THE MODIFICATION OF PROTEIN METABOLISM OF SUNFLOWER PLANTS UNDER SALINE STRESS

Maria Duca and Ana Bârsan*)

ABSTRACT

The action of salinity with chlorides and sulfates on hydro- and saline-extractible protein content, fractionated according to the principle of their solubility in different solvents has been studied at three genotypes of *Helianthus annuus* L. (SW-501 cms, RW-637 Rf and F_1 hybrid) under controlled conditions of plant cultivation. It has been established that the prime reaction of plants to the stressing action is accomplished by the modification of the protein metabolism – by the increase of the hydro-soluble proteins and the decrease of the saline soluble proteins. The quantitative modifications of the protein complex as the response to the stress factors depend on the substance nature and on the investigated genotype. It has been found that under normal conditions, F_1 hybrid is characterized by the more balanced content of the hydro- and saline-extractable fractions. The lower protein content in plants supposed to stress at the all stages of development proves that the protein metabolism of plants grown under conditions of salinity is deranged.

Key words:, protein, salinity, stress, sunflower

INTRODUCTION

Soil salinity represents a major problem for agriculture in the whole world. According to the data from professional literature, at the present moment about two millions km^2 of soil used for growing crops have a high content of salts (Flowers et al., 1986).

Different types of salinity create specific conditions for the functional activity of the plant organism and have a negative impact on the growth and development of plants, causing serious damages to agricultural production. In this context the study of the plant organism responses to the action of salinity is of a present interest. Knowledge about the biochemical reactions of the whole organism to extreme factors would permit the elaboration of the optimal conditions for growth and development that do not disturb the homeostatic state and in some cases help to prevent the stress (Andreeva et al., 1992).

Plants grown on saline soils withstand a multi-factorial influence.

It is especially mentioned the toxic and osmotic action of salts on the plant organism (Franko and Melo, 2000; Udovenko and Goncearova, 1982). It has been ascertained that plants withstand in a larger measure a toxic action when salted with chloride and an osmotic action when salted with sulphate. The last one gets a significant importance only under the action of the high salt concentrations. That is why the toxicity level of salts is a decisive factor for the existence of plants in the soils with a low concentration of salts where the plant water supply is not disturbed.

Plant osmoregulation and resistance under conditions of saline stress are achieved due to the existence of various mechanisms of adaptation to salinity.

These mechanisms are of differrent nature and are connected with the activity of the plant organism at the different levels of structural organization. The mechanisms of halophyte plant adaptation consist in the selective capacity of plants to extract, to accumulate, to distribute and to eliminate ions (Neamþu, 1983; Tarhon, 1993), while the resistance level of glicophyte plants to the saline substrate is different for each species (Makeinse and Leshen, 1994).

As a response to saline stress both halophyte and glicophyte plants change their biochemical and physiological activity by accumulation of substances with a protective role such as proline, betaine, poliamines, easy soluble sugars and proteins. Easy soluble organic substances take part in the process of cell metabolism regulation and in the biochemical reactions of plants to the stress (Junghietu, 1991; Kuzneþov et al., 1990, Franko and Melo, 2000; Neamþu, 1983).

The study of adaptive-protective reactions based on the modification of plant metabolism

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under controlled conditions has a particular role in elucidating the plant resistance to salinity. One of the cellular processes with a high sensitivity to salinity is the process of protein synthesis (Blehman, 1987; Levit, 1972;).

At the present moment, the results of many scientific researches highlight notable quantitative modifications in the polypeptide complex of the cells under the stress of long and short duration. In the majority of cases, researchers study the quantitative modifications of summary proteins and certify a decrease of protein compounds under the influence of various stress factors. The authors explain the decrease of protein content in the tissues by the blockage of biosynthesis processes as well as by the activation of hydrolytic reactions that under the stress conditions prevail over the processes of biosynthesis. But, sometimes it is found out an increase of protein content in the vegetal tissues especially under the action of high concentration of salts (Udovenko and Goncearova, 1982). It is supposed that this phenomenon is determined by the concentration of proteins in the cells because the processes of growth are repressed much strongly than the processes of protein synthesis under salinity conditions.

In Udovenko's opinion (1979) the above-mentioned contradictions are caused by the research of the action of factors of various nature and power of manifestation on the different groups of plants at the different stages of ontogenetic development of plant organism. Thus the necessity to estimate the modifications of certain parameters that serve as indicators of plant resistance and the comparison of the values of these parameters at least in two regimes - optimal and extreme ones at the same level of ontogenetic development have a major importance in studying the stress resistance of plants.

According to data from professional literature (Kobanov and Cervina, 1973; Vovciuc et al., 1991), an important parameter that characterizes the reaction of plants to the stress factors and their resistance to the stress may be the content of different fractions of proteins. The composition and the ratio of protein fractions represent an important indicator of protein complex of the vegetal organism. It shows the metabolic activity and the resistance of the plants to the external factors.

That is why our purpose was to study the protein metabolism in sunflower plants under the action of salt of various nature. During the investigations lot of biochemical analyses have been carried out in order to determine the fractional content of protein under the action of high salt concentration at the different stages of ontogenetic development of plants.

MATERIALS AND METHODS

The following sunflower lines served as research material: SW-501 cms, RW-637 Rf and F_1 hybrid with these lines. Sunflower seeds were planted in vegetation dishes with sand. The moisture of the substrate was 70%. As a nutritive medium served the nutritive medium with macroelements recommended in the profesional sources (Boldor et al., 1983). The stressing action on the plants has been produced with NaCl (0.5%), Na_2SO_4 (0.7%). The dishes were placed into the growing room at the temperature of 23-25°C taking into account the photoperiodic reaction of the studied crop.

The protein content was determined by means of Bradford method (Bradford, 1976) and it was evaluated in milligrams of protein per gram of dry material (mg/g d.m.).

The received results were processed statistically by means of standard methods (Dospehov, 1972).

RESULTS AND DISCUSSION

The unfavourable influence of soil salinity on plants is determined by a number of factors such as: concentration, the chemical nature of salt, environmental conditions, species and the physiologic state of investigated plants (Kusnerenko, 1992).

As a result of many researches it was ascertained that at the different ontogenesis stages the plants show a different sensibility to salinity, their sensibility being much higher during the juvenile period (Udovenko et al., 1971; Udovenko, 1979). Numerous researches show that under high salinity conditions the processes of growth and synthesis are repressed and due to this fact plant development under the influence of salinity is delayed (Stroganov et al., 1970; Stroganov, 1973). But under the same extreme conditions different crops, wild species and even varieties, show a different reaction – the fact that speaks about different levels of resistance.

The results of our previous investigations proved the negative action of salts on the growth of sunflower plants. The plants grown in media with a high content of salts had a smaller germinative faculty and energy, a lower height and a less accumulated fresh biomass in comparison with the control plants (Duca et al., 2001).

The experiments carried out by us showed that the saline substrate, with high concentrations of chloride and sulphate retarded the growth rate of the plants. Thus the plants from the control variant emerged on the third day after sowing while the plants from saline variants emerged with a delay of four days. This fact shows that during different periods of growth, plants are at different stages of development. That is why the quantitative determination of protein fractions has been made: on the 10th day after sowing when both the plants from the control variants as well as those from the salted ones were at the stage of cotyledon development; on the 14th day after sowing when the plants from the control variant had the first pair of leaves well developed and the plants from the salted variants remained behind the development and were at the stage of cotyledon development; on the 18th day after sowing when the plants from the stressed variants had two well developed leaves. The last determination has been made in order to compare the modifications caused by saline stress in the vegetal organism with the control, when the plants were at the same stages of development.

The data from table 1 show that at the stage of cotyledon development the plants from the control lot had a different content of proteins. The highest protein content was ascertained at the hybrids from the first generation while the line SW-501 cms was characterized by a lower content of proteins in comparison with the hybrid as well as with RW-637 Rf line.

At the same time it was established that at this stage of development the main compounds of protein complex are represented by the albumins and globulins.

The percentage of the albumins at SW-501 cms line was 56, at RW-637 Rf, 58 and at F_1 hybrid, 49. The percentage of globulins was 32; 39 and accordingly 47 while the glutenin percentage constituted about 10 of the total protein content. That is why further we have studied the influence of salinity on the compounds with a higher rate in tissues.

The data from table 2 show that at the initial stage of stress action the salinity of the substrate with sodium chloride and with sodium sulphate caused the increase of water-soluble

Table 1. The content of protein fractions in sunflower plants grown under optimal conditions of cultivation, at the stage of cotyledon development (mg/g d.m.)

Genotypes	Albumins		Globulins		Glutelins	
	mg/g d.m.	%	mg/g d.m.	%	mg/g d.m.	%
SW–501 cms	102.7±2.9	56	72.1±0.4	32	8.4±0.5	12
RW–637 Rf	118.0±2.2	58	66.1±1.2	39	18.4±2.5	3
F_1	135.0±1.4	49	130.1±0.7	47	9.6±0.7	4

Table 2. The modification of protein content under the action of stress factors at the stage of cotyledon development (mg/g d.m.)

Parameters	Genotypes	Control	NaCl (0.5%)	Na_2SO_4 (0.7%)
Albumins	SW-501 cms	102.7±2.9	128.5±1.5*	159.5±2.8*
	RW-637 Rf	118.0±2.2	164.8±1.0*	176.9±0.7*
	F_1	135.0±1.4	143.3±2.1	171.4±2.0*
Globulins	SW-501 cms	72.1±0.4	35.1±0.6*	52.5±0.5*
	RW-637 Rf	66.1±1.2	45.0±0.5*	61.6±1.4
	F_1	130.1±0.7	33.9±0.6*	62.3±0.6*

* - Significant for P<0.05

protein fractions (albumins) at the all studied genotypes.

A much obvious increase of albumin content was noticed at the paternal lines. Thus at SW-501 cms line sodium chloride increased the albumin content with 25% while at RW-637 Rf line it increased with 39% the content of water – soluble proteins. Sodium sulphate increased with about 50% the content of albumins at both sunflower lines. At the hybrid from the first generation it was ascertained an insignificant increase just with 6% when salted with NaCl and an increase with 27% when salted with Na_2SO_4. In Udovenko's opinion (1979, 1982), the stress reactivity of the plants may be characterized by the variations degree of the parameters that determine the resistance of the organisms. At the resistant plants these parameters modify less while at the sensitive plants they undergo more profound changes. Thus the lower variation of albumin content certified by us testifies a higher stability of heterozygote forms under the action of unfavourable factors while the albumin content increase may be characterized as the primary reaction of plants to the stress, albumins having the role of increasing plant resistance to unfavourable factors, because the presence of easy soluble proteins in the protoplasm contributes to osmoregulation (Junghietu, 1991; Niu et al., 1997).

In contrast to albumins the content of which increases under the action of saline stress, the content of salt soluble fraction of proteins (globulins) undergoes a considerable decrease. A much pronounced decrease of 74% under the action of NaCl and 52% under the action of Na_2SO_4 was attested at the heterozygote form (Table 2, Figure 1). Also, a much pronounced decrease of this indicator was ascertained at SW-501 cms line in comparison with RW-637 Rf line.

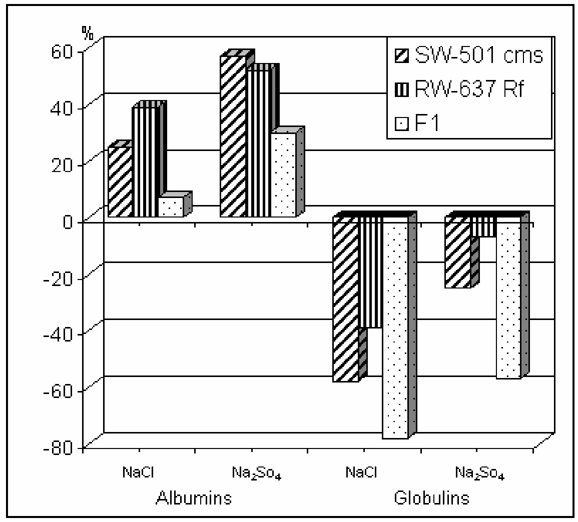

Figure 1. The effect (%) of stress action on the protein content at the stage of cotyledon development

At the next stages of development salinity of the substrate lead to the decrease of albumin and globulin content at the studied forms. As we have mentioned before, the results of numerous researches also attest the decrease of protein content under the action of different stress factors.

The analyses of the results obtained at the stage of development of two leaves of control plants and the stage of cotyledon development of the plants exposed to stress attest the decrease of albumins and globulins content at all studied forms (Table 3, Figure 2). This fact denotes the intensification of degradation processes under conditions of saline stress. Thus under the action of salts in concentrations that cause states of stress in the vegetal

Table 3. The modification of protein content under the action of stress factors at the stage of cotyledon development (mg/g d.m.)

Parameters	Genotype	Control	NaCl (0.5%)	Na_2SO_4 (0.7%)
Albumins	SW-501 cms	160.4±4.9	127.2±1.2*	145.4±2.3
	RW-637 Rf	186.4±5.5	138.8±2.8*	164.1±3.3*
	F_1	165.7±5.1	100.4±1.4*	84.8±2.4*
Globulins	SW-501 cms	95.4±3.3	45.1±2.1*	62.5±1.9*
	RW-637 Rf	110.3±2.9	60.7±1.4*	68.0±2.7*
	F_1	77.4±2.0	33.0±0.8*	57.1±1.5*

* - Significant for $P<0.05$

of cotyledon development of two leaves of sunflower plants (mg/g d.m.)

Parameters	Genotypes	Control	NaCl (0.5%)	Na_2SO_4 (0.7%)
Albumins	SW-501 cms	160.4±4.9	142.9±3.3*	170.6±0.2
	RW-637 Rf	186.4±5.5	168.8±0.6*	177.7±1.3
	F_1	165.7±5.1	134.0±1.7*	162.8±0.7
Globulins	SW-501 cms	95.4±3.3	49.1±0.4*	34.3±0.2*
	RW-637 Rf	110.3±2.9	45.8±2.4*	35.2±0.4
	F_1	77.4±2.0	37.7±1.0*	57.1±0.7*

* - Significant for P<0.05

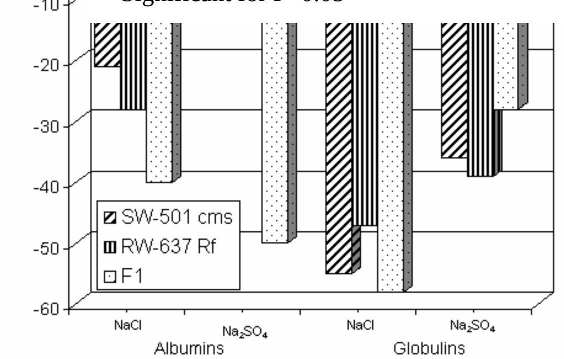

Figure 2. The effect (%) of stress action on the protein content of sunflower at the stage of development of two leaves at the control plants and at the stage of cotyledon development at plants grown under conditions of salinity

organism, the action that exceeds the plant reaction norm, prevails the processes of degradation.

At the next stage of research there were ascertained: significant differences of albumin and globulin content under the action of NaCl (0.5%), insignificant differences of albumin content and significant differences of globulin content under the action of Na_2SO_4 (0.7%) in comparison with the control. This proves that the harmful action of sodium chloride on plants is more pronounced in comparison with sulphate. At the same time it is noticed that when plants supposed to stress reached the stage of development of two leaves, the albumin content modifies less in comparison with the control, while the globulin content is about two times less (Table 4, Figure 3).

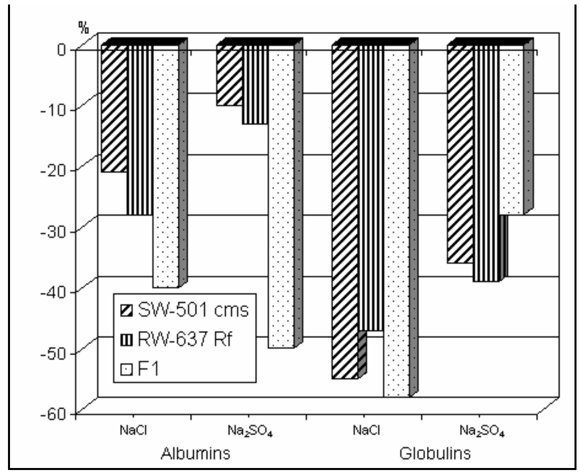

Figure 3. The effect (%) of stress action on the protein content of sunflower at the development stage of two leaves

The obtained results confirm the data from the professional literature according to which the salinity of the substrate deranges the protein metabolism by intensifying the catabolic processes. Some authors mention that the substrate salinity influences the protein me tabolism by intensifying the content of nucleic acids. Under conditions of salinity takes place especially the decrease of RNA content, while the DNA synthesis is repressed less due to its blockage by histonic protein and its passing into a stable non-active functional state. The decrease of the functional activity of nuclear DNA leads to the deceleration of metabolic reactions and to the repression of synthesis processes, first of all the protein biosynthesis. This on its turn slows down the biomass accumulation rate of plants and the size of organs.

CONCLUSIONS

At the initial stages of salinity action on plants the albumin content in tissues increases. The increase of water soluble protein content can be seen as the primary reaction of plants to the impact of salt.

The decrease of water soluble and salt soluble protein content at the next stages of action of high salt concentrations points to the prevalence of degradation processes under the action of the stress.

The lower protein content in the plants supposed to stress at all stages of development proves that the protein metabolism of plants grown under conditions of salinity is deranged.

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Table 1. The content of protein factions in sunflower plants grown under optimal conditions of cultivation, at the stage of cotyledon development (Mg/g d.m.)

Genotype	Albumins		Globulins		Glutelins	
	Mg/g d.m.)	%	Mg/g d.m.)	%	Mg/g d.m.)	%
SW-501 ASC	102.7±2.9	56	72.1±0.4	32	8.4±0.5	12
RW-637 Rf	118.0±2.2	58	66.1±1.2	39	18.4±2.5	3
F_1	135.0±1.4	49	130.1±0.7	47	9.6±0.7	4

Table 2. The modification of protein content under the action of stress factors at the stage of cotyledon development (mg/g d.m.)

Researched parameter	Genotype	Control	NaCl (0.5%)	Na_2SO_4 (0.7%)
Albumins	SW-501 ASC	102.7±2.9	128.5±1.5*	159.5±2.8*
	RW-637 Rf	118.0±2.2	164.8±1.0*	176.9±0.7*
	F_1	135.0±1.4	143.3±2.1	171.4±2.0*
Globulins	SW-501 ASC	72.1±0.4	35.1±0.6*	52.5±0.5*
	RW-637 Rf	66.1±1.2	45.0±0.5*	61.6±1.4
	F_1	130.1±0.7	33.9±0.6*	62.3±0.6*

* - Significant for P<0.05

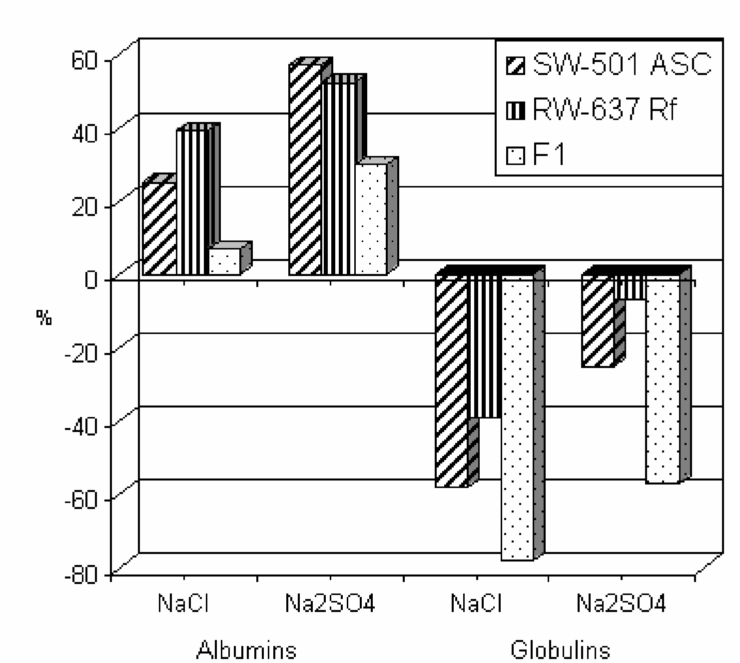

Figure 1. The effect (%) of stress action on the proteins content at the stage of cotyledon development

Table 3. The modification of protein content under the action of stress factors at the stage of cotyledon development (mg/g d.m.)

Researched parameter	Genotype	Control	NaCl (0.5%)	Na_2SO_4 (0.7%)
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	F_1	165.7±5.1	100.4±1.4*	84.8±2.4*
Globulins	SW-501 ASC	95.4±3.3	45.1±2.1*	62.5±1.9*
	RW-637 Rf	110.3±2.9	60.7±1.4*	68.0±2.7*
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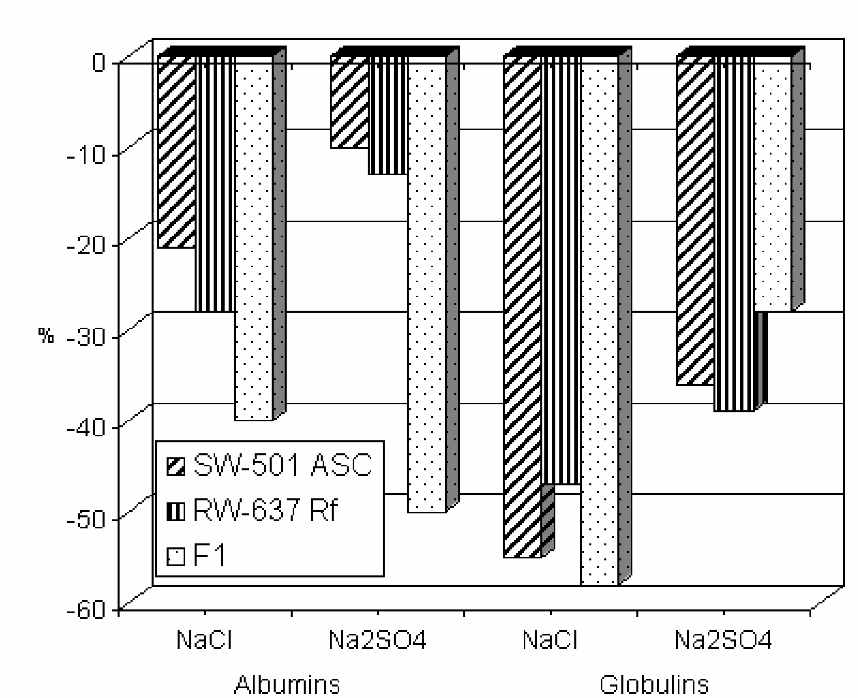

Figure 2. The effect (%) of stress action on the proteins content of sunflower at the stage of development of two leawes at the control plants and at the stage of cotyledon development at plants grown under conditions of salinity.

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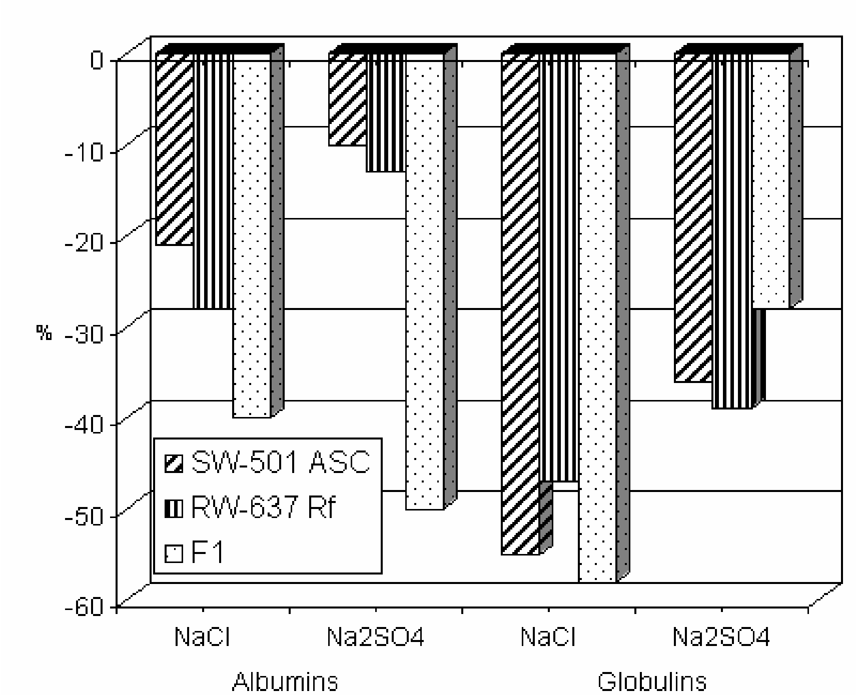

Figure 3. The effect (%) of stress action on the proteins content of sunflower at the stage of development of two leawes.