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WATER LOSS FROM EXCISED LEAVES IN A COLLECTION OF *TRITICUM AESTIVUM* AND *TRITICUM DURUM* CULTIVARS

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ABSTRACT

Excised leaf water loss has been suggested as a technique to identify cereal genotypes that loose less water through cuticle and incompletely closed stomata, mainly during the night, and are therefore more adapted to dry environments. Initial water content (IWC) and water loss after 4 hours (WL1) and during the period from 4 to 24 hours (WL2) following excision was measured in a collection of forty four cultivars of common wheat (*Triticum aestivum* L.) and durum wheat (*T. durum* L.), including cultivars from different regions and with contrasting performance under drought. Environmental conditions of the two years of study had a large influence on IWC, WL1 and WL2. Differences between cultivars were significant for all parameters, but interaction between cultivars and years was strong for water loss, suggesting that readings from one year might not be meaningful for other environmental conditions. Genotype*Environment interaction for IWC was less important. Common wheat cultivars adapted to dryer conditions generally had lower IWC, while durum wheat cultivars had highest IWC. Water loss during the first 4 hours after excision was negatively correlated, or was not correlated with water loss during the next 20 hours. Water loss through cuticles, as expressed by water loss from excised leaves, did not represent a major mechanism determining known genotypic differences in drought resistance in the field. Further improvements of the techniques for measuring water loss from excised leaves are needed to reduce the interaction with environmental conditions, in order to make this approach more useful for breeding drought resistant wheat.

Key words: wheat, water loss, excised leaves.

INTRODUCTION

Skoss (1955) and Daly (1964) attempted to classify cuticular properties according to climate or habitat, on the basis of increasing xerophytic character or in relation to gross differences in environmental conditions, as for example between sun and shade conditions. Skoss (1955) showed that decreasing soil water potential increased deposition of wax and cutine on the leaves and reduced water loss. Plants growing in environments differing in prevailing humidity exhibit variation in traits associated with regulation of water loss, particularly cuticle and stomata properties (Grantz, 1990). Cuticular permeability has been related to the quantity and quality of cuticular waxes rather than to thickness of the cuticle itself (Schonherr, 1982; Grantz, 1990).

The ability of a plant to survive severe water deficits depends on its ability to restrict water loss through the leaf epidermis after stomata attain minimum aperture. The non-stomatally controlled water loss through the leaf epidermis, named epidermal or residual transpiration may comprise up to 50% of total transpiration in water-stressed wheat plants during the day and 100% during the night. Epidermal transpiration, estimated gravimetrically, on excised leaves has shown promise for differentiating drought resistance among wheat cultivars (Sabour et al., 1997). Several workers have reported the existence of a significant positive correlation between yield and flag water retention in durum wheat (Clarke and McCaig, 1982a).

Studies have indicated that water status of intact leaves (Schonfeld et al., 1988) and excised leaves (Dedio, 1975; Kirkham et al., 1980; Jarad and Konzak, 1983; Clarke and McCaig, 1982b; Winter et al., 1988; Clarke and Townley-Smith, 1986) may be related to drought resistance. Clarke et al. (1989) reported that low rate of water loss from excised leaves was positively correlated with grain yield under drought. The efficacy of

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RWL for selection of durum genotypes for adaptation to dry environments was compared with visual scoring of other traits in approximately 4300 accessions from the ICARDA germplasm collection (Clarke et al., 1991).

Low rate of water loss (RWL) from excised leaves has been suggested for screening wheat (*Triticum* spp.) genotypes for adaptation to dry growing conditions (Clarke, 1992). Balotă et al. (1995) found large variation among Romanian wheat cultivars for cuticular transpiration, cultivars with known good performance under drought having low or medium IWC and low WL.

The objective of this research is to investigate genotypic variation among Romanian and some foreign winter wheat cultivars for excised leaves water loss and to add more information on excised-leaf water loss in hexaploid and tetraploid wheat as a physiological trait related to drought resistance.

MATERIAL AND METHODS

47 cultivars (in 2009) and 50 cultivars (in 2010) from a large collection of wheat cultivars from Romania and other countries, selected to represents several ecotypes, adapted to regions that are contrasting for water availability, were used for studying water loss from excised leaves. Forty four of these cultivars were common to both years and are presented in this paper (Table 1).

Table 1. Origin and characteristics of wheat cultivars included in the study in 2009 and 2010

CULTIVAR	Species	Origin	Status
Plainsman V.	*T. aestivum*	USA	Old cultivar *
MV. Toborzo	*T. aestivum*	Hungary	Released cultivar
MV. Magdalena	*T. aestivum*	Hungary	Released cultivar
MV. Mazurka	*T. aestivum*	Hungary	Released cultivar
Dropia	*T. aestivum*	Romania	Released cultivar
Izvor	*T. aestivum*	Romania	Released cultivar*
Pobeda	*T. aestivum*	Bulgaria	Released cultivar
Evropa90	*T. aestivum*	Serbia	Released cultivar
Skopjanka	*T. aestivum*	Serbia	Released cultivar
Bankuti 1201	*T. aestivum*	Hungary	Old cultivar
MV16	*T. aestivum*	Hungary	Released cultivar
Flamura 85	*T. aestivum*	Romania	Released cultivar
Fundulea 4	*T. aestivum*	Romania	Released cultivar
Lovrin 34	*T. aestivum*	Romania	Released cultivar
Elida	*T. aestivum*	Serbia	Released cultivar
Balada	*T. aestivum*	Moldova	Released cultivar
Milenka	*T. aestivum*	Serbia	Released cultivar
Radika	*T. aestivum*	Serbia	Released cultivar
Jiana	*T. aestivum*	Romania	Line
TX86A5606	*T. aestivum*	USA	Near isogenic line
TX86A8072	*T. aestivum*	USA	Near isogenic line
TX88A6880	*T. aestivum*	USA	Near isogenic line
Apullicum	*T. durum*	Bulgaria	Old cultivar
Gergana	*T. durum*	Bulgaria	Released cultivar
F00030G	*T. aestivum*	Romania	Line
F05503G	*T. aestivum*	Romania	Line
Giura 31-4	*T. aestivum*	Romania	*Aegilops* introgression line
Alex	*T. aestivum*	Romania	Released cultivar
00X0090-54	*T. aestivum*	USA	Breeding line*
Dacia	*T. aestivum*	Romania	Released cultivar
Ceres	*T. aestivum*	Romania	Released cultivar
Fundulea 29	*T. aestivum*	Romania	Released cultivar
Litera	*T. aestivum*	Romania	Released cultivar
Miranda	*T. aestivum*	Romania	Line
Monada	*T. aestivum*	Romania	Line
Arieşan	*T. aestivum*	Romania	Released cultivar
Apache	*T. aestivum*	France	Released cultivar
Ain Abid	*T. aestivum*	Alger	Released cultivar
Hiddab	*T. aestivum*	Alger	Released cultivar
Bidi17	*T. durum*	Alger	Local cultivar*
M.Ben Bachir	*T. durum*	Alger	Local cultivar*
Vitron	*T. durum*	Spain	Released cultivar
Murga	*T. aestivum*	Mexic	Line
CMSS99Y03439	*T. aestivum*	Mexic	Line

*) previously reported as drought resistant

This collection included:

- 16 Romanian cultivars with various performance under drought, from Izvor described as having best drought resistance among Romanian wheat cultivars to Fundulea 4, adapted to a more humid climate (Mustățea et al., 2009; Săulescu et al., 2006);
- 3 near-isogenic lines developed at Texas A&M University, characterized as different in their performance under drought (Balota et al., 2008);
- 2 *Triticum aestivum* and 3 *Triticum durum* spring cultivars grown on significant acreage in various regions of Algeria, under diverse conditions of water stress (Boufenar and Zaghouan, 2006; Younes, 2009);
- Plainsman V., an old US cultivar, and 00X0090-54 a Kansas breeding line, described as drought resistant (Farshadfar et al., 2001; Sears, R.G., *personal communication*);
- several cultivars from Hungary, Serbia and Moldova, assembled in a collection for studying stress resistance, in the frame of SIERANET project.

Six flag leaves for each replication were detached from field plots and the excised leaves were transported to the laboratory within 30 minutes and weighed to obtain the initial water content (IWC).

Leaves were then wilted for 4 hours under laboratory conditions (20°C, in the dark), and weighed to obtain W_{4h}. Water loss after 4 hours of wilting was obtained using the formula:

$$WL_{4h} = (IWC - W_{4h})/DW$$

Leaves were then wilted for other 20 hours at 20°C and re-weighed to obtain W_{24h}. After that leaves were oven-dried at 70°C to obtain the dry weight (DW).

Water loss during the period between hour 4 and hour 24 was estimated using the formula:

$$WL_{4\text{-}24h} = (W_{4h} - W_{24h})/DW$$

where DW is the dry weight.

RESULTS AND DISCUSSION

The initial water content of freshly harvested leaves was significantly influenced by both years and cultivars. The interaction between cultivars and years was also significant, but the effect of cultivars was significant when tested both against the error and the interaction variance (Table 2).

Table 2. ANOVA for initial water content of wheat leaves

Source of variation	df	MS	F (against IA)	F (against error)
Cultivars	43	6511.30	**2.05**	**15.41**
Years	1	140334.24	**44.30**	**332.10**
IA Cultivars*Years	43	3167.43		**7.49**
Error	440	422.56		

F values written in bold are significant at P<0.05.

Average initial water content was slightly higher in 2009 than in 2010 (235.4% and 200.4) and varied among cultivars from 198.4 to 290.8 in 2009 and from 157.6 to 257.9 in 2010 (Table 3).

Lowest initial water content was found mainly in US Great Plains cultivars (the Texas lines, the Kansas line 00X0090-54, the cultivar Plainsman V., two CIMMYT lines, as well as in three Romanian cultivars. All of these have been previously described as having relatively good performance under drought.

Highest initial water content was found in three durum wheat cultivars, with various performance under drought (Bidi 17 and Apullicum described as drought resistant and Gergana described as less resistant), as well as in a French and two Hungarian common wheat cultivars. Highest initial water content among Romanian cultivars was found in Fundulea 4, previously described as being less resistant to drought.

Therefore, one can notice a tendency for cultivars more adapted to drought to have a lower water content of fresh leaves.

Table 3. Initial water content of wheat leaves (% from dry matter)

No.	Cultivar	Initial water content (% from dry matter)		
		2009	2010	Average
22	TX88A6880	203.9	164.2	184.1
20	TX86A5606	198.4	172.3	185.3
29	00X0090-54	211.4	164.6	188.0
33	Litera	208.6	170.9	189.8
44	CMSS99Y03439	193.6	186.1	189.8
1	Plainsman V.	228.6	157.6	193.1
21	TX86A8074	210.7	182.6	196.6
19	Jiana	208.2	186.2	197.2
43	Murga	209.5	185.4	197.4
6	Izvor	235.4	166.3	200.9
8	Evropa90	238.8	168.3	203.5
12	Flamura 85	227.7	179.9	203.8
34	Miranda	207.9	205.8	206.8
26	F05503G	240.1	175.7	207.9
41	Mohamed B. Bachir	234.3	182.2	208.3
32	Fundulea 29	228.3	194.0	211.1
16	Balada	232.1	190.6	211.3
11	MV16	221.9	204.3	213.1
42	Vitron	245.1	185.9	215.5
36	Arieşan	229.0	208.2	218.6
18	Radika	218.1	222.3	220.2
17	Milenka	216.5	225.0	220.8
5	Dropia	239.6	202.0	220.8
28	Alex	238.5	203.9	221.2
31	Ceres	222.5	220.4	221.5
35	Monada	235.8	207.6	221.7
38	Ain Abid	232.2	212.3	222.3
10	Bankuti 1201	218.1	226.4	222.3
25	F00030G	247.4	198.3	222.9
27	Giura 31-4	233.8	212.4	223.1
14	Lovrin 34	250.0	198.6	224.3
39	Hiddab	249.3	201.3	225.3
9	Skopjanka	264.7	187.6	226.2
30	Dacia	235.8	216.5	226.2
2	MV Toborzo	271.7	189.6	230.6
7	Pobeda	238.2	231.0	234.6
13	Fundulea 4	274.3	195.3	234.8
15	Elida	235.9	234.3	235.1
4	MV. Mazurka	258.8	219.6	239.2
3	MV. Magdalena	258.0	220.7	239.4
37	Apache	262.3	228.4	245.4
24	Gergana	272.0	235.0	253.5
40	Bidi17	278.3	239.4	258.9
23	Apullicum	290.8	257.9	274.3
	Average	235.4	200.4	217.9
	LSD 5%	24.0	23.0	23.5

Water loss during the first 4 hours after leaf excision, as well as water loss during the next 20 hours, were very much influenced by years, but also by cultivars and the interaction between cultivars and years (Table 4). The effect of cultivars was significant when tested against error, but not when tested against the interaction between cultivars and years.

Excised leaves lost on average 32.2% from their initial water content in the first 4 hours after excision and 49.8% during the next 20 hours, i.e. a total of 82.0% from the initial water content in 24 hours.

The average total loss during 24 hours was similar in the two years (82.6% in 2009 and 81.3% in 2010). However, the water loss rate varied from one year to another, the loss during the first 4 hours being only 23.3% in 2009 and 42.5% in 2010, while the loss during the next 20 hours was 59.3% in 2009 and only 39.8 in 2010 (Table 5).

There were significant differences between the tested cultivars for water loss in both intervals and in both years, but these differences are generally not consistent in the two years of testing and do not seem to be associated with known differences in drought resistance. For example, water loss during first 4 hours was not significantly different in the Romanian cultivars Fundulea 4 and Izvor, known for their contrasting behaviour under drought, and the US cultivar Plainsman V. had higher water loss than the French cultivar Apache. These results suggest that water loss through cuticles as expressed by water loss from excised leaves, does not represent a major mechanism determining genotypic differences in drought resistance in the field.

Our data suggest that differences between cultivars in water loss from excised leaves are influenced by their initial water content, but this influence was dependent on environment. In 2010 the initial water content was significantly correlated with water loss in the first 4 hours after excision, while in 2009 the initial water content was significantly correlated with water loss during the interval from 4 to 24 hours (Table 6 and Figures 1 and 2).

Table 4. ANOVA for water loss from excised leaves

Source of variation	df	Water loss during first 4 hours			Water loss during the next 20 hours		
		MS	F (against IA)	F (against error)	MS	F (against IA)	F (against error)
Cultivars	43	3460.307	1.159241	**18.17433**	1173.004	0.715837	**5.221662**
Years	1	137540.3	**46.07753**	**722.3936**	516465.2	**315.1778**	**2299.06**
IA Cultivars*Years	43	2984.976		**15.67779**	1638.647		**7.294485**
Error	440	190.3953			224.6419		

F values written in bold are significant at $P<0.05$.

Table 5. Water loss from excised leaves, expressed as percentage from the initial water content

No.	Cultivars	2009			2010			Average		
		% WL 4h	% WL 4-24h	% total WL	% WL 4h	% WL 4-24h	% total WL	% WL 4h	% WL 4-24h	% total WL
40	Bidi17	14.8	47.7	62.5	51.3	33.7	84.9	31.6	41.2	72.9
8	Evropa90	20.8	66.4	87.1	25.6	29.1	54.7	22.8	50.9	73.7
4	MV. Mazurka	14.3	56.4	70.7	46.4	31.3	77.7	29.1	44.9	73.9
9	Skopjanka	16.4	57.2	73.6	45.1	29.1	74.3	28.3	45.5	73.9
3	MV. Magdalena	14.7	57.2	71.9	40.9	36.5	77.5	26.8	47.7	74.5
23	Apullicum	16.9	48.7	65.6	50.8	34.3	85.1	32.8	42.0	74.8
24	Gergana	14.9	54.0	68.9	47.3	35.7	83.0	29.9	45.5	75.4
19	Jiana	23.8	58.0	81.9	30.3	37.9	68.2	26.9	48.5	75.4
18	Radika	21.1	55.6	76.7	30.9	44.2	75.1	26.0	49.9	75.9
13	Fundulea 4	18.9	55.9	74.8	31.6	48.5	80.1	24.2	52.8	77.0
15	Elida	20.1	57.0	77.1	43.4	36.6	80.0	31.7	46.9	78.6
33	Litera	21.7	61.0	82.7	28.3	45.4	73.7	24.7	54.0	78.7
29	00X0090-54	21.0	59.1	80.2	32.5	44.9	77.3	26.0	52.9	78.9
14	Lovrin 34	18.0	60.5	78.5	34.9	45.9	80.7	25.5	54.0	79.5
2	MV. Toborzo	17.9	62.8	80.7	29.3	48.5	77.8	22.6	56.9	79.5
42	Vitron	18.3	57.4	75.7	52.8	32.5	85.4	33.2	46.7	79.8
32	Fundulea 29	20.8	60.8	81.6	39.9	38.2	78.0	29.6	50.4	80.0
44	CMSS99Y03439	26.3	62.0	88.3	26.6	44.9	71.5	26.4	53.6	80.1
43	Murga	22.6	58.2	80.9	25.9	53.4	79.4	24.2	56.0	80.2
25	F00030G	20.8	61.3	82.1	44.7	36.7	81.4	31.5	50.4	81.8
38	Ain Abid	20.9	60.1	81.0	31.0	52.3	83.3	25.7	56.4	82.1
41	Mohamed B. Bachir	21.2	55.9	77.0	30.8	58.3	89.1	25.4	56.9	82.3
37	Apache	24.0	60.9	84.9	36.5	43.1	79.7	29.8	52.6	82.4
7	Pobeda	20.5	59.4	79.9	56.4	28.7	85.1	38.2	44.3	82.5
39	Hiddab	28.8	60.9	89.6	29.3	46.9	76.3	29.0	54.6	83.7
31	Ceres	22.0	63.0	85.0	52.2	30.7	83.0	37.0	47.0	84.0
6	Izvor	21.2	65.5	86.8	27.0	53.2	80.2	23.6	60.4	84.0
21	TX86A8074	23.0	59.7	82.8	47.3	38.9	86.2	34.3	50.1	84.4
17	Milenka	32.8	58.2	91.0	41.2	37.2	78.3	37.1	47.5	84.5
26	F05503G	23.7	63.4	87.1	36.7	46.8	83.5	29.2	56.4	85.6
34	Miranda	23.3	63.8	87.1	58.4	26.6	84.9	40.7	45.3	86.0
16	Balada	30.4	58.2	88.6	34.5	48.3	82.7	32.2	53.7	86.0
1	Plainsman V.	32.1	54.8	86.9	32.9	52.1	85.0	32.4	53.7	86.1
36	Arieşan	29.0	60.7	89.7	57.1	25.4	82.5	42.4	43.9	86.3
22	TX88A6880	24.2	60.4	84.5	45.6	43.2	88.9	33.7	52.7	86.5
28	Alex	27.0	60.7	87.8	60.8	24.9	85.8	42.6	44.2	86.8
20	TX86A5606	29.0	59.0	88.0	38.1	48.0	86.1	33.3	53.9	87.1
5	Dropia	20.9	66.9	87.8	59.8	27.1	86.9	38.7	48.7	87.4
12	Flamura 85	37.7	52.0	89.6	39.2	46.9	86.1	38.4	49.7	88.1
27	Giura 31-4	26.6	63.9	90.5	59.5	26.0	85.5	42.3	45.8	88.1
11	MV16	33.0	58.9	91.9	38.8	45.5	84.3	35.8	52.5	88.3
35	Monada	28.9	61.9	90.8	65.2	22.2	87.4	45.9	43.3	89.2
30	Dacia	28.5	63.5	91.9	67.7	20.8	88.5	47.3	43.0	90.3
10	Bankuti 1201	33.7	60.4	94.1	64.4	27.1	91.5	49.4	43.4	92.8
	Average	23.3	59.3	82.6	42.5	38.8	81.3	32.2	49.8	82.0
	LSD 5%	6.0	9.8	8.2	7.3	7.1	7.2	6.3	8.1	7.7

Table 6. Correlation between initial water content and water loss of excised leaves

	2009	2010	Average
Water loss during the first 4 hours	-0.22	**0.71**	**0.45**
Water loss during the next 20 hours	**0.70**	0.01	**0.41**

Correlation coefficients written in bold are significant at $P<0.05$.

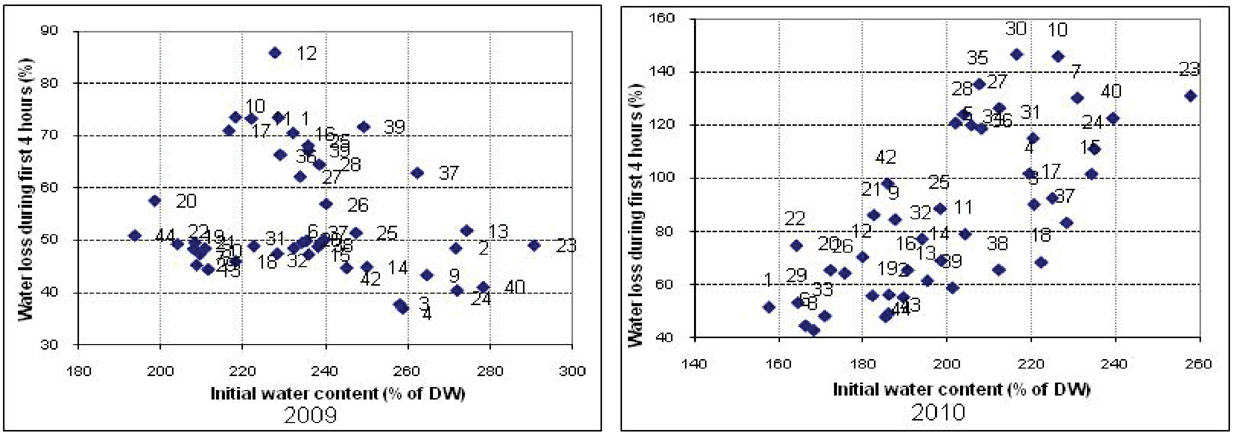

Figure 1. Relationship between initial water content and water loss during the first 4 hours after excision

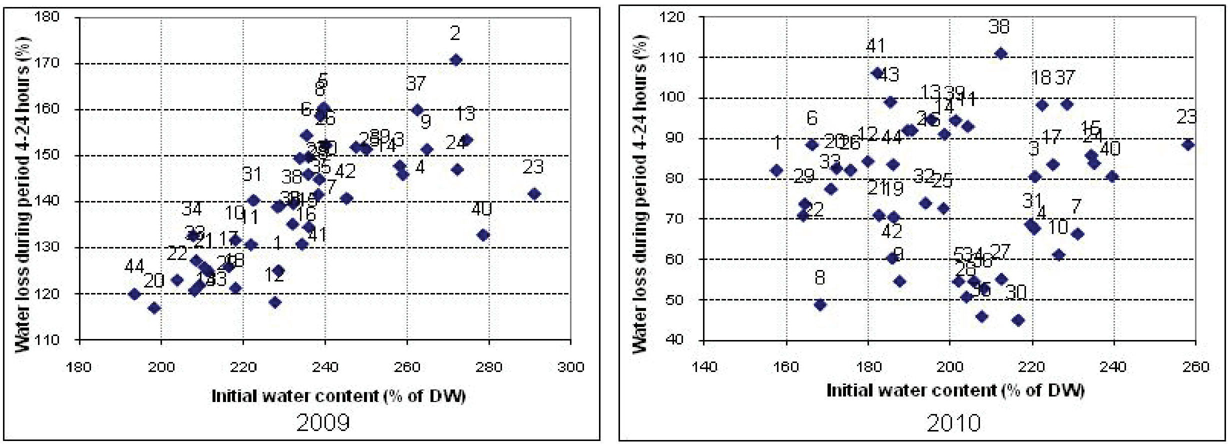

Figure 2. Relationship between initial water content and water loss during the period from 4 to 24 hours after excision

Cultivars that lost more water during the first 4 hours after excision generally lost less water during the next 20 hours of wilting. The negative correlation between these readings being significant in 2010 and on average for the two years (Figure 3).

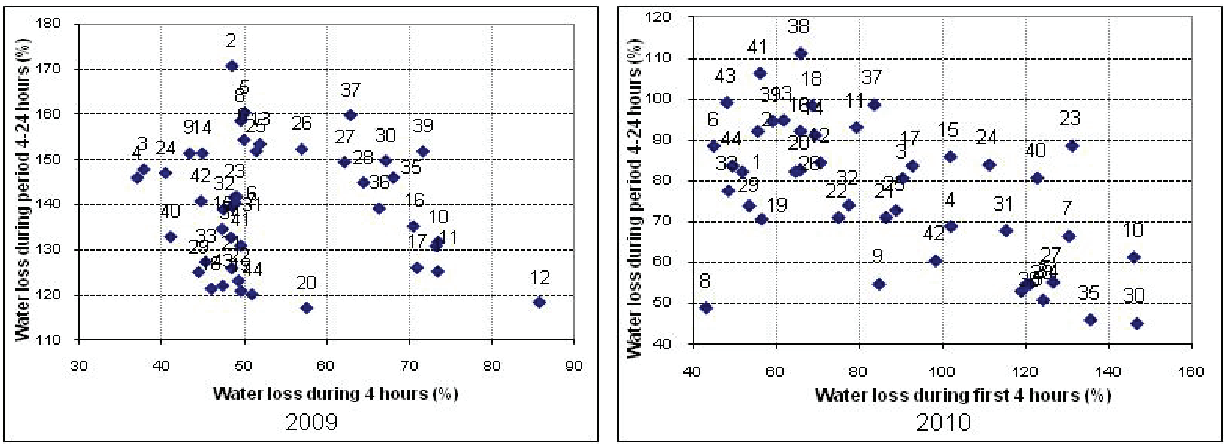

Figure 3. Relationship between water loss during first 4 hours after excision and water loss during the following 20 hours after excision

CONCLUSIONS

Environmental conditions of the two years of study had a large influence on the water content of freshly harvested leaves and on water loss from excised leaves.

Differences between cultivars from a diverse collection of common and durum wheat were significant for initial water content and for water loss from excised leaves after 4 hours and during the next 20 hours.

Interaction between cultivars and years was strong for water loss, suggesting that readings from one year might not be meaningful for other environmental conditions. Further improvements of the techniques for measuring water loss from excised leaves are needed to reduce the interaction with environmental conditions.

Genotype*Environment interaction for initial water content was less important, Common wheat cultivars adapted to dryer conditions generally had lower initial water content, while durum wheat cultivars had highest initial water content.

Water loss during the first 4 hours after excision was negatively correlated, or was not correlated with water loss during the next 20 hours.

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