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PROLINE CONTENT AND THE CONDUCTIVITY TEST AS SCREENING METHODS FOR FROST TOLERANCE OF WINTER WHEAT

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Introduction

Studies of hardiness are essential in evaluating new cultivars of winter wheat. The general trend of research is to replace or to complete field testing by laboratory procedures.

It is advantageous for breeding to identify biochemical markers or some physiological indicators, which can be used as simple indicators of frost tolerance.

Free proline as a biochemical marker for frost tolerance in potato, barley and wheat has been discussed (Van Swaaij et al., 1986; Dobslaw and Bielka, 1988; Dörffling et al., l990). Frost tolerance of wheat estimated as relative injury by the conductivity test is another method which is used in frost tolerance testing (Dexter, 1930, 1932; Hömö, l994).

In the present investigation we studied the frost tolerance of fifty winter wheat cultivars hardened in natural conditions, as well as their frost tolerance when hardened in controlled conditions.

The purpose of our study was to find out whether a correlation existed between the frost tolerance of plants hardened in natural conditions and proline content of plants hardened in controlled conditions for 50 cultivars.

Material and Methods

Fifty cultivars of winter wheat were studied. Some of them were with known frost tolerance: Odesskaia 51 and Dropia (very resistant), Rapid and Fundulea 29 (resistant), Iulia, Padinka and P.K.B. Sunce (middle resistant), Libellula (very sensitive), Colina (sensitive) and other inbreed lines developed by Romanian breeders with unknown frost tolerance.

Experiment I

The plants were hardened in natural conditions, in a vegetation house. In order to evaluate frost resistance we used a direct method.

Winter survival was estimated in January and February. For frost resistance evaluation plants were taken out from the soil, washed and cut 2.5 cm above and below the tillering node. Plants of each entry were placed in plastic tags and exposed to frost treatment in a growth chamber (at –11°C for 18 hours). After that the plants were transplanted in wooden boxes and let to recover in a phytotron at 18–20°C for 10–12 days.

The effect of the frost treatment was estimated by evaluating each plant using a scale from 1 to 10 (individual scoring = S.I.) where 10 = complete necrosis and 1 = no necrosis; also a scale from 1 to 5 (direct scoring = D.S.) where 5 = all plants were green and 1 = all plants were dry. The cultivars of winter wheat studied were classified into classes of resistance using necrosis index values (N.I.= S.I./D.S.) and the scale presented in Table 1.

Table 1. Ranking of winter wheat genotypes after N.I.

Class of resistance	Necrosis index
Very resistant	0.20–0.66
Resistant	0.67–1.20
Middle resistant	1.21–2.00
Sensitive	2.00–4.50
Very sensitive	4.50–10.0

Experiment II

The plants were hardened in controlled conditions (2°C, 10 hours photoperiod) for two weeks.

Free proline content was measured according to the Bates method (1973). Control plants remained under non-hardening conditions.

A conductivity test was also performed after the Dexter (1932) and Blum (1982) methods.

The samples for assay consisted of control (unstressed but hardened plants) and treated (plants hardened and exposed to freezing treatment). The temperature was gradually reduced with a cooling rate of 2°C per hour to –10°C and held for 18 hours. The control samples were without frost treatment.

Frost tolerance of the wheat genotypes studied was evaluated as percentage injury according to the following formula: % injury=1– $(1–T_l/T_2)/(1–C_l/C_2)$, where T and C refer to mean of treatment conductivity and control, respectively, and the notation 1 and 2 refer to initial and final conductivity, respectively.

Results and Discussion

In 1994, the autumn air temperature was normal averaging approximately 10°C during October and 3°C during November (Fig. 1). These conditions were favourable

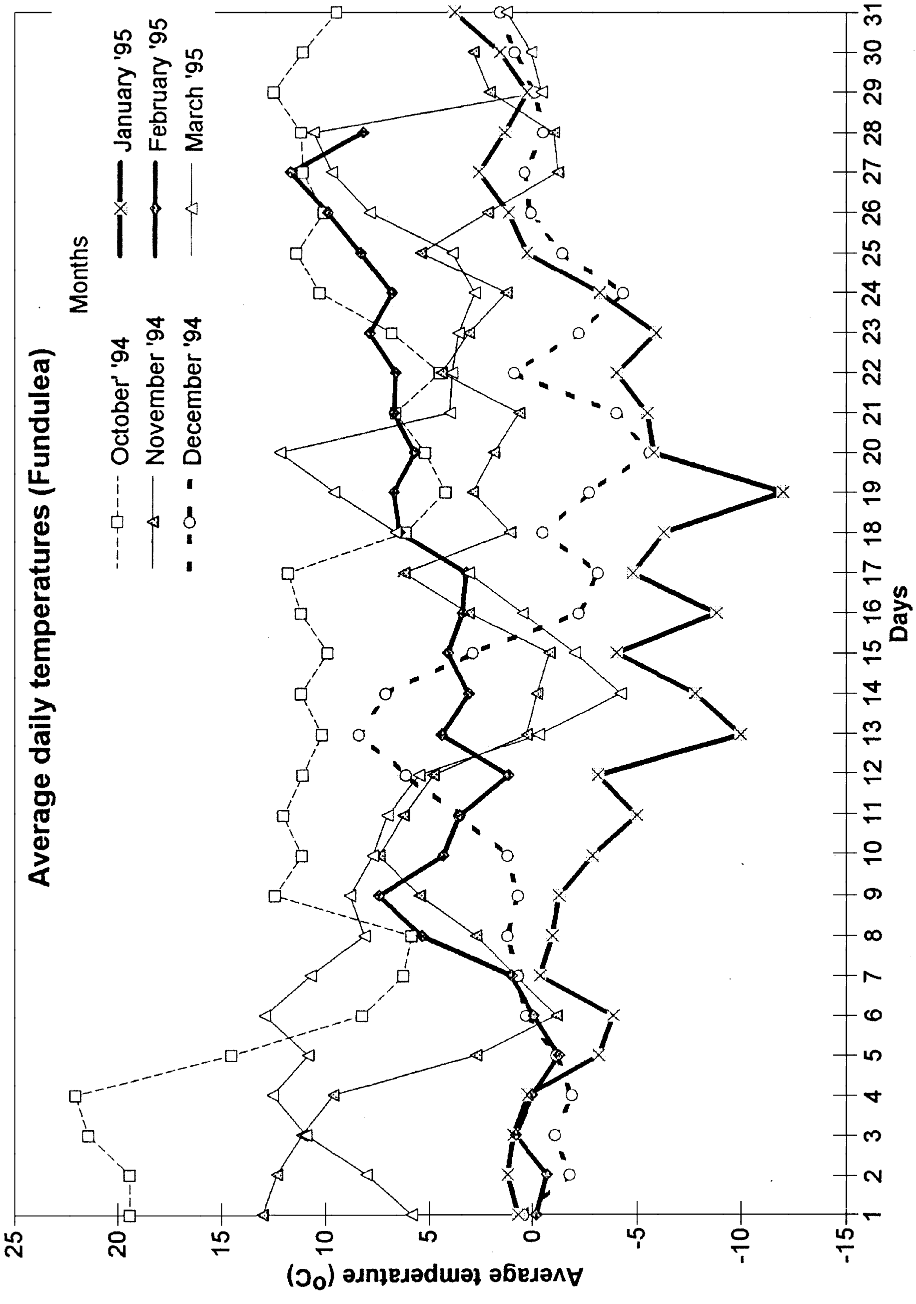
Average daily temperatures (Fundulea)
Months
October' 94
November '94
December '94
January '95
February '95
March '95
Average temperature (oC)
Days

Fig. 1

for growth and hardening of winter wheat, so the plants developed normally and were hardened. It is know that low temperature combined with short days provide ideal conditions for hardening of winter wheat.

The frost resistance potential of the winter wheat cultivars included in the study, determined by the direct method and expressed by the Necrosis index is shown in Table 2. In January it varied from very resistant (Odesskaia 51 and Dropia) to sensitive (Libellula). The frost resistance potential in February was similar with few exceptions (Lovrin 50 and A.F. 93-1) which were resistant in January but only middle resistant in February.

Table 2. Necrosis index variation and class of resistance to frost of 50 winter wheat cultivars

No. Crt.	Genotype	Necrosis index January	Class of resistance	Necrosis index February	Class of resistance
1	Odesskaia 51	0.44	Very resistant	0.58	Very resistant
2	Flamura 85	0.60	Very resistant	0.71	Resistant
3	4105 W1-121	0.60	Very resistant	0.73	Resistant
4	Dropia	0.60	Very resistant	0.74	Resistant
5	Fundulea 29	0.75	Resistant	0.75	Resistant
6	AF 93-3	0.75	Resistant	0.75	Resistant
7	AF 92-2	0.91	Resistant	0.91	Resistant
8	AF 92-1	0.94	Resistant	0.94	Resistant
9	7012 W1-1	0.90	Resistant	0.96	Resistant
10	6041 W2-1	0.90	Resistant	0.96	Resistant
11	AF 93-1	0.98	Resistant	1.21	Middle resistant
12	7019 W1-1	1.00	Resistant	1.04	Resistant
13	AF 93-2	1.10	Resistant	1.08	Resistant
14	AF 92-4	1.08	Resistant	1.08	Resistant
15	Rapid	1.08	Resistant	1.08	Resistant
16	F. 7926	1.08	Resistant	1.08	Resistant
17	201 R2-111	1.10	Resistant	1.11	Resistant
18	219 S3-14	1.10	Resistant	1.11	Resistant
19	6555 W1-11	1.10	Resistant	1.11	Resistant
20	Fundulea 4	1.10	Resistant	1.11	Resistant
21	24 T2-2	1.13	Resistant	1.13	Resistant
22	445 T5-4	1.13	Resistant	1.13	Resistant
23	24 T5-1	1.13	Resistant	1.13	Resistant
24	444 T6-2	1.15	Resistant	1.15	Resistant
25	201 R2-112	1.20	Middle resistant	1.20	Middle resistant
26	4993 W2-111	1.20	Middle resistant	1.21	Middle resistant
27	808 S3-12	1.20	Middle resistant	1.23	Middle resistant
28	24 T7-2	1.20	Middle resistant	1.25	Middle resistant
29	143 T3-1	1.20	Middle resistant	1.51	Middle resistant

No. Crt.	Genotype	Necrosis index January	Class of resistance	Necrosis index February	Class of resistance
30	Lovrin 50	1.10	Resistant	1.51	Middle resistant
31	4549 W2-121	1.50	Middle resistant	1.52	Middle resistant
32	F.7905	1.50	Middle resistant	1.54	Middle resistant
33	AF 92-3	1.50	Middle resistant	1.55	Middle resistant
34	456 S1-12	1.50	Middle resistant	1.56	Middle resistant
35	378 T1-1	1.56	Middle resistant	1.56	Middle resistant
36	F.7307	1.67	Middle resistant	1.67	Middle resistant
37	Padinka	1.70	Middle resistant	1.75	Middle resistant
38	Iulia	1.77	Middle resistant	1.77	Middle resistant
39	4549 W4-03	1.77	Middle resistant	1.77	Middle resistant
40	6022 W2-121	1.78	Middle resistant	1.77	Middle resistant
41	Plai	1.78	Middle resistant	1.78	Middle resistant
42	669 T1-1	1.78	Middle resistant	1.78	Middle resistant
43	219 S3-12	1.80	Middle resistant	1.85	Middle resistant
44	P.K.B. Sunce	1.80	Middle resistant	1.97	Middle resistant
45	Colina	1.90	Sensitive	2.05	Sensitive
46	6508 W2-12	2.20	Sensitive	2.20	Sensitive
47	F.7161 W1-3	2.50	Sensitive	2.80	Sensitive
48	444 T5-3	2.78	Sensitive	2.78	Sensitive
49	143 T1-1	2.98	Sensitive	3.98	Sensitive
50	Libellula	4.20	Sensitive	5.20	Sensitive
		LSD 5%=0.31		LSD 5% = 0.23	

These changes were probably related to fluctuations in the temperatures to which the plants were subjected prior to freezing. During February abnormal high temperatures were registered. These different cultivar responses to fluctuating temperatures may reflect different thermal characteristics of the hardening and dehardening processes for resistant and susceptible cultivars.

Although the genotypes differed in their frost resistance they showed a similar response as concerns relative injury of non-hardened plants (Table 3).

Table 3. Cell membrane stability of 50 winter wheat cultivars

No. Crt.	Genotypes	Plants non-hardened	Plants Hardened 14 days	Plants Hardened 35 days
		% Injury	% Injury	% Injury
1	**2**	**3**	**4**	**5**
1	Odesskaia 51	87.3	28.66	25.10
2	Flamura 85	78.8	29.33	27.35
3	4105 W1-12101	78.8	29.00	27.25
4	Dropia	84.6	28.66	26.63

1	2	3	4	5
5	Fundulea 29	85.00	29.33	27.53
6	AF 93-3	98.20	31.00	29.16
7	AF 92-2	75.20	36.66	31.35
8	AF 92-1	85.14	37.00	30.31
9	7012 W1-1	91.50	35.00	30.40
10	6041 W2-1	99.00	35.66	31.28
11	AF 93-1	84.10	40.00	33.08
12	7019 W1-1	87.20	41.66	35.25
13	AF 93-2	93.00	63.00	32.98
14	AF 92-4	91.20	40.33	32.98
15	Rapid	93.00	37.00	33.77
16	F 7926	84.00	38.00	34.31
17	201 R2-111	87.00	40.66	38.10
18	219 S3-14	82.20	47.00	39.20
19	6555 W1-11	91.50	30.66	33.40
20	Fundulea 4	87.00	36.66	33.52
21	445 T5-4	87.00	43.33	33.06
22	24 T2-2	87.00	35.66	35.41
23	24 T5-1	87.00	36.33	40.50
24	444 T6-2	87.10	39.10	38.25
25	201 R2-112	87.10	59.33	39.51
26	4993 W2-1111	87.00	41.66	42.30
27	808 S3-12	87.00	53.33	40.75
28	24 T7-2	87.00	55.66	35.41
29	143 T3-1	87.00	41.66	41.56
30	Lovrin 50	73.34	59.00	41.36
31	4549 W2-12112	73.34	46.66	45.28
32	F 7905	73.50	45.00	42.10
33	AF 92-3	73.34	41.33	41.33
34	456 S1-12	73.50	48.00	43.23
35	378 T1-1	73.00	36.66	30.32
36	F 7307	73.15	50.00	49.00
37	Padinka	82.34	49.00	48.95
38	lulia	92.68	49.00	48.90
39	4549 W4-03	92.60	45.00	45.17
40	6022-W2-121	92.61	45.77	45.77
41	Plai	92.68	43.66	42.70
42	669 T1-1	92.68	41.00	49.40
43	219 S3-12	93.00	45.00	45.48
44	P.K.B. Sunce	92.68	49.66	40.66
45	Colina	84.34	40.66	40.66
46	6508 W2-12	100.00	36.33	30.77
47	F 7161 W1-3	100.00	45.66	43.66
48	143 T4-1	100.00	41.66	41.56
49	444 T5-3	98.00	43.83	43.33
50	Libellula	100.00	53.10	50.21
		LSD=3.15	LSD=2.45	LSD=2.70

Table 4. Free proline content of 50 winter wheat cultivars

Nr.Crt.	Genotypes	Non-hardened µM Proline/g FW	Hardened at 2°C – 14 days µM Proline/g FW
1	Odesskaia 51	3.50	162.45
2	Flamura 85	3.10	109.03
3	4105 W1-12101	3.75	134.10
4	Dropia	4.10	114.77
5	Fundulea 29	3.20	99.60
6	AF 93-3	3.20	133.40
7	AF 92-2	2.25	115.30
8	AF 92-1	5.20	105.10
9	7012 W1-1	4.25	93.70
10	6041 W2-1	4.50	67.50
11	AF 93-1	4.70	90.10
12	7019 W1-1	3.75	102.15
13	AF 93-2	3.70	50.67
14	AF 92-4	4.05	52.89
15	Rapid	3.25	81.53
16	F 7926	3.20	87.20
17	201 R2-111	3.90	65.30
18	219 S3-14	4.20	71.60
19	6555 W1-11	4.10	73.80
20	Fundulea 4	3.25	83.90
21	445 T5-4	3.20	57.08
22	24 T2-2	3.50	70.50
23	24 T5-1	5.50	60.60
24	444 T6-2	5.20	53.90
25	201 R2-112	3.90	51.50
26	4993 W2-1111	4.10	53.20
27	808 S3-12	4.20	54.25
28	24 T7-2	3.75	54.25
29	143 T3-1	3.20	64.00
30	Lovrin 50	3.90	51.00
31	4549 W2-12112	4.10	57.55
32	F 7905	6.10	55.10
33	AF 92-3	5.75	48.00
34	456 S1-12	4.20	48.80
35	378 T1-1	3.75	57.00
36	F 7307	5.20	60.00
37	Padinka	3.10	42.00
38	lulia	4.10	35.00
39	4549 W4-03	5.20	39.00
40	6022 W2-121	3.25	133.40
41	Plai	4.12	40.00
42	669 T1-1	3.20	34.00
43	219 S3-12	3.70	32.30
44	P.K.B. Sunce	3.10	34.00
45	Colina	3.20	27.00
46	6508 W2-12	3.75	39.00
47	F 7161 W1-3	3.10	37.00
48	143 T4-1	3.50	40.00
49	444 T5-3	3.25	23.00
50	Libellula	3.10	11.70
		LSD 5% = 2.55	LSD 5% = 11.75

The relative injury of cell membranes fluctuated during the hardening period. There was a tendency toward decreasing amount of ions in the effuse and a corresponding decrease in the conductivity during cold acclimation. Two weeks of hardening decreased significantly relative injury at 28–30% for the resistant genotypes while the susceptible genotypes had a relative injury over 60%.

Free proline content in non-hardened plants was very low (3–4.5 µM/g F.W.) in all genotypes (Table 4). After two weeks of hardening the proline content increased in all cultivars. The resistant cultivars accumulated higher levels of proline (80–163 µM Proline/g F.W.) than the susceptible ones (11–27 µM/g F.W.). An exception was the middle resistant 6022 W1-121 variety which had a higher proline content and in the resistant AF 93-2 and AF 92-4 which had a low content of proline.

The levels of free amino acids, especially proline, increased during cold acclimation, this being correlated with frost tolerance in several species (Hömmö, 1994).

The correlation between frost tolerance and proline content of winter wheat after two weeks of hardening in controlled conditions was highly significant ($r = -0.71^{***}$, Table 5). This fact offers a possibility for improving frost tolerance in winter wheat by selecting “high proline plants” by means of *in vitro* technique, as it has been successfully demonstrated (potato: Van Swaaij et al., 1986; barley: Dobslaw and Bielka, 1988; wheat: Tantu and Dörffling, 1991; Dörflling et al., 1993, 1994).

Table 5. Relationship between frost resistance expressed by necrosis index, proline content and relative injury of cell membranes at –10°C of 50 winter wheat cultivars

	% injury (hardened–two weeks)	% injury (hardened–five weeks)	Proline content (hardened–two weeks)
Necrosis index	0.29 y = 3.84x + 35.48	0.69**; y = 8x + 26.35	– 0.71***; y = –39.81x + 124.3
% injury (hardened–two weeks)			– 0.45*; y = – 1.85x + 147.92
% injury (hardened–five weeks)			– 0.67***; y = – 3.20x + 188.7

The results of the conductivity test were correlated with proline content ($r = -0.67^{***}$, Table 5) and with the frost potential in natural conditions ($r = 0.69^{***}$, Table 5).

Conclusions

Frost tolerance of winter wheat hardened in natural conditions and evaluated as necrosis index was correlated with frost resistance of winter wheat hardened in controlled conditions and evaluated by proline content or conductivity test.

The correlation between frost tolerance and proline accumulation in young leaves and during the first phase of hardening offers the possibility of a rapid screening to frost tolerance of new winter wheat cultivars (after only two weeks) eliminating the need for a freezing treatment.

Five weeks of hardening at 2°C and ten hours photoperiod are sufficient for screening the frost tolerance of winter wheat using conductivity test. For treatment temperature was gradually reduced with a cooling rate of 2°C per hour to –10°C and held for 18 hours.

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