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Comparison of ethanol productions of maize (*Zea mays* L.) HTF hybrids and conventional hybrids from Croatian breeding programs

Usporedba u proizvodnji etanola iz kukuruza (*Zea mays* L.) između HTF hibrida i hibrida iz hrvatskih oplemenjivačkih programa

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ABSTRACT

The aim of this study was to determine the differences in grain and ethanol yields between hybrids obtained from breeding programs in the Republic of Croatia and introduced HTF (High Total Fermentable) maize hybrids. The trial was conducted at three sites (Osijek, Beli Manastir and Zagreb) for two consecutive years (2012 and 2013) in three plant densities (60,000, 70,000 and 80,000 plants/ha). Pioneer HTF hybrids for the ethanol production were used as controls to assess the productivity of ethanol of hybrids from the Agricultural Institute Osijek and Bc Institute d.d. The study of ethanol production was carried out by using the Lemuz method. According to ANOVA statistical analysis, statistically significant differences in grain and ethanol yields were not found between HTF hybrids and hybrids of domestic breeding companies. Statistically significant difference was found in ethanol yield by sites ($P<0.05$) with highest average ethanol yield determined at Beli Manastir and the lowest in Osijek. Site × year × plant density interaction showed statistically significant difference in ethanol yield between hybrids and the most productive was the OS378 hybrid. Ethanol production of hybrids designated for this purpose did not produce higher ethanol yields than domestic hybrids from the same amount of grain.

Keywords: yield, plant density, site, growing season, ethanol production

SAŽETAK

Cilj istraživanja bio je utvrditi razliku u proizvodnji zrna i etanola iz kukuruza između domaćih hibrida kukuruza koji se uobičajeno koriste za proizvodnju zrna i uvezenih HTF (High Total Fermentable) deklariranih hibrida za proizvodnju etanola. Poljski pokus je postavljen na 3 lokacije (Osijek, Beli Manastir, Zagreb) dvije godine uzastopno (2012, 2013) u tri gustoće sklopa (60,000, 70,000 i 80,000 biljaka/ha). Hibridi tvrtke Pioneer, deklarirani kao "HTF" hibridi za proizvodnju etanola iz zrna kukuruza korišteni su kao kontrola za procjenu produktivnosti etanola iz domaćih hibrida Poljoprivrednog instituta Osijek i Bc Instituta d.d. Laboratorijska proizvodnja etanola utvrđena je Lemuz metodom. Sukladno ANOVA statističkoj analizi nisu utvrđene statistički značajne razlike u prinosu zrna i etanola između HTF hibrida i hibrida domaćih oplemenjivačkih kuća. Statistički značajna razlika utvrđena je u prinosu etanola po lokalitetima ($P<0.05$) gdje je prosječno najveći prinos etanola ostvaren u Belom Manastiru, a najmanji u Osijeku. Interakcija lokalitet × godina × gustoća biljaka imala je statistički značajan utjecaj na prinos etanola između hibrida, a najproduktivniji je bio hibrid OS378. Deklarirani namjenski hibridi za proizvodnju etanola nisu proizveli više etanola u odnosu na domaće hibride iz iste količine zrna kukuruza.

Ključne riječi: prinos, gustoća sklopa, lokacija, vegetacijska sezona, proizvodnja etanola

INTRODUCTION

Maize is a source material for many products and is used in many ways around the world. Although it belongs to the raw materials of the first generation of production of biofuels from renewable energy sources (Aro, 2016) in recent times, or the last 15 years, maize is increasingly used in ethanol production (Ranum et al., 2014). The United States and Brazil play leading roles in the production and use of bioethanol as a fuel in the world, accounting for 84% of global production in 2018 (RFA 2019 Ethanol Industry Outlook).

In Croatia, to date, there has been no targeted breeding program for the selection of maize for ethanol production. Maize breeding is carried out in the Republic of Croatia by using only conventional methods. Dien et al. (2002) states that there is no difference in ethanol yield between conventional hybrids and hybrids obtained using biotechnology. In the last ten years, seeds producing companies have significantly increased the number of hybrids on the market intended for ethanol production. Researchers from Pioneer seed company found that certain hybrids yield more ethanol per unit mass than other hybrids and labeled them “HTF” (High Total Fermentable). In her paper, Zoeller (2008) states that HTF hybrids produced 3.6% more ethanol than control.

Modern maize hybrids may differ in properties that are important for ethanol production. It is assumed that different hybrids will be differently productive in terms of ethanol production (Mladenović-Drinić et al., 2011). The number of plants per unit area (plant density) is a factor that is significant in maize production, and according to the literature, it can be concluded that different hybrids have shown different reactions in different plant densities in terms of grain yield (Tollenaar and Wu., 1999; Ahmad et al., 2008; Luca and Tabara, 2010; Moussavi et al., 2011). Furthermore, some studies have concluded that maize production in higher plant population can also provide an increase in grain yield per unit area (Douaglas et al., 1982). Hybrids with higher starch content also have higher ethanol yields (Milašinović et al., 2007), and the influence of hybrids and plant density on starch content has been observed (Mahanna, 2011). The hybrid itself, with its genetic predisposition, generates a greater or lower proportion of starch or protein whose ratio is generally inversely proportional (Idikut et al., 2009). A study of the influence of the level of applied agricultural technology has led to the conclusion that it does not effect the grain composition and ethanol yield (Jukić et al., 2003).

The aim of this study was to determine the differences in grain and ethanol yield between non-declared hybrids obtained from breeding programs in the Republic of Croatia and those foreign maize hybrids designated as HTF type.

MATERIALS AND METHODS

Field trials

Field trials were conducted during two growing seasons (2012 and 2013) and at three sites (Osijek, Beli Manastir and Zagreb). Soil types at the research sites were: fluvisol, chernozem and luvic calcocambisol. The pH of the soil at the sites where the research was conducted ranged from 6.68 to 7.10 (KCl). Humus content at the sites where the research was conducted was from 1,74 to 2,67%. Fertilization was made on the basis of soil tests and climate properties. Depending on the soil tests and climate properties, 160 to 200 kg/ha N, 80 to 110 kg/ha P_2O_5 and 120 to 180 kg/ha K_2O were used in the fertilization. Nutrients were added before fall tillage, pre-plant and through top-dressings. Previous crops at the sites where the research was conducted were: winter wheat, sugar beet and spring barley. Production practices were similar at all locations with ploughing, harrowing and application of soil seed harrow. Selected maize hybrids were grown under intensive production conditions (Svečnjak et al., 2004). Identical field trials were performed at all sites in four replications by random block design. The main factors in the trial were plant density, hybrid/seed company, year and site. In the field trials, a total of six maize hybrids were sown: PR37Y12 and PR35F38 (Pioneer Hi-Bred International, Inc., Johnston, Iowa, United States), OS378 and OS515 (Agricultural Institute Osijek, Osijek, Croatia) and Bc344 and Bc532 (Bc Institute d.d., Rugvica, Croatia). Hybrids from breeding programs of the Bc Institute d.d. and Agricultural Institute Osijek were selected as high-yield hybrids in its maturity groups, and hybrids from Pioneer Seed as declared HTF hybrids for maize ethanol production. Field trials were sown in three different planting densities (60,000, 70,000 and 80,000 plants/ha). The basic plot was consisted of four rows (row spacing was 0.7 m) 8 meters long, while the accounting plot was consisted of two middle rows 8 meters long. The sowing was done with a four-row pneumatic sowing machine "TC 2700" (Winersteiger AG, Ried im Innkreis, Austria). Harvest was carried out at the stage of physiological maturation (the appearance of a black layer in more than 50% of ears) (Abendroth et al., 2011) using a two-row harvester "Nurserymaster Elite" (Wintersteiger AG, Ried im Innkreis, Austria) specialized for micro-trials. At the harvest, grain yield and moisture content were determined, and a certain amount of grain was taken for further (laboratory) analysis (5 kg). The yields obtained were converted to 14% of moisture.

Weather conditions

The experiment was set in 2012 and 2013. Due to their climatic differences, the sites where the experiment was set up can be divided into two parts as west (Zagreb) and the east (Osijek and Beli Manastir), and weather conditions are recorded at Osijek and Zagreb-Maksimir stations. On east sites (Osijek) during vegetation period (from April to September) average monthly air temperature in 2012 and in 2013 were above than in long-term average except for September 2013. Also, on east sites in April, June, July and august in both years the amount of precipitation was lower than in long-term average. In august 2012 at east sites the amount of precipitation was just 4 mm in comparing with 59,3 mm how much it has fallen in the long-term average (Figure 1).

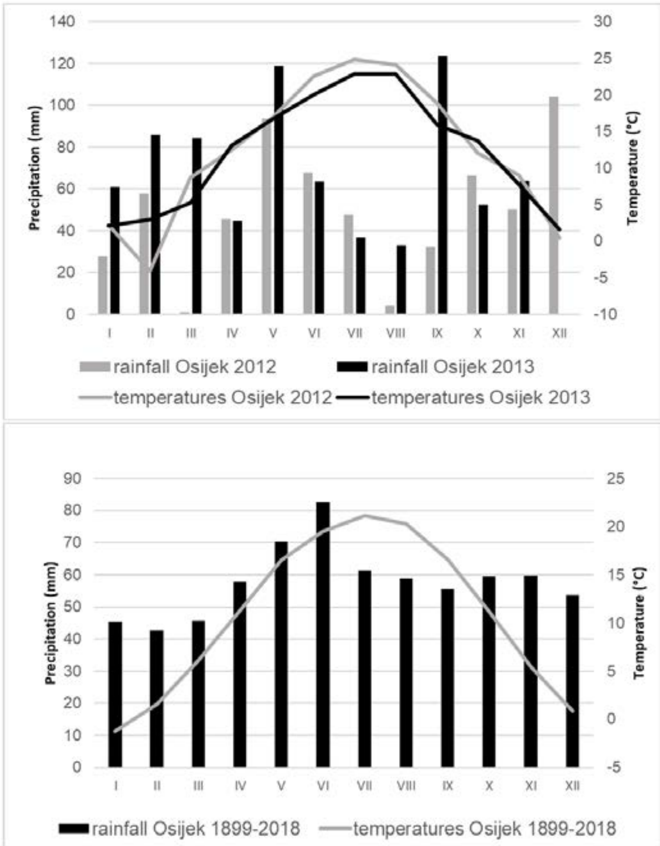


Figure 1. Seasonal rainfall and temperatures at Osijek in 2012 and 2013 and seasonal rainfall and temperatures in long-term average at Osijek

On west site (Zagreb) similar to the east sites, during vegetation period (from April to September) average monthly air temperature in 2012 and in 2013 were above than in long-term average except for September 2013. On west site in April and July in both years the amount of precipitation was lower and in May and September was higher than in long-term average. A large difference in the amount of precipitation was found for the August, when in 2012 much less precipitation fell than in the August 2013 (Figure 2).

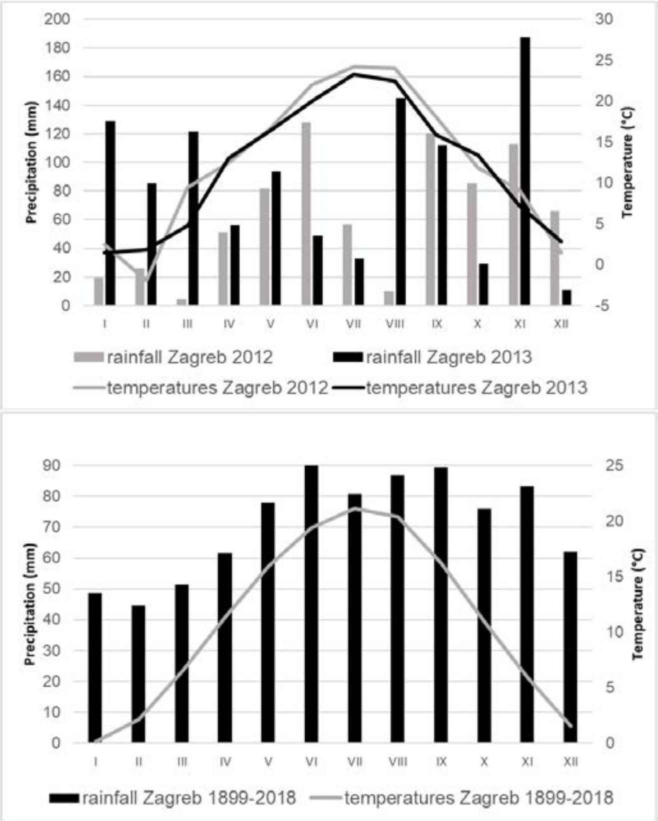


Figure 2. Seasonal rainfall and temperatures at Zagreb in 2012 and 2013 and seasonal rainfall and temperatures in long-term average at Zagreb

Laboratory analysis

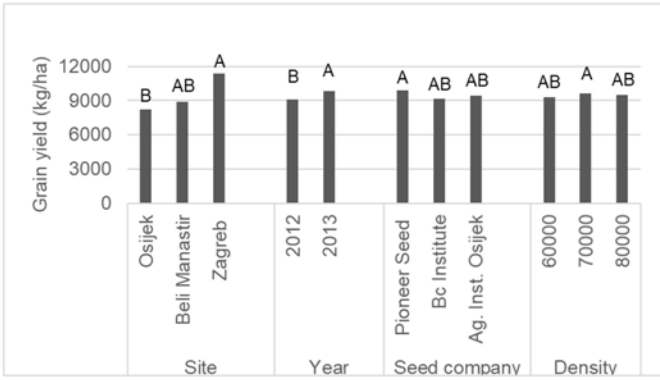
The analysis of maize grain samples taken at harvest were carried out at the Central Laboratory of the Departmant of Agroecology and Environmental Protection at the Faculty of Agro-biotechnical Sciences in Osijek. Prior to analysis, grain samples from which analysis were made were prepared by sample divider “Model 5” (Rationel Kornservice A/s, Esbjerg, Denmark). The size of the working samples depended on the type and procedure of the analyses. Preparation of samples involved drying in a drying oven “NI00CF” (ELE International, Leighton Buzzard, UK) at 130 °C to constant weight and grinding on a hammer mill “Cemotec 1090” (Foss A/s, Hillerød, Denmark), with sieves 3 mm in diameter. The grounded samples were used to determine ethanol yield. Hydrolysis of starch suspension was performed using α-amylase (Termamyl, SC DS, Novozymes, Denmark) and glucoamylase (Attenuzyme Core, Novozymes, Denmark). The fermentation phase was carried out using Fleischmann's yeast solutions, Fenton, MO, USA for 64 hours. Ethanol yield was determined by the gravimetric method (Lemuz et al., 2009) wherein the average sample weighed 25 g.

The results of the experiment were statistically processed in Microsoft Excel and SAS 9.3 software package (SAS Institute Inc., 2011). The ANOVA by ranks test, and multiple comparisons of mean ranks for factors (Fisher's LSD test), were carried out when more than two groups have been compared. The statistical hypothesis were tested at significance level $P = 0.05$.

RESULTS AND DISCUSSION

Plant analyses

The highest average grain yield was on the site Zagreb (11,360.1 kg/ha), and the lowest average grain yields were achieved in Osijek (8,192.2 kg/ha) and ANOVA statistical analyses showed that this difference was statistically significant as well as the difference between years (Figure 3).

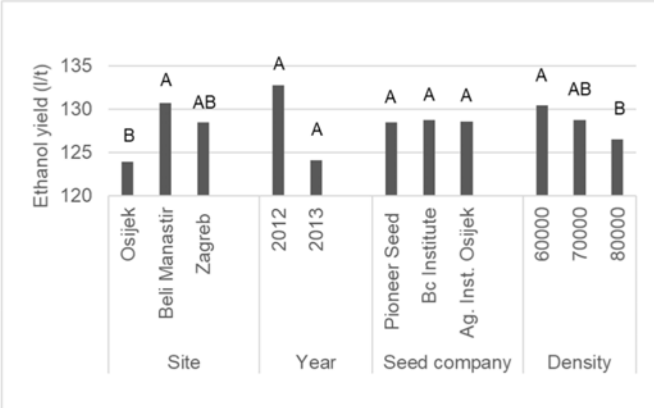


Bars labeled with different letters within each site, year, seed company and plant density are significantly different at $P<0.05$ (LSD test)

Figure 3. Grain yield in average depending on site, year, seed company and plant density

Namely, in 2013, there was a statistically higher average yield (9,848 kg/ha) than in 2012 (9,116.6 kg/ha), which was influenced by better agro-climatic conditions and better ameliorative properties of the soil for the cultivation of maize. Since production conditions play a big role in generating higher yields, according to Kovačević et al. (2013), alleviation of drought stress and extreme high air-temperature stress for maize is possible by irrigation in critical stages of maize growth, by growing of more tolerant genotypes and by adequate soil management. So, the highest grain yields were achieved by Pioneer hybrids (9,883.8 kg/ha), followed by the Agricultural Institute Osijek hybrids (9,407.7 kg/ha), while the Bc Institute d.d. hybrids had the lowest yield (9,155.3 kg/ha). The highest average grain yield was achieved at a plant density of 70,000 plants/ha (9,620.5 kg/ha). The lowest average grain yield was achieved at plant density of 60,000 plants/ha (9,302.6 kg/ha), which is partly in line with the statement (Murányi, 2015) that the hybrids used in the studies achieve maximum grain yield in assemblies of between 80,000 and 90,000 plants/ha. No statistically significant differences were found between these data in our study (Figure 3).

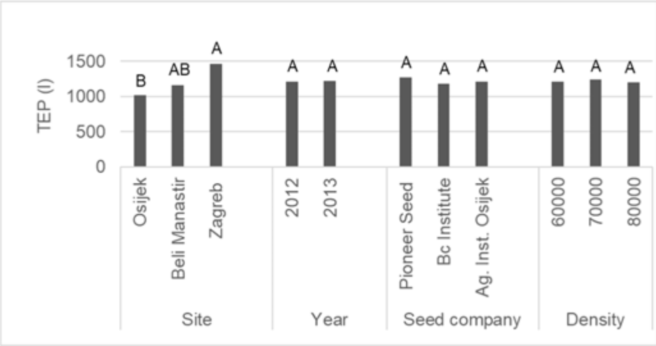
Also, no statistically significant difference was found in this study between HTF hybrids and hybrids of domestic breeding companies in terms of ethanol productivity. The highest average yield of ethanol was on the site Beli Manastir (130.72 l/t), and the lowest ethanol yield was achieved at the Osijek site (123.91 l/t). The yield of ethanol in 2012 (132.79 l/t) was not significantly higher than in 2013 (124.08 l/t) although the total grain yield was significantly higher in Zagreb (Figure 4). According to Bothast and Schlicher (2005) Pioneer's research make efforts to generate hybrids more conducive to the ethanol production process characterized by high total fermentables (HTF) and result in ethanol yields up to 4% greater than a bulk commodity. The statistically significant highest average ethanol yield was achieved at a plant density of 60,000 plants/ha (130.45 l/t). Furthermore, Szambelan et al. (2020) found that higher ethanol production was achieved using lower plant density of maize and sorghum (*Sorghum bicolor* L.). The same authors stated that the plant density has a significant impact on the quality of the raw material for processing which is related to the results of our research. Ethanol yields, in our research, were significantly lower than usual because according to Sharma et al. (2016) in maize grain processing plants ethanol yields can achieve up to 430 l/t. The same authors stated that such ethanol yields depend on production practices, equipment settings, residence times, concentrations, maintenance schedules, equipment conditions, environmental conditions, the composition and quality of the maize kernel itself, the location where the maize was grown, as well as the growing season in which maize was produced.



Bars labeled with different letters within each site, year, seed company and plant density are significantly different at $P<0.05$ (LSD test)

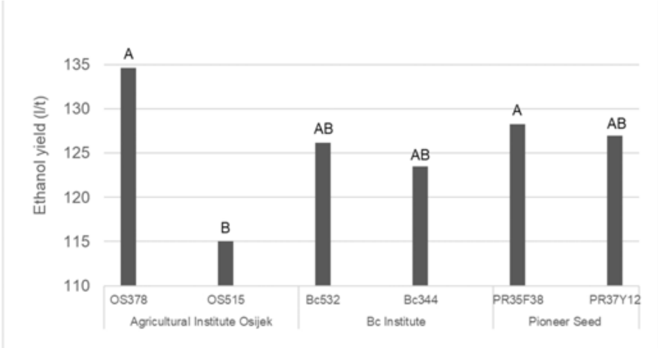
Figure 4. Ethanol yield in average depending on site, year, seed company and plant density

Since the same amount of grain was always used in the laboratory ethanol analyses, it was necessary to calculate the total amount of ethanol produced (TEP), which represents the amount of ethanol (l) produced from the total amount of raw material produced per unit area. The highest TEP was determined on Zagreb site (1,459.77 l). Due to better production conditions, more ethanol was produced in 2013 (1,221.94 l), and due to the higher average grain yield, more ethanol was produced by Pioneer hybrids (1,269.67 l). The largest TEP was achieved at density of 70,000 plants/ha (1,238.93 l) but a statistically significant difference was found only between sites (Figure 5). However, in our research the problem was the lower yield by certain hybrids and sites, so there was less raw material for further ethanol production. The foregoing indicates that an increase in maize grain yield may produce more ethanol but does not affect the potential of maize grain to increase ethanol yield from the same maize grain amount.



Bars labeled with different letters within each site, year, seed company and plant density are significantly different at $P<0.05$ (LSD test)

Figure 5. The total amount of ethanol produced depending on site, year, seed company and plant density



Bars labeled with different letters within each hybrid are significantly different at $P<0.05$ (LSD test)

Figure 6. Average ethanol yield for six hybrids cultivated on three experimental sites in three plant densities in two years (2012 and 2013)

As mentioned earlier, ANOVA showed that the difference in ethanol yield was determined by sites ($P<0.05$) with the highest average ethanol production achieved in Beli Manastir and the lowest in Osijek (Figure 4). Similar results were obtained by Zhan et al. (2003), who compared the influence of growing conditions on the ethanol yield using 8 varieties of sorghum and concluded that the location of sorghum cultivation significantly affected the yield of ethanol.

These differences in ethanol yields are probably due to the agroclimatic conditions of sites and soil properties. According to Hao et al. (2010) biofuel production requires understanding relationships of soil properties and topography with variation of biofuel production within sites. The same authors stated that ethanol yield was significantly positively correlated with total soil C, N, and organic C probably due to nutrient availability associated with these variables. Pioneer hybrids declared as HTF hybrids for maize ethanol production were employed as control hybrids for the estimation of ethanol production of domestic hybrids from the Agricultural Institute Osijek and Bc Institute d.d. The site × year × plant density interaction showed statistically significant difference in ethanol yields between hybrids (Figure 6). The most productive was the OS378 hybrid, which leads us to the conclusion that some maize hybrids created in breeding programs in the Republic of Croatia can be competitive in the global market of maize seeds intended for ethanol production.

CONCLUSIONS

The results obtained from this study is part of a doctoral dissertation, which is nearly to be completed, and indicate the potential of ethanol production from Croatian maize hybrids. Namely, hybrids designated for ethanol production do not produce higher ethanol yields than non-designated hybrids with the same yield levels, but overall have a higher grain yield, which provides a greater amount of raw material for ethanol production per unit area. At the same time, the results show that there are maize hybrids created in the Republic of Croatia have great potential for ethanol production and it can be realized in different environmental conditions of production. Also, from the position of the Republic of Croatia, these results offer great potential in terms of applying the principles of the European green deal (2019) through the use of innovative technologies in agricultural production. Considering that this is the first such research in Croatia, studies should be continued in the direction of studying other factors that can have an impact on the production of maize for ethanol production. So, these results were used to set up a field trial in eastern Croatia in 2020, which included 54 different maize hybrids currently used for grain production. Ethanol yield will be determined based on the grain yields of the samples, and the results might be of both empirical and practical importance. Also, future research needs to go towards defining the use of domestic hybrids for ethanol and for food production.

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