

## Full Length Research Paper

# The effects of zinc (Zn) and C<sup>14</sup>-indoleacetic acid (IAA) on leaf senescence in *Helianthus annuus* L.

Serap Sağlam-Çağ\* and Yener Okatan

Department of Botany, Faculty of Science, İstanbul University, 34134 Süleymaniye-İstanbul, TURKEY.

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**Sequential leaf senescence is defined as a kind of programmed death events which is an important process in growth of plant. This study aimed to explore the sequential leaf senescence rate due to indoleacetic acid and lack of zinc (-Zn). Therefore, the effect of zinc and indole-3-acetic acid on senescence which occurs in *Helianthus annuus* (sunflower) cotyledons was analyzed. It was found that in cotyledons of seedlings grown in Hoagland solution which was prepared without addition of zinc senescence is delayed. It was recognised that in case of <sup>14</sup>C indoleacetic acid (IAA) which was given from apical tip not reaching the root and cotyledons, senescence does not occur in cotyledons. It was studied to get more information about physiological system of sequential leaf senescence.**

**Key words:** Sequential leaf senescence, cotyledon, zinc, <sup>14</sup>C indole-acetic acid (IAA), *Helianthus annuus* (sunflower).

## INTRODUCTION

Senescence is the final phase of plant vegetative and reproductive development, preceding the widespread death of cells and organs (Schmid et al., 1999; Guiboileau et al., 2010; Caswell and Salguero-Gomez, 2013). It has long been known that hormones regulate the progression of leaf senescence (Fletcher and Osborne, 1965; Misra and Biswal, 1980; Noodén and Leopold, 1988; Jibrán et al., 2013). In the process of senescence, destruction cases occur more than synthesis. From point of that view, definition of senescence is the process which increases destruction cases in cell and causes the plant to die.

The analysis made on leaf cells shows that during senescence consecutive metabolic events occur. These

events can be ordered as the synthesis of proteolytic enzyme (Colin and Thimann, 1972; Cheng and Kao, 1984; Hörtensteiner and Feller, 2002), the start of destruction of membrane proteins caused by these enzyme's activities, the decrease of quantity of protein and total nitrogen in the cell (Krul, 1974; Peterson and Huffaker, 1975; Peoples and Dalling, 1978, Prakash et al., 2001; Hopkins et al., 2007; Kaplan-Dalyan and Sağlam-Çağ, 2013), the acceleration of chlorophyll destruction (Peoples et al., 1980; Rodoni et al., 1997; Hörtensteiner, 2006; Darnel et al., 1990) and lipid destruction (Dhindsa et al., 1982; Harwood et al., 1982; Thompson et al., 1998; Hebelers et al., 2008). It is accepted that transportation of nutrients in other leaves

\*Corresponding author. E-mail: serapcag63@yahoo.com or sercag@istanbul.edu.tr. Tel. +90 212 455 57 00 (26807). Fax: +90 212 522 65 62.

starting from the oldest organ to the youngest supports the nutrient drain hypothesis. According to another hypothesis called 'signal' hypothesis, a signal which is thought to be synthesized by developing seeds, is being transported to old leaves and causes senescence as a result of catabolic reactions. According to this hypothesis, if the signal center is eliminated, senescence does not occur (Lindoo and Noodén, 1977). But the above mentioned signal has been displayed that it could not be isolated (Moore, 1979; Ridge, 1991). It is obviously known that the cause of all biochemical events during senescence are related to gene expression (Draper, 1969; Sanders and Write, 1995; Hörtensteiner, 1997; Distelfeld et al., 2014). The meanings of these chemical events come into being only with the researches made by plant physiologists on plant's physiology. While it is being said that auxins (Wareing and Seth, 1967; Kahanak et al., 1978; Lim et al. 2003) delay senescence, researches made recently indicate that auxins (Palni et al., 1988; Lu et al., 2001; Gören and Sağlam-Çağ, 2007) accelerate the senescence. Otherwise, Noh and Amasino (Noh and Amasino, 1999) detected that auxin represses transcription of some genes whose expression is correlated with senescence.

## MATERIALS AND METHODS

*Helianthus annuus* L. seedlings were grown in intensity of 6000 lux light, under 12 h photoperiod and  $26 \pm 2^\circ\text{C}$ .

### Designation of senescence degree

To determine the senescence which occur in cotyledons of *H. annuus* quantitatively the method improved and used for soya bean's Anoca variety-show by Lindoo and Noodén (1976) was adapted to *H. annuus* cotyledons. For chlorophyll designation Arnon (1949) method was used. To determine total nitrogen quantity a method, formed with combination of Kjeldahl method and spectrophotometric measurement method was used (Lindoo and Noodén, 1976). The zinc quantity in the material was designated with atomic absorption spectrophotometer (AAS).

### Giving IAA to the cut ends of the decapitated seedlings

One to two days before senescence starts in cotyledons, seedlings were decapitated by being cut approximately 3 cm above the internodium cotyledons.  $10^{-5}$  M. IAA solution (treated) or water (control) was applied to decapitated surface.

### Giving $^{14}\text{C}$ -IAA to the seedlings on the top buds and nodium leaves

$10^{-5}$  mol.  $^{14}\text{C}$ -IAA (specific activity:40 mCi / mmol.) 1 drop 1% tween-20 was added per 1 ml. 60 and 120  $\mu\text{l}$  from IAA solution was dropped on plant's top bud, 80 and 320  $\mu\text{l}$  was dropped on nodium leaves. To hinder indolacetic acid's photo oxidation, this process was made when the plants were passing to dark period. The whole organ, of which radioactivity will be enumerated in  $\beta$  counter was

prepared accordingly. Counting value of the material per 5 min (cp5m=count per 5 min) was calculated.

### Statistic evaluation of the results

Standard deviation estimate was made to evaluate the results obtained from experiment and control groups statistically. In case of the number obtained when  $\pm$  values of differences between the result's square's total sum's square root is multiplied with three is found to be smaller than the difference between values, the difference is decided to be significant statistically.

## RESULTS

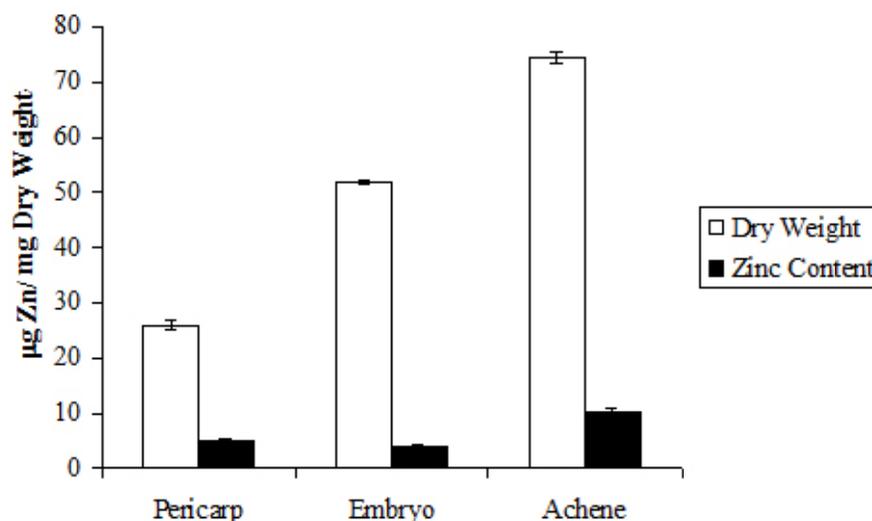
As it is known that zinc provides indole-acetic acid (IAA) stabilization and in case of zinc deficiency quantity of IAA decreases, this mineral's effects on plant growing and cotyledon senescence were analysed. The seedlings forming the experiment group were grown in Hoagland solution which does not include zinc and was diluted in 1/8 ratio (Table 1). Senescence delayed in cotyledons of seedlings in -Zn solution. Besides with the ingathering, when cotyledon senescence in plants grown with the existence of zinc (control) is 50% according to plastochron index (28<sup>th</sup> day), total chlorophyll and total nitrogen quantities in cotyledons of all seedlings belonging to experiment or control group (Table 2). The quantity of the zinc which is thought to exist in the seed naturally was measured with AAS (Figure 1). Nineteen days old *H. annuus* seedlings were divided into 4 groups. First group plants were intact (control). Other group plants were given IAA, NAA and H<sub>2</sub>O from truncated end being decapitated from under 2nd internodium. After this process the speed of senescence occurring in cotyledons was observed (Figure 2). In cotyledons of plants to which IAA and NAA applied senescence occurred quickly just like it occurs in cotyledons of intact plants. However, in a great majority of plants having a process with H<sub>2</sub>O, cotyledons remained green. Senescence did not occur in the cotyledons of the 17 days old experiment and control seedlings which were exposed to the same process and application. To determine first which organ as a target indolacetic acid after being produced in the plant is transported, 120  $\mu\text{l}$  from  $10^{-5}\text{M}$   $^{14}\text{C}$ -IAA+tween 20 upon top bud of the plants was dropped in. After dark period for 12 h, the quantity of  $^{14}\text{C}$ -IAA in the roots and cotyledons of the plants was stated (Table 3). Radioactivity existing in the root is found to be more than 20 times more than the radioactivity in cotyledons. From the values obtained it was understood that IAA given from top bud was transported quickly to the root. On the other hand, from the middle of the first internodium's of the seedlings, a part, approximately 1 cm was boiled with hot water vapour on the 17 day 60  $\mu\text{l}$   $10^{-5}$  M-IAA+tween 20 was dropped in the top bud of the plants of which cotyledons was just 100% green on the 32 day. After dark period for

**Table 1.** The effect of Zn (105 mg/L) on the green area (%) of the cotyledons from-day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Day	Average green area Percentage [Control (+ Zn)]	Day	Average green area Percentage [Control (- Zn)]
17	100.00 ± 0.00	20	100.00 ± 0.00
22	78.00 ± 0.26	23	73.93 ± 1.62
25	56.34 ± 1.42	27	49.45 ± 1.23
29	32.73 ± 2.46	30	21.61 ± 1.72
33	00.00 ± 0.00	34	00.00 ± 0.00

**Table 2.** The effect of Zn (105 mg/L) on the chlorophyll and nitrogen content of the cotyledons from 28 day *H. annuus* seedlings grown in 1/8 Hoagland solution.

Hoagland	mg N / g cotyledon	%	mg chlorophyll / cotyledon	%
Hoagland (- Zn)	129.603 ± 4.256	100	0.0449 ± 0.005	100
Hoagland (+ Zn)	108.109 ± 2.562	83.4	0.0382 ± 0.002	74.2

**Figure 1.** Dry weight and Zn quantity in pericarp, embryo and achene.

12 h, radioactivity in different organs of the plants was measured (Table 4).

As the radioactivity difference counted in organs under the 1 internodium's boiled part was insignificant statistically, it was understood that radioactivity can pass downwards from boiled area in trace quantity. Besides, although it was found on boiled part, on the leaves of the 2 internodium a statistically significant quantity of radioactivity could not be found. It was stated that radioactivity had accumulated in a great quantity on the boiled part of internodium.

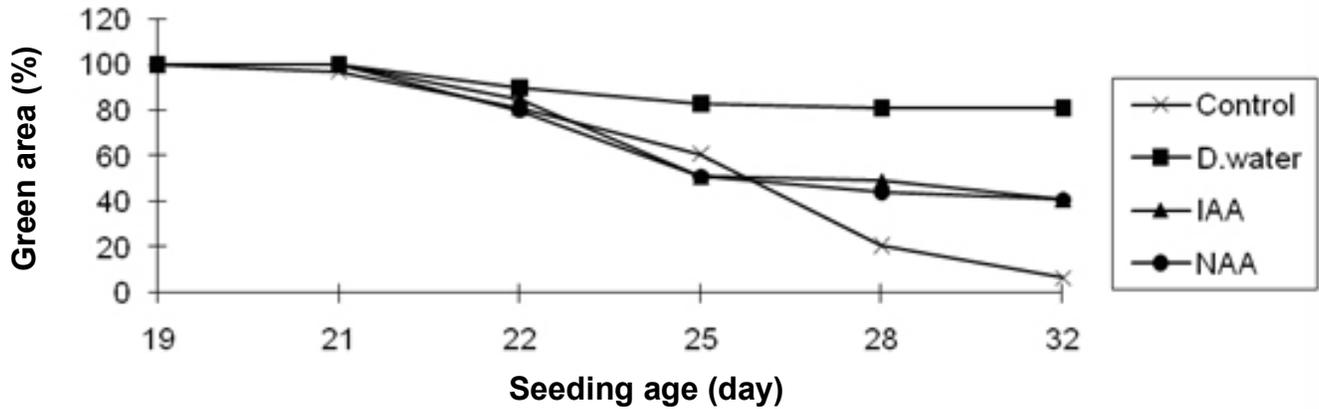
To the first leaves (second nodium leaf) after cotyledon of 19 days old plants totally 80µl <sup>14</sup>C-IAA, 40 per each, was applied. After plants being ingathered on different

days (Avery et al., 1937; Lindoo and Noodén, 1976; Papadopoulus et al., 1985; Noodén and Leopold, 1988), radioactivity on root and 3. nodium leaves was measured (Table 5).

It was recognised that <sup>14</sup>C-IAA applied to 2 nodium leaves was transported to root first and then from there by xylem, was transported to the leaves making transpiration.

## DISCUSSION

In this research, the effects of zinc and indolacetic acid, which is a growing hormone, on senescence was



**Figure 2.** Green area (%) of cotyledons of 19 days old *H. annuus* seedlings which were decapitated below the second internode and treated with  $10^{-5}$  M IAA,  $10^{-5}$  M NAA or  $H_2O$  from the truncated end.

**Table 3.**  $^{14}C$  amounts in the roots and cotyledons of *H. annuus* seedlings treated with  $10^{-5}$  M  $^{14}C$ -IAA (\* Significant).

Organ	Count / 5 min	Radiation of background	Difference
Cotyledon	299.85 ± 31.25	288.36 ± 3.015	11.49
Root	875.41 ± 29.09	288.36 ± 3.015	587.05 *

**Table 4.**  $^{14}C$  amounts in different organs of *H. annuus* seedlings treated with  $10^{-5}$  M  $^{14}C$ -IAA (\* Significant).

Organ	Count / 5 min	Radiation of background	Difference
2nd node leaves	306.92 ± 7.05	288.36 ± 3.015	18.56
First internode	1039.33 ± 57.63	288.36 ± 3.015	750.97 *
100% Green cotyledon	299.87 ± 7.66	288.36 ± 3.015	11.51
Hypocotyl	304.00 ± 11.82	288.36 ± 3.015	15.64
Root	313.00 ± 10.64	288.36 ± 3.015	24.64

**Table 5.**  $^{14}C$  amounts in the roots and 3<sup>rd</sup> node leaf of *H. annuus* seedlings leaf on different days after  $10^{-5}$  M  $^{14}C$ -IAA treatments on the 2<sup>nd</sup> nodium. (\* Significant).

Day	Root (Count / 5 min)	3rd nodium leaf (count / 5 min)	Radiation of background	Difference (root)	Difference (3 rd nodium leaf)
20	318,44 ± 11.627	302,67 ± 3.567	288.36 ± 3.015	30.08	14.31 *
21	489,11 ± 34.310	352,22 ± 10.97	288.36 ± 3.015	200.75*	63.86 *
22	454.00 ± 45.287	342.00 ± 14.09	288.36 ± 3.015	165.64 *	53.64 *
25	485,67 ± 11.794	712,89 ± 131.27	288.36 ± 3.015	197.31 *	424.53 *

searched. In a research (Ray and Choudhuri, 1981), it was supported that hormones (IAA, GA, Kinetin) plays the most important role in transporting nutrients to seeds that develops as an endogenous hormone resource. It is known that the deficiency of the hormones which prevents senescence (for ex: cytokinin) may cause senescence. Palni et al. (1988) mentions that auxin has

an effect on cytokinin's metabolism and this effect is actualized by oxidase enzyme. While some researchers (Jacobs and Cready, 1967; Sanchez-Bravo et al., 1991) declare that indolacetic acid localize in cortex, vascular tissue and pith, auxin is transported in vascular and epidermal tissues, other researchers (Bangerth, 1994; Eköf et al., 1995; Li et al., 1995; Shimizu-Sato et al., 2009)

emphasized that intact plants's cytokinins in xylem exudate are under the control of polar auxin transportation system. Hare and Staden (1994) expressed that cytokinin catabolism which becomes true with the activity of sitokinin, a specific enzyme oksidase realizes death in plant tissue and moreover stated that auxin plays the role of allosteric systematizer increasing this enzyme's activity.

It is known that indolacetic acid is synthesized in the end of stem and zinc provides the stabilization of indolacetic acid (Skoog, 1940; Takaki and Kushizaki, 1970). In the early development phase of the plants of which endogen IAA quantity was decreased by being grown in zinc deficiency, IAA that is under the control of the quantity of zinc in the seed has such a quantity that it delays the senescence but can provide growing. But zinc which is given with Hoagland solution in addition to the zinc quantity in the seedling may be impulsive in senescence or may delay growing because of its toxic effects on some enzyme systems related to growing. Likewise, Sağlam-Çağ and others (Sağlam-Çağ et al., 2004) emphasized that senescence was delayed in excised cotyledons in the solution lacking zinc. In that research, in the existence or deficiency of zinc, IAA which can be controlled endogenously was held to be responsible for the change in senescence's speed. In some experiments which  $^{14}\text{C}$ -IAA was used (Hew et al., 1967), it was noticed that IAA given from truncated end of the stem goes through stem axis quickly and don't enter to leaves. Also, in this research it was found that  $^{14}\text{C}$ -IAA was transported to root without touching at leaves. Moreover, as  $^{14}\text{C}$ 's internodium does not goes through boiled part, it was noticed that it could not reach the root and cotyledons and senescence didn't occur in cotyledons.

We can assert that sequential leaf senescence is related to the occurrence of metaxylem after protoxylem and in this event, with IAA's effect on xylem formation, it may come on the scene. Just before senescence, although senescence occurred when IAA was applied from truncated top, cotyledons remained green when IAA was applied in early phase. Researchers (Shimomura et al., 1988; Jones et al., 1989; Jones, 1994) indicated that there are 2 different receptors in plasma membrane connecting IAA and in recent years it was determined that first one of these receptors isolated is related to cell growing but then any absolute information about second IAA receptor's function wasn't given (Darnel et al., 1990; Cooper, 1997).

As a result of our research, we saw that zinc, providing IAA stabilization accelerates senescence; in the researches made with  $^{14}\text{C}$ , as its internodium does not go through boiled part  $^{14}\text{C}$ -IAA given from apex, it can not reach root and cotyledons and senescence does not occur in cotyledons. It became certain that it was transported to the root without touching at leaves and this transportation is made by parenchymatic living tissues not xylem. This research indicated that senescence signal may be indoleacetic acid or a substance like indoleacetic acid.

## Conflict of interests

The author(s) have not declared any conflict of interests.

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