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CORRELATION OF MORPHOLOGICAL AND PHYSIOLOGICAL SUGAR BEET TRAITS WITH ETHANOL PRODUCTION FROM FRESH ROOT AND MOLASSES

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ABSTRACT : Environmental pollution as a basic challenge of man at this century along with pollution increasing followed by high oil products changes to serious problem. Nowadays ethanol as a clean energy source and reliable have been focused by scientists. Studying the relation of ethanol production with some morphological and physiological traits in sugar beet varieties, experiment was conducted with 10 varieties in complete block design with 3 replications at Khoy - Iran agricultural research station in 2009. Bio-ethanol was produced by *Saccharomyces cerevsiae* fermentation from root and molasses of sugar beet varieties. The results showed significant positive correlation between root, raw sugar and white sugar yields with ethanol production from sugar beet roots. Molasses sugar also showed high positive correlation. But ethanol production from molasses, the highest correlation belongs to potassium and sodium impurities of root. There was significant high negative correlation between molasses ethanol yield with raw extract purity. The IR2 variety with 9285 lit/ha was superior in root ethanol production and highest production of molasses ethanol observed with fodder beet variety of 7112 with 804.3 lit/ha. It seems commercial traits of sugar beet is suitable for sugar production and so for bio-ethanol production.

Key words: Ethanol production, molasses, *Saccharomyces cerevisiae,* sugar beet

INTRODUCTION

Because of environmental concerns over the use and depletion of nonrenewable fuel sources, together with the increasing price of oil and instabilities in the oil markets, there is a need to search for energy substitutes. Bio-ethanol is now considered a profitable commodity by its increasing use as renewable energy source and car fuel [21]. Sugar beet juice and molasses are the main substrates for ethanol production in Iran because of its high sugar content and availability. Sugar beet as also a major economic crop that was covered about 320000 hectares in 2010. It has been already recommended as one of the best raw materials for ethanol production by Iran government [22]. In order to make this process economic, it is essential to produce ethanol at low cost and high efficiency.

β. vulgaris is the only species of agricultural importance in this small family; it includes sugar and fodder beets and mangels [6]. Sugar beets are main crop grown in many states of Iran in different climatic conditions, from the hot climate in Khozestan to the colder climates of Azarbyjan and Khorasan. Sugar beet byproducts like beet pulp and molasses commonly was used as animal feed or further processed to extract more sugar [6]. More than ever before, the interest in ethanol fermentation is growing.

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Ethanol is considered to be the cleanest liquid fuel alternative to fossil fuels. Amazing advances have been made towards the technology of homemade, or home brewed ethanol [3].
Ethanol has high octane, which is most commonly used as a gasoline additive and or extender. Up to now, methyl tertiary butyl ether (MTBE) has been used to increase fuel octane, and is the primary gasoline additive in the Iran and many other countries which is created new difficult for man about air pollution and other problems [14]. While it possible increasing benzene octane by adding some ethanol without any problem. This way is very inexpensive, no pollution and high efficiency.
More recently, several countries have banned the use of MTBE as a gasoline additive, due to problems with the environment, such as groundwater contamination [10]. With both the banning of MTBE in some countries, and the surging prices for petroleum based fuels, the need for ethanol production in the most countries is dramatically increasing. Sugar Beet Ethanol could be part of the answer.
Even though the fermentative process for ethanol production is well known, the production costs are still the key impediment wide use of ethanol as fuel. Therefore, the development of a fermentation process using economical carbon sources is important for the biofuel ethanol production on a commercial scale [16]. Many studies have been done that focus on production improvement and decreasing its costs [15, 5, 13 and 12].
Since the cost of raw materials make up 55–75% of the final alcohol selling price, alcohol production from low priced materials has become an important area of investigation [2]. There is a need to assay the merit of fresh root and raw sugar or molasses for potential alcohol fuel production. Molasses, a by-product of the sugar industry, represents a ready and renewable source for ethanol fermentation because of its high availability and low cost. Molasses may vary somewhat in composition but usually contains about 50–55% fermentable sugar. For economic [4] and scientific [3] reasons, some techniques have been reported for microbial fermentation with high produce from sugar beet molasses. Sugar beet is an obvious choice in the quest for cost effective bio-ethanol production.
Bio-ethanol production a comparison among common crops of the temperate region shows that the net energy gain of ethanol from sugar beet exceeds those of wheat, corn and oil seed rape [11, 18].
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 which have correlation with ethanol production.

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 depended on bioethanol production, this experiment was carried out with 10 beet varieties in the statistical form of RCBD[2] with three replications during 2008 in Khoy - Iran agricultural research station. Ten varieties of beets including sugar beet, fodder beet, multi-germ, mono-germ and too diploid, triploid and tetra-ploid that they were the most appropriate type for the season and region. The studied parameters was include morphological and physiological traits such as green biomass, fresh and dry weight of root, leaves, petiole, root yield, sugar content, white sugar content, sugar yield, molasses yield, nitrogen, sodium and potassium impurities, syrup purity, LAI, length of root and green cover percentage was recorded. In the end of growth season, all of root yield and crown was harvested and then samples was taken for recording some characteristics in laboratory. Fresh sugar beet root, molasses and raw sugar were used for producing ethanol in laboratory.

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Ethanol Production from Fresh Root

Amount of 20 kg of fresh sugar beet roots from each treatment after harvest and washing the extracts were prepared. Juice of beets was derived by extracting slice of beets. The sugar is extracted by juicing the beets and boiling with water. Now the solution is called "mash" and it could be fermented and then distilled to extract the ethanol [9]. Ethanol fuel can be used in most converted engines if it has an alcohol percentage greater than 80%. Due to ethanol's strong ability to bind with water, no still can remove the last 5% of water [14]. Therefore the highest possible yield from a still was 95% ethanol.

Juicing Beets

After washing the beets followed by slicing them to tiny bits (1 mm × 7 mm) were extracted for each treatment and yielded some juice and morsel of pulp. Then 5 liters of water was used for washing pulp sake emitting sugar from that.

Fermenting Beets

Mash was heated to 87 ºC for 20 minute for sterilization. Mash temperature came down to 27 ºC and then was added 10g of Yeast that had been prepared already. The Original type of S. cerevisiae PTCC[3] 5269[4] was obtained from the Persian Type Culture Collection of Yeast Cultures, Tehran, Iran. The Yeast (S. cerevisiae) was a special strain that we had tested to produce up to 20% alcohol in 48 hours. When fermenting mash, it is important to achieve the highest alcohol percentage possible so more alcohol can be collected once distilled [1]. Once yeast was added we agitated the solution for 20 minute to increase yeast activation then placed on lid and air lock. Was let the mash ferment for 72 hours hoping to get all sugar converted to alcohol.

Distilling Beet Mash

The pulp which floated to the top of the fermented mash was scraped off, and then the rest of the mash was transferred to the boiler of the still. The distillation began with an initial still temperature of 20 ºC. After 90 minute of heating on a burner the still temperature reached to 78 ºC. Distillate began flowing out of the still and was collected at an Erlenmeyer. As time went on, and temperature of the still increased, alcohol was extracted from the boiler. When increased temperature above 78.5 ºC, more water percentage in the distillate that was being collected, and thus less over all alcohol of distillate being collected. As ethanol and water vapor rose up the column from the boiler, the vapor encounters scrubber pads that cooled in temperature from bottom to top. The digital thermometer was used for determining still temperature. It was placed above the top of the stacked scrubber pads to read the temperature of the vapors passing through the scrubber pads and on to the condenser. Thus temperatures closest to 78.5 ºC will have the highest percent of ethanol since 78.5 ºC which is the vapor point of ethanol.

Ethanol Production from Molasses

All process of sugar producing was done for each treatment and was purveyed molasses. Then was produced ethanol from these molasses in following way. Molasses of different varieties contained 45-53 % fermentable sugar. The special Yeast (S. cerevisiae) was maintained on malt agar medium which consisted of 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 [20]. The medium used to grow cells for a free cell inoculum and for cell immobilization contained per liter of distilled water: molasses, 200 g/L; urea, 1.00 g; MgSO4, 0.3 g; and NH4PO4, 0.3 ml; K2SO4 0.3 g [19 and 20].

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4

The pH was adjusted to 5.6 with 0.5 M citric acid and autoclaved for 15 min at 121°C. Inoculums was prepared by putting 1 ml of yeast with sampler and added to 100 ml of the above medium. After 30 hours cultivation at 35°C the culture contained approximately 5 × 108 cells per ml.

RESULTS

In the experiment of ethanol production from fresh root, total amount of distillate collected was recorded for each treatment with 94 % alcohol. Sugar beet roots had sugar content around 10-18 %. So the theoretical yield of ethanol produced is around 50 % alcohol per weight of sugar and almost 15 % for fresh root. Sugar beet juice and sugar beet molasses are the substrates for yeast fermentation in Iran that can use last for 6 months. An important characteristic of the ethanol production process is its quality of feedstock, 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 is dependent, among many factors, on the fermentative capacity of the yeast population [7] and the resistance of those industrial cells to stress conditions [8 and 9]. Amount substance of feedstock that is converted to ethanol by Zymogene such as sugar content would be very important [9].

Analyze of variance for sugar beet physiological and morphological treats (tables No. 1 and 2) showed that these selected varieties were had very large diversity. Varieties were different point of view all recorded traits such as root yield, sugar content, sugar yield, impurities, syrup purity, molasses yield, root length, leaves number, leaf area index, fresh and dry weight of root and crown. This difference was normal completely because they had multifarious type of beet instance N, E and Z types and or sugar beet and fodder beet and too diploid, triploid and tetraploid. As this difference was being foresighted that be different ethanol yield form each varieties and it happened.

Table No 1: Analyze of Variance for Sugar Beet Physiological Treats

Source	df	Root Yield	Sugar Yield	White Sugar Yield	Sugar Content	White Sugar Content	K	NA	N	Alkality	Syrup Purity	Molasses Yield
Rep	2	9.499	1.143	0.694	2.044	1.550	0.418	2.338	0.359	0.241	5.965	0.074
Factor	9	** 242.502	** 12.966	** 15.059	** 20.123	** 30.527	** 6.668	* 1.058	* 1.097	* 2.441	** 267.791	** 0.906
Error	18	26.209	1.882	1.633	3.014	3.068	0.620	0.430	0.432	0.679	7.883	0.118

* and **: Correlations are significant at 0.05 and 0.01 probability respectively

Table No 2: Analyze of Variance for Sugar Beet Morphological Treats

Source	df	Root Length	leaves Number	LAI	fresh weight of Crown	fresh weight of Root	Dry Weight of Crown	Dry Weight of Root
Rep	2	4.656	0.511	0.782	227325.409	10360299.296	1.561	3.256
Factor	9	** 19.466	** 21.256	** 7.065	** 6480669.513	** 3285549.009	** 18.822	** 12.760
Error	18	1.159	0.515	1.026	37917.038	473814.065	3.092	2.664

* and **: Correlations are significant at 0.05 and 0.01 probability respectively

Means Comparisons of Some Treats in Varieties of Sugar and fodder Beet was shown in table No 3. Fodder beet cultivars had greater root weight and less sucrose content than sugar beet.

In this experiment the most ethanol yield from root relevant to which variety that had the most root yield with high sugar content (table No 3). The most root yield and sugar yield relevant to mono-germ and triploid sugar beet cultivar (14.51 ton ha-1) and ethanol yield for this cultivar was 9285 lit ha-1 while the lowest sugar yield relevant to investigated two fodder beet cultivars (6.53 and 8.03 ton ha-1) and ethanol yield for these cultivars was 4974 and 4394 lit ha-1. It is full vivid that fodder beet cultivars had potassium, sodium and nitrogen impurity therefore they had more molasses yield and ethanol yield form molasses too. But this upraising cannot legitimize planting of fodder beet varieties for economic reasons.

Table No 3: Means comparisons of some treats in varieties of sugar and fodder beet

Variety	Root Yield	Sugar Yield	White Sugar Yield	Sugar Content	K	NA	N	Molasses Yield	Ethanol from Root	Ethanol from Molasses
9634	57.33 C	9.68 BC	8.59 B	16.9 A	3.893 B	1.883 BC	2.230 ABC	1.090 D	6194 BC	311.8 D
7233	57.86 C	9.9 BC	8.637 B	17.12 A	4.48 B	2.11 BC	2.337 ABC	1.263 CD	6334 BC	360.6 CD
191	58.14 C	10.37 BC	9.273 B	17.85 A	4 B	1.68 C	2.417 ABC	1.097 D	6637 B	313.7 D
BR1	61.68 BC	10.14 BC	8.647 B	16.43 A	4.81 B	2.207 BC	2.933 AB	1.493 BCD	6489 BC	426.6 BCD
9597	71.35 B	11.4 B	9.59 B	16.03 A	4.417 B	3.05 AB	2.757 AB	1.810 BC	7294 B	516.5 BC
IR2	87.49 A	14.51 A	12.42 A	16.58 A	4.56 B	2.537 ABC	2.600 AB	2.090 B	9285 A	597.8 B
37RT	65.67 BC	10.78 B	9.23 B	16.45 A	4.423 B	2.62 ABC	2.367 ABC	1.553 BCD	6901 B	443.3 BCD
19669	68.13 B	10.05 BC	8.85 B	14.75 A	3.78 B	1.693 C	1.897 BC	1.203 CD	6436 BC	343.8 CD
7211	66.60 BC	8.03 CD	5.753 C	11.59 B	7.21 A	2.197 BC	1.170 C	2.020 B	4974CD	576.8 B
7212	69.40 B	6.527 D	4.05 C	9.9 B	8.2 A	3.523 A	3.420 A	2.817 A	4395 D	804.3 A

*Means followed by similar letters are not significantly different at 5% levels of probability

The results of correlations between some morphological and physiological traits with ethanol production from fresh root in sugar beet cultivars have been shown in table No 4. Raw sugar yield and white sugar yield had the positive highest and significant correlation with ethanol production. In this reason must be considered these two characteristics in order to breeding sugar beet variety that it is suitable for high ethanol production ability. Because of negative relationship between root yield and sugar content selection on the base of these treats can be deceptive but sugar yield is just real. As there are high Correlations of sugar yield with root yield and sugar content thus can tell these treat are very important. Potassium impurity in root had negative significant correlation with ethanol yield but there hadn't significant correlation with other impurities. Length and dry weight of root from morphological treats had positive significant correlation with ethanol yield. As production of ethanol from root is done via fermentation by S. cerevisiae it could be concluded that in this experiment, cellulosic compounds such as number of leaves, leaf area index, fresh and dry weight of crown hadn't any role in ethanol production. This case is approved with any significant correlation between ethanol yield and treats with cellulosic compounds in sugar beet.
Analyses of Correlation between some sugar beet traits with ethanol production from molasses have been shown in table No 5. These results indicated that ethanol yield had high positive relation with root yield, potassium, and sodium and nitrogen impurity. And it had negative significant correlate with sugar content, white sugar content, Syrup Purity, Fresh and dry Weight of leaves and petiole, Leaf Area Index. As potassium, sodium and nitrogen impurity had positive high effect on molasses sugar and so on amount of molasses production thus these treats are important in ethanol production from molasses. But if sugar beet tilling has two intents, sugar and too ethanol, in this state K, Na and N impurity can be reduce white sugar yield. Ethanol production had significant negative correlation with raw extract purity and it might be because of negative relationship between above impurities with raw extract purity. Length of Root, Number of Leaves and sugar yield hadn't significant correlation with ethanol production from molasses.

Table (4): Correlation of morphological and physiological sugar beet traits with ethanol production from fresh root

	Ethanol yield from root	Root Yield	Sugar Yield	White Sugar Yield	Sugar Content	White Sugar Content	**K** impurity	**Na** impurity	**N** impurity	Syrup Purity	Molasses Yield
RY	.548**	1									
SY	.995**	.534**	1								
WSY	.968**	.375*	.969**	1							
SC	.724**	-.179	.727**	.828**	1						
WSC	.695**	-.201	.702**	.830**	.985**	1					
K	-.506**	.079	-.514**	-.675**	-.666**	-.761**	1				
Na	-.035	.395*	-.057	-.218	-.360	-.461*	.336	1			
N	.207	.169	.166	.070	.112	-.012	.220	.556**	1		
Purity	.573**	-.196	.591**	.755**	.829**	.907**	-.901**	-.586**	-.293	1	
MY	-.097	.565**	-.117	-.342	-.579**	-.694**	.785**	.741**	.500**	-.854**	1
RL	.544**	.258	.542**	.584**	.434*	.469**	-.555**	-.153	.045	.528**	-.280
LN	.194	.063	.185	.174	.167	.136	-.092	.171	.236	.112	.037
L A I	-.225	-.573**	-.224	-.102	.213	.244	-.247	-.265	-.033	.253	-.442*
F WC	-.229	-.574**	-.229	-.093	.208	.253	-.305	-.288	-.077	.282	-.490**
D WC	.349	-.074	.355	.427*	.477**	.499**	-.510**	-.139	.029	.518**	-.389*
D W R	.561**	.320	.575**	.582**	.382*	.407*	-.513**	.005	-.005	.491**	-.211

* and **: Correlations are significant at 0.05 and 0.01 probability respectively

RL: Root length, LN: Leaves number, LAI: Leaf Area Index, FWC: Fresh Weight of Crown, DWC: Dry Weight of Crown, DWR: Dry Weight of Root

Table 5: Correlation of Some Sugar Beet Traits with Ethanol Production from Molasses

Treats	**Ethanol yield from molasses**	**K** impurity	**Na** impurity	**N** impurity
Root Yield	**0.566****	0.079	0.395 *	0.169
Sugar Yield	**-0.117**	-0.514 **	-0.057	0.166
White Sugar Yield	**-0.341**	-0.675 **	-0.218	0.070
Sugar Content	**-0.578****	-0.666 **	-0.360	0.112
White Sugar Content	**-0.694****	-0.761 **	-0.461 *	-0.012
potassium impurity	**0.785****	1	0.336	0.220
sodium impurity	**0.740****	0.336	1	0.556 **
nitrogen impurity	**0.500****	0.220	0.556**	1
Syrup Purity	**-0.854****	-0.901**	-0.586**	-0.293
Molasses Yield	**1.000****	0.785**	0.741**	0.500**
Length of Root	**-0.280**	-0.555**	-0.153	0.045
Number of Leaves	**0.036**	-0.092	0.171	0.236
Leaf Area Index	**-0.442***	-0.247	-0.265	-0.033
Fresh Weight of Crown	**-0.490****	-0.305	-0.288	-0.077
Dry Weight of Crown	**-0.390***	-0.510**	-0.139	0.029
Dry Weight of Root	**-0.210**	-0.513**	0.005	-0.005

* And **: Correlations are significant at 0.05 and 0.01 probability respectively

CONCLUSION

Bio-ethanol production from sugar beet via fermentation technology is promising as an alternative fuel. In order to attain ethanol production from sugar beet and byproducts via fermentation how have efficiency and too knowing correlation between some morphological and physiological treats with ethanol production. This aspect in the other scientist researches had been done lesser in the world yet specially ethanol produce from fresh sugar beet root. Was observed several cultivars had different ethanol production potentially correlated with treats. In fact this research got a pattern in order to breeding sugar beet varieties particularly for ethanol production. Based on the analysis of experiment data, ethanol production from raw beet juices had more efficiency than fermentation of molasses. Into all of investigated cultivars, Sugar beet varieties produced more ethanol per hectare than fodder beet. Sugar beet varieties had more root yield and too sugar content than fodder beet which this two characteristics had basic role in ethanol production. Adapted sugar beet hybrids have 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, has less bulk to transport, more extractable sugar per unit mass, and resistance to prevalent sugar beet diseases [17].

Totally beet insemination can be different relative need of society and country politics. If country cane provides sugar necessary form other ways, in this state sugar beet can be a suitable source for bio ethanol with 9285 litter per hectare. But if sugar beet is planted in order to provide sugar alone, in this case can be produce ethanol from molasses as byproducts.

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