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Original scientific paper

PATH COEFFICIENT ANALYSIS OF QUALITY OF TWO-ROW SPRING BARLEY

Novo PRŽULJ[1], Vojislava MOMČILOVIĆ[1] and Jovan CRNOBARAC[2]

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[2]Faculty of Agriculture, Novi Sad, Serbia

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Malting quality is composed of numerous interacting traits with a high complexity concerning their biochemical and genetic basis**.** Malt extract is key indicator of barley malting quality and it is a mega-trait since it is influenced by a number of independent component traits. Understanding genetic and non-genetic factors that effects grain quality and grain yield is crucial in developing new cultivars, seed and mercantile production. Path analysis is one of the reliable statistical techniques which allow separation of the direct effect of each component trait on malt quality from the indirect effects caused by the interdependence component trait. The aim of this study was to investigate spring two-row barley quality as mega-trait depending on the component traits in the conditions of the Pannonian environments. Regression analysis with extract (EXT) as dependant and other traits (yield-YIL, test weight-TW, grain weight-GW, grading-GRA, grain protein concentration-GPC, viscosity-VIS, Kolbach index-KOL, Hartong number-HAR) as independent traits was performed out. Simple coefficient of correlations were calculated between independent traits and EXT in all pair combination and then used as inputs for path coefficient analysis. The quadratic curve fitted the best relationship between EXT and the independent traits. EXT was in positive ($P<0.01$) relationship with GW, GRA, KOL, and HAR with simple correlation coefficient of 0.47, 0.42, 0.39 and 0.50, respectively and in negative ($P<0.01$) relationship with GPC and VIS with simple correlation coefficient of -0.72 and -0.51, respectively. Path analysis explained more than 70% of the variation in EXT of which 34.3% was determined by direct negative path coefficient ($P<0.01$) of GPC without significant any indirect path effect. VIS negatively directly, ($P<0.01$) and negatively indirectly via GPC effected EXT. KOL did not have significant direct effect on EXT, but had rather prominent indirect effect via GPC, VIS and HAR. HAR positively

Corresponding author: Novo Pržulj, Institute of Field and Vegetable Crops,Maksima Gorkog 30, 21000 Novi Sad, Serbia,Tel: 021 4898 220, fax: 021 4898 222,e-mail: novo.przulj@ifvcns.ns.ac.rs

directly (P<0.01) and positively indirectly via GPC effected EXT. The direct effect of VIS and HAR determined 13.0% and 14.1% of the variation, respectively.

Key words: Barley (*Hordeum vulgare* L.), malt, extract, quality, path coefficient analysis, correlation, regression

INTRODUCTION

Quality of barley grain is quantitative and complex trait that is determined by genetic and environmental factors (LU *et al*., 1999). Some authors considered quality as a mega-trait because it is influenced by a number of independent component traits (EMEBIRI, 2010; MOLINA-CANO *et al*., 2000). Malt extract is key indicator of barley malting quality (MOLINA-CANO *et al*., 2000; FOX *et al*., 2003). Relationships between component traits can be different, from absent of significant relationship till almost functional relationship and interaction in compensatory patterns (LU *et al*., 2000; GARCIA DEL MORAL *et al*., 2003).

Malting quality is dependent upon both the physical and chemical properties of mature grain and the enzymes synthesized during germination. Important malting quality traits include grain size (grain weight and grain plumpness), grain protein content, malt extract and diastatic power (LU *et al*., 1999). Grain size is an important trait for any barley breeding program. Ideally, large grain size would be one of the primary grain quality objectives as smaller grains generally have lower levels of starch and higher protein levels, thus reducing the energy that could be utilised for any end use (COVENTRY *et al*., 2003; FOX *et al*., 2006). Large grains generaly have increased levels of starch and therefore more potential extract. However, excessively large grain could impact on malt quality particularly on the rate of water hydrationand modification during malting (FOX *et al*., 2003). Grain weight was also reported to be related to malt extract (SWANSTON and MOLINA-CANO, 2001), so that, these characteristics are closely correlated with malt quality.

Barley protein inherited quantitatively and in itself rather complicated and as such has important role in grain structure and complex interaction with quality. High protein level is in strong correlation with low starch content and thus low extract content (FOX *et al*., 2003). Barley with too low protein content (<9%) is also unacceptable for production good malt, but in the Pannonian conditions usually there is no problem with low barley protein content (PRŽULJ *et al*., 1998). Viscosity depends of the breakdown of β-glucan during malting. Viscosity cannot be related to a single trait within barley and high level of viscosity reduces the efficiency of breweries (FOX *et al*., 2003). The Kolbach index is an indicator of proteolytic modification of the malt and reflects protein solubilized during malting and preparation of the congress mash. Free amino nitrogen, amino acid composition and Kolbach index can fully show the degradation of protein. The optimum steeping and germination time and temperature are the key factors influencing the protein modification (GRUJIĆ *et al*., 2005). Kolbach index was calculated using the ratio of soluble protein to malt protein. The determination of the Hartong at 45°C is the most revealing result because at this temperature, the proteolytic and cytolytic activity of enzyme is maximum. Higher numbers are given by better modified malts where is value < 30 means bad malt, 30- 36 insufficient malt, 36-40 malt possessing a satisfactory enzymatic quantity and > 40 good malt, rich in enzymes.

Understanding genetic and non-genetic factors effecting grain quality and grain yield is crucial in the developing new cultivars. From previous studies it is known that malting quality traits and yield are under strong effect of non-genetic factors first of all environments and nitrogen fertilizer (SCHELLING *et al*., 2003; PETTERSSON and ECKERSTEN, 2007; PRŽULJ and MOMČILOVIĆ, 2008; MARCONI *et al*., 2010). Seasonal quality variation is mostly consequence of the effect of high temperatures or water deficit during grain filling (PASSARELLA *et al*., 2005). The temperature effects during grain filling are attributed to direct effects on the grain, either reducing starch synthesis or decreasing grain size due to effects on grain development itself. Consequences of unfavorable factors are decreased physical grain properties, higher grain protein concentration and finally lower malt quality.

In the most of the previous studies involving malting quality the traits were analyzed independently (PRŽULJ and MOMČILOVIĆ, 2008; EMEBIRI, 2010). Single trait analysis has advantage of calculation but disadvantage for explanation of multivariate structure of the data. Due to cause-effect relationships between variables in a complex system simple correlations analysis may not provide reliable information of the importance of each component trait in determining mega-trait. Path analysis is one of the reliable statistical techniques which partitions correlation coefficients into direct and indirect effects and allows separation of the direct effect of each component trait on malt quality from the indirect effects caused by the interdependence component trait (GUILLEN-PORTAL *et al*., 2006).

The aim of this study was to investigate spring two-row barley quality as mega-trait depending on the component traits in the conditions of the Pannonian environments.

MATERIALS AND METHODS

Field trial and grain analysis. The eight two-row spring barley cultivars were studied during a seven-year period (1998-2004) in the location of Novi Sad (45°20'N, 15°51'E, 86 m asl). A complete randomized block design with three replications was used. Plots 5m x 1m in size with 10 cm between rows were sown at the rate of 400 germinable grains m^{-2}. The growing practices applied were those regularly used for large-scale spring malting barley production. Grain yield (YIL) was determined for combine-harvested plots in each of the three replications with correction to 130 g kg^{-1} moisture. After that, grains were sieved on Sortimat machine (Pfeuffer) and the grading percentage (GRA, %), i.e., grains retained on a 2.5 mm and 2.8 sieves (I class) was recorded and used for physical grain property determination, grain protein concentration (GPC, g 100^{-1}g dm) and micro-malting. Test weight (TW, kg hl^{-1}) was determined by Perten Inframatic 9200 NIR Analyser. Grain weight (GW, g) was determined from graded samples by measuring three sets of 300 grains per plot and expressed as weight of 1000 grains. Grain N concentration (GNC) obtained by Kjeldahl analysis and protein concentration expressed on dry weight basis was estimated by multiplying grain N by 6.25. Micro-malting according to a procedure recommended by the European Brewery Convention (Analytica-EBC, 1998) was conducted four months after harvesting using the micromalting plant Seeger, Germany. After malting viscosity (VIS, mP.s), Kolbach index (KOL, %), Hartong number (HAR, VZ 45°C) and malt extract (EXT, % dm) were determined.

Statistical analyses. Regression analysis with EXT as dependant and other traits as independent variables was performed out. Simple coefficient of correlations were calculated between YIL, TW, GW, GRA, GPC, VIS, KOL, HAR and EXT in all pair combination and then used as inputs for path coefficient analysis. Path coefficient analysis was performed to partition the correlation coefficient, r_{ij}, into direct and indirect effects. The EXT was the dependent variable and other traits were considered as independent (Figure 1). The Genstat v10 procedures and programs were used for data processing.

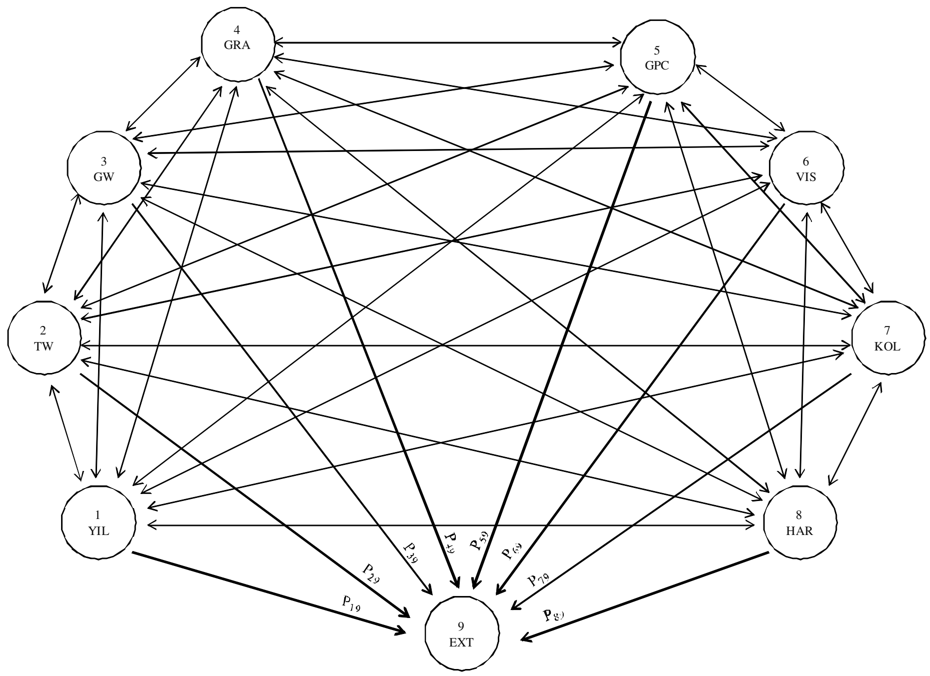

Figure 1. Path coefficient diagram showing the interrelationships among malt extract (9-EXT) as the dependent variable and (1-YIL), test weight (2-TW), grain weight (3-GW), grading (4-GRA), grain protein concentration (5-GPC), viscosity (6-VIS), Kolbach index (7-KOL) and Hartong number (8-HAR) as the independent variable. The single-headed arrows indicate direct effects, and the double headed arrows indicate simple correlation coefficients.

The following simultaneous equations were solved in order to determine path coefficient, P_{ij} (subscripts designate nine characters):

$r_{19}=P_{19}+r_{12}P_{29}+r_{13}P_{39}+r_{14}P_{49}+r_{15}P_{59}+r_{16}P_{69}+r_{17}P_{79}+r_{18}P_{89}$

$r_{29}=r_{12}P_{19}+P_{29}+r_{23}P_{39}+r_{24}P_{49}+r_{25}P_{59}+r_{26}P_{69}+r_{27}P_{79}+r_{28}P_{89}$

$r_{39}=r_{13}P_{19}+r_{23}P_{29}+P_{39}+r_{34}P_{49}+r_{35}P_{59}+r_{36}P_{69}+r_{37}P_{79}+r_{38}P_{89}$

$r_{49}=r_{14}P_{19}+r_{24}P_{29}+r_{34}P_{39}+P_{49}+r_{46}P_{59}+r_{46}P_{69}+r_{47}P_{79}+r_{48}P_{89}$

$r_{59}=r_{15}P_{19}+r_{25}P_{29}+r_{35}P_{39}+r_{45}P_{49}+P_{59}+r_{56}P_{69}+r_{57}P_{79}+r_{58}P_{89}$

$r_{69}=r_{16}P_{19}+r_{26}P_{29}+r_{36}P_{39}+r_{46}P_{49}+r_{56}P_{59}+P_{69}+r_{67}P_{79}+r_{68}P_{89}$

$r_{79}=r_{17}P_{19}+r_{27}P_{29}+r_{37}P_{39}+r_{47}P_{49}+r_{57}P_{59}+r_{67}P_{69}+P_{79}+r_{78}P_{89}$

$r_{89}=r_{18}P_{19}+r_{28}P_{29}+r_{28}P_{39}+r_{48}P_{49}+r_{58}P_{59}+r_{68}P_{69}+r_{78}P_{79}+P_{89}$

In the equation $r_{19}=P_{19}+r_{12}P_{29}+r_{13}P_{39}+r_{14}P_{49}+r_{15}P_{59}+r_{16}P_{69}+r_{17}P_{79}+r_{18}P_{89}$, P_{19} is the direct effect of trait 1 (YIL) on trait 9 (EXT) and this is path coefficient. Value of $r_{12}P_{13}$ represents indirect effect of trait 1on 9, via 2, value of $r_{13}P_{39}$ represents indirect effect of trait 1 on 9 via 3 and so on. Similar explanations refer to the other equations.

RESULTS AND DISCUSSION

EXT is the most important trait whether selecting potential new malting varieties or trading malt. The quality of the EXT is influenced by growing conditions, temperature, fertilizer, available nitrogen or moisture (COLLINS *et al*., 2003). These factors do not impact on extract directly but rather effect grain traits that influence extract, predominantly protein and starch level and composition. Many papers reported some relationships between EXT and properties of grain and malt (LU *et al*., 2000; COVENTRY *et al*., 2003; MOLINA-CANO *et al*., 2004). We tried by regression analysis to determine the relationships in our research (Figure 2). The quadratic curve fitted the best relationship between all pairs of regressed traits. Although in all plotted relations the coefficient of determination was low and non significant some trends could be noticed. Obviously EXT decreased with increasing value of GPC (Figure 2e) and VIS (Figure 2f). It could be state that barleys with TW of 71-74kg hl^{-1} and GW 45-50g give the highest EXT (Figure 2b, c). There was a trend of positive regression of EXT against YIL, KOL and HAR (Figure 2a, g, h).

To be included in growing by farmers and use for malting by maltsters malting barley varieties must be both high yielding and with good quality. Yield and quality are mega-traits, which are defined by their “own” component traits, mutual traits for yield and quality, and environment. Thus, yield is defined by its principal components; grains number per square meter and grain weight, but in same time there is a positive regression of YIL on TW and GRA. TW does not have big importance in either yield or quality but it is still good indicator of both of them. GW and GRA are important physical property of grain and indicator of grain quality for malting, with positive relationship with EXT (FOX *et al*., 2003).

Briefly, YIL positively correlated with TW ($P<0.05$), GW ($P<0.01$) and GRA ($P<0.01$) (Table 1). GW had positive correlation ($P<0.01$) with TW and GRA. GRA had positive correlation ($P<0.01$) with TW. These positive relationships should enhance selection for improved malting quality in breeding programs.

In our study simple correlation showed an absence significant relationship of GPC with YIL and TW, while the relationship with GW and GRA was negative ($P<0.05$). Higher YIL decreased and higher GPC increased VIS ($P<0.01$) what are appropriate relationships for breeding of malting barley. GPC is a major determinant of malting quality in barley, as it influences any aspects of the malting and brewing processes (EMEBIRI *et al*., 2005). Malting barley with a high protein content results in lower extracts for the brewer. It also slows down water uptake during steeping, potentially affecting final malt quality. Major storage protein in barley is hordein which comprises of 40-50% of total grain protein (FOX *et al*., 2003). Hordein is soluble in aqueous alcohol and comprises four fraction designated D, C, B and A. The diversity in the hordein fraction is useful in varietal identification (MOLINA-CANO *et al*., 2004). Each of four hordein fractions has specific relationship to EXT (FOX *et al*., 2003).

Higher TW, GW and GRA are generally desirable features of malting barley, but with some limits. Indeed, the cultivars with medium size of grain (41-44g) suit better for malting because they soak uniformly and rapidly (FOX *et al*., 2003), that means very heavy grains are more required for grain components decomposition. That explains the negative correlation of protein breakdown with TW and GW (Table 1). KOL was also in negative correlation (P<0.01) with GPC and VIS. The determination of the HAR at 45° is the most revealing result because at this temperature, the proteolytic and cytolytic activity of enzyme is maximum. So, a negative correlation (P<0.01) of HAR with GPC and VIS and positive with KOL is expected. In this research there was no significant correlation of HAR with YIL and grain properties.

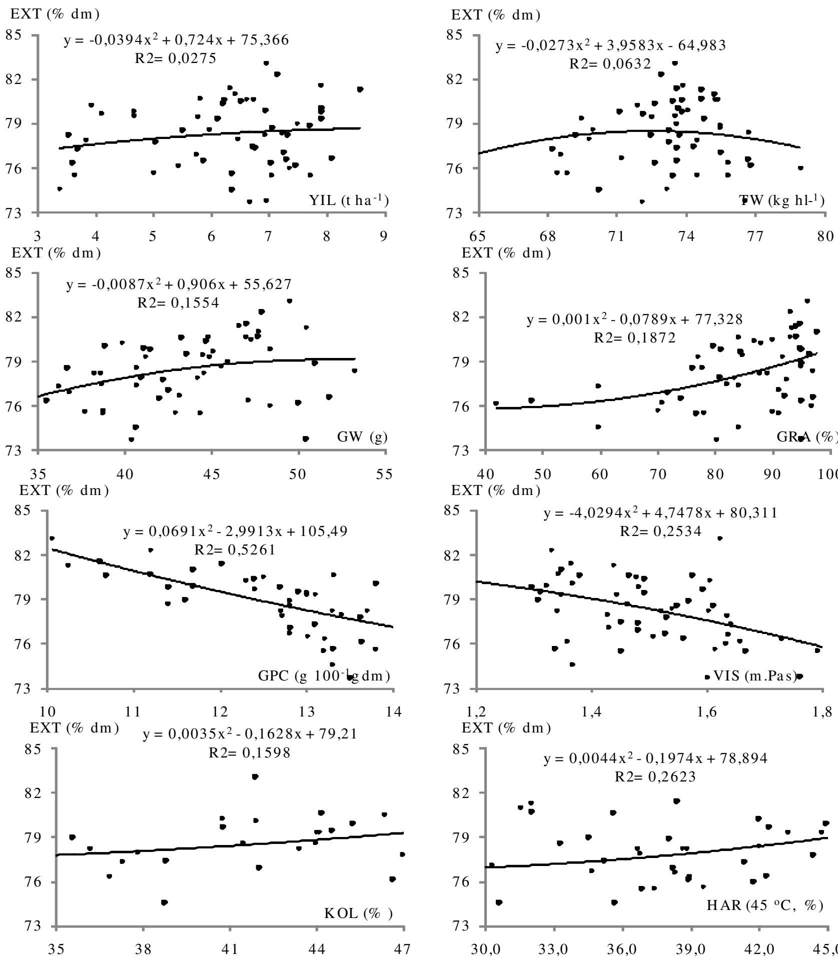

Figure 2. Relationship between yield (YIL), test weight (TW), grain weight (GW), grading (GRA), grain protein concentration (GPC), viscosity (VIS), Kolbach index (KOL), Hartong number (HAR) as independent and malt extract content (EXT) as the dependent variable in eight two-row spring barley cultivars in 7 years (1998-2004) period

Table 1. Simple correlation coefficients between yield (YIL), test weight (TW), grain weight (GW), grading (GRA), grain protein concentration (GPC), viscosity (VIS), Kolbach index (KOL), Hartong number (HAR) and malt extract content (EXT) in eight two-row spring barley cultivars in 7 years (1998-2004) period.

	YIL	TW	GW	GRA	GPC	VIS	KOL	HAR
TW	0.302*							
GW	0.394**	0.745**						
GRA	0.402**	0.666**	0.747**					
GPC	-0.049	0.008	-0.277*	-0.302*				
VIS	-0.425**	0.141	0.033	-0.149	0.387**			
KOL	-0.153	-0.482**	-0.301*	-0.192	-0.354**	-0.444**		
HAR	-0.084	-0.108	0.054	0.060	-0.271*	-0.304*	0.650**	
EXT	0.162	0.124	0.370**	0.421**	-0.722**	-0.506**	0.386**	0.499**

Relationship of EXT with YIL and TW was not significant (P>0.05) (Table 1). However, the relations of EXT with grain physical properties that are important for malt quality, GW and GRA, are significantly positive (P<0.01). Higher GPC and VIS significantly (P<0.01) decreased EXT content while higher HAR marked higher EXT.

Path analysis explained more than 70% of the variation in EXT of which 34.3% was determined by direct negative path coefficient (P<0.01) of GPC (Table 2). In this study GPC did not have a significant indirect path effect. MOLINA-CANO *et al*. (2004) found that correlation of EXT with KOL depended of the environments. In Canada environments EXT highly negatively correlated with GPC and positively with KOL, whereas in Spain it is weakly correlated with GPC and positively with KOL. So, it is evident that intensity of negative correlation between EXT and GPC depends also on growing conditions and due to that high GPC does not always mean low EXT. In MOLINA-CANO *et al*. (2004) study it was found that the Canadian-grown barleys, despite showing lower protein and hordein contents, produced malt of inferior quality than their Spanish-grown counterpart, which, overall, produced higher levels of degrading enzymes (amylolytic, proteolytic and possibly, cytolytic) during germination.

Table 2. Direct (DPC) and indirect (IPC) path coefficients between yield (YIL), test weight (TW), grain weight (GW), grading (GRA), grain protein concentration (GPC), viscosity (VIS), Kolbach index (KOL), Hartong number (HAR) on malt extract content (EXT) in eight two-row spring barley cultivars grown during seven growing seasons (1998-2004) period.

	DPC on	IPC on EXT									
	EXT	YIL	TW	GW	GRA	GPC	VIS	KOL	HAR	PCD	TCD
YIL	-0.0617		0.0034	0.0591	0.0501	0.0233	0.1092	0.0022	-0.0237	-0.0100	0.7076
TW	0.0114	-0.0186		0.1117	0.0831	-0.0038	-0.0362	0.0070	-0.0305	0.0014	
GW	0.1499	-0.0243	0.0085		0.0932	0.1316	-0.0085	0.0044	0.0152	0.0555	
GRA	0.1247	-0.0248	0.0076	0.1120		0.1435	0.0383	0.0028	0.0169	0.0525	
GPC	-0.4752**	0.0030	0.0001	-0.0415	-0.0377		-0.0995	0.0051	-0.0764	0.3431	
VIS	-0.2570*	0.0262	0.0016	0.0049	-0.0186	-0.1839		0.0064	-0.0857	0.1300	
KOL	-0.0145	0.0094	-0.0055	-0.0451	-0.0239	0.1682	0.1141		0.1833	-0.0056	
HAR	0.2820*	0.0052	-0.0012	0.0081	0.0075	0.1288	0.0781	-0.0094		0.1407	

In our research VIS negatively directly (P<0.01) and indirectly via GPC effected EXT. HAR positively directly (P<0.01) and positively indirectly via GPC effected EXT. The direct effect of VIS and HAR determined 13.0% and 14.1% of the variation, respectively. Contrary to the significant simple correlation between KOL and EXT, KOL did not have significant direct effect on EXT, but had rather prominent indirect effect via GPC, VIS and HAR.

There is evidence of a difference in the negative effect of barley protein upon the final malt extract yield when going from the north to the south of Europe (MOLINA-CANO *et al*., 2000). Indeed, contrasting temperature and daylight conditions across the crop life cycle were thought to be responsible for the recorded differences, since in south barley is winter and in north spring crop. MOLINA-CANO *et al.* (2004) found that different hordein protein composition and ß-glucan effected different patterns of malt extract development.

Malting quality is composed of numerous interacting traits with a high complexity concerning their biochemical and genetic basis (FOX *et al*., 2003). In most instances, the traits are conditioned by interaction of polygenes, so called quantitative trait loci (QTLs). To accelerate the breeding of cultivars with improved malting quality, molecular markers and genetic linkage maps were used to localize these loci. Thus, in recent years, the elite barley gene pool was extensively used for numerous QTL studies on malting quality (EMEBIRI *et al*., 2005; PANOZZO *et al*., 2007; EMEBIRI 2010).

CONCLUSION

Malt extract, as a key indicator of barley malting quality, is mega-trait because it is influenced by a number of component traits. Grain protein concentration is a major determinant of malting quality in barley and **m**alting barley with high grain protein content results in lower extract. Barleys with higher test weight, grain weight and grading give generally malt with higher extract content. The relationship of malt extract with grain properties highlighted direction and success of improving spring barley quality.

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PATH KOEFICIJENT ANALIZA KVALITETA DVOREDOG JAROG JEČMA

Novo PRŽULJ[1], Vojislava MOMČILOVIĆ[1] i Jovan CRNOBARAC[2]

[1]Institut za ratarstvo i povrtarstvo, Novi Sad, Srbija
[2]Poljoprivredni fakultet, Novi Sad, Srbija

Izvod

Kvalitet pivskog ječma određuje veliki broj međusobno povezanih osobina, kompleksne biohemijske i genetička osnove. Ekstrakt slada je osnovni pokazatelj kvaliteta pivskog ječma i može se smatrati mega osobinom**,** pošto zavisi od delovanja velikog broja drugih osobina. Poznavanje genetičkih i negenetičkih faktora pod čijom kontrolom se nalazi kvalitet i prinos pivskog ječma suštinsko je za stvaranje novih sorti i uspešnu semensku i merkantilnu proizvodnju. Path analiza je pouzdana statistička metoda koja omogućava razdvajanje direktnih i indirektnih efekata nezavisnih osobina na mega osobinu ili mega osobine. Indirektni efekti su posledica međuzavisnosti i zajedničkog delovanja nezavisnih osobina**.** Cilj ovoga istraživanja je proučavanje ekstrakta slada, kao mega osobine, u zavisnosti od osnovnih osobina zrna i slada, koje su u određenoj vezi sa sadržajem ekstrakta u sladu. Istraživanje je obavljeno u periodu 1998-2004 na osam sorti dvoredog jarog ječma u agroekološkim uslovima Panonske nizije. Odnos između sadržaja ekstrakta u sladu (EXT) kao zavisne osobine i prinosa (YIL), zapreminske mase (TW), mase hiljadu zrna **(**GW), sortiranja (GRA), sadržaja proteina u zrnu (GPC), viskoziteta (VIS), Kolbah indeksa (KOL), Hartong broja (HAR) kao nezavisnih osobina određeni su regresionom analizom, prostom korelacijom i path analizom**.** Kvadratna regresija je najbolje predstavljala funkcionalnu determinisanost EXT navedenim nezavisnim osobinama, mada nisu utvrđene statisički značajne vrednosti koeficijenta determinacije**.** Vrednosti prostih koeficijenta korelacije između EXT s jedne strane i GW, GRA, KOL, i HAR sa druge strane bile su pozitivne ($P<0.01$) i iznosile su 0.47, 0.42, 0.39 i 0.50, respective. EXT je bio u negativnoj ($P<0.01$) korelacionoj vezi sa GPC, -0.72 i VIS, -0.51. Path analiza je objasnila 70% varijabilnosti kod EXT, od čega je 34.3% bilo pod uticajem negativnog direktnog path koeficijenta GPC, koji nije imao značajnih indirektnih efekata. VIS je imao značajan ($P<0.01$) direktan efekat na EXT i indirektan efekat preko GPC. KOL nije imao značajan direktan efekat na EXT ali su bile visoke vrednosti indirektnih efekata KOL preko GPC, VIS i HAR. HAR je imao značajan pozitivan ($P<0.01$) direktan efekat na EXT i indirektan efekat preko GPC. Pod uticajem VIS bilo je 13.0%, a pod uticajem HAR 14.1% od ukupne varijabilnosti EXT.

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