Effect of nitrogen fertilization and of the genotype on the yield, and nitrogen and β-glucans content in oats (Avena sativa L.) grains

ABSTRACT

Oat grain contains relatively high amounts of dietary fiber, particularly β-glucans. This is an advantage since it represents a significant potential in the production of human prebiotic, nutraceutical and functional foods. The aim of this work was to evaluate the effect of genotype and nitrogen (N) applied through the growing, on the concentration of grain β-glucans, nitrogen, and yield. For this purpose, seven commercial varieties, Turquesa, Obsidiana, Karma, Avemex (Cevamex), Chihuahua, Paramo y Menonita were sowing in the Universidad Autónoma del Estado de México, located north of Toluca city (19°15′33″N, 99°39′38″W, 2640 m.a.s.l.) in Mexico, and the fertilization treatments consisted of 60, and 120 kg N·ha⁻¹. β-glucans content and yield and some of its main components were quantified in harvested grain. Results showed that N dose increases the β-glucans content in the Turquesa Paramo and Menonita genotypes and in the other four, the content of this dietary fiber decreases. The grain yield increases with the N dose, although each genotype responds differently to this factor. This parameter is better explained by the number of grains obtained per surface than by the unit grain weight. Grain yield is produced in the tillers and in this part of the architecture of the plants is where the effect of N dose is focused.

Keywords: Nitrogen dose; β-Glucans; Oat; dietary Fiber.
INTRODUCTION

Oats (Avena sativa L.) is a grass and is the sixth most-produced cereal worldwide, as its grains are used for human nutrition or fodder for animal feed (Hoffman, 1995). According to FAO (2017), the annual global production of oats is approximately 22.5 million tons obtained in 9.7 million hectares, with average yields of 2.3 t/ha-1 and a range from 0.7 to over 7 t/ha-1.

Oat grain contains a considerable amount of soluble dietary fiber (DF) mostly constituted by glucose polysaccharides called β-glucans, which produce a high viscosity when finding in aqueous solutions. Recent studies have reported that the concentrations of these compounds range between 2.0 and 5.0 g per 100 g of oat devoid of the bran (Asima et al., 2017).

DF, and especially the β-glucans in this cereal, have been shown to have significant potential to reduce the risks of cardiovascular diseases, type 2 diabetes Mellitus, gastrointestinal disorders, and some cancers, due to their prebiotic and antioxidant properties (Martínez-Villaluenga and Peñas, 2017). The diet is poor in DF - especially in urban areas - due to the low intake of vegetables, which often does not meet the recommendations of FAO: at least five servings (80 g each) of fruits and vegetables per day (Villanueva Carvaljal et al., 2013).

Since only a minority of the world’s population reaches this goal, and due to the fact that this problem is of particular importance in low-income social centers, a viable alternative is to include these nutrients in the diet by enriching food products with extracts obtained in extensive agricultural crops. These crops, such as oats, can synthesize more DF than conventional fruits and vegetables grown in similar environments and in a shorter period.

Since the environment, genotype, and plant nutrition affect the composition, yield, and components of the oat grain (Güller, 2003), it is likely that the concentration and characteristics of prebiotic compounds, such as β-glucans, are also influenced by these factors.

For example, on the one hand, Lim et al. (1992) reported high variability in the concentration of these compounds in various oat cultivars, and Ajithkumar et al. (2005) reported variations in their molecular weight and rheological properties. On the other hand, Anker-Nilssen et al. (2008) found that in barley, higher temperatures during the grain filling stages resulted in a higher content of β-glucans, a higher molecular weight, and a higher viscosity of the derived aqueous solutions.

In addition, the highest levels of these polysaccharides have been observed in hot and dry climates (Saastamoinen, 1995) and, conversely, the lowest levels were found in cold and humid climates (Brunner and Freed, 1994). Even so, the study of genotypic and environmental effects on the production of DF, essentially β-glucans, in oats has been poorly addressed and focusing agronomic research on this cereal from this perspective could generate viable alternatives to increase its cultivation and the economic value of grain production.

In order to propose oats as a source of ingredients for the production of functional and nutraceutical foods, our objectives in this work were to (i) verify the existence of genetic variability for yield and its main components, and (ii) to evaluate the concentration of N and β-glucans in this cereal, given environmental variations dominated by the availability of nitrogen in the soil.

MATERIALS AND METHODS

Description of the experimental site

The experiment was prepared during the summer-autumn 2014 cycle at the Faculty of Agricultural Sciences, Autonomous University of the State of Mexico [Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México], located north of the city of Toluca (19° 15’ 33” N, 99° 39’ 38” W, 2640 m.a.s.l.), Mexico. The climate is classified as sub-humid semi-cold with summer rains, annual rainfall ranging from 800 to 1300 mm, temperature from 8 to 14°C.

The plots were prepared in a 400 m² area with pellic vertisol type soil, clay-loam texture. The pH of this soil was equal to 6.6, with 6.7% organic matter, 35 kg ha-1 of N, 123 ppm of P and 564 ppm of K, measured at 0.6 m depth. According to the determination of soil fertility of NOM-021-SEMARNAT-2000 (2002), it is classified as neutral, with average levels of organic matter and inorganic nitrogen, and high levels of phosphorus and potassium.

Field activities and treatments

Seeds were hand planted at a rate of 120 kg ha-1 (approximately 350 seeds per m²), at a depth of 4 to 5 cm, in rows separated by 20 cm. The experimental plot consisted of six 5-meters long rows, separated by 0.20 m (6 m2).

For the measurements, we considered an area of 0.4 m² constituted by two central rows (length: 1 m) as an effective plot. Seven oat varieties were used as genetic material: Turquesa (Villaseñor-Mir et al., 2009), Obsidiana (Espitia-Rangel et al., 2007), Karma (Espitia Rangel et al., 2001), Avenex (Villaseñor-Mir et al., 2001), Chihuahua (INIA, 1971), Paramo (Sudermann, 1975) and Menonita (Salmerón-Zamora, 2002).

Nitrogen (N) treatments were 60 and 120 kg ha-1, which are similar to the lowest and highest doses used by the producers. In the first case, it was administered at the time of sowing and in the second, it was divided: 60 kg at sowing and 60 kg at tillering. Urea (46% N), 60 kg ha⁻¹ of triple calcium superphosphate (46% P₂O₅) and 30 kg ha⁻¹ potassium chloride (60% K₂O) were used.

Weeds were manually controlled throughout the crop cycle. No pests and/or diseases were reported during the growth period. The treatments had a factorial arrangement of 2 doses of N and 7 varieties of oats (14 treatments),
which were distributed in a randomized complete block design with three repetitions.

**Harvest and grain analysis**

Specimens were manually harvested 164 days after emergence. In each experimental unit, plants were harvested in 1 linear meter of the two central furrows. The stems of each plant were separated in main stem and tillers, after which they were dried in a forced air oven at 60 °C for 72 hours.

Using the grain obtained from the main stems and from the tillers, we calculated grain yield (g m-2), number of grains per m2 and individual grain weight (mg grain-1) for each fraction. That is, we obtained a value for each of these variables was obtained using the grains from the main stem, and another value using grains from the tillers.

We determined the content of β-glucans in each grain sample, using the K-TDFR enzyme kit purchased from Megazyme (Megazyme International Ireland Limited) (Hollmann, et al., 2013), and total nitrogen using the Kjeldahl method (Nielsen, 2017). We performed an analysis of variance (ANDEVA) using the obtained data, according to the linear model used. Means were compared using the Tukey test (with the help of the Statistical Analysis System software V.6.12, USA).

**RESULTS AND DISCUSSION**

**Yield of grain and some of its components**

The effect of the dose of N, the genotype, and the interaction between these two factors was highly significant for yield and number of grains, but not for the individual grain weight, whose variability only depended on the genotype. Similarly, the concentration of N and β-glucans in the grain was more associated with the genotype than to the nitrogen fertilization (Table 1).

The variability we report in the yield and the number of grains can be mainly explained by the effect of the genotype (26.1 and 38.5%), of the total sum of squares, respectively, compared to the effect of the dose of N that only accounted for a marginal percentage (14.6 and 7.97%, respectively). In the case of individual grain weight, this variability was also explained by the genotype effect (63.8%), since the effect of the dose of N only contributed with 4.2%.

The variability in the compounds evaluated in the grain is mostly attributed to the different genotypes. By itself, this factor integrated 28.5 and 39.8% of the sum of squares in ANDEVA for nitrogen and β content-glucans, respectively. The results obtained for this dietary fiber are accordance to the results reported by Humphreys et al. (1994), who did not find a significant effect between nitrogen fertilization and β-glucans content in the grains of four varieties of oats.

Table 2 shows the comparison of means for the dose of N and for the genotypes in each of the variables that define some performance components. On the one hand, the increase in the dose of N led to a yield increase of about 19%, and the number of grains also increased similarly, but at a rate of 16%. There were no significant effects on the remaining variables with the increase in nitrogen fertilization. On the other hand, after a correlation analysis of the number of grains vs. yield we found that for every 21.45 units of increase in the number of grains there was one unit of increase in yield (R2 = 0.84).

In addition, no other significant result was obtained with any other variable studied (Figure 1). At large, results suggest that in the evaluated genotypes grain yield was better explained by the number of grains obtained per m2 than by their individual weight (Table 2). According to these results, nitrogen in the grain was not affected by the dose of N and its variations essentially depended on the genotype. Nitrogen in the grain is the basis of the calculation of protein and dietary fiber, represented here by β-glucans.

On the one hand, the yield of oats was closely related to the number of grains per unit area, possibly associated with a greater number of panicles and grains per panicle, as it occurs in wheat and barley (Albrizio et al., 2010; Slafer et al., 2014). On the other hand, studies in wheat and barley have shown that there is a negative correlation between grain yield and its nitrogen concentration. This is mainly due to a process of dilution of N as grain size increases (Abeledo et al., 2008; Zhao et al., 2014). The results in Table 2 and Figure 1 suggest that in oats there is no significant correlation between these two variables.

<table>
<thead>
<tr>
<th>Source</th>
<th>GL</th>
<th>Yield</th>
<th>Number of grains</th>
<th>Individual weight of grains</th>
<th>Nitrogen in grains</th>
<th>β-glucans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>14023**</td>
<td>42.63** (2.5)</td>
<td>20.30** (3.4)</td>
<td>0.084** (8.91)</td>
<td>0.129**</td>
</tr>
<tr>
<td>A: Nitrógen</td>
<td>1</td>
<td>203845**</td>
<td>133.88 (7.7)</td>
<td>24.67** (4.2)</td>
<td>0.000** (0.03)</td>
<td>0.059**</td>
</tr>
<tr>
<td>B: Genotype</td>
<td>6</td>
<td>365512**</td>
<td>664.85 (38.5)</td>
<td>378.21** (63.8)</td>
<td>0.269 (28.5)</td>
<td>1.538*</td>
</tr>
<tr>
<td>A*B</td>
<td>6</td>
<td>406051**</td>
<td>382.98 (22.2)</td>
<td>16.62** (2.8)</td>
<td>0.231* (24.51)</td>
<td>0.513**</td>
</tr>
<tr>
<td>Error</td>
<td>26</td>
<td>410085 (29.3)</td>
<td>503.71 (29.1)</td>
<td>153.34 (25.9)</td>
<td>0.359 (38.05)</td>
<td>1.622 (42.0)</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>1399520</td>
<td>1728.05</td>
<td>593.15</td>
<td>0.944</td>
<td>3.862</td>
</tr>
</tbody>
</table>

Table 1. Sum of squares of ANDEVA, and their significance and contribution to some components of oat yield.

Effect of nitrogen fertilization and of the genotype on the yield, and nitrogen and β-glucans content in oats.
Table 2. Average values of Yield, Number of grains, Individual weight of grains, Nitrogen in grain, and β-glucans content in 7 oat genotypes grown in 2 doses of N, under heavy weather conditions in Toluca, Mexico.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Genotype</th>
<th>Yield (g-m⁻²)</th>
<th>Number of grains (103 m⁻²)</th>
<th>Weight of one grain (mg)</th>
<th>Nitrogen in grain (g/100 g)</th>
<th>β-Glucans (g/100 g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avemex</td>
<td>632.25⁴⁺</td>
<td>16.9100⁴⁺</td>
<td>37.6¹⁺</td>
<td>2.15⁺</td>
<td>3.62⁺</td>
</tr>
<tr>
<td></td>
<td>Chihuahua</td>
<td>607.38⁺⁺⁺⁺</td>
<td>20.1214⁺⁺⁺⁺</td>
<td>30.33⁺⁺⁺⁺</td>
<td>1.93⁺⁺⁺⁺</td>
<td>3.26⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>Karma</td>
<td>764.25⁺⁺⁺⁺</td>
<td>26.3406⁺⁺⁺⁺</td>
<td>28.92⁺⁺⁺⁺</td>
<td>1.99⁺⁺⁺⁺</td>
<td>3.45⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>Menonita</td>
<td>561.63⁺⁺⁺⁺</td>
<td>18.5413⁺⁺⁺⁺</td>
<td>30.26⁺⁺⁺⁺</td>
<td>2.15⁺⁺⁺⁺</td>
<td>3.47⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>Obsidiana</td>
<td>675.92⁺⁺⁺⁺</td>
<td>20.6491⁺⁺⁺⁺</td>
<td>32.86⁺⁺⁺⁺</td>
<td>1.98⁺⁺⁺⁺</td>
<td>3.93⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>Paramo</td>
<td>568.71⁺⁺⁺⁺</td>
<td>15.5999⁺⁺⁺⁺</td>
<td>36.03⁺⁺⁺⁺</td>
<td>2.02⁺⁺⁺⁺</td>
<td>3.54⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>Turquesa</td>
<td>828.21⁺⁺⁺⁺</td>
<td>26.4991⁺⁺⁺⁺</td>
<td>31.14⁺⁺⁺⁺</td>
<td>1.98⁺⁺⁺⁺</td>
<td>3.60⁺⁺⁺⁺</td>
</tr>
<tr>
<td></td>
<td>DMS 95%</td>
<td>149.04</td>
<td>5.22</td>
<td>2.88</td>
<td>0.14</td>
<td>0.30</td>
</tr>
</tbody>
</table>

| Doses of N (kg-ha⁻¹) | 60        | 592.95⁺⁺⁺⁺    | 18.8805⁺⁺⁺⁺              | 31.68⁺⁺⁺⁺                | 2.03⁺⁺⁺⁺                   | 3.59⁺⁺⁺⁺               |
|                     | 120       | 732.29⁺⁺⁺⁺    | 22.4513⁺⁺⁺⁺              | 33.22⁺⁺⁺⁺                | 2.03⁺⁺⁺⁺                   | 3.52⁺⁺⁺⁺               |
|                     | LSD 95%   | 79.67          | 2.79                      | 1.54                     | 0.07                       | 0.16                   |

**LSD**: Least significant difference at confidence: 95%

Figure 1. Correlations between variables associated to yield and composition of oats' grains.

As Figure 2 shows, the response to the N dose was different according to the oat genotype. The interaction was significant in the estimation of yield, number of grains, and nitrogen in grain. The genotype Turquesa, followed by Chihuahua, was the one that best responded to fertilization because it produced the highest yield and number of grains. This effect was not significant in the remaining genotypes. When analyzing nitrogen in grain, genotypes Obsidiana and Turquesa presented a more noticeable response, where fertilization played a beneficial role. In Chihuahua, we observed a decrease in this component of the grain protein, associated with the increase in the dose of the fertilizer.

The non-significance of the effect of the dose of N on the synthesis of β-glucans in the oat grain (Table 1) is explained by the interaction N-dose*genotype (Figure 2). Figure 2e shows that in 4 genotypes (Chihuahua, Avemex, Obsidiana-
Figura 2. Interaction Genotype*Dose of N in oats.
na, and Karma) the dose of N caused a significant reduction in this prebiotic component of the grain.

In contrast, genotypes Turquesa (in which the yield responded in a highly significant way to nitrogen fertilization), Paramo, and Menonita presented similar increases in this compound. These results suggest that, depending on the cultivated genotypes, nitrogen fertilization promotes the increase of the β-glucans content in the oat’s grain or, on the contrary, it reduces it.

On the one hand, this is consistent with the results obtained by Güler (2003), who found that high levels of N significantly increased the content of β-glucans in barley, and by Noworolnik et al. (2014), who also reported that variations in the content of insoluble fiber in barley did not depend on nitrogen fertilization. On the other hand, Welch et al. (1991) found high variability in the effect of nitrogen fertilization on the concentration of β-glucans in the grain or modern and wild oats crops. In addition, they also did not observe, as in the case of this study, a correlation between grain protein and these polysaccharide compounds.

Relationship between tillers and main stem

Figure 3 represents the fraction of the total number of obtained grains per square meter in the tillers when compared to the fraction of grain yield in this plant structure (Figure 3a) and to the relationship between the weight of the grain from the tillers and the weight of the grains from the main stem (Figure 3b).

This figure also shows with arrows the effect of the dose of N on these relationships. The distribution of the components that define the yield in tillers and in the main stem varied between genotypes and with fertilization. Genotypes Turquesa, Obsidiana, Chihuahua and, to a lesser extent, Páramo presented a significant increase in the number of grains in the tillers with the dose of N, which implies that the fraction corresponding to the main stem decreased.

At the same time, the fraction of the yield corresponding to the tillers also increased, especially in Páramo, Turquesa, and Obsidiana (Figure 3a). It was also evident genotypes Karma, Menonita, and Avmex presented a very small response to nitrogen fertilization when compared to the other genotypes. These results coincide with those reported by Peltonen-Sainio and Järvinen (1995) who found that dwarf oats, tillers contributed to 27% of the grain yield.

Figure 3b indicates that when the grain fraction in tillers was low, the size of the grains in tillers was relatively smaller than those of the main stems. On the contrary, when that fraction tended to one, the weight of the grains in the tillers tended to increase. According to this figure, no values of this ratio equal to or greater than one were observed, which implies that the grains in tillers were lighter than those of the main stem.

It is important to note how the increase in the yield of Páramo with the dose of N was explained by the increase in the weight of the grains from the tillers. But in the case of Karma and Menonita, these genotypes did not respond or, on the contrary, the nitrogen fertilization increased the weight of the grains in the main stem.

These results suggest that oats tillering plays an important role in grain yield and nitrogen fertilization plays a fundamental role in these structures, probably due to its effect on their survival. However, the genotype component must be taken into account since tillering capacity is variable in this plant species. Also, it is important to note that Peltonen-Sainio and Järvinen (1995) concluded that high planting densities reduced tillering in oats, although they shorten the length of the main stem and show reductions in yield and its components.

CONCLUSIONS

According to the results obtained in this work, the differences in grain yield were mainly due to the genotype and, secondarily, to nitrogen fertilization. Both factors manifested significantly in the number of grains per m2.

The tillering capacity of oats allowed the tillers to account for most of the yield, and tillers were the organs that concentrated the effect of fertilization. The data obtained allowed us to demonstrate that oats (like other winter cereals) responds to N through the number of grains per m2. The response to fertilization in the content of β-glucans and nitrogen in the grain was not uniform among cultivars, and the effect of the interaction prevailed over that of the dose of N.

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REFERENCES


**Figure 3.** Fraction of the total grains obtained (per square meter) corresponding to tillers vs the fraction of the grain yield, typical of this structure of the plant (A), and vs the relationship between the weight of the grain from tillers and the weight of grain from the main stem (B).


