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Proline accumulation in some barley genotypes exposed to drought

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Abstract The objective of this work was to evaluate the changes in the amino acid, proline accumulated in plants under stress conditions. The osmotic stress were induced by PEG 6000 solution with the osmotic potential (-2.72 Bars, -4.48 Bars -7.35 Bars) using method suggested by Michael and Kaufman (1973). The results of this study showed that water deficit led to generally high free proline levels. From this parameters stand point the genotypes with a high drought tolerance correlated with high values of proline was presented by cultivars:, Andrei, Dana, Mădălin, Lyric.

Key words

drought tolelance, winter barley, proline contents

Water deficit is the most important factor in yield reduction in the semiarid regions [6, 13,14,]. Positive correlation with relative yield performance of genotipes in reinfal condition is a start point for identification of characters related to drought tolerance [6].

In response to water stress, plants accumulate low molecular weight compounds (compatible solutes), such as proline, betaine and sugar alcohols [19]. Accumulation of these compounds contributes to enhancing water stress tolerance by generating an osmotic driving force. Therefore, much recent research has been focused on improving the ability to produce proline [5, 25, 8, 11, trehalose [12] and betaine [9] .

The amino acid proline contains a secondary amino group and is thus unique among the proteinogenic amino acids. Proline plays a crucial role for cellular metabolism both as a component of proteins and as free amino acid. Due to its cyclic structure, proline has a restricted conformational flexibility, which determines the arrangement of the peptide chain around it, and as a consequence leads to stabilization or destabilization of secondary structures of protein conformation. In addition to its role in primary metabolism as a component of proteins, the free amino acid proline is one of the most widely distributed compatible solutes that accumulate in plants and bacteria during adverse environmental conditions such as drought, high salinity or low temperatures. The role of proline as compatible solute and the importance of proline metabolism under stress conditions in plants have been a field of intense research and the current state of knowledge including novel findings and open questions is covered by several recent and excellent reviews [29]. The level of free proline varies considerably in different plant organs and is usually higher in reproductive organs than in vegetative tissues [7]. High concentrations of proline have been reported in plant organs during endogenously controlled dehydration, e.g. in seeds or pollen (see below). Furthermore, a high leaf-to-root ratio for proline has been found in several plant species including Arabidopsis, lentil and common bean . In barley, the preferential accumulation of proline in epidermis and vascular bundles was only observed under stress conditions [30].

Material and Methods

Experiment were conducted in the laboratories of the Faculty of Horticulture and Forestry Timisoara, Departament of the Genetic Enginering in agriculture. The biological material used in this study was represented by a collection of genotypes of whinter barley made by romanian and foreign kinds and double haploid lines. Double haploid lines used in these experiments were provided by ICDA Fundulea and obtained by Bulbosum method, adapted for greenhouse and field conditions. The control taken was represented by the cultivar Dana that presented a good drought tolerance being well adapted to the environmental conditions of the Banat Plain. Seed were initially treated with 1,5% sodium hypochlorite for 15 min., residual chlorine was eliminated by throught washing of the seeds with distilled water. The seed were germinated in culture dish with perlit. The experiment was conducted under normal (0 Bars) and drought stress (-2.72 Bars,-4.48 Bars, and – 7.35Bars) conditions created with the help of Polyethylene- glychol (PEG6000) by the method suggested by Michael and Kaufman (1973). The stress tratament was induced after 15 day from germination and data were collected after 3days, 10 days respectively 17 days after stress induction. Proline was determined according to the method described by Bates et al. (1973).

Results and Discussions

Sometimes weather conditions in the field do not allow evaluation of drought resistance of the genotypes and therefore is necessary to use one or more laboratory methods by which to induce a stress field similar to the field conditions and to allow the characterization of genotypes in terms of resistance to drought.

It is well known that the proline contents in leaves of many plants get enhanced by several stresses including drought stress [15, 16,4,1, 20, 22,24]. Thus, we monitored the proline levels in leaves of barley genotypes during drought. According to this technique, the cultivars with a hight proline content manifest a high drought tolerance.

The experimental results obtained after 3 days of water stress induced by PEG 6000 at different osmotic pressures are presented in Table (1).Basic content registered in normally hydrated proline variety was between 0.6804 mg / g in variety Adi and 0.7295 mg / g in variety Lyric. On the medium variant V1(-2.72 Bars) the genotypes showed value in proline content ranged between 0.655 mg/g in genotypes Adi and 0.7486 mg/g in variety Secura. Most genotypes were superior to the control. On the medium variant with the osmotic pressure (-4.48 Bars) the proline content accumulated in the studied genotypes was between 0.691 mg / g in Plaisant and 0.819 mg/ g in the Regal variety, the result obtained in this genotype was significantly superior to the control. The stronger the stress` action, the amount of proline accumulated was higher, with values between 0.7142 mg / g at Lyric and 0.8242 mg / g at Regal. One more time it may be noted that the Regal variety was significantly higher to the control.

The results obtained with PEG6000 in water stress conditions after 10 days of stress induction are presented in Table 2. After a period of 10 days from induction of water stress, the proline content accumulated in the studied genotypes, on variant V1 was ranged between 0.6689 mg /g in variety Tas and 0.7263mg / g in variety Andrei. Compared to the control most genotypes were superior in terms of the amount of proline accumulated, the results being significantly positive probably due to a reaction of the genotypes to adjust under the action of stress factor. On the medium variant V2 the amount of proline accumulated ranged between 0.659 mg / g in variety Adi and 0.784 mg / g in variety Andrei. In the case of the variant in which the osmotic pressure applied was (-7.35 Bars) the genotypes superior to the control were Andrei, Compact, Djerbel, Plaisant, Dina, DH260/12, the results obtained being ensured in statistical terms.

After a long period of hydric stress (17 days) the average values of free proline content shows an increasing in its value with increased drought (Table 3). Thus on medium variable V1 the proline content was ranged between 0.691mg / g in the variety Tas and 1.18 mg / g in Andrei this genotype being significantly superior to the control. With a high content of proline were also noted the genotypes Plaisant and Lyric. On the medium variable V2 proline content ranged from 0.724 mg/g in Adi and 1.200 mg / g in Andrei. In this situation the only genotype that exceeded significantly the control was variety Andrei. On the medium variable V3 (-7.48Bars) the content of proline ranged between 0.754 mg /g in Adi and 1.632 mg / g in Andrei.

After the period of water stress applied to the studied genotypes it can be observed that they had a different behavior in terms of accumulated proline. However, some of them have presented stability over the test period, such as genotypes Andrei, Dana, Orizont, Madalin, Dina, Lyric, they recorded a higher content of proline and proved more tolerant to the action water stress. Comparing the studied genotypes it can be seen that the double haploid lines showed lower values of proline content than the varieties. This increase in free proline content due to water deficit has been reported by many authors [4]. Some authors [2, 19, 26]. reported that free proline accumulation is very important in enhancing osmotic stress tolerance in plants.

It can be concluded that the ability of some genotypes to accumulate high amounts of free proline as a result of water stress shows a high tolerance to drought. Complex role of proline on plant response to stress conditions were confirmed by numerous studies based on transgenic plants [10, 17]. For example, in wheat was found that the proline is higher in tolerant varieties than in susceptible ones[21]. Proline may improve tolerance to stress in different ways, having the capacity to protect the integrity of proteins preventing their aggregation [23] and protects the nitrate-reductase under osmotic stress [27]. In this respect,) found that proline is involved in mechanisms of tolerance to oxidative stress, which is the main strategy of plants to avoid the negative effects of water stress.

Table 1

Accumulation of proline after three days

Nr crt	Genotipul	meen mg/g VO	%	diference	Signif	meen mg/g V1	%	diference	Signif	meen mg/g V2	%	diference	Signif	meen mg/g V3	%	diference	Signif
1	DANA	0,7122	100,0000	0,0000		0,6606	100,0000	0,0000		0,801	100,000	0,000		0,813	100,000	0,000	
2	ORIZONT	0,7006	98,3850	-0,0115	o	0,6555	99,2319	-0,0051		0,728	90,889	-0,073	ooo	0,758	93,252	-0,055	ooo
3	PRECOCE	0,6883	96,6465	-0,0239	ooo	0,7314	110,7125	0,0708	***	0,773	96,586	-0,027	ooo	0,777	95,507	-0,037	ooo
4	ADI	0,6804	95,5445	-0,0317	ooo	0,6552	99,1807	-0,0054		0,793	99,096	-0,007	-	0,807	99,193	-0,007	ooo
5	MADALIN	0,7107	99,7910	-0,0015	o	0,6885	104,2195	0,0279	***	0,749	93,594	-0,051	ooo	0,758	93,277	-0,055	ooo
6	ANDREI	0,6974	97,9290	-0,0147	ooo	0,7138	108,0497	0,0532	***	0,748	93,399	-0,053	ooo	0,754	92,778	-0,059	ooo
7	REGAL	0,6843	96,0860	-0,0279	ooo	0,6848	103,6664	0,0242	***	0,819	102,290	0,018	***	0,825	101,440	0,012	***
8	COMPACT	0,7214	101,2920	0,0092	***	0,6981	105,6840	0,0375	***	0,732	91,473	-0,068	ooo	0,754	92,695	-0,059	ooo
9	DJERBEL	0,6996	98,2330	-0,0126	ooo	0,7068	106,9949	0,0462	***	0,712	88,954	-0,088	ooo	0,723	88,925	-0,090	ooo
10	LYRIC	0,7295	102,4415	0,0174	***	0,7223	109,3401	0,0617	***	0,693	86,621	-0,107	ooo	0,714	87,844	-0,099	ooo
11	PLAISANT	0,6846	96,1335	-0,0275	ooo	0,7277	110,1595	0,0671	***	0,691	86,311	-0,110	ooo	0,718	88,301	-0,095	ooo
12	TAS	0,7046	98,9455	-0,0075	ooo	0,6956	105,2964	0,0350	***	0,716	89,483	-0,084	ooo	0,738	90,731	-0,075	ooo
13	SECURA	0,6768	95,0410	-0,0353	ooo	0,7486	113,3240	0,0880	***	0,799	99,831	-0,001	ooo	0,820	100,874	0,007	***
14	DINA	0,7039	98,8410	-0,0083	ooo	0,7161	108,3979	0,0555	***	0,755	94,312	-0,046	ooo	0,773	95,116	-0,040	ooo
15	DH19/1	0,6937	97,4065	-0,0185	ooo	0,7074	107,0870	0,0468	***	0,754	94,143	-0,047	ooo	0,775	95,307	-0,038	ooo
16	DH 254-10	0,7008	98,4040	-0,0114	ooo	0,6886	104,2399	0,0280	***	0,748	93,450	-0,052	ooo	0,770	94,691	-0,043	ooo
17	DH260-18	0,7122	100,0000	0,0000		0,6929	104,8851	0,0323	***	0,733	91,608	-0,067	ooo	0,754	92,769	-0,059	ooo
18	DH 260-12	0,7059	99,1165	-0,0063	ooo	0,7178	108,6540	0,0572	***	0,718	89,698	-0,083	ooo	0,746	91,788	-0,067	ooo
19	DH 261 22	0,7108	99,8100	-0,0014		0,7069	107,0154	0,0463	***	0,719	89,864	-0,081	ooo	0,726	89,275	-0,087	ooo
			DL5%	0,0014			DL5%	0,0134			DL5%	0,010			DL5%	0,003	
			DL1%	0,0018			DL1%	0,0179			DL1%	0,013			DL1%	0,005	
			DL0.1%	0,0024			DL0.1%	0,0236			DL0.1%	0,017			DL0.1%	0,006	

Table2

Accumulation of proline after then days

Nr crt	Genotypes	meen mg/g VO	%	diference	Signif	meen mg/g V1	%	diference	Signif	meen mg/g V2	%	diference	Signif	meen mg/g V3	%	diference	Signif
1	DANA	0,692	100,000	0,000		0,681	100,000	0,000		0,727	100,000	0,000		0,743	100,000	0,000	
2	ORIZONT	0,681	98,407	-0,011	-	0,673	98,828	-0,008	ooo	0,717	98,678	-0,010	ooo	0,747	100,574	0,004	***
3	PRECOCE	0,694	100,244	0,002	-	0,675	99,126	-0,006	ooo	0,718	98,837	-0,009	ooo	0,739	99,454	-0,004	ooo
4	ADI	0,696	100,567	0,004	**	0,706	103,665	0,025	***	0,659	90,590	-0,068	ooo	0,662	89,144	-0,081	ooo
5	MADALIN	0,699	100,909	0,006	***	0,715	104,936	0,034	***	0,697	95,896	-0,030	ooo	0,718	96,685	-0,025	ooo
6	ANDREI	0,707	102,049	0,014	***	0,726	106,633	0,045	***	0,784	107,847	0,057	***	0,827	111,385	0,085	***
7	REGAL	0,687	99,177	-0,006	ooo	0,708	103,894	0,027	***	0,711	97,798	-0,016	ooo	0,723	97,386	-0,019	ooo
8	COMPACT	0,685	98,925	-0,007	ooo	0,702	103,089	0,021	***	0,701	96,426	-0,026	ooo	0,768	103,361	0,025	***
9	DJERBEL	0,684	98,779	-0,008	ooo	0,704	103,357	0,023	***	0,678	93,215	-0,049	ooo	0,755	101,603	0,012	***
10	LYRIC	0,679	97,997	-0,014	ooo	0,694	101,897	0,013	***	0,726	99,851	-0,001		0,734	98,789	-0,009	ooo
11	PLAISANT	0,672	97,000	-0,021	ooo	0,707	103,854	0,026	***	0,752	103,397	0,025	***	0,750	101,002	0,007	***
12	TAS	0,674	97,284	-0,019	ooo	0,669	98,192	-0,012	ooo	0,705	96,947	-0,022	ooo	0,726	97,714	-0,017	ooo
13	SECURA	0,668	96,492	-0,024	ooo	0,681	100,010	0,000	-	0,703	96,715	-0,024	ooo	0,734	98,743	-0,009	ooo
14	DINA	0,683	98,652	-0,009	ooo	0,725	106,386	0,044	***	0,716	98,520	-0,011	ooo	0,758	102,013	0,015	***
15	DH19/1	0,668	96,512	-0,024	ooo	0,710	104,241	0,029	***	0,727	100,037	0,000		0,750	100,956	0,007	***
16	DH 254-10	0,701	101,300	0,009	***	0,701	102,900	0,020	***	0,714	98,167	-0,013	ooo	0,788	106,065	0,045	***
17	DH260-18	0,715	103,312	0,023	***	0,715	104,916	0,034	***	0,718	98,725	-0,009	ooo	0,733	98,616	-0,010	o
18	DH 260-12	0,689	99,443	-0,004	ooo	0,692	101,619	0,011	***	0,701	96,407	-0,026	ooo	0,778	104,699	0,035	***
19	DH 261 22	0,713	102,941	0,020	***	0,715	105,026	0,034	***	0,727	100,047	0,000	-	0,763	102,732	0,020	***
			DL5%	0,003				DL5%	0,003		DL5%	0,004			DL5%	0,0019	
			DL1%	0,004				DL1%	0,004		DL1%	0,005			DL1%	0,0026	
			DL0.1%	0,005				DL0.1%	0,005		DL0.1%	0,007			DL0.1%	0,0034	

Table 3

Accumulation of proline after seventeen days

Nr crt	Genotypes	meen mg/g VO	%	diference	Signif	meen mg/g V1	%	diference	Signif	meen mg/g V2	%	diference	Signif	meen mg/g V3	%	diference	Signif
1	DANA	0,784	100,000	0,000	-	0,966	100,000	0,000	-	0,976	100,000	0,000	-	0,978	100,000	0,000	-
2	ORIZONT	0,743	94,770	-0,041	-	0,795	82,287	-0,171	ooo	0,881	90,222	-0,095	ooo	0,880	89,936	-0,099	-
3	PRECOCE	0,728	92,914	-0,056	ooo	0,840	86,908	-0,127	ooo	0,870	89,122	-0,106	ooo	0,857	87,541	-0,122	-
4	ADI	0,715	91,258	-0,069	ooo	0,726	75,139	-0,240	ooo	0,724	74,187	-0,252	ooo	0,754	77,032	-0,225	ooo
5	MADALIN	0,700	89,284	-0,084	ooo	0,832	86,068	-0,135	ooo	0,841	86,168	-0,135	ooo	0,953	97,408	-0,025	-
6	ANDREI	0,704	89,802	-0,080	ooo	1,181	122,243	0,215	***	1,200	122,962	0,224	***	1,632	166,772	0,653	***
7	REGAL	0,703	89,776	-0,080	ooo	0,756	78,247	-0,210	ooo	0,766	78,452	-0,210	ooo	0,767	78,364	-0,212	-
8	COMPACT	0,751	95,803	-0,033	-	0,729	75,475	-0,237	ooo	0,731	74,901	-0,245	ooo	0,769	78,634	-0,209	-
9	DJERBEL	0,703	89,655	-0,081	ooo	0,730	75,552	-0,236	ooo	0,777	79,562	-0,200	ooo	0,776	79,339	-0,202	-
10	LYRIC	0,683	87,099	-0,101	ooo	0,780	80,768	-0,186	ooo	0,785	80,432	-0,191	ooo	0,809	82,668	-0,170	-
11	PLAISANT	0,734	93,644	-0,050	ooo	0,748	77,393	-0,218	ooo	0,785	80,376	-0,192	ooo	0,767	78,337	-0,212	-
12	TAS	0,672	85,761	-0,112	ooo	0,691	71,519	-0,275	ooo	0,734	75,178	-0,242	ooo	0,784	80,161	-0,194	-
13	SECURA	0,675	86,098	-0,109	ooo	0,751	77,701	-0,215	ooo	0,783	80,154	-0,194	ooo	0,787	80,390	-0,192	-
14	DINA	0,736	93,964	-0,047	ooo	0,776	80,271	-0,191	ooo	0,858	87,861	-0,119	ooo	0,869	88,813	-0,110	-
15	DH19/1	0,702	89,629	-0,081	ooo	0,778	80,558	-0,188	ooo	0,785	80,451	-0,191	ooo	0,862	88,094	-0,117	-
16	DH 254-10	0,686	87,571	-0,097	ooo	0,712	73,655	-0,255	ooo	0,742	76,024	-0,234	ooo	0,790	80,764	-0,188	-
17	DH260-18	0,729	93,098	-0,054	ooo	0,741	76,686	-0,225	ooo	0,756	77,394	-0,221	ooo	0,757	77,347	-0,222	ooo
18	DH 260-12	0,682	87,021	-0,102	ooo	0,747	77,281	-0,220	ooo	0,753	77,087	-0,224	ooo	0,777	79,457	-0,201	-
19	DH 261 22	0,728	92,910	-0,056	ooo	0,729	75,398	-0,238	ooo	0,736	75,435	-0,240	ooo	0,766	78,309	-0,212	-
			DL5%	0,042				DL5%	0,016		DL5%	0,013			DL5%	0,218	
			DL1%	0,056				DL1%	0,022		DL1%	0,017			DL1%	0,293	
			DL0.1%	0,074				DL0.1%	0,029		DL0.1%	0,022			DL0.1%	0,386	

Conclusions

According to results, it can be concluded that plants in drought stress time, make changes in some of their physiological and biochemical features. One of these responses is proline accumulation in barley varies between tolerant and sensitive genotypes. The higher efficiency of the proline accumulation in genotypes can be considered as one of the factors responsible for its tolerance to drought.

From the material taken in study, it can be observed that genotypes Andrei, Dana, Madalin, Lyric, Orizont have accumulated a higher amount proline, proving to be more tolerant to the action of stress. Genotypes that have accumulated a lower amount of proline were variety Adi, Tas, double haploid lines DH 260/12, 250/10 DH, DH, these proved to be more susceptible to water stress.

Aknowlegement

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