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Role of Zinc Deficiency in Photosynthetic Pigments and Peroxidase Activity of Tomato Seedlings

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Abstract

Zinc (Zn) deficiency is occurring in different climate regions and almost in all countries. Zinc deficiency is a common micronutrient deficiency in plants and causes severe reductions in crop production. In this study the effect of zinc deficiency on pigment content and peroxidase activity in early growth phase of tomato seedlings was investigated. For this purpose, Hoagland solutions with or without zinc ($ZnCl_2$) were used as growth medium to determine the possible effects of zinc deficiency. In order to elucidate the physiological consequences of zinc deficiency; anthocyanin, carotenoid, and chlorophyll content and peroxidase enzyme activity were measured in roots, hypocotyls, and cotyledons of 8 day-old plants. Zn deficiency promoted anthocyanin and carotenoid accumulation in hypocotyl and cotyledon tissues of tomato seedlings. Furthermore, Zn deficiency enhanced peroxidase activity especially in the root and hypocotyl tissues of tomato seedlings.

Keywords: *Chlorophyll, Peroxidase, Tomato, Zinc, Zinc deficiency*

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Introduction

Zinc (Zn) is one of the essential micronutrients playing a significant role in many vital metabolic processes (Rout and Das 2003; Aravind and Prasad 2005a, b). For instance, Zn is a cofactor for several enzymes such as anhydrases (Aravind and Prasad 2004a; 2005c), dehydrogenases, oxidases and peroxidases (Vallee and Auld 1990; Aravind and Prasad 2003). Furthermore, Zn could be also involved in chlorophyll formation by taking part in the regulation of cytoplasmic concentrations of nutrients. For example, Fischer (1997) reported that Mg-deficiency decreased chlorophyll concentration in beans, and Welkie et al. (1990) recorded that there was a linear relationship between leaf-Fe and chlorophyll content in peppers. Zn could also increase the biosynthesis of chlorophyll and carotenoids ultimately proving beneficial for the photosynthetic machinery of the plant system (Aravind and Prasad 2004b). Conclusively, Zn is a must for plant development. Zinc deficiency is a major global problem hindering plant cultivation, and this problem is especially exacerbated in acidic calcareous soils which is the most common soil type in arid and semi-arid regions of the world (Cakmak 2000; Hacisalihoglu et al. 2004). Zinc deficiency reduces plant growth and inhibits photosynthesis in a wide variety of plants including maize (Wang and Jin 2005) and rice (Wenrong et al. 2008). Since the most parts of Turkey lie in arid or semi-arid climatic zones, zinc deficieny is one of the most serious problems restricting plant yield, particularly in Central Anatolia. Although Turkish soils are rich in total Zn content, the bioavailability of Zn is extremely low due to the highly acidic nature of soils and results in stunted plant growth and reduced yields (Eyüpoğlu et al. 1994; Gülser et al. 2004).

Peroxidases have various physiological roles in plant cells and participate in many reactions including lignification, cross linking of cell elongation and phenol oxidation, all linked to growth reductions (Mocquot et al. 1996). Peroxidase activity shows a close correlation with changes in physiological processes such as, respiration, photosynthesis and transpiration, and therefore has the potential to serve as a sensitive indicator of compromised metabolic activity (Verkleij and Schat 1990; Aravind and Prasad 2005a). Inhibition of photosynthesis by some stressors in higher plants is well documented. Reduction in the levels of photosynthetic pigments, including chlorophylls a and b and accessory pigments such as carotenoids, on exposure to biotic or abiotic stressors have been observed in many species (Macfarlane and Burchett 2001; Thao and Yanyun 2005; Lau et al. 2006). The inhibition of both photosystem II and photosystem I generating lipid peroxidation from active oxygen radicals, may also result in the destruction of photosynthetic pigments and thylakoid membrane structure (Droppa and Horvath 1990). Anthocyanins are best known as the characteristic red, blue, and purple of plant tissues. They belong to the widespread class of phenolic compounds collectively named flavonoids, produced in the cytoplasm and then transported into the vacuole (Harborne 1988; Shirley 1996). The synthesis of anthocyanins is induced by UV-B (Wilson et al. 1998, Warren et al. 2003), nutrient deficiency (Pinto et al. 1999), low temperature (Rabino and Mancinelli 1986), water deficit (Nogués et al. 1998), and heavy metal stress (Ling-Peng et al. 2006). Thus, both peroxidases and pigments may have the potential to be employed as sensitive indicators of biotic or abiotic stressors and may predict subsequent events at the organism level (Dietz et al. 1999).

The main objective of this study was to investigate the effects of zinc deficiency on pigment content and peroxidase activity in the early growth phase of tomato seedlings. In this study, we determined quantitatively the change in chlorophyll, carotenoid, anthocyanin, and peroxidase activity which will be expected to shed light on the role of pigment in tomato seedlings and zinc deficiency.

Material and Methods

Tomato plants (*Lycopersicon esculentum* Mill.) were grown in standard Hoagland and Arnon (1938) medium, with Zn [+(Zn), $ZnCl_2$, control] and without Zn [-(Zn), treatment]. After sterlization with 70 % ethanol, tomato seeds were imbibed at room temperature for 24 hours. Subsequently, the seeds were washed with distilled water and transferred into petri dishes and wrapped into the filter papers wetted with appropriate nutrient solutions (+Zn and -Zn). Next, each petri dish was watered with distilled water for every two days. Experimental plants were grown for 12 hours of light and 12 hours of dark cycles in 25 ±1 °C and 8000 lux light intensity. For analyses, roots, hypocotyls and cotyledons of the plants were collected at appropriate times.

After the 8 days of growth under the above mentioned conditions the same method was employed as in Manchinelli et al. (1990) for the determination of anthocyanin amounts in the cotyledons and hypocotyls of 8 day-old seedlings. For the determination of chlorophyll and carotenoid amounts in the same tissues, the same approach was utilized as explained by Parsons and Strickland (1963). Finally, peroxidase enzyme activity in the roots, cotyledons and hypocotyls were calculated by the absorbance ratios at 470 nm in 0.1 mM phosphate buffer (pH 5.8), 15 mM guaiacol and 5 mM H_2O_2 solutions (Birecka et al. 1973).

One-way ANOVA test was employed for statistical analyses of the obtained data ($P<0.01$). Vertical bars show standard errors.

Results

The anthocyanin content in the hypocotyls and cotyledons of the tomato seedlings grown in the medium without $ZnCl_2$ was much higher than the control (Fig. 1).

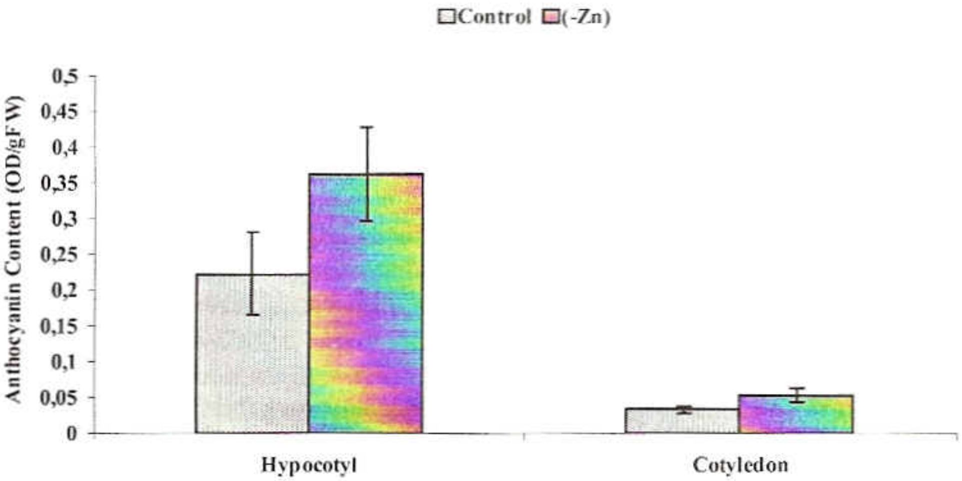

Figure 1. The amount of anthocyanin, hypocotyls, and cotyledons of 8 day old tomato seedlings grown in normal Hoagland solution containing zinc (control) and without zinc (-Zn).

As for chlorophyll, it was observed that the amount of chlorophyll in the hypocotyls of seedlings grown in -Zn increased compared to the control plants. When we compared the level of chlorophyll in the cotyledon tissues of plants grown in control medium and in Hoagland solution without -Zn, the total amount of chlorophyll was almost equal, but there was some difference in the relative ratios of chlorophyll a and chlorophyll b (Table 1 and Fig. 2).

Table 1. Chlorophyll contents (μg/g FW) of hypocotyls, and cotyledons of tomato seedlings grown in normal Hoagland solution containing zinc (control) and without zinc (-Zn).

	Hypocotyl			Cotyledon		
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	95,2 ± 6,8	46,7 ± 5,5	141,9 ± 9,7	642,1 ± 11,1	409,4 ± 16,6	1051,5±21,9
(-Zn)	112,7 ± 10,1	57,9 ± 7,4	170,6 ± 15,1	575,3 ± 39,4	477,9 ± 42,9	1053,2±30,3

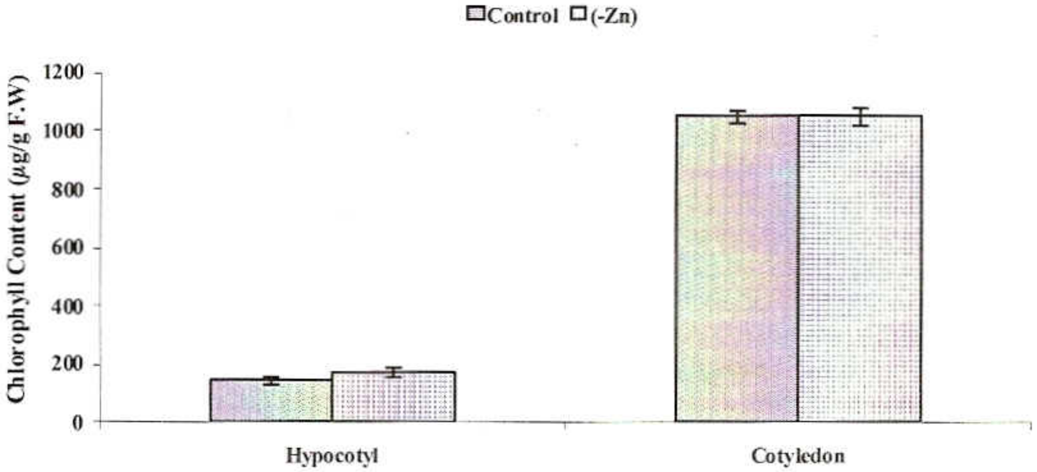

Figure 2. Total chlorophyll contents of hypocotyls, and cotyledons of tomato seedlings grown in normal Hoagland solution containing zinc (control) and without zinc (-Zn).

There was an increase in the carotenoid level of the seedlings grown in Hoagland solutions containing -Zn (Fig. 3).

The peroxidase enzyme activity of tomato seedlings which were grown -Zn was increased in comparison to the control plants. The peroxidase activity in the root and hypocotyl tissues of tomato seedlings grown in the -Zn medium was noticeably higher than the control (Fig. 4).

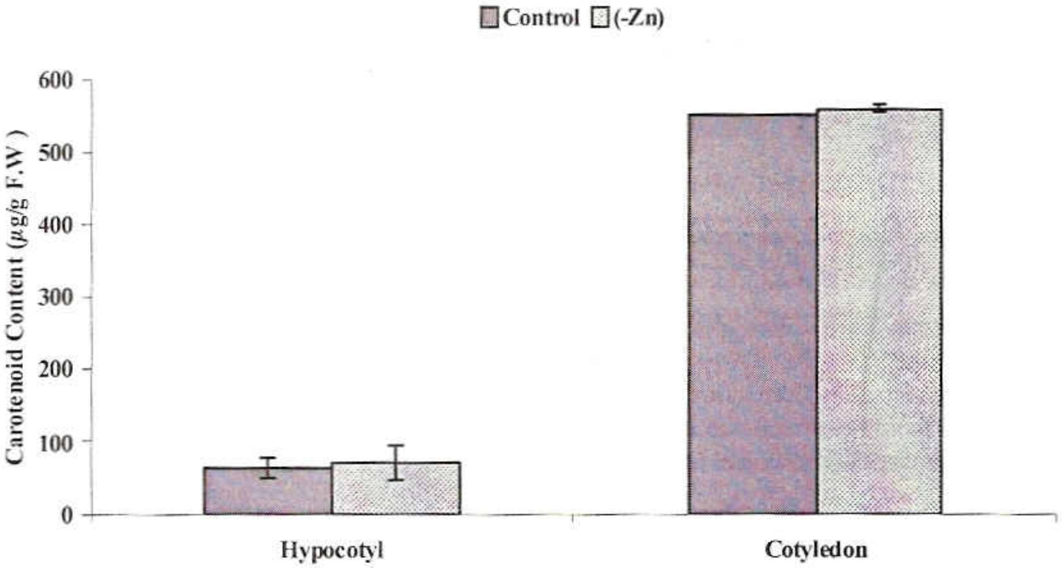

Figure 3. Total carotenoid contents of hypocotyls, and cotyledons of tomato seedlings grown in normal Hoagland solution containing zinc (control) and without zinc (-Zn).

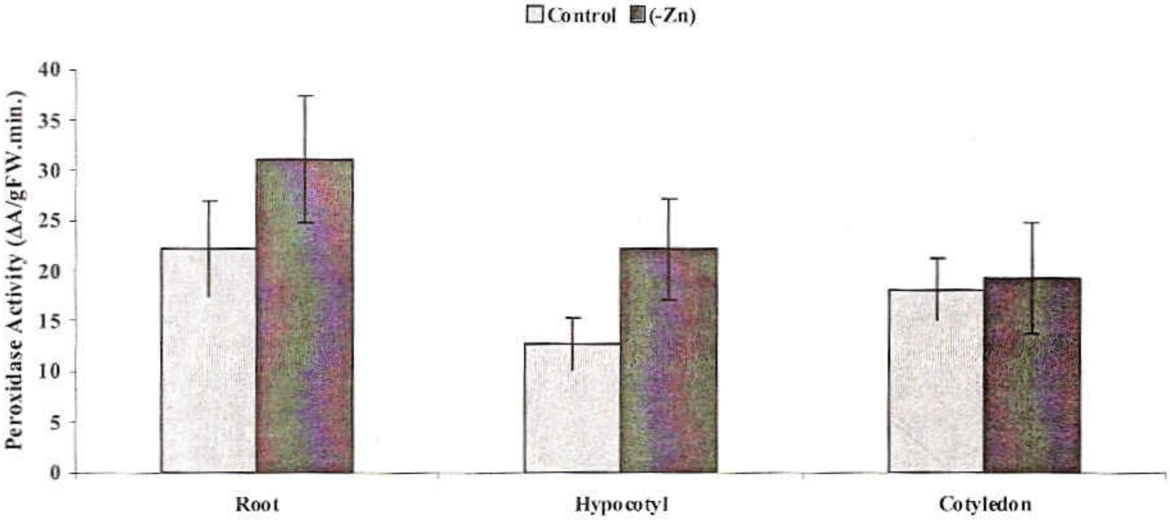

Figure 4. Peroxidase activitiy (ΔA/g FW.min.) of roots, hypocotyls, and cotyledons of tomato seedlings grown in normal Hoagland solution containing zinc (control) and without zinc (-Zn).

Discussion

Anthocyanins have long been considered to act as a screen against UV-B light because the latter induced reddening. More recent studies have found that anthocyanins are produced in response to various types of stress, including metal stress (Chalker-Scott 1999; Hale et al. 2001). In general, anthocyanins are believed to increase the antioxidant response of plants in order to uphold the regular physiological status in tissues directly or indirectly affected by biotic or abiotic stressors (Yamasaki 1996; Rice-Evans et al. 1997; Neill et al. 2002). Gülçin et al. (2005) also reported that total anthocyanins from *Perilla pankinensis* had strong antioxidant activity, reducing power, superoxide anion scavenging, hydrogen peroxide scavenging, and metal chelating activities when compared to standard antioxidant compounds such as α-tocopherol and trolox. We observed that the absence of zinc in the medium caused the augmentation of anthocyanin in both hypocotyls and cotyledons of the tomato seedlings. It is a known fact that plants defend themselves against a variety of hostile environmental conditions by inducing the biosynthesis of certain chemicals such as anthocyanins and antioxidants, and by hyping the activity of certain enzymes (Weckx and Clijsters 1997; Mazhoudi et al. 1997). Thus, from the results of our trials, we can claim that zinc deficiency is a stress factor and it increases the anthocyanin contents of tomato seedlings (Fig. 1).

Despite the amount of chlorophyll in the hypocotyl tissues of the tomato seedlings, seedlings grown in the -Zn medium were higher relative to the control, the chlorophyll amount in the cotyledon tissues of these seedlings was not very different than the control but there was some difference in the relative ratios of chlorophyll a and chlorophyll b (Table 1, Fig. 2). Wenrong et al. (2008) reported zinc deficiency caused extensive declines in leaf chlorophyll content and ratios of chl a:b . Also, Hu and Sparks (1991) found that the zinc deficiency reduces the amount of chlorophyll in the leaves of Stuart pecans. In one other experiment, it is shown that the application of exogenous zinc to leaves of tomato plants caused accumulation of chlorophyll content of leaves at both low and high concentrations (Kaya and Higgs 2002). However, we reached a contradicting result in this experiment; chlorophyll content of cotyledons of the plants grown without zinc was almost same with the values obtained from the control plants (Table 1, Fig. 2).

We denoted that the total carotenoid content of the tomato seedlings grown in media containers without zinc, increased (Fig. 3). It is shown that the decreased carotenoid levels observed in Cd-treated *Ceratophyllum demersum* plants were well maintained with the addition of Zn. (Aravind and Prasad 2004b). Carotenoids are known to quench the oxidizing species and triplet state of the chlorophyll and other excited molecules in the pigment bed, which are seriously involved in disrupting metabolism through oxidative damage to cellular components (Candan and Tarhan 2003). It could be hypothesized that relative change in the carotenoid content in the various tissues could be as result of physiological response caused by zinc deficiency.

It is shown that the peroxidase enzyme activity of tomato seedlings grown without $ZnCl_2$, was increased in comparison to the control plants (Fig. 4). Zinc is known to have a stabilizing and protective effect on the biomembranes against oxidative and peroxidative damage, loss of plasma membrane integrity and also alteration of the permeability of the membrane (Aravind and Prasad 2003). Marschner and Cakmak (1989) had shown an enhancement in the peroxidative damage of crucial cell components (such as membranes, enzymes, etc.) in plants grown under zinc deficiency. Subsequently, Cakmak and Marschner (1993) reported a decline in the activities of various antioxidant enzymes with the exception of guaiacol peroxidase (POD).

The most common plant pigments are chlorophyll, carotenoid, and anthocyanin. The content of chlorophyll, carotenoid, and anthocyanin as well as their relative amount determine color and appearance of plants (Abbott 1999) and serve as markers of health. In conclusion, our results showed that zinc deficiency induced anthocyanin and carotenoid augmentation, and peroxidase enzyme activity either in hypocotyls or cotyledons of tomato seedlings. Anthocyanin and POD may be involved in internal detoxification mechanisms of the hypocotyls and cotyledons against zinc deficiency.

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