EVALUATION OF CELL MEMBRANE INJURY IN CARAWAY (*CARUM CARVI* L.) GENOTYPES IN WATER DEFICIT CONDITIONS

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ABSTRACT

The aim of the study was to evaluate the resistance of caraway genotypes to water deficit based on the estimation of cell membrane stability (CMS) in leaves using polyethylene glycol (PEG) test. In 2007 and 2008, 25 selected caraway genotypes, originating from European botanical gardens (18), cultivars (2) and our own breeding strains (5), were tested. The plant material was collected from the experimental field. The obtained results showed highly significant differences in cell membrane injuries (p=0.001) among investigated genotypes.

The rank of genotypes in membrane injury index in 2007 was similar to that of 2008. Caraway genotypes originating from Warsaw (49.4%), Cracow (45.3%), Reykiavik (39.9%), Berlin (23.8%), Wisley (22.7%) and strains 9/2 (23.7%), 60/8 (22.2%) exhibited a high level of injury, which showed weak CMS and their high sensitivity to drought. The lowest extent of membrane injury was observed in genotypes originating from Bayreuth (4.2%), Ulm (4.4%), Cluj (5.5%), Lousanne (6.8%) and cultivar 'Kończewicki' VI/4 (6.2%), which proves low sensitivity of these genotypes to water deficit and cell membrane stability. These genotypes may be used in further breeding program to improve caraway resistance to drought.

KEY WORDS: caraway, genotypic variability, membrane injury, PEG test.

INTRODUCTION

Caraway (*Carum carvi*) is one of the most important medicinal plants in Poland cultivated over an area of 8000 ha. The yield of caraway fruit depends on the size of leaves rosette and root thickness reached in the first year of its vegetation. Fertilization and soil's moisture are the major factors that affect proper formation and growth of these parts of the plant (Węglarz 1998). Due to poor rainfall for the last few years in Poland the moisture in the soils has decreased and that has affected growth and yielding of many crops, including caraway (Ryszkowski et al. 1996). Drought affects almost all plant functions and is one of the major factors limiting growth and crop production every year in many parts of the world (Ludlow and Muchow 1990; Hall 2001; Farooq et al. 2009). Caraway breeding program should be focused on obtaining new cultivars resistant to drought, which may improve its yields in water-limited conditions what is very important from the economic point of view (Toxopeus and Lubberts 1998). This new cultivar should perform high productivity and stable yield during droughts. Perfect genotype combines resistance to water deficit with good yield in drought conditions (Cativelli et al. 2008).

Water deficit causes changes in almost all the cell processes, which affect plant growth and development (Bray 1997; Chaves et al. 2003; Farooq et al. 2009). The first visible reaction to water deficit is the loss of turgor caused by cell dehydration. Dehydration causes a series of membrane modifications, which results from a slow down in lipid biosynthesis and acceleration of break-up (Pham Thi et al. 1990). These modifications occur mainly in drought sensitive plants and lead to a loss of semipermeable properties of the cell membrane, which is the main reason of the metabolic injuries developed in water stressed plants (Simon 1974; Pham Thi et al. 1985; Hubac et al. 1989).

Therefore, the integrity and stability of cell membranes (CMS) in water deficit conditions can be considered a possible adaptive value indicative of stress resistance (Blum 1996; Dhanda et al. 2004). Cell membrane stability may be determined through estimation of the extent of cell membrane injury in desiccated leaf-tissue fragments. This technique is based on desiccation of leaf fragments in vitro with a polyethylene glycol solution (PEG) and subsequent measurement of electrolyte leakage into aqueous medium (Sullivan 1971). The method can be used for a large number of samples and may be applicable to rapid evaluation of drought resistance in a large number of genotypes.

In the current study CMS was evaluated in the collection of caraway genotypes of different origin. Our objective was to identify genotypic variability of this trait and to use it to select the most promising genotypes in further breeding program to improve caraway resistance to drought.

MATERIALS AND METHODS

The investigated caraway collection consisted of 25 objects: 18 populations originated from botanical gardens of: Bayreuth, Berlin, Bonn, Cluj, Göttingen, Jena, Cracow, Lousanne, Nantes, Poznań, Prague, Reykiavik, Riga, Salzburg, Ulm, Warsaw, Wisley, Wroclaw. Moreover, two cultivars 'Rekord' (Czech Republic) and 'Kończewicki' represented by 2 strains (6, 7) and four strains 9/2, 9/7, 9/12, 60/8 were obtained in the Institute of Medicinal Plants of Poznań.

The plant material was collected from the experimental field in 2007 and 2008. However, in 2008 there was lack of the following genotypes: Warsaw, Cracow, Wisley, strains 9/7, 60/8 and strain nr 7 of 'Kończewicki' cultivar, because of lack of seed germination. The collection was established on field plots without repetitions. The number of plants in the investigated objects oscillated from 20 to 100. In April, caraway seeds were sown in a greenhouse and then 5-10--leaves plantlets were planted out in 45×45 cm spacing. In July, when plants had developed full rosette, one leaf from each plant (five plants for each object) was collected to determine cell membrane stability (CMS). From each compound leaf, eight central leaflets were collected and used for estimation.

Membrane injury was measured according to the method described by Sullivan (1971). This technique is a modification of the method developed by Dexter et al. (1932). Leaflets were washed three times in 10 cm^3 of de-ionised water in order to remove surface adhered electrolytes, and put into 50 cm^3 flask. Four leaflets were submerged in 10 cm^3 of 20% PEG 600 solution (water deficit) and kept at temperature of 10°C for 24 h. Next, four leaflets were submerged in de-ionised water (control) and kept at temperature of 10°C for 24 h. Then, both the groups of leaflets were quickly washed three times with de-ionised water and finally submerged in 10 cm^3 of water. All samples were kept at temperature of 10°C for 24 h and then warmed to 25°C, shaken and the electrical conduction of effusion was measured. The tissues were killed by autoclaving for 15 min, cooled down to 25°C, and electrical conduction of the effusion was measured once again. Membrane injury (%) was evaluated as an injury index in percentage, according to the Sullivan (1971) formula:

$$MI = 1 - \frac{1-(T1/T2)}{1-(C1/C2)} \times 100,$$

where $C1$ and $C2$ represent conductivity values of the control samples before and after autoclaving, respectively; $T1$ and $T2$ represent conductivity values for PEG treated samples before and after autoclaving, respectively.

A two-way analysis of variance was carried out to determine the effects of genotypes, years and the genotypes × years interaction on the variability in cell membrane injury. The least significant differences for cell membrane injury were calculated. Homogeneous groups for cell membrane injury were determined on the basis of least significant differences by Duncan's test (Duncan 1955). The relationship between cell membrane injury in 2007 and 2008 was estimated using a correlation coefficient. Coefficients of variation of cell membrane injury for each genotype were calculated. The broad-sense heritability coefficients were calculated (Falconer and Mackay 1996). Data were analyzed using the statistical package GenStat v. 7.1. (Payne et al. 2003).

RESULTS

Two-way variance analysis (Table 1) showed a significant effect of all the differential factors (genotype, year) and their interaction. There were highly significant differences in cell membrane injuries (p=0.001) among investigated genotypes (Table 2). The highest cell membrane injury was noticed in leaves that came from plants originating from: Warsaw (49.4%), Cracow (45.3%), Reykiawik (39.9%), Berlin (23.8%), strain 9/2 (23.7%), Wisley (22.7%) and strain 60/8 (22.2%), which proves high sensitivity of these genotypes to water deficit, whereas the leaves of the following genotypes: from Bayreuth (4.2%), Ulm (4.4%), Cluj (5.5%), cultivar 'Kończewicki' VI/4 (6.2%) and Lousanne (6.8%) showed the lowest index of injury and their cell membranes exhibited greater stability. These genotypes can be recognized as highly resistant to water deficit at the cell level in the investigated caraway collection. In general, in 2008 (av.=9.91%) the lower index of cell membrane injury for most genotypes was noticed compared to 2007 (av.=18.93%).

In the second year (2008) of investigation the objects were more similar to each other – there were three homogeneous groups marked, though, in 2007, there were seven groups. Both in each year of the investigations and for two-year means, strains 9/2, 60/8, 'Kończewicki' VI/4, 'Kończewicki' VII/4 and populations from Prague, Poznan, Wi-

TABLE 1. Analysis of variance for cell membrane stability.

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F-statistics
Genotypes	24	33778.43	1407.43	35.59***
Years	1	2145.5	2145.5	54.26***
Genotype × Years	18 (6)	2246.55	124.81	3.16***
Residual	172 (28)	6801.19	39.54	
Total	215 (34)	37499.99		

*** $p<0.001$

() – the number of missing observation

TABLE 2. Mean values and coefficients of variation [%] of caraway cell membrane stability in 2007 and 2008.

Genotypes	2007		2008		Mean values[2]
	Mean	CV[1]	Mean	CV[1]	
Riga	24.782cde	75.11	18.25bc	21.39	21.516defg
Warsaw	49.446g	14.45	*	*	49.446i
Cracow	45.256fg	14.29	*	*	45.256hi
Reykiavik	48.98g	5.57	30.982c	8.953	39.981h
Nantes	29.384e	40.8	10.49ab	37.52	19.937cdefg
Salzburg	27.858de	41.84	11.818ab	38.71	19.838cdefg
Berlin	34.548ef	22.37	13.03ab	4.427	23.789g
9/2	23.708cde	22.01	*	*	23.708g
9/7	14.902abcd	35.92	12.154ab	17.23	13.528abcdef
9/12	13.52abc	23.43	8.82ab	31.89	11.17abc
60/8	22.228bcde	50.8	*	*	22.228efg
‘Kończewicki’ VI/4	7.64a	41.54	4.736a	54.35	6.188ab
‘Kończewicki’ VII/4	12.456abc	58.87	*	*	12.456abcd
Prague	8.334a	39.74	8.334ab	39.74	8.334ab
Poznan	15.644abcd	77.41	13.104ab	29.09	14.374bcdefg
Wisley	22.732bcde	62.2	*	*	22.732fg
Göttingen	10.234ab	38.43	7.092ab	34.01	8.663ab
Wroclaw	7.402a	84.05	5.162a	39.28	6.282ab
Bayreuth	5.942a	58.32	2.466a	20.6	4.204a
‘Rekord’	8.056a	36.24	6.553ab	18.28	7.3047ab
Cluj	7.898a	27.08	3.092a	40.1	5.495ab
Jena	6.484a	34.07	7.713ab	44.91	7.099ab
Ulm	5.166a	31.8	3.62a	26.97	4.393a
Lousanne	6.686a	8.885	6.942ab	56.97	6.814ab
Bonn	13.892abc	58.18	11.798ab	23.41	12.845abcde
Generally	18.927B	80.52	9.915A	70.7	14.985

$LSD_{0.001}$ for Genotypes: 9.415
$LSD_{0.001}$ for Years: 2.663
$LSD_{0.001}$ for Genotypes × Years: 13.315
[1] – CV – coefficient of variation
[2] – means followed by the same letters are not significantly different

sley, Göttingen, Wroclaw, Bayreuth, ‘Rekord’, Cluj, Jena, Ulm, Lousanne and Bonn assembled in a homogeneous group. The analysis of correlation for means between years showed that differences in the extent of damage of cell membranes among studied genotypes ranged in both years similarly. Statistically significant ($p<0.001$) correlation of mean values of stability of membranes in individual years of the investigations ($r=0.873$) was found (Fig. 1).

Considerable genotype variability in cell membrane injury was observed. Thus, variability coefficients of the observed trait were 80.52 and 70.70 respectively for 2007 and 2008 year. As for variability of individual genotype, a large range of variability coefficients was obtained: from 5.57% (Reykiavik) to 84.05% (Wroclaw) in the first year of the experiment and from 4.427% (Berlin) to 56.97% (Lousanne) in the second year. The population that came from Reykiavik deserves special consideration; it showed low variability of the observed trait in both years (Table 2).

Heritability of cell membrane stability in the second year was somewhat lower ($h^2=0.49$) than in the first year ($h^2=0.57$).

DISCUSSION

Considerable variability in cell membrane injury (4.2--49.4%) in desiccated leaf-tissue of the collected caraway genotypes indicated their different membrane injury. Genetic variability of membrane injury, measured with this technique, was also revealed in barley, wheat, maize, sorghum and orchard-grass (Blum and Ebercon 1981; Premachandra and Shimada 1987a-c; Premachandra and Shimada 1988; Premachandra et al. 1989; Bandurska and Gniazdowska-Skoczek 1995). Sullivan and Ross (1979) showed that the degree of cell membrane stability in water deficit conditions in sorghum correlated well with the stress tolerance of other plant processes such as proteins and enzyme resistance to denaturation, chloroplast activity and the maintenance of photosynthesis by intact plant tissue. Moreover, CMS measured in naturally dehydrated excised wheat leaves was compatible with the CMS measured by means of PEG test (Premachandra and Shimada 1988). On the basis of relationships among physiological traits such as root-to-shoot length ratio, osmotic potential, leaf water content CMS is an important trait in predicting the drought resistance of a particular genotype. The usefulness of polyethylene glycol (PEG) test for assessing drought resistance was examined by many authors (Blum and Ebercon 1981; Premachandra et al. 1989, 1990; Dhanda et al. 2004). So, the differences in CMS of the examined caraway genotypes, which imply the differences in resistance to water deficit at the cell membrane level, may be useful for predicting their resistance at the plant level.

Cell membrane sensitivity to dehydration depends on its chemical structure, which is largely genetically determined (Pham Thi et al. 1990). However, this trait can be modified

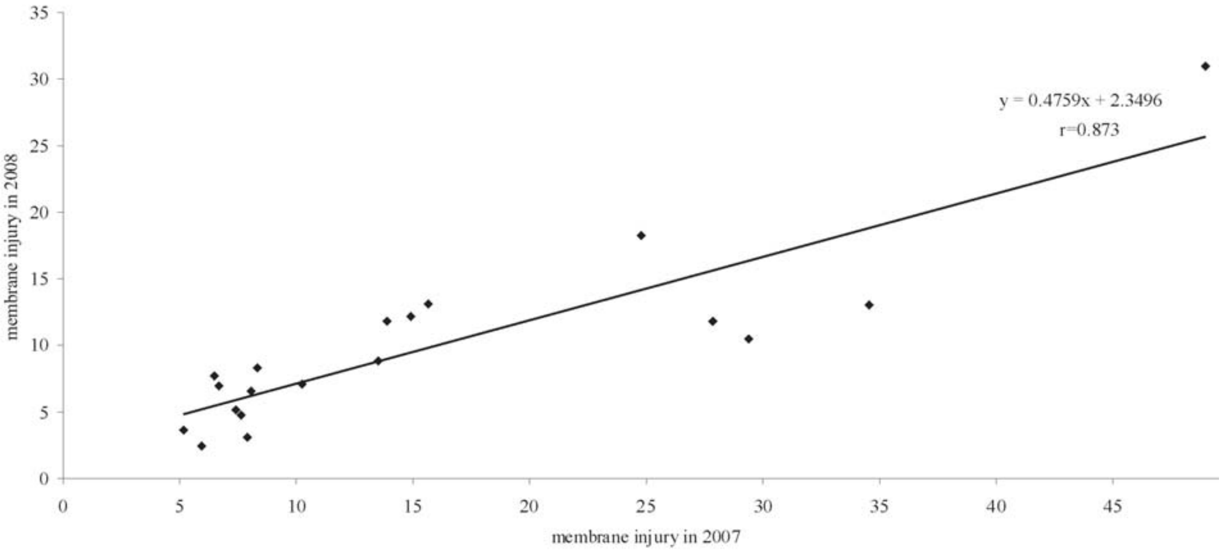

Fig. 1. The relationship between cell membrane injury in 2007 and 2008.

by different factors. The injury value obtained in PEG test was dependent on the growth stage of the plant as well as the position of the leaf on the stem (Blum and Ebercon 1981; Premachandra and Shimada 1987a; Bandurska and Gniazdowska-Skoczek 1995). So, it is important to collect for measurements leaves of the same physiological age. The plants cultivated in moderate drought conditions were characterized by a lower degree of cell membrane injury than well watered ones (Blum and Ebercon 1981; Premachandra and Shimada 1987a; Bandurska and Gniazdowska-Skoczek 1995; Dhanda et al. 2004). This phenomenon is called the cell membrane adjustment to water stress. In the present study, the mean membrane injury index for the examined caraway collection was reduced in 2008 in comparison with 2007. It should be noticed that the extent of cell membrane injury decreased especially in genotypes characterized by high index (over 20%) of injury. The observed differences in the extent of injury index between 2007 and 2008 are probably an effect of different growth conditions. The major difference between these two years is a very favourable water regime and air temperature in 2007 in comparison with low rainfall and higher temperature in 2008. The worse moisture conditions in 2008 probably created better resistance to water deficit, which was manifested by the lower membrane injury. Blum and Ebercon (1981) noticed a similar reaction in different wheat genotypes.

The investigated caraway genotypes differed in terms of susceptibility to water deficit at the cell membrane level. These differences might be determined by genetic traits such as membrane chemical structure or by their acclimatization ability. However, much more useful for caraway breeding program are those genotypes which are characterized by a genetically determined high cell membrane stability index.

Caraway genotypes with low mean object-oriented values and high values of variability coefficients are very interesting. Such kinds of genotypes are very suitable as material for promising hybrids in further breeding program. In our experiments such genotypes are the population from Wroclaw and the cultivar 'Kończewicki' VI/4, with their very high variability coefficients, particularly the population from Wroclaw in the first year of the experiment. However, populations with the lowest variability were characterized by large values of the observed trait (Reykiavik). Genotypes with a low value of the index and a low value of variability coefficient are the other interesting group of the objects. In this case, it can be expected that genotypes will exhibit stability in further years of experiment, although such kinds of genotypes were not observed in the presented experiments. The population from Ulm was interesting because its variability coefficient was not the lowest, but moderate in both years of the experiment.

Heritability of CMS was not very high, so further investigation of the tested caraway genotypes should be continued to extend the knowledge about *Carum carvi* L.

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