardation

Treatment	Chlorophyll mg gm fresh wt.$^{-1}$	Percent	Percent of pre-senescent leaves
Dark	0.598	100	27
+ Calcium 0.1 mM	0.694	116	31
0.5 mM	0.884	148	40
1.0 mM	0.870	146	39
5.0 mM	1.016	170	46
10.0 mM	1.148	192	52
40.0 mM	1.182	198	53
+ BAP 10^{-5}M	1.930	323	87
Continuous white light	1.018	170	46

phosphatidylinositol metabolism was tested for its effect on leaf senescence, and was found to be a potent retardant of senescence. The senescence retarding effect of serotonin increased gradually upto a concentration of 40 mM as shown in Table 6.

Regulation of Superoxide Dismutase Activity by Phytochrome

As shown in Table 7, when 12 day old (6 days dark + 6 days continuous white light) excised leaves floated on water for 6 days were irradiated with red light (10 min) a 64% increase in enzyme activity over the dark control was observed. An immediate exposure to 10 min far-red light reversed the red light effect. The data given in Table 7 indicated that the activity of superoxide dismutase during senescence is determined by the quality of final irradiation. Thus, the photo-reversibility of superoxide dismutase activity indicated the involvement of phytochrome in the regulation of superoxide dismutase activity.

To study dose response, light of different duration was given. The maximum increase in the

TABLE 6

Effect of serotonin on retardation of leaf senescence

Treatment		Chlorophyll mg gm fr. wt.$^{-1}$	Percent
Dark		0.518	100
+ Serotonin	1.25 mM	0.898	173
	2.5 mM	0.915	177
	5.0 mM	1.089	210
	10.0 mM	1.191	230
	20.0 mM	1.548	299
	40.0 mM	1.784	344

TABLE 7

Effect of phytochrome and calcium on superoxide dismutase activity in senescent leaves. Dark-absolute value is $\Delta OD= 0.00724$ mg protein^{-1}.

Treatment	Percent Enzyme Activity
Dark	100
Red 10 min	164
Far-red 10 min	91
Red 10 min + Far-red 10 min	118
Red 10 min + Far-red 10 min + Red 10 min	167
Ca^{2+} 10 mM	121

activity of SOD i.e., 113% over dark control was recorded with the treatment of red light of 10 min duration. The further increase in duration of red light decreased the activity of SOD as shown in Table 8.

Activation of Animal Superoxide Dismutase by Ca^{++} and Animal Calmodulin In Vitro

As shown in Table 9, a concentration of 20 μM of calcium showed 25% increase in the activity of 1 μg SOD over the EGTA control. Calmodulin (2.5 ug) when tested along with calcium showed an increase in SOD activity of 150% over EGTA control. The same amount of calcium and calmodulin, showed about 109% increase in SOD activity over EGTA control. The calmodulin inhibitor, compound 48/80 (6.67 $\mu g/ml$) showed an increase of only 59% in SOD activity over the EGTA control.

Effect of Metal Cations

To check the effect of other metal cations on chlorophyll content during leaf senescence, the

TABLE 8

Effect of red light duration on superoxide dismutase activity in senescent leaves. The dark absolute value of enzyme activity is $\triangle OD = 0.000187$ mg protein^{-1} or $\triangle OD = 0.002405$ gm fr. wt^{-1}. Dark level is taken as 100%.

Treatment	Percent Enzyme Activity	
	mg protein^{-1}	gm fr. wt^{-1}
Dark	100	100
Red 10 min	213	145
15 min	117	97
20 min	74	71

TABLE 9

In vitro effect of calcium and calmodulin on animal
superoxide dismutase activity. SOD was obtained
commercially from Sigma Chemical Co., MO. With the
addition of 1 μg SOD to +EGTA (-SOD) extract, about
58% inhibition in NBT reduction was noticed. This
value is taken as 100%.

Treatment	Percent SOD Activity
EGTA + SOD (1 μg)	100
Ca^{2+} (20 μM) minus EGTA	125
Ca^{2+} (20 μM) + Calmodulin (2.5 μg)	250

effect of Ni, Cu and Ag was tested at different concentrations (Table 10) and others at 15 mM concentration (Table 11). The response with respect to different cations was varied. Nickel showed increase in retention of chlorophyll but this response, unlike the calcium effect was not concentration dependent as from 5 to 20 mM the response was almost similar. This was the situation with copper also, although with this cation the response was less than with nickel (Table 10). Silver showed inhibitory effect. Among other ions (Table 11) the maximum effect was noted with zinc.

Effect of Calcium, Nickel and Red Light on Lipid Peroxidation

The formation of senescence products which absorb light at 350 nm and fluoresce at about 500 nm was observed to be more in case of senescing leaves kept in dark. The senescing excised leaves kept in continuous white light showed very low levels of fluorescent compounds, the relative fluorescence intensity/mg protein was about 0.9% of the dark control (Table 12).

TABLE 10

Effect of nickel, copper and silver on chlorophyll level
during senescence of leaves

Treatment		Chlorophyll mg gm fr. wt.$^{-1}$	Percent
Dark		0.631	100
+ $NiCl_2$	5 mM	1.680	266
	10 mM	1.804	286
	15 mM	1.452	230
	20 mM	1.692	268
+ $CuCl_2$	5 mM	1.238	196
	10 mM	1.187	188
	15 mM	1.055	167
	20 mM	0.997	158
+ $AgNO_3$	5 mM	0.504	80
	10 mM	0.444	70
	15 mM	0.385	61
	20 mM	0.384	64
Continuous white light		1.536	243

TABLE   11

Effect of inorganic cations on chlorophyll level
during senescence of leaves

Treatment	Chlorophyll mg gm fr. wt.$^{-1}$	Percent
Dark	0.598	100
+ KCl 15 mM	0.602	101
+ AlCl$_3$ 15 mM	0.940	157
+ BaCl$_2$ 15 mM	1.118	187
+ MnCl$_2$ 15 mM	0.989	165
+ MgCl$_2$ 15 mM	0.715	120
+ ZnCl$_2$ 15 mM	1.481	248

TABLE 12

Effect of calcium and nickel on lipid peroxidation
as measured by fluorescence in leaf extracts

Treatment	Relative fluorescence intensity/mg protein	Percent
Dark	214	100
Ca^{2+} (10 mM)	21	9.8
Ni (10 mM)	11867	5545
Continuous white light	1.818	0.850

When the divalent cations Ca^{++} and Ni^{++}, which were found to act as senescence retardants, were tested for their role in the formation of fluorescence products, Ca^{++} showed a relative fluorescence intensity/mg protein, of about 9.8 when compared to dark control (100), whereas Ni^{++} showed a very high relative fluorescence intensity/mg protein of about 5545 as shown in Table 12.

The relative fluorescence intensity/mg protein was minimum in pre-senescent leaves, and showed a relative value of 23% over the dark control (100). The treatment of red light, for 1 min gave a relative fluorescence intensity of 40% and the extent of such fluorescence intensity decreased when the duration of red light treatment was increased from 1 min to 5 min and showed a relative value of 33 with 5 min red light treatment (Table 13).

TABLE 13

Effect of duration of red light on lipid peroxidation
as measured by fluorescence in leaf extracts

Treatment	Relative fluorescence intensity/mg protein	Percent
Dark	141	100
Red 1 min	57	40
2 min	52	37
5 min	46	33
Pre-senescent	33	23

DISCUSSION

The action of light in delaying leaf senescence
has been reported by several authors (see Biswal and
Biswal, 1984). However, the precise role of light in
this process is controversial. It is argued whether
the action is photosynthetic or photomorphogenetic.
The present work was aimed at looking into the photo-
morphogenetic aspects especially with respect to the
involvement of the pigment phytochrome, and the
mechanism of its action. Further, the involvement
of calcium together with other ions was also tested
in this process. Some of the results obtained in
this direction are discussed in this chapter.

Role of Phytochrome

Since the first demonstration of non-photo-
synthetic effects on leaf senescence by light many
papers have appeared, but none in C_4 plants. In the
present study, it was demonstrated, by monitoring
pigment breakdown, that light acts via phytochrome
in retarding leaf senescence. The effect of brief
irradiation of red light was totally reversed by
far-red light (upto the dark level) which was again

nullified by red light (Table 2 and 3). The increase
in red light was more with chlorophylls than with
carotenoids. It also followed that carotenoids
degraded slower than the chlorophylls compared to
pre-senescent leaves; red light treatment for 10 min
was almost as effective as leaves kept in continuous
white light. In the present study measurements of
phytochrome have not been made but in other studies
it was also proved that there is an effective control
by phytochrome in retarding chlorophyll loss (Biswal
et al., 1983a).

The mechanism of phytochrome action at present
is still not clear, less so in retarding senescence.
In view of recent reports on the mediation of phyto-
chrome responses by polyamines and calcium, these two
were tested in the present system.

Polyamine, Calcium and Serotonin Effects

Polyamines have been recorded as the inhibitors
of leaf senescence, besides having many other effects
(Smith, 1985). The regulation of endogenous polyamine
levels by phytochrome has also been reported (Kaur-
Sawhney et al., 1982). When added exogenously, all

three, spermine, spermidine and putrescine retarded chlorophyll breakdown in <u>Sorghum</u> leaves, in the present study. Whereas spermidine was most effective at 1 mM concentration, the others showed maximum effectiveness at 10 mM concentration. And these effects were more than that obtained with white light alone.

It has been proposed that calcium is the possible mediator of phytochrome action (Roux, 1983). When tested at a wide range of concentrations, it inhibited chlorophyll breakdown and at 5 mM concentration gave as much protection as obtained under continuous white light (Table 5), even when compared with the pre-senescent leaves. Calcium has been shown earlier to decay senescence in corn leaf discs, together with cytokinins (Poovaiah and Leopold, 1983). Even in present study benzylaminopurine delayed senescence and this effect was much higher than that of calcium. The biochemical basis of these effects of Ca^{2+} are not well worked out.

Serotonin is known to regulate the levels of cytoplasmic calcium through inositide metabolism at

membrane level in animal systems (Fain and Berridge,
1979). In our plant system it showed a senescence
retarding effect. Although serotonin has not been
reported to be present in plant systems, its action
at membrane level may be analogous in animals as well
as plants. However, a quantitative study of different
phosphoinositides of plant plasma membrane treated
with physiological levels of serotonin would give
conclusive results in determining the role of serotonin
in the regulation of intracellular calcium concentration.

Free Radicals and Senescence

It is known that the degradative reactions during
the process of leaf senescence result in accumulation of
different kinds of free radicals that further accelerate
degradative reactions. The role of free radicals in
senescence has long been in debate as the question whether
the free radicals are the cause or the consequence of
senescence process is yet to be resolved. However, the
fact that there is an increase in the level of free
radicals during senescence is well established one. In
our study, we have not measured directly the production
of free radicals during senescence, however, we checked

on the extent of lipid peroxidation by the formation of senescence products. It was observed that in the absence of light more peroxidation occurred which in turn might lead to more production of free radicals. Increasing duration of red light decreased lipid peroxidation.

Phytochrome and Calcium Effects on Superoxide Dismutase

It was reported earlier (Leshem et al., 1981) that retardation of leaf senescence by cytokinin was because of its ability to quench directly the free radicals. Free radicals mostly participate in the form of activated O_2 species such as superoxide ($O_2^{-\bullet}$) or H_2O_2 and are controlled by protective mechanisms like superoxide dismutase, catalase and peroxidases (Elstner, 1982). In the present investigation, we wished to find out if light has any effect on superoxide dismutase and thereby regulating senescence. Interestingly, it was found that the enzyme activity was higher in light grown leaves and that this effect was controlled by phytochrome, as a clear red, far-red photoreversible phenomenon was obtained.

To check if phytochrome was involved in superoxide dismutase control through Ca^{2+} and calcium binding protein calmodulin, the enzyme activity was tested in Ca^{2+} grown leaves. Calcium increased the activity by only 21% (Table 7). Since in present study, we did not purify superoxide dismutase from plant source, the effect of Ca^{2+} and calmodulin was tested with animal enzyme *in vitro* (Table 9) and both were found to increase the enzyme activity. This activation by animal calmodulin was reversed with EGTA, a Ca^{++} chelating agent. However, more refined experiments with plant leaf extracts, extracted in presence of EGTA, and experiments with plant calmodulin would give conclusive results without any ambiguity.

Although, these results are of interest, a direct correlation between free radicals and initiation of senescence will have to be established in view of some work where correlations were not obtained (Kar and Feierabend, 1984).

Role of Other Metal Ions

From the results discussed above it seems that calcium could mimick the effects of phytochrome, totally or partially, both in retarding chlorophyll breakdown as

well as in controlling superoxide dismutase activity.
The question arises, is this effect specific? We
looked into the effect of a number of metal cations
at different concentrations and found many of them
were able to prevent breakdown of chllrophyll; of
these nickel was very effective. However, when tested
for other parameter Ni^{2+} gave different results.

Nickel was observed to act differentially with
regard to different senescence parameters. It has
maximum retardation effect on chlorophyll breakdown
whereas it had stimulatory effect on proteolysis,
when compared to dark control. The level of fluore-
scent products formed during senescence was also
maximum in Ni^{++}-treated senescing leaves. Thus, except
in the case of chlorophylls, Ni^{++} had many senescence
promoting effects. The actual mechanism of chlorophyll
protection, specifically by Ni^{++} remains to be elucidated.

The Working Model

The data obtained suggests that the initiation
of the process of membrane lipid peroxidation would
lead to the formation of superoxide radicals. These

radicals inturn would then hasten the process of
senescence which could be measured as pigment
breakdown, protein breakdown or any other response.
In light, which in present case is acting via phyto-
chrome, the increase in superoxide dismutase activity
would scavenge the free radicals, thus delaying the
process of breakdown. The phytochrome would probably
increase the enzyme activity through calcium and
calmodulin, which, however, remains to be elucidated
conclusively with the plant system. While other metal
ions also slow down the senescence process, their
mechanism of action might be different than phyto-
chrome or calcium.

Although light has been shown to work through
phytochrome system in the regulation of leaf senescence
the possible contribution by photosynthetic system in
this process can not be ignored. In essence, light
might have a very general effect whereby it regulates
several processes so as to finally protect senescing
leaves from degradative processes. No other regulatory
substance either physical or chemical has an ability to
totally mimick the effect of continuous white light.
Light is, thus, almost an integral regulatory agent
in plants.

SUMMARY

The following points have come to light during the present investigation on leaf senescence in a C_4 plant, Sorghum bicolor.

1. The breakdown of chlorophyll is slower in light than in darkness.

2. A definite involvement of phytochrome in the retardation of leaf senescence is shown.

3. There is a possible involvement of Ca^{2+} as mediator of phytochrome response in retarding chlorophyll breakdown.

4. Serotonin, which is a neurotransmitter, retarded leaf senescence. Its effect could be mediated via regulation of calcium levels in the cytoplasm by controlling phosphoinositide cycle.

5. Polyamines also retarded chlorophyll breakdown.

6. Superoxide dismutase activity was found to be controlled by phytochrome and calcium. This enzyme has not been shown earlier to be phytochrome regulated.

7. Animal superoxide dismutase activity seems to be regulated by calcium and calmodulin.

8. Both red light and Ca^{2+} decrease lipid peroxidation as evident by fluorescence data.

9. Besides calcium other metal ions also retarded senescence. Differential effect of Ni^{++} during leaf senescence was seen, on chlorophyll and on lipid peroxidation.

BIBLIOGRAPHY

Abeles, F.B. 1973. Ethylene in plant biology. Academic
    Press, New York.

Adams, D.O. and Yang, S.F. 1979.   Ethylene biosynthesis :
    Identification of 1-aminocyclopropane-1-carboxylic
    acid as an intermediate in the conversion of methio-
    nine to ethylene. Proc. Natl. Acad. Sci.(USA) 76 :
    170-174.

Aharoni, N. and Lieberman, M. 1979. Ethylene as a regula-
    tor of senescence in tobacco leaf discs. Plant
    Physiol. 64 : 801-804.

Aharoni, N. and Richmond, A.E. 1978. Endogenous gibberellin
    and abscisic acid content as related to senescence of
    detached lettuce leaves. Plant Physiol. 62: 224-228.

Aharoni, N., Lieberman, M. and Sisler, H.D. 1979a. Patterns
    of ethylene production in senescing leaves. Plant
    Physiol. 64 : 796-800.

Aharoni, N., Anderson, T.D. and Lieberman, M. 1979b.
    Production and action of ethylene in senescing leaf
    discs. Plant Physiol. 64 : 805-809.

Altman, A. 1982. Retardation of radish leaf senescence by
    polyamines. Physiol. Plant. 54 : 189-193.

Apelbaum, A., Ickson, A.C., Burgoon, A.C. and Lieberman, M.
    1982. Inhibition of polyamines of macromolecular
    synthesis and its implication for ethylene production
    and senescence processes. Plant Physiol. 70: 1221-1223.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts.
    Polyphenoloxidase in Beta vulgaris. Plant Physiol.
    24 : 1-15.

Beevers, L. 1968. Growth regulator control of leaf senescence in leaf discs of Tropaeolum majus. In: Biochemistry and Physiology of Plant Growth Substances (Eds. Wightman, F. and Setterfield, G.) pp. 1417-1435, Runge Press, Ottawa.

Biswal, U.C. and Sharma, R. 1976. Phytochrome regulation of senescence in detached barley leaves. Z. Pflanzen-physiol. 80 : 71-73.

Biswal, U.C., Bergfeld, R. and Kasemir, H. 1983a. Phyto-chrome mediated delay of plastid senescence in mustard cotyledons. Changes in pigment contents and ultra-structure. Planta 157 : 85-90.

Biswal, U.C., Choudhary, N.K. and Biswal, B. 1983b. Degrada-tion of plastid membranes during senescence. In: Indian Review of Life Sciences (Edited by Sen, D.N. and Kumar, S) Vol. 3, pp. 33-61.

Biswal, B., Choudhary, N.K., Sahu, P. and Biswal, U.C. 1983c. Senescence of detached fern leaves. Plant Cell Physiol. 24 : 1203-1208.

Biswal, U.C., Kasemir, H. and Mohr, H. 1982. Phytochrome control of degreening of attached cotyledons and primary leaves of mustard (Sinapis alba L.) seedlings. Photochem Photobiol. 35 : 237-241.

Cheug, S.-H., and Kao, C.H. 1983. Localised effect of polyamines on chlorophyll loss. Plant Cell Physiol. 24 : 1463-1468.

Cheug, S.-H. and Kao, C.H. 1984a. Senescence of rice leaves. 10. The effect of metal chelators. Bot. Bull. Acad. Sci. (Taipei) 25 : 87-94.

Cheug, S.-H. and Kao, C.H. 1984b. Senescence of rice : II. Inhibition of protease activity by γ,γ'-dipyridyl. Bot. Bull. Acad. Sci. 25 : 95-102.

Chin, T.Y. and Beevers, L. 1970. Changes in endogenous
    growth regulators in Nasturtium leaves during senes-
    cence. Planta 92 : 178-188.

Colohoun, A.J. and Hillman, J.R. 1972. The effects of
    abscisic acid in senescence in leaf discs of radish,
    Raphanus sativus L. Planta 105 : 213-224.

Dai, Y.-R. and Galston, A.W. 1981. Simultaneous phyto-
    chrome-controlled promotion and inhibition of arginine
    decarboxylase activity in buds and epicotyls of
    etiolated peas. Plant Physiol. 67 : 266-269.

Debata, A. and Murthy, K.S. 1981. Effect of growth regulat-
    ing substances on leaf senescence in rice. Oryza 18 :
    177-179.

DeGreef, J.A., Butler, W.L., Roth, T.F. and Frederieq, H.
    1971. Control of senescence in Marchantia by phytochrome
    Plant Physiol. 48 : 407-412.

Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A. 1981. Leaf
    senescence : Correlated with increased levels of membrane
    permeability and lipid peroxidation and decreased levels
    of superoxide dismutase and catalase. J. Exp. Bot. 32 :
    93-101.

Dhindsa, R.S., Plumb-Dhindsa, P. and Reid, D.M. 1982. Leaf
    senescence and lipid peroxidation : Effect of some phyto-
    hormones and scavengers of free radicals and singlet
    oxygen. Physiol. Plant. 56 : 453-457.

Dieter, P. and Marme, D. 1980. Calmodulin activation of
    plant microsomal Ca^{2+} uptake. Proc. Natl. Acad. Sci.
    (USA) 77 : 7311-7314.

Dogra, J.V.V. and Tiwary, M. 1983. Comparative effects of
    some imidazole derivatives on starch content in detached
    maize leaves. Phyton. Rev. Int. Bot. Exp. 43: 227-230.

Elkenawy, M. 1984. Hormonal changes associated with leaf senescence in cotton (Gossypium barbadense). Physiol. Planta. 62 : 593-598.

Elstner, E.F. 1982. Oxygen activation and oxygen toxicity. Ann. Rev. Plant Physiol. 33 : 73-96.

Esser, K. and Keller, W. 1976. Genes inhibiting senescence in Ascomycetes Podospora anserina. Mol. Gen. Genet. 144 : 107-110.

Evan-Chen, Z., Mattoo, A.K. and Goren, R. 1982. Inhibition of ethylene biosynthesis by aminoethoxyvinylglycine and by polyamines shunts label from 3,4-(^{14}C)-methionine into spermidine in aged orange peel discs. Plant Physiol. 69 : 385-388.

Fain, jJ.N. and Berridge, M.J. 1979. Relationship between phosphatidylinositol synthesis and recovery of 5-hydroxy-tryptamine-responsive Ca^{2+} flux in blowfly salivary glands. Biochem. J. 180 : 655-661.

Ferguson, I.B. 1983. Calcium stimulation of ethylene production induced by 1-aminocyclopropane-1-carboxylic acid and indole-3-acetic acid. Journal of Plant Growth Regulation. 2 : 205-214.

Ferguson, I.B., Watkins, C.B. and Harman, J.E. 1983. Inhibition by calcium on senescence of detached cucumber cotyledons: Effect on ethylene and hydroperoxide production. Plant Physiol. 71 : 182-186.

Ferguson, I.B. 1984. Calcium in plant senescence and fruit ripening. Plant Cell and Environment. 7 : 477-489.

Fletcher, R.A. 1975. Regulation of leaf and fruit senescence by gibberellic acid. In : Gibberellins and Plant Growth (Ed. Krishnamoorthy, N.H.) pp. 183-188. John Wiley & Sons Inc., New York.

Galston, A.W. 1983. Polyamines as modulators of plant development. Biosci. 33 : 383-388.

Gepstein, S. 1982. Light induced proton secretion and the relation to senescence of oat leaves. Plant Physiol. 70 : 1120-1124.

Gepstein, S. and Thimann, K.V. 1980. Changes in abscisic acid content of oat leaves during senescence. Proc. Natl. Acad. Sci. (USA) 77 : 2050-5053.

Gepstein, S.and Thimann, K.V. 1981. The role of ethylene in the senescence of oat leaves. Plant Physiol. 68: 349-354.

Goren, R., Palavan, N. and Galston, A.W. 1982. Separating phytochrome effects on arginine decarboxylase activity from its effect on growth. J. Plant Growth Regul. 1 : 61-73.

Grossman, K. and Jung, J. 1984. The influence of new terpenoid analogues of ABA on stomatal movement and leaf senescence. Z. AckerPflanzenbau. 153 : 14-22.

Halloran, G.M. and Boydell, C.W. 1967. Wheat chromosomes with genes for vernalization responses. Cand. J. Genet. Cytol. 9 : 632-639.

Halevy, A.H. and Mayak, S. 1981. Senescence and post harvest physiology of oat flowers. Horticulture Reviews. 3 : 59-143.

Hemantarajan, A. and Garg, O.K 1984. Effect of cytokinin and several inorganic cations on the polyamine content of barley endosperms. Indian J. Exp. Bot. 22 :379-381.

Jana, J. and Choudhuri, M.A. 1984. Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plants. Water, Air and Soil Pollution. 21: 1-4.

Kao, C.K. and Yang, S.F. 1983. Role of ethylene in the senescence of detached rice leaves. Plant Physiol. 73 : 881-885.

Kar, M. and Feierabard, J. 1984a. Changes in the activity of enzymes involved in amino acid metabolism during the senescence of detached wheat leaves. Physiol. Plant. 62 : 39.

Kar, M. and Feierabend, J. 1984b. Metabolism of activated oxygen in detached wheat and rye leaves and its relevance to the initiation of senescence. Planta 160 : 385-391.

Kasemir, H. and Mohr, H. 1982. Coaction of three factors controlling chlorophyll and anthocyanin synthesis. Planta 156 : 282-288.

Kasemir, H. 1983. Light control of chlorophyll accumulation in higher plants. In: Encyclopaedia of Plant Physiology (Eds. Shropshire, W. and Mohr, H.) Vol. 16, pp. 662-686.Springer -Verlag, Berlin.

Kaur-Sawhney, R., Shih, L.M., Flores, H.E. and Galston, A.W. 1982. Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol. 69 : 405-410.

Koll, F., Odile, B., Keller, A.M., Corinne, V. and Leon, B. 1984. Ethidium bromide rejuvenation and senescent cultures of Podospora anserina : Loss of senescence-specific DNA and recovery of normal mitochondrial DNA. Curr. Genet. 8 : 127-134.

Kumar, K.B., Khan, P.A. 1984. Levels of DNA and of deoxy-ribonucleolytic activity in ragi (Eleusine coracana) leaves during senescence. Biol. Plant. (Prague) 26 : 174-180.

Leshem, Y.Y. 1981. Oxy free radicals and plant senescence. What's New in Plant Physiology? $\underline{12}$: 1-4.

Leshem, Y.Y., Wurzburger, jJ., Grossman, S. and Frimer, A.A. 1981. Cytokinin interaction with free radical metabolism and senescence : Effects on endogenous lipoxygenase and purine oxidation. Physiol. Plant. $\underline{53}$: 9-12.

Leshem, Y.Y., Sridhara,S., and Thompson, J.E. 1984. Involvement of calcium and calmodulin in membrane deterioration during senescence of pea foliage. Plant Physiol. $\underline{75}$: 329-335.

Liaaen-Jensen, S. and Jensen, A. 1971. Quantitative determination of carotenoids in photosynthetic tissues. Methods Enzymol. $\underline{23}$: 586-602.

Lieberman, M. 1979. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. $\underline{30}$: 533-591.

Malek, L. and Cossins, E.A. 1983. Senescence, fusion development and fusion germination in nitrate-deficient and sulfate deficient Spirodela polyrrhiza: Relationships between nutrient availability and exogenous cytokinins. Can. J. Bot. $\underline{61}$: 1887-1897.

Marx, G.A. 1968. Influence of genotype and environment on senescence in peas, Pisum sativum. Bioscience. $\underline{18}$: 505-506.

Mohr, H. and Shropshire, W.Jr. 1983. An introduction to photomorphogenesis for the general reader. In: Encyclopaedia of Plant Physiology (Eds. Shropshire, W.Jr. and Mohr, H.) Vol. 16, pp. 24038, Springer-Verlag, Berlin.

Mondal, R. and Choudhuri, M.A. 1984b. Interaction of
    phytohormones with hydrogen peroxide metabolism
    and senescence of excised leaves of some 3-carbon
    pathway and 4-carbon pathway plants. Biochem.
    Physiol. Pflanzen. (BPP) 179 : 463-471.

Morison, J.I.L. and Jarvis, P.G. 1983. Direct and
    indirect effects of light on stomata. I. In
    Scotpines and sitka spruce. Plant Cell Environ.
    6 : 95-101.

Mozer, T.J. 1980. Control of protein synthesis in barley
    aleurone layers by the plant hormones, gibberellic
    acid and abscisic acid. Cell. 20 : 479-485.

Muhitch, M.J., Edwards, L.A. and Fletcher, J.S. 1983.
    Influence of diamines and polyamines on the
    senescence of plant suspension cultures. Plant
    Cell Rep. 2 : 82-84.

Nejidat, A., Itai, C. and Roth-Bejerano, N. 1983. Stomatal
    response to ATP mediated by phytochrome. Physiol.
    Plant. 57 : 367-370.

Nooden, L.D. 1980. Senescence in the whole plant. In:
    Senescence in Plants (Ed. Thimann, K.V.) pp. 219-258,
    CRC Press Inc., Boca Raton FL.

Nooden, L.D. and Leopold,A.C. 1978. Phytoohormones and
    and endogenous regulation of senescence and abscision.
    In : Polyhormones and related compounds. A compre-
    hensive treatise. Vol. II (Eds. Lethem, D.S., Goodwin,
    P.B. and Higgins, T.J.V.) Elsevier/North-Holland,
    Amsterdam.

Osborne, D.J. 1967. Hormonal regulation of leaf senescence.
    Symp. Soc. Exp. Biol. 21 : 179-213.

Osborne, D.J. 1968. Hormonal mechanisms regulating senescence and abscission. In: Biochemistry and Physiology of Plant Growth Substances (Eds. Wightman, and Setterfield, G.) pp. 815-840. The Runge Press, Ottawa.

Osborne, D.J. and Hallaway, M. 1964. The auxin 2,4-dichloro-phenoxy acetic acid as a regulator of protein synthesis and senescence in detached leaves of Prunus. New Phytol. 63 : 334-347.

Osiewacz, H.D. and Esser, K.1984. The mitochondrial plasmid of _Podospora_ _anserina_ : A mobile intron of ta mito-chondrial gene. Curr. Genet. 8 : 299-306.

Paliyath, G., Poovaiah, B.W., Munske, G.R. and Magnuson, J. 1982. Effect of calcium on membrane properties of apples during senescence. Hort. Sci. 17 : 530 (abstr.).

Paranjothy, K. and Wareing, P.F. 1971. The effect of abscisic acid, kinetins and 5θfluorouracil on ribonucleic acid and protein synthesis in senescing radish leaf discs Planta 99 : 112-119.

Peason, J.A., Thomas, K. and Thomas, H. 1978. Nucleic acids from leaves of a yellowing and a non-yellowing variety of _Festuca_ _pratensis_. Planta 144 : 85-87.

Pfeiffer, H. and Kleudgen, H.K. 1980. Investigations on the phytochrome control of senescence in the phyto-synthetic apparatus of _Hordeum_ _vulgare_ L. Z. Pflanzen-physiol. 100 : 437-445.

Pierce, R.O., Knowles, P.F. and Donald, A.P. 1984. Inheritance of delayed leaf senescence in soybean. Crop. Sci. 24 : 515-517.

Pjon, C.J. 1984. Temperature dependent inhibition action of Alpha-Alpha-Dipyridyl and cycloheximide on the senescence of maize leaves. Plant and Cell Physiol. 25 : 1103.

Poovaiah, B.W. and Shekhar, V.C. 1978. Effects of calcium infiltration on golden delicious apples on fruit firmness and senescence. Hort. Sci. 13 : 37 (abstr.).

Poovaiah, B.W. and Leopold, A.C. 1973a. Defferral of leaf senescence with calcium. Plant Physiol. 52: 236-239.

Poovaiah, B.W. and Leopold, A.C. 1973b. Inhibition of abscission with calcium. Plant Physiol. 51: 848-851.

Poovaiah, B.W. and Leopold, A.C. 1976. Effects of inorganic salts on tissue permeability. Plant Physiol. 58 : 182-185.

Proebsting, W.M., Davies, P.J. and Marx, G.A. 1977. Evidence for a graft-transmissible substance which delays apical senescence in *Pisum sativum*. Planta 135 :93-94.

Rao, L.V.M., Datta, N., Guha-Mukherjee, S., and Sopory, S.K. 1983. The effect of blue light on the induction of nitrate reductase in etiolated excised maize leaves. Plant Sci. Lett. 28 : 39-47.

Ray, S., Mondal, W.A. and Choudhuri, M.A. 1983. Regulation of leaf senescence, grain filling and yield of rice (*Oriza sativa* cultivar Jaya) by kinetin and abscisic acid. Physiol. Plant. 59 : 343-346.

Richmond, A.E. and Lang, A. 1957. Effect of kinetin on protein content and survival of detached *Xanthium* leaves. Science. 125 : 650-651.

Rohwer, F. and Schierle, J. 1982. Effect of light on
ethylene production : red light enhancement of
1-aminocyclopropane-1-carboxylic acid concentration
in etiolated pea shoots. Z. Pflanzenphysiol. 107 :
295-300.

Roux, S.J. 1983. A possible role for Ca^{2+} in mediating
phytochrome responses. In : The Biology of Photo-
reception (Eds. Cosens, D.J. and Vince-Prue, D.)
pp. 561-580, Cambridge Univ. Press, U.K.

Roy, D., Pathak, D.N. and Singh, R. 1983. Effects of
centrophenoxine on the antioxidative enzymes in
various regions of the aging rat brain. Exp.
Gerontol. 18 : 185-189.

Satler, S.O. and Thimann, K.V. 1983. Metabolism of oat
leaves during senescence. VII. The interaction of
carbon dioxide and other atmospheric gases with light
in controlling chlorophyll loss and senescence.
Plant Physiol. 71 : 67-70.

Sen, N.K., Patra, H.K. and Mishra, D. 1984. Phytochrome
regulation of biochemical and enzymatic changes during
senescence of excised rice leaves. Z. Pflanzenphysiol.
113 : 95.

Sharma, R., Sopory, S.K. and Guha-Mukherjee, S. 1976.
Phytochrome regulation of peroxidase activity in
maize. Plant Sci. Lett. 6 : 69-75.

Shih, L.-M., Kaur-Sawhney, R., Fuhrer, J., Samanta, S.
and Galston, A.W. 1982. Effects of exogenous, 1,3-
diaminopropane and sperimidine on senescence of oat
leaves. Plant Physiol. 70 : 1582-1596.

Smith, T.A. 1985. Polyamines. Ann. Rev. Plant Physiol. 36 : 117-143.

Srivastava, S.K., Vashi, D.J. and Naik, B.I. 1983. Control of senescence by polyamines and guanidines in young and mature barley (Hordeum vulgare) leaves. Phytochemistry 22 : 2151-2154.

Steinitz, B., Cohen, A. and Leshem, B. 1980. Factors controlling the retardation of chlorophyll degradation during senescence of detached statice (Limonium sinuatum) flower stalks. Z. Pflanzenphysiol. 100 : 343-349.

Stoddart, J.L. and Thomas, H. 1982. Leaf Senescence. In : Encylopedia of Plant Physiology (Eds. Boulter, D. and Parthier, B.) Vol. 14A, pp. 592-636. Springer Verlag, Berlin.

Thimann, K.V. 1978. Senescence. In: Controlling factors in plant development (Eds. Shibroka, H., Funiya, M., Katsumi, M. and K Takivots, A.) pp. 19-43, Bot. Mayazine, Tokyo, Special Issue 1.

Thimann, K.V. and Satler, S.O. 1979. Relations between leaf senescence and stomatal closure : senescence in light. Proc. Natl. Acad. Sci. (USA) 76 : 2295-2298.

Thomas, H. 1977. Ultrastructure, polypeptide composition and photochemical activity of chloroplasts during foliar senescence of a non-yellowing mutant genotype of Festuca pratensis. Planta 137 : 53-60.

Thomas, H. and Stoddart, J.L. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (Festuca pratensis). Plant Physiol. 56 : 438-441.

Thomas, H. and Stoddart, J.L. 1977. Biochemistry of leaf senescence in grasses. Ann. Appl. Biol. 85 : 461-463.

Thomas, H. and Stoddart, J.L. 1980. Leaf senescence. Ann. Rev. Plant Physiol. 31 : 83-111.

Thomas, T.N., Priest, D.G. and Zemp, J.W. 1976. Distribution of superoxide dismutase in rat brain. J. Neurochem. 27 : 309-310.

Tucker, D.J. 1981. Phytochrome regulation of leaf seneseence in cucumber and tomato. Plant Sci. Lett. 23 : 103-108.

Veluthami, K. and Poovaiah,B.W. 1984. Calcium-promoted protein phosphorylation in plants. Science 223 : 167-169.

Virgin, H.I. 1966. Carotenoid synthesis in leaves of wheat after irradiation by white light. Physiol. Plant. 19 : 40-46.

Wardle, K. and Short, K.C. 1983. Stomatal responses and the senescence of leaves. Ann. Bot. 52 : 411-412.

Wareing, P.F. and Thompson, A.G. 1976. Rapid effects of red light on hormone levels. In: Light and Plant Development (Ed. Smith, H.) pp. 285-294, Butterworths, London.

Woolhouse, J.W. 1967. The nature of senescence in plants. Symp. Soc. Exp. Biol. 21 : 179-213.

Whyte, P. and Luckwill, L.C. 1966. A sensitive bioassays
    for gibberellins based upon retardation of leaf
    senescence in Rumex obtusifolius. Nature 210 ፡
    1360.

Yoshida, Y. 1961. Nuclear control of chloroplast activity
    in Elodea leaf cell. Protoplasma 54 ፡ 476-492.