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# CONTROL OF SENESCENCE BY POLYAMINES AND GUANIDINES IN YOUNG AND MATURE BARLEY LEAVES

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**Key Word Index**—*Hordeum vulgare*; Gramineae; barley; polyamines; guanidines; chlorophyll; peroxidase; phosphomonoesterase; senescence.

**Abstract**—Loss of chlorophyll and protein during senescence was more rapid in young (8-day-old) than in mature (45-day-old) barley leaves. Chlorophyll loss was retarded by putrescine, spermidine, spermine, guanidino acetic acid and guazatine in young but not in mature leaves. Protein breakdown and increase in amino nitrogen were not affected by these compounds in young or mature leaves. Dodine had no effect on chlorophyll loss but accelerated protein breakdown. Increase in peroxidase activity during senescence was potentiated by polyamines and guanidines except for dodine which decreased it. Phosphomonoesterase activity was decreased by polyamines and guanidines in young but not in mature leaves.

## INTRODUCTION

Senescence in leaves involves a series of degradative events leading to nucleic acid and protein breakdown, disintegration of chloroplast structure, and loss of chlorophyll and photochemical activities along with a marked increase in a number of hydrolytic enzymes [1, 2]. Several metabolites and growth regulators have been studied for their effect on protein metabolism and structure and photochemical properties of chloroplast during senescence [1–8]. Kinetin, for instance, retards the loss of protein and chlorophyll [9–11] and also preserves the chloroplast structure [9]. Inorganic cations, especially $Ca^{2+}$, also retard senescence due to their role in maintaining membrane integrity [12]. Several studies have shown that polyamines, apart from their role in growth and development, are also involved in stress-related processes such as senescence [13–17]. They stabilize protoplasts [15], thylakoid membranes of chloroplasts [14] and retard the increase in RNase and protease [16]. The protective effect of these compounds was thought to be due to their cationic nature. More recent reports have indicated that membrane stabilization by polyamines may be associated with their binding to membrane phospholipids [17–20]. The role of polyamines in controlling senescence is also supported by the findings that their concentration decreases in senescing tissues [21–22]. Guanidino compounds, which are structurally related to polyamines, are known to destabilize the membranes and the effect is reversed by polyamines [20, 23]. Dodine and guazatine, the two guanidino fungicides [24, 25], are very potent membrane destabilizers [20, 23]. If senescence is associated with membrane destabilization which could be prevented by polyamines then guanidino compounds may be expected to potentiate it.

Most of the studies on detached leaf senescence with polyamines have been carried out using comparatively young (1- to 2-week-old) plants and no study seems to have been done on mature plants. Maturation of the tissues may modify the membrane function even before senescence becomes apparent. It was therefore of interest to study the effect of polyamines and guanidines during senescence of young and mature barley plants to determine whether tissue age could alter their response. The results of these studies are reported in the present paper.

## RESULTS AND DISCUSSION

The leaves from 8- and 45-day-old plants were analysed at the time of excision for chlorophyll, protein and amino nitrogen content. The 45-day-old mature leaves had 75% more chlorophyll than young 8-day-old leaves. Although the amino nitrogen content was the same in both leaves, the protein content in mature leaves was only 70% of that in young leaves. During senescence the chlorophyll content decreased rapidly in young leaves (Table 1). After 48 hr of senescence it was 50% and decreased further to 25% of the initial value in 72 hr. A similar decrease has been reported by Cohen *et al.* [13] in 7-day-old barley leaf discs during the same periods of senescence. In mature leaves the decrease of chlorophyll was much slower (*ca* 40% in 72 hr). The polyamines putrescine, spermidine and spermine caused complete retention of chlorophyll in young leaves, even at 72 hr. Out of the guanidino compounds tested, guanidino acetic acid (GAA) and guazatine retarded the chlorophyll loss, though not to the same extent as polyamines. Dodine, however, had very little effect. In mature leaves none of the compounds had any effect. Protein breakdown during senescence was more rapid in young than in mature leaves. None of the compounds tested except dodine had any effect on protein breakdown in young or mature leaves. Dodine, however, enhanced protein breakdown in both young and mature leaves. The amino nitrogen content, which is a measure of protease activity, was the same in both young and mature leaves at the initiation of the experiment but increased continuously

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Table 1. Effect of polyamines and guanidines on chlorophyll, protein and amino nitrogen content in senescing barley leaves

| Treatment | Chlorophyll (%) hr* | | | Protein (%) hr* | | | Amino nitrogen (%) hr* | | |
|---|---|---|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| | | | 8-day-old | | | | | | |
| Control | 83 | 49 | 25 | 78 | 56 | 35 | 150 | 300 | 600 |
| Putrescine | 108 | 101 | 99 | 82 | 62 | 39 | 142 | 300 | 625 |
| Spermidine | 100 | 91 | 90 | 80 | 63 | 35 | 148 | 305 | 600 |
| Spermine | 101 | 96 | 95 | 80 | 56 | 38 | 165 | 300 | 615 |
| Guanidino acetic acid | 86 | 81 | 62 | 81 | 63 | 40 | 150 | 310 | 575 |
| Dodine | 84 | 53 | 39 | 63 | 48 | 29 | 175 | 305 | 575 |
| Guazatine | 85 | 76 | 74 | 76 | 63 | 31 | 175 | 320 | 600 |
| | | | 45-day-old | | | | | | |
| Control | 81 | 71 | 58 | 79 | 71 | 61 | 175 | 300 | 265 |
| Putrescine | 82 | 71 | 64 | 84 | 74 | 66 | 125 | 175 | 250 |
| Spermidine | 83 | 75 | 63 | 84 | 71 | 66 | 100 | 175 | 250 |
| Spermine | 85 | 75 | 64 | 84 | 74 | 64 | 100 | 175 | 245 |
| Guanidino acetic acid | 83 | 66 | 55 | 82 | 70 | 66 | 100 | 250 | 250 |
| Dodine | 82 | 68 | 58 | 74 | 58 | 54 | 100 | 225 | 300 |
| Guazatine | 81 | 74 | 65 | 89 | 79 | 67 | 125 | 225 | 230 |

The values are % of the control at the initiation of the experiment, i.e. 0 hr. All compounds were used at 1 mM.

*Duration following excision.

with the period of senescence. In young leaves it was 6 times more at 72 hr but in mature leaves it increased up to 48 hr and there was no further increase thereafter. Polyamines and guanidines had no effect on the amino nitrogen content in young leaves but all of them decreased it during 24 and 48 hr of senescence in mature leaves.

It is believed that as a result of their binding polyamines may stabilize the membrane structure and thereby prevent or delay senescence. From the present study it appears that the membrane structure in the young leaves may be different from that of mature leaves, thus giving a differential response to polyamines and guanidines at the two stages. Guanidino compounds which act as membrane destabilizers [20, 23], and whose effect is counteracted by polyamines were expected to promote senescence but they also delayed it, except in the case of dodine which appeared to be toxic. It is generally assumed that compounds which delay senescence will also cause a retardation in protein breakdown along with chlorophyll. Polyamines which delay senescence have been reported to cause retention of protein in radish leaves [17]. Cohen *et al.* [13], however, reported that in barley leaves polyamines had no stabilizing effect on the soluble proteins. Spermidine and spermine rather caused a further decrease at 1 mM. In the present study also, none of the compounds had any retarding effect on protein breakdown. Amino nitrogen content was also not affected by these compounds in young leaves but in mature leaves they decreased it during 24 and 48 hr of senescence. Our results on amino nitrogen content in young leaves are in contrast to the observations of Kaur-Sawhney and Galston [16] in oat leaves where the increase in amino nitrogen content was retarded by polyamines.

Peroxidases have been reported to increase during senescence [26–28]. In the present study the peroxidase levels were almost identical in young and mature leaves at the start of the experiment. Peroxidase activity increased with senescence in both young and mature leaves (Table 2) and all the polyamines and guanidines except dodine further increased it. Dodine, however, decreased it in both young and mature leaves. Esterases have been reported to decrease during senescence [29]. In the present study senescence had very little effect on phosphomonoesterase in young leaves but decreased this enzyme in mature leaves. All the polyamines and guanidines except dodine caused a small but significant (20–30%) decrease in esterase activity over the control value in young but not in mature leaves. Dodine continuously decreased the esterase activity in both young and mature leaves during senescence.

From these studies it appears that the effect of polyamines and guanidines on senescence parameters depends on the age of the tissue. The differential response obtained by these compounds at the two stages may be due to the differences in their membrane properties. Further, although polyamines protect chloroplasts and prevent the loss of chlorophyll, they are unable to prevent the increase in peroxidase activity during senescence. Peroxidase thus does not appear to be directly associated with senescence, as has also been suggested by Kar and Mishra [27]. It may be of interest to mention that in the present study peroxidase activity is potentiated by both polyamines and guanidines during senescence but in maize scutellum slices we found that the membrane-bound peroxidase is decreased by polyamines and increased by guanidines (unpublished observations).

## EXPERIMENTAL

*Plant material.* Barley (*Hordeum vulgare* L.) plants were grown in garden soil under field conditions of natural light and dark periods. The first leaf from 8- and 45-day-old plants was excised,

Table 2. Effect of polyamines and guanidines on peroxidase and phosphomonoesterase activity in senescing barley leaves

| Treatment | Peroxidase (%) hr* | | | Phosphomonoesterase (%) hr* | | |
|---|---|---|---|---|---|---|
| | 24 | 48 | 72 | 24 | 48 | 72 |
| | 8-day-old | | | | | |
| Control | 148 | 184 | 133 | 84 | 83 | 80 |
| Putrescine | 174 | 224 | 193 | 88 | 77 | 80 |
| Spermidine | 165 | 201 | 170 | 85 | 62 | 60 |
| Spermine | 187 | 206 | 186 | 81 | 68 | 60 |
| Guanidino acetic acid | 180 | 221 | 201 | 85 | 72 | 64 |
| Dodine | 156 | 120 | 91 | 66 | 59 | 50 |
| Guazatine | 168 | 194 | 155 | 85 | 70 | 69 |
| | 45-day-old | | | | | |
| Control | 123 | 142 | 171 | 62 | 58 | 44 |
| Putrescine | 164 | 199 | 251 | 62 | 58 | 49 |
| Spermidine | 143 | 196 | 246 | 71 | 56 | 46 |
| Spermine | 155 | 209 | 242 | 70 | 55 | 48 |
| Guanidino acetic acid | 131 | 202 | 266 | 64 | 53 | 44 |
| Dodine | 120 | 141 | 158 | 37 | 34 | 28 |
| Guazatine | 145 | 168 | 221 | 59 | 52 | 44 |

The values are % of control at the initiation of experiment, i.e. 0 hr. All compounds were used at 1 mM.

*Duration following excision.

washed with $H_2O$ and blotted lightly with filter paper. The upper and lower quarter portions of the leaf were removed and the middle half portion was cut into pieces of 1 cm length. These pieces (500 mg) in triplicate were floated on 10 ml $H_2O$ or test solns in sterile Petri dishes kept in the dark at 22°. A sample was analysed for various parameters immediately after excision and the remaining at intervals of 24, 48 and 72 hr after washing them in $H_2O$.

A 5% (w/v) homogenate was prepared in 0.1 M NaPi buffer, pH 6.8. The homogenate was passed through 2 layers of cheesecloth and used for enzyme assays. Chlorophyll was extracted and estimated according to the method of ref. [27], protein by the method of ref. [30] and amino nitrogen by the method of ref. [31]. Peroxidase activity was assayed essentially according to the method of ref. [32]. The assay system consisted of 2.5 ml of 50 mM NaOAc buffer (pH 5.5), 0.1 ml of 0.25% *o*-dianisidine, 0.1 ml of 3% $H_2O_2$ and enzyme (0.1 ml) in a total vol. of 4 ml. After incubation for 1 min at room temp., the reaction was terminated with 0.5 ml of 5 N $H_2SO_4$ and the *A* of the coloured complex was measured at 400 nm. Phosphomonoesterase was assayed according to the method of ref. [33]. The assay system consisted of 1 ml of 50 mM NaOAc buffer (pH 5.5), 5 μmol of *p*-nitrophenyl phosphate and 0.1 ml enzyme in a total vol. of 2 ml. After incubation at 37° for 15 min, the reaction was terminated by the addition of 3 ml of 0.5 M NaOH. The *A* of the *p*-nitrophenol formed was measured at 420 nm.

*Chemicals.* Putrescine (2 HCl), spermidine (3 HCl), spermine (4 HCl) and guanidino acetic acid were purchased from Sigma. Guazatine (acetate) was donated by KenoGard AB, Sweden and dodine (acetate) (Melprex Tech.) was donated by Cyanamid of Great Britain, London. Other chemicals of analytical grade were purchased locally.

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