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Biochemical &Morfological Responses Of Zea Mays To Drought Stress In Green House

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Drought is considered as one of the most important limiting factors for oil seed plant zea mays 732;growth and productivity in Iran. On the basis of root and air organ dry weight as affected by drought stress exerted by polyethylene glygohle 6000 (PEG 6000) , out of cultivars of 732 seeds corn.
Their reponses to low water potential with respect to changes in activity of the antioxident enzyme ascorbate peroxides, guagohtl peroxides , and its translocations .
To the shoots and production of osmoprotection proline and soluble sugars were analyzed and compared .
In low water potential increase ascorbate peroxidase activity , constitutively the enzyme activity was significantly higher in the corn732 A significant increase in root proline content ;
Although the total soluble sugars in both cultivars tended to increase under low water potential The amounts accumulated were mostly comparable in both constitutively higher ascorbate peroxidase activity along with the higher rates of proline accumulation and total soluble sugars are taken as part of the mechanisms which confer drought tolerance to the zea mays cultivars.

Key words – Drought stress – zea mays – ascorbate peroxide, proline , soluble sugars .

Introduction

Water deficit stress due to drought , salinity or extremes in temperature is the main limiting factors for plant growth and productivity resulting in large economic losses in many regions of the words . [1]
Plants respond to water stress through anumber of biochemical , physiological and developmental changes [2,3] These in clude decrease in photosynthetic carbon assimilation due to stomatal closure and losses in chloroplast activity [4] , down – regulated of PSII activity [5] increase in O2 consumption by mehler-peroxidase reaction and photorespiration [6,7] increase in leaf ABA concentration and induction of many stress – responsive genes by ABA [3,8] , modification of the lipid matrix of the plasma membrain and the changes in the physical organization of the membrane [9] , and accumulation of osmoprotectants such as sugar; alcohols; amino acids and organic acids [10] .
The type of responses observed depend on several factors such as severity and duration of the stress and plant genotype .
Oilseed zea mays (corn) is an important agricultural crop grown for its edible oil The meal that remains after oil extraction has value as a source of protein for the livee- stock feed industry [11].
In Iran , The production of the oil seed plant is limited by soil salinity and drought .
Therefore , development of varieties or selections with increased salinity and drought tolerance is important for growing this economical plant in regions where wateris limited understanding the biochemistry and physiology of sunflower adaptation to Drought stress will be help develop varieties with enhanced stress tolerance The observations That water deficiency causes the chloroplasts of wheat to reduce oxygen to superoxide[12]
Promoted Mc kersie et al .[9] to hypothesis that plants over expressing antioxidants might have Improved water deficit tolerance. In the present work , zea mays 732 cultivar were examined for their tolerance to drought strees exerted by polyethylene glycohl 6000(PEG 6000). on the basis of leaft shoot organ and root dry weight the relatively tolerant and sensitive cultivavs were selected and were further analyzed for their responses to water stress by : (1)
Compairing the activity of antioxidant enzyme ascorbate peroxidase ; 2)

The extent of osmoprotectants production .

2- MATERIALS AND METHODS

a) plant growth and stress application:

seeds of zea mays732 were supplied by the Agricultural Research Experimental station in fars province , Iran. seeds were surface sterilized in 10% (v/v) sodium hypochlorite for 10 minutes , followed by several washes with distilled water .

seeds were germinated on vermiculate in the dark at 25^0c and uniform ten – days old seedlings were transferred to half strength Hogland nutrient solution in plastic containers .

after 7-10 days , water stress was induced by adding PEG 6000 to the nutrient solutions;The Michel [13] equation was used to calculate the solutions water potential . to minimized osmotic stock , PEG was raised to the desired levels by a stepwise addition of 13 gr PEG at 8 hr interval.

The secdlings were maintained in a growth room with 16: 8 hr Light – dark regime , and illuminance of 9×10^3 Lux at $25 \pm 3^\circ c$. seedlings were harvested after 9 days and separated into root and shoots where needed , plant materials were dried for at Least 24 hrs in an oven set at $70^\circ c$.

b) Enzyme extraction and assay

to determine ascorbate peroxidase activity , 250 mg of mature fresh Leaf tissues were sampled from 13 day old plants grown in different external water potentials Tissues were homogenized using an ice cold mortar and pestle in 5 ml of cold grinding buffer consisting of 50 mM potassium phosphate buffer PH=7,2.2 Mm ascorbate and 1.mM EDTA. The homogenate was centrifuged at 10.000 g for 5 minutes and supernatant was assouyed for ascorbate peroxidase activity as described by chen and Asada [14] . The reaction mixture in a final volume of 1 ml consisted of 100 mM potassium phosphate buffer PH= 7.0 , 0.22 Mm ascorbate 0.3Mm H_2O_2 and enzyme extract .

The masure in A290 due to oxidation of the ascorbic acid by H_2O_2 was monitored using a shimadzo double – beam spectrophotometer model uv- 160A.

To determine Guaicol peroxidase activity 250 mg of mature fresh Leaf tissues were sampled from 13 days old plants grown in different external water potential . Tissue were homogenized using an ice cold mortar and pestle in 5 ml of cold grinding buffer consisting of 100 Mm potassium phosphate PH= 6.00

0.2 M guaicol 0.03 M H_2O_2

The homogenate was centrifuged at 4.000 g for 10 minutes and supernatant was assayed for guaicol peroxidase activity as described by Mac- Adam . [ ]

The reaction mixture in a final volume of 1mL consisted of 100 mM potassium phosphate buffer PH= 7.0 50 wl guaicol wl , 50 ml H_2O_2 and 50ml enzyme extract .

The masure in A436 due to oxidation of the guaicol peroxidase activity as decribed by H_2O_2 was monitored using a shimadzo double – beam spectrophotometer model UV- 160 A.

C) Determination of praline and total soluble sugars

For praline determination , 0.3 g of fresh root tissue was taken from plants grown as described above and praline content was measured according to Bates et al .[1,5]

Total soluble sugar was extracted from 0.3 g of mature fresh Leaves with 70% (V/V) boiling ethanol treated with chloroform to separate cloyophyll , and the amount of soluble sugar was measured as described by prakash and prathapsenan [16]

3-Results And Discussion

a)screening cultivars for drought tolerance water stress stimulated root growth in most cultivars when each cultivar was grown in solutions with different water potentials (0,-0.3and -0.5Mpa) ,the average root dry weight of -5 Mpa cultivar was the most increased but The average root dry weignt of 0 Mpa cultivar was decreased .

water stress reduced shoot growth in all treatment.

Respectively and their responses to water stress with respect to ascorbate peroxidase , guacol peroxidase activity , proline and soluble sugar accumulation were studied .

table 1. Effect of Drought strees on root and leaf dry weight of 3 treatment of Zea mays
each value is percent dry weight relative to control

Treatment	0	-3	-5	-7
Root	113 Ba	131 Ba	135 Ba	147 Bb
Leaf	50 Bb	44 Bb	44 Ab	35 Cb

b) Effect of water stress on ascorbate peroxidase
Althought in both tolerant and sensitire cultivars , the decrease in external water potential affected leaf ascorbate peroxidase activity significantly (fig ..1) , indeed the enzyme activity in -5Mpa cultivar was constitutively higher in all treatment .different types of environmental stresses exert at least part of their effects by causing oxidative damage .
conesquently the plant antioxidant defense systems and their roles in protecting plants against stresses have attracted considerable interest SOD –transgenic alfa lfa plants tended to show less injury form water deficit stress [9] Thus these results seem to support the hypothesis that tolerance in oxidative stress plays an important role in adapting plants to adverse enviromental condition [9.17]
In leaves of *Allium schoenoprasumL.* , drought increased the specific activity of ascorbate peroxidase by 29% [18]. similar findings were reported by tanaka et al . [19]. for spinach leaves and by Castillo [20] for sedum album leaves under mild water stress .
In salt tolerant plant species higher constitutive Levels and a significant increase in ascorbate peroxidase (21)

Since water deficit stress restricts co2 assimilation , the formation of a superoxide radical by the transfer of electrons from PSI to molecular oxygen (Mehler reaction) is enhanced [7] A superoxide radical is rapidly scavenged by superoxide dismutases thus producing H2O2 Increased superoxide dismutases and ascorbate peroxidase activities or higher constitutive level of these enzymes promote the removal of reactive oxygen species (ROS) , thus conferring higher drought resistance to plants
c) proline and total soluble sugars as effected by water stress drought stress up to -0.3 and -0.5 Mpa resulted in a significant increase in leaf proline content of both cultivars but the increase was more pronounced in -5Mpa rather than 0 cultivar [ ]
a common response to water deficit in plants is the accumulation of osmo protectants such as proline and glycin betaine [17,24]
Genotype variation in proline content have been reported in sorghum and wheat under moisture stress [25].

leaf proline content of 3 Treatment of PEG in Zea mays

PEG Mpa	Leaf proline (Mm/g fresh weight)
0.0	68.46 Aa
-0.3	70.47 Aa
-0.5	73.00Ab
-0.7	70.00 Aa

Total soluble sugars tends to increase , which is probably due to mobilization of reserved polysaccharides , by decreasing external water potential in 3 treatment [fig]
At -3.0 Mpa total soluble sugar was higher in -7Mpa
High resistance to several stresses has also been related to an increase in polysynthesis

Increased polyoltransport , both in phloem and xylem occurs frequently as a result of salt and drought stresses [10]

It is becoming clear that plants have developed several pathways to produce and accumulate arrange of osmoprotectants as defens mechanisms against salinity and drought stresses .

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