TWO CONSECUTIVE PHASES OF WATER STATUS MODIFICATION IN PLANTS LEAVES UNDER ACTION OF LOW TEMPERATURES

DOUĂ FAZE CONSECUTIVE DE MODIFICARE A STATUSULUI HIDRIC AL FRUNZELOR PLANTELOR SUB ACȚIUNEA TEMPERATURILOR SCĂZUTE

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Abstract. *Under controlled conditions, which imitate late-spring cold weather and frosts, there has been investigated water status of the grapes leaves on the initial stage of sprouts intensive growth. There have been found two phases of water status modification under low-temperature stress. Within the plants tolerance limits low temperature action leads to the increase of water contents in tissues due to the free water, situated in apoplast and vacuoles, at the water contents decrease in cytoplasm. At temperatures, which cause deteriorations, there takes place the total water decrease, as well of free water in symplast, conditioned probably by the cell membranes integrity deterioration. There are discussed the peculiarities of leaves water status in regard of their genotypic and phenotypic resistance to low-temperature stress.*

Rezumat. *În condiții modelate ce imitau temperaturile scăzute şi îngheţurile târzii de primăvară s-a studiat statusul hidric al frunzelor plantelor de viță de vie la începutul fazei de creştere intensivă a lăstarilor. Au fost evidențiate două faze consecutive în modificarea statusului hidric al frunzelor condiționată de stresul termic. În diapazonul toleranței plantelor acțiunea temperaturilor scăzute provoacă sporirea hidratării țesuturilor, apei libere localizată în apoplast şi vacuole pe fondalul diminuării hidratării citoplasmei; în diapazonul temperaturilor deterioratoare se atestă o diminuare evidentă a conținutului de apă totală, inclusiv în simplast, condiționată de deteriorarea sistemului membranar. Se discută particularitățile statusului hidric al frunzelor în funcție de rezistența genotipică şi fenotipică a plantelor la acțiunea temperaturilor scăzute.*

Low temperatures and late frosts during spring time represent an important and specific factor in regard of plants cryoresistance problem. In contrast to the mode of frost bear during winter time the surviving of perennial plants under conditions of low temperatures and late spring frosts could be ensured by their capacity to maintain stability of metabolic processes integration and coordination and structural-functional integrity. At the temperature modification plants react through modification of membrane structure and composition, flux of ions and metabolites between cellular compartments, enzymes functioning *(Daniel Come, 1992; Shizuo Ioshida, 1994; Milon F.George and M.J.Burke, 1977)*, cytoplasm acidity *(Sing J., Laroche A, 1988)*, structure and function of photosynthetic apparatus *(Denis P. Maxwell, Stefan G. Trick, Huner N.P.A., 1994)*, water exchange *(Dument Vincent, 1992)*. Traditionally water state in

cells is determined by estimation of its retention capacity using solutions with different water potential (the dehydration force). Extension of investigations upon mechanisms of water retention, the barrier function of cellular membranes, deterioration of water conductibility of the complexes plasmolemma – cell wall, appreciated as a non-specific effect caused by the action upon plants of different unfavorable factors *(Ionenco I.F., Goleadina L.V., 1999*), demonstrates that information about water state in plants supposed to the low temperatures action on the molecular level is insufficient. On the base of special literature analysis and own experimental results we assume that the main integrated deterioration factors conditioned by the action of low temperatures and late spring frosts could be divided in functional disorders of the organisms and derangements of cellular and subcellular structures as the result of water freezing; plants tolerance under present conditions is mainly determined by the thermal gradient of the main parts of metabolism, their capacity for reparation as well as by the limits of the supercooling temperature of water in tissues, which exceeding leads to freezing. Coming from these premises there have been studied the peculiarities of water state in grape plants under conditions of hypothermal stress.

MATERIALS AND METHODS

Investigations have been realized at the beginning of the sprouts' intensive growth phase of different varieties of vine according to their ecologico-geographical origin, grown in phytotron under conditions, which imitated low temperatures and late spring frosts. The temperature has been gradually decreased by 2-5 °C/hour, from 18-25 °C down to 2, 0, -2, -3, -5 °C after that the temperature was gradually brought to the initial gradation (fig.1). Via regulation of the plants' exposition duration at the different gradations of the low temperatures there have been imitated different situations of the hypothermal stress and after that the samples for analyses were collected.

For more detailed studying of the water compartmentalization modification aspects in leaves' tissues there has been carried out model experiment with cultivation of two soy varieties: Mida and Soier 3, plants of which were supposed to the action of low temperature of 2°C during 48 hours.

The components of water statuse have been detemined by the method of NMR-relaxation of water protons *(Carr H.Y., Purcell E.M., 1954; Harciuc O., Aksionov S., Chirilov A., Toma S., 2003).* Water fractions content has been calculated using the range of echo-signals registration of 30-80 msec and 3-8 msec..

RESULTS AND DISCUSSIONS

In the model experiment with soy plants there has been revealed that low temperatures action leads to the redistribution of water between different fractions (according to its localization). Within plants' tolerance low temperatures action conditions increase of water quantity in apoplast and vacuoles (free water) and simultaneous decrease of water in cytoplasm, although the total water content in tissues remains almost on the same level or even slightly higher. In plants supposed to the action of temperatures that provoke deteriorations the tissue dehydration takes place as well as the considerable decrease of free water (fig.2, tab.1). In our experiments there has been proved that temperature of 2^0C was tolerant for soy plants

cv. Soier (with higher resistance) and deteriorative for those of cv. Mida. Increase of free water content, as primary reaction of plants could have an indirect protective character, because additional water quantity containing more thermal energy needs a longer period of time for leaves temperature decreaseto the deterioration level. Infiltration of soy plants' leaves (non-resistant cv. Mida), supposed to the low temperatures action with paramagnetic solutions during long time leads to the considerable modifications of the NMR-relaxation of water protons curves: delay in echo-signals attenuation simultaneously with their amplitude decrease. The analysis of these modifications demonstrates that after action of cold in leaves' cells with reduced content of free water, mainly vacuolar, vacuoles maintain the reparation capacity but not at the same level with control plants that have not been stressed. The increase of protons relaxation time of water remained in symplast indicates on two possible effects: fusion of undamaged vacuoles in bigger vacuole formations or diminution of water permeability in complexes plasmalemma – cell wall.

Table 1

Modification of some water status parameters in soy plants leaves under low temperatures action

Temperature	Total water		Free water
	mg/mg m.u (NMR)	% m.v	mg/mg m.u (NMR)
cv. Mida			
	1,95	66,10	1,25
2 ^{o}C	1,32	56,90	0,68
cv. Soier 3			
15 ^{o}C	1,83	64,76	0,97
2 ^{o}C	1,92	65,75	1,07

The character of NMR-relaxation of water protons curves at cv. Soier 3 (resistant) soy plants leaves infiltrated with paramagnetic solution after action of cold reveals the increase of permeability of the complexes plasmalemma – cell wall for water; these plants demonstrate higher capacity to retain water in leaves' tissues that permits them not only to maintain water homeostasis but to increase their watering.

On the base of comparative analysis of these peculiarities of water protons NMR-relaxation curves as indexes of primary deterioration provoked by cold accompanied by the major loss of symplast water there could be assumed the fusion of vacuolar compartments during the reparation process by infiltration of water status in the leaves' tissues. Using the NMR-relaxation method in study of interrelation of water state and functional characteristics of membranes in roots of wheat plants *Şvaleova A.L. (2001)* has established that high permeability of cellular membranes towards water has positive role in maintenance of plants tolerance to the low temperatures action.

It is necessary to mention that increase in free water content at the low temperatures action is more pronounced during darkness period that has an adaptive character that has been demonstrated by us in experiments with vine plants supposed to the action of frost (fig.3). During night frosts (-2^{0}C), in special repeated ones repeated ones increases leaves' hydration but on the cellular level takes place the increase of vacuoles' dimensions and sharply decreases water quantity with extremely rapid relaxation of water protons echo spin. Under this experiment condition the

biologic result of the extended action of cold on the vine plants becomes apparent through deterioration of sprouts' tops at some plants that leads to the division of lot in phenotypes with higher and lower (more sensitive) resistance.

During the cold season there takes place the increase of dry substance per unit of foliar surface. At first this phenomenon occurs at sensitive phenotype achieving the maximal values after 22 hours of experiment at temperature of 0^0C, while at the resistant phenotype the maximum of compression occurs on 44th hour of the experiment at temperature of -2^0C.

After reestablishment of the initial temperature conditions the diminution of the leaf's density takes place, although at non-resistant phenotype to a lesser degree that indicates more significant deteriorations of the water status provoked by the low temperatures action. The further decrease of temperature till -5^0C, imitating of the heavy frosts, causes in both investigated varieties Muscat Iantarnyi and Cardinal, sharp loss of the essential free water (mainly vacuolar) quantity during a rather short period of time (fig.4). We assume that such dynamics of free water (mainly vacuolar) loss in leaves is provoked by the irreversible deterioration of the restrictive cells' membranes.

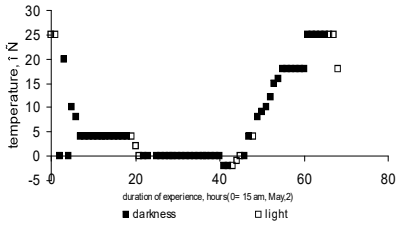

Fig 1 -Temperature conditions during an experiment

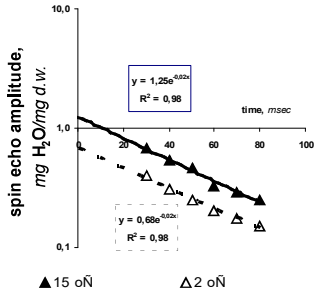

Fig.2 - Character of NMR-relaxation of water protons from soy plants' leaves supposed to the hypothermal stress action. cv.Mida (free water content)

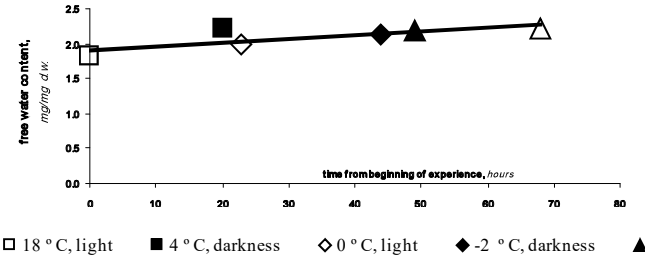

Fig.3 - Free water content dynamics in vine plants' leaves supposed to the low temperatures action. S. Cardinal.

As we observe at fig.4, after this stage of deterioration the recovery of optimal temperature conditions for the leaves' water status doesn't occur anymore. In

the previous work *[13]* in order to identify physiologic consequences provoked by the disorganization of metabolic processes and mechanic deteriorations during water crystallization there has been determined tissues freezing temperature with usage of sensors specially elaborated for that (fig.5). The obtained data demonstrate that at the beginning of vegetation young leaves of vine could remain in state of supercooling till -3, -4^{0}C *[13]*. There could occur just some differences according to variety but they are not essential. Temperatures of leaves' tissues freezing are situated in limits of -2,7 ÷ -3,5^{0}C. After general biological reaction at -3^{0}C the withering of sprouts tops and young leaves takes place, but at -5^{0}C plants are completely frozen in special in the upper part and young leaves and only completely formed leaves remain non-frozen. After this phase of the frost action the deceased water potential of leaves brought back to the initial temperature (-2,2 Mpa against -0,6 Mpa at leaves before frost action) reflects water binding by the denaturized biological structures because the free water is completely absent.

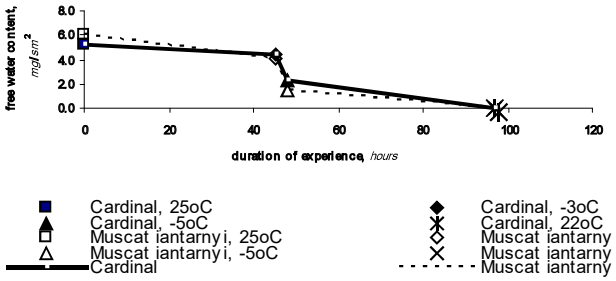

Fig. 4 - Modification of the free water content in leaves of the vine plants var.Cardinal şi Muscat iantarnyi) under the action of deteriorative temperatures (-5^{o}C).

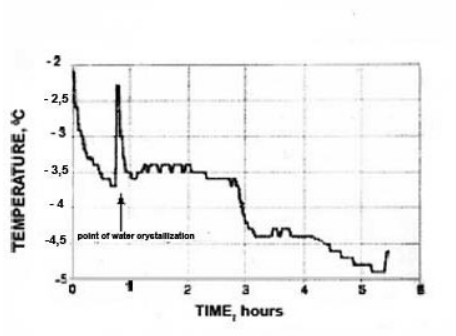

Fig.5 -Temperature of sprouts tissues freezing in vine plants. var. Cardinal

CONCLUSIONS

1. At the action of low temperatures on the growing plants the modifications of the water state are characterized by 2 phases: a) action of low temperature within plants tolerance limit leads to the increase of leaves watering, in special of the free water situated in apoplast and vacuoles, while the cytoplasm watering decreases; b) in range of the deteriorative temperatures the diminution of the total water content takes place including in symplast.
2. Unlike to the plants that enter the repose period late in the autumn when water loss is positive factor that favors adaptation against winter frosts, for plants that actively vegetate during the late spring period loss of free water by leaves represents a negative factor and characterizes by deteriorative action of low temperatures that finally leads to the total drying and loss of leaf.

REFERENCES

1. Carr H.Y., Purcell E.M., 1954 **-** *Effects of diffusion on free procession in nuclear magnetic resonance experiments* , v.94, p.630-638.
2. Daniel C., 1992 **-** *Les vegetaux et le froid. Hermann.* Editeurs des sciences et des arts. Paris.
3. Dument V., 1992 - *Le compartement du vignoble charentais suite aux degats du gel du 21 avril 1991*. Pays Cognac, **122**, p.31-33.
4. Harciuc O., Aksionov S., Chirilov A., Toma S., 2003 **-** *Metodă de determinare a continutului de apa in țesuturile vegetale.* BOPI, **1**, p.35-36.
5. Maxwell D.P., Trick S.G., Huner N.P.A., 1994 - *Growth at low temperature mimics high-light acclimation in Chlorella vulgaris.* Plant Physiology, **105(**2**),** 535-545**.**
6. Milon F.George and Michael J.Burke, 1977 **-** *Cold hardiness and deep supercooling in xylem of Shagbark Hickory.* Plant Physiology, **59(**2**),** 319-325**.**
7. Sing J., Laroshe A., 1988 - *Freezing tolerance in plants: a biochemical overview. Biochem.* And cell.Biol.,**66** (6), 650-657.
8. Shizuo Ioshida, 1994 - *Low-temperature Induced Cytoplasmic Acidolis in Cultured Mung Bean* (Vigna rasdiata I./Milczek) Cells. Plant Physiology, **104(**4**),** 1131-1138**.**
9. Ionenco I. F., Goleadina LV.,1999 - *Necotorye osobenosti mejcletocinogo perenosa vody pri stressovyh vozdeistvieah po danym impulisnogo metoda RMN*. 4-i sezd Obşcestva fiziologov Rossii. Москва,4-9октября 1999: tez..docl., v.1, M.,1999,s..367.
10. Chirilov A.F., Toma S.I., Harciuc O.A.., Cozmic R.A., Baştovaia S.I., Cleiman E.I., 2003 **-** *Necotorye fiziologiceschie posledstvia u vinogradnyh rastenii, vyzvanye vozdeistviem pozdne-vesenih zamorozcov.* Izvestia Academii nauc Moldovy. Biologicescie, himiceschie i celiscohozeaictvenye nauchi, 2(291),c.27-33.
11. Şvaleova A.L.,2001 - *Sostoeanie vody i ego vlieanie na funcționalinye haracteristichi membran v semenah pşeniţy (Triticum aestrivum L.) i prorostcah pri razlicinyh fiziologiceschih uclovieah: avtoreferat dis. na soisc.uci.step.doct.biol.nauc*, M., MGU, 32стр.