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Endogenous polyamine levels and flooding-enhanced leaf senescence of tobacco

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Changes in levels of nicotine and polyamines in leaves and rates of leaf senescence in response to flooding treatment by a low-alkaloid line (LA 5) and normal alkaloid (Speight G-70) tobacco plants were compared. Leaves from LA 5 plants contained 12-, 13-, 2- and 6-fold less nicotine, putrescine, spermidine and spermine, respectively, than those from Speight G-70 at the start of the experiment (7 days after topping). Flooding treatment resulted in an increase in levels of putrescine in both LA 5 and Speight G-70 plants. Flooding did not affect the levels of spermidine and spermine. Leaf senescence of LA 5 and Speight G-70 was promoted by flooding. However, the senescence syndrome in untreated leaves of LA 5 was not as pronounced as that of Speight G-70. Neither was the rate of senescence of flood-treated LA 5 leaves faster than that of flood-treated Speight G-70 leaves. It appears that endogenous polyamines may not play a significant role in the control of leaf senescence or flood-enhanced leaf senescence of tobacco plants.

Key words: flooding; *Nicotiana tabacum*; nicotine; polyamines; senescence

Introduction

Aliphatic polyamines (putrescine, spermidine and spermine), recently recognized as a new class of plant growth substances, are present in all plants examined to date. Several reports have shown that polyamines can retard leaf senescence [1–5] and that endogenous levels of polyamines decrease with advancing leaf age [6–8]. However, several other examples have also been reported of systems where polyamine levels did not decrease during senescence [9–11]. There are many reports of accelerated leaf senescence in flooded plants [12–17]. It is not known whether polyamines play a significant role in regulating flooding-enhanced senescence of leaves.

LA 5 is a low-alkaloid line of tobacco, whereas tobacco cultivar Speight G-70 contains high levels of nicotine. Putrescine is an intermediate in the synthesis of nicotine in tobacco [18]. It is also known that spermidine and spermine require putrescine during synthesis [19]. Thus, LA 5 and Speight G-70 tobacco leaves may differ in levels of polyamines. In this paper, changes in levels of nicotine and polyamines in leaves and rates of leaf senescence in response to flooding treatment by LA 5 and Speight G-70 tobacco plants were compared.

Materials and Methods

Seedlings of *Nicotiana tabacum* (cv. Speight G-70 and line LA 5) were selected 30 days after sowing the seeds in plastic trays containing sandy loam. Each tray received an optimum amount of water every day. The seedlings were then grown in a greenhouse, each in a small plastic bag (25 cm^2). After 30 days, the seedlings were again selected for uniformity and transplanted to pots (5 dm^2). Each pot contained a plant and received 30 g of a com-

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pound fertilizer ($N-K_2O-P_2O_5$, 7:21:21). Each treatment had four plants which were then placed in the field.

Topping (decapitation) at onset of flowering is a standard practice in the production of tobacco. One of the main effects of topping is a higher alkaloid content in leaves [20]. Flooding treatment started at 47 days after transplanting, i.e. 107 days after sowing or 7 days after topping. Tobacco plants were flooded by maintaining the level at 1 cm above the soil surface. Untreated control plants were allowed to receive the optimum amount of water. At the times indicated, the two lowest leaves of each plant were collected for chemical analysis. Each treatment was repeated four times. All experiments described here were repeated twice. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Chlorophyll was determined according to Wintermans and De Mots [21] after extraction in 96% (v/v) ethanol. For protein extraction, leaf samples were homogenized in a mortar and pestle in 25 mM sodium phosphate buffer (pH 7.5). The extracts were centrifuged at 17 000 × *g* for 20 min and the supernatant fractions were used for determination of protein by the method of Lowry et al. [22].

For polyamine extraction, leaf samples were homogenized in a mortar and pestle in 5% perchloric acid. Polyamine levels were determined using high performance liquid chromatography after benzoylation as described previously [11].

A high performance liquid chromatographic technique developed by Saunders and Blume [23] was used to quantitate the levels of nicotine in tobacco leaves. The procedure involves an aqueous extraction of the milled air-dried leaf samples followed by separation of the nicotine on a reversed-phase C18 column with a mobile phase of 40% methanol containing 0.2% phosphoric acid buffered to pH 7.25 with triethylamine.

Results

Table I shows the changes of nicotine levels in the two lowest leaves of Speight G-70 and LA 5 plants during flooding. Leaves from the low-alkaloid (LA 5) plants contained much less (about 12-fold) nicotine than did respective leaves from Speight G-70 at the start of the experiment (7 days after topping). In control leaves, nicotine levels of

Table I. Effects of flooding on levels of nicotine in tobacco leaves of LA 5 and Speight G-70

Time (days)		Nicotine (nmol/g dry wt.)	
		LA 5	Speight G-70
0		244 ± 45	2866 ± 191
1	Control	311 ± 36	3891 ± 174
	Flooded	332 ± 43	2791 ± 189
2	Control	337 ± 20	3920 ± 169
	Flooded	249 ± 50	3991 ± 348
4	Control	261 ± 39	3904 ± 212
	Flooded	235 ± 58	3939 ± 159

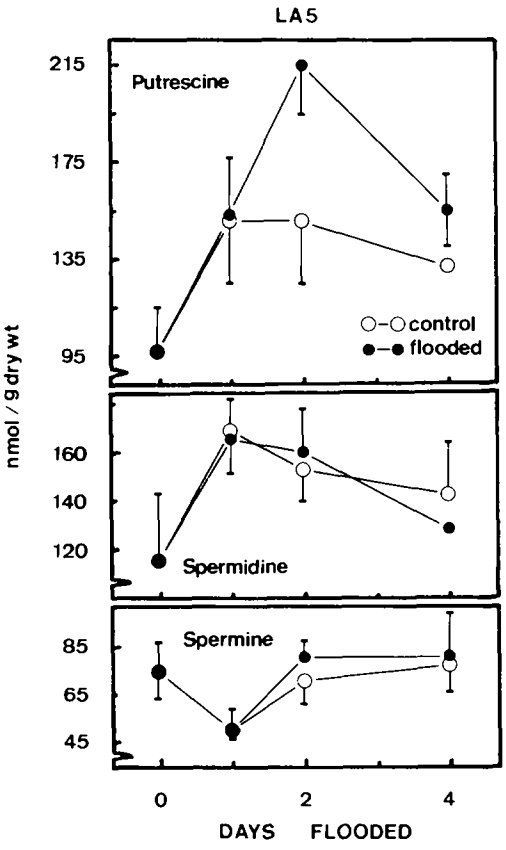

Fig. 1. Effects of flooding on levels of polyamines in LA 5 tobacco leaves. Means ± S.E., four replicates.

Speight G-70 increased at 1 day after the start of experiment and then remained unchanged, whereas that of LA 5 increased on day 2 but decreased slightly on day 4. Flooding treatment resulted in lower levels of nicotine in leaves of Speight G-70 and LA 5 than in the controls on days 1 and 2, respectively.

It is known that nicotine requires putrescine during synthesis [18]. It is expected that levels of polyamines in leaves of tobacco genotypes with different levels of nicotine may differ. Madsen et al. [24] demonstrated that total free polyamine levels were higher in leaves of low-alkaloid Burley 21 tobacco plants than in Burley 21. In contrast, we observed that low-alkaloid LA 5 plants had lower total free polyamine levels than Speight G-70. At the start of experiment, the two lowest leaves from LA 5 plants contained 13-, 2- and 6-fold less putrescine, spermidine and spermine, respectively, than those in Speight G-70 (Figs. 1 and 2).

In control leaves of LA 5 plants, levels of putrescine and spermidine increased at 1 day after starting the experiment and remained unchanged thereafter, whereas the level of spermine, in general, did not change significantly throughout the entire duration of the experiment (Fig. 1). Also, no significant change in levels of putrescine, spermidine and spermine in control leaves of Speight G-70 was observed during a 4-day experimental duration (Fig. 2). Flooding treatment resulted in a higher level of putrescine in leaves of LA 5 than in the controls (Fig. 1). Flooding also resulted in a higher level of putrescine in leaves of Speight G-70

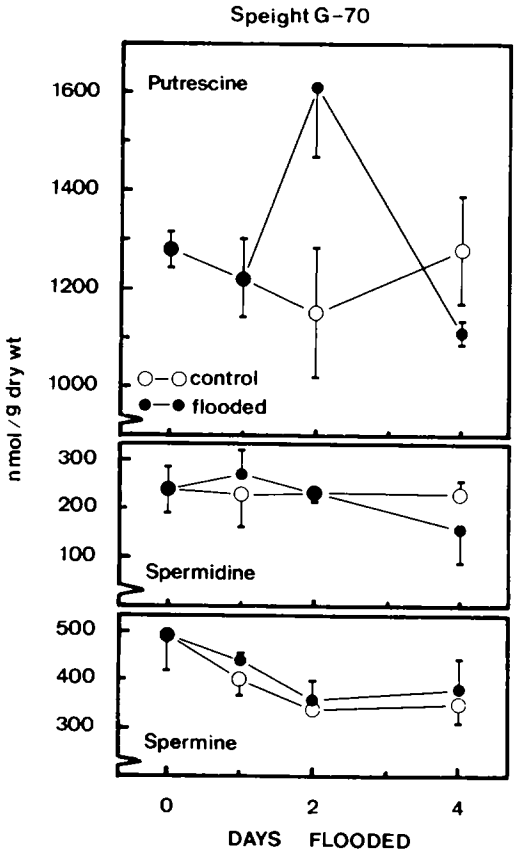

Fig. 2. Effects of flooding on levels of polyamines in Speight G-70 tobacco leaves. Means ± S.E., four replicates.

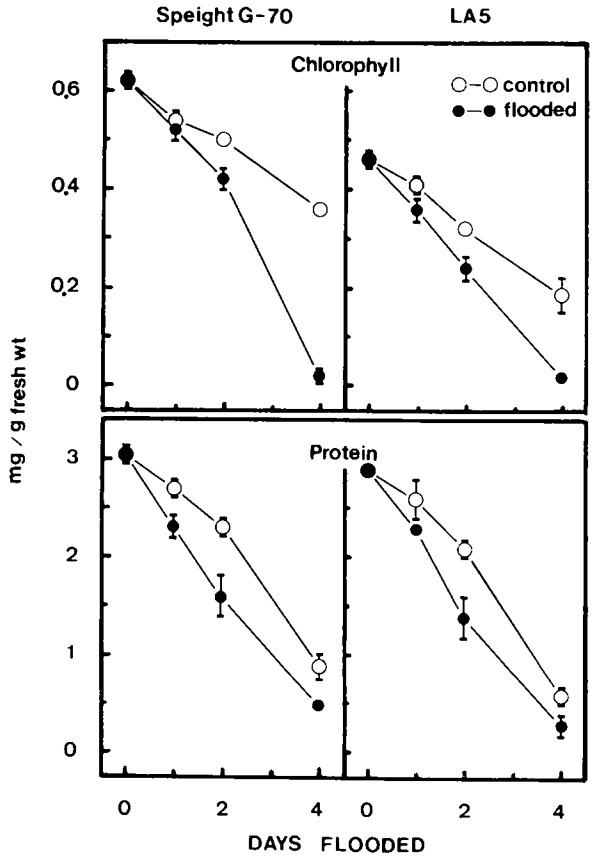

Fig. 3. Effects of flooding on levels of chlorophyll and protein in LA 5 and Speight G-70 tobacco leaves. Means ± S.E., four replicates.

than those in untreated controls at 2 days after the start of the experiment (Fig. 2). However, flooding did not affect the levels of spermidine and spermine in LA 5 and Speight G-70 leaves.

The senescence of tobacco leaves was followed by measuring the decrease in levels of chlorophyll and protein. Figure 3 shows the effects of flooding on the levels of chlorophyll and proteins. In control leaves, the decrease of protein and chlorophyll levels was evident at 1 day after the start of experiment in LA 5 and Speight G-70. Flooding resulted in an enhancement of leaf senescence, as judged by levels of chlorophyll and protein, of LA 5 and Speight G-70. Neither control leaves nor flooding-treated leaves of LA 5 senesced at a faster rate than the corresponding leaves of Speight G-70.

Discussion

Putrescine, spermidine and spermine are all present in tobacco leaves. The present investigation showed that low-alkaloid LA 5 tobacco leaves had much lower levels of nicotine and polyamines than Speight G-70. Since nicotine, spermidine and spermine require putrescine during synthesis [18,19], the low levels of nicotine, spermidine and spermine in leaves of LA 5 plants may be due to the low levels of putrescine.

Our results show that flooding enhances leaf senescence of LA 5 and Speight G-70 tobacco leaves. These results are in agreement with earlier reports [12–17]. It has been shown that exogenous application of polyamines retard senescence of detached leaves [1,4,5]. If levels of endogenous polyamines play a functional role in the regulation of senescence of untreated tobacco leaves, the senescence syndrome of untreated leaves of LA 5, which contained very low polyamine levels, would be expected to be more pronounced than that of Speight G-70 plants having high polyamine levels. However, this was not observed. Neither the senescence rate of control leaves nor of flood-treated leaves of LA 5 was faster than the corresponding leaves of Speight G-70. It is concluded that endogenous polyamines are unlikely to be the factor responsible for the senescence of untreated or flood-treated tobacco leaves. The present investigation provides support for the suggestion of Birecka et al. [9] that endogenous polyamines may have effects different from the possibly non-specific effects frequently reported for exogenous polyamines.

Exposure of plants to a variety of stress conditions (such as salt, acid and potassium deficiency) often results in an accumulation of putrescine [25]. The accumulation of putrescine induced by flooding has not been demonstrated. In this paper, we provide evidence that flooding results in an accumulation of putrescine. It has been shown that putrescine is accumulated in plant tissues when the oxygen availability becomes limiting for growth [26]. Flooding conditions impose an oxygen shortage on roots, due to rapid microbial consumption of existing oxygen and the low rate of diffusion of oxygen through water. It seems that the accumulation of putrescine induced by flooding may be attributed, at least in part, to oxygen deficiency of roots. There is considerable evidence in the literature to support a role for putrescine in the tolerance of plants to stress, but there is also accumulating evidence associating increased putrescine level with the development of the symptoms of stress [25]. The physiological role of putrescine under flooding stress remains to be investigated.

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