African Journal of Biotechnology Vol. 10(21), pp. 4450-4456, 23 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2173
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Variation for carbon isotope ratio in a set of emmer (*Triticum dicoccum* Schrank) and bread wheat (*Triticum aestivum* L.) accessions

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Accepted 12 January, 2011

A high level of drought tolerance is an important objective in breeding new generation wheat varieties. A group of six landraces of the emmer wheat (*Triticum diccocum* Schrank), as well as two landraces, two old cultivars and two modern varieties of bread wheat (*Triticum aestivum* L.) were evaluated for their ability to discriminate ^{13}C, a trait related to stomatal closure and consequently, an important component tolerance to drought. Three emmer wheat landraces and one of the bread wheat landraces has the lowest. Therefore, emmer wheat may play the role of a donor of the tolerance to drought in wheat breeding programs targetting drought prone conditions.

Key words: Emmer wheat, bread wheat, drought tolerance, carbon isotope ratio, discrimination.

INTRODUCTION

Lack of water is one of the main factors limiting the productivity of crops (Peleg et al., 2005), in particular bread wheat (Hoffmann and Burucs, 2005). The occurrence of serious drought has been relatively low in Europe and has been consequently poorly considered in breeding programs (Bucur and Savu, 2006). Global climate change is modifying the perspectives and water requirements needed now to be considered in the development of a new generation of cultivars. Not only potential yield but also stability of yield under a large range of climatic conditions have to be increasingly taken into account. In important crops such as wheat, a screening of cultivars and landraces leading to an identification of potential donors of drought tolerance is relevant and urgent. This requires a comprehensive exploration of potential genetic resources and an in-depth understanding of their adaptive mechanisms and responses to water stress (Peleg et al., 2005).

Wheat landraces are often tolerant to local stress factors (Ehdaie et al., 1988; Skovmand and Reynolds, 2000; Davood et al., 2004; Reynolds et al., 2007). Emmer wheat landraces were chosen for our research purposes as this wheat species is referred in the scientific literature to be resistant to drought (Zaharieva et al., 2010). Cultivated emmer wheat, *Triticum diccocum* (syn. *Triticum turgidum* L. subsp. *diccocum* (Schrank) Thell.), is a tetraploid species (BA-genomes) belonging to *Triticum* genus (Zaharieva et al., 2010). It is one of the earliest domesticated plants and has been a staple crop over millennia (Nesbitt and Samuel, 1996). The resistance to drought of some landraces of this species is based on the fact that, the growing of emmer wheat has been carried out in less favourable farming conditions and in dry areas (Marconi andZ, 2005). It has been grown, for example, in

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Table 1. List of varieties.

Field number	ECN (identificator)	BCHAR (taxonomical code)	Name of variety	Origin[1]	Botanical clasification[2]
T. dicoccum Schrank					
D1	01C0200117	412064	Horny Tisovnik	CZ	*rufum* subsp. *dicoccum* conv. *dicoccum* SCHUEBL
D2	01C0200947	412048	Ruzyně	un	*rufum* subsp. *dicoccum* conv. *dicoccum* SCHUEBL
D3	01C0201262	412051	Tapioszele 1	un	*serbicum* subsp. *asiaticum* conv. *serbicum* A. SCHULZ
D4	01C0201282	412017	Tapioszele 2	un	*rufum* subsp. *dicoccum* conv. *dicoccum* SCHUEBL
D7	01C0203989	412013	Kahler Emmer	D	*dicoccum* subsp. *dicoccum* conv. *dicoccum*
D10	01C0204501	412013	No. 8909	un	*dicoccum* subsp. *dicoccum* conv. *dicoccum*
T. aestivum L.					
S23	01C0204158	635100	Kundan	IND	var. *meridionale* MANSF.
P2	01C0200051	635104	Rosamova přesívka	CZ	*milturum* (ALEF.) MANSF.
K4	01C0200008	635090	Praga	CZ	*lutescens* (ALEF.) MANSF.
K17	01C0200100	635090	Jara	CZ	*lutescens* (ALEF.) MANSF.
M6	01C0204800	635090	Vánek	D	*lutescens* (ALEF.) MANSF.
M10	01C0204877	635000	SW Kadrilj	S	-

Remarks: [1]Origin: CZ = Czech Republic; D = Germany; IND = India; S = Sweden; un = origin unknown; [2]Clasification according: Dorofeev VF, Filatenko AA, Migušova EF (1980). Opredelitel pšenicy, Leningrad, 105 p.

the extreme montaneous conditions of the Pyrenees and Alps (Bareš et al., 2002), in Italy (Stagnari et al., 2008) and Spain (Zaharieva et al., 2010). Some local forms of this species are still cultivated in Ethiopia (Eticha et al., 2006), the arid climatic areas of the Balkans, Turkey and Caucasus (Reddy et al., 1998) and in the drought prone areas of India and Yemen (Zaharieva et al., 2010).

Because the direct testing of genotypes of crops in dry conditions is quite expensive, other indirect methods of selection and evaluation have been proposed such as the discrimination of $^{13}CO_2$ (Farquhar and Richards, 1984; Ehdaie et al., 1991; Boutton 1991; Farquhar and Loyd, 1993; O´Leary, 1993; Araus et al., 2002). The values of discrimination $^{13}CO_2$ is to possible to present as carbon isotope ratio (δ) (negative value) or carbon isotope discrimination (Δ) (positive value). The discrimination $^{13}CO_2$ may be represented by the difference between ^{13}C and ^{12}C content in the standard and experimental sample. A negative correlation between carbon isotope dicrimination and the efficiency of the integrated transpiration has been reported (Rebetzke et al., 2002). This method has consequently been proposed as an indirect method for estimating water-use efficiency evaluation (WUE) (Araus et al., 2002).

The objective of this study was to explore emmer wheat as a source for improving drought resistance in cultivated wheats. In this paper we report; (1) the results of carbon isotope discrimination, represented by ratio as a tool for the selection of prospective drought resistant varieties and (2) a comparison of carbon isotope ratio between emmer and landraces and modern varieties of bread wheat.

MATERIALS AND METHODS

The plant material used in the present study came from the Gene bank of the Research Institute of Crop Production (RICP) in Prague-Ruzyně (Prague). It comprised six accessions of emmer wheat (*T. dicoccum* Schrank) and two landraces, two old varieties and two modern varieties of bread wheat (*Triticum aestivum* L.). More information about eco-geographical origin are given in Table 1. Varieties were sown in a randomized complete block design on the experimental parcels of the RICP in Prague and the University of South Bohemia in České Budějovice (CB) in 2007, 2008 and 2010. The seeding rate was adjusted to a density of 350 germinable grains per m^2. Years were characterised by higher temperatures compared with the mean year temperatures (Figure 1). 2007 was the warmest year and the highest temperature was registered at the station in Prague in 2008 (higher by 1°C than in an average normal year in July). In April 2007, there was a precipitation deficiency which considerably decreased field emergence. The precipitation was balanced and stable in the following season. The year 2008 was characterised by an average level of precipitation. The year 2010 was characterised by a slightly lower temperature, whereas the precipitation rate in the growing period was above average.

Samples (grains) were taken from fully-ripened harvested plants from small-plot trials. They were dried and they achieved a constant weight. These dry samples were powderized and burnt in an oxygenized atmosphere. The produced gases were separated, cleaned and reduced. The Thermo-conductive detector (TCD) which were in the elementary analyzer, (EuroEA 3028-HT called Eurovector) indicated the total proportion of elements. The separated gases went through the mass spectrometer (IRMS Isoprime) where each gas type containing the isotopes was diverted into the magnetic field according to its molecular weight. The analyses of isotopes, found in the plant samples, were carried out with the following instruments: Eurovector elemental analyser for IRMS (EuroEA 3028-HT) and Isotope ratio mass spectrometer (IRMS Isoprime). The international standards, PDB for $^{13}C/^{12}C$,

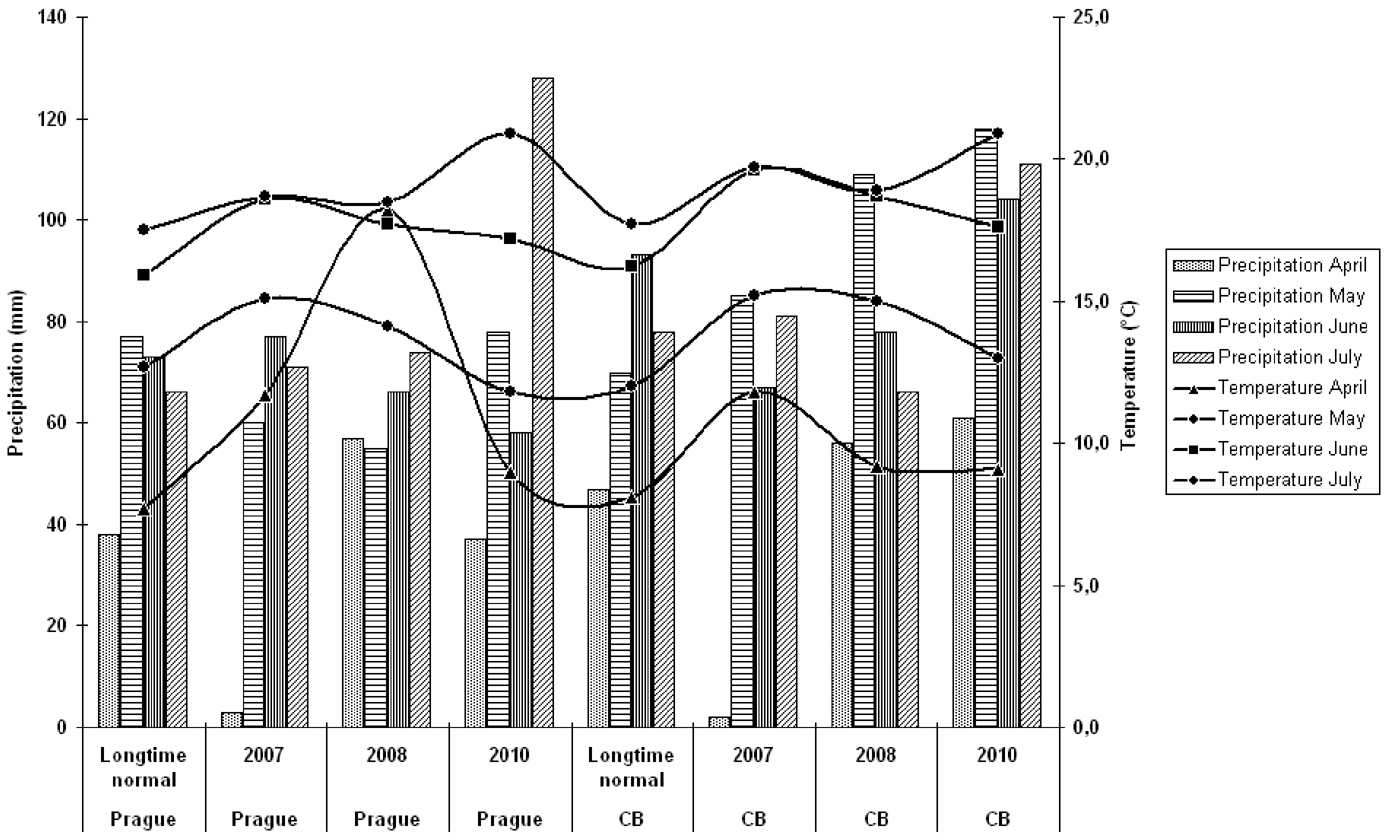

Figure 1. Precipitation (mm) and temperature (°C) characteristics of the experimental stations.

were applied to the indication of isotopes in the samples. $\delta^{13}C$ (‰) characteristic was used as an indicator of ^{13}C discrimination; the values were compared with the standard (Pee Dee limestone from South Carolina was compared with $^{13}C/^{12}C$ = R standard = 0.011237 (= 1.1237 %). $\delta^{13}C$ = (R sample/R standard – 1) x 1000 ($\delta^{13}C$ in ‰). $\delta^{13}C$ = -8.0‰ in CO_2 atmosphere, $\delta^{13}C$ = -25 to -30 ‰ in the dry matter of C3 plants). The plants, grown in water-friendly and favourable conditions, are usually characterised by $\delta^{13}C$ ratio close to -30‰.

The statistical data evaluation was carried out via the Statistica 9.0 program. Instruments of basic statistics, analysis of variance and the evaluation of potential influence of factors and interactions, ANOVA was applied there. The LSD (least significant differences), testing statistical method allowed a preliminary mutual comparison of the cultivars and classification or division of the cultivars into statistically different groups according to the attained values.

RESULTS

Lower $\delta^{13}C$ values were noted in the case of the emmer wheat accessions and some bread wheat ones (land-races in particular) (Figure 2 and Table 2). The emmer accessions Ruzyně, Kahler emmer, No. 8909 and the bread wheat landrace Rosamova přesívka had $\delta^{13}C$ values significantly lower than most of the other tested accessions. Conversely, both modern control bread wheat varieties, Vánek ($\delta^{13}C$ = -26.60‰) and SW Kadrilj ($\delta^{13}C$ = -26.80‰), were characterised by the highest $\delta^{13}C$ values (Figure 3).

Locality and year also had a significant influence on $\delta^{13}C$ values. In general, the evaluated varieties had lower $\delta^{13}C$ values (Table 3) at České Budějovice station (CB) in 2007 (Table 2) likely to be related to a lower precipitation rate (Figure 1). Emmer wheat and bread wheat landraces were characterised by a hiher suscpetibility to local conditions in a particular year, whereas the modern bread wheats highly significantly responded to changes in year conditions.

DISCUSSION

Nowadays, emmer wheat is a minor crop, cultivated mainly in isolated marginal areas. Its main value lies in its ability to give good yields on poor soil and its resistance to fungal diseases such as stem rust that is prevalent in wet areas. The fact that some populations have also proven to be particularly tolerant to drought and heat stress (Zaharieva et al., 2010) is one of the arguments to use emmer wheat in breeding programs or to grow it directly. Because of some previous studies with major used tetraploid wheat species, *T. turgidum*, subsp. *durum* and presented its lower predisposition to drought in

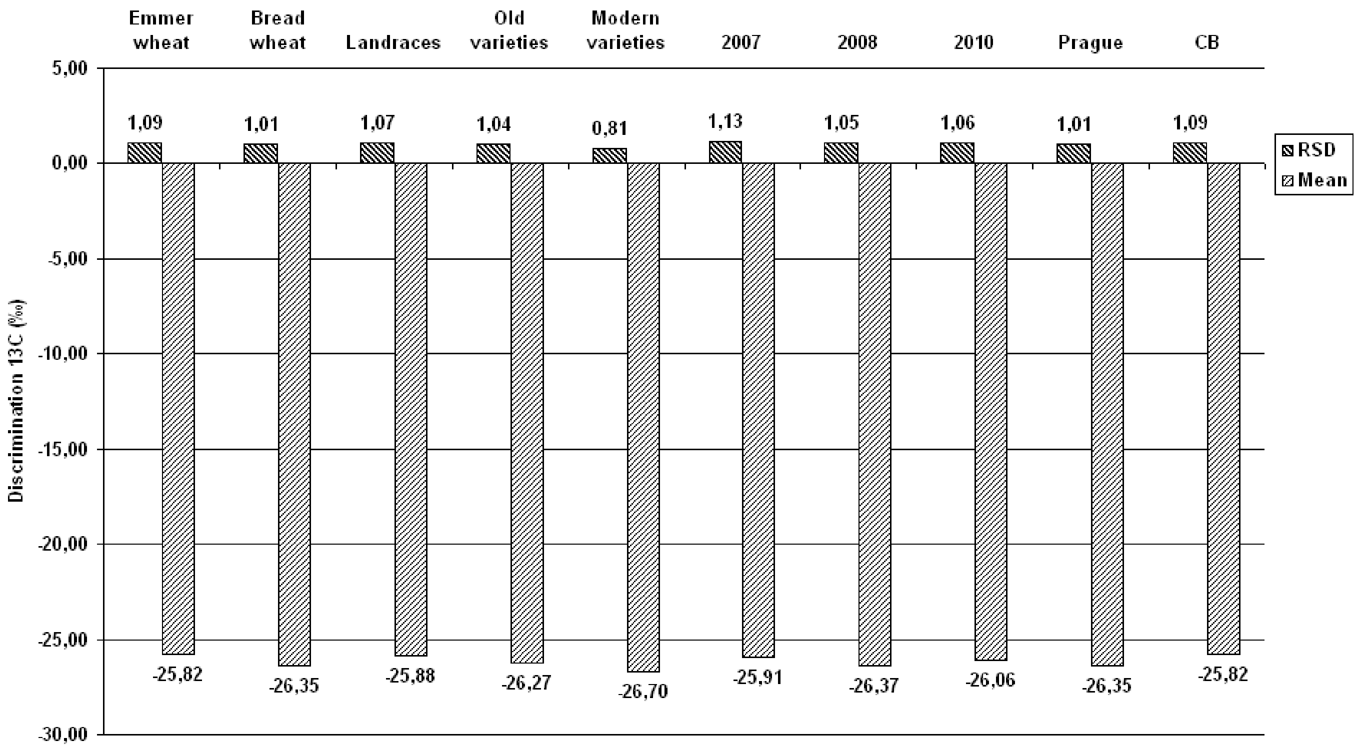

Figure 2. $\delta^{13}C$ (‰), differences in groups of varieties, year and locality.

Table 2. Results of the evaluation of $\delta^{13}C$ (‰) (mean of two replications + SD) and LSD test.

Variety		Mean + SD + LSD*	Locality		Year		
			Prague	CB	2007	2008	2010
Horny Tisovnik	D1	-26.01 ± 0.92 [abc]	-25.62 ± 0.86	-26.39 ± 0.78	-25.39 ± 0.32	-26.19 ± 1.29	-26.43 ± 0.43
Ruzyně	D2	-25.63 ± 1.21 [a]	-24.93 ± 0.96	-26.33 ± 1.06	-24.76 ± 0.28	-25.72 ± 1.80	-26.41 ± 0.26
Tapioszele 1	D3	-26.08 ± 0.96 [abc]	-25.68 ± 0.82	-26.47 ± 0.88	-25.48 ± 0.43	-26.28 ± 1.40	-26.46 ± 0.27
Tapioszele 2	D4	-25.91 ± 1.14 [ac]	-25.10 ± 0.88	-26.72 ± 1.47	-24.70 ± 0.18	-26.18 ± 1.89	-26.87 ± 0.73
Kahler Emmer	D7	-25.53 ± 1.01 [a]	-25.13 ± 0.97	-25.94 ± 0.97	-24.72 ± 0.09	-25.66 ± 1.45	-26.22 ± 0.31
No. 8909	D10	-25.74 ± 1.02 [a]	-25.10 ± 0.89	-26.38 ± 0.60	-25.18 ± 0.62	-25.74 ± 1.47	-26.30 ± 0.05
Kundan	S25	-26.06 ± 1.19 [abc]	-25.80 ± 0.87	-26.33 ± 0.93	-25.01 ± 0.35	-26.35 ± 0.74	-26.83 ± 0.12
Rosamova přesívka	P2	-25.67 ± 1.08 [a]	-25.02 ± 0.95	-26.31 ± 0.82	-24.81 ± 0.45	-25.64 ± 1.37	-26.55 ± 0.30
Praga	K4	-26.46 ± 0.90 [bcd]	-25.84 ± 0.78	-27.08 ± 1.24	-25.20 ± 0.30	-26.97 ± 1.24	-27.22 ± 0.55
Jara	K17	-26.48 ± 0.91 [bcd]	-25.87 ± 0.57	-27.09 ± 0.76	-25.77 ± 0.39	-26.66 ± 1.18	-27.02 ± 0.50
Vánek	M6	-26.60 ± 0.69 [bd]	-26.45 ± 0.66	-26.76 ± 0.73	-26.22 ± 0.20	-26.09 ± 0.22	-27.50 ± 0.30
SW Kadrilj	M10	-26.80 ± 0.93 [d]	-26.31 ± 0.75	-27.29 ± 0.86	-26.00 ± 0.32	-26.90 ± 1.19	-27.50 ± 0.23
	Mean		-26.35 ± 1.01	-25.82 ± 1.09	-25.91 ± 1.13	-26.37 ± 1.05	-26.06 ± 1.06

Remark: * statistically significant $0.01 \leq p \leq 0.05$.

comparison to diploid and hexaploid wheat (Khazaei et al., 2009; 2010), that is why we evaluated tetraploid emmer wheat.

Utilization of bread wheat landraces is limited by their low productivity rate (Ehdaie et al., 1988, 1991). Generally, besides the resistance to serious wheat diseases and some specific parameters of production, a higher adaptibility to changing environmental conditions and local stress factors such as drought is one of the reasons for growing and use bread wheat landraces in breeding programs (Ehdaie et al. 1988; Skovmand and Reynolds, 2000; Reynolds et al., 2007).

Drought tolerance is subject to a complexity of features. Nevertheless, the regulation of gas exchange through

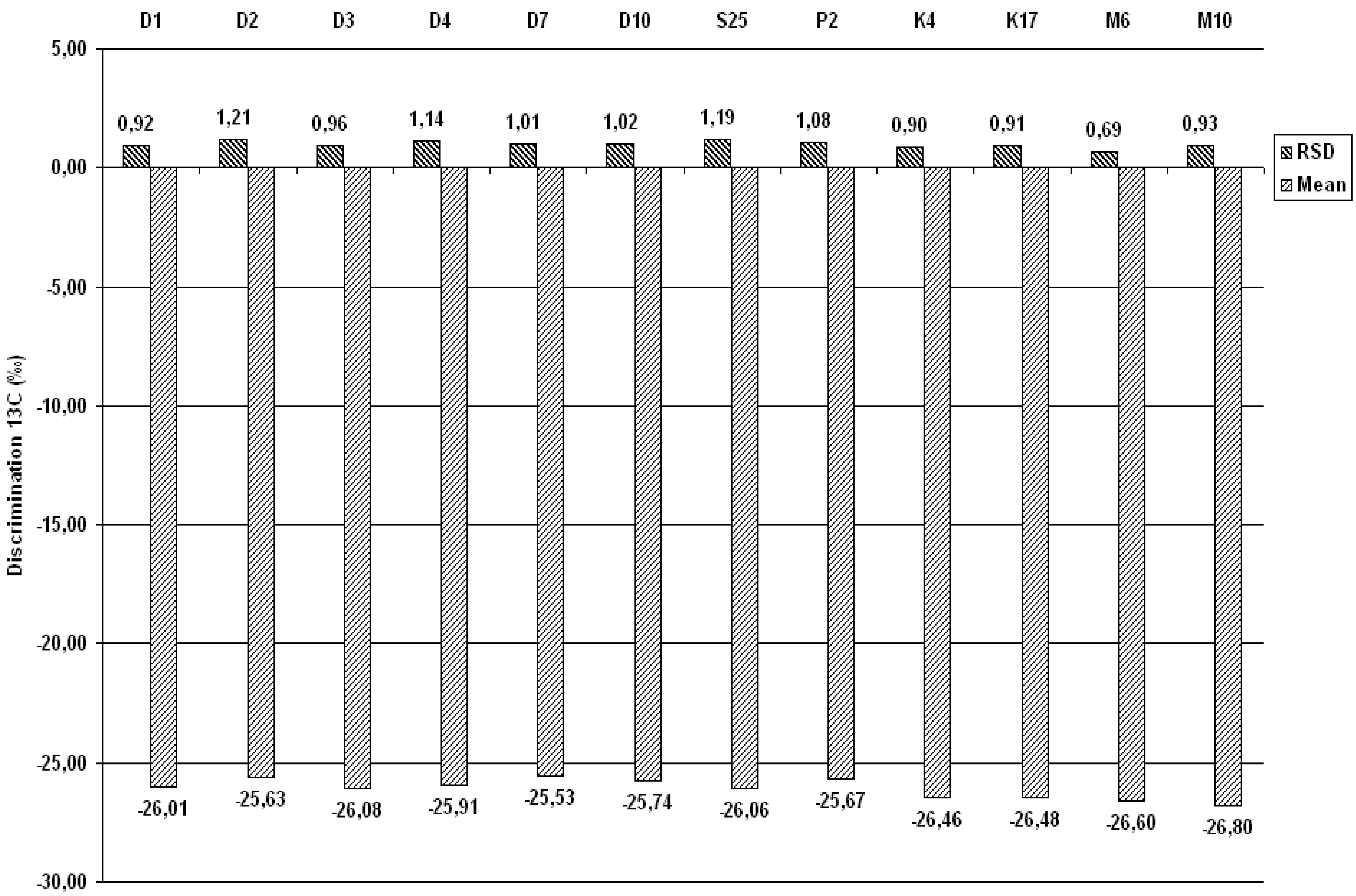

Figure 3. $\delta^{13}C$ (‰), differences in varieties.

Table 3. Examination of the contribution of factors (variety, locality, year) and its interactions to $\delta^{13}C$ via the analysis of variance (ANOVA).

Factor		Emmer wheat			Bread wheat			Landraces			Old varieties			Modern varieties		
		DF	MS	TV (%)	DF	MS	%TV	DF	MS	TV (%)	DF	MS	TV (%)	DF	MS	TV(%)
Variety	(1)	5	0.84	1.14	5	3.05	5.45	7	0.81**	0.88	1	0.00^{ns}	0.00	1	0.34**	2.47
Locality	(2)	1	33.18	44.88	1	23.13	41.30	1	40.26**	43.78	1	13.56**	53.51	1	3.74**	27.20
Year	(3)	2	18.51	25.04	2	23.26	41.54	2	27.89**	30.33	2	9.00**	35.52	2	6.18**	44.95
1x2		5	0.63	0.85	5	0.76	1.36	7	0.67**	0.73	1	0.00^{ns}	0.00	1	0.98**	7.13
1x3		10	0.35	0.47	10	0.68	1.21	14	0.33**	0.36	2	0.69**	2.72	2	0.88**	6.40
2x3		2	20.09	27.17	2	4.83	8.63	2	21.62**	23.51	2	2.03**	8.01	2	0.79**	5.75
1x2x3		10	0.29	0.39	10	0.26	0.46	14	0.34**	0.37	2	0.02^{ns}	0.08	2	0.81**	5.89
error		72	0.04	0.05	72	0.03	0.05	96	0.03	0.03	24	0.04	0.16	24	0.03	0.22

Remark: * statistically significant $0.01 \le p \le 0.05$; ** statistically highly significant $p \le 0.01$; ^{ns}not significant.

stomata plays a major role (Gloser and Prášil, 1998). Boutton (1991), Farquhar and Loyd (1993), O´Leary (1993), Monneveux et al. (2005) and Ferrio et al. (2007), postulated that the methods based on carbon isotope discrimination can provide useful information on stomatal regulation. The results of our research showed that, the tested emmer wheat varieties were characterised by the lower values of $\delta^{13}C$. Concerning the whole period of the three-year trials and both localities, the difference between the lowest value of $\delta^{13}C$, Kahler emmer ($\delta^{13}C$ = -25.74‰) and the highest one, Tapioszele 1 ($\delta^{13}C$ = -26.08‰), was almost 0.55‰. However, important differences in drought tolerance were detected among emmer wheat landraces. The values of $\delta^{13}C$ were also strongly influenced by the rate of the precipitation each year and locality. The varieties with lower δ^{13}C values in arid conditions (in 2007) also had lower δ^{13}C values in wet conditions (2010 was a year characterised by an abundance of precipitation).

Among the tested and evaluated bread wheat varieties, the bread wheat landrace Rosamova Přesívka, was classified in the same statistical group as the emmer wheat varieties. Although, Rosamova přesívka is a landrace coming from central Europe, which was not affected by such a serious drought in the past as it is nowadays (Nicolescu et al., 2010; Bucur et al., 2010), this intermediate form was characterised by a high rate of tolerance to drought. Davood et al. (2004) state a quite high rate of drought tolerance in the wheat landraces. Experiments carried out on landraces and modern semi-dwarf varieties of wheat confirm that, landraces are adapted to the regular types of stress occuring in their growing areas. Landraces, when confronted to drought are usually characterised by a higher level of production of plant matter (Skovmand and Reynolds, 2000) probably because they have better ability to absorb water through the root system (Reynolds et al., 2007). Kundan, the second evaluated landrace has a slightly higher δ^{13}C value. The differences in the rate of drought tolerance between the wheat landraces are also demonstrated in the study by Ehdaie et al. (1988), who found many more or less tolerant genotypes among the landraces and bread wheat varieties.

The bread wheat varieties Praga and Jara released during the last mid-century and the modern varieties SW Kadrilj and Vánek had higher δ^{13}C values. The breeding of spring wheat forms is not strongly focused on tolerance to drought in our country.

Conclusions

The evaluation of the group of landraces, obsolete cultivars and modern emmer and bread wheat varieties, demonstrated that most emmer wheat varieties as well as one bread wheat landrace had lower δ^{13}C. The results of the trials and studies show that, emmer wheat is an interesting crop for growing in dry conditions. Certain genetic resources may also serve as donors of drought tolerance in the breeding process. The results also show that, the indirect δ^{13}C method may be used for the screening of the large complex accessions of genetic resources.

ACKNOWLEDGEMENTS

This work was supported by the research project no. MSM 6007665806 of The Ministry of Education, Youth and Sports of the Czech Republic and the research project no. NAZV QH82272 of the National Agency for the Agricultural Research of the Ministry of Agriculture of the Czech Republic.

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