



**SZENT ISTVÁN UNIVERSITY**

**The effect of ecological factors on the  
technological properties of barley and malt**

Thesis of Ph.D. dissertation

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# Gödöllő

2011.

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• **INTRODUCTION AND AIMS**

Barley is the fourth most abundant crop grown around the world. It is produced for human consumption, animal feed, pharmaceuticals, and alcoholic beverage products (BANTAYEHU, 2009). Significant amount of barley is used for brewing. Two-thirds of malt is made from spring barley, one-third is from winter barley. In Hungary, spring barley is used primarily due to its better chemical indicators. Winter malting barley production is increasingly spreading in Europe. The main reason of this is that winter barley is the most resistant plant to drought. It tolerates very high temperature occurred in recent years, including drought periods and atmospheric drought also (MURÁNYI et al., 2008).

The beer industry has special and strict quality requirements. Barley grown for the purpose of brewing has to meet different requirements such as germination, purity, moisture content, seed size, homogeneity and health. The barley is constantly exposed to biotic and abiotic factors that determine fertility, yield as well as qualitative indicators. The climatic risk can inhibit the production of spring and winter barley. Quality is changing year by year depending on the weather and sowing time. It is therefore necessary to breed resistant varieties that are sufficient to satisfy the needs of the brewing industry.

My aim was to investigate and evaluate the chemical and technological properties of winter barley varieties which have higher yield and are more tolerant to drought and diseases. I made the evaluation depending on the weather. The results were compared with the brewing properties of spring barley strains. During the experiment, two brewing barley varieties (KH KORSÓ and VANESSA) and several strains grown in conventional farming were investigated, in order to determine the suitability for industrial use. I analysed barley seeds, malt and wort, then the results were evaluated. The experimental results showed that between 2005 and 2007, which spring and winter barley strains were inadequate regarding to the brewing properties. Knowledge of these attributes and the interactions between the traits and cropping technologies of winter and spring barley has a very important role for barley breeders and producers.

My additional objective was to provide microbiological results of barley soil and heavy metal tolerance of *Saccharomyces cerevisiae* strains occurred in barley rhizosphere. Soil life and soil biological activity plays important roles in metabolic processes, roundabout systems of elements and prevention of environmental and sanitary problems by the bioaccumulation of heavy metals. During

our research, we determined CO<sub>2</sub> production of rhizosphere microorganisms, soil enzyme activities and occurrence of microorganisms in spring and winter barley rhizosphere.

My research hypotheses were as follows:

- Determination of the effect of ecological changes on barley production, and brewing properties
  - Monitoring and evaluation of the interaction between malting barley varieties and strains occurred in conventional farming and ecological factors.
  - Microbial analysis of winter and spring barley soil (respiration, soil microbiological properties, soil activity) to determine the effects of those ecological factors which influence the rhizosphere.
  - Malting of the selected barley varieties and strains to determine the technological properties of malt.
- Determination of the effect of ecological changes on the brewing properties of malt
  - The physical and chemical analysis of malt according to EBC (European Brewery Convention) regulation to determine the effects of ecological factors and those properties which are influenced by these factors.
  - Assessment of the interaction between the technological properties of malt and weather.
  - Heavy metal tolerance of fermenting *Saccharomyces cerevisiae*.
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## **2. LITERATURE**

- **Effect of ecological changes on barley production, and brewing properties**

Barley, malt and beer properties are affected by a number of environmental / climatic factors (VERMA and NAGARAJAN, 1996). Although all of the properties of brewing barley genotypes are determined by the environmental factors, temperature and rainfall are also determining factors (MOLINA-CANO et al., 1997). The protein content of barley is highly influenced by environmental factors, and this is a problem when it comes to malting barley (BERTHOLDSSON, 1999). 11.5% protein content is difficult to reach, as this property is highly influenced by the environment or other factors of barley production (SMITH, 1990). The weather (GRANT et al., 1991), including drought (BIRCH et al., 1997), and high temperature and drought together (SAVI and NICOLAS, 1996) increases the protein content of barley. Where such circumstances occur, there will be higher protein,

in each variety. Increasing N fertilizing causes higher diastatic power and lower extract content (WANG et al., 2007). QI et al. (2006) and MOLINA-CANO (2000) suggest that N fertilizing correlating with barley total protein content. Effect of N fertilizing on the properties of malt is depending on the traits of the different varieties. It was investigated, that using N fertilizers in different rates, significant differences can be experienced regarding to malt extract, Kolbach, diastatic power, and protein content among varieties, but there were not significant differences regarding to viscosity. It means that higher N fertilizers decreased extract content but increased diastatic power. Regarding to the time of N application, there was significant difference between diastatic power and protein content, but there was not difference among extract content, Kolbach and viscosity (WANG et al., 2007). CHEN et al. (2006) investigated the effect of time of N application on the properties of malt. They suggested that by applying N fertilizers in late periods, quality of malt such as extract content, and Kolbach will be deteriorated, however, diastatic power increases mildly. Barley protein content is depending on the amount of N, however, it is dependent upon the production site and the season (CONRY, 1995; 1997). The form of applied N and its interaction with soil water content is also an important factor (McTAGGART and SMITH, 1992). Drought before flowering decreases N uptake during the vegetation period therefore it decreases yield, however, yield can also be lowered by water deficiency in the root zones (SALEKDEH et al., 2009). Due to the less seeds, more N will be available during grain filling therefore the barley protein content will increase. Weather has the biggest influence on grain filling. Drought occurring in this period can decrease seed sortiment (LEISTRUMAITÉ et al., 2009). Time of sowing has a significant influence on the brewing properties of barley regarding to Kolbach, viscosity, diastatic power and protein content, however, there were not significant correlation regarding to the extract content (WANG et al., 2007). Temperature during earing and grain filling can also influence barley protein content, especially when the daily maximum temperature reaches 32°C (WARDLAW and WRIGLEY, 1994). Yield can be concluded from daily average temperature and relative humidity, protein content can be judged by only the relative humidity.

- **Malting properties of barley**

The effectiveness of malting barley production is primarily determined by the appropriate use of varieties. For barley growers and the barley processing industry, this is the cheapest factor (PSOTA et al., 2009). In order to use malting barley for malting and brewing, a number of quality requirements must be met. Approximately there are 10 to 15 physical and chemical parameters which characterizes barley, malt, or wort. Currently there is not exact wording of the brewing quality of malt. The main reason of this is that beer with different quality parameters are produced around the world (FILICHKIN et al., 2010). Essential task is to compare the new winter malting barley genotypes to the technological properties of spring malting barley. The most widely accepted parameters, which determine the properties of brewing barley: the grain size, grain weight,  $\beta$ -glucan content, protein

content, friability,  $\alpha$ -amylase activity, viscosity, and soluble nitrogen content (FOX et al., 2003). VERMA et al. (2008) suggest that either positive or negative correlation can occur between barley extract (mass per storage, sorting, protein, hull) and malt properties (friability, consistency, wort viscosity, filtration time, Kolbach). Strong correlation was found between extract and friability, but these two parameters are negatively correlated with protein content of barley, the nitrogen content of malt and malt  $\beta$ -glucan content (GIANINETTI et al., 2005). The higher protein content is also linked to other malting traits. Protein content above 11.5% results lower starch content and less alcohol, but less than 9.5% protein causes that the yeasts will not have sufficient amount of N available (PETTERSSON and ECKERSTEN, 2007).

- **Heavy metal uptake by *Saccharomyces cerevisiae***

Environmental pollution as a consequence of industrial processes is one of the most important problems which has to be solved and regulated. (YURTSEVER, et al., 2009). Unfortunately, the industrial activities are coupled with agriculture, because the utilized agrochemicals such as pesticides and fertilizers, organic manures and sewage sludge also present as sources of pollution (TÓTH et al., 2008). Industrial waste water can contain the following heavy metals: Pb, Cr, Cd, Ni, Zn, As, Hg, Cu and Ag (SHAREEF, 2009). It has been reported that *S. cerevisiae* can uptake and accumulate Cu, Cd, Pb, Zn, Cr and Ni ions (CHEN and WANG, 2007). PASTARNAKIEWICZ (2006) investigated *S. cerevisiae* growth on medium treated with cadmium. He found that higher than 50  $\mu\text{M}$   $\text{Cd}^{2+}$  concentration inhibited yeast growth. The inhibition was reduced in presence of  $\text{Ca}^{2+}$  ion. BLACKWELL et al. (1998) found that  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  reduced the toxicity of  $\text{Mn}^{2+}$  ion on *S. cerevisiae* strains.

### 3. MATERIALS AND METHODS

- **Examined samples**

The plant material for winter barley and spring barley were C and D strains, and came from the area of Fleischmann Rudolf Research Institute. The experiment was carried out in random block design, in four repetitions, between 2005 and 2007. The application rate was 4.5 to 5 million/ha. The parcel size was 5  $\text{m}^2$  of winter barley, 10  $\text{m}^2$  of spring barley. One plot consisted 10 rows. Distance between the rows were 11.1 cm, distance between the plants were 2-2.5 cm. For comparison and evaluation of the results, standards were used. In the case of winter barley they were KH KORSÓ and VANESSA, in the case of spring barley they were SCARLETT and PASADENA.

- **Description of the weather in the experimental years**

2005. autumn started with sunny and dry weather. The mean temperature in October ( $11^{\circ}\text{C}$ ) was above the average of many years ( $10.2^{\circ}\text{C}$ ) in Kompolt. Rainfall was less than the average. Average rainfall of 100 years in Kompolt was 43.3 mm, but in this year it was only 14.8 mm. The beginning of November was rather dry, the amount of rain was 33.9 mm in the second half of the month. The first half of December was mild, but from the middle of the month rainy and dry periods was exchanging. The last days of the year were rainy (75.2 mm), which amount was above the average rain of 100 years (40 mm). January and February in 2006 was colder than the average and rainfall was much less in Kompolt than in December. 2006. February was cold ( $-2.6^{\circ}\text{C}$ ), but in the second half warmer period occurred. This month was rainy (50.1 mm). Rainfall was 20 mm more than the average of 100 years. In the winter, extreme conditions did not develop, which would have caused the frost of sowings. In March, mean temperature ( $3.2^{\circ}\text{C}$ ) was below of the average of 70 years ( $4.7^{\circ}\text{C}$ ). The month was rather rainy (44.6 mm). In April, rainfall was less (35 mm) than the average of 100 years (40.3 mm). May was rather mutable in Kompolt, temperature was around the average of 70 years ( $15.2^{\circ}\text{C}$ ), but cooling occurred in the last period of the month. The first part of June was rather cold ( $19.2^{\circ}\text{C}$ ), and rainy (170.4 mm). Average rainfall of 100 years was 69.9 mm. July was hot ( $23.2^{\circ}\text{C}$ ) with less rain (55.6 mm). Average of 100 years: 58.4 mm. Plants were not dried out because there were enough humidity in the soil from June. In this year we find the exchanging of cooling and warming, dry and wet periods. Nevertheless we can conclude that the meteorological characteristics of the test period did not exceed the normal values of Hungary, and extreme events did not occur. 2006. October was  $2^{\circ}\text{C}$  warmer than the average of 70 years. Rainfall was only 11.9 mm in comparison with the average of 100 years (43.3 mm). November was rather mild ( $3.5^{\circ}\text{C}$ ), and dry (6.4 mm). Mean temperature in December ( $6.5^{\circ}\text{C}$ ) was higher than the average of 70 years ( $4.6^{\circ}\text{C}$ ). Amount of rain was much less: 3.2 mm in comparison with 40 mm of average of 100 years. 2007 was a drought year. Winter precipitation was low, and during the growing season in March, rainfall was 16 mm more than the average of many years (49 mm). April was unusually dry, 3.1 mm was raining compared to the average of 100 years (40.3 mm). May and June was unusually warm,  $2-3^{\circ}\text{C}$  higher than the 70-year average. Rainfall was also less – especially in June – comparing to the average of many years. Average was 69.9 mm, but in this year, rainfall was only 36.5 mm.

- **Barley preparing for malting**

After harvesting, barley has to be stored immediately under appropriate circumstances. Storing has to be carried out at low temperature ( $10^{\circ}\text{C}$ ) and in dry conditions (13-14% humidity) so that moisture content of barley could be kept between 12.0-12.5%. At the first step, barley sortiment has to be measured and only seeds above 2.5 mm will be used for further analysis. Subsequently, barley protein and thousand corn weight is measured. In accordance with the requirements of EBC, barley above 12.0% protein content will not be malted. Germination energy (GE) is measured 3 weeks after

harvesting in case of spring barley, 6 weeks after harvesting in case of winter barley. Those materials will be malted of which germination energy is more than 95% after three days.

- **Determination of malting properties of barley**

Determination of malting quality was carried out according to EBC requirements. Rules and methods has been set in the note of ANALYTICA-EBC published by European Brewing Convention.

- **Malting**

500 g – 2.5 mm or above – barley seeds were set in Australian „Phoenix Biosystem” Micromalting Equipment. Malting in case of spring barley: 5 hours steeping at 14.5°C; 19 hours resting at 14.5°C; 4 hours steeping at 14.5°C; 20 hours resting at 14.5°C; 10 minutes steeping at 14.5°C; 23 hours 50 minutes resting at 14.5°C; 72 hours germination at 14.5°C; 16 hours kilning til 50°C; 1 hour kilning til 60°C; 1 hour kilning til 70°C; 5 hours kilning til 80°C; 15 hours kilning til 25°C.

Malting in case of winter barley: 5 hours steeping at 14.5°C; 19 hours resting at 14.5°C; 4 hours steeping at 14.5°C; 20 hours resting at 14.5°C; 1 hour steeping at 14.5°C; 23 hours resting at 14.5°C; 72 hours germination at 14.5°C; 12 hours kilning til 55°C; 1 hour 30 minutes kilning til 60°C; 1 hour 30 minutes kilning til 65°C; 1 hour 30 minutes kilning til 70°C; 1 hour 30 minutes kilning til 75°C; 4 hours kilning til 80°C; 15 hours kilning til 25°C. Malting was carried out according to the Malting Institute Brno of Research Institute of Brewing and Malting, Prague.

- **Determination of brewing properties of malt**

Determination of brewing properties of malt was carried out according to EBC and the following parameters were analysed: friability (%), moisture content (%), extract (%), difference of extract (%), protein content (%), soluble nitrogen content (%), Kolbach (%), viscosity (mPa\*s), saccharification rate (minute), colour (EBC), turbidity (EBC), filtration (minute).

- **Microbiological characterization of barley soil**

- **Determination of CO<sub>2</sub> emission**

For measuring CO<sub>2</sub> emission, 0,5 kg soil was set into 2 l glass container. 50 cm<sup>3</sup> plastic tube containing 10 mol NaOH was placed into the middle of the soil to capture the improving CO<sub>2</sub>, then the container was closed. NaOH solution was titrated by 1 mol HCl solution then CO<sub>2</sub> volume was calculated which disengaged during soil respiration (FERNANDES et al., 2005).

- **FDA enzyme activity**

For measuring FDA activity, method worked out by ZELLES et al. (1991), and modified by SCHNÜRER and ROSSWALL (1982) was used. Fluorescein concentration (µg fluorescein·g<sup>-1</sup> dry soil·hour<sup>-1</sup>) was determined by spectrophotometer at 490 nm.

- **Occurrence of soil microorganisms**

Plate count technique was done to estimate the total aerobic bacterial numbers, aerobic spore-forming bacteria, actinomycetes and fungi, cellulose decomposers (HENDRICKS et al., 1995) and phosphate solubilizers (GOLDSTEIN, 1986) in the rhizosphere. Plants were carefully uprooted and washed with water followed by washing with sterile 0.85% saline water. 10 g of the roots was cut, then it was placed into 90 cm<sup>3</sup> sterile physiological salt solution. Suspension was diluted til 10<sup>-7</sup>.

Total microbial number occurred in rhizosphere, number of spore-forming, actinomycetes and microscopical fungi was determined by using selective media (SZEGLI, 1979). During this process, 1 cm<sup>3</sup> samples (from 10<sup>-4</sup> to 10<sup>-7</sup>) was plated on King-B, Nutrient, Nutrient + crystal violet, Nutrient + cyclohexidine (100 µg<sup>-1</sup>·cm<sup>3</sup>), EMB, triptone-glucose-yeast extract, Martin-Bengal Rose, maltextract, PDA, Jensen, Küster-Williams, Actinomycetes (DIFCO), *Trichoderma* selective agar media.

Microorganisms were incubated in 28°C (bacteria for 48 hours, Actinomycetes, fungi and yeast for 3-5 days) on media mentioned above. Isolated microbes were classified by morphological properties (colour, shape, appearance, size), according to morphotype and ability to spore-forming. Representative cells were selected from each morphotype which were cleaned then identified. Aerob heterotrophic bacterial isolates were identified by morphology of the cells, Gram-staining, spore-staining, oxidase-catalase reaction, oxidation and fermenting of glucose, and moving and pigmentation.

Determination of rhizosphere microorganisms was carried out according to NAUTIYAL and DION (1990), determination of *Pseudomonas* was carried out according to LLOYD-JONES et al. (2005). The determined bacteria were controlled by using BBL Crisystal™ method according to HOLT et al. (1994). Fungi strains were determined by macro and micromorphological properties according to the study of DOMSCH et al. (1980). Characters of the cells were described by macromorphological determination, micromorphological properties were identified according to microscopical traits (BÁNHEGYI et al., 1985). Yeasts were determined by using „API 20C of AUX bio-Merieux” system, and method by DEÁK (1998).

- **Examination of microbial phosphate solubility**

Investigation of phosphate solubility was carried out on the medium by GOLDSTEIN (1986). Dicalcium-phosphate agar was inoculated, and those strains had phosphate solubility which produced pure rings around their cells.

- **Characterization of cellulose decomposer microorganisms**

For determination of cellulose decomposing, we inoculated cellulose agars (HENDRICKS et al., 1995) by using two types of media (PDA: fungi, Nutrient agar: bacteria), which contained CMC-Congo-red (carboximetyl-cellulose congo-red) substrate. Enzyme production was stopped by

hydrochloric acid and those strains had the ability to cellulose decomposing which produced violet blue rings around their cells.

- **Heavy metal tolerance of *Saccharomyces cerevisiae***

- **Maintaining and cultivation**

Winter and spring barley was grown under glasshouse conditions for 50 days. Applied soil was brown forest soil originated from Debrecen University, Research Institute of Nyíregyháza (pH: 5.9; salt: 0.17%; CaCO<sub>3</sub>: 3.1%; humus: 2.54%; NO<sub>3</sub>-N: 2.3; Zn: 1.7; Cu: 1.4; Mn: 55 mg·kg<sup>-1</sup>) and contained aerob sewage sludge in 50% (w/w), from Nyíregyháza. Sewage sludge contained (mg·kg<sup>-1</sup> dry material): Cd: 2.3; Cu: 110.4; Ni: 21.6; Pb: 66.9; Ca: 2133; K: 1716; Mg: 2507. Total dry material: 53%, pH(H<sub>2</sub>O): 6.9.

Plants were carefully uprooted and washed with water followed by washing with sterile 0.85% saline water. Then the roots were cut down. Soil–root suspension and its dilutions made by using sterile distilled water were shaken on 150 rpm, for 1 hour, the inoculated media was incubated for 48 hours, at 28°C. Isolation was carried out on YPDA medium contained 10 g/l yeastextract, 10 g/l peptone, 20 g/l dextrose and 20 g/l agar. PH was adjusted to 5.8, then it was autoclaved at 121°C, for 15 minutes, on 1 atm pressure. 100 µg/ml cycloheximide and 30 µg/ml benomile (3 mg benomil was dissolved in 1 ml 96% ethanol then cooled until it reached 55°C and added to sterile YEPD media) were added to the media for inhibit the growing of other microorganisms. Selected yeasts were listed into different categories according to their morphologies (colour, structure, appearance, size) and microscopical properties (cell shape and reproduction), then their morphotypes were determined. 1 strain was selected from each type which was further analysed. Most of the isolates were identified as *S. cerevisiae* or other *Saccharomyces* species – according to their morphologies or their reactions with AUX bioMerieux system API 20C (DEÁK, 1998).

Six strains of 32 strains of *S. cerevisiae* were selected according to ecophysiological properties (siderophore production, growing ability on medium of high glucose concentration, acid tolerance, and growing ability at different temperatures). Two strains (NSS5099 and NSS7002) were isolated from spring and winter barley rhizosphere. In a test tube containing fresh culture on the agar mentioned above, a biomass was washed out with physiological solution. Yeast cultures were grown aerobically. Under sterile conditions, 40 ml broth of the above medium was placed into a 100 ml Erlenmeyer flask and 5 ml yeast cell suspension was inoculated in it to obtain 5 mg dry weight of yeast/l. The cultivation was carried out on an orbital shaker at 150 rpm and 28°C, for 2 days. Maintaining was carried out at 4°C, on YPDA medium. To examine the effect of the heavy metal ions on the strains *S. cerevisiae*, Cu, Cd, Pb and Ni ions were added as CuSO<sub>4</sub>, Cd(NO<sub>3</sub>)<sub>2</sub> and Pb(NO<sub>3</sub>)<sub>2</sub> and NiSO<sub>4</sub> to the nutrition medium in different concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 µM). Solutions of metal ions were sterilized by using 45 µm biological membrane.

- **Determination of minimal inhibitory concentration (MIC)**

Heavy metal tolerance of yeast cells was determined by minimal inhibitory concentration. Heavy metals were added to YPDA medium in different concentration (0, 10, 20, 40, 80, 160, 320, 640  $\mu\text{M}$ ) to reach 0–640  $\mu\text{M}$ . Petri dishes were divided into two and yeast was inoculated into the dishes with heavy metal and into the control dishes, too. Petri dishes were incubated at 28°C, for 2 days. Then minimal inhibitory concentration (MIC) was determined.

- **Determination of cell growth in different heavy metal concentrations**

The yeast cell growth of NSS5099 and NSS7002 was studied in YEPD broth medium which contained (0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500  $\mu\text{M}$ ) concentrations of Cu, Cd, Pb and Ni salts ( $\text{CuSO}_4$ ,  $\text{Cd}(\text{NO}_3)_2$ ,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{NiSO}_4$ ) at 28 °C. The concentration of yeast cells was monitored by optical absorbance at 610 nm. The amount of dry biomass in the suspension was determined by samples taken at different intervals from the medium, which were then centrifuged at 4000 rpm for 10 min. Then the precipitate was dried at 85°C until it reached the constant weight.

- **Effect of Ca, Mg and K on yeast tolerance at different heavy metal concentrations**

The experiment was carried out as mentioned above, and the rate of the yeast cell growth was measured after the application of  $\text{Ca}(\text{HCO}_3)_2$ ,  $\text{MgSO}_4$ , and  $\text{K}_2\text{SO}_4$  at 50, 75, and 150 mM, respectively to all cultures at different concentrations of the investigated heavy metal at 28°C.

- **SUMMARY**

It has been more than 7 years that Fleischmann Rudolf Breeding Research Institute in Kompolt, Hungary has been analyzing to determine the technological properties of malting barley genotypes. These analysis help to breed those species which are the most appropriate for brewing (winter and spring malting barley) besides to achieve conventional aims such as yield, disease resistency, etc. Breeding of winter and spring malting barley with excellent technological properties can be detained by climatical factors. Quality is changing a year by reason of weather and sowing time. The Institute started researches regarding breeding of barley species which have both excellent growing parameters and technological properties.

Particularly spring barley is used for malting and brewing. Our aim was to analyse and determine chemical and technological properties of those winter barley strains which have higher yield and better resistance against drouth and diseases, on the grounds of weather conditions. Our results were compared with the properties of spring barley.

During our research, two winter malting barley species (KH Korsó and Vanessa) and several barley strains were analyzed which were produced under conventional circumstances. Our aim was to

determine whether these strains are convenient for industrial using. Our results showed which winter and spring barley strains had good technological properties between 2005 and 2007. We determined, both from field and laboratory experiments, that malt prepared from winter barley does not differ from that malt which was prepared from spring barley, that is winter barley can be qualified for brewing. Knowledge of these attributes and the interactions between the traits and cropping technologies of winter and spring barley has a very important role for barley breeders and producers. Winter barley could be one of those crop plants which can be produced effectively in the European Union. The costs of producing it are lower and could be profitable under unfavourable conditions, too.

Soil life and soil biological activity plays important roles in metabolical processes, roundabout systems of elements and prevention of environmental and sanitary problems by the bioaccumulation of heavy metals. During our research, we determined CO<sub>2</sub> producing of rhizosphere microorganisms, soil enzyme activities and occurrence of microorganisms in spring and winter barley rhizosphere. We determined that soil respiration and enzyme activity is higher in spring barley rhizosphere in 2006 than in winter barley rhizosphere. The highest density of microorganisms was measured in spring barley rhizosphere, in 2006 and 2007. Many strains were belonged to *Saccharomyces* genus.

We examined, how *S. cerevisiae* - which has important role in wort fermenting – tolerates heavy metal pollution in soils. Tolerance against heavy metal pollution of two *S. cerevisiae* strains (NSS5099 and NSS7002) were examined. We studied multiplication of the two strains on a substrate in which Cu<sup>2+</sup>-, Pb<sup>2+</sup>-, Cd<sup>2+</sup>- or Ni<sup>2+</sup>-ions were added in 50 μM concentration. The toxicity of the examined heavy metals on yeast strains in decreasing order: Cu<sup>2+</sup> > Pb<sup>2+</sup> > Cd<sup>2+</sup> > Ni<sup>2+</sup>. Cu<sup>2+</sup>, Pb<sup>2+</sup> or Cd<sup>2+</sup> in 350 μM concentrations and Ni<sup>2+</sup> in 450 μM concentrations reduced the cell numbers by 50%, after 48 hours incubation. When 50 mM Ca(HCO<sub>3</sub>)<sub>2</sub>, 75 mM MgSO<sub>4</sub>, or 150 mM K<sub>2</sub>SO<sub>4</sub> was added into the substrate before heavy metal rationing, toxicity of these elements decreased and more cells stayed alive. Toxicity of heavy metals in 350 and 450 μM concentrations were reduced by the metal salts by 40%. Our results (TÓTH et al., 2008) showed that NSS7002 is more eligible to purify the polluted soils by heavy metals than NSS5099.

In recent years, many studies have been made on the accumulation of heavy metal ions by microorganisms. The tolerance and uptake of heavy metals by microorganisms has received much attention because of their potential application in bioremediation. The properties of microbes make them very useful in monitoring soil contamination. Toxic heavy metals cause a serious threat to the environment, so it is a major ecological challenge to remove heavy metals from contaminated soils. It was determined that *S. cerevisiae* occurred in barley rhizosphere reduces the heavy metal content therefore can prevent the plant from metal pollution due to its excellent bioaccumulation properties.

- **THE EVALUATION OF HYPOTHESES AND SUGGESTIONS**

We experienced significant effects of environmental factors on crop yield and malt quality. The temperature during grain filling has a huge impact on malt quality. If the high temperature or drought - or both - shortens the period of filling, it also results reduction in yield. The optimum temperature during the grain filling is about 15-18°C. Precipitation has greater impact on malt quality than high temperature and drought. For winter barley, it is better if April and May is rainy, but 2007 had warm and dry spring. Rainfall was 0-10 mm in April, which caused faster vegetation.

Malt quality in 2006 was said to be favourable than in 2007, which resulted weak quality. The dry and mild winter, and the warm and dry spring in 2007 had a negative effect on winter barley. In this year, yield of all the crops decreased, and was less than in 2006. Yield of barley in 2007 was about 3% less than in 2006. Year of 2007 was unfavourable to barley growing. Conditions in Kompolt met the requirements of growing malting barley. Yield and sortiment was rather high, protein content was low.

Breeding of malting barley is for obtaining resistant strains with higher yield, and also with good malting quality. The best method for selection is the micromalting process, of which disadvantage is the high number of samples, and small amount of material. Therefore the breeders take other traits into account for which micromalting is unnecessary. These can be the following: thousands corn weight, seed size, seed morphology, protein content, and expected extract content by using NIR technics. During our research, barley seeds of both low protein content were malted. Barley of higher protein content is not suitable for brewing because extract content reduces and has negative effects on beer quality. Barley seeds with similar protein content can differ from each other regarding to their malting qualities. Protein content is changing depending on the weather. It can be influenced by climatic factors and the application rate of N. We drew the conclusion that most of the barley strains (winter and spring) from Kompolt is appropriate for malting and brewing.

Proper brewing quality of barley can be produces at mild temperatures and higher rainfall areas. Weather has the biggest effect on grain filling. Drought can result decreasing of seed sortiment (LEISTRUMAITÉ et al., 2009). SHAKHATREH et al. (2001) suggest that appropriate moisture content under grain filling has positive effect on yield, but in dry conditions yield of varieties with long vegetation period will be lower.

Winter and spring barley production plays a significant role in our country, with growing area of 300 - 400.000 ha. In Hungary, winter barley (feed and malting barley) can be one of the best economically cultivated plant in the European Union. It tolerates warmer climate and drought which occurs more often. Winter barley tolerates best drought (MURÁNYI et al., 2008).

In recent years, many studies have been made on the accumulation of heavy metal ions by microorganisms. The tolerance and uptake of heavy metals by microorganisms has received much attention because of their potential application in bioremediation. The properties of microbes make them very useful in monitoring soil contamination. Toxic heavy metals cause a serious threat to the environment, therefore it is a major ecological challenge to remove heavy metals from contaminated soils. It was determined that *S. cerevisiae* occurred in barley rhizosphere reduces the heavy metal content therefore can prevent the plant from metal pollution due to its excellent bioaccumulation properties. Our results showed that NSS7002 is more eligible to purify the polluted soils by heavy metals than NSS5099 (TÓTH et al., 2008). More researches are needed to be done to optimize heavy metal uptake from aqueous solutions and to investigate yeasts heavy metal absorbance and their usage in bioremediation. Consequently, appearance of the metal is more determining in adsorption than the type of cells.

## • **NEW SCIENTIFIC FINDINGS**

Winter and spring barley production plays a significant role in Hungary. Earth's warming climate is expected to result in drier and changeable conditions. Our aim was to provide results about the technological traits of spring and winter barley and to make conclusions about their usage in the brewing industry. We made some biochemical and microbiological analysis in connection with barley soil and heavy metal tolerance of two *Saccharomyces cerevisiae* strains occurred in the rhizosphere.

We concluded that:

- 6.1. In spite of the warm and dry conditions of Kompolc, high yield and relatively low protein content can be approached in malting winter barley. In conventional farming and soil with high acidity, considerably high productivity can be reached regarding to winter barley. Technological traits of malt made from winter barley is similar to those made from spring barley therefore winter barley can be used in the brewing industry.
- 6.2. Winter barley can tolerate warm climate and drought (year of 2007) due to its excellent ecological properties therefore – by breeding appropriate varieties – it can be produced for malting and brewing.
- 6.3. Soil respiration and soil biological activity is higher in spring barley than in winter barley rhizosphere and higher in 2006 than in 2007. Differences measured in rhizosphere enzyme activity and soil respiration is due to the different climatic conditions. We determined, that the rate of soil respiration, FDA activity together with the increasing numbers of microorganisms is higher when soil is treated with sewage sludge.

- 6.4. We determined that *S. cerevisiae* occurred in barley rhizosphere reduces the heavy metal content therefore can prevent the plant from metal pollution due to its excellent bioaccumulation properties. More researches are needed to be done regarding to measuring heavy metal content of plants and soil.
- Our results showed that *S. cerevisiae* strain NSS7002 is more eligible to purify the polluted soils by heavy metals than *S. cerevisiae* strain NSS5099.
- 6.5. When 50 mM Ca(HCO<sub>3</sub>)<sub>2</sub>, 75 mM MgSO<sub>4</sub>, or 150 mM K<sub>2</sub>SO<sub>4</sub> was added into the substrate before heavy metal rationing, toxicity of these elements decreased.

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ISBN 80-7040-847-2.

#### **KNOWLEDGE DISTRIBUTION ARTICLES**

- Murányi I., **Tóth N.**, Bódi Z. (2008): Őszi és tavaszi árpa nemesítése és eredményei a kompolti Fleischmann Rudolf Kutatóintézetben. *Értékálló Aranykorona*. **8**: 12-14.
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