# Suitability of winter triticale varieties for bioethanol production in Latvia

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**Abstract.** The research was carried out at the State Stende Cereals Breeding Institute in experimental years 2007/08 and 2008/09. The subject of the research was the suitability of seven winter triticale varieties and lines for bioethanol production. Two supplementary fertilization rates of nitrogen were used (N 100 kg ha<sup>-1</sup> and N 140 kg ha<sup>-1</sup>). The correlations of characteristics of different varieties were evaluated. During the research the analysis of grain quality (starch and crude protein content) was carried out. The theoretical bioethanol outcome (L t<sup>-1</sup>) and yield (L ha<sup>-1</sup>) were calculated by using the indicators of starch content. The results of the research show that the winter triticale variety 'Dinaro' and line 9402-3 with the highest theoretically calculated bioethanol outcome (489.4 L t<sup>-1</sup> and 486.5 L t<sup>-1</sup>) and the highest starch content of the grains (681.19 g kg<sup>-1</sup> and 677.13 g kg<sup>-1</sup>) were the most suitable for the bioethanol production in Latvian conditions. No substantial effect of the fertilization methods on the starch content in grains and on the theoretically calculated ethanol outcome was observed.

Keywords: winter triticale, crude protein, starch, bioethanol

## **INTRODUCTION**

The use of renewable energy resources is one of the preconditions for sustainable rural development. It affects the rural economy and regional development favourably, ensures independence from the import of energy resources and facilitates the diversification of rural entrepreneurship. Bioethanol is produced from biomass and/or biologically degradable waste particles and is used as a biofuel. Bioethanol is being used more and more broadly as a substitute for fossil fuels in various countries. Currently a small amount of bioethanol is added to petrol (up to 5%).

The cereal varieties that are suitable for bioethanol production are high-yield varieties that can provide good yields with reduced fertilization levels, thus diminishing production costs, and ensuring high starch content and lower protein content of grains. There are several criteria for choosing raw materials for bioethanol production: price and yield of the raw material, ethanol outcome, starch content, pest and disease resistance, suitability for soil and weather conditions, harvesting transportation and storage options as well as the usability of by-products.

Having carried out the research on triticale suitability for bioethanol production in the Czech Republic, Krejčirova & Capouchova (2008) acquired data on the starch content of different triticale varieties. The starch content and yield differed depending upon the variety, location and year of growing. The starch content of the same variety ranged from 673.3 to 693.9 g kg<sup>-1</sup>. The yield of the same variety ranged from 7.50 to 7.90 t  $ha^{-1}$ .

S. Wang and his colleagues indicated in 1997 that the outcome of bioethanol from winter triticale grains is  $362-367 \text{ L t}^{-1}$  by 14% of grain humidity. Thus, triticale is a good alternative for ethanol production. According to the data of Kučerov (2007) the ethanol outcome from winter triticale can be influenced by several factors: first, the choice of variety, because different varieties provide different ethanol outcomes; secondly, the choice of geographical location and annual agro-ecological differences. Cereals are used for bioethanol production throughout the world. Cereals with high starch content are a suitable raw material for production of industrial alcohol (Clarke et al, 2008). Triticale is one of the most suitable winter cereal crops for bioethanol production in Latvia, where a wide range of triticale varieties are grown, but there is little research on their use for bioethanol production.

The main task of our research was investigation of the suitability of winter triticale varieties and lines for bioethanol production, using different nitrogen rates.

# MATERIALS AND METHODS

The research on suitability of winter triticale lines and varieties for ethanol production was carried out at the State Stende Cereals Breeding Institute in 2007–2009. The research was carried out on sod-podzolic loam soil that had the following characteristics:  $pH_{KCL}$  5.3–6.7, humus content 2.1–2.6%, P<sub>2</sub>O<sub>5</sub> 193–251 mg kg<sup>-1</sup>, K<sub>2</sub>O 172–189 mg kg<sup>-1</sup>. Field experiments were carried out in breeding crop rotation fields in 4 repetitions with 12 m<sup>2</sup> plot area. The research was carried out as a 3-factor experiment. Factor A was the experimental year. Factor B was the supplementary fertilization using two rates: B1 N 100 (70+30) kg ha<sup>-1</sup>, B2 N 140 (70+70) kg ha<sup>-1</sup>. The total amount of fertilizer was divided into two portions, which were applied first when the vegetation process began in spring and during the 31st to 32nd stage of plant development. Factor C was variety ('SW Falmoro', 'Dinaro', 'Collegial', 'Woltario', 'SW Valentino', line 9405-23, line 9402-3). The sowing rate was 400 germinating grains m<sup>-2</sup>. The sowing time was the second decade of September. The complex fertilizer N6-P26-K30 in the amount of 300 kg ha<sup>-1</sup> was used as the main fertilizer. Triticale was harvested in the second decade of August.

Meteorological data of the vegetation and hibernation periods of winter crops were collected from the Stende hydro-meteorological station. Multi-annual average temperature and precipitation measurements were defined as an average. Meteorological conditions were variable in the research years; the average daily temperature and precipitation are indicated in Table 1.

The analysis of the grain quality was carried out in the cereals technology and agro chemistry laboratory of the State Stende Cereals Breeding Institute where the crude protein and starch content were determined by using INFRATEC ANALYS 1241.

Month	Average dai	Average daily air temperature, °C		Precipita		
	2007/2008	2008/2009	Average	2007/2008	2008/2009	Average
September	11.8	10.5	11.4	92.6	44.9	75
October	7.2	8.2	6.6	76	108.5	71
November	1.6	2.6	1.8	85.7	108.4	63
December	2	0.4	-2	43.3	42.6	47
January	-0.2	-2.5	-4.6	40.1	67.6	37
February	1.7	-3.6	-4.7	51.3	21.9	26
March	1	-0.5	-1.5	69.4	55.1	29
April	6.5	6.6	4.3	45.1	11.5	37
May	10.2	11	10.2	16.1	28.8	45
June	14	13.5	14.2	72	94.3	57
July	15.9	17.1	16.3	95.6	147.5	87
August	15.9	15.7	15.5	159.5	83.4	87

**Table 1.** Temperature and precipitation compared with meteorological average during 2007–2009.

The formulas recommended by the Institute of Microbiology and Biotechnology of the University of Latvia, were used in order to calculate the theoretical ethanol outcome (L  $t^{-1}$ ) and yield (L  $ha^{-1}$ ). Theoretical outcome of ethanol from starch was as follows:

$$E t = \frac{C \times 1.11 \times 2 \times 46}{180.16}$$
 (1), where

Et-theoretical outcome of ethanol, g g<sup>-1</sup>,

1.11 – factor for starch recalculation into glucose,

2 – stechiometrical factor for the summary reaction equation of glucose conversion into ethanol,

46 – molar mass of ethanol,

180.16 - molar mass of glucose,

 $C - starch, g g^{-1}$ .

Outcome of ethanol, L t<sup>-1</sup>

$$E = \frac{E}{0.789} \times 1000$$

(2), where

 $\begin{array}{l} E-\text{outcome of ethanol, L }t^{\text{-1}},\\ E_t-\text{acquired outcome of ethanol, g }g^{\text{-1}},\\ 0.789-\text{ethanol density, g }ml^{\text{-1}}. \end{array}$ 

Ethanol yield L ha-1

$$Er = E \times R$$
 (3), where

 $E_r$  – ethanol yield, L ha<sup>-1</sup>, E – outcome of ethanol, L t<sup>-1</sup>, R – grain harvest, t ha<sup>-1</sup>. MS Excel program was used for data statistical processing by the methods of three-factor analyses with replication (A – years, B – increased N fertilizer rates, C – winter triticale varieties) using the methodology of Dospehov (1979). The test of statistically significant differences ( $\gamma_{0.05}$ ) at Fischer criterion (*F*) and density of factors influence ( $\eta^2$ , probability < 0.05) were used for the analyses of means differences.

# **RESULTS AND DISCUSSION**

Meteorological conditions in the experimental year 2007/08 were more favourable for growing winter triticale and contributed to high crop harvests. In the autumn of year 2008/09 the level of precipitation was higher whereas in spring, during the growth period of the winter crops, there was a lack of humidity. It substantially (p < 0.05) affected the formation of crops. The average yield in 2008/09 was lower by 3.52 t ha<sup>-1</sup> in comparison to the 2007/08 periods.

Applying a supplementary nitrogen fertilizer in the amount of 100 kg ha<sup>-1</sup>, the average winter triticale yield in both years was 11.27 t ha<sup>-1</sup>, while the average triticale yield did not substantially (p > 0.05) increase when the amount of nitrogen fertilizer was increased by 40 kg ha<sup>-1</sup>. This indicates that winter triticale varieties do not respond to increased amounts of nitrogen. The winter triticale variety 'Dinaro' provided the highest yields in both research years and with both methods of supplementary fertilization. The results are compiled in Table 2.

		2007/2008	8	/ 4	_		
Varieties and lines	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	Average in two years ( $\gamma_{0.05}$ = 0.45)
SW Falmoro	11.70	13.30	12.50	10.11	8.80	9.45	10.98
SW Valentino	12.01	13.03	12.52	10.27	10.06	10.16	11.34
Dinaro	15.48	15.62	15.55	10.55	10.13	10.34	12.95
Line 9405-23	13.51	13.38	13.44	9.58	8.63	9.10	11.27
Line 9402-3	14.57	14.52	14.54	9.78	9.18	9.48	12.01
Woltario	12.40	12.97	12.68	9.12	9.34	9.23	10.96
Collegial	9.68	10.31	9.99	9.07	8.54	8.80	9.40
Average $(\gamma_{0.05} = 1.24)$	12.76	13.30	13.03	9.78	9.24	9.51	11.27

**Table 2.** Yield (t ha<sup>-1</sup>) of the winter triticale varieties and lines.

The yield of winter triticale was substantially (p < 0.05) affected by the factor A – experimental year and by factor C – the variety. A substantial (p < 0.05) effect was also caused by the correlation of the year and the amount of fertilization, as well as the correlation of the year and the genotype (Table 3). The research of Czech scientists also showed that the yield of winter triticale was affected by the variety and the year (Kučerov, 2007).

Factors	Grain yield	Protein content	Starch content	Bioethanol yield
Experimental year – factor A	63.24*	21.30*	38.78*	61.71*
Increased N fertilizer rates – factor B	0.00	1.90*	0.11	0.00
Winter triticale varieties – factor C	20.49*	40.94*	39.68*	22.99*
Interaction AB	1.49*	7.29*	1.02*	1.41*
Interaction AC	9.41*	7.87*	14.71*	9.75*
Interaction BC	0.55	0.99	0.82*	0.48
Interaction ABC	0.82*	0.88	0.24	0.66
Effect of unexplored factors	3.93	17.09	4.52	2.93

**Table 3.** The influence of factors on the winter triticale grain and bioethanol yield, and quality indices,  $\eta$ %, 2007–2009.

 $*F > F_{0.05}$ 

Suitability of variety for ethanol production is indicated by its starch content. The outcome of ethanol is calculated by the starch content of grains. The research data showed that the winter triticale variety 'Dinaro' and the line 9402-3 had the highest starch content. The change in the amount of supplementary fertilizer did not cause substantial (p > 0.05) changes in starch content of the winter triticale genotypes. The highest starch content was produced in 2007/08, ranging from 629.5 to 694.0 g kg<sup>-1</sup>, when the amount of supplementary nitrogen fertilizer was 100 kg ha<sup>-1</sup>. With the amount of supplementary nitrogen fertilizer being 140 kg ha<sup>-1</sup>, the starch content of the winter triticale varieties and lines ranged from 636.0 to 693.75 g kg<sup>-1</sup>. In the period of 2008/09 the average starch content of the winter triticale grains was 4% lower (Table 4).

Starch content of the winter triticale grains was substantially (p < 0.05) affected by the experimental year and the genotype. The correlation of the year and the amount of fertilizer and the correlation of the year and the variety or line also substantially (p < 0.05) influenced the results (Table 3).

Scientists from other countries have found that the quality of grains is determined by different correlated factors: meteorological conditions, genetic characteristics of the variety, fertilization with nitrogen, and others (Swanston et al., 2007). It is also confirmed by this research.

		2007/200	8				
Varieties and lines	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	Average in two years $(\gamma_{0.05} =$ 4.35)
SW Falmoro	683.25	684.25	683.75	656.75	649.75	653.25	668.50
SW Valentino	677.50	683.75	680.63	650.25	644.00	647.13	663.88
Dinaro	694.00	693.75	693.88	671.50	665.50	668.50	681.19
Line 9405-23	682.00	687.00	684.50	651.00	650.75	650.88	667.69
Line 9402-3	693.25	691.50	692.38	669.75	654.00	661.88	677.13
Woltario	677.50	679.75	678.63	644.25	644.75	644.50	661.56
Collegial	629.50	636.00	632.75	646.50	643.25	644.88	638.81
Average $(\gamma_{0.05} = 12.08)$	676.71	679.43	678.07	655.71	650.29	653.00	665.54

**Table 4.** The starch  $(g kg^{-1})$  content of the winter triticale grains.

The Czech scientists ascertained that the grain quality indicators – crude protein and starch – of the same winter triticale variety differ depending on growing conditions (Kučerov, 2007). Such results were also acquired in the research of the State Stende Cereals Breeding Institute.

The results of the research showed that the winter triticale variety 'Dinaro' and line 9402-3 that had substantially higher starch content also had substantially lower crude protein content. This variety and line are more suitable for bioethanol production in Latvian conditions. The crude protein content of the winter triticale varieties and lines was substantially (p < 0.05) affected by the experimental year and the amount of supplementary fertilizer as well as the variety or line. Substantial (p < 0.05) influence was yielded by the correlation of the experimental year and the amount of fertilizer, as well as the correlation of the experimental year and the variety or line (Table 3). An increased amount of nitrogen fertilizer (N 100 kg ha<sup>-1</sup>) affected only the winter triticale variety 'Falmoro', causing a substantial (p < 0.05) average increase of the crude protein during both experimental years (Table 5).

	2007/200	8		2008/2009	9		Average in two years $(\gamma_{0.05}=$ 3.29)
Varieties and lines	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	
SW Falmoro	101.50	105.00	103.25	109.75	117.25	113.50	108.38
SW Valentino	104.75	102.25	103.50	106.00	113.50	109.75	106.63
Dinaro	93.00	89.25	91.13	100.75	106.25	103.50	97.31
Line 9405- 23	109.50	105.50	107.50	111.75	116.50	114.13	110.81
Line 9402-3	96.50	93.00	94.75	100.00	108.75	104.38	99.56
Woltario	107.25	103.25	105.25	109.75	116.50	113.13	109.19
Collegial	111.75	111.50	111.63	107.25	111.25	109.25	110.44
Average $(\gamma_{0.05}=9.15)$	103.46	101.39	102.43	106.46	112.86	109.66	106.04

**Table 5.** The crude protein  $(g kg^{-1})$  content of the winter triticale grains.

The most important quality indicators that have to be analyzed in order to assess the suitability of varieties for bioethanol production are starch and crude protein content of grains. In order to identify starch and crude protein correlations, the collected data were subjected to linear regression analysis (Fig. 1).



Figure 1. Starch and crude protein correlations for winter triticale (p < 0.05).

Starch and crude protein content correlations are described by the regression equation y = -1.963x+873.7. The determination factor  $R^2 = 0.584$  shows that the changes in crude protein content by 58% of the cases affects the starch content of winter triticale grains. There is a 95% possibility that these indicators have a fairly close correlation. The ethanol outcome of triticale grains was calculated theoretically, made on the basis of starch content of grains by using formulas 1 and 2.

The mathematical data processing, using the theoretically calculated ethanol outcome from a ton of grains, shows that a substantial (p < 0.05) influence was exercised by the variety of winter triticale and the experimental year. The correlation of the experimental year and the variety was also of great importance (Table 3).

It was estimated that the average amount of ethanol that can be obtained from the examined winter triticale varieties and lines in two years was 478.14 L t<sup>-1</sup>. According to the research of S. Wang and his colleagues (1997) the outcome of bioethanol from winter triticale grains was 362-367 L t<sup>-1</sup>, which is lower than the research results acquired in Stende (Table 6). The highest theoretically calculated outcome of ethanol was acquired from the winter triticale variety 'Dinaro' and line 9402-3 that had the highest starch content.

-	2007/200	8		2008/200	2008/2009			
Varieties and lines	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	Average in two years ( $\gamma_{0.05}$ = 3.13)	
W Falmoro	490.9	491.6	491.2	471.8	466.8	469.3	480.3	
SW Valentino	486.7	491.2	488.9	467.2	462.7	464.9	476.9	
Dinaro	498.6	498.4	498.5	482.4	478.1	480.3	489.4	
Line 9405- 23	489.9	493.6	491.8	467.7	467.5	467.6	479.7	
Line 9402-3	498.0	496.8	497.4	481.2	469.8	475.5	486.5	
Woltario	486.7	488.3	487.5	462.8	463.2	463.6	475.3	
Collegial	452.2	456.9	454.6	464.5	462.1	463.3	458.9	
Average $(\gamma_{0.05} = 8.68)$	486.2	488.1	487.1	471.1	467.2	469.1	478.14	

**Table 6.** The bioethanol outcome  $(L t^{-1})$  of the winter triticale varieties and lines.

Analyzing the theoretically calculated outcome of ethanol, it was observed that the increase of supplementary nitrogen fertilizer caused no substantial changes (p > 0.05).

One of the most important indicators is ethanol yield per hectare. Both yield of the variety or line and the starch content of the grains of the certain variety or lines are important for acquisition of higher ethanol outcome per hectare. The theoretical calculation was done by using formula No. 3.

Calculating the ethanol yield per hectare, it was observed that a substantial influence (p < 0.05) was exercised by the growing variety and the experimental year.

The correlation of both previously mentioned factors as well as the correlation of the experimental year and the amount of supplementary fertilizer also had a substantial (p < 0.05) influence on yield (Table 3).

The average theoretically calculated ethanol yield was 5416 L ha<sup>-1</sup> in both years. The meteorological conditions in the experimental year 2007/08 were more favourable for harvesting high amounts of crops, thus the ethanol yield (L ha<sup>-1</sup>) was by 1906 L ha<sup>-1</sup> higher in experimental year 2007/08 (Table 7).

	2007/2008			2008/2009	2008/2009			
Varieties and lines	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	N 100 kg ha <sup>-1</sup>	N 140 kg ha <sup>-1</sup>	Average year	Average in two years $(\gamma_{0.05} =$ 211.45)	
SW Falmoro	5745.6	6539.5	6142.5	4767.7	4103.8	4435.8	5289.2	
SW Valentino	5844.9	6398.5	6121.7	4796.8	4653.3	4725.1	5423.4	
Dinaro	7719.4	7784.6	7752.0	5090.0	4842.4	4966.2	6359.1	
Line 9405- 23	6618.9	6601.7	6610.3	4479.4	4032.7	4256.0	5433.2	
Line 9402-3	7255.9	7213.2	7234.6	4703.7	4312.9	4508.3	5871.4	
Woltario	6034.2	6332.9	6183.5	4220.2	4326.7	4273.5	5228.5	
Collegial	4375.7	4711.6	4543.6	4210.4	3944.7	4077.6	4310.6	
Average ( $\gamma_{0.05} =$ 586.9)	6227.8	6511.7	6369.8	4609.7	4316.6	4463.20	5416.5	

**Table 7.** Bioethanol yield (L ha<sup>-1</sup>) of the winter triticale varieties and lines.

The highest theoretically calculated ethanol yield (L ha<sup>-1</sup>) was acquired from the winter triticale variety 'Dinaro', the average of which was 6359 L ha<sup>-1</sup> in both years. This variety was characterized by high yield as well as by high starch content of grains.

### CONCLUSIONS

Evaluating the suitability of winter triticale varieties and lines for bioethanol production, the research at the State Stende Cereals Breeding Institute indicates that the most suitable winter triticale variety and line for bioethanol production is the variety 'Dinaro' (489.4 L t<sup>-1</sup>) and the line 9402-3 (486.5 L t<sup>-1</sup>) that had a high theoretically calculated outcome of ethanol. The winter triticale variety 'Dinaro' had also the highest theoretically calculated ethanol yield (6359.1 L ha<sup>-1</sup>).

Neither the supplementary fertilization methods using nitrogen nor yield (L ha<sup>-1</sup>) of the examined winter triticale varieties and lines had a substantial (p > 0.05) influence on the theoretically calculated outcome of ethanol (L t<sup>-1</sup>).

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