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Effect of Drought Stress on Nucleic Acids Content Changes in Maize Varieties

[1]M. Shiri and [2]R.T. Aliyev
[1]Agricultural and Natural Resources Research Center of Ardebil, Iran
[2]Genetic Resources Institute of Azerbaijan National Academy of Sciences, Baku, Azerbaijan

Abstract: The DNA and RNA analysis can be useful to the selection of genotypes containing drought tolerance and good properties in the breeding program. So, this research was carried out on 38 maize (*Zea mays* L.) varieties to investigate the effect of drought stress on genome structure and its changes in drought tolerant, semi-tolerant and susceptible maize verities. For classifying maize varieties to three groups (drought tolerant, drought semi-tolerant and susceptible groups), seed germination ability was used under artificial water stress condition and in laboratory condition. Artificial water stress was created with sucrose solution at 10 atm. Then, one variety was chosen from each group (drought tolerant, semi-tolerant and susceptible groups). In drought tolerant, semi-tolerant and susceptible maize verities, stabile DNA, labile DNA, residual DNA and RNA were extracted. The results showed that the rate of labile DNA and RNA was increased 22.9 and 48.1%, respectively in tolerant variety under drought stress condition. Whereas in susceptible variety, the rate of those was decreased 35.9 and 17.2% under drought stress condition, respectively.

Key words: Maize, nucleic acids, drought stress, breeding programe, variety, Iran

INTRODUCTION

Maize (*Zea mays* L.) is an important crop to which a large extent of cultivable land has been allocated. As regard of under cultivation area, the amount of production and yield per area, maize ranks 3rd in the world after wheat and rice. Using genetic resources and increasing the tolerance of genotypes against biotic and a-biotic environmental stresses will increase the production (Shiri, 2011).

Improving the drought tolerance had been studied by many researchers. Previous achievements were not such notable due to the complexity of drought tolerance improvement, insufficient genetic variation for drought tolerance, complex interaction of drought with environmental factors and lack of effective selection techniques (Shiri *et al*., 2010a, b). Currently with advances in germplasm improvement, evaluation techniques for genetic heredity and using molecular markers, the improvement of drought tolerance and other a-biotic stresses has been facilitated (Shiri, 2011).

Drought tolerance liking other environmental stresses in higher plants is a complex genetic and physiologic trait. Most plant processes which are critical in drought tolerance have little inheritance and show a continual variation and are also under the influence of environmental conditions (Shiri, 2011). 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 (Abbasov and Aliyev, 2008).

Plant adaptation to unfavorable environmental conditions depends 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 stable DNA is inactive part of DNA and located in heterochromatin which it closely connected with histons (Abbasov and Aliyev, 2008; Rahimli *et al*., 2011). Tolerance 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 (Abbasov and Aliyev, 2008).

Corresponding Author: M. Shiri, Agricultural and Natural Resources Research Center, Oltan, Pars Abad-e-Moghan, Ardebil Province, I.R. Iran

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; Abbasov and Aliyev, 2008; Rahimli *et al*., 2011). Aliyev and Abbasov (2004) and Abbasov and Aliyev (2008) 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 Rahimli *et al*. (2011) in barely varieties under drought stress conditions.

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

MATERIALS AND METHODS

This study was carried out on 38 maize (Table 1) local varieties in 2008. For classifying maize varieties to three groups (drought tolerant, semi-tolerant and susceptible groups), seed germination ability was used under artificial water stress condition and in laboratory condition. Artificial water stress was created with sucrose solution at 10 atm. Then one variety was chosen from each group (drought tolerant, semi-tolerant and 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 (dH_2O) 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.

Table 1: Name of studied varieties

Cod	Varieties	Cod	Varieties
1	Naxchevan (A11(I))	20	Qusar (Kf-58 (II))
2	Naxchevan (A11(II))	21	Lankaran (Kf-59 (I))
3	Astara (Kf-4)	22	Lankaran (Kf-59 (II))
4	Kepez (Kf-9)	23	Jalilabad (Kf-60 (I))
5	S.Zaqatala 27 (Kf-10)	24	Lankaran (Kf-60 (II))
6	Shirvan (Kf-23)	25	Shamkir (Kf-61 (I))
7	S.Mirvari AZE (Kf-31)	26	Shamkir (Kf-61 (II))
8	S.Mondoaim (Kf-37)	27	Beylaqan (Kf-62 (I))
9	Zaqatala-68 (Kf-38)	28	Beylaqan (Kf-62 (II))
10	S.Kx-65ALM (Kf-33)	29	Zaqatalk (247)
11	Absheron (Kf-52)	30	Aq Zaqatalk248
12	Ykruyan (Kf-54)	31	Goy Zaqatalk250
13	Berde (Kf-55(I))	32	Zaqatala420
14	Berde (Kf-55(II))	33	Zaqatala380
15	Qusar (Kf-56(I))	34	Zaqatala486
16	Qusar (Kf-5(II))	35	Zaqatala NP-38
17	Qusar (Kf-57(I))	36	ZaqatalaNP-42
18	Qusar (Kf-57(II))	37	ZaqatalaF-522TOPH722
19	Qusar (Kf-58(I))	38	Zaqatalk shakil-1

The first was irrigated with 100 mL of distilled H_2O and the second with 100 mL of PEG (60 g PEG/L dH_2O: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. 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. 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 according to Konarev and Tyuterev (1970).

RESULTS AND DISCUSSION

Because of un-uniformity of soil environment and difficult of environment factors control in field conditions, laboratory investigations, especially seed germination ability has special importance in the evaluation of drought tolerance. The studied variety divided to three groups based on germination ability under artificial water deficit condition (Fig. 1).

According to germination ability of maize varieties under artificial drought stress and control condition, varieties No. 12, 35, 8, 11, 36, 18, 38 and 1 had the highest rate of germination under drought stress condition and considered as drought tolerant varieties. Whereas, varieties No. 6, 16, 30, 2, 24, 29, 32, 19, 37, 13, 22, 25 and 9 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 (Table 2 and Fig. 1). In order to detail changes in chromatin structure caused by drought stress among studied varieties maize, No. 38, 7 and 37 varieties were chosen as drought tolerant, semi-tolerant and susceptible varieties, respectively.

The obtained result of on nucleic acids content showed that in drought tolerant variety, No. 38 variety, the amount of labile DNA which is the active portion, increased 22.9% under drought condition in comparison to control but in drought susceptible variety, No. 37 variety, the amount of labile DNA (free DNA) decreased 35.9% under drought condition in comparison

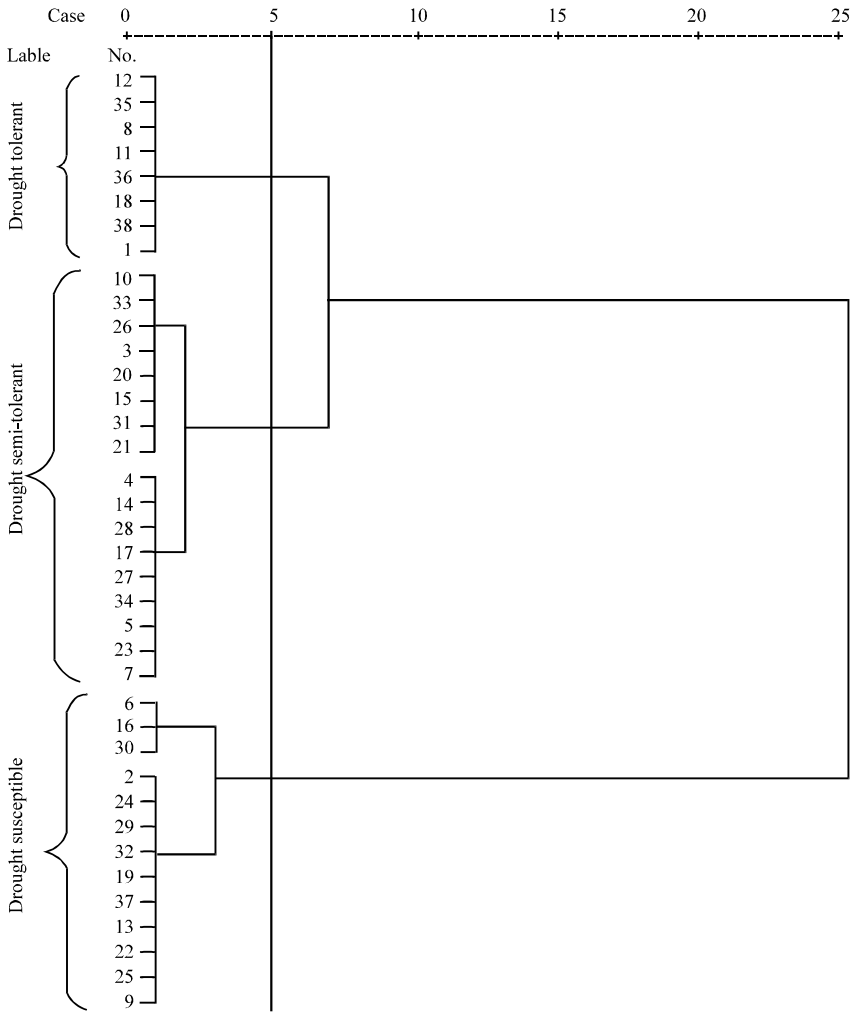

Fig. 1: Dendrogram for 38 maize varieties based on germination ability under drought stress created saccharose solution with 10 atm

Table 2: Seed germination ability under control and drought stress created saccharose solution with 10 atm and its changes by drought stress

Varieties	Control (No.)	Drought stress (No.)	Variation (%)
1	48	42.00	87.50
2	47	16.00	34.04
3	50	24.33	48.67
4	44	31.33	71.21
5	46	28.67	62.32
6	45	9.00	20.00
7	45	28.67	63.70
8	49	39.67	80.96
9	48	21.67	45.14
10	48	27.67	57.64
11	50	39.33	78.67
12	33	27.33	82.83
13	48	20.00	41.67
14	50	35.67	71.33
15	49	26.00	53.06
16	46	8.00	17.39
17	50	36.67	73.33
18	46	40.00	86.96

Table 2: Continue

Varieties	Control (No.)	Drought stress (No.)	Variation (%)
19	44	17.00	38.64
20	42	20.67	49.21
21	46	25.33	55.07
22	50	20.67	41.33
23	50	31.33	62.67
24	46	15.33	33.33
25	50	21.33	42.67
26	50	29.33	58.67
27	46	30.67	66.67
28	50	35.33	70.67
29	44	12.67	28.79
30	42	6.00	14.29
31	46	24.00	52.17
32	39	11.67	29.91
33	50	28.67	57.33
34	24	16.00	66.67
35	33	27.33	82.83
36	21	16.67	79.37
37	48	18.33	38.19
38	40	34.67	86.67

Table 3: The effect of drought (PEG) stress on RNA and DNA fractions (mg/100 g of fresh weight) in selected maize varieties

Drought tolerant degree	Treatments (%)	RNA	DNA fractions		
			Labile	Stabile	Residual
Tolerant variety	Control	267.72±16.79	32.19±2.27	13.12±0.31	1.42±0.31
	Drought stress (induced with PEG)	396.52±16.64	39.54±1.6	19.89±2	1.06±0
	Variation	48.1	22.9	51.2	-25
Semi-tolerant variety	Control	257.6±21.08	28.82±0.77	18.89±2.27	0.709±0.08
	Drought stress (induced with PEG)	348.6±27.75	34.87±2.11	20.75±1.2	1.419±0.15
	Variation	35.3	20.7	9.6	100
Susceptible variety	Control	169.28±16.86	14.54±3.62	11.17±1.84	0.665±0.13
	Drought stress (induced with PEG)	140.21±7.47	9.31±0.92	7.98±0.53	0.8±0.2
	Variation	-17.2	-35.9	-28.6	26.7

to control. The increasing of labile DNA was 20.7% in drought semi-tolerant variety (No. 7 variety). In case of RNA content, in drought tolerant variety, No. 38 variety, the amount of that increased 48.1% under drought condition in comparison to control but in drought susceptible variety, No. 37 variety, the amount of RNA decreased 17.2% under drought condition in comparison to control. In drought semi-tolerant variety (No. 7 variety) increased the amount of labile DNA was 35.3% (Table 3). The rate of RNA and DNA in a cell shows the transcription amount of DNA (Smith and Grierson, 1982). Aliyev and Abbasov (2004) and Abbasov and Aliyev (2008) 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 Rahimli *et al.* (2011) in barely variety 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, No. 38 variety, the amount of stable DNA (DNA bound loosely to histones), increased 51.2% and the amount of residual DNA, decreased 25% under drought condition in comparison to control but in drought semi- tolerant variety, No. 7 variety, the amount of those increased 9.6 and 100% under drought condition in comparison to control, respectively. Whereas in drought tolerant variety, the amount of stable DNA, decreased 35.9% and the amount of residual DNA, increased 26.7% under drought condition in comparison to control (Table 3).

CONCLUSION

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 drought stress influence.

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