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SUNFLOWER LINES AND HYBRIDS RESPONSE TO WATER STRESS

M. Petrović, R. Kastori, D. Škorić and N. Petrović

University of Novi Sad, Faculty of Agriculture, Institute of Field and Vegetable Crops, Novi -Sad, Yugoslavia

SUMMARY

The response of four hybrids and their lines to water stress was tested in conditions of water culture. Water stress was induced by PEG. Plant reaction to water stress was estimated at the basis of nitrate reductase activity, proline accumulation, transpiration intensity, and relative water content. According to the results obtained it may be concluded as follows.

Nitrate reductase activity, free-proline concentration, transpiration intensit,y and relative water content significantly differed among the tested hybrids and lines. Water stress reduced nitrate reductase activity, transpiration intensity and relative water content, and increased free-proline concentration in all hybrids and lines, although not equally. The greatest change was observed in the concentration of free-proline which increased in all tested hybrids and lines approximately 14 times, while nitrate reductase activity was lower by approximately 50% than that in the control plants.

Nitrate reductase activity and free-proline accumulation which suffered great modification in plants under water stress, may be considered as the two parameters which indicate sunflower resistance to drought. However, this should be examined in field conditions, as well.

Dependence between the examined morphological parameters and the response of the hybrids to water stress was not discovered.

Key words: Water stress, nitrate reductese activity, free proline, relative water content, transpiration intensity, morphologic characteristics, sunflower lines and hybrids.

INTRODUCTION

Water is an ideal environment for life processes, because of its numerous physical and chemical qualities. Consequently, water deficit brings far-reaching changes in plant metabolism (Hsiao, 1973). The problem of water deficiency and plant resistance to drought is interesting academically and important practically, since plant growth and development and the distribution of plant species are restricted on a global scale because of drought. Therefore, great attention had been paid to this ecological factor for a long time. The problem of drought was further emphasized by the deficiency of high quality irrigation water, the recent introduction of high yielding genotypes, and the application of chemicals and other agricultural practices aimed at increased yield.

Plant resistance to water deficiency may be based on morphological, anatomical, and physiological characteristics. These characteristics are specific not only for individual species but for genotypes as well (Planchon, 1987). Therefore, it is important to know the metabolic changes in plant as well as in individual species and genotypes for a better understanding of plant resistance to water deficiency and in breeding genotypes for drought resistance (Monti, 1987). A number of indirect methods and new techniques have been developed for the evaluation of plant water status and drought resistance in last decade (O'Toole et al., 1984; Turner, 1986; Cruiziat, 1989). The aim of this investigation was to characterize the water stress in different lines and hybrids of young sunflower plants by physiological and biochemical parameters.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse on four sunflower hybrids and their lines: NS-H-43 (♀Ha 22 x RHA-SNRF), NS-H-26-RM (♂cms V 8931-3-4 x RHA-58), NS-Helios (♀Ha-BCPL x RHA-NK) and NS-H-55 (♀Ha 74 x RHA-C-D), by the method of water culture.

Seed germination was carried out in quartz sand in a thermostat at 25°C. After cotyledones appeared, the seedlings were transferred into the Reid-York (1958) nutrient medium and grown to the phase of three–leaf pairs. After this period, the plants were exposed to water stress, which was induced by polyethylene glycol solution (PEG-6000) of 10 bars osmotic value. The plants were under water stress for three days. The second leaf pair was used for all tests, except for transpiration intensity, which was assesed in intact plants.

Fre -proline was extracted from fresh plant material by 3% sulfosalicylic acid and stained with acid-ninhydrin reagent and glacial acetic acid. Spectrophotometric readings were done on a Beckman, M-25 at 520 nm (Bates et al., 1973).

Nitrate rectuase activity was determined *in vivo* in phosphorus buffer pH 7.4 which contained 0.25 mol KNO_3 dm^{-3}, and sulphanilamide and N-(1-Naphthyl) ethylenediamine dihydrochloride (Witt, 1975). Transpiration intensity was determined gravimetrically during the period from 9 a.m. to 1 p.m. at the temperature of 25°C. Relative turgidity (RWC) was measured according to the method described by Duniway (1973). Disks of 1 g were cut from interveinal parts of lamina with a cylinder 1.0 cm in diameter. The leaf disks were weighed and put into Petri dishes containing water and maintained at room temperature for 4 hours. After that, they were blotted dry, weighed, and dried at 105°C in a dryer to determine total water content. Leaf saturation was calculated according to the results obtained. Total root area was determined according to the method of Sabinin and Kolosova (Sarić et al., 1986). The results were statistically processed calculating the least significant difference.

RESULTS AND DISCUSSION

The effect of water deficit on enzymic system activity, especially on nitrate reductase, has started to be studied recently (Sirvastava, 1980, Hadžitašković-Šukolović et al., 1980, Petrović et al., 1987). Nitrate reductase is an essential enzyme in primary nitrate assimilation. Numerous experiments proved that the activity of this enzyme dicreased significantly in conditions of water deficit, so that nitrate reductase activity reflects the state of plant water schedule. This assumption is proved by the results in Table 1. On average, nitrate reductase activity was reduced by 50% in all lines and hybrids. It should be emphasized that the tested lines and hybrids differently responded to water deficit so that nitrate reductase activity was different as well. The smallest and the highest changes in nitrate reductase activity in the conditions of water deficit were recorded in lines Ha-BCPL, RHA-NK and Ha 22, and hybrids NS-H-26-RM, NS-H-43 and NS-H-55, respectively. According to Zhelyuk et al., (1985) both water deficit and excess in soil negatively affect nitrate reductase activity.

There is a number of assumptions about the unfavourable effect of water deficit on nitrate reductase activity. Bearing in mind that the activity of this enzyme depends on substrate concentration, i.e., NO_3^- in tissues; the reduction of its activity can occur due to lower NO_3^- ions uptake from the environment in conditions of water deficit. Water deficit can also cause conformational changes in the enzyme, i.e., its reversible denaturation, etc. According to the results achieved by many authors (Morila et al., 1973; Hisao, 1973; etc.), the fundamental reason for low enzymic activity in conditions of water deficit is the decrease of functionally capable polyribozomes, which results in slow protein synthesis and changes in enzymic activity. Bearing in mind unfavourable effect of water deficit on photosynthesis and plant energy change, the reason for decreased nitrate reductase activity in conditions of water deficit is probably the deficit of reduction eqivalents indispensable for nitrate reduction.

The plants exposed to water stress accumulated five times higher quantity of free proline than the plants optimally supplied with water (Table 1). Many authors registered this phenomenon earlier (Gring, 1979; Mukherjee, 1980). It was supposed that this is a result of several biochemical processes, such as activity of proline synthesis, inhibition

Table 1. Effect of water stress on nitrate reductase activity and free proline concentration in sunflower hybrids and lines

Hybrids and lines (A)	Nitrate reductase activity Mmol NO_2 g^{-1} DM h^{-1}		Free proline concentration $\mu g\ g^{-1}$ DM	
	Control (B)	Stress (B)	Control (B)	Stress (B)
NS-H-43	95.5	37.0	164	682
♀Ha 22	116.2	75.5	229	1343
♂RHA-SNRF	61.4	26.3	197	1162
NS-H-26-RM	102.3	38.7	292	1154
♀ CMS V 8931-3-4	140.0	82.7	385	2054
♂ RHA-58	92.1	41.2	325	1686
NS-Helios	104.8	58.3	285	1762
♀Ha-BCPL	88.9	68.4	228	1338
♂ RHA-NK	111.5	67.5	255	1510
NS-H-55	107.3	45.7	197	1007
♀ Ha-74	87.8	44.0	218	1458
♂ RHA-C-D	104.3	45.3	204	1320

	Nitrate reductase activity		Proline concentration	
LSD for	5%	1%	5%	1%
Factor A	15.35	20.46	19.49	25.98
Factor B	6.26	8.35	7.96	10.61

of its oxidation, decreased synthesis and increased protein hydrolisis (Shiralipour and West, 1984; Kueh et al., 1984). The increase of free proline concentration in conditions of water deficit was different in individual lines and hybrids. Free proline concentration increased most in the lines Ha 74 and RHA-C-D and the hybrid NS-Helios and least in the hybrids NS-H-26 RM, NS-H-43 and NS-H-55.

The results obtained showed significant differences among the tested genotypes with respect to proline accumulation in conditions of water deficit. Some authors suggested free proline accumulation in conditions of water deficit to be used as an indicator of plant drought resistance in breeding plants for drought resistance (Singh et al., 1972; Palfi and Pinter 1980; etc.).

Transpiration intensity was different in the tested sunflower hybrids and lines. Compared with the control, it decreased by 58.7% in stress conditions and in all tested genotypes (Table 2). In the presence of PEG, the highest transpiration decrease was detected in those lines (RHA-58, Ha-BCPL and RHA-NK) and hybrids (NS-H-26-RM) which had the most intensive transpiration. Less intensive transpiration which occures in the presence of PEG is a result of a lower difference in water potential between the solution and root system. In such conditions, water uptake and transpiration are slower.

Relative water content decreased significantly in stress conditions in all lines and hybrids (Table 2). It was lowest in the hybrids NS-H-43 and NS-H-26-RM.

Table 2. Water stress effect on transpiration intensity and relative water content in sunflower hybrids and lines

Hybrid and line (A)	Transpiration intensity g H_2O dm^{-2} h^{-1}		Relative water content %	
	Control (B)	Stress	Control	Stress
NS-H-43	1.806	833	84.2	69.2
♀Ha 22	1.630	778	85.5	74.5
♂RHA-SNRF	1.790	802	84.9	73.4
NS-H-26-RM	2.212	695	83.9	70.1
♀ CMS V 8931-3-4	1.895	868	85.2	75.3
♂ RHA-58	2.410	915	84.2	74.1
NS-Helios	1.859	892	85.8	73.1
♀ Ha-BCPL	2.103	788	85.9	77.0
♂ RHA-NK	1.980	703	86.1	72.1
NS-H-55	1.851	748	85.4	74.4
♀ Ha-74	1.624	807	85.1	73.1
♂ RHA-C-D	1.813	749	84.4	74.2

	Nitrate reductase activity		Proline concentration	
LSD for	5%	1%	5%	1%
Factor A	28.9	38.5	2.76	3.86
Factor B	11.8	15.7	1.27	1.50
Interaction AxB	40.9	54.5	3.90	5.20

When relative water content is decreased, diffusive stomatal resistance is increased and transpiration is decreased (Sen Gupta et al., 1989). The correlation between relative water content and transpiration intensity was not found in our examination. However,

Tab. 3 Morphological characteristics of the tested sunflower hybrids and lines

Hybrid and lines	Stem height (cm)	Stem diameter (mm)	Leaf area ($cm^2.plant^{-1}$)	Root area ($m^2.plant^{-1}$)	Dry matter mass (mg.plant-1)				Mass of above-ground part/root mass	Leaf area /root area x 10^3
					Stem	Leaf	Root	Plant		
NS-H-43	30.8	5.1	120	0.529	306	246	86.2	638	6.4	22.7
♀Ha 22	26.3	4.9	116	0.449	270	264	82.9	616	6.4	25.8
♂RHA-SNRF	28.3	4.7	93	0.574	227	183	61.8	471	6.6	16.2
NS-H-26-RM	30.2	6.0	133	0.595	328	230	80.1	638	6.9	22.4
♀CMS V8931-3-4	19.3	4.5	104	0.492	190	207	81.7	478	4.8	21.1
♂RHA-58	22.1	3.7	86	0.495	159	168	73.7	447	4.4	17.0
NS-Helios	30.0	5.1	123	0.558	234	256	92.2	582	5.3	22.0
♀Ha-BCPL	21.2	4.0	110	0.496	185	287	64.8	590	7.3	22.2
♂RHA-NK	24.5	5.0	115	0.500	242	243	86.6	559	5.6	23.0
NS-H-55	25.2	3.8	138	0.490	232	265	74.9	571	6.6	28.2
♀Ha 74	29.9	4.3	139	0.507	261	252	90.2	603	5.7	27.4
♂RHA-C-D	28.7	3.9	99	0.523	185	186	51.0	422	7.3	18.9
AVERAGE	26.4	4.6	115	0.517	235	232	77.2	552	6.1	21.2
LSD 5%	1.0	0.33	5.1		8.3	10.0	4.8			
1%	1.5	0.45	6.9		11.3	13.5	6.5			

genotypes which had the lowest relative water content in the presence of PEG had also the highest decrease of nitrate reductase activity and the lowest proline accumulation.

The tested sunflower hybrids and lines had significantly different morphological characteristics (Table 3). Total leaf and root system areas and their interrelationships are specially significant for water schedule. According to the results obtained, it can be concluded that there is no dependence between the tested morphological parameters and the physiological and biochemical reactions (nitrate reductase activity, proline accumulation, transpiration intensity, and relative water content) of the tested sunflower hybrids and lines to water stress.

Water stress caused significant changes in the tested physiological indicators, with different reaction of individual hybrids and lines. The assumption about the usability of physiologic parameters in evaluation of sunflower resistance to drought is supported by the above-mentioned facts. However, it has to be kept in mind that plant resistance to unfavourable ecological factors and thus to drought is a complex characteristic. Therefore, before making a final decision about the reliability of an indicator of plant resistance to extreme environmental conditions, it is necessary to test it in natural conditions.

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RESPUESTA DE LINEAS Y SUS HIBRIDOS AL STRES HIDRICO

RESUMEN

La respuesta de cuatro híbridos y sus líneas al estrés hídrico fue testada en condiciones de cultivo hidropónico. El estrés hídrico fue inducido por PEG. La reacción de las plantas al estrés hídrico fue estimada en base a la actividad de nitrato reductasa, acumulación de prolina, intensidad de transpiración y contenido relativo de agua. De acuerdo con los resultados obtenidos puede ser concluido lo siguiente: La ectividad de nitrato reductasa, concentración líbre de prolina, intensidad de transpiración y contenido relativo de agua difirieron significativamente entre los híbridos y líneas testados. El estrés hídrico redujo la actividad de nitrato reductasa, intensidad de transpiración y contenido relativo de agua e inoremento de concentración de prolina líbre en todos los híbridos y líneas testadas aunque no igualmente. Los mayores cambios se encontraron en la concentración de prolina libre que se incrementó en todos los híbridos testados.

RÉPONSE DE LIGNÉES FIXÉES ET D'HYBRIDES DE TOURNESOL AU STRESS HYDRIQUE

RESUMÉ

La résponse de quatre hybrides et de leurs lignées à un stress hydrique a été testée en condition de culture hydroponique. Le stress hydrique a été induit par le PEG. La réaction des plantes au stress hydrique a été jugée sur l'activité nitrate réductase, l'accumulation de proline, l'intensité de transpiration, et la teneur relative en eau (RWC). Les résultats obtenus conduisent aux conclusions sulvantes: L'activité nitrate réductase, la concentration en proline libre, l'intensité transpiratoire et la teneur relative en eau différent significativement entre génotypes testés. Le stress hydrique réduit l'activité nitrate réductase, l'intensité transpiratoire et la teneur relative en eau, et augmente la concentration en proline libre chez tous les hybrides et lignées testés, mais de facon inégale. La modification la plus importante observée concerne la conentration en proline libre, qui a augmenté chez tous les génotypes d'envrion 14 fois, tandis que l'activité nitrate réductase a été réduite d'envrion 50% par rapport aux plantes témoins. L'activité nitrate réductase et l'accumulation de proline libre qui ont été sensiblement affectées chez les plantes soumises au stress hydrique peuvent être considérées comme deux paramétres qui indiquent la resistance du tournesol à la sècheresse. Toutefois, ceci devrait être examiné en condition de plein champ. Aucune relation entre les paramètres morphologiques étudiés et la réponse au stress hydrique des hybrides testés n'a été miso on évidence.