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Effects of Different Flooding Periods on Some Histochemicals of *Zea mays* L. Seedlings

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Abstract: Flooding stress has many important morphological and biochemical effects on plants. Because of the importance of determination the effects of flooding on plants and understanding of the tolerance mechanisms, in this research 4 day old *zea mays* L. (*cv. single cross 704*) seedlings were exposed to 4, 7 and 10 days flooding stress. At the end of each treatment the roots and shoots of seedlings were harvested separately. To show the histological effects of flooding on plants, cross section of roots and shoots were studied with light microscope. There was no clear change in the tissues structures of leaves and stems of different treatments in comparison with controls, but in the roots of plants the aeranchyma had been developed under stress condition especially in the mesocotyl region. The roots of flooded plants grow towards the soil surface despite positive geotropism of control roots. The chlorophyll a and b content and the ratio of chlorophylls a/b have been decreased but the amounts of soluble sugars have been increased in both the roots and shoots of seedlings. We conclude that flooding influences plants growth and life and development of aeranchyma and vegetative roots help plants to adapt itself to stress condition. So it is very important to know which plants are sensitive or tolerant and what are tolerance mechanisms in different plants to succeed in agricultural efforts.

Key words: Flooding, aerenchyma, soluble sugar, *Zea mays*, seedlings, histochemicals

INTRODUCTION

Flooding occurs when all of the pores of the soil are saturated with water and the amount of soil gases as oxygen have reduced highly because the diffusion of O_2 in water is 10000 times lower than the air (Ram *et al*., 2002). Plants require a free exchange of atmospheric gases through the soil for their natural root growth and metabolism. Reduction of oxygen below the optimum levels (hypoxia) is the most common form of stress in wet soils that causes the roots of plants submerge in water while the shoots are out in atmosphere. In such condition, because of low oxygen in soil, respiration rate of roots reduces and plants maintain their energy from fermentative metabolism in roots to survive (Liao and Lin, 2001; Mohanty *et al*., 1993). Photosynthetic capacity has also been shown to be significantly inhibited in flooding-intolerant plants (Liao and Lin, 2001).

Adaptive mechanisms to secure a renewed supply of oxygen to flooded root tissues include the development of aerenchyma that allows oxygen to move from the aerobic shoots to the anaerobic roots. Aerenchyma differs in origin among species and may be either lysigenous or schizogenous. Lysigenous aerenchyma develops as a consequence of senescence of specific cells followed by their autolysis and disintegration, whereas schizogenous aerenchyma develops by cell separation and cell division (Igamberdieve *et al*., 2005). In monocots such as maize and rice, natural lysigenous aerenchyma develops in the roots cortex toward the endodermis, abow the root tip, in regions where cells growth has been completed (Jackson, 1985). In *Rumex* species a new aerenchymatous root system develops near the soil surface in response to flooding. This mechanism has supported oxygen concentrations for more than 50% of the total root respiration at hypoxia condition (Laan *et al*., 1990).

MATERIALS AND METHODS

In this experiment, the seeds of *Zea mays*, L. (cv. Single cross 704) were obtained from Agricultural Research Center of Urmia, they were cleaned, selected by size, washed with water and detergent and finally with distilled water. The seeds are incubated in 25°C to germinate, after 3 days the seedlings were transferred to pots filled with vermiculate in controlled condition with 16/8 h light/dark photoperiod and 25/23°C day/night temperature. After 24 h, the seedlings were flooded for 4, 7 and 10 days. At the end of each treatment the roots and shoots of seedlings were harvested separately, to show the effect of flooding on the plants, the changes of the amounts of soluble sugars and chlorophylls contents and the anatomical changes of the roots and shoots tissues were studied.

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Anatomical studies: To prepare cross-sections of the shoots and primary roots, the roots and shoots of all treatments and controls were taken and prepared to do a paraffin-cut section for anatomic observation by the method of Lin and Yeh (1996) and then they were studied by light microscope.

Determination of chlorophyll: Chlorophylls were extracted from the seedings using Lichentaller method (Zhang and Kirkham, 2003). Chlorophyll contents of youngest leaf of plants after extraction in acetone were measured spectrophotometerically at 664 and 647 nm. And chlorophyll amounts were calculated using following formula:

$$\mathrm{Chl}_a = 12/25\,A_{664} - 2/79\,A_{647}$$
$$\mathrm{Chl}_b = 21/51\,A_{647} - 5/10\,A_{664}$$

Determination of soluble sugars: Phenol-sulfuric method was used to determine soluble sugars. According to this method 0.5 g of the roots and shoots was measured separately. After rubbing they were filtered, 2 mL from each sample was taken, 1 mL 5% phenol was added then 5 mL 98% sulfuric acid was added to the samples. After coolness and complete colour emergence of the solutions, sugar contents were maintained by using spectrophotometer at 485 nm (Hsu *et al*., 2000).

Statistical analysis: Data analysis was done using Analysis of Variance Method ANOVA1, comparing the mean of data by Duncan test and the values indicate the mean of 3 replicates ±SE (Heim *et al*., 1990).

RESULTS AND DISCUSSION

Anatomical changes of flooding: The results of tissues studies of the roots and shoots of plants exposed to different periods of flooding indicated that there was no clear change in leaf and stem tissues; while in the flooded roots aerenchyma has been developed in comparison with controls (Fig. 1). Aerenchyma formation creates an internal gas exchange channel from the aerobic shoot to the hypoxic roots. Air enters through stomata of leaves or lenticels on the stem and passes through the network of aerenchyma channels to the submerged roots. Oxygen consumption in the roots creates a negative pressure gradient that draws air by mass flow to the roots, which in rice has been measured about 20 mL h^{-1} (Raskin and Kende, 1985).

Another typical symptom of flooding in this research was hypertrophic growth in the mesocotyl of 10 days flooded seedlings (Fig. 2). This growth that appears as a swelling at the area between the base of the stems and roots are important because of the transitional role of mesocotyl in carrying O_2 from shoots to roots. The reason for these changes depends on the radial cells division and their expansion and is often accompanied by cell collapse and aerenchyma formation. It is consequently considered to be an adaptive mechanism that causes increased air diffusion from shoots to roots (Visser and Voesenek, 2004).

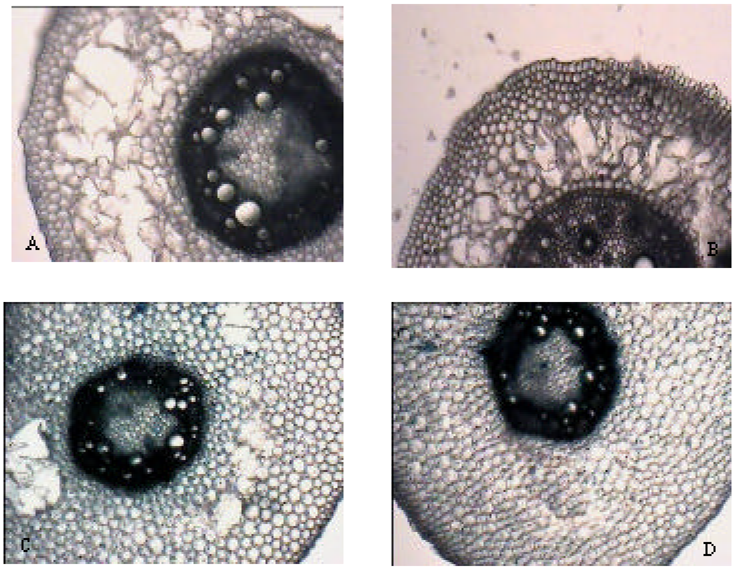

Fig. 1: Cross-sections of *Zea mays* roots, (A) aerenchyma developing in flooded root seedlings for 10 days (×50) in comparison with controls (B). (C) The initial aerenchyma formation in flooded root seedlings flooded for 4 days (×50) in comparison with controls (D)

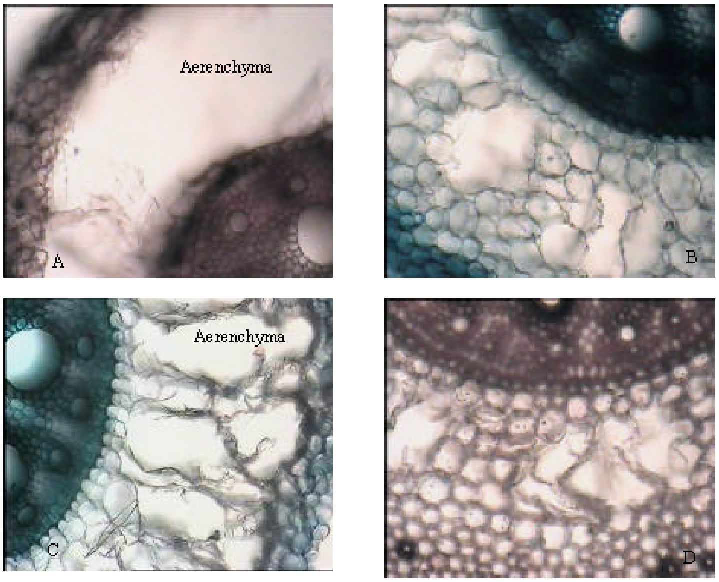

Fig. 2: Cross- sections of *Zea mays* seedlings mesocotyles, (A) aerenchyma developing in flooded mesocotyle seedlings for 10 days (×100) in comparison with controls (B). (C) Abnormal mesochotyle at 7 days after flooding (×100) in comparison with control (D)

Another early symptom of flooding stress involves reorientation of growth processes; for example, roots of flooded plants tend to become negative geotropism. They grow upwards (Fig. 3) and are able to receive more air from the soil surface (Jackson and Drew, 1984).

The third change is the development of adventitious roots in flooded plants (Fig. 3). Flooding often causes malfunctioning of roots formed prior to flooding, even in wetland species. This may eventually lead to the death of a considerable part of the root system and a fast replacement by well-adapted adventitious roots that contain more aerenchyma than the original roots (Visser *et al*., 1996).

Changes of the chlorophyls contents: The amount of chlorophyll a and b in the leaves of flooding treated plants was found to be significantly lower than controls (Fig. 4). Reduction of chlorophyll contents in hypoxia stress is probably due to the slowly synthesis and fast destruction of chlorophyll pigment (Ashraf *et al.*, 2003). The ratio of chlorophyll b/a has also been decreased (Fig. 4). This reduction is because that the sensitivity of chlorophyll b against flooding stress is more than chlorophyll a (Zaidi *et al.*, 2003). Previous studies suggest that chlorophyll b as a main part of photosystems breaks down higher than chlorophyll a under stress conditions (Mauchamp and Methy, 2004).

Fig. 3: The roots of flooded *Zea mays* plants tend to become negatively gravitropic and adventitious roots develop against flooding

The soluble sugars contents: Soluble sugars levels in flooded roots and shoots have been increased more than controls (Fig. 5). A high level of fermentative metabolism in roots has been shown to be important for plant survival because it supplies a high enough energy charge that can sustain metabolism in roots (Mohanty *et al.*, 1993). Thus, maintaining adequate levels of fermentable sugars in flooded roots is undoubtedly important for long term survival of plants during flooding. The changes of soluble sugars in roots of *zea mays* during flooding periods are agreed strongly with studies on alfalfa by Barta (1988) and Castonguay *et al.* (1993) that reports the root starch can be mobilized and converted to soluble sugar at the early stages of flooding. The starch levels in roots of flooded corn were found to decrease markedly during the early flooding stages (Su *et al.*, 1998) may cause the soluble sugars to increase.

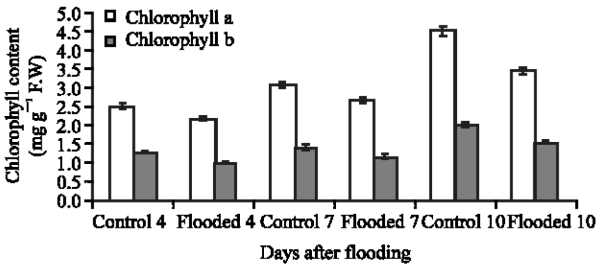

Fig. 4: The changes of total chlorophyll a and b content of *Zea mays* L. Seedlings exposed to different periods of flooding (during 4, 7 and 10 days).The values represent the mean of three replicates ±SE

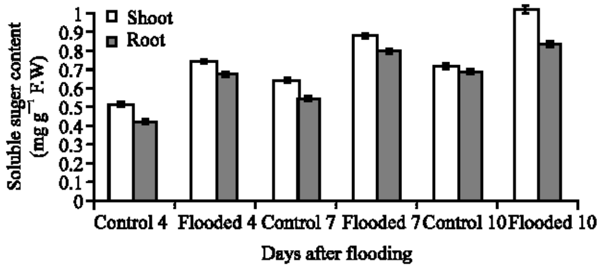

Fig. 5: The changes of total soluble sugars of *Zea mays* L. Seedlings roots and shoots exposed to different periods of flooding(during 4, 7and 10 days).The values represent the mean of three replicates ±SE

Analyses of soluble sugar in roots revealed that the amounts of soluble sugar has increased 1.5-2 fold as compared controls during the early stage of flooding, but by increasing flooding period duration this ratio has decreased and the amount of sugars gradually decreased and finally has reached to the levels similar of the controls (Fig. 5). Since consumption of soluble sugar under fermentative metabolism during flooding condition has been increased (Mohanty *et al*., 1993) and under hypoxia, starch accumulation in leaves has been attributed to a reduced rate of translocation of carbohydrates from leaves to roots (Barta, 1987) which apparently causes the carbohydrate demands to decrease (Hsu *et al*., 1999).

CONCLUSION

There are vast flooded areas in the world and plants respond differently to flooding stress. So it is important to understand, how plants especially major crops as *Zea mays* behave against low external O_2 concentration and adapts its growth and metabolism over the short- and long-term flooding stress. Moreover, we founded that the flooding stress causes some anatomical, morphological and biochemical changes in plants; development of aeranchyma and adventious roots are more recessive factors that increases hypoxic tolerance in *zea mays.*

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