

COMPARATIVE ELECTROPHORETIC PATTERNS OF ALBUMINS/ GLOBULINS EXTRACTED FROM DRY GRAINS AND GREEN MALTS OF BARLEY VARIETIES

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SUMMARY

Applicability of electrophoretic separations of albumins/globulins, followed by non-specific protein staining and specific glycoprotein and aminopeptidase detection has been examined for barley variety discrimination. Albumins/globulins extracted from dry grains and green malt of six barley varieties were separated by polyacrylamide gel electrophoresis (10 % T, pH=8.9) of native and sodium dodecyl sulfate denatured proteins, and by isoelectric focusing in pH gradient of 3.5-9.5 and 4.0-6.5. Analysis of dry grain extracts gave better results than green malt extracts. Obtained data indicate that SDS-PAGE of albumins/globulins and IEF in pH gradient 3.5-9.5 followed by Coomassie Blue staining could be useful in Croatian barley variety discrimination. Angora barley could be clearly distinguished from the other varieties which were grouped as follows: Rodnik/Sladoran, Barun/Rex, and Martin. Glycoprotein patterns did not improve the recognition of individual varieties. Broad specificity, phenylalanine and leucine preferring, and arginine specific aminopeptidase were not found as applicable markers for discrimination of examined barley varieties.

Key-words: barley variety discrimination, electrophoretic separations, glycoprotein detection, aminopeptidase activity detection

INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) at acidic pH and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of barley seed storage proteins, hordeins, have been widely applied for barley variety discrimination (Cooke, 1995; Wrigley, 1995; White and Cooke, 1992). However, their use is limited to variety grouping, while full differentiation could not be achieved (Lamelland and Briand, 1990; Weiss et al., 1991b; Cooke, 1995; Wrigley, 1995; Bernardo et al., 1997; Binneck et al., 2002; Alvarez et al., 2006; Pan et al., 2007; Sipahi et al., 2010; Strelec et al., 2011). Therefore, useful modifications of standard separation methods or additional differentiation markers are desired. The search for them could include: (i) analysis of different stages of barley grain development, (ii) modification of extraction procedures, (iii) analysis of other protein fractions such as albumins/globulins, (iv) increasing resolution power of gels using gradient gels, (v) use of other electrophoretic separations such as isoelectric focusing or two dimensional-electrophoresis, and (vi) detection

of specific group of proteins such as glycoproteins or various enzymes (Strelec et al., 2011; Finnie and Svensson, 2009; Cooke, 1995; Zhang and Jones, 1995; Görg et al., 1992; Weiss et al., 1992a; 1992b; 1991a; Ott and Scandalios, 1978).

Analysis of albumins/globulins for barley varieties discrimination had limited capability, as reported by Weiss et al. (1991b). Electrophoretic patterns did not show significant differences between 20 German barley varieties, but allowed their grouping by glycoprotein patterns. Vaz et al. (2004) succeeded to group lupine varieties by electrophoretic separation of their globulins, and glycoprotein detection. Use of enzymes pattern profiling in variety differentiation has shown limited applicability. Görg et al. (1992) found α -amylase isoenzymes as suitable markers for barley malting quality, but differentiation of varieties by

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α -amylase isoenzymes wasn't promising. Lallemand and Briand (1990) succeeded to arrange 208 French barley cultivars in 25 groups analyzing isoenzymes of acid phosphatases and esterases from barley leaves, Binneck et al. (2002) grouped 5 Brazilian barley varieties in 3 groups by barley seed esterase patterns, Bernardo et al. (1997) succeeded to arrange 222 Spanish barley varieties into 14 groups by analyzing esterase isoenzyme patterns of barley leaves, while Ott and Scandalios (1978) grouped 50 maize varieties in 3 groups by aminopeptidase activity pattern.

Data regarding possibility of Croatian barley varieties discrimination by albumin/globulin, glycoprotein and enzyme electrophoretic analysis are lacking. Therefore, in this work electrophoretic patterns of barley albumins/globulins protein fraction obtained by several procedures and use of standard staining, specific glycoprotein and aminopeptidase staining were studied. The study included two different stages of barley development, dry grain and green malt.

MATERIALS AND METHODS

Barley samples and green malt production

The experiments were performed with six two-rowed winter barley (*Hordeum vulgare* L) varieties. Five of them (Sladoran, Rodnik, Rex, Martin and Barun) were domestic varieties created at the Agricultural Institute Osijek, Croatia, while one variety (Angora) was foreign variety originated from Germany, bred by Saatucht Josef Breun, GdbR-Herzogenaurach. Barley grains (50 g) were surface-sterilized with 1% sodium hypochlorite solution (20 minutes), rinsed several times with 0.5 M sodium chloride solution and then with distilled water. After steeping at 24 °C during 24 hours, the grains were germinated for 3 days in the darkness at 24 °C. Developed green malt barley was collected and used for protein extraction.

Protein extraction

Ten grains of dry seed or green malt of each variety were frozen and disintegrated in liquid nitrogen using mortar and pestle. The obtained powder was mixed with 4 mL of 50 mM Tris-HCl buffer (pH=8.0) and protein extracted during 1 hour at 4 °C by vortexing (30 s) each 15 minutes. The extracts were clarified by centrifugation at 20 000xg for 20 min at 4 °C and: (i) used immediately for isoelectric focusing, (ii) mixed with equal amount of 375 mM Tris-HCl buffer (pH=8.9) containing 0.006 % Bromphenol Blue for native PAGE, (iii) mixed with equal volume of SDS sample buffer (125 mM Tris-HCl buffer (pH=6.8) containing 4 % SDS, 20 % glycerol, 0.2 M DTT, and 0.02 % Bromphenol Blue) and processed as described before (Strelec et al., 2011).

Electrophoretic separations

The electrophoretic separations were performed as native PAGE, SDS-PAGE and IEF repeating each

separation at least twice. Horizontal native PAGE, pH=8.9, was performed according to the instruction for Anodal Electrophoresis with Native Buffer Kit pH 8.9 (Amersham Biosciences, Sweden). Albumins/globulins (10 μ g) applied into precast application slots were stacked at 300 V and 20 mA for 10 minutes and resolved during 60 minutes at 900 V and 50 mA. After electrophoresis gels were stained with Coomassie Brilliant Blue G-250 in phosphoric acid according to Neuhoff et al. (1985), or used for aminopeptidase detection. SDS-PAGE was performed using vertical system and gels cast according to user manual Vertical electrophoresis, Hoefer SE 600 Ruby (Amersham Biosciences, Sweden). Albumins/globulins (40 μ g) were separated during 3.45 h at 600 V and 60 mA (conditions for 2 gels). The separated proteins were: (i) electroblotted onto PVDF membrane for glycoprotein detection or (ii) stained with Coomassie Blue. Isoelectric focusing was performed on horizontal system using Ampholine PAGplate with pH gradient of 3.5-9.5 and 4.0-6.5. Prior to sample application, gels were prefocused 20 minutes at 700 V and 20 mA at 10 °C. Samples (15 μ g) were applied near cathode using sample application pieces. Separation of proteins in gels with pH gradient of 3.5-9.5 was performed as previously described by Strelec et al. (2011). Similar conditions of separation were applied when IEF gels with pH gradient of 4.0-6.5 were used; stepwise increase of voltage (800, 1200, 1500 V) every 30 minutes at constant 20 mA current, and IEF finishing by 30 minutes band sharpening at 2000 V and 20 mA. After focusing the gel was immediately: (i) fixed in 10 % trichloroacetic acid solution for 45 minutes and stained with Coomassie Blue, (ii) used for aminopeptidase detection or (iii) used for transfer of proteins onto PVDF membrane by pressure blotting.

Protein blotting

For SDS-PAGE separated proteins transfer to PVDF membrane was performed using semidry electroblotting technique according to Khyse-Andersen (1984). Focused proteins from IEF gels were transferred onto PVDF membrane by pressure blotting technique according to Desvaux et al. (1990) and Reinhart and Malamud (1982). After 90 min transfer at room temperature membrane was washed 5 minutes in deionized water, air-dried, and stored until detection.

Glycoprotein detection

Glucose or mannose rich glycoproteins of albumin/globulin protein fraction transferred to PVDF membrane were detected using optimized ConA/HRP procedure for glycoprotein staining according to Weiss et al. (1991a).

Aminopeptidase activity detection

After native PAGE or IEF, gel was sliced lengthwise and each lane divided in 3 mm slices. The slices

were incubated in 935 μL of 50 mM Tris-HCl buffer (pH=7.5) containing 0.1 mM CaCl_2 for 20 minutes at 37 $^\circ\text{C}$ and then to each sample 65 μL of 1.36 mM 2-naphthylamides (Phe, Leu or Arg) were added. After 1 hour for IEF gels or 2 hours for native PAGE gels, the enzyme reaction was stopped with addition of 200 μL of freshly prepared Fast Blue B (FBB) salt solution and developed colour intensity was measured after 10 minutes at 530 nm (Strelec et al., 2009).

RESULTS AND DISCUSSION

Native PAGE of albumins/globulins and aminopeptidases detection

When albumins/globulins extracted from barley dry grains and green malt, were separated by native PAGE (pH=8.9) and stained with Coomassie Brilliant Blue G-250, numerous bands could be observed in the upper part of the gel. However, the obtained protein patterns were more or less identical for the examined barley varieties (Fig. 1). The only exceptions were variety Angora and Martin with additional band in albumins/globulins pattern of dry grains (marked by arrows on Fig. 1., No. 2 and 7 respectively). These differences disappeared in green malts, where no significant deviations of protein patterns among varieties could be seen. A lack of differences between varieties could be expected, since albumin/globulin protein fraction mainly consists of enzymes, enzyme inhibitors, translation inhibitors and non-specific lipid transfer proteins, whose amino acid sequences are highly conserved during phylogenesis (Finnie and Svensson, 2009; Weiss et al., 1991b). Eventual slight deviations caused by single nucleotide polymorphism cannot be detected with native PAGE.

Analysis of aminopeptidase activities in gel slices after native PAGE (pH=8.9) of barley protein extracts using Phe-, Leu-, and Arg-2NA as substrates, revealed the existence of two aminopeptidases: AP of lower mobility that cleaved all three substrates with similar efficiency, and AP of higher mobility that was specific for arginine (data not shown) being in accordance with our previous findings (Strelec et al., 2009). However, enzyme pattern as detected with all three substrates was identical for all examined varieties. Previously reported slight difference of aminopeptidase activities in dry grain and green malt extracts (Strelec and Vitale, 2005; Strelec et al., 2001) could not be clearly seen by the applied method. Preservation of aminopeptidases pattern might also be due to previously mentioned conserved amino acid sequences.

Thus native PAGE (pH=8.9) Coomassie Blue stained albumins/globulin electrophoretic patterns, as well as subsequent aminopeptidase activity detection, could not be a promising method for Croatian barley variety discrimination.

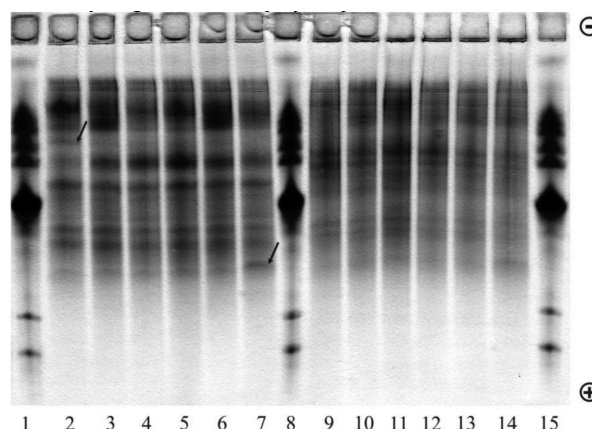


Figure 1. Native PAGE (10 % T, pH=8.9) of albumins/globulins extracted from barley dry grains and green malts with 50 mM Tris-HCl buffer (pH=8.0). Vertical lanes present: pl protein standards (1, 8, 15); dry grains (2-7); green malts (9-14) of barley varieties: Angora (2, 9); Barun (3, 10); Rodnik (4, 11); Sladoran (5, 12); Rex (6, 13); Martin (7, 14). Arrows indicate differences between varieties

Slika 1. PAGE u 10 % T gelu pH 8,9 albumina/globulina ekstrahiranih iz suhoga zrna i zelenoga slada ječma pomoću 50 mM Tris-HCl pufera (pH=8). Stupci: pl standardi (1, 8, 15); suho zrno (2-7); zeleni slad (9-14) sorti ječma: Angora (2, 9); Barun (3, 10); Rodnik (4, 11); Sladoran (5, 12); Rex (6, 13); Martin (7, 14). Strelice pokazuju razlike među sortama

SDS-PAGE of albumins/globulins and glycoprotein detection

Separation of barley albumins/globulins by SDS-PAGE in 10 % T gel with staining by Coomassie Blue, revealed existence of numerous protein bands in the range of molecular masses between 10 kDa and 97 kDa (Fig. 2). The observed protein patterns were similar, but some differences between varieties existed, both in dry grain and green malt extracts. Angora barley could be easily distinguished from other varieties by poorly expressed protein band of molecular mass of 45 kDa (marked by arrow on Fig. 2., No. 2). Other varieties could be grouped by their high similarities of protein patterns. One group consisted of varieties Barun and Rex, which could be distinguished from the others by lack of protein band of 27 kDa molecular mass (marked by arrows on Fig. 2., No. 3 and 6). The other group consisted of varieties Rodnik (No. 4), Sladoran (No. 5) and Martin (No.7) among which variety Sladoran had highly expressed protein band of molecular mass of 41 kDa. Similar discrimination and grouping of barley varieties could be based on patterns of albumin/globulin fraction extracted from green malts (Fig. 2. No. 9-14), but observed differences were less pronounced.

Electrophoretic patterns of albumin/globulin protein fraction from dry grains and from green malts of each individual variety were not identical. Differences

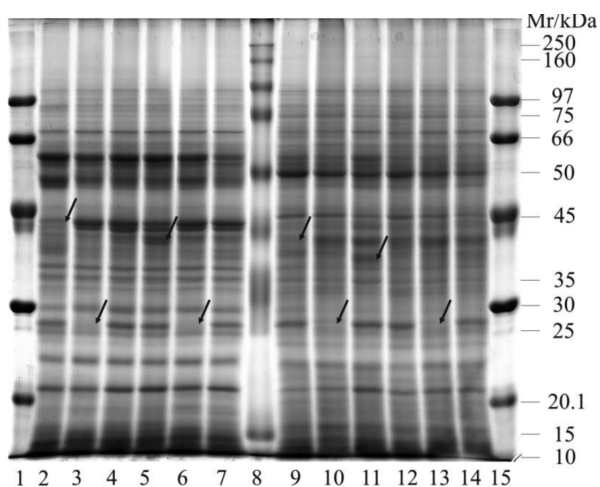


Figure 2. SDS-PAGE (10 % T) of barley albumins/globulins extracted from barley dry grains and green malts with 50 mM Tris-HCl buffer (pH= 8.0). Vertical lanes present: low molecular weight protein standards (1, 15); wide range molecular weight protein standards (8); dry grains (2-7); green malts (9-14). Barley varieties: Angora (2, 9); Barun (3, 10); Rodnik (4, 11); Sladoran (5, 12); Rex (6, 13); Martin (7, 14). Arrows indicate differences between varieties

Slika 2. SDS-PAGE u 10 % T gelu albumina/globulina ekstrahiranih iz suhoga zrna i zelenoga slada ječma pomoću 50 mM Tris-HCl pufera (pH=8,0). Stupci: niskomolekularni standardi proteina (1, 15); standardi protein širokoga raspona molekulskih masa (8); suho zrno (2-7); zeleni slad (9-14). Sorte ječma: Angora (2, 9); Barun (3, 10); Rodnik (4, 11); Sladoran (5, 12); Rex (6, 13); Martin (7, 14). Strelice pokazuju razlike među sortama

in protein pattern between these two phases of grain physiological state were expected due to degradation of seed storage proteins and biosynthesis of novel proteins during green malt development (Bewley and Black, 1985).

When the same proteins (albumins/globulins) after SDS-PAGE were electroblotted onto PVDF membrane and specifically stained for high mannose/glucose glycoprotein moiety by ConA/HRP method, the obtained protein patterns (Fig. 3) differed significantly from patterns obtained by Coomassie Blue staining illustrating that not all present proteins have glycoprotein moiety. Albumin/globulin protein fraction of barley dry grains contained five major glycoprotein bands corresponding to approximate molecular mass of 64, 59, 53, 35 and 29 kDa (Fig. 3., Nos. 1-6), as well as 27 kDa band which only occurred in Angora variety (No. 1). Additionally, multiple weak glycoprotein bands were distributed over molecular mass range between 10 and 160 kDa. The observed glycoprotein patterns of individual barley varieties were very similar (Fig. 3). Only the variety Angora could be clearly distinguished from the other varieties by additional glycoprotein band at 27 kDa (marked by arrow on Fig. 3.,

No. 1). Albumin/globulin protein fractions of green malts (Fig. 3., Nos. 8-13) had increased multiplicity of weak glycoprotein bands in the range of molecular mass between 35 and 96 kDa, and reduced intensity of major glycoprotein bands. Also, 27 kDa glycoprotein present in dry grain extract of Angora variety disappeared in green malt. This points to its intensive degradation during malting and makes glycoprotein detection useful for Angora recognition. However, the same method was not useful for other examined barley varieties discrimination.

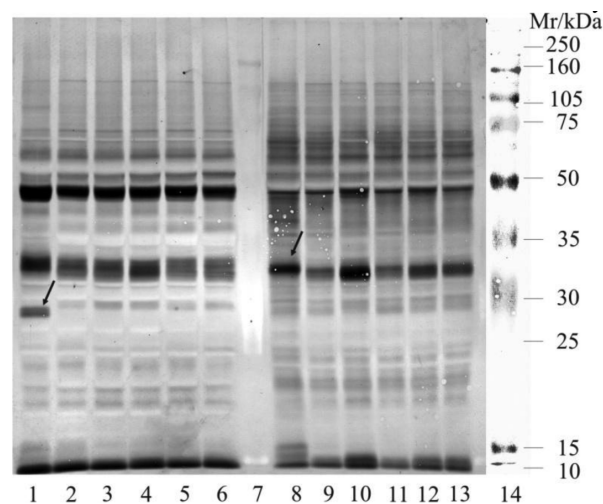


Figure 3. Detection of glycoproteins by ConA/HRP method in barley albumin/globulin protein fraction after SDS-PAGE (10 % T) and blotting onto PVDF membrane. Vertical lanes present: prestained wide range molecular weight protein standards (14); protein standard for ConA/HRP staining specificity control (7); dry grains (1-6); green malts (8-13). Barley varieties: Angora (1, 8); Barun (2, 9); Rodnik (3, 10); Sladoran (4, 11); Rex (5, 12); Martin (6, 13). Arrows indicate differences between varieties

Slika 3. Detekcija glikoproteina pomoću ConA/HRP metode u albuminsko-globulinskoj frakciji proteina nakon SDS-PAGE (10 % T) i prijenosa proteina na PVDF membranu. Stupci: obojani standardi proteina širokoga raspona molekulskih masa (14); proteini za kontrolu specifičnosti metode detekcije (7); suho zrno (1-6); zeleni slad (8-13). Sorte ječma: Angora (1, 8); Barun (2, 9); Rodnik (3, 10); Sladoran (4, 11); Rex (5, 12); Martin (6, 13). Strelice pokazuju razlike među sortama

Possible use of albumin/globulin fraction and SDS-PAGE with Coomassie Blue staining for differentiation of six Croatian barley varieties, and the lack of their differences in glycoprotein electrophoretic pattern, are not in accordance with the findings of Weiss et al. (1991b). The authors reported the lack of significant differences in albumin/globulin pattern of 20 German barley varieties after SDS-PAGE and silver staining, and much better differentiation by subsequent glycoprotein detection. This might indicate

differences of the examined German and Croatian barley varieties.

Isoelectric focusing of albumins/globulins followed by glycoprotein and aminopeptidase detection

Separation of albumins/globulins of dry grains and green malts of examined barley varieties by isoelectric focusing in pH gradients of 3.5-9.5 and 4.0-6.5 followed by Coomassie Blue staining was not proven useful in barley variety discrimination.

Multiplicity of protein bands ranging in their isoelectric points from 4.0 to 6.55 could be observed in IEF gels with pH gradient of 3.5-9.5 (Fig. 4a). Obtained protein patterns of individual varieties were similar, but allowed varieties distribution into two groups by differences in band intensities of proteins with isoelectric point of 7.5 (marked by arrows on Fig 4a). The first group comprised of Rodnik (No. 3) and Sladoran (No. 4), and the second one of the other varieties.

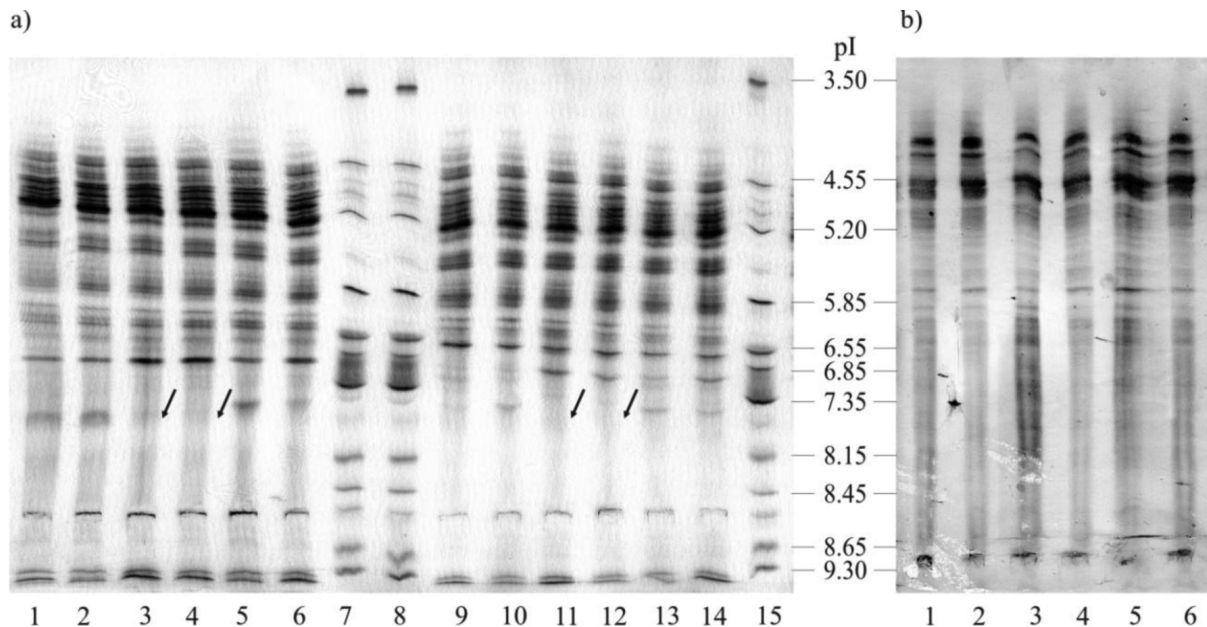


Figure 4. IEF in pH gradient of 3.5-9.5 of barley albumins/globulins extracted from dry grains and green malts with 50 mM Tris-HCl buffer (pH= 8.0). (a) Proteins stained with Coomassie Brilliant Blue G-250, (b) After IEF proteins blotted onto PVDF membrane and glycoproteins stained by ConA/HRP method. Vertical lanes present: protein pI standards, pH=3-10 (7, 8, 15); dry grains (1-6); green malts (9-14). Barley varieties: Angora (1, 9); Barun (2, 10); Rodnik (3, 11); Sladoran (4, 12); Rex (5, 13); Martin (6, 14). Arrows indicate differences between varieties

Slika 4. Izoelektrično fokusiranje u gradijentu pH 3,5-9,5 albumina/globulina ekstrahiranih iz suhoga zrna i zelenoga slada ječma pomoću 50 mM Tris-HCl pufera (pH=8,0). (a) Proteini obojani pomoću Coomasie modrila, (b) proteini iza IEF preneseni na PVDF membranu i glikoproteini obojani ConA/HRP metodom. Stupci: pI standardi pH=3-10 (7, 8, 15), suho zrno (1-6); zeleni slad (9-14). Sorte ječma: Angora (1, 9); Barun (2, 10); Rodnik (3, 11); Sladoran (4, 12); Rex (5, 13); Martin (6, 14). Strelice pokazuju razlike među sortama

Separation in narrower pH gradient of 4.0-6.5 (Fig. 5a) improved resolution of protein bands, but the obtained protein patterns were identical for all the examined varieties. When albumins/globulins were separated by IEF in pH gradient of 3.5-9.5 they were blotted to PVDF membrane and specifically stained for glycoprotein moiety. The obtained protein patterns (Fig. 4b) differed significantly from the pattern obtained by Coomassie Blue staining, but no differences between varieties could be observed.

Analysis of aminopeptidase activities in gel slices after IEF in pH gradient of 4.0-6.5 of proteins extracted from dry grains and green malt, by using Phe-, Leu-, and Arg-2NA as substrates, revealed the existence of three aminopeptidases of different isoelectric points

(pI). They are AP of pI=4.4 splitting Phe- and Leu-2NA, AP of pI=4.6 splitting Arg-2NA and AP of pI=4.9 that hydrolysed all three substrates (Fig. 5b). Those data are consistent with previous reports on the presence of arginyl-AP, Phe and Leu preferring, and broad specificity AP in dry grains and green malt of barley varieties (Strelec et al., 2009). The lack of differences between varieties in their aminopeptidases pattern after IEF occurred probably due to previously suggested conservation of amino acid sequences during phylogenesis. Obtained data indicate that IEF of barley albumin/globulin protein fraction in pH gradients of 3.5-9.5 and 4.0-6.5 combined with Coomassie Brilliant Blue G-250, glycoprotein or aminopeptidase activity detection does not present a promising method for Croatian barley varieties discrimination.

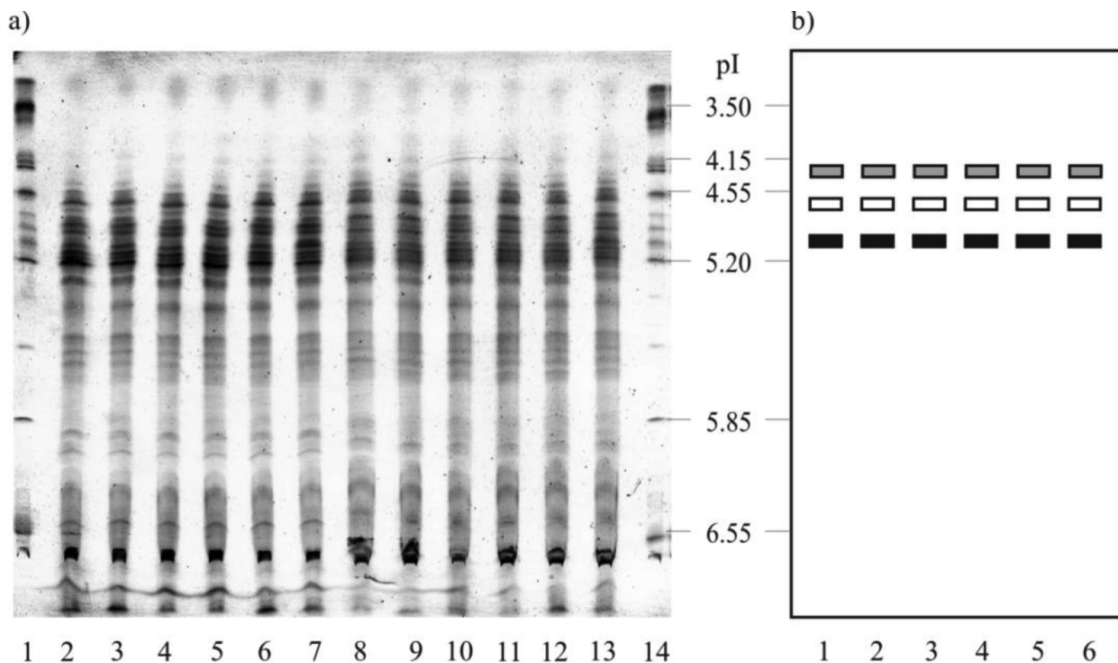


Figure 5. IEF in pH gradient of 4.0-6.5 of barley albumins/globulins extracted from dry grains and green malts with 50 mM Tris-HCl buffer (pH= 8.0). (a) Proteins stained with Coomassie Brilliant Blue G-250, (b) schematic presentation of aminopeptidase zymograms. Vertical lanes present: protein pI standards, pH=3-10 (1, 14), dry grains (2-7) green malts (8-13). Barley varieties: Angora (2a, 8a, 1b); Barun (3a, 9a, 2b); Rodnik (4a, 10a, 3b); Sladoran (5a, 11a, 4b); Rex (6a, 12a, 5b); Martin (7a, 13a, 6b). Grey boxes - aminopeptidase detected with Phe- and Leu-2NA; white boxes - aminopeptidase detected with Arg-2NA, black boxes - aminopeptidases detected with Arg-, Leu- and Phe-2NA as substrates

Slika 5. Izoelektrično fokusiranje u gradijentu pH 4,0-6,5 albumina/globulina ekstrahiranih iz suhoga zrna i zelenoga slada ječma pomoću 50 mM Tris-HCl pufera (pH=8,0). (a) Proteini obojani pomoću Coomasie modrila, (b) shematski prikaz zimograma aminopeptidaza. Stupci: pI standardi pH=3-10 (1, 14), suho zrno (2-7); zeleni slad (8-13). Sorte ječma: Angora (2a, 8a, 1b); Barun (3a, 9a, 2b); Rodnik (4a, 10a, 3b); Sladoran (5a, 11a, 4b); Rex (6a, 12a, 5b); Martin (7a, 13a, 6b). Sivi kvadrati - aktivnost detektirana uz Phe- i Leu-2NA; bijeli kvadrati - aktivnost detektirana uz Arg-2NA; crni kvadrati - aktivnost detektirana uz Arg-, Leu- i Phe-2NA. Strelice pokazuju razlike među sortama

CONCLUSION

The applicability of various one-dimensional electrophoretic methods for barley variety discrimination using their albumins/globulins fraction from dry grains and green malt, and glycoproteins and aminopeptidase electroforegrams visualisation of six barley varieties have been investigated. In all the separation experiments, analysis of dry grain protein extracts gave better results than green malt extracts suggesting their selection for application. Aminopeptidase activities towards Phe-, Leu, and Arg-2NA as substrates were not found as reliable markers for discrimination of examined barley varieties. Barley varieties could be partially discriminated by albumin/globulin protein pattern after SDS-PAGE (10 % T) and subsequent Coomassie Blue staining. This implies that SDS-PAGE of albumins/globulins could be proven useful in Croatian barley variety discrimination.

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ELEKTROFORETSKI PROFIL ALBUMINA/GLOBULINA EKSTRAHIRANIH IZ SUHOGA ZRNA I ZELENOGA SLADA SORTI JEČMA

SAŽETAK

Ispitana je mogućnost primjene različitih elektroforetskih metoda razdvajanja albumina/globulina slijeđenih nespecifičnim bojanjem proteina, te specifičnom detekcijom glikoproteina i aminopeptidaza za razlikovanje sorti ječma. Albumini/globulini ekstrahirani iz suhoga zrna i zelenoga slada šest sorti ječma razdijeljeni su poliakrilamid gel elektroforezom nativnih uzoraka (10 % T, pH=8,9), poliakrilamid gel elektroforezom u prisutnosti natrijeva dodecil-sulfata te izoelektričnim fokusiranjem u gradijentima pH 3,5-9,5 i pH 4,0-6,5. Ekstrakti proteina iz suhoga zrna davali su bolje rezultate od onih iz zelenoga slada. Rezultati upućuju da bi razdjeljivanje albumina/globulina pomoću SDS-PAGE i izoelektričnoga fokusiranja u gradijentu pH 3,5-9,5 slijeđeno bojanjem proteina pomoću Coomassie Blue moglo poslužiti za razlikovanje hrvatskih sorti ječma. Sortu Angora moguće je, nedvojbeno, razlikovati od ostalih sorti koje se mogu grupirati u skupine kako slijedi: Rodnik/Sladoran, Barun/Rex te sorta Martin. Bojanje glikoproteina ne osigurava razlikovanje ispitanih sorti. Detekcija aktivnosti aminopeptidaze široke specifičnosti, aminopeptidaze koja preferira fenilalanin i leucin, te arginil-aminopeptidaze po provedenom elektroforetskom razdvajanju nije se pokazala pogodnom metodom za razlikovanje ispitanih sorti ječma.

Ključne riječi: razlikovanje sorti ječma, elektroforetska razdvajanja, detekcija glikoproteina, detekcija aktivnosti aminopeptidaza

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