# Retardation of Tulip Shoot Senescence by Auxin

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**Abstract**

**In our studies we found that application of indole-3-acetic acid (IAA) at a concentration of 0.1, 1.0 and 2.0% in lanolin paste in the place of removed flower bud at the beginning of flowering of tulips, in the presence of leaves or after excision of all leaves, greatly retarded the chlorophyll degradation and delayed both stem and leaves senescence. The interaction of auxin with ethephon (ethylene), abscisic acid (ABA) and benzyladenine (BA) on tulip stem and leaves senescence was also studied. At the beginning of flowering IAA was applied in lanolin paste in the place of removed flower, and water, ethephon, ABA or BA were applied to the second leaf sheath using a soaked cotton wick. It was found, on the basis of morphological observation and chlorophyll changes, that senescence occurred at first in tulips treated with ABA, followed by ethephon and water. Senescence processes stimulated by ABA and ethephon were greatly delayed after application of IAA. BA applied alone delayed senescence of plant. In all experiments auxin retarded the chlorophyll degradation and high concentrations of IAA (1.0 or 2.0%) were more effective, even 34 days after treatment stems were still stiff and green. From our results it is clear that auxin, IAA, applied exogenously, is an important anti-senescence factor of tulip shoot.**

## INTRODUCTION

It is well known that auxins play an important role in tulip growth and development. The leaves and gynoecium provide auxins which control the elongation growth of the stem (Op den Kelder et al., 1971; Hanks and Rees, 1977; Saniewski and De Munk, 1981; Banasik and Saniewski, 1985). Elongation of the stem and leaves of tulips is due almost entirely to the elongation of cells produced during early stages of flower bud development (Gilford and Rees, 1973). Excision of all leaves and flower bud in the early stage of tulip growth almost totally inhibited stem growth and application of auxin in the place of removed flower bud induced stem growth (Saniewski and De Munk, 1981). It was shown that only basipetal (polar) transport of IAA or its metabolites is responsible for the induction of tulip stalk elongation (Banasik and Saniewski, 1985). Then, it has been suggested that the elongation of all the internodes in tulips is controlled by interaction of endogenous auxins and gibberellins (Okubo and Uemoto, 1985; Okubo et al., 1986; Saniewski, 1989; Saniewski and Kawa-Miszczak, 1992; Rietveld et al., 2000).

In our previous studies we found that application of IAA at a concentration of 0.1 and 1.0% in lanolin paste in the place of removed flower bud at the beginning of flowering of tulips, after excision of all leaves, greatly retarded the chlorophyll degradation and delayed stem senescence (Kawa-Miszczak et al., 1999). Auxin transport inhibitors, 2,3,5-triiodobenzoic acid (TIBA) and naphthylphthalamic acid (NPA) at a concentration of 0.2 and 0.5% in lanolin paste, applied 1 cm below IAA treatment or in the middle of 4$^{th}$ (upper) internode, evidently diminished the retardation of tulip stem senescence by IAA (Kawa-Miszczak et al., 2000).

In the present work the interaction of auxin, indole-3-acetic acid (IAA), with ethephon (ethylene), abscisic acid (ABA) and benzyladenine (BA) in tulip stem and leaves senescence were studied.

## MATERIALS AND METHODS

'Apeldoorn' tulip bulbs of a circumference of 11-12 cm were stored at 18-20$^{o}$C

until 14 October 2001 after lifting, and then were transferred into cold dry storage at 5°C. On 4 March 2002 the bulbs, after removal of the dry scales, were individually planted into pots and cultured in a greenhouse at 18-20°C under prevailing light conditions. On 20 March 2002, at the beginning of flowering (flower bud fully coloured), flowers were removed and lanolin only or IAA in lanolin paste was applied in the place of removed flower, and water, ABA, ethephon or BA soaked with cotton wick were applied daily to the second leaf sheath for 5 days.

The following treatments were applied:

- control, lanolin and water; ABA 40 $mgL^{-1}$; ethephon 40 $mgL^{-1}$; BA 20 $mgL^{-1}$,
- IAA 0.1% and water; IAA 0.1% and ABA 40 $mgL^{-1}$; IAA 0.1% and ethephon 40 $mgL^{-1}$; IAA 0.1% and BA 20 $mgL^{-1}$,
- IAA 2.0% and water; IAA 2.0% and ABA 40 $mgL^{-1}$; IAA 2.0% and ethephon 40 $mgL^{-1}$; IAA 2.0% and BA 20 $mgL^{-1}$.

Twenty five plants were used in each treatment. The chlorophyll content in the acetone extracts was determined using the Bruinsma method (Bruinsma, 1963). The middle part of every internode and disk of a circumference of 20 mm from middle part of every leaf were used for chlorophyll determination. Chlorophyll content was calculated for 1 cm internode explants and for disk of leaf explants. Five plants were used for determination of chlorophyll in each treatment.

## RESULTS AND DISCUSSION

Senescence is an important developmental process in plants that eventually leads to the death of whole plant, organ, tissue and cell through highly regulated, endogenously controlled degenerative processes. Cellular and molecular events contributing to visual symptoms of plant senescence (yellowing, desiccation) include chlorophyll break-down, chloroplast disintegration, a decline in photosynthesis, loss in the ability to accumulate proteins and nucleic acids, loss of plasma membrane structure with increase in permeability (Smart, 1994; Chandlee, 2001). Many different activities (e.g. enzymes) and genes contribute to the overall senescence syndrome. Internal senescence-inducing factors appear to be hormonal in nature. The initiation and progression of plant senescence can be influenced by various external factors (environmental stresses, including darkness, desiccation, temperature, wounding, detachment and pathogen attack). The senescence processes can be stimulated or retarded by plant growth regulators treatment.

In our previous studies we found that application of IAA at a concentration of 0.1 and 1.0% in lanolin paste in the place of removed flower bud at the beginning of flowering of tulips, after excision of all leaves, greatly retarded the chlorophyll degradation and delayed stem senescence (Kawa-Miszczak et al., 1999). Higher concentration of auxin (IAA 1.0%) was more effective, even 34 days after treatment stems were still stiff and green.

In the present studies IAA was applied at a concentration of 0.1 and 2.0% in the place of removed flower bud at the beginning of flowering with the presence of leaves. The auxin retarded the chlorophyll degradation in internodes (Table 1 and 3) and leaves (Table 2 and 3). 22 days after treatment, when control plants (lanolin + water) were fully dry, chlorophyll content in internodes and leaves of tulips treated with high concentration of auxin (IAA 2.0% and water) remained on high level (Table 1, 2, 3). 27 days after treatment tulips treated with IAA 0.1% were fully dry and those treated with IAA 2.0% were still stiff and green with the exception of 1$^{st}$ – lower leaf in which yellowing has began (morphological observation). In control plants (lanolin only in the place of removed flower) with water, ABA, ethephon or BA applied to the second leaf sheath it was found, on the basis of morphological observation and chlorophyll changes, that senescence occurred at first in tulips treated with ABA, followed by ethephon and water. Cytokinin, benzyladenine (BA) delayed senescence of plant if applied alone. Senescence processes stimulated by ABA and ethephon were greatly delayed after application of IAA (Table 1, 2, 3 and Fig. 1).

From our previous and present results it is clear that auxin, IAA, applied

exogenously, is an important anti-senescence factor of tulip shoot.

Many studies have clearly implicated cytokinins and ethylene as significant regulators in the senescence program in leaves and other plant tissues including fruits and flower (Smart, 1994; Gan and Amasino, 1997). Cytokinins delay leaf senescence in many species and may cause regreening of yellow leaves in some species. Ethylene, like cytokinins, is thought to play a prominent role in regulation of senescence. It has the opposite effect to cytokinins as it accelerates many of the physiological changes normally associated with leaf senescence. ABA accelerates the senescence of leaf discs of some species, but the response is genotype-dependent. Some reports have indicated that auxins may also be able to delay senescence (Sacher, 1959; Osborne and Hallaway, 1964). Noh and Amasino (1999) showed that cytokinin, auxin and sugars can repress developmental senescence at the molecular level. However, there are numerous cases in which auxins are not very powerful in their effects on senescence, and high concentrations have to be used compared with cytokinins. Generally, auxins do not delay senescence and they may even promote the process through the stimulatory effect on ethylene biosynthesis (Smart, 1994). Our previous results (Saniewski et al., 1990) showed that when auxins were applied in the early stage of tulip growth, after excision of flower bud and leaves, higher concentrations of auxins promoted internodal elongation less, increased stem thickening and stimulated ethylene production more than low concentrations of auxin. Silver thiosulphate (STS), an inhibitor of ethylene action, stimulated growth of all internodes and decreased their thickening induced by high concentrations of auxin, with or without small effect on the production of ethylene in the earlier stages of stem elongation (Saniewski et al., 1990). Tulip stem growth induced by low concentration of IAA, after removal of flower bud and all leaves, was inhibited by ethephon applied on different internodes, and STS completely reversed the inhibitory effect of ethephon on stem elongation (Saniewski and Kawa, 1988). It was also found that STS retarded the chlorophyll degradation in tulip stems and counteracted the stimulatory effect of ethephon on the process (Kawa and Saniewski, 1983). It seems that in tulips the inhibitory effect of IAA on stem senescence is independent from ethylene.

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## Tables

Table 1. The effect of IAA, ABA, ethephon and BA on chlorophyll "a" content (μg $cm^{-1}$) in tulip stem internodes. IAA in lanolin paste or lanolin only was applied in the place of excised flower, and water, ABA (40 mg $L^{-1}$), ethephon (40 mg $L^{-1}$) or BA (20 mg $L^{-1}$) was applied to the second leaf sheath on 20 March 2002; analysis were made on April 2, 5 and 11.

| Treatments | April 2 | | | | April 5 | | | | April 11 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Internodes | | | | | | | | | | | |
| | I - basal | II | III | IV | I - basal | II | III | IV | I - basal | II | III | IV |
| Lanolin + water | 17.6 bc | 10.5 b | 7.8 b | 3.8 b | 7.6 a | 5.4 b | 3.1 a | 0.4 a | dry | dry | dry | dry |
| Lanolin + ABA | 11.3 a | 4.5 a | 1.8 a | 0.7 a | 4.9 a | 1.7 a | 0.7 a | 0.4 a | dry | dry | dry | dry |
| Lanolin + ethephon | 14.9 ab | 6.0 a | 3.6 a | 0.5 a | 7.1 a | 4.3 ab | 1.3 a | 0.3 a | dry | dry | dry | dry |
| Lanolin + BA | 24.5 de | 18.8 cd | 14.2 c | 1.7 ab | 16.8 bc | 17.0 de | 17.9 cd | 0.4 a | 7.2 a | 9.2 ab | 15.6 b | 0.3 a |
| IAA 0.1% + water | 14.9 ab | 15.7 c | 14.0 c | 15.2 c | 19.5 cd | 17.8 de | 10.6 b | 10.9 b | 7.8 a | 5.9 ab | 5.9 a | 14.4 d |
| IAA 0.1% + ABA | 24.3 de | 18.7 cd | 14.0 c | 15.4 c | 15.0 b | 12.1 c | 8.1 b | 11.5 b | 6.5 a | 3.1 a | 4.2 a | 7.6 b |
| IAA 0.1% + ethephon | 21.9 cd | 18.5 cd | 14.9 c | 14.3 c | 16.8 bc | 15.0 cd | 9.6 b | 11.4 b | 8.9 a | 5.3 ab | 6.0 a | 13.1 cd |
| IAA 0.1% + BA | 20.4 cd | 23.9 ef | 19.1 d | 14.3 c | 17.7 bc | 16.8 de | 17.7 cd | 10.6 b | 8.8 a | 11.5 b | 14.1 b | 9.0 bc |
| IAA 2.0% + water | 18.4 bc | 21.3 de | 18.0 d | 21.6 de | 22.8 e | 25.5 g | 16.8 c | 20.1 d | 24.1 d | 27.4 d | 22.1 c | 20.0 e |
| IAA 2.0% + ABA | 26.9 ef | 24.2 ef | 22.0 e | 22.6 de | 22.5 de | 23.0 fg | 15.6 c | 18.9 cd | 26.1 d | 24.6 cd | 21.2 c | 25.7 f |
| IAA 2.0% + ethephon | 29.6 f | 27.1 f | 22.0 e | 23.8 e | 19.3 cd | 20.3 ef | 18.0 cd | 16.6 c | 21.3 c | 26.4 d | 22.8 c | 23.7 ef |
| IAA 2.0% + BA | 25.1 de | 24.8 ef | 17.8 d | 20.0 d | 18.2 bc | 23.9 g | 21.8 d | 18.2 cd | 18.3 b | 19.7 c | 16.3 b | 20.6 e |

In columns. means followed by the same letter are not significantly different at P=0.05 according to Duncan's test; values are calculated separately for each internode and for each day.

Table 2. The effect of IAA, ABA, ethephon and BA on chlorophyll "a" content (µg disk$^{-1}$) in tulip leaves. IAA in lanolin paste or lanolin only was applied in the place of excised flower, and water, ABA (40 mg L$^{-1}$), ethephon (40 mg L$^{-1}$) or BA (20 mg L$^{-1}$) was applied to the second leaf sheath on 20 March 2002; analysis were made on April 2, 5 and 11.

| Treatments | April 2 | | | April 5 | | | April 11 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaves | | | | | | | | |
| | 1st - lower | 2nd | 3rd | 1st - lower | 2nd | 3rd | 1st - lower | 2nd | 3rd |
| Lanolin + water | 102.6 cd | 107.9 bc | 113.2 cde | 36.2 a | 68.4 b | 62.8 b | dry | dry | dry |
| Lanolin + ABA | 35.9 a | 79.8 a | 44.2 a | 22.4 a | 21.4 a | 16.6 a | dry | dry | dry |
| Lanolin + ethephon | 67.7 b | 81.9 a | 83.1 b | 24.6 a | 62.3 b | 50.0 b | dry | dry | dry |
| Lanolin + BA | 100.5 cd | 113.2 bcd | 114.5 cde | 89.0 cd | 107.1 c | 112.1 cde | 15.5 a | 60.8 bc | 101.4 bc |
| IAA 0.1% + water | 97.4 cd | 102.2 b | 110.3 cd | 102.5 de | 110.0 c | 108.8 cd | 11.2 a | 35.7 a | 74.8 a |
| IAA 0.1% + ABA | 98.8 cd | 110.6 bcd | 101.0 c | 77.9 bc | 100.5 c | 96.2 c | 12.4 a | 48.9 ab | 66.9 a |
| IAA 0.1% + ethephon | 77.8 bc | 126.1 def | 127.6 ef | 71.9 b | 98.8 c | 102.0 cd | 16.6 a | 67.1 bcd | 74.3 a |
| IAA 0.1% + BA | 121.8 d | 116.0 bcd | 125.7 def | 96.6 de | 105.7 c | 118.5 def | 36.2 b | 72.8 cd | 95.1 b |
| IAA 2.0% + water | 111.2 d | 119.0 cde | 135.2 fg | 109.2 e | 115.8 cd | 121.7 def | 78.6 e | 99.1 ef | 132.7 d |
| IAA 2.0% + ABA | 114.4 d | 120.5 cde | 130.9 f | 99.1 de | 114.7 cd | 131.4 ef | 55.3 c | 83.5 de | 114.7 cd |
| IAA 2.0% + ethephon | 125.6 d | 134.2 f | 149.3 g | 97.9 de | 106.8 c | 113.5 cde | 68.2 d | 97.5 ef | 120.9 d |
| IAA 2.0% + BA | 114.0 d | 129.6 ef | 133.2 f | 105.2 de | 126.9 d | 137.6 f | 80.5 e | 108.7 f | 129.7 d |

In columns, means followed by the same letter are not significantly different at P=0.05 according to Duncan's test; values are calculated separately for each leaf and for each day.

Table 3. The effect of IAA, ABA, ethephon and BA on chlorophyll "b" content in tulip stem internodes (µg $cm^{-1}$) and leaves (µg $disk^{-1}$). IAA in lanolin paste or lanolin only was applied in the place of excised flower, and water, ABA (40 mg $L^{-1}$), ethephon (40 mg $L^{-1}$) or BA (20 mg $L^{-1}$) was applied to the second leaf sheath on 20 March 2002; analysis were made on April 2, 5 and 11; data presented only from analysis made on April 5.

| Treatments | April 5 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Internodes | | | | Leaves | | |
| | I - basal | II | III | IV | 1st - lower | 2nd | 3rd |
| Lanolin + water | 5.4 b | 3.8 ab | 2.8 ab | 1.3 a | 13.1 a | 22.7 bc | 21.2 b |
| Lanolin + ABA | 1.9 a | 2.2 a | 1.9 a | 1.3 a | 10.2 a | 9.4 a | 8.9 a |
| Lanolin + ethephon | 1.0 a | 5.3 bc | 2.7 ab | 1.2 a | 12.8 a | 19.0 b | 17.8 b |
| Lanolin + BA | 10.4 de | 7.0 cd | 8.3 de | 1.1 a | 33.1 c | 36.5 de | 37.5 cde |
| IAA 0.1% + water | 10.2 de | 9.2 de | 6.5 cd | 4.6 b | 35.8 cd | 40.5 e | 36.2 cd |
| IAA 0.1% + ABA | 7.0 bc | 6.0 bc | 4.7 bc | 6.1 cd | 31.6 c | 28.2 cd | 30.7 c |
| IAA 0.1% + ethephon | 10.0 de | 9.5 de | 6.3 cd | 5.1 bc | 26.0 b | 35.4 de | 36.7 cd |
| IAA 0.1% + BA | 12.1 e | 10.4 ef | 9.2 f | 4.2 b | 35.6 cd | 37.0 de | 40.2 def |
| IAA 2.0% + water | 12.2 e | 12.4 g | 9.3 f | 8.4 f | 39.4 d | 39.1 e | 42.1 def |
| IAA 2.0% + ABA | 8.8 cd | 11.0 ef | 8.5 f | 7.6 ef | 34.0 c | 37.1 de | 44.6 ef |
| IAA 2.0% + ethephon | 12.4 e | 12.1 ef | 8.7 ef | 6.6 de | 36.7 cd | 34.5 de | 37.5 cde |
| IAA 2.0% + BA | 12.6 e | 12.3 fg | 9.7 f | 7.2 de | 39.9 d | 44.0 e | 47.6 f |

In columns, means followed by the same letter are not significantly different at P=0.05 according to Duncan's test; values are calculated separately for each internode and for each leaf.

**Figures**

Fig. 1. Auxin retards tulip shoot senescence. At the beginning of flowering in the place of removed flower was applied (from left to right): lanolin, IAA 0.1%, IAA 2.0%. The photograph was taken 19 days after treatment.