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## Triticale for Bioenergy Production

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### Abstract

The promotion of renewable energy represents a target of the European 2020 strategy for growth.

Plant biomass and organic wastes from agriculture represent an effective resource to be exploited for a sustainable rural development, optimizing the land use, diversifying rural entrepreneurship.

Cereals are considered a promising biomass producing crop in temperate regions of Europe to be used for both fuel alcohol and biogas production.

In particular, triticale shows a number of advantages such as high grain yield even in marginal environments, tolerance to drought, tolerance to more acid soils, low susceptibility to biotic stresses and is known to have reduced production costs.

The characteristics of triticale were reviewed, focusing on bio-energy applications.

Furthermore, data from a two-year experiment carried out in Italy using nine triticale lines grown in marginal areas close to Bracciano, Italy, were reported. A bread wheat variety selected for bio-energy application, EW9, were also included for a more complete analysis. Traits such as day-to-heading, plant height, number of plants, number of spikes, grain yields were analysed. Preliminary results concerning biogas potential of biomass consisting of triticale hay harvested at milky-dough phase were also measured and results are reported.

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## 1. Introduction

The promotion of renewable energy represents a target of the European 2020 strategy for economical growth and sustainable competitiveness. Plant biomass and organic wastes from agriculture represent an effective resource to be exploited for a sustainable rural development, optimizing the land use, diversifying rural entrepreneurship and producing energy and new income.

Among crops, triticale is considered particularly promising as biomass source in temperate regions of Europe and protocols are developed for producing bio-alcohol and biogas.

Triticale (X *Triticosecale* Wittmack) is a human-made crop, being a hybrid small grain produced crossing wheat and rye. The name “triticale” derives from the combination of the scientific classifications of the two genera involved, that is, wheat (*Triticum*) and rye (*Secale*). The triticale hybrids are all amphidiploid, which means the plant is diploid for two crossed genomes, thus is an allotetraploid. The first crosses were attempted in Scotland in the late 1870s, the first true result was obtained by Rimpau in 1888, but the first commercial releases, available for producers, dates back 1960s. The CIMMYT Triticale Improvement Program started in 1964 and today CIMMYT is the principal supplier of improved germoplasm for national and international programmes worldwide.

Generally, triticale combines traits of the rye genome, as the rusticity, disease and environmental tolerance (including soil conditions), to traits of wheat, as the high yield potential the good grain quality. Therefore, it is a crop which is particularly suited for marginal environments characterized by high acidity “sub-standard” soils, water shortage and located at a high altitude or where disease pressure is high. Triticale is known to have lower production costs in comparison to other crops, lower susceptibility to biotic stresses, thus reducing requirements for chemical protection and fertilization, and results in high grain yield and large biomass production even in marginal environments not usable for food crops.

Furthermore, triticale improves the environment because of its ability to reduce leaching into groundwater acting as a soil improver, due to its extensive root system which is able to bind erosion-prone soil and provides a good substrate for conversion into subsoil organic carbon by soil microbes (Salmon et al., 2004). In addition, triticale is important as a rotation crop for the reduction of soil pests, (e.g. nematodes).

Although a new crop, the benefits of triticale production are enormous and an ever increasing acreage is used for growing it, in more than 30 countries. In these last 20 years, triticale is assuming more and more importance in relationship with the increasing needs to find new lands for agriculture and with the growing interest of possible industrial uses as feedstock in bio-energy production. Current production is concentrated in Europe with nearly 90% of the world production and ~7 million acres harvested annually. US production is nearly 1 million acres, with the majority of the planted acres used for forage and pastures (Mergoum et al., 2009).

In Italy, the first registered national variety was Mizar (1979), followed by Rigel (1983), both developed at ENEA. These two triticale varieties are characterized by high yields and high pest resistance and are still cultivated in Italy. Ever since, in Italy, new varieties have been released and more and more programmes for genetic improvement are underway by private seed producers.

Currently, it is grown mainly as a feeding crop, but increasing attention is given to bio-energy production. In particular, triticale is considered a good feedstock for ethanol production but increasing attention is paid to biogas production by anaerobic digestion. At global level limited sources of fuel and energy have given a strong input to study and develop biomass production and bio-energy crops (Cardona and Sanchez, 2007). Several protocols with different type of pre-treatment for de-restructuring plant biomass and recovering high yield of sugar and lignin-cellulose molecules have been studied to better design a specific and efficient protocol for triticale variety (Belkacemi et al., 1998; Chen et al., 2007).

The aim of this study was to compare the yields and some biochemical parameters related to the bio-energy production of eight triticale elite lines in a two-year experiment in marginal soil. These lines were constituted at CIMMYT (MX) and were growth in Italy for the first time. For comparison, an Italian triticale variety, Magistral, and an Italian bread wheat variety, EW9, constituted as energy crop, were also studied as Italian witnesses. Preliminary results concerning biogas potential of biomass consisting of triticale hay harvested at milky-dough phase were also measured and results are reported.

## 2. Materials and Methods

### 2.1. Materials and Field Experiments

Eight elite lines of triticale were provided from CIMMYT (MX). Field data collected at CENEB-CIMMYT (Obregon, MX) during their field experiments were also kindly provided. Triticale Magistral was kindly provided by Agrarian Faculty, University of Foggia, IT and bread wheat EW9 was provided by Società Produttori Sementi (Bo, IT).

Italian field experiments were carried out during two crop seasons, 2010-2011 and 2011-2012, in marginal areas close to Bracciano, Italy. In season 2010-2011, trials were conducted in a marginal area near the Cupinoro dump (42.0472°N, 12.1670°E, Bracciano Ambiente srl - Bracciano, Italy). In season 2011-2012, trials were conducted in another marginal area near Castel Giuliano (42.0631°N, 12.1402°E, non-profit organization AaIS, Bracciano, Italy). Experimental design consisted of completely randomized rainfed fields with two replications. Soil chemical analyses were carried out using standard methods to assess composition and quality. Nitrogen was supplied in both seasons at sowing and tillering phases.

Some agronomic traits were analysed during the two seasons, mainly at the phenological period ranging from booting to milk phase.

The crop biomass was harvested at the milk-phase, when early kernel formation occurs, starting as a milky fluid that increases in solids as the milk stage progresses and becomes physiologically mature at the hard dough stage, before final ripening (usual agronomic harvesting).

Aerial part of plants were harvested, measured, sampled and then air-dried and stored, following Laboratory Analytical Procedures (Hames et al., 2008).

### 2.2. Characterization and anaerobic digestion of feedstocks

The total solids, ash contents and other parameters of the fresh untreated and air-dried feedstock were determined by Laboratory Analytical Procedures (Sluiter et al., 2005; Sluiter et al., 2008).

A bomb calorimeter (Mod. Parr 6725 semi-micro calorimeter equipped with Parr 6772 calorimetric thermometer, Parr Instrument Company, USA) was used to determine the calorific value, following manufacturer procedure.

Potential biogas yield (BPP) was estimated by an anaerobic test in batch. Anaerobic organisms to be used as inoculum came from an anaerobic digestion plant located at Nepi, Italy (Azienda Palombini) and samples were collected during the stable methanogenic phase ( $\text{CH}_4 > 60\%$ ). It was biochemically characterized before use and, in case, adjusted to meet the following required analytical values: dry matter  $< 3\%$ , volatile fat acids (VFA)  $< 2000$  mg expressed as acetic acid/l and  $\text{N-NH}_4 < 3000$  mg expressed as N/l. The following protocol was used: 4.34 g dry matter (or equivalent total fresh biomass on a 105°C dry weight basis), 262.5 g inoculum and 140 ml distilled water were mixed in 500 ml vials. Blanks consisted of same total weight (406.84 g) of inoculum. The vials were sealed with aluminium-faced gray rubber stoppers, purged by flowing argon in and out and put at 37°C in oven. For 75 days, the biogas production was evaluated by allowing gas to expand in a syringe and measuring the final volume, during a specific time-course experiment. Initially samples were collected daily but frequency became infrequent with progressing of experiment. The composition of sampled volumes was analyzed by gas-chromatography (Dani DPC 1000, DANI Instruments spa, IT). Experiment was carried out in duplicate and two blanks were added for normalizing the results.

Statistical analysis of data was carried out by R (version 3.0.2 extended via Agricolae package).

## 3. Results and discussion

### 3.1. Environmental features

The two growing seasons showed quite different environmental conditions. Season 2010-2011 was characterized by a heavy rainfall, mainly on November (294.4 mm), December (137 mm) and March (165.4mm) and an average temperature of 7.5°C and a minimum average temperature of 6.3°C, in winter. In season 2011-2012, the winter was

relatively dry, with snow in February and abundant rainfall in April (91mm) and May (133mm) and was characterized by an average temperature of 7.3°C and a minimum temperature average of 3.6°C.

The soil chemical analyses in the two fields showed comparably values (data not shown), compatible with marginal soils, even if Castel Giuliano resulted poorer, in total organic matter and available nitrogen, and heavier, due to the presence of clay.

### 3.2. Field data and agronomic trait evaluation

Evaluation of agronomic traits of the eight elite lines is shown in Table 1. Data resulted from experimental fields carried out in Obregon (MX) during three seasons (2007-2009) in two environments (full and reduced irrigation).

Table 1. Data from Mexico, averages of three year experiments carried out in triplicate, in two environmental conditions, full irrigation (white colour) and reduced irrigation (grey colour). GY=grain yields; TWT=test weight; KWT=Kernel weight; PHT=plant height.

Line	PHT (cm)	GY (t/ha)	TWT (kg/ha)	KWT (g)	PHT (cm)	GY (t/ha)	TWT (kg/ha)	KWT (g)
1	123	7.34	761	43.2	99	4.31	775	43.1
2	116	7.25	757	46.6	99	4.34	754	42.8
3	115	7.36	755	42.7	99	4.48	741	39.0
4	124	7.55	771	49.4	105	3.98	776	42.8
5	121	7.44	766	48.3	98	3.96	758	42.3
6	108	7.49	721	57.4	88	4.11	725	43.7
7	117	7.35	740	44.6	93	3.55	734	41.7
8	120	7.49	738	42.0	90	3.44	721	33.8

Agronomic results from experiments carried out in Italy in 2010-2011 and 2011-2012 seasons are reported in Table 2.

The comparison between Mexican and Italian data is not completely affordable due to the different harvest time (full maturation versus milk phase), environments and measured traits, in relationship with the different purpose of the two field experiments (agronomic evaluation versus biogas from biomass). Thus, plant height is the unique common trait, even if it refers to two different growing stages: milk phase (generally early May) and final maturation (generally end of June). Thus, values in Italy resulted lower than in Mexico, at full irrigation condition and more comparable to values observed in Mexico, at reduced irrigation, probably due to the effect of the two abiotic stresses coming from reduced irrigation (Mexico) and poorer soils (Italy).

Analysing data obtained from Italian fields (Table 2), in 2011-2012, the effect of cold is clear in the delayed heading stage (on average 50 days), while late spring raining induced second tillering, thus explaining the lower spike/plant ratio, both in comparison with 2010-2011 data.

It is worth of noting that in 2011-2012 season, the overall total harvested biomass was not significantly lesser than previous one (data not shown, from combined years analysis), in spite of the lower quality of soil.

The analysed lines appeared statistically significant different, in both seasons as resulting by ANOVA. In particular, the height of EW9 (line 10) resulted significantly the shortest, an expected result related to species, bread wheat versus triticale.

In the 2010-2011 season, total mass of the lines 3 and 6 was affected by a particularly poor crop establishment, not in relationship with seed quality, because seeds from the same stock produced a total mass on the average in the 2011-2012 season.

Table 2. Data from Italy, 2010-2011 (white colour) and 2011-2012 (grey colour) seasons. DTH=days to heading; PHT=plant height. Lines 1-8 represent the CIMMYT elite lines; line 9 stands for Magistral and line 10 stands for EW9. Total Mass refers to the total weight of biomass of the two replications. Means with the same letter are not significantly different.

Line	DTH (days)		PHT (cm)		Spikes/Plants ratio		Total Mass (kg)	
1	106.5 <sup>ab</sup>	152 <sup>b</sup>	88.5 <sup>ab</sup>	96.0 <sup>a</sup>	4.7 <sup>ab</sup>	2.9 <sup>b</sup>	4.3 <sup>cde</sup>	4.24 <sup>b</sup>
2	104 <sup>abcd</sup>	149 <sup>b</sup>	94.0 <sup>a</sup>	96.5 <sup>a</sup>	4.8 <sup>ab</sup>	3.6 <sup>a</sup>	6.4 <sup>bcd</sup>	8.0 <sup>a</sup>
3	107.5 <sup>a</sup>	154 <sup>ab</sup>	80.8 <sup>abc</sup>	94.5 <sup>a</sup>	5.4 <sup>ab</sup>	3.4 <sup>ab</sup>	2.0 <sup>e</sup>	5.59 <sup>ab</sup>
4	99.5 <sup>d</sup>	150 <sup>b</sup>	93.5 <sup>a</sup>	89.5 <sup>ab</sup>	3.7 <sup>abc</sup>	3.0 <sup>ab</sup>	4.6 <sup>cde</sup>	5.4 <sup>ab</sup>
5	101 <sup>cd</sup>	153 <sup>b</sup>	88.8 <sup>ab</sup>	96.0 <sup>a</sup>	5.6 <sup>a</sup>	3.6 <sup>ab</sup>	4.1 <sup>cde</sup>	5.0 <sup>ab</sup>
6	102 <sup>bcd</sup>	148 <sup>b</sup>	74.5 <sup>bc</sup>	80.0 <sup>bc</sup>	5.2 <sup>ab</sup>	4.5 <sup>ab</sup>	2.1 <sup>de</sup>	5.54 <sup>ab</sup>
7	102 <sup>bcd</sup>	154 <sup>ab</sup>	92.0 <sup>ab</sup>	96.0 <sup>a</sup>	3.3 <sup>bc</sup>	2.5 <sup>ab</sup>	7.9 <sup>abc</sup>	5.82 <sup>ab</sup>
8	101 <sup>cd</sup>	150 <sup>b</sup>	95.0 <sup>a</sup>	96.5 <sup>a</sup>	3.4 <sup>abc</sup>	2.4 <sup>ab</sup>	7.2 <sup>bc</sup>	4.89 <sup>ab</sup>
9	105.5 <sup>bcd</sup>	154 <sup>b</sup>	97.8 <sup>a</sup>	97.5 <sup>a</sup>	1.5 <sup>c</sup>	2.7 <sup>b</sup>	12.2 <sup>a</sup>	3.99 <sup>b</sup>
10	104.5 <sup>abcd</sup>	160 <sup>a</sup>	68.3 <sup>c</sup>	69.0 <sup>c</sup>	3.4 <sup>abc</sup>	4.2 <sup>b</sup>	10.2 <sup>ab</sup>	4.48 <sup>b</sup>

On the contrary, Italian triticale line Magistral (line 9) and EW9 (line 10) seemed to be particularly affected by environmental conditions in the 2011-2012 season, showing a very large loss of productivity, with reduction up to 25% and 50%, respectively. The same is true for lines 7 and 8 in less extension, while the total mass of line 2 remained quite high and stable in both seasons, showing even an increase.

As a conclusion of overall evaluations carried out in the two Italian crop seasons, the line 2 was considered the most promising line. It is worth of noting, that the same line 2 was considered at CIMMYT the most interesting of the whole group, but this information was received by us only after our experiments and analyses.

In Table 3 results of additional biomass characterization are reported.

Table 3. Data from Italy, 2010-2011 (white colour) and 2011-2012 (grey colour) seasons. TS%=total solids; M%=moisture. Means with the same letter are not significantly different.

Line	Calorific Value		TS%		M%		Ash%	
	kJ/g		fresh mass		fresh mass			
1	17.14 <sup>a</sup>	17.61 <sup>a</sup>	37.95 <sup>ab</sup>	35.61 <sup>a</sup>	62.05 <sup>ab</sup>	64.4 <sup>a</sup>	7.44 <sup>a</sup>	6.72 <sup>a</sup>
2	17.63 <sup>a</sup>	18.17 <sup>a</sup>	35.02 <sup>ab</sup>	36.27 <sup>a</sup>	64.99 <sup>ab</sup>	63.73 <sup>a</sup>	5.75 <sup>cd</sup>	5.49 <sup>a</sup>
3	17.19 <sup>a</sup>	17.54 <sup>a</sup>	34.88 <sup>ab</sup>	36.81 <sup>a</sup>	65.13 <sup>ab</sup>	63.19 <sup>a</sup>	7.06 <sup>ab</sup>	5.89 <sup>a</sup>
4	17.54 <sup>a</sup>	18.23 <sup>a</sup>	35.67 <sup>ab</sup>	36.23 <sup>a</sup>	64.33 <sup>ab</sup>	63.78 <sup>a</sup>	6.08 <sup>bcd</sup>	5.28 <sup>a</sup>
5	17.50 <sup>a</sup>	17.85 <sup>a</sup>	37.95 <sup>ab</sup>	37.98 <sup>a</sup>	62.05 <sup>ab</sup>	62.02 <sup>a</sup>	6.55 <sup>abc</sup>	5.89 <sup>a</sup>
6	17.72 <sup>a</sup>	17.89 <sup>a</sup>	36.26 <sup>ab</sup>	35.75 <sup>a</sup>	63.75 <sup>ab</sup>	64.25 <sup>a</sup>	7.18 <sup>ab</sup>	5.86 <sup>a</sup>
7	17.44 <sup>a</sup>	17.72 <sup>a</sup>	39.26 <sup>a</sup>	40.10 <sup>a</sup>	60.75 <sup>b</sup>	59.91 <sup>a</sup>	5.26 <sup>d</sup>	5.36 <sup>a</sup>
8	17.23 <sup>a</sup>	17.37 <sup>a</sup>	36.74 <sup>ab</sup>	37.47 <sup>a</sup>	63.26 <sup>ab</sup>	62.54 <sup>a</sup>	7.41 <sup>a</sup>	5.85 <sup>a</sup>
9	17.35 <sup>a</sup>	18.1 <sup>a</sup>	38.58 <sup>ab</sup>	38.19 <sup>a</sup>	61.42 <sup>ab</sup>	61.82 <sup>a</sup>	5.85 <sup>cd</sup>	6.51 <sup>a</sup>
10	17.22 <sup>a</sup>	17.78 <sup>a</sup>	33.89 <sup>b</sup>	38.04 <sup>a</sup>	66.11 <sup>a</sup>	61.97 <sup>a</sup>	7.28 <sup>a</sup>	6.36 <sup>a</sup>

Concerning biochemical results, values are comparable to data reported in literature. Calorific values resulted not significantly different among lines in both seasons. It is worthwhile noting that the values of other parameters were significantly different between lines in the 2010-2011 season, while in the 2011-2012 season, the values appeared homogeneous among lines. These results suggested that environmental differences, mainly soil, could have an influence on these parameters.

### 3.3. Potential biogas yields

As an example, the representation of gas production during the time-course experiment of line 7 is shown in Fig. 1. Normalized values are calculated on both dry matter and volatile substance bases.

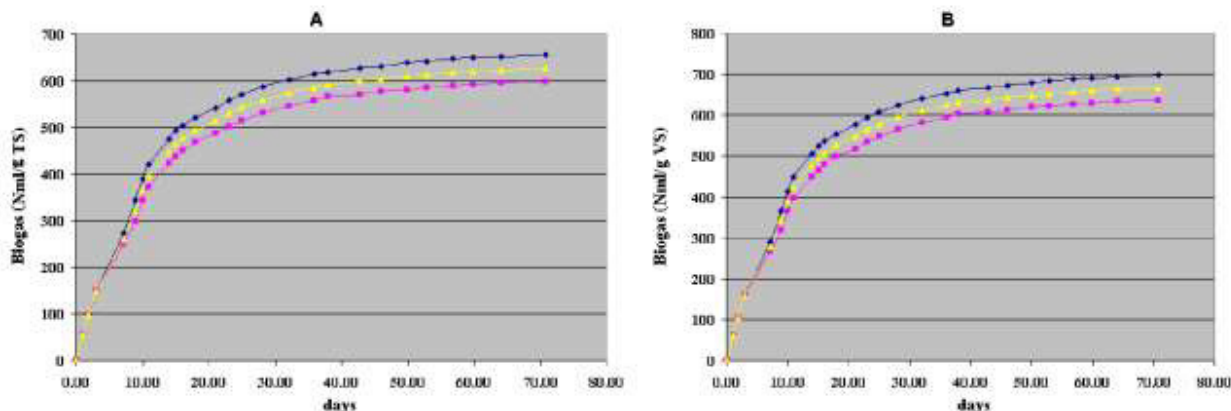


Fig. 1. Evolution of biogas production during the time of experiment. Values are Normalized by subtracting blanks. Pink and blue colours refer to duplicate samples; in yellow colour the average value is represented. (A) total biogas expressed with reference to dry mass; (B) total biogas expressed with reference to volatile substance

Potential biogas yields after 75 days of anaerobic digestion experiment are shown in Table 4, referred to both dry matter and volatile substance, and ANOVA analysis was carried out on both representations. Methane represented roughly 57% of total biogas in all samples. Lines resulted significantly different in their potential, being lines 7 and 9 the best performers and line 4 the worst one. The differences are significant, but their extent is moderate and became negligible when the total harvested masses are considered. Effectively, line 2 is about 25% more productive than the best one of remaining lines (line 7), but it is just about 8% less productive in potential biogas yield, in comparison with the same line 7.

Table 4. Potential biogas yields of the 10 lines after 75 days of anaerobic digestion. Values refer to duplicate samples, their mean and SD. Means with the same letter are not significantly different.

Lines	Total Biogas Nml/g dry matter				Total Biogas Nml/g VS			
	min	mean	max	SD	min	mean	max	SD
1	545	586.5 <sup>ab</sup>	628	58.69	577	620.2 <sup>ab</sup>	664	61.79
2	564	580.0 <sup>ab</sup>	596	22.63	592	609.3 <sup>ab</sup>	626	23.99
3	578	585.2 <sup>ab</sup>	592	10.22	612	619.3 <sup>ab</sup>	627	10.64
4	530	530.8 <sup>b</sup>	532	1.40	569	570.4 <sup>b</sup>	571	1.50
5	605	611.4 <sup>a</sup>	617	8.44	645	651.4 <sup>ab</sup>	658	8.99
6	545	571.3 <sup>ab</sup>	598	37.58	583	611.3 <sup>ab</sup>	640	40.20
7	599	627.2 <sup>a</sup>	656	40.43	637	667.1 <sup>a</sup>	698	43.01
8	577	580.0 <sup>ab</sup>	583	4.17	619	622.5 <sup>ab</sup>	626	4.47
9	590	621.0 <sup>a</sup>	652	43.76	640	673.7 <sup>a</sup>	707	47.48
10	540	555.7 <sup>ab</sup>	571	22.04	577	594.1 <sup>ab</sup>	611	23.56

Even if the yields of biogas from a particular feedstock vary according to dry matter content, length of time in the digester, the type of anaerobic digestion system and the conditions in the digester, a comparison with literature data shows a very good agreement with our results. In Table 5, some data from the Official Information Portal on Anaerobic Digestion (<http://www.biogas-info-co.uk/biogas-yields.html>) are reported. The most relevant crops are shown, for comparative purposes.

Table 5. Biogas yields of different crops based on values from "Biogas from Energy Crop Digestion" by the International Energy Agency (IEA).

Crop	Biogas Yield m3/tonne
Potatoes	276-400
Rye grain	283-492
Sorghum	295-372
Grass	298-467
Triticale	337-555
Barley	353-658
Hemp	355-409
Wheat grain	384-426

#### 4. Conclusions

Triticale is the only man-made crop and just over 130 years of breeding, it is still in the process of evolving not only as a species but also in its utilization. Triticale has potential as an alternative crop for various end-uses in a wide range of environments; breeding programs worldwide are going on for improving abiotic stress resistance in marginal lands (acid, alkaline, sandy, element deficient or toxic soils), biotic resistance, the food and feed quality, with the aim of developing new triticale varieties for specific end-uses.

As the selection of the most interesting genotypes is important in view of bio-energy application, a study was carried out comparing elite lines from CIMMYT and two Italian varieties. To our knowledge, this is the first time these CIMMYT lines were grown in Italy. They were not adapted to Italian environment and were not specifically selected for marginal soils, thus our results as a whole are encouraging. In particular, line 2 appears the most promising and adaptable, due to its improving in mass yields even in the relatively unfavourable environmental conditions of the second season. Really, in a third experimental field carried out in 2013-2014, line 2 remained the best performer, further improving mass yields (data not shown) and in comparison with well adapted Italian lines, line 2 proves to be more resistant to biotic and abiotic environmental stresses.

Further experiments are planned in order to investigate the efficiency of these lines for ethanol production.

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