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SUPEROXIDE DISMUTASE IN SPRING BARLEY CARYOPSES

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Abstract

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Superoxide dismutase (SOD) activity was determined in caryopses of spring barley grown in field trials in 2004–2006. A total set under study included five malting varieties with hulled grain, three waxy hull-less and hulled varieties (of US origin), seven lines formed by crossing of the above given varieties and four hull-less lines of Czech origin. SOD activity was determined by a modified method using a Ransod diagnostic kit (RANDOX). The method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. Statistically significantly higher activity was measured in the variety Nordus (131 $U.g^{-1}$ d.m.) and line ME1 (128 $U.g^{-1}$ d.m.) compared to the other varieties/lines (66–111 $U.g^{-1}$ d.m.). The line ME1 had significantly higher SOD activity in grain versus its parental varieties Kompakt (83 $U.g^{-1}$ d.m.) and Krona (78 $U.g^{-1}$ d.m.). The results of this study proved the availability of varieties/lines with a higher SOD content, the antioxidant effect of SOD can improve quality of beer and food made from barley.

Keywords: antioxidant, enzyme, superoxide dismutase, SOD, hull-less, spring barley, Ransod

INTRODUCTION

In 1969, McCord and Fridowich (1969) discovered metaloenzyme superoxide dismutase (SOD, E. C. 1.15.1.1), to be a highly active defense system effective against free oxygen radicals.

Superoxide dismutase acts as an antioxidant (Fridovich, 1986), its primary function is to convert dangerous superoxide free radicals to oxygen and less reactive hydrogen peroxide (Bamforth, 1983; Fridovich, 1995; Halliwell, 2002; Hasan and Fridovich, 1981; Johnson and Giulivi, 2005; Meng *et al*., 2007; Packer, 2002). Its main role is to protect cellular membranes against free radical damage protecting thus plants especially under unfavorable growing conditions.

SOD was found nearly in all aerobic organisms (McCord *et al*., 1971), in some aerotolerant anaerobes (Tally *et al*., 1977) and obligatory anaerobes (Hewit and Morris, 1975). Presumably, longer living animals have a high level of this enzyme, the highest level of SOD being detected in humans (Johnson and Giulivi, 2005).

In the barley caryopsis, the highest SOD activity was found in the embryo, lower quantity in the aleurone layer. In oats, a higher rate of the total SOD activity (62%) was detected in the endosperm, while in maize, the embryo contains even ten-time higher SOD level than the endosperm (Giannopolitis and Ries, 1977). According to this study, SOD is present in roots, shoots, seeds of wheat, maize and peas. SOD in grain and green malt is relatively thermally stable but mashing destroys it. Quantity of SOD in barley grain differs depending on the variety and locality (Bamforth, 1983; Belcrediová *et al*., 2006; Havlová, 1999). In addition, activity of this enzyme may be affected by the change of malting conditions. SOD activity increases with the number of days of germination to the fourth or fifth day of germination and from the sixth day the values slightly decline or do not change any more (Bamforth, 1983; Havlová, 1999). Meng *et al.* (2007) reported that SOD activity

also declined with a longer steeping time of barley and higher temperature of malt kilning. Clarkson and Large (1992) found that SOD activity after kilning of malt for lager beer was twice as high as in pale malt and Boivin *et al.* (1993) stated that also special malts and mainly chocolate malt exhibited a strong antioxidant activity.

The activity of SOD in barley grain was measured in the range of 73–115 $U.g^{-1}$ d.m. (Havlová, 1999; Boivin, 2001). Activity in malt was higher, in the range of 88–154 $U.g^{-1}$ d.m. (Belcrediová *et al.*, 2006). The activity of this enzyme (428–494 $U.g^{-1}$ d.m.) was found the highest in barley green biomass (Březinová Belcredi *et al.*, 2007). Boivin (2001) suggested that SOD from barley could be used as an antioxidant in facial cosmetics.

The activity of SOD is stimulated by an increased superoxide production, which may be reflected in changes of oxidation parameters. A small amount of superoxide formed during malting generates a highly reactive hydroxyl radical (Meng *et al.*, 2007). This hydroxyl radical is considered a principal initiator of harmful effects on the biochemical systems of cells; it reduces stability of organoleptic beer characters (Bamforth and Parsons, 1985). The presence of superoxide anion radical in a barley caryopsis or malt and beer may affect lipide peroxidation, polysaccharide degradation, enzyme inactivation and it can result in the reduction of yeast vitality, decline in colloidal stability, change of color, formation of undesirable beer off-flavors developed during storage (Boivin, 2001; Havlová, 1999; Meng *et al.*, 2007). SOD content in grain is sufficient for the prevention of free radicals during germination, drying and malting (Bamforth and Parsons, 1985). Reduced SOD activity results in the insufficient removal of the superoxide, which leads to damage of the organism by reactive oxygen species (Racek and Holeček, 1999). Březinová Belcredi *et al.* (2007) studied the effect of application of zinc in a form of zinc sulphate and zinc oxide on SOD activity in barley grain.

MATERIAL AND METHODS

Material

Samples of two-rowed spring barley used can be split into four groups:

Malting varieties: donors of high yield level with hulled grain registered in the Common Catalogue of Varieties of Agricultural Plant Species (*Amulet, Kompakt, Krona, Nordus,* and *Tolar*).

Genetic resources: *Wabet*, *Wanubet,* and *Washonubet* (further only resources) are donors of so-called waxy type of starch (i.e. non-standard rate of amylase and amylopectin) with high contents of β-glucans and vitamin E. Donors of the waxy type of starch were obtained with kind help of Prof. C. W. Newman (Monatana State University, Bozeman, MT USA). These three resources are waxy isolines of the variety *Betzes*.

I: *List of lines and abbreviations*

Lines	Abbreviation
Kompakt x Krona	ME1
Krona x Kompakt	ME2
Kompakt x Wabet	ME3
Wabet x Kompakt	ME4
Krona x Wanubet	ME5
Wanubet x Krona	ME6
Wabet x Washonubet	ME7

Lines formed by crossing of the two above given groups of donors: lines (of F_8–F_{10} generation) of spring barley, bred at the Department of Crop Sciences, Plant Breeding and Plant Medicine, Faculty of AgriSciences, Mendel University in Brno (MENDELU, CR). The malting varieties (*Kompakt, Krona*) and waxy resources (*Wabet, Wanubet, Washonubet*) were used as parental materials. List of the lines is given in Tab. I.

Hull-less KM lines for food and feed use: materials denoted as *KM* (*KM2084, KM2283, KM1057, AF Lucius*) were bred at the Agricultural Research Institute in Kroměříž and its subsidiary Agrotest fyto, within the research projects of MA CR – NAZV as the initial genetic resources of hull-less barley for the final diversified use of grain. The line *KM1910* was registered for growing in the CR and EU in 2009 under the name *AF Lucius* (a legally protected variety).

Description of the Experimental Locality

In 2004–2006, seeds were sown in plots in a field experiment with a randomized block design in three replications in the MENDELU farm in Žabčice. This experimental farm is situated in the maize production area at an altitude of 184 m above sea level. The weather in this area is warm, with a mean temperature of 9.3 °C and the average temperature during the vegetation period (April–July) 15.3 °C. The locality is moderately dry, with a mean rainfall of 480 mm per year; 221.7 mm during the vegetation period (April–July).

Methods of Determination

All samples were ground on the laboratory mill Super Jolly SJ 500 (Mezos, CR) and homogenized. Milled barley grain (25 g) was added into a container and mashed with 225 ml of deionized water (45 °C) in the mashing device (1-Cube, CR) using the MEBAK method (1997). Subsequently, the samples were cooled to 25 °C for 30 min and filtered for at least one hour. The samples were stored according to Bamforth's method (Bamforth 1983) for 18 h at 4 °C. Afterwards diluted 1:14 in phosphate buffer (0.01 $mol.l^{-1}$, pH 7.0) so that percent inhibition reaction varied between 30–60% of the inhibition on the y-axis of the calibration curve. Next steps were identical both for the prepared samples and standards (S1–S6). The Ransod kit (RANDOX

II: *Analysis of variance and variance components for superoxide dismutase in barley grain (2004–2006)*

Source of variability	d.f.	MS	Variance of components [%]
Varieties/lines	18	2191.1***	46.2
Years	2	2026.0***	6.6
Interactions:			
Varieties/lines × years	36	537.7***	43.0
Error	57	25.1	4.2

Note: ***P ≤ 0.001

Laboratories Ltd., Great Britain) was used according to instruction and was described in Březinová Belcredi *et al.* (2007). Phosphate puffer used to dilute samples of barley grains was used as a reference sample.

The method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The SOD activity was determined by the degree of inhibition of this reaction measured by absorbance at 505 nm and 37 °C.

Calculation of SOD activity and repeatability were published in Březinová Belcredi *et al.* (2007).

Statistical Analysis

The data acquired from the analyses of SOD activity were assessed using the analysis of variance (ANOVA) with dual interactions between the factors using the STATISTICA program version 7.0 (StatSoft, Inc. Tulsa, Oklahoma, USA) and multiple comparison of mean values using Fischer's test (LSD test) at P = 0.05. Genetic variability was calculated from variation rates and this rate was checked with F-test.

Two samples from each variety/line were used for chemical assays each year.

RESULTS

The results were evaluated by the analysis of variance (Tab. II) with a subsequent test of differences of mean values (Tab. II). Variability of SOD activity was statistically highly significantly affected by a genotype, year of growing and genotype/year interaction both in all years and the individual years. As indicated by the calculated variance components (Tab. II), the effect of the variety/line was 46% and the interaction between varieties/lines and year was 43%. Weather in the individual growing years contributed to variability of SOD activity only by 7%. Genetic variability of varieties/lines did not affect SOD activity with

III: *Average activity of superoxide dismutase in barley grain in 2004–2006*

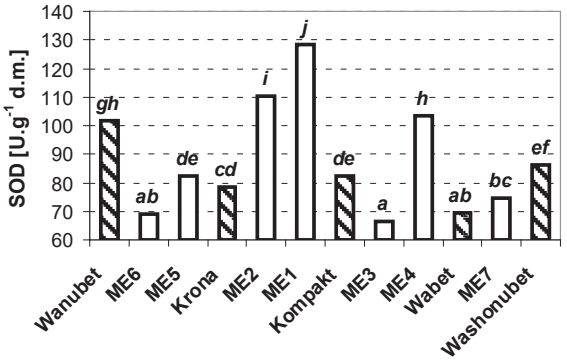
Varieties/lines	SOD [U.g^{-1}]				
	2004	2005	2006	$\bar{x}_{2004-2006}$	± SD
ME3	74.49 ab	61.35 ab	63.46 bc	66.43 a	7.57
ME6	80.81 bc	77.09 cde	49.63 a	69.17 ab	15.87
Wabet	65.71 a	84.22 def	58.81 abc	69.58 ab	12.13
ME7	92.73 de	68.96 bc	61.87 bc	74.52 bc	15.06
Amulet	75.69 ab	78.84 cdef	77.34 d	77.29 cd	2.40
Krona	123.49 i	56.00 a	55.55 ab	78.35 cd	35.39
ME5	88.97 cd	77.75 cdef	80.06 d	82.26 de	8.94
Kompakt	94.69 def	87.52 f	65.28 c	82.50 de	15.38
Washonubet	100.76 efg	75.36 cd	82.50 d	86.21 ef	11.90
KM2084	103.01 efg	87.58 f	79.39 d	89.99 f	11.01
KM2283	109.09 gh	87.63 f	96.73 e	97.82 g	9.97
KM1057	119.12 hi	86.98 ef	98.46 e	101.52 gh	14.74
Wanubet	101.94 efg	106.67 gh	96.52 e	101.71 gh	4.91
ME4	129.32 i	105.24 gh	76.30 d	103.62 h	24.10
Tolar	104.48 fg	100.43 g	111.52 f	105.48 hi	5.73
AF Lucius	105.13 fg	83.73 def	131.19 g	106.68 hi	21.42
ME2	102.67 efg	102.98 gh	125.90 g	110.52 i	12.17
ME1	125.66 i	110.00 gh	149.74 h	128.47 j	18.14
Nordus	120.70 i	112.51 h	161.05 i	131.42 j	23.59

Note: Different letters in table mark statistically significant average values at P = 0.05

respect to the studied years statistically significantly, while genetic variability affected by interactions was statistically highly significant.

In terms of mean SOD activity (for the three studied years, Tab. III), the malting variety *Nordus* (131 U.g^{-1}) and line *ME1* (123 U.g^{-1}) had statistically significantly higher SOD activity compared to all other studied varieties/lines in the set. Statistically significantly lower SOD activity was recorded only in the line *ME3* (66 U.g^{-1}) compared to the other ones, it did not differ statistically significantly only from the line *ME6* (69 U.g^{-1}) and resource *Wabet* (70 U.g^{-1}). The reciprocal lines *ME1, ME2* (128 and 111 U.g^{-1}), and line *ME4* (104 U.g^{-1}) had statistically significantly higher SOD activity compared to the parental varieties *Krona* (78 U.g^{-1}), *Kompakt* (83 U.g^{-1}), and *Wabet* (Fig. 2). The line *ME3* (66 U.g^{-1}) had statistically significantly lower SOD activity compared to the parental variety *Kompakt* but it did not differ significantly from the parental genotype *Wabet* (Fig. 2). SOD activity of the resource *Wanubet* (102 U.g^{-1}) was significantly higher compared to the reciprocal lines *ME5* and *ME6* (82 and 69 U.g^{-1}), the other parental variety *Krona* differed statistically from the line *ME6* (69 U.g^{-1}) but it did not differ from the reciprocal line (82 U.g^{-1}, Fig. 2). The variety *AF Lucius* (107 U.g^{-1}) did not differ statistically significantly from the lines *ME2, ME4, KM1057* (Tab. III), and varieties *Tolar* and *Wanubet* (102–111 U.g^{-1}). The line *KM2084* (90 U.g^{-1}) had statistically significantly lower SOD activity compared to other *KM* lines (107–98 U.g^{-1}) but it did not differ significantly from the resource *Washonubet*. The malting variety *Kompakt* (83 U.g^{-1}) did not differ statistically significantly from the varieties *Krona, Amulet, Washonubet*, and the line *ME5* (86–77 U.g^{-1}). The average SOD activity in the set was 93 U.g^{-1}.

Statistically significantly higher SOD activity was measured in the varieties/lines in 2004 (101 U.g^{-1}) compared to 2005 and 2006 (87 and 91 U.g^{-1}). Based on the Walter-Lieth's climate diagram (Fig. 1), we can assume that a higher SOD activity could be supported by wetter weather in June (the grain filling period) and wetter weather in July 2004



2: *Comparison of the average activity of superoxide dismutase in lines and their parental varieties (2004–2006)*
Note: Different letters in table mark statistically significant average values at P = 0.05

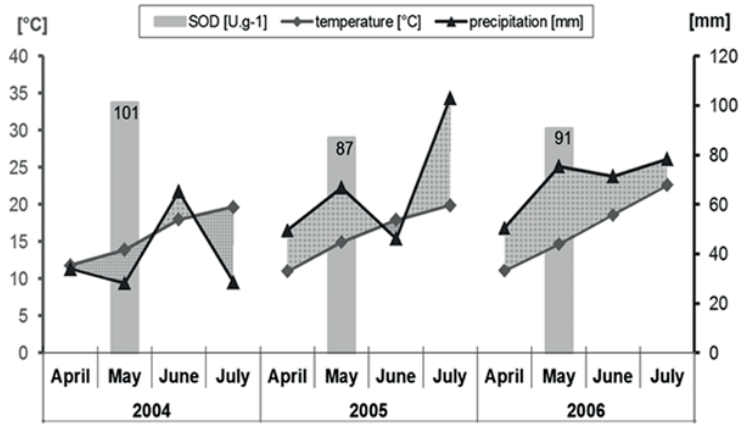
(during ripening). On the contrary, in 2005, June was dry and July wet. In 2005, SOD activity in barley varieties/lines was statistically significantly lower than in 2004 and 2006, this could be caused by a higher precipitation sum during May and July.

Despite the variability caused by the weather conditions, SOD activity was statistically significantly higher in the variety *Nordus* and line *ME1*, from which the line *ME2* did not differ statistically significantly in 2005. Conversely, the resource *Wabet* (Tab. III) belonged to the materials with the lowest SOD activity (50–66 U.g^{-1}) measured in the individual years.

The results of this study proved the availability of varieties/lines with a higher SOD content, the antioxidant effect of SOD can improve quality of beer and food made from barley.

DISCUSSION

Diagnostic kits (Ransod, RANDOX, Great Britain) for the assessment of SOD activity are based on the spectrophotometric determination introduced by McCord and Fridovich (1969). Havlová (1999) compared this technique with the previously used Bamforth's method (Bamforth, 1983) and found that the Ransod set showed by 5–20% higher SOD



1: *Course of weather in the growing period of 2004–2006 and its effect on SOD activity*

activity in the identical samples. Sample preparation was the same for both the methods. The difference in the values of activities was probably caused by a higher sensitivity of the detection system of the Ransod kit based on the formation of formazan compared to the detection system using cytochrom *c* in the original technique. It was also found that cytochrom *c* was not an ideal color indicator and results could be affected by other enzymes such as cytochrom *c* oxidase and cytochrom *c* peroxidase.

The mean SOD activity for the period monitored (2004–2006) was in the range of 66–131 U.g^{-1}. Authors Bamforth (1983), Boivin *et al.,* (1993), Březinová Belcredi *et al.* (2008) reported a slightly higher SOD activity in malt from the varieties/lines (88–187 U.g^{-1}) compared to the SOD activity in grain. SOD activity may rise during germination. Malting probably leads to activation of the antioxidant systems in barley grain (Boivin *et al.,* 1993). According to Bamforth (1983), higher SOD activity was measured in wheat flour and soybean (56 U.g^{-1}, 640 U.g^{-1}, and resp.) compared to barley grain, this was also confirmed by our results. This author determined lower SOD activity in maize and rice flakes (4.3 U.g^{-1}).

Level of SOD activity was affected most significantly by the genetically conditioned variability of plant materials. The highest activity of SOD in barley grain was found in the reciprocal lines of the parental varieties *Kompakt* and *Krona* and the malting varieties *Nordus* and *Annabell*. Our results suggest that SOD activity in barley grain depends on a barley variety; a similar conclusion was also made by authors Boivin *et al.* (1993), Březinová Belcredi *et al.* (2008), Ehrenbergerová *et al.* (2009) and Havlová (1999). Markedly higher SOD activity was measured in a green biomass of three barley varieties (428–494 U.g^{-1}) compared to the grain analyzed in this study. SOD activity depends not only on the genotype but also on the locality and in green biomass on the growing phase and sample preservation (Ehrenbergerová *et al.,* 2009). The comparison of years – course of weather during the growing period – showed that years 2004, 2005 and 2006 differed in SOD activity significantly. The drier weather in 2004 during the grain-filling period and maturation favorably affected SOD activity in ripe caryopses. Besides the effects of years, authors also reported a significant difference in SOD activities in the studied localities Březinová Belcredi *et al.* (2008).

CONCLUSION

The activity and variability of superoxide dismutase in grain of 19 spring barley varieties/lines were determined (2004–2006). The SOD activity was assessed using the Ransod diagnostic set (RANDOX Laboratories Ltd., Great Britain). This kit commonly used for the determination of SOD in blood was modified for the assessment of SOD activity in barley grain. The values of SOD activity are given in grain dry matter.

The results of this study proved the availability of varieties/lines with a higher SOD content, the antioxidant effect of SOD can improve quality of beer and food made from barley.

We can state that in SOD activity, statistically highly significant genotype differences between the spring barley varieties/lines, differences caused by the effect of year and mutual genotype-year interactions were detected. Variability of SOD activity was caused mostly by the barley genotypes (46%), further genotype-year interactions (43%) and to the least extent by years (7%).

The malting variety *Nordus* (131 U.g^{-1}) and line *ME1* (123 U.g^{-1}) had statistically significantly higher average SOD activity compared to the other studied varieties/lines. A significantly higher SOD activity compared to the parental varieties *Krona* (78 U.g^{-1}) and *Kompakt* (83 U.g^{-1}) was detected in the lines *ME2* (111 U.g^{-1}), *ME1* (128 U.g^{-1}), and *ME4* (104 U.g^{-1}). The average SOD activity in the set was 93 U.g^{-1}. Addition of barley varieties or lines with high SOD activity into various antioxidant mixtures indicates that their preventive use can help to prolong life; it may reduce the number of premature disablements and deaths (Boivin 2001, Qingming *et al.,* 2010). Consumption of antioxidants in their natural form is also recommended by the American Heart Association.

Consumption of green biomass of barley rich in antioxidants, barley grain or other intermediate products formed during brewing can thus play a role in healthy nutrition.

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