



Individual and combined effects of pre- and post-anthesis temperature on protein composition of two malting barley cultivars



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ABSTRACT

The present study has investigated the individual and combined influence of pre- and post-anthesis temperatures and cultivars on the protein composition in barley grains. Two barley cultivars were grown in soil and hydroponic systems in daylight chambers with different pre- and post-anthesis temperatures. Size exclusion (SE)-HPLC was used to evaluate the protein composition in mature barley grains. The results showed that individual and interactive effects of pre- and post-anthesis temperatures and cultivar variations influenced protein composition in the barley grains. Pre-anthesis temperature greatly affected the amounts of total sodium dodecyl sulphate (SDS) extractable proteins (TOTE) and explained 30% of the variation in TOTE. The barley cultivars accounted for 20% of the variation in TOTE. Variation in malting barley cultivars was found to influence the SDS extractable small monomers (41% of the variation). Percentage of SDS un-extractable polymeric proteins in total amount of polymeric proteins (%UPP) was governed by post-anthesis temperature, accounting for 11% of the variation and cultivar differences accounted for 7% of the variation. Thus, the climatic conditions during the specific growing period and a choice of cultivars played a major role in determining the protein composition and ultimately the malting quality of spring barley.

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1. Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal crops grown worldwide reaching 54 million hectares and a total production of 152 metric tons in 2010 (FAO, 2011). Malting barley is specifically used for production of beer and other alcoholic beverages, where certain end-use quality criteria are of importance; e.g.

List of abbreviations: eSMP, SDS extractable small monomeric proteins; GMP, Grain maturation period; GPC, Grain protein concentration; LTP, Lipid transfer proteins; Mon/Pol, Monomers/Polymers; SAS, Statistical analysis software; SDS, Sodium dodecyl sulphate; SE-HPLC, Size exclusion-high performance liquid chromatography; TOTE, total SDS extractable proteins; TOTU, total SDS un-extractable proteins; uSMP, SDS un-extractable small monomeric proteins; %LargeUPP, percentage of large unextractable polymeric proteins in total large polymeric protein; %LUMP, percentage of large unextractable monomeric protein into total large monomeric proteins; %SUMP, percentage of small unextractable monomeric protein into total small monomeric proteins; %UPP, percentage of SDS-unextractable polymeric proteins in total polymeric proteins.

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grain protein concentration (GPC) and protein composition (Evans et al., 1999). In most countries of the world, the acceptable range of GPC in barley grains for malting purposes has been suggested to be in between 9.5 and 11.5% (Palmer, 2000). The concentration of proteins in the barley grain is not stable and the fluctuation is mainly due to variations in environmental, cultivation and genotypic factors (Pettersson et al., 2006). Barley cultivars with similar GPC may differ greatly in malting quality. Therefore, it is suggested that protein composition may play a more important role in determining the malting quality than GPC (Wang et al., 2007).

Protein composition of barley is affected by a number of environmental factors, e.g. temperature, precipitation, fertilizer etc., and from these factors temperature is known to be of significance (Zhang et al., 2001). Pre- and post-anthesis temperatures are well known to affect maturation time and protein composition in wheat (Malik, 2012); however, few studies are available in malting barley. Studies available on the effect of temperature on protein composition of barley have mainly been carried out in field conditions (Wang et al., 2007). Due to large soil and environmental variations in the field, the impact of pre- and post-anthesis temperature on the protein composition of barley is rather little characterized. Fluctuations in the GPC can also be attributed to un-predictable differences in nitrogen mineralization in the soil (Dessureault-Rompré et al., 2010). Temperature has been found to increase the

mineralized nitrogen in the soil and ultimately GPC in the barley grains (Andersson and Holm, 2011). Thus, the grain protein composition might also be affected by mineralized nitrogen in the soil. Therefore, in order to investigate the effects of temperature separately from the effects of mineralized nitrogen on protein composition, use of hydroponic cultivation with controlled nitrogen supply can be one good option (Andersson and Holm, 2011).

The aim of the present investigation was to study the individual and interactive effects of pre- and post-anthesis temperatures on protein composition of two spring malting barley cultivars, genetically differing in maturation times and GPC. Further the aim was to study if differences in the cultivation systems, i.e. soil or hydroponic, create differences in the protein composition of two spring malting barley cultivars.

2. Materials and methods

2.1. Cultivars and cultivations

To obtain differences in protein composition in mature barley grains, spring malting barley cultivars with varying genetic backgrounds, i.e. differences in maturation time and GPC, were chosen. Two (two-row) spring malting barley cultivars, Henley (medium tall, medium maturation time, high grain weight and medium to high GPC) and NFC Tipple (medium short, long maturing time, high grain weight, low GPC) were investigated. Seeds of the cultivars Henley and NCP Tipple were provided by Scandinavian Seed, Linköping (Sweden) and Lantmännen SW Seed, Svalöv (Sweden), respectively.

Plants were grown in controlled climatic daylight chambers in the Biotron. Two independent daylight chambers were used. The day/night temperatures in the chambers were set to 18/12 °C (for low temperature chamber) and 23/17 °C (for high temperature chamber). The relative humidity in both temperature chambers was kept at 70%. Two cultivation media, soil and hydroponic, were investigated with application of equalized nitrogen amount in the plants.

In the hydroponic cultivation, barley seedlings were placed on black Styrofoam plates, which floated continuously on aired nutrient solution in black 2 dm³ beakers. Four plants of the same cultivar were used in each beaker. The nutrient solution was replaced every week and the nitrogen supply was controlled through a daily dose according to Andersson et al. (2004). Soil cultivation was carried out in boxes with eight plants of the same cultivar according to Andersson and Holm (2011).

On average, the plants reached anthesis at day 62 after sowing and that day was set to change from pre-anthesis temperature treatment to post-anthesis treatment. The temperature combinations were high pre-anthesis/high post-anthesis (high/high); high pre-anthesis/low post-anthesis (high/low); low pre-anthesis/high post-anthesis (low/high), and low pre-anthesis/low post-anthesis (low/low).

2.2. Protein analysis

The plants were harvested at full maturity. To determine the amount and size distribution of polymeric and monomeric proteins, the grain samples from each treatment were lyophilized. Thereafter, size exclusion high performance liquid chromatography (SE-HPLC) was used with a two step extraction procedure according to Johansson et al. (2005). The SE-HPLC chromatograms of both sodium dodecyl sulphate (SDS) extractable (e) and SDS unextractable (u) proteins were divided into two main parts representing polymeric (PP) and monomeric proteins (MP) (Fig. 1). Each of the PP and MP parts was further divided into two parts

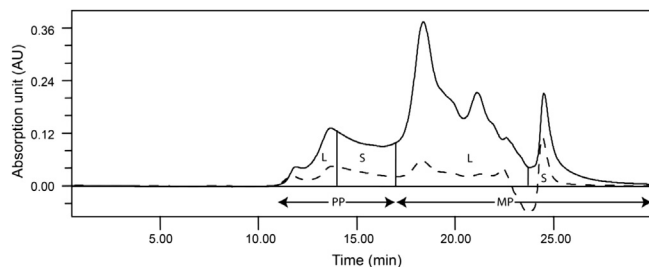


Fig. 1. SE-HPLC chromatogram of SDS-extractable proteins (—) and SDS-unextractable proteins (---), respectively. The chromatogram was divided into two main parts comprising polymeric proteins (PP) and monomeric proteins (MP), respectively. Each main part of the chromatogram was subdivided into two parts [designated as Large (L) and small (S)].

representing the large (L) and small (S) proteins in each part. The areas of different protein fractions were calculated according to Johansson et al. (2008):

- eSMP = SDS extractable small monomeric proteins
- uSMP = SDS un-extractable small monomeric proteins
- TOTE = total SDS extractable proteins
- TOTU = total SDS un-extractable proteins
- %UPP = percentage of un-extractable polymeric protein in total polymeric protein = $[(uLPP + uSPP)/(uLPP + uSPP + eLPP + eSPP)] \times 100$
- %LargeUPP = percentage of large unextractable polymeric proteins in total large polymeric protein = $[uLPP/(uLPP + eLPP)] \times 100$
- %LUMP = percentage of large unextractable monomeric protein into total large monomeric proteins = $[uLMP/(uLMP + eLMP)] \times 100$
- %SUMP = percentage of small unextractable monomeric protein into total small monomeric proteins = $[uSMP/(uSMP + eSMP)] \times 100$
- Mon/Pol (Monomers/Polymers) = $[(eLMP + eSMP + uLMP + uSMP)/(eLPP + eSPP + uLPP + uSPP)]$

2.3. Statistical analyses

SAS release 9.1 (SAS, 2004) was used for statistical analysis. Data evaluation was done by Spearman rank correlation and analysis of variance procedure using a general linear model. The hydroponic cultivation consisted of eight replicates (eight beakers with four plants) of each combination of cultivar and temperature treatment, while the soil cultivation consisted of five replicates (boxes with eight plants), respectively. For the protein analyses, two replicates were analysed from each replicate of each treatment at maturity. In order to explain the percentage effect of the variables such as cultivations, cultivars and pre- and post-anthesis temperature as well as combinations of these on the protein composition, regression analysis was performed similarly as in Malik et al. (2013).

3. Results

3.1. Effect of pre- and post-anthesis temperature on amount and size distribution of proteins

Significantly higher amounts of TOTE, TOTU and %LUMP were observed at high pre-anthesis as compared to low pre-anthesis temperature, in both cultivation systems (Tables 1 and 2). High pre-anthesis temperature resulted in significantly higher amounts of eSMP, uSMP and %SUMP at soil cultivation and significantly lower

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