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The Effects of Water Stress on the Growth of Corn and the Activity Dynamics of NADP–Dependent Isocitrate Dehydrogenase Enzyme in the Leaf and Root T issues

Naila Aliyeva, Ziyeddin Mamedov
Department Biophisic and Biochemystry, Faculty of Biology, Baku State University, Azerbaijan
Corresponding Author: naila.aliyeva.bk.2018@gmail.com

Abstract

Developmental dynamics of the NADP-dependent isocitrate dehydrogenase enzyme has been studied (NADP-ICDH, EC 1.1.1.42) as the reducing potential of the cell for the development of corn sprouts through water stress conditions (water shortage and over water stress) which has a great role in forming NADPH pool. It was determined that the activity of NADP-ICDH enzyme increases both in root and leaf tissues significantly for the effects of water stress and over water stress induces the activity of enzyme more sharply than water shortage stress. The activity degree of enzyme in all three variants manifests itself higher in leaf tissues than root tissues. It seems NADP is required for the corn seedlings in order to eliminate stress complications and NADP-ICDH enzyme is directly involved in its synthesis.

Keywords: corn seedlings, water stress, over water, water shortage

Introduction

Water stress is manifested as water shortage and over water. This case includes main abiotic stress-based group that makes a serious ecological problem for the developmental productivity of plants. Water shortage is more widespread in nature than over water. One of the main reasons of water shortage is global heating. Plenty of water is used due to a 2 C^0 drop in temperature during a year. On the other hand water demand increases for the grows of population number year –after- year/ water demand speeds up year after the year due mainly to population growth increase. Climatologists predict that the number of the people under water stress will increase

twice for water demand in 2050 compared to 2010's. Nowadays water usage in agriculture accounts 70% of general water demand on the Earth. As well as 60 % increase in food demand will increase water usage too till 2050 year (Boretti et al., 2019). Salinization of suitable lands on the Earth causes the reduction of water storage capacity of the soil that results disruption of the plant's water supply. Sub-optimal water supply is disrupted for the physiology and biochemical changes in a plant during the water shortage (Jain et al., 2019). The main response to the water shortage of plants is aimed to protect it by reducing transpiration. Further, the synthesis of the abscisic acid increases and this causes the closing of mouthpieces (Munemasa et al., 2013). Although this and such other mechanisms can protect a plant preventing water loss on the first stage of the stress, generally metabolism in plants gets weak due to the weakening of photosynthesis in the next growing level of a plant and water shortage causes the destruction of a plant.

On the other side, water evaporation causes over rain under high temperature in tropical and subtropical countries and this leads to over water stress in plants as a result of overflow of water balance (Ashraf., 2012). In addition, over water stress is both a stress factor as the result of regular rain and overflow or over-irrigation, and hence this effect can be more fatal than water shortage for plants. Thus, diffusion gets weak at the result of low oxygen in the soil during the overflow and toxic substances are formed in roots under anaerobic conditions. Leaf withering and/or root rot, in a word, underdeveloped plants appear in this case.

So, both forms of water stress make/cause obstacles in the growing of plants as well as corn plants, especially in the level of sprout (Song et al., 2019). Plants activate the defense mechanism in order to be protected from the influence of stress. The main based component of this is to make the enzymes NADPH pool which forms reducing potential of cells. NADPH is considered as one of the widespread components in nature that possesses high energy, forms basis of reducing potential of cells, plays a great role in biosynthesis process, and has a central place in metabolism (Corpas et al., 2014). NADP-dependent isocitrate dehydrogenase (NADP-ICDH, EC 1.1.1.42) enzyme that makes NADPH in living organism whose activity has been determined in corn sprouts, playing an important role in the defense of plants from stress. This enzyme splits isocitrate into α-ketoglutarate and CO_2 gas. The reaction is observed by forming NADPH. There are both mitochondrial and cytoplasmic forms of the enzyme in eukaryotic cells. The enzyme acts on non-photosynthetic cells, or on the darkest stage of metabolism of photosynthetic cells (Leterrier et al., 2012).

It should be noted that ferredoxin -NADP-reductase is considered the main enzyme making NADPH, forming its pool on the light stage of photosynthetic cells. NADP metabolites which realize reducing coenzyme function and enzymes which

make out its synthesis in plants have a great role in their defense and adaptation to extreme conditions (Ying et al., 2008). Taking all into consideration, activity dynamics of NADP-ICDH enzyme are related to the development of corn sprouts in extreme conditions which have been created by water stress and are aimed to be studied in this research work/ and fall into the core of this study.

Materials and methods

Object of experiments and germination of seeds

Experiments have been done on the *Pride (Gurur)* genotype of corn (*Zea mays* L.) that was brought from the Institute of Genetic Resources of the Azerbaijan National Academy of Sciences. Corn seeds have been disinfected in a 3% solution of hydrogen peroxide for five minutes in order to get rid of pathogens. Then they were soaked in Petri Dish by washing in distilled water during a day and the next day they were replaced in a plant growing device with phytotron principle (MLR Plant Growth Chamber) by planting in vegetation containers under 24-27 C^0 temperature. After the observation of sprouts on the soil, the controlled variants were/variant was irrigated by distilled water once on the third day of the experiment. The second variant, created for the aim of over water, was irrigated twice a day,but the third variant firstly was irrigated once every two days in order to create drought, after six days it was irrigated once every three days, but the irrigation process was stopped through the next days. The biometric indicator of the plant and the calculation of the activity of NADP-dependet ICDH enzyme were registered once every four days.

Making of enzyme preparation

100 mM Tris-HCl buffer (pH 9) that contains 5mM $MgCl_2$, 2 mM EDTA, 14 mM merchaptoethanol, 5% polyvinylpirolidone (PVP), 1% polyethylene glycol (PEG) is used as extract solution. Tissue: extract solution was taken in a ratio of 1 gr:4 ml. The obtained homogenate was firstly centrifuged 1000 gr, then 9000 gr, after it was centrifuged fast. The supernatant part of the solution was taken and it was used as enzyme preparation.

The indication of the activity of NADP-ICDH enzyme

The activity was calculated at 340 nm wavelength according to NADP reduction speed under 24 C^0 temperature on the spectrophotometer of MRS (Israel) by the spectrophotometric method. 50 mM Tris-HCl (pH 8,2) which contains 2,5 mM $MgCl_2$, 2 mM-D, L- isocitrate and 0,5 mM NADP and its solution were used as a reaction environment. Enzyme preparation was added to this solution and the

reaction started by adding NADP to the reaction environment and the measurement repeated four or five times.

Results and discussion

It is known that there are serious abiotic stress factors that reduce the growing and productivity of plants, so water stress has got an important place among such kinds of cases. Although the drought, as the result of water shortage, is widespread, water stress manifesting as over water problem causes the incomplete growing and destruction of plants too. If we take into account that ecological factors have laxative effects on all the creatures/ living creatures in nature, including plants, year-by-year, it is necessary to eliminate abiotic stress for future and water stress too. Meanwhile, studying how to make defense mechanism in order to create stress resistant sorts is one of the important issues. If we consider that environmental change is regular, then this task stays actual. Bringing this fact into considering this fact, we have tried to study the influence of the defense system of corn plants in the condition of water stress which is one of the factors appearing with the influence of environmental changes.

Biometric indication of the growing of the root and trunk sprouts of the corn, grown under the artificial water condition, has been introduced in the following table as a control variant.

Table 1. Growing indications of corn sprouts under the condition of control and water stress (by sm).

Variants	4 days'	8 days'	12 days'
Control	leaf 6.2±03 root 3.1±02	leaf 9.4±04 root 4.5±03	leaf 13.6±04 root 5.9±03
Water shortage stress	leaf 4.6±01 root 2.5±01	leaf 6.1±01 root 3.2±01	leaf 8.1±02 root 4.1±01
Over water stress	leaf 3.9±01 root 2.1±01	leaf 5.0±01 root 2.9±01	leaf 6.3±01 root 3.3±01

As seen in the table, the leaves of the sprouts under the control variant have been increased 2,1 times compared to the fourth day for 12 days, but the growing of the root system has been increased 1,9 times. Both the growing of the leaves and the root system have been slow down significantly under the influence of the both forms of water stress. So, similar figures for the plant's leaves constitute 1,7 times, but this figure is 1,6 times for the root system. It means that informal water supply of the plant in the both forms of water stress has disrupted the functions in the tissues of

the leaf and root system by effecting the metobolism of the plant, and as a result, it caused to reduce the biometric indications of them.

The next table describes the dynamics of the activity of NADP-dependent isocytrate dehydrogenase enzyme on the homogenate, prepared from the root and leaf tissues of corn plants in the condition of control and water stress.

Variants	First	4 days'	8 days'	12 days'
Control leaf				
root	86.1 ± 3.2	97.6 ± 3.1	109.7 ±3.5	122.5 ± 4.1
	78.5 ± 2.3	89.0 ± 3.8	99.7 ± 3.4	110.8 ± 3.8
Water				
shortage leaf	-	103.5 ± 4.1	124.3 ± 3.8	139.5 ± 4.0
root	-	99,6 ± 4.3	111.3 ± 4.2	124.1 ± 4.1
Over water				
leaf root	-	114.7 ± 2.9	137.6 ± 3.3	147.8 ± 4.1
	-	101,2 ± 4.1	117,1 ± 3.6	128.2 ± 3.7

Table 2. The description of the activity dynamics of NADP_ICDH enzyme in the tissues of the leaf and root systems in the condition of control and water stress related to the growth of corn sprouts.

As it can be seen/As shown in the table, the activity of NADP-ICDH enzyme, related to the corn sprouts, is increasing significantly both in the cells of the leaf and root systems. Both forms of water stress cause the next induction of the activity of the enzyme. We can say that/We can conclude that according to the table, over water stress activates the enzyme more strongly than water shortage stress in both tissue cells. The activity degree of the enzyme in the leaf tissues shows itself more than root tissues in three variants. It seems that NADPH is required for corn sprouts to eliminate the complications of stress conditions and NADP-ICDH enzyme participates in its synthesis directly.

Conclusion

The activity dynamics of NADP-dependent isocitrate-dehydrogenase enzyme in the root and leaf tissues during the growth period of corn plants in the condition of control, over water and water shortage and the biometric indicators of the plant have been observed. It is determined that the activity dynamics of NADP-dependent isocitrate- dehydrogenase enzyme is followed by increasing sequence with the influence of water stress both in the root and leaf cells. The activity of enzyme in the leaf tissues is higher than root tissues. The influence of stress is negative on the

growth of the biometric indicators of corn plants, as well. The growing of the plant's leaves is noticeably slower than the roots in both forms of water stress compared to the control condition.

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