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The reaction of *Carthamus tinctoriuos* L. Metabolism to the Drought Stress and the Resistance Rate in the Plant

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ABSTRACT

In this research the effect of drought stress to the proline density, soluble sugars and the activities of the Guaiacol Peroxidase and Catalase has been considered. The plants have been grown in the Perlite environment including Hoagland and different concentrations of Polietilen Glycol 6000. The plants have been kept in the growing room with a 16 hours brightness and 8 hours darkness light diet in 2± 25 centigrade degree. The rate of Proline, soluble sugars and the activities of Guaiacol Peroxidase and Catalase antioxidant enzymes has been determined by the spectrophotometer. As the Proline drought stress increased the sample group was faced with a meaningful increase in soluble sugars rate which the increase rate in the resistant plants was more than that of the sensitive plants in all cases.

The consideration of Guaiacol Peroxidase and Catalase enzymes activities rate in the sensitive and resistant plants roots and stem indicated that these enzymes in resistant plants were more active than the sensitive ones. As the environment stresses increased the activity of reactive oxygen species, this also decreased the plants growing process as well. We can therefore suggest the increasing of Antioxidant enzymes activity and also the soluble smolites as the mechanisms of resistance to the drought stress for the resistant plants.

Key words: *Carthamus tinctorious*, Drought stress, resistance rate.

INTRODUCTION

The botany properties of *Carthamus tinctoriuos*

Carthamus tinctoriuos L. is from Compositae or Asteraceae family and it is in Carthamus type which has 19 species. *Carthamus tinctoriuos* L. was known in India, Egypt and Iran and has been used there since the old time.

There is plenty of wild *Carthamus tinctoriuos* L. in addition to the agricultural species in Iran. We can claim that Iran genetically is one of the richest countries in possessing *Carthamus tinctoriuos*.

This plant has alternative leaves and an inflorescence with 5 free stamens. Its lower two-carpel ovary includes an egg cell.

Carthamus tinctoriuos L. is a xerophytic plant which enables it to live in tropics areas. This plant is used as a calmative medicine to relieve the tension. And it also maintains the heart and liver and helps the blood circulation works well.

The oil seeds include plenty of fats inside the cotyledon or endosperm. The composition of fat and the fatty acids of oily products is not fixed therefore can be influenced by genotype and environment. Normally the *Carthamus tinctoriuos* L. seed's oil includes 6-8% Palmitic Acid, 2-3% Stearic Acid, 16-20% Oleic Acid and 71-75% Linoleic Acid. Among the oily products, the *Carthamus tinctoriuos* L. oil has the least rate of saturated fatty acids.

In 2000 Chen and his colleagues reported the decreasing of Phosphatidylcholine caused by the drought conditions, so that the water stress stimulated the movement of choline to the biosyntheses of Osmotichom Glycine – Thebaine of the osmotic regulators released by the corn leaves influenced by the stress[7-31].

Cell membranes are the main goal in the environment stresses. Lipids are the main components of membrane. Changes in lipids components caused by drought stress help the membrane keep its integrity and maintain the churning process[8-44].

The resistance of *Carthamus tinctoriuos* L. against drought stress depends on its structure changes. These changes in plants structure let them control their membranes cyanite not only by a suitable rearrangement in Glycerolipids but also by regulating the composition of the unsaturated fatty acid[9-50].

Increasing the rate of drought might limit the biochemical processes of stabilization of photosynthesis CO_2. [10-71]

The extra light energy controlled by Chlorophene has not been used therefore it destroyed PSII by over rehabilitating of the photosynthesis electron transfer chain and increasing the production of reactive oxygen species (ROS)[11-13].

The omission of ROS has been occurred by the lipophilic antioxidant molecules such as Tocopherol, Carotene B and the hydrophilic antioxidants like Ascorbic Acid, Glutathione and also the antioxidant enzymes such as Superoxide dismutase, Glutathione Peroxidase and Catalase.

The enzymes which involve in detoxification of ROS work more actively in drought stress. And this increasing of the enzymes might be related to the increasing of plants resistance against drought stress[12-22].

Ascorbate Peroxidase (APX) and Guaiacol Peroxidase (GPX) play a role in detoxifying H_2O_2. Ascorbate Peroxidase is highly interested in omitting H2o2. [13-83].

The significant enzyme of Catalase catalyzes the Conversion of H2o2 to hydrogen peroxide. The plant catalases are located only in Peroxisome and Glyoxysome. As the catalase activity decreases the transgenic plants become more sensitive against drought stress[14-143].

If the defense system of antioxidant stops working, this may cause some oxidative damage in the cell organization such as proteins, DNA and membrane oils[13-15].

MATERIAL AND METHODS

The *Carthamus tinctoriuos* L. cultivar seed named PI, Mec, LRV5151, IL111, 279, 2811 and Padideh (phenomenon) were provided from the research center of Dashtestan, Boushehr Province, Iran.

The mentioned seeds have been soaked in water for 4-5 hours then they were disinfected by javel water for 10 minutes and were washed with normal water for several times, finally the seeds were washed by distilled water.

The solutions with osmotic potential (including PEG) were provided with zero control and -0.5, -12, -5 and -10 bar[16-82].

Providing Hoagland $\frac{1}{2}$ trition solution: Hoagland nutrition solution with $\frac{1}{2}$ power has been used as basic nutrition solution.

The examination of drought stress effect on peroline amino acid rate in the leaves and root of the sensitive and resistant cultivars of *Carthamus tinctoriuos* L.

We used bates method in order to measuring peroline[17].

The required materials and solutions

In this examination we used Ninhydrin, Acetic Acid, 6 molar Phosphoric Acid, 3% Sulfur Salicylic Acid, Peroline Amino Acid and Toluene.

Providing Ninhydrin solution

We added 30 milliliters of acetic acid and 20 milliliters of 6 molar phosphoric acid to 1.25 gram

of Ninhydrin, and after these materials were completely dissolved, the solution was maintained in the refrigerator.

After providing the samples the light absorption rate in 520 nanometers wave-length was measured by spectrophotometer.

Considering the effect of dehydration stress on the soluble sugars rate in the leaves of the sensitive and resistant cultivars of *Carthamus tinctoriuos* L.

The soluble sugars have been extracted and measured in accordance with Nelson method.

The required materials and solutions

In this examination we used 70 % of Atanol solution, distilled water, Chloroform, Alkaline Copper Reagent and Arsenomolybdute solution.

After providing the samples the light absorption rate in 520 nanometers wave-length was measured by spectrophotometer and the rate of soluble sugars in sample plant and the pioneer crops has been determined by the use of standard curve.

We used concentrations of 0, 20, 40, 60, 80, 100 glucose micrograms in milliliter in order to provide the standard curve.

We used non-glucose test tube to set up the machine.

The examination of dehydration stress effect on Guaiacol Peroxidase enzyme extracted from the leaves and roots of the sensitive and resistant cultivars of *Carthamus tinctoriuos* L.

The required materials and solutions

In this examination we used 0.2 molar of Guaiacol, 3% H_2O_2 molar solution, 0.1 molar buffer phosphate with PH = 6 and KCl solid chloride potassium.

The 15-day seedlings were used to extract the enzyme. Then the light absorption rate changes have been recorded and compared with each other in per 15 seconds for a period of 5 minutes through the spectrophotometer in the wave-length of 436 nanometers in order to measuring the activities of Guaiacol Peroxidase enzyme in the sample and pioneer plants.

The examination of drought stress effect on Catalase enzyme extracted from the leaves and roots of the sensitive and resistant cultivars of *Carthamus tinctoriuos* L.

The required materials and solutions

200 millimolar of buffer phosphate potassium with PH = 7, Triton-X-100 with the concentration of 0.1 %, 30 millimolar of Hydrogen Peroxidase solution.

The 15-day seedlings were used to extract the enzyme

Decreasing in the rate of light absorption in the wave-length of 240 nanometers in per 5 seconds for a period of 1.5 minute was measured by the use of spectrophotometer.

The changes in light absorption rates have been drawn according to the time and the lines slope was determined.

RESULTS

The effect of osmotic potentials caused by PEG 6000 on the proline rate of sensitive and resistant cultivars of *Carthamus tinctoriuos* L. roots against dehydration stress.

The rate of proline in -5 bar osmotic potential

In comparison with the sample group the rate of proline has increased meaningfully in resistant cultivar (IL 111).

But this increasing in sensitive cultivar (LRV 5151) is not meaningful.

In -10 bar osmotic potential, the proline rate has highly increased in both cultivars and there is a meaningful deference between other pioneer crops.

The effect of osmotic potential caused by PEG 6000 on the proline rate extracted from airolic organ of sensitive and resistant cultivars of *Carthamus tinctoriuos* L. against dehydration stress.

In different potentials the rate of proline in both cultivars leaves (IL111 and LRV5151) is significantly more than the rate of proline in the cultivars roots.

The rate of proline in -5 bar osmotic potential in both cultivars is several times more than that of the sample group.

In -10 bar osmotic potential in comparison with the pioneer leaves the rate of proline in sensitive and resistant leaves has increased infinitely, which this increasing rate was observed in resistant cultivar more.

The effect of osmotic potentials caused by PEG 6000 on the rate of soluble sugars in sensitive and resistant cultivars of *Carthamus tinctoriuos* L. against drought stress.

As the osmotic potential increased (specially in negative rate) the rate of soluble sugars increased too. In -10 bar osmotic potential the rate of increasing is more than the pioneer crops. (image 4-4)

In -10 bar osmotic potential the rate of soluble sugars in resistant plant has been changed from 0.75 milligrams into 10.50 milligrams per 1 gram of wet weight in sample plants.

In sample plants and -10 bar osmotic potential the rate of soluble sugars in sensitive plants respectively are 0.5 and 7 milligrams per 1 gram of wet weight.

It is necessary to mention that as the osmotic potential was negated more the rate of soluble sugars of the leaves increased as well.

The effect of osmotic potential caused by PEG 6000 on Guaiacol Peroxidase enzyme extracted from the sensitive and resistant cultivars roots of *Carthamus tinctoriuos* L. against drought stress.

The effect of different osmotic potentials on Guaiacol Peroxidase enzyme of the roots indicated that Guaiacol Peroxidase enzyme in resistant plant is more active that the sensitive one. And in both cultivars there was no significant statistical difference between other pioneer crops.

The effect of osmotic potential caused by PEG 6000 on Guaiacol Peroxidase enzyme extracted from the sensitive and resistant cultivars leaves of *Carthamus tinctoriuos* L. against drought stress.

In resistant plants IL111, only in -10 bar osmotic potential the activity of the enzyme significantly differed from other pioneer crops, so that the enzyme activity in -10 bar osmotic potential has become 8.5 times the pioneer crop.

In comparing the two cultivars any significant statistical difference was not observed between other pioneer crops.

The effect of osmotic potentials caused by PEG 6000 on catalase enzyme activity extracted from the sensitive and resistant cultivars roots of *Carthamus tinctoriuos* L. against dehydration stress.

In resistant cultivar IL111 the rate of catalase stracted from the root has been significantly increased in -5 and -10 bar osmotic potential especially in -10 bar osmotic potential.

Therefore by contrasting the activity of catalase enzyme in resistant and sensitive cultivars, we came to this conclusion that the enzyme activity in resistant cultivar is always more than sensitive one.

The effect of osmotic potentials caused by PEG 6000 on catalase enzyme activity extracted from the sensitive and resistant cultivars leaves of *Carthamus tinctoriuos* L. against drought stress.

In -5 bar osmotic potential the enzyme activity has increased to 81 percent and in -10 bar osmotic potential the enzyme activity was more than 2.5 time the sample plants.

Although the catalase enzyme activity also reduced in -1 bar osmotic potential in the sensitive cultivar LRV 5151, like the resistant cultivar the enzyme activity increased in -5 and -10 bar osmotic potentials.

By comparing the enzyme activity in both

cultivars leaves we observed that the catalase enzyme in resistant cultivar was more active than in the sensitive one among all of the pioneer crops.

DISCUSSION

The results obtained from considering the effect of dehydration stress caused by PEG 6000 in the food solution of Hoagland and Perlite on *Carthamus tinctoriuos* L.,

Indicates that

1. As the osmotic potential (specially in negative rate) increases the Proline extracted from roots and leaves increases too.
2. Increasing of the osmotic potential will increase the rate of soluble sugars in the leaves.
3. As the rate of osmotic potential increases the activity of Guaiacol Peroxidase enzyme and catalase enzyme which are two types of antioxidant enzymes increases too.

If we come to this conclusion that the increasing of proline rate or the soluble sugars rate on the cell surface is regarded as a factor to adapting or resisting against the dehydration, adding some external proline to the plants that are less resistant than the resistant plants and in comparison with the resistant plants they cannot supply proline, can we guarantee their growing conditions like the resistant plants? And can we protect them from the harmful effects of dehydration?

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