Article

Response of oat genotypes to infection by *Puccinia graminis* f. sp. *Avenae* in the high valleys of Mexico

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Abstract

Stem rust of oats, caused by *Puccinia graminis* f. sp. *avenae*, has increased its incidence and severity significantly in temporal plots distributed in the high valleys of Mexico. The objective of this study was to determine the response of 28 temperate oats genotypes to *Puccinia graminis* f. sp. *avenae* infection under field conditions, during the spring-summer 2013 and 2014 cycles. A completely randomized experimental design was used with four replications, and an arrangement of treatments in divided plots. The large plots were five planting dates and the small plots consisted of the 28 oat genotypes. The response variable was the percentage of stem rust damage in each of the genotypes, from the beginning of the disease to the end of each crop cycle. The variety of oats most resistant to stem rust was Diamond R-31, followed by Nodaway, AB177 and Teporaca. While the most susceptible varieties were Guelatao, Tulancingo and Ópalo. Likewise, planting dates 1 (June 23) and 2 (June 30) presented the lowest percentages of disease severity. Additionally, it was observed that the oat grain yield decreases severely in the presence of stem rust and without being controlled with any fungicide.

Keywords: *Avena sativa*, *Puccinia graminis* f. sp. *avenae*, fungicide, severity.

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Introduction

In Mexico, the area planted with oats (*Avena sativa* L.) has increased, although not enough is produced to supply the demands. The area planted with oats in Mexico in 2014 was 833 221 ha distributed in all the agricultural areas of the country (SIAP, 2015). Due to its adaptation to diverse environmental conditions, oats are considered an alternative crop in the high valleys of Mexico and in the semi-arid north-central region, particularly when the onset of the rainy season is delayed or the temperature is low, as planting Traditional crops, such as maize (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.), are put at risk (Villaseñor-Mir *et al*., 2003).

It is important to indicate that oats are an excellent option for the productive reconversion of low productivity lands with livestock aptitude in the regions in which the growing season is short and which are currently used in the production of traditional crops; however, for this crop to become widely used, it is necessary to have varieties suitable for the production of forage and grain with a set of agronomic and phytopathological attributes that minimize the negative effect of the incidence of stem rust (*Puccinia graminis* f. sp. *avenae*) and crown rust (*Puccinia coronata* var. *avenae*), as well as the occurrence of early frosts and intermittent drought (Villaseñor-Mir *et al*., 2003).

Worldwide, *Puccinia graminis* f. sp. *avenae* is the most destructive pathogen of oat cultivation, since it has caused severe epidemics in the main producing areas of South Africa (van Niekerk *et al*., 2001), Australia (Keiper *et al*., 2006; Hake *et al.*, 2008), Canada (Gold-Steinberg *et al*., 2005; Michell and Fetch, 2011), Sweden (Berlin *et al*., 2013) and China (Li *et al*., 2015). In Mexico, stem rust is the disease that most affects the production of oats, since it can diminish the yield up to 50%, because this pathogen affects from the stage of seedling until the filling of the grain, besides they use highly susceptible varieties (Leyva-Mir *et al*., 2013).

Likewise, the disease is especially important in spring-summer cycles in central Mexico (Leyva-Mir *et al*., 2013; García-León *et al*., 2015), which is why these environments may not be suitable for seed production and autumn-winter production in the Mexican Bajío becomes an option (Bobadilla-Meléndez *et al*., 2013).

The genetic improvement of oats in Mexico began in 1960 and since then the Valley of Mexico Experimental Field (CEVAMEX) of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP) has been the main station where the release of varieties. To date, it has made more than 30 varieties available to farmers, which have been the basis of national production (Villaseñor-Mir *et al*., 2009). Likewise, the selection criteria of the genetic improvement program of oats in Mexico, have focused mainly on, the greater production of grain, resistance to stem rust, good response in conditions of temporary limitation (drought) and greater tolerance to lodging (Jiménez-González, 1992; Villaseñor-Mir *et al*., 2009).

The sowing season defines not only the yield and other agronomic aspects of the crop, but also the expression of some attributes of quality, presence or absence of harmful organisms, for which the one that allows obtaining the best yield and quality must be carefully selected (Forsberg and Reeves, 1995). In addition, the sowing season is associated with the duration of the day, solar radiation and temperature, so it is important to identify the most suitable areas for the growth, development and production of the crop. For this reason, the optimal date should be determined in each locality, which will depend on the climate, the incidence of pests and diseases, as well as other factors (Bobadilla-Meléndez *et al*., 2013).

The objective of this study was to determine the response of 28 temperate oats genotypes from the high valleys of Mexico to *Puccinia graminis* f. sp. *avenae* infection, in five sowing dates during the Spring-Summer 2013 and 2014 cycles.

Materials and methods

Study site

The experiment was carried out during the spring-summer (S-S) cycles of 2013 and 2014, in the Valley of Mexico Experimental Field (CEVAMEX), belonging to the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), located in Chapingo, State of Mexico, Mexico at 19° 29' north latitude and 99° 53' west longitude, at a height of 2250 meters above sea level, with an average annual rainfall of 640 mm and 15 °C average annual temperature (García, 1981).

Establishment of the experiment

Twenty-eight varieties of oats belonging to the CEVAMEX collection were evaluated under temporary conditions on five sowing dates and with a spacing of 07 days between each date. The first sowing date was June 23, the second on June 30, the third on July 7, the fourth on July 14 and the fifth was on July 21, of the S-S 2013 and 2014 cycles. The preparation of the land consisted of a fallow and a harrowing step with the purpose that the ground would remain soft and thus obtain a uniform germination. Seeding was done manually, at a density of 60 kg ha^{-1}. The presence of the pathogen was allowed to occur naturally, because the environmental conditions where the experiment was carried out are highly favorable for the development of the disease.

We used a completely randomized experimental design with four replications, and an array of treatments in divided plots. The large plots were the five sowing dates and the small plots were constituted by the 28 varieties. Twenty-eight experimental units were established, consisting of four rows with 5 m long × 0.3 m wide, and four repetitions were made of each.

The evaluations were carried out only in the two central rows of each experimental unit and the response variable was the percentage of damage caused by stem rust in each of the oat varieties from the time the disease was disease present until the end of each cycle of the culture. It is worth mentioning that when analyzing the data for a date, the experimental design was completely random; however, when all the dates were analyzed together, a design was chosen in divided plots (Montgomery, 2003).

The incidence and severity data were recorded in all varieties until the end of each cycle; the evaluations were every 8 days in 5 dates and in all varieties.

Variables evaluated

The disease was presented at the beginning of September 2013 and 2014, as of this date the data collection began. In each variety and in each sowing date, the severity was evaluated visually every 8 days until the commercial maturity of the culture using the modified scale of Peterson *et al*. (1948), which consists in taking values of disease severity from 01 to 100%.

On the other hand, the performance of each of the varieties on each date was evaluated. At the end of the 2013 and 2014 crop cycles, 10 stems were harvested at ground level for each variety and in each of the five planting dates. Later the samples were threshed to obtain the grain yield using an analytical scale, and in this way the grain yield of each of the varieties was compared on each date. In addition, the weight of 1 000 seeds without fungicidal treatment was compared against the weight of 1 000 seeds of the same varieties, but treated with the fungicide tebuconazole (Folicur®, Bayer) at a concentration of 2 mL of commercial product per L of water, sprinkling in total form to the plant during a date of the crop cycle.

Finally, the flowering days were determined by counting the days elapsed from sowing until the time when 50% of the spikelets of each variety reached flowering. While, the days to commercial maturity were obtained by counting the days elapsed from sowing until the moment when the pedicle of the panicle turned yellow.

Statistical analysis

The data obtained from the percentage of severity of all the samplings, in the different sowing dates and in all the varieties during each crop cycle, were adjusted to a model of progress of the epidemic of exponential type with the Curve Expert 2® program. Likewise, with this model an area under the curve of the progress of the disease (ABCPE) was calculated as an integrative parameter using Scientific Work Place 5®. A T test was carried out to determine if there were differences in the ABCPE values of the different varieties in the 5 sowing dates and in both cycles. With the statistical package SAS (Version 9.1) an analysis of variance (Anova) and mean comparisons were performed using the minimum significant difference test (DMS) (α= 0.05), by date (completely random) and an analysis of variance and comparisons of means between dates (divided plots).

Results and discussion

Variety response to infection

The disease was presented at the beginning of September in both years, time when the first pustules were observed on leaves and stems of the susceptible varieties. On the first date the first symptoms were observed and it was disseminated to the other dates. According to the T test, the ABCPE of the two cycles evaluated did not have significant differences; in such a way that the average obtained from the two cycles was used for the analyzes. The Anova carried out with all the varieties in the five sowing dates showed significant differences (p= 0.05).

When comparing means with DMS, it was observed that the variety with the lowest ABCPE in the five sowing dates was Diamante R-31 (Table 1), which coincided with Jiménez-González (1992), who described this variety as one of the most resistant to stem rust for own genes of resistance to different races. Also, the Nodaway, AB177 and Teporaca varieties were also resistant to the infection caused by *P. graminis* f. sp. *avenae*, coinciding with what was indicated by Salmeron-Zamora (2001), in the case of the Teporaca variety.

Table 1. Values of area under the disease progress curve (ABCPE) in 28 oat genotypes in 5 planting dates during the Spring-Summer 2013 and 2014 cycles.

Variety	Date 1	Date 2	Date 3	Date 4	Date 5	Mean (combined)
Guelatao	2390.5	2600.5	2776.7	2758	2450	2595.1 a
Tulancingo	2397.5	2671.7	2473.3	2676.3	2467.5	2537.3 ab
Ópalo	2327.5	2460.5	2444.2	2438.3	2397.5	2413.6 abc
Cuauhtemoc	1976.3	1814.2	2279.7	2310	2390.5	2154 abcd
Texas	1629.8	2117.5	2437.2	2362.5	1820	2073 abcde
Pampas	2100	2000.8	1925	2024.2	2310	2072 abcde
Perla	1855	1853.8	2251.7	1894.7	2135	1998 bcdef
Cevamex	1918	2172.3	1919.2	1890	2047.5	1989.4 bcdefg
Huamantla	1890	1556.3	1953	2191	1888.8	1896 cdefgh
Paramo	1935.5	1505	1761.7	2013.7	1731.3	1789.4 defghi
Chihuahua	1830.5	1369.7	2123.3	1534.2	2088.3	1789 defghi
Gema	1697.5	2173.5	1895.8	1575	1417.5	1752 defghi
Babicora	1260	1258.8	1545.8	1890	2286.7	1648 defghij
Obsidiana	1620.5	1235.5	1685.8	1709.2	1843.3	1619 defghij
Tarahumara	1423.3	1305.5	1806	1627.5	1468.8	1526.2 efghijk
Karma	1277.5	1433.8	1370.8	1790.8	1585.5	1491.7 fghijk
Raramuri	1155	1655.5	1674.2	1715	1120	1464 fghijk
Bachiniva	1165.5	1334.7	1510.8	1295	1884.2	1438 ghijkl
Cusihuiriachi	1298.5	1159.7	1265.8	1388.3	1872.5	1397 hijkl
Putnam61	1225	1451.3	1761.6	1295	1242.5	1395.1 hijkl
Papigochi	1018.5	1387.2	1036	1540	1709.2	1338 ijklm
Turquesa	1114.2	1398.8	1212.2	1155	1289.2	1233.9 ijklm
Juchitepec	990.5	990.5	1281	1556.3	1136.3	1191 jklm
Menonita	1008	1194.7	1012.7	1061.7	1610	1177 jklm
Teporaca	1018.5	808.5	850.5	1193.5	1060.5	986.3 klm
AB177	791	779.3	826	1057	980	886.7 l mn
Nodaway	598.5	780.5	784	955.5	940.3	811.8 mn
Diamante R-31	413	402.5	434	434	423.5	421.4 n

*= Means with the same letter in each column are statistically equal (α= 0.05).

The importance of knowing varieties of oats that can serve as a source of resistance to stem rust is key to breeding programs, in addition to the use of resistant varieties is the most used strategy for the control of this disease (Mariscal-Amaro *et al*., 2010; Leyva-Mir *et al*., 2013).

The varieties that were most susceptible and had a higher ABCPE were Guelatao, Tulancingo and Opalo. In the case of the Tulancingo variety, it was deferred with that indicated by Castro-Melendrez and Jiménez-González (1981), who considered it to be moderately resistant to stem rust, however, in this investigation it was one of the most susceptible varieties during the two cycles evaluated. Meanwhile, in the case of the Guelatao and Ópalo varieties, our results coincided with those reported by Jimenez-González (1992), who reported these varieties as highly susceptible to stem rust.

Comparison between planting dates

According to the Anova, in the five sowing dates (p= 0.05), there were highly significant differences. Likewise, the comparison of means with DMS (Table 2) indicated that planting dates 1 and 2 had the lowest values of ABCPE (severity of stem rust), coinciding with Tovar (1974), who assured that there is an effect of different dates of sowing on the yield before the attack of stem rust on varieties of oats.

Table 2. Area under the disease progress curve (ABCPE) for each of the 5 sowing dates of 28 oat genotypes during the Spring-Summer 2013 and 2014 cycles.

Planting dates	ABCPE
Date 5 (July 21)	1657.58 a*
Date 4 (July 14)	1643.08 a
Date 3 (July 7)	1619.13 a
Date 2 (June 30)	1471.38 b
Date 1 (June 23)	1431.63 b

*= Means with the same letter in each column are statistically equal (DMS, 0.05).

When analyzing the interactions between each one of the varieties and in all planting dates, it was observed that, in most varieties of oats, dates 1 and 2 could be considered as the best, since they obtained lower ABCPE. It should be noted that some exceptions were found, such as the Gema and Cevamex varieties, where the best sowing date was date 4, as well as the Pampas variety, in which the best sowing date was 3, while in the Raramuri variety was date 5. This shows that there are varieties that are more susceptible to stem rust on certain dates and for this reason the yields of a variety on different dates will depend on the environmental conditions that the disease has to damage the cultivation and decrease the yield, as mentioned by Leyva-Mir *et al.* (2004).

The results generated in this study can be considered to make decisions about the optimal time to carry out the sowing and thus obtain a higher yield of these 28 oat genotypes under rainfed conditions in the High Valleys of Mexico. Similarly, Bobadilla-Meléndez *et al.* (2013) verified that planting dates, varieties and densities have a direct influence on the productivity and physical quality of the seed.

Percentage of damage in the last evaluation

Oat varieties evaluated showed an increase in severity from the first to the fourth planting date. Stem rust caused lower percentage of damage in earlier dates compared to later dates that is to say to earlier dates, the oat varieties can escape the attack of rust (Table 3). The above, agrees with the work of Smith (1992), who found that one of the best methods of control of stem rust in oats is to choose the right time to sow and avoid late varieties.

Table 3. Percentage of disease severity caused by *Puccinia graminis* f. sp. *avenae* in each of the 28 oat genotypes in the 5 sowing dates evaluated during the Spring-Summer 2013 and 2014 cycles.

Variety	Severity (%)					Average (%)
	Date 1	Date 2	Date 3	Date 4	Date 5	
Texas	61	77	88	90	77	78.6
Nodaway	25	29	28	40	38	32
AB177	30	28	30	38	40	33.2
Putnam61	65	55	80	67	56	64.6
Perla	70	65	85	83	85	77.6
Ópalo	90	85	90	92	90	89.4
Chihuahua	66	50	78	63	80	67.4
Cuauhtémoc	71	67	83	85	88	78.8
Guelatao	93	83	90	93	95	90.8
Tulancingo	90	87	90	88	90	89
Juchitepec	40	38	45	60	45	45.6
Huamantla	75	56	66	78	68	68.6
Diamante R-31	15	15	16	16	15	15.4
Tarahumara	53	48	55	72	51	55.8
Paramo	75	55	65	74	61	66
Gema	80	70	85	80	65	76
Babicora	55	51	65	75	85	66.2
Cusihuiriachi	45	46	55	68	82	59.2
Papigochi	37	51	40	70	65	52.6
Raramuri	55	58	70	80	50	62.6
Karma	50	51	52	80	63	59.2
Cevamex	78	76	73	85	88	80
Menonita	43	46	40	50	70	49.8
Bachiniva	48	53	58	67	77	60.6
Obsidiana	63	48	68	82	77	67.6
Turquesa	50	54	53	50	65	54.4
Pampas	75	73	77	80	85	78
Teporaca	35	30	33	40	43	36.2

The variation in the percentage of damage between varieties shows that each genotype contains genes that give it resistance against the attack of stem rust. For this case we can point out that the Guelatao, Tulancingo, Ópalo, Cuauhtémoc, Texas, Pampas and Perla varieties were the varieties of oats most susceptible to stem rust. While the less susceptible varieties were Diamante R-31, Nodaway, AB177 and Teporaca.

It is important to point out that the majority of oat varieties cultivated in Mexico are susceptible to stem rust, coinciding with Li *et al*. (2015), who mentioned that the majority of the varieties produced in China and worldwide are susceptible to the pathogen and that they vary widely in the level of response to the disease.

Fungicide treatment

The comparison of the average weight of the 5 sowing dates without fungicide and the date with fungicide of the 1 000 seeds indicated that the yield of grain in the presence of stem rust in all oat varieties tends to decrease when the disease is present, because the fungus disturbs the normal source-demand relationships of the plant by sucking the assimilated ones that go to the developing seeds (Table 4). Smith (1992) found that when *P*. *graminis* f. sp. *avenae* disturbs the epidermis, the plant may dry up, so that the flow of sap does not reach the spike and as a result prematurely matures forming less seed and less weight of the grain.

Table 4. Average weight of 1 000 seeds of 28 varieties of oats harvested during 5 dates of the spring-summer 2013 and 2014 cycles, with and without fungicide treatment.

Varieties	With fungicide 1 date	Without fungicide x̄ of 5 dates	(%) of loss
Texas	3.3	2.36	28
Nodaway	4.5	3.9	13
Ab-177	4.6	3.96	14
Putnam 61	4.2	3.12	26
Perla	3.4	2.38	30
Ópalo	3.1	1.5	52
Chihuahua	4	3.36	16
Cuauhtémoc	4	2.74	32
Guelatao	3.5	2.78	21
Tulancingo	3.2	2.56	20
Juchitepec	4	3.5	13
Huamantla	3	2.42	19
Diamante R-31	3.5	3.32	5
Tarahumara	3.2	2.6	19
Paramo	5	3.74	25
Gema	4	2.9	28
Babicora	3.4	3.12	8
Cusihuiriachi	4.1	3.8	7
Papigoche	4.4	3.74	15

Varieties	With fungicide 1 date	Without fungicide x̄ of 5 dates	(%) of loss
Raramuri	4.4	3.2	27
Karma	4.1	3.16	23
Cevamex	4.8	3.18	34
Menonita	3.7	3.04	18
Bachiniva	4.4	3.18	28
Obsidiana	4.1	3.24	21
Turquesa	4.4	3.4	23
Pampas	4.3	3.16	27
Teporaca	4.1	3.52	14

In the case of the Opalo variety, which is highly susceptible to infection by *P*. *graminis* f. sp. *avenae*, a 52% reduction in yield was found compared to the treatment with fungicide application. This coincided with May *et al*. (2014), who recommended that the application of fungicide for the control of rusts in oats, should only be carried out in highly susceptible varieties. According to Murray (2007), the time of application of the fungicides is critical for the success in the control of the disease. Also, the treatments can be applied as treatments to the seed, mixed with fertilizers or sprinkled on the crop.

It should be mentioned that the percentage of loss of yield in some varieties of oats did not coincide with the percentage of susceptibility or damage caused by stem rust, such is the case of the Guelatao variety, which was one of the varieties most susceptible to rust. Stem, although it was not the most lost performance. The above coincided with Epstein *et al*. (1988) and Leyva-Mir *et al.* (2004), who mentioned that there are oat genotypes that are highly susceptible to infection by stem rust but that show an acceptable yield because of their high yield potential.

Days to flowering

The varieties with less days to flowering were Diamante R-31 (resistant to rust) and Parámo. This is important, because the earlier a variety is, in addition to choosing an appropriate planting time, it can reduce the risk of loss from stem rust (Smith, 1992). For this reason, the variety Diamante R-31 can be an alternative as a source of resistance if you want to make a cross with some variety of yielding oats but susceptible to stem rust.

The Opalo variety showed to be one of the later varieties in days to flowering and one of the most susceptible to stem rust, which indicates that this type of varieties is more difficult to escape from the attack of this phytopathogenic fungus, even and when the sowing dates are advanced (Figure 1). The flowering days allow us to choose the earliest varieties when the purpose of the production of these varieties is for forage, but when they are required for grain you need to know the days to maturity and thus calculate the planting date in which you can get rid of the attack of stem rust.

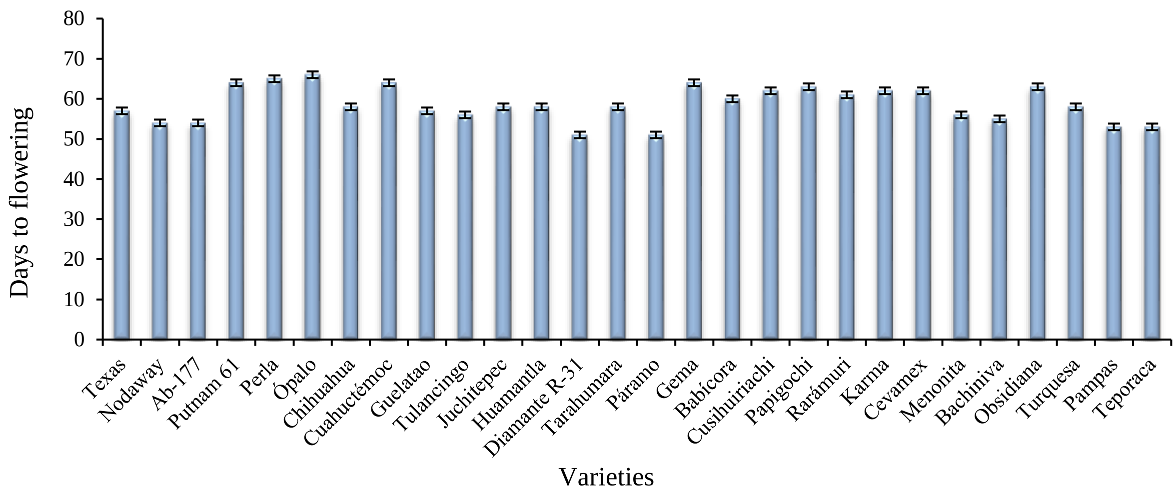

Figure 1. Days to flowering of 28 varieties of oats planted under storm in the high valleys of Mexico during the spring-summer 2013 and 2014 cycles.

Days to maturity

It was observed that the variety Diamante R-31 was one of the varieties most resistant to rust, although its cycle was later compared to the earliest varieties (Figure 2), which behaved as highly susceptible, which indicated that the best way to control this disease is by generating oat varieties that contain horizontal resistance. Similarly, Cornide *et al.* (1993) mentioned that horizontal resistance is genetically polygenic and has as its main characteristic the lack of specificity before a series of races, which is why it delays the development of an epidemic or new race.

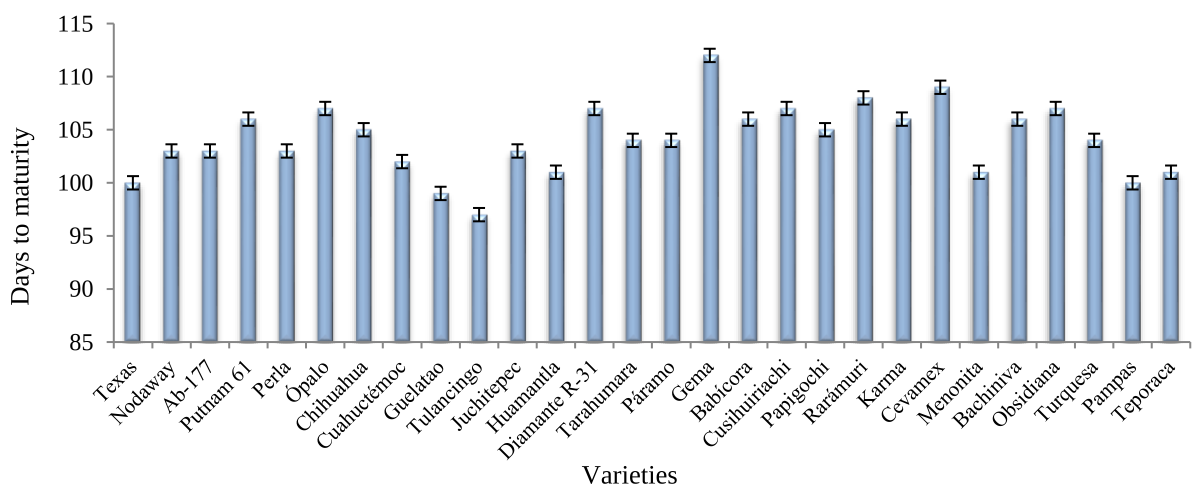

Figure 2. Days to maturity of 28 varieties of oats planted under rainy season in the high valleys of Mexico during the spring-summer 2013 and 2014 cycles.

On the other hand, in the susceptible and early varieties, the yields will decrease unless they are sown in times where they manage to escape the attack of the rust, which will be the periods when this disease does not have the adequate conditions to develop and consequently severely damage the crop.

Conclusions

The best sowing dates for temporary oats were the dates of June 23 and 30, 2013 and 2014, since they were the dates with the lowest severity of stem rust caused by *Puccinia graminis* f. sp. *avenae*.

The variety of oats most resistant to stem rust in the 5 sowing dates evaluated was Diamante R-31, which is also early, so it is inferred that it can have minor genes of durable resistance. While, the varieties most susceptible to stem rust in the 5 planting dates were the varieties Tulancingo, Guelatao and Ópalo.

On the other hand, it was observed that the yield of oats in the presence of stem rust and without being controlled with any fungicide, severely decreases grain yield.

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