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Full Length Research Paper

Sugar beet genotype effect on potential of bioethanol production using *Saccharomyces cerevisiae* fermentation

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In order to study the effect of genotype on sugar beet ethanol production potential, ten beet varieties including diploid, triploid and tetraploid, multigerm, monogerm sugar beet and fodder beet were planted in randomized complete block design (RCBD) with three replications in Khoy Agricultural Research Center for two years (2008 to 2009). Some morphological and physiological traits were recorded. The fresh root and raw sugar of each treatment were fermented to ethanol using *Saccharomyces cerevisiae*. Results showed that, genotype had a remarkable effect on the ethanol production potential. Significant difference (at the 1% level) in ethanol was observed among the beet varieties in both ethanol production methods. In addition, more significant differences in most morphological and qualitative traits among the varieties studied were observed. Variation on ethanol production were intensively related to the chemical composition of root, especially sugar content, potassium impurity, syrup purity and some characteristics such as root dry matter and root length. Bioethanol production was enhanced by increasing the sugar content and root yield in sugar beet. Sugar beet varieties contained less root yield and more sugar content had higher potential for ethanol production than the fodder beet varieties.

Key words: Bioethanol, *Saccharomyces cerevisiae* , sugar beet, raw sugar

INTRODUCTION

Oil crises occurrence in the 1970 decade due to consumption increase over production, and the extraordinary increase of oil price, persuaded industrial countries to have different approach on the energy issue. Reduction of fossil fuel resources, commencement of environmental concerns and improvement of social living standards under different conventions and protocols like *Kyoto* protocol, led to direct human use of clean fuels (Miyamoto, 1997). Industry leading manufacturers in the world and producers attempt to replace petrochemical materials with agricultural products. Nowadays, bio-energy forms about 8% of the total annual energy used in the United States (Anonymous[c], 2010). Governments and industries make use of various initiatives associated with bio-energy production, increase of production efficiency and decrease of production cost (National research council, 2000). Ethanol or ethyl alcohol, unlike past decades that was only used in few industries, is considered as one of the important and strategic commodities in many countries and yet the production volume of this substance is increasing day by day. More than 80% of the existing ethanol in the world is used as fuel consumption and its by-products, and 20% of the remaining is used for traditional applications in industries such as medical, health and cosmetics, paint and resin (Anonymous[a], 2010). Brazil and United States are the world's leading producer of ethanol. In 2008, United States produced 9 billion U.S. liquid gallons of ethanol fuel and 10.6 billion U.S. liquid gallons of ethanol fuel in 2009. This represents around 55% of the world's total ethanol production (Anonymous[a], 2010). Ethanol also can be considered as an agricultural sector stabilizer, for

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example, if ethanol conversions factories are enable to produce ethanol from sugar beet, potato and maize, we will not be faced with difficulty of production increase of these products in different periods (Parvin, 2010).

Currently, more than 90% of the existing ethanol in the world is produced as bio-ethanol and 7% synthetically. In fact, until the late 1980 a noticeable proportion of ethanol in the world was artificially produced from petrochemicals, but after this decade new investment has not been done in this case. It is obvious that each country based on the dominant culture and climate conditions can make use of some agricultural products for producing ethanol. For an example, more than 20% of the nation's total corn supply is consumed for ethanol production in United States (Anonymous[a], 2010), while ethanol is produced in Iran, countries like South America, Brazil, India, Pakistan and Thailand and some European countries from sugar beet and cane molasses. Meanwhile, countries such as Russia, Canada, China, Australia and some European countries use wheat for ethanol production (Venturi and Venturi, 2003).

Sugar beet (*Beta Vulgaris* L.) is a sucrose-rich product that has many industrial applications in the today world. Beets are planted from northern latitudes of 30 to 60° and from Cairo in Egypt, to Helsinki in Finland and generally, in Europe, Africa and North America and from the height of zero to over 2000 meters above sea level (Khajehpour, 2006). Beet has a relatively wide consistency range with an extensive resistance to water stress, salinity and cold. Among agricultural products, sugar beet is a plant that has allocated itself a special place in supplying a part of energy required for human, in particular, in the third world countries that do not have access to other energy sources and it is considered as one of the key of economic, agricultural and national components (Cook and Scott, 1998).

Today, sugar beet provides 40% of world sugar trade within less than 200 years progress (USDA, 2008). Sugar beet cultivation in 41 countries is estimated as 8.1 million hectares. In the United States, every year about 0.5 million hectares of lands are planted with sugar beet, Germany with more than 0.4 million hectares and Italy and England, each with more than 0.2 million hectares are placed in next ranks (USDA, 2008). Different researches have been done on ethanol production from sugar beet. Theurer et al. (1987) evaluate different varieties of sugar and fodder beets in different weather conditions with respect to ethanol production potential. They stated that the amount of ethanol produced from sugar beet is more than fodder beet. Gibbons and Westby (1987) did a study with the aim of optimizing ethanol production from different sizes of fodder beet slices and achieved 4.83% of ethanol.

Venturi and Venturi (2003) and Koga (2008) compared agricultural products of temperate regions and concluded that, energy consumption efficiency of sugar beet for producing ethanol is much higher than those of wheat, corn and oil seeds. A research conducted in Turkey about ethanol production from sugar beet varieties showed that this country has enough potential to supply energy required for domestic consumptions and independence from imports of fossil fuels (Icoz et al., 2009).

With respect to pure carbohydrates, sugar beet has very high harvest index, because either the aerial part or underground part of sugar beet can be used to ferment and produce ethanol. On the other hand, sugar beet compared with other agricultural products, can be harvested in a three-month period and this provides a good chance for ethanol production (Chatin et al, 2004).

In a research conducted in the UK, bio-ethanol values produced from different crops were studied and it was announced that 2688 and 5250 L of bio-ethanol were produced from every hectare of wheat and sugar beet farms, respectively (Anonymous[b], 2010). In another study in India, 0.38 and 0.126 L of ethanol were produced from every kg of sugar and fresh sugar beet root, respectively (Blazek, 2007).

Srivastava et al. (2008) observed significant differences between different varieties with respect to bio-ethanol production and announced that the varieties with more root yield and higher sugar percentage are more promising for ethanol production. Kosaric et al. (1983) compared two types of yeast including *Saccharomyces cerevisiae* and *Saccharomyces diastaticus* for bio-ethanol production from fresh sugar beet root and announced that the yeast of *S. cerevisiae* had more efficiency.

Undoubtedly, ethanol production from renewable materials and biomass mostly depends on physico-chemical properties of the materials, pre-treatment manner, efficiency of zymogenic microorganisms and fermentation conditions such as initial concentration of sugar, PH, temperature, microbial density and fermentation time (Camire and Camire, 1994; linko et al., 1983; Zhan et al., 2003). Increase of fermentable biomass amount is an issue that must be studied by different varieties with respect to fermentation efficiency.

Plant breeding strategies can be implemented to improve biomass yield, biomass quality, biomass conversion efficiency, resistance to diseases and pests, sugar content and other characteristics associated with ethanol production. This study investigated the ethanol production potential in some beet genotypes and its relation with some characteristics such as root yield and sugar content.

MATERIALS AND METHODS

The quality and quantity of the ethanol produced from sugar beet is strongly dependent on variety. In order to evaluate some characteristics of sugar beet varieties that affect Bioethanol production, this experiment was carried out with ten beet varieties in randomized complete block design (RCBD) with three replications for two years (2008 to 2009) in Khoy Agricultural Research Station, Iran. Ten beet varieties including 8 sugar beets and 2 fodder beets were the most appropriate varieties with respect to plantation season and region. Some traits including green biomass, fresh and dry weight of root, leaves and petiole, root yield, sugar content, white sugar content, sugar yield, molasses, nitrogen, sodium and potassium impurities, syrup purity, leaf area index (LAI), length of root and green cover percentage were measured and recorded. At the end of the growth season, all roots and crowns were harvested and then samples were taken for recording some characteristics in the laboratory. Fresh sugar beet root and raw sugar were used for the ethanol production in the laboratory.

Preparing of yeast culture

The special yeast (*S. cerevisiae*) was maintained on malt agar medium [contains yeast extract (3 g); malt extract (3 g); peptone (5 g); glucose(10 g); agar (20 g); all dissolved in 1 L of distilled water and adjusted to pH 5.6] accordingly (Zayed and Foley, 1987). Inoculum was prepared from 1 ml of yeast with 100 ml of the earlier stated medium. After maintaining for 30 h in an incubator at 35°C, the culture contained approximately 5×10^8 cells per ml.

Production of ethanol from fresh sugar beet root

After washing the roots of 20 kg of each treatment, using cutting machine, the roots were cut into slices with a thickness of 1.5 mm and a length of 5 to 7 cm (Mesbahi, 2003). Then, the slices were kept in warm water (60°C) for 2 h with agitator timer till their syrup leaked into the water (Jones et al., 1981). After filtration of the total solution, the remaining syrup was separated from pulp by pressure. The sap was boiled for 2 h in a vacuum till its concentration reached the sufficient limit and also, its microbial contamination was destroyed. Then the sap was fermented under 35°C and at pH 5.5 for 72 h (Srivastava et al., 2008) using a special strain of *S. cerevisiae* Persian type culture collection (PTCC) 5269 obtained from the Persian type culture collection of yeast cultures, Tehran, Iran. After fermentation, the obtained solution was filtered and then purified with a distiller apparatus.

Production of raw sugar from sugar beet

For the production of raw sugar, 20 kg beet root from each treatment after washing with water was sliced to the dimensions of 1.5 mm in thickness and 5 to 7 cm in length (Mesbahi, 2003). In whole, the current syrup in the slices after rinsing with pure water of almost the same volume and temperature of 75°C was exited by a press machine. In order to refine the obtained solution, lime and CO_2 were added, respectively. In this stage, the pH increased to about 11 (Clarke, 1988). The solution was maintained in a closed container for at least an hour with a gentle agitation at 85°C to some of the impurities deposit. Then the solution was transferred to a pre-provided apparatus with special tubes passing steam until it was concentrated up to 70% at 120°C (Mesbahi, 2003). Some sugar powder was added to the produced syrup in vacuum at temperature below 100°C and it was kept at the same temperature till sugar crystals were formed and enlarged. After cooling the mixture, it was centrifuged at 1800 rpm for 5 min and the produced sugar was weighed and kept after drying. The remaining syrup was weighed as molasses (Mesbahi, 2003).

Production of ethanol from raw sugar

The sugar produced from each treatment with 25% concentration was dissolved in distilled water at 40°C. The obtained solution was fermented for 72 h under the earlier mentioned temperatures and pH for all the treatments by using suitable population density of *S. cerevisiae*. After filtering the produced solution, it was purified up to 94% by using distiller apparatus (Koga, 2008).

Statistical analysis

Ethanol concentration was determined by specific gravity method (AOAC, 1990). Ethanol was analyzed using HPLC system with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) to compare the results with earlier method. The mobile phase was 5 mm H_2SO4 pumped at a flow rate of 0.6 ml/min. Data acquisition and analysis were performed using the SHIMADZU EZ START 7.1.1 software. The results reported were based on specific gravity method. Analysis of variance (ANOVA), least significant difference (LSD) and comparison of means were done using ASA (SAS, 1995).

RESULTS AND DISCUSSION

In the experiment of the ethanol production from fresh sugar beet root, the total amount of distilled alcohol was recorded for each treatment with 94% alcohol. Sugar beet roots had around 10 to 18% sugar for different varieties. So the theoretical yield of the produced ethanol was almost 15% for fresh sugar beet root and around 50% for sugar. An important characteristic of the ethanol production process is its feedstock quality, which makes it susceptible to contamination by non-*S. cerevisiae* yeasts. The most important aspect of the fuel-ethanol fermentation is ethanol yield or more generally the industrial yield. It depends on the fermentative capacity of the yeast population (Grote and Rogers, 1985) and their resistance to stress conditions (Bai et al., 2008).

Analysis of variance

Analysis of variance for sugar beet physiological and quali-tative characteristics for two years showed that, there was a significant difference among the different varieties with respect to ethanol production potential from fresh root and raw sugar. Varieties also showed significant differences in some recorded traits including root yield, sugar content, sugar yield, nitrogen, potassium and sodium impurities, syrup purity and molasses yield. This difference was completely normal because the various types of beet including N, E and Z types and/or sugar and fodder beet and also diploid, triploid and tetraploid were applied in this study. These differences resulted in different ethanol yields of the varieties.

Combined analysis of variance for morphological traits for the two years showed that root length, leaf number, leaf area index (LAI), dry weight of roots and crowns had significant differences ($p < 0.01$).

Comparison of means

Comparison of some traits of beet varieties showed that

Table 1. Comparison of some traits of beet varieties

Variety	Root Yield	Sugar Yield	White Sugar Yield	Sugar Content	K	NA	N
9597	57.33 c	9.68 bc	8.59 b	16.9 a	3.89 b	1.88 bc	2.23 abc
7233	57.86 c	9.90 bc	8.63 b	17.12 a	4.48 b	2.11 bc	2.34 abc
Shirin	58.14 c	10.37 bc	9.27 b	17.85 a	4.00 b	1.68 c b	2.42 abc
BR1	61.68 bc	10.14 bc	8.65 b	16.43 a	4.81 b	2.21 bc	2.93 ab
7112	71.35 b	11.40 b	9.59 b	16.03 a	4.42 b	3.05 ab	2.76 ab
IR2	87.49 a	14.51 a	12.42 a	16.58 a	4.56 b	2.54 abc	2.60 ab
37RT	65.67 bc	10.78 b	9.23 b	16.45 a	4.42 b	2.62 abc	2.37 abc
19669	68.13 b	10.05 bc	8.85 b	14.75 a	3.78 b	1.69 c	1.90 bc
Fd1	66.60 bc	8.03 cd	5.75 c	11.59 b	7.21 a	2.20 bc	1.17 c
Fd2	69.40 b	6.53 d	4.05 c	9.9 b	8.20 a	3.52 a	3.42 a

Numbers followed by similar letters are not significantly different at 5% level of probability.

Table 2: Comparison of beet varieties regarding ethanol production potential

Variety	Ethanol from Root (L Ton–1)	Ethanol from Root (L ha–1)	Ethanol from Raw Sugar (L ha–1)
9597	107.10 a	6194 bc	4725 b
7233	108.40 a	6334 bc	4750 b
Shirin	113.20 a	6637 b	5100 b
BR1	104.80 a	6489 bc	4756 b
7112	102.40 a	7294 b	5275 b
IR2	105.60 a	9285 a	6829 a
37RT	105.70 a	6901 b	5077 b
19669	93.64 a	6436 bc	4868 b
Fd1	74.57 b	4974 cd	3164 c
Fd2	63.25 b	4395 d	2228 c

Numbers followed by similar letters are not significantly different at 5% level of probability.

in the bioethanol production from fresh root of beet, cultivar of IR2 had the highest root yield with 87.49 ton/h and the highest ethanol yield with 9285 L/h (Tables 1 and 2). Also, IR2 with 12.42 ton/h of white sugar yield had higher yield than the rest of the varieties, while fodder beet varieties (Fd1 and Fd2) with 5.75 and 4.05 tone/h of white sugar yield, respectively, had the lowest yield and these varieties had the lowest bioethanol yield (Table 1). The fodder varieties had impurities amounts of nitrogen, potassium and sodium. Hence, the amount of molasses production in these varieties also was more than the rest (data not shown).

Theurer et al. (1987) evaluated sugar beet and fodder beet varieties, and declared that sugar beet with 8640 L/h of ethanol production, compared with 6380 L/h fodder beet, had more potential for ethanol production.

Ethanol production from raw sugar, IR2 with 6829 L/h had the highest value, while the fodder beet varieties with 3164 and 2228 L/h had the lowest ethanol yield (Table 2). According to the qualitative analysis of raw sugar obtained from the different varieties, it can be stated that, the quality of sugar in different varieties had no difference and so ethanol production was related to quantity of the produced sugar for each variety. The amounts of potassium and nitrogen impurities of the fodder varieties were comparable with those of other varieties. Significant difference between the two years of the tests was not observed in terms of our studied traits. Regional weather data also showed that, no large changes in climate parameters were observed during the years 2008 and 2009 (data not shown).

Correlations of some sugar beet traits with ethanol production

Correlation between some sugar beet traits with ethanol production from the fresh root indicated that, ethanol yield had high positive correlation with the sugar yield, white sugar yield, sugar content, white sugar content, syrup purity and dry weight of root. Also, it had significant negative correlation with molasses sugar (Table 3).

The correlation between some sugar beet traits with ethanol production from raw sugar is shown in Table 3. The results indicated that, ethanol yield had high positive correlation with sugar yield, white sugar yield, sugar

Table 3. Correlation of some beet traits with ethanol production from fresh root and raw sugar.

	Ethanol from Fresh Root (L/Ton)	Ethanol from Raw Sugar (L/H)
Root Yield	-	0.575 **
Sugar Yield	0.727 **	0.969 **
White Sugar Yield	0.838 **	0.986 **
Sugar Content	0.921 **	0.828 **
White Sugar Content	0.985 **	0.830 **
Potassium Impurity	0.266	-0.475 *
Sodium Impurity	0.340	-0.218
Nitrogen Impurity	0.112	0.070
Syrup Purity	0.829 **	0.655 **
Molasses Sugar	-0.628 **	-0.342
Root Length	-0.002	0.584 **
Number of leaves	-0.128	0.174
Leaf Area Index	0.007	-0.102
Fresh Weight of Crown	0.038	-0.093
Dry Weight of Crown	-0.037	0.427 *
Dry Weight of Root	0.369 *	0.582 **

**& * represent correlations with significant levels of 0.01 and 0.05, respectively.

content, white sugar content, root yield, syrup purity, root length, dry weight of root and crown. Also, it had significant negative correlation with potassium impurity. Sodium and nitrogen impurities, number of leaves and leaf area index (LAI) had no significant correlation with ethanol production.

In this study, *S. cerevisiae* was used for the fermentation and the used sugar type was sucrose that can be converted to bioethanol. Hence, cellulose tissue in fresh beet root cannot have great role in ethanol production (Singh et al., 1995). Therefore, the high correlation between ethanol yield and white sugar in this study seems to be reasonable. Variations of 32 to 43% for ethanol yields were observed among the 10 beet varieties. The effect of genotype on ethanol production is related to both the chemical composition and morphological properties of the beet root samples, with a stronger effect observed for chemical composition such as sugar content and impurities. Ethanol production increased as sugar content increased, whereas the ethanol production decreased as nitrogen and potassium impurities increased. Further research is needed to test a broad number of varieties across a wide range of growing conditions to further evaluate the effects on ethanol fermentation yields. Both sugar content and root yield were strongly correlated with ethanol yield based on regression analysis using single chemical composition. The relationship between sugar content and theoretical percentage of ethanol yield indicated that, sugar content had significant effect on ethanol yield (Table 3).

Zhan et al. (2003) studied a number of varieties of grain and fodder sorghum and found that the chemical composition and physical properties of grain are very important factors on ethanol production potential.

Conclusions

Bioethanol production from sugar beet via fermentation technology is a promising fuel alternative. In order to produce ethanol from sugar beet and by-products via fermentation, it is important to know the correlations between some morphological and physiological traits with ethanol production. The novelty of this study compared with other investigations is to produce ethanol from fresh sugar beet root. It was observed that, several varieties had different ethanol production potentials highly correlated with root quantitative and qualitative traits. In fact, this study highlighted breeding of sugar beet varieties particularly for ethanol production. Based on the analysis of the experimental data, ethanol production from fresh root has more efficiency than fermentation of raw sugar. Among all of the investigated varieties, sugar beet varieties produced more ethanol per hectare than fodder beet. Sugar beet varieties had more root yield and sugar content than fodder beet and these two characteristics play basic role in ethanol production. The adapted sugar beet hybrids showed better promise than fodder beet as a fuel crop in the USA, since sugar beet produces an equal or greater quantity of fermentable sugar, it has less bulk to transport and more extractable sugar per unit mass (Theurer et al., 1987).

The results obtained from this research showed that, a limited number of beet genotypes had significant effects on the potential of ethanol production. A significant difference ($p < 0.01$) was observed in ethanol yield among varieties in both methods of ethanol production. Also, highly significant differences were observed in the morphological and physiological traits among the varieties. Genotype variation effects were found on ethanol yield, that is, highly related to the chemical composition of roots, especially sugar content, potassium impurity, crude syrup purity and some morphological characteristics such as root length and root dry matter. In addition, ethanol production increased as sugar content and root yield increased.

When sugar beet was compared with other sugar crops, its irrigation requirement was less and so, it was suitable in our agricultural system. It needs more ethanol from beet genotypes and support from private sugar industry from all over the world. This subject may create a new vista.

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