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Effect of Drought and Phytohormones on Nucleic Acids Content in Drought Tolerant and Susceptible Barley Varieties

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ABSTRACT

Molecular studies have shown that several genes with various functions are induced by drought stresses and that various transcription factors are involved in the regulation of stress-inducible genes. So, this research was carried out on 12 barley (*H. nutans* Schubl.) varieties, in order to investigate the effect of drought stress and phytohormones on genome structure and its changes in drought tolerant and susceptible barely varieties. In drought tolerant and susceptible barely varieties, stabile DNA, labile DNA, residual DNA and RNA were extracted. The results showed that the rate of Stabile DNA, Labile DNA and RNA was increased in tolerant variety under drought stress condition. Whereas, in susceptible variety; the rate of those was decreased under drought stress condition. Gibberellic acid plus Kinetin complex induced remarkable increasing on DNA and RNA content in drought susceptible variety, whereas it had little effect on those in drought tolerant variety.

Key words: Barley, nucleic acids, drought, PEG, Gibberellic acid, kinetin

INTRODUCTION

Yield of crops is determined by many processes originating in the genetic information of the nucleus, chloroplast and other organelles of the higher plant cell. The information controls the synthesis of structural proteins and enzymes which synthesize other cell components. Biotic and a-biotic stresses cause changes in normal physiological functions of all plants, including economically important cereals as well. Drought stress, which is a natural stress factor, has the highest percentage with 26% part when the usable areas on the earth are classified in view of stress factors (Shiri *et al.*, 2009). Barley is a crop of major economic importance and also a model species for genetics and physiology (Koornneef *et al.*, 1997). Barley is grown under varying agro-climatic situations. It is an important crop grown worldwide for food, feed and forage. Due to hardy nature, superior nutritional and medicinal importance barely is being considered as highly needed crop of present era. It has superior nutritional qualities due to presence of beta-glucan (an anticholesteral substance), acetylcholine (a substance which nourishes our nervous system and recover memory loss), easy digestibility (due to low gluten content) and high lysine, thiamin and riboflavin (Chand *et al.*, 2008). The barley is an important cereal crop in Azerbaijan. Drought negatively effects barely growth and development in the world and Azerbaijan and causes a sharp decrease of barely productivity.

Plant adaptation to unfavorable environmental conditions depend on normal synthesis of protein. DNA structure and its function have the key role in normal protein synthesis. DNA-strand consists of different parts, which they have different structure and functions, Such as labile DNA and stabile DNA. Labile DNA is active part of DNA and located in euchromatin. This part of DNA has genetics code to synthesize protein but stabile DNA is inactive part of DNA and located in heterochromatin which it closely connected with histons (Shiri *et al.*, 2009).

Tolerant of crops to stress factors depends on functional state of genome under stress condition. Genome activity and genetic ordering mechanism is connected with the structural condition of the DNA (Shiri *et al.*, 2009).

Although, many studies have been done on plants under water deficit, but there were a few studies on the genetic structure of plants (Aliyev *et al.*, 2000; Aliyev and Abbasov, 2004; Shiri *et al.*, 2009). Aliyev and Abbasov (2004) investigated genome structure under drought and salinity stress. They concluded that the rate of stabile DNA, labile DNA and RNA was increased in tolerant variety under stress condition but in susceptible variety; the rate of those was decreased under stress condition. The similar results were obtained by Shiri *et al.* (2009) in maize hybrids under drought stress conditions.

Phytohormones are among organic molecules which markedly influence many metabolic reactions of plants when present in only minor quantities. In all the crops, phytohormones mainly play important roles in transmission of information within a plant. Thus as basis for cultivation and breeding of crops, the elucidation of physiological effects of phytohormones is essential (Kojima, 2001).

Aliyev *et al.* (2000) and Pustoraytova (1990) stated that GA₃ has an important role in the adaptation and resistance of plants to water deficit.

The aim of this study is to investigate structural and functional changes caused by stress factors in barley genome chromatin, as well as, to gain some knowledge about the mechanism of this effect.

MATERIALS AND METHODS

This study was carried out on 12 barley (*H. nutans* Schubl.) varieties named as Huseyn-1, Arpa-47, Arpa-84, Nutans-303, Nutans-67/91, Arpa-43, Jalilabad-19, Arpa-77, Nutans-86-35/18, Arpa-59, N-Selection and Nutans-80-34/14. This experiment was done at Genetic Resources Institute of Azerbaijan National Academy of Sciences in 2008.

For classifying barley varieties to two groups (drought tolerant and drought susceptible groups), seed germination ability was used under artificial water stress condition in laboratory condition. Artificial water stress was created with sucrose solution at 10 atm. Then, one variety was chosen from each group (drought tolerant and drought susceptible groups). Then, for studying genomic structure, the stabile DNA, labile DNA, residual DNA and RNA were extracted from selected variety. The seeds were kept in distilled water (dH₂O) overnight and germinated in plastic pots (20 cm in diameter) containing air-dried greenhouse soil under natural light at room temperature. Pots containing seedlings were divided into two equal groups after 5 days. The first was irrigated with 100 mL of distilled H₂O and the second with 100 mL of PEG (60 g PEG/L d H₂O: 05 atm.) twice a day at 12 h intervals. The PEG was used to create drought stress. At the end of 48 h, for extraction of nucleic acids, 2 g fresh leaves of seedlings were collected from each group. The rest of the seedlings irrigated with dH₂O were kept as a control group and the rest of the seedlings irrigated with PEG were divided into two groups. The first group was irrigated with 100 mL of dH₂O and the second group with 100 mL of gibberellic acid (GA₃) plus Kinetin (50+50 mg L⁻¹) twice

a day at 12 h intervals over four days. Leaf samples from these groups were randomly collected after 96 h the onset of stress (72 h after the relief of stress). To reduce sample variation, all measurements were performed on the second and third leaves of seedlings and samples were collected in four replicates.

Total cell DNA and RNA were isolated by Konarev and Tyuterev (1970) and Alekseev (1973) methods. Nuclear nucleic acids were extracted by the gradual fractionation method (Konarev and Tyuterev, 1970). The gradual application of varying ionic power forms the basis of this method, allowing the separation of labile chromatin DNA (free DNA), stable chromatin DNA (DNA bound loosely to histones) and residual chromatin DNA (DNA bound strongly to histones). Stable chromatin, labile chromatin, residual chromatin and RNA content was determined by ultraviolet absorbency difference at 270 and 290 nm wave-length as mg mL^{-1} according to Konarev and Tyuterev (1970).

RESULTS AND DISCUSSION

The studied variety divided to three groups based on germination ability under artificial water deficit condition according to cluster analysis by ward method (Fig. 1). Discriminate function analysis confirmed the classifying correctness in cluster analysis (data not shown). According to cluster analysis based on germination ability of barely varieties under artificial drought stress and control condition, Huseyn-1, Arpa-84, Arpa-83 and Arpa-47 had the highest rate of germination under drought stress condition and considered as drought tolerant varieties. Whereas, N-Selection, Arpa-59 and Nutans-80-34/14 had the least rate of germination under drought stress condition and considered as drought susceptible varieties and the rest of varieties had moderate rate of germination under drought stress condition (Fig. 1).

The result of on nucleic acids content showed that in drought tolerant variety, Huseyn-1, the amount of labile DNA, which is the active portion, increased 21.9% under drought condition after 24 h in comparison to control but in drought susceptible variety, N-Selection, the amount of labile DNA (free DNA) decreased 23.81% under drought condition in comparison to control. In case of RNA content, in drought tolerant variety, Huseyn-1, the amount of that increased 16.5% under drought condition in comparison to control but in drought susceptible variety, N-Selection, the amount of RNA decreased 8.13% under drought condition in comparison to control (Fig. 2a, b). The rate of RNA and DNA in a cell shows the transcription amount of DNA (Smith and Grierson, 1982). Aliyev and Abbasov (2004) investigated genome structure under drought and salinity stress. They concluded that the rate of stabile DNA, labile DNA and RNA was increased in tolerant variety under stress condition but in susceptible variety; the rate of those was decreased under stress condition. The similar results were obtained by Shiri *et al.* (2009) in maize hybrids under drought stress conditions. Transformation from stabile DNA to labile DNA is possible within cell during morphogenetic processes which it could be affected by genetic and environmental stress (Aliyev *et al.*, 2000). Therefore, the ability of tolerant varieties in suffering stress is because of their potential to increase the rate of labile DNA and RNA content, thereby increasing protein synthesis.

In drought tolerant variety, Huseyn-1, the amount of stable DNA (DNA bound loosely to histones), residual DNA and total DNA increased 13.8, 29 and 19.9%, respectively under drought condition in comparison to control but in drought susceptible variety, N-Selection, the amount of those decreased 36.4, 34 and 30.45%, respectively under drought condition in comparison to control (Fig. 2a, b).

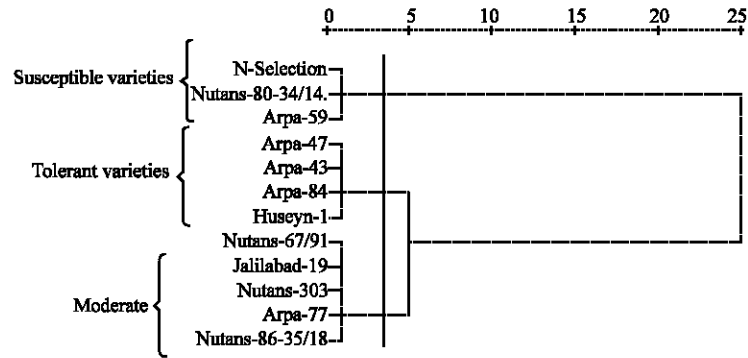


Fig. 1: Dendrogram for 12 barley varieties based on germination ability under drought stress created saccharose solution with 10 atmosphere pressure

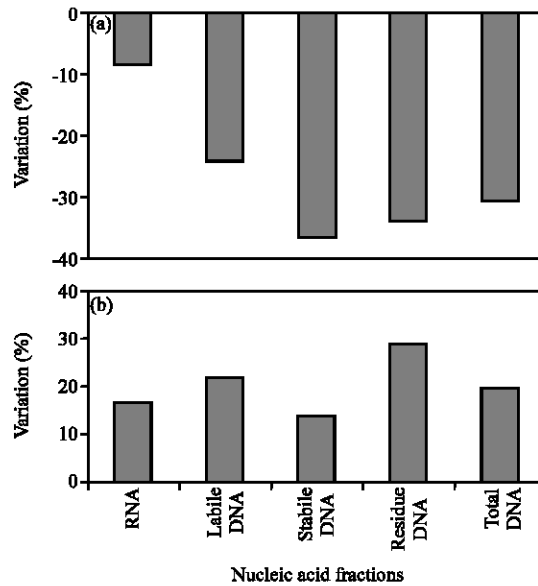


Fig. 2: Variation (%) of RNA and DNA fractions in drought stress condition (created with PEG) in comparison to control (a) in drought susceptible variety (N-Selection) and (b) in drought tolerant variety (Huseyn-1)

In the seedlings applied Gib+Kin phytohormones complex following PEG application, the DNA content increased in comparison to the water applied following PEG seedlings. The increasing in the amount of labile, stable DNA , residual DNA and RNA was 38.5, 46.9 , 50.9 and 30.1%, respectively in drought tolerant variety and was 21.1, 20.9 , 34.8 and 37.9%, respectively in drought susceptible variety (Fig. 3a, b). The similar results were obtained by Aliyev *et al.* (2000). Thus, it is suggested that Gib+Kin complex activates the synthesis of nucleic acids and strengthens the resistance of plants to water deficit. In the study carried out by Shang *et al.* (1994), it was determined that the foliar application of plant growth regulator increases the resistance of wheat seedlings to the water deficit. This increase can be explained by the increase in the active portion of chromatin DNA. These observations are interpreted as phytohormones molecules (Gib+Kin complex) bind to histones chemically and remove the repressing effect of histones on genes.

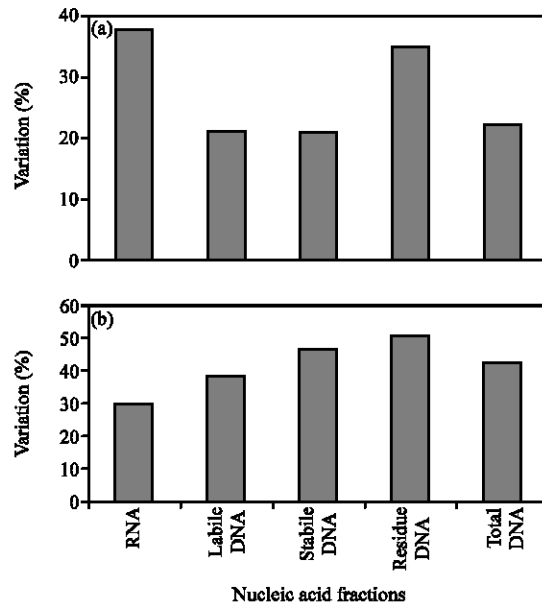


Fig. 3: Variation (%) of RNA and DNA fractions in application of Gib+Kin phytohormones complex following PEG in comparison to irrigation with dH₂O following PEG (a) in drought susceptible variety (N-Selection) and (b) in drought tolerant variety (Huseyn-1)

After application of phytohormones (Gib+Kin) complex following drought stress, genome activation increased both in tolerant and susceptible varieties, resulting in the increase of RNA and DNA fractions. Therefore, Gib+Kin complex application alleviates drought stress effect by activating the synthesis of nucleic acids and allows the maintenance of normal functions of plant cells.

Results obtained from this investigation showed that changes occurred in genome structure and functioning can be accepted as stress resistance indices of plants and can be used in explanation of plant resistance and molecular-genetic mechanisms of phytohormone influence.

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