Fungal Biology 123 (2019) 481–488

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Productivity and flavor of diverse genotypes of *Ustilago maydis* “cuitlacoche” for human consumption

Vladimir Castañeda de León [a,*,1], Daniel Martínez-Carrera [a], Porfirio Morales [a], Mercedes Sobal [a], Abel Gil-Muñoz [a], Patricia Severiano-Pérez [b], Hermilo Leal-Lara [b]

[a] *Colegio de Postgraduados (CP), Campus Puebla, Biotecnología de Hongos Comestibles, Funcionales y Medicinales & Recursos Fitogenéticos, Apartado Postal 129, 72001, Puebla, Mexico*

[b] *Universidad Nacional Autónoma de México (UNAM), Departamento de Alimentos y Biotecnología, Facultad de Química, Ciudad Universitaria, Coyoacán, CDMX, 04510, Mexico*

ARTICLE INFO

Article history:
Received 10 September 2018
Accepted 10 April 2019
Available online 16 April 2019

Corresponding Editor: Angel Medina

Keywords:
Controlled production
Edible fungi
Infected galls
Maize
Sensory evaluation
Virulence

ABSTRACT

Maize plants infected by *Ustilago maydis* develop galls known as “cuitlacoche”, a food product appreciated in the Mexican gastronomy. The virulence of different *U. maydis* isolates was assessed, as well as the development of the infection on one commercial maize variety. Sporidia were isolated of wild galls collected in Mexico. Sexual compatibility patterns were determined using the Fuzz reaction, showing a 1:1:1:1 segregation of mating type specificities. Ten *U. maydis* compatible strains were selected on the basis of their virulence, namely: four wild-type compatible sporidia, one multi-teliosporic strain, two hybrids between wild-type and tester strains, and three tester strains. Maize plants of a commercial hybrid (Tornado XR™) were inoculated with these strains of *U. maydis*, using a randomized complete block experimental design. Phenological and phenotypic characteristics of plants, as well as production, quality and sensory attributes of the resulting galls, were evaluated. Greater yields of galls were recorded in tester strains (incidence >90 %, severity >80 %, productivity >12 t/ha), a hybrid strain (EM1-6 × FB1) [incidence 82.6 %, severity 51.8 %, productivity 5.6 t/ha] and a wild-type strain (EM4-10 × EM2-4) [incidence 68.2 %, severity 44.0 %, productivity 4.8 t/ha]. Wild-type strains showed better flavor, characterized by less bitterness and acidity, but prevailing sweet, umami and maize flavor.

© 2019 British Mycological Society. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Three types of inocula have been used to induce artificial infection of maize ears (*Zea mays* L.) by *Ustilago maydis* (DC.) Corda, in order to produce galls: 1) Teliospores, 2) Mixed sporidia derived from multi-teliosporic cultures, and 3) Compatible haploid sporidia (yeast-like form). Inoculation with teliospores resembles natural infection, which occurs randomly at a rate lower than 10 %. In this case, suitable environmental and nutrimental conditions are needed for germination of teliospores to produce sporidia, then recognition and mating of genetically compatible sporidia has to take place, leading to the formation of infectious dikaryotic hyphae, followed by invasion and mycelial proliferation in meristematic plant tissues, and finally, development of galls full of teliospores (Castañeda de León et al., 2016). Furthermore, maize infection only take place during a short period of time, just before fertilization of maize ovules by pollen grains (Snetselaar et al., 2001; Pataky and Chandler, 2003) or stigma senescence (Bassetti and Westgate, 1993a,b; Pataky and Richter, 2007). If mixed sporidia derived from multi-teliosporic cultures is used as inoculum, infection efficiency decreases significantly due to genetic variability. In general, inocula from teliospores and mixed sporidia show unpredictable infections of maize ears, including low incidence and severity rates. By contrast, the use of inoculum containing two compatible haploid isolates (*a1 bx* × *a2 by*) of *U. maydis* leads to predictable maize ear infection, as well as high rates of incidence and severity (du Toit and Pataky, 1999; Pataky and Chandler, 2003).

The existence of resistant and susceptible maize varieties to the natural infection of *U. maydis* was documented by Pataky et al. (1995). The susceptibility of maize varieties to artificial infection, however, is also influenced by different variables of the plant

* Corresponding author.
E-mail address: vcastanedadeleon@yahoo.com (V. Castañeda de León).
[1] Doctoral scholarship (no. 49140) from the National Council of Science and Technology (CONACYT) at CP, Campus Puebla, Mexico.

https://doi.org/10.1016/j.funbio.2019.04.003
1878-6146/© 2019 British Mycological Society. Published by Elsevier Ltd. All rights reserved.

pathosystem and the inoculation technique, such as plant vigor, pollination rate, development of the stigma, inoculum quantity and quality, weather conditions, the method of inoculation and the skills of persons doing inoculations (Pataky, 1991; Pope and McCarter, 1992a, b; Zimmerman and Pataky, 1992; du Toit and Pataky, 1999). Overall studies have demonstrated that the artificial inoculation of maize ears by *U. maydis* is a complex and multifactorial process and, accordingly, important aspects of the plant pathosystem have not yet been approached. In this study, we carried out the controlled production of “cuitlacoche” in order to produce edible galls for human consumption. Different strains of *U. maydis* were used to infect maize ears in the State of Mexico, Mexico. Compatible sporidia were isolated from native genetic resources (wild-type) of *U. maydis* to inoculate a commercial maize hybrid, including reference strains. The virulence of strains was assessed, galls yields characterized and their quality studied by sensory evaluation.

2. Materials and methods

2.1. Strains and isolation of sporidia of U. maydis

Maize ears sowing natural smut galls, “cuitlacoche”, were obtained from the central region of Mexico, four from State of Mexico (EM) and two from State of Puebla (Pu). Haploid sporidial cultures were isolated from each gall sample by germination of a single teliospore. A multi-teliosporic strain was also obtained by germinating several teliospores according to Holliday (1974). All isolates were maintained at room temperature on sterile petri dishes containing bacteriological agar (18 g/L) plus malt extract (15 g/L). Fuzz reaction tests were performed on complete agar media with activated charcoal to determine mating-type specificities of haploid sporidia (Banuett and Herskowitz, 1989). Cultures of wild-type haploid sporidia were mated in all possible combinations and reaction patterns at the inter-crossings zone were observed for the presence of Fuzz phenotype, under the microscope (Zeiss, Germany) after 24 h, 48 h, and 72 h. *U. maydis* tester strains FB1 and FB2 were provided by F. Banuett (University of California U.S.A.), PK-*a1 b1* and PK-*a2 b2* by J. Pataky (University of Illinois U.S.A.) and CP-436 and CP-437 by D. Martínez-Carrera (CP, Campus Puebla, Mexico). All strains are deposited at Centre for Genetic Resources of Edible, Functional, and Medicinal Mushrooms (CP, Campus Puebla, Mexico).

2.2. Infective capacity of U. maydis strains under greenhouse conditions

EM and Pu wild-type haploid isolates were evaluated by inoculating plant seedlings of a native maize (*Z. mays*), “cacahuacintle” variety, under greenhouse conditions at the Faculty of Chemistry (UNAM). Maize seedlings were inoculated with compatible pairings of parental mating-type specificities and their recombinants, and compatible pairings of parental specificities and tester strains either FB1 (*a1 b1*) or FB2 (*a2 b2*). Inocula were prepared according to Pataky and Chandler (2003) in liquid media with 15 g/L malt extract. After 14 d emergence, maize seedlings were injected with 3 mL inoculum (>10^6 c.f.u./mL), using a hypodermic syringe, at 6 cm from the stem base of the plant (three repetitions). Strain virulence was evaluated according to the incidence of infection (infected seedling/inoculated seedling), followed by periodic visual inspection for the presence of galls with teliospores. Maize seedlings inoculated with tester strains and non-inoculated seedlings were used as positive and negative controls, respectively.

2.3. Virulence and productivity of U. maydis strains in a field plot

Field experiments were carried out at the community of San Andrés de Nicolás Bravo, in the Municipality of Malinalco, State of Mexico (18°46′12″ N, 99°27′20″ O; 1200 m altitude). Local climate is warm, sub-humid with summer and winter rains A(W), 34 °C maximum temperature, 24 °C average temperature, 14 °C minimum temperature, and the average annual precipitation is 1000 mm. The commercial maize hybrid Tornado XR™ (CERES Seeds, México) was planted on January 30, 2015. Plants were detasseled as soon as tassels came out. Two d after silks appeared, primary ears were inoculated with 3 mL inoculum through the silk channel, with a 5 mL automatic syringe (Henke-Sass Wolf, model HSW Eco-matic, Germany), connected to a plastic bottle (2.5 L) by a hose. Ten strains (treatments) were studied along with negative controls, using a randomized complete block experimental design. Three replicates were made per treatment. Each pair of compatible *U. maydis* strains, or a multi-teliosporic strain, was inoculated in 72 plants, which were distributed in two contiguous rows (5 m length by 0.50 m width). Each row had 18 bushes (two plants per bush), the distance between each bush was 30 cm. A total of 720 plants were inoculated per experimental replica. Twelve central maize bushes (24 maize ears) were assessed, as they represented the useful plot in each treatment of 72 plants. Phenological and phenotypic characteristics of maize plants were evaluated, including male and female flowering time, detasseling, inoculation and harvesting period, plant height, ear (maize-cob) insertion, and size of infected ears. The production of galls was evaluated measuring infection incidence (infected plants/inoculated plants), infection severity (5 %, 25 %, 50 %, 75 % and 100 %, coverage of galls in the ear), and average yield [galls (g)/ear harvested]. The weighted severity was calculated considering the distribution of severities among all inoculated plants, according to the following formula:

$$\text{Weighted severity} = \frac{(No.\,Plants\,100\%)\times 100 + (No.\,Plants\,75\%)\times 75 + (No.\,Plants\,50\%)\times 50 + (No.\,Plants\,25\%)\times 25 + (No.\,Plants\,5\%)\times 5}{Total\,number\,of\,plants}$$

Average yield (g) was determined by the total weight of galls produced in the useful plot, considering maize ears showing more than 50 % severity. The weighted yield per inoculated plant was calculated from the average yield, considering that the production of galls (g) is reduced according to the degree of severity, in the case of ears showing 50 %, 25 % and 5 % severity. Productivity (t/ha) was calculated considering 60,000 plants/ha. Characteristics associated to “cuitlacoche” quality, such as protection of the ears by husk leaves was rated from 1 to 5 for each ear, where: 1 = ear galls uncovered throughout the ear, 2 = 50 % of the ear galls covered, 3 = 70 % of the ear galls covered, 4 = 90 % of the ear galls covered, 5 = ear galls completely covered by husk leaves. Husk leaves were removed and quality of galls were rated from 1 to 5, where: 1 = ≤ 1.5 cm small galls, unsuitable for cuitlacoche, 2 = *ca.* 2.5 cm galls slightly larger than kernels of maize, but too small for high quality cuitlacoche, 3 = *ca*. 3.5 cm medium-sized galls, 4 = *ca*. 4.5 cm mixture of medium, and large-sized galls, and 5 = ≥ 6 cm predominantly large galls best suited for cuitlacoche (Castañeda de León and Leal Lara, 2012). Data were analyzed by a simple analysis of variance and the Tukey's multiple range tests ($\alpha < 0.01$) using the Statistical Analysis System (SAS, 2007) software. Temperature and relative humidity were recorded during experiments using a Vantage Pro2 Plus meteorological station, model 6152 (Davis Instruments, U.S.A.).

2.4. Sensory evaluation of "cuitlacoche"

A flash profile sensory analysis (Dairou and Sieffermann, 2002) of edible smut galls from *U. maydis* was performed at the Sensory Evaluation Laboratory, Faculty of Chemistry (UNAM). A group of 10 panelists used a 10 cm non-structured scale. Each panelist generated a list of terms to describe sensory attributes. Panelist discussed all terms and generated by means of consensus, a final list of descriptors for the quantification of attributes.

Samples were prepared before analysis. Fresh "cuitlacoche" was obtained from each strain studied. Maize ears full of gall were harvested, on average, 20 d after inoculation. Complete galls were then separated, selected, and placed in containers. Galls from every sample were disinfected in a solution [1 L H_2O + 8 drops Microdyn™ (Tavistock, Bahamas)] for 15 min, rinsed out with tap water, and drained. Thereafter, galls were homogeneously cooked in electric pan (Black&Decker™, U.S.A.) at 215 °C for 10 min.

Six cooked galls per sample were served on dishes labeled with random codes for sensory evaluation by each panelist. Samples were randomized. Water and neutral biscuits were given to panelist after each evaluation. The following taste attributes were studied: acid, bitter, astringent, sweet, maize, earthy, and umami. Data were processed using the Generalized Procrustes Analysis (GPA) (Dijksterhuis, 1996).

3. Results

3.1. Genotypes of U. maydis haploid strains isolated from wild galls

Haploid sporidial cultures were isolated from six different locations in central Mexico. After pairing sporidial cultures from each gall sample in all possible combinations, mating-type specificities were identified according to the Fuzz reaction. In all cases, a 1:1:1:1 segregation was obtained and confirmed by the χ^2 ($\alpha = 0.01$) test (Table 1).

Table 1
Ustilago maydis haploid strains isolated from wild galls collected from corn fields in central Mexico.

Wild Galls	*Ustilago maydis* haploid stranis (Nr)					x^2 for 1:1:1:1 distribution
	Total	Classification by mating type				
		a1b1	a2b2	a1b2	a2b1	
EM1	20	3	6	7	4	2.3
EM2	17	7	2	5	3	4.7
EM3	20	11	1	5	3	10.0
EM4	13	3	2	6	2	3.8
P1	14	2	3	3	6	2.7
P2	19	4	3	7	5	1.7

x^2 Values = 11.34 (a = 0.01). Higher x^2 values do not correspond to a 1:1:1:1 distribution.

3.2. Selection of U. maydis infective genotypes

Infection was observed after inoculation of maize seedlings with tester strains (FB, CP). Infection was also produced by most pairings of parental types of EM and Pu wild-type haploid isolates, and by pairings between parental types and tester strains FB1 or FB2. Chlorosis and red pigmentation were recorder 5–6 d after inoculation, whereas galls full of teliospores developed after 12–14 d. Virulence was evaluated in this greenhouse study and 10 strains showing the higher and more consistent incidence of infection were selected for further experiment in the field plot (Table 2).

3.3. Agronomic characterization, virulence and productivity of selected strains of U. maydis

Selected strains were used for the controlled production of "cuitlacoche". Weather conditions during development of the infection (1–14 d after inoculation) were a minimum temperature of 20 °C and a maximum temperature of 35 °C. These temperatures were higher than those recorded during the harvesting period (18–31 d after inoculation), showing a minimum of 18 °C and a maximum of 31 °C. Relative humidity fluctuated from 26 % to 77 % during development of the infection, increasing up to 95 % at harvesting (Fig. 1).

Statistical analysis of phenological and phenotypic characteristics of the Tornado XR™ maize hybrid, which was inoculated with ten selected strains of *U. maydis*, indicated no significant differences ($P < 0.01$) in variables studied during the complete production cycle. Gall maturation took place between days 18 and 23 after inoculation (average: 20 d) [Table 3]. Significant differences ($P < 0.01$) were recorded among selected strains of *U. maydis* in the production of galls. A highly significant correlation was found between incidence and weighted severity (r = 0.904, $P < 0.0001$) as well as between weighted severity and weighted yield (r = 0.956, $P < 0.0001$) for all strains. Tester strains PK (97 %), FB (93 %) and CP (91 %) presented greater incidence that wild-type isolates and one of the hybrids (Fig. 2). The weighted severity of tester strains was 82 % (PK), 84 % (FB) and 82 % (CP), which were greater (35 %–51 %) than those of the best performing wild-type and hybrid strains (Fig. 3). The weighted yields of tester strains were 187 g/plant (PK), 191 g/plant (FB) and 233 g/plant (CP), which were also superior than the average of wild-type (45 g/plant) and hybrid (77 g/plant) strains (Fig. 4). Thus, productivity of tester strains ranged from 11 to 14 t/ha, about 8 t/ha (on average) above wild-type and hybrid strains (Fig. 5). In the group of wild-type and hybrid strains, better results in production variables were recorder in the hybrid EM1-6 × FB1 (83 % incidence, 52 % weighted severity, 93 g/plant of weighted yield) and the wild-type strain EM4-10 × EM2-4 (68 %

Table 2
Ustilago maydis strains selected for infection of corn ears under field conditions.

Strain (Nr)	Pairings	Source
1	CP-436 × CP-437	CP (Tester strain)
2	FB1 × FB2	FB (Tester strain)
3	PK-a1b1 × PK-a2b2	PK (Tester strain)
4	EM1-6 × EM1-10	EM (Wild Gall)
5	EM2-4 × EM4-10	
6	EM2 multi-teliosporic	
7	P1-6 × P1-16	P (Wild Gall)
8	P2-17 × P2-23	
9	EM1-6 × FB1	Hybrids (Wild Gall × Tester strain)
10	EM3-6 × FB2	

All matings showed 100 % infection in corn seedlings after 14 d inoculation (by triplicate).
All matings were a1b1 × a2b2, except multi teliosporic strain Nr 6.

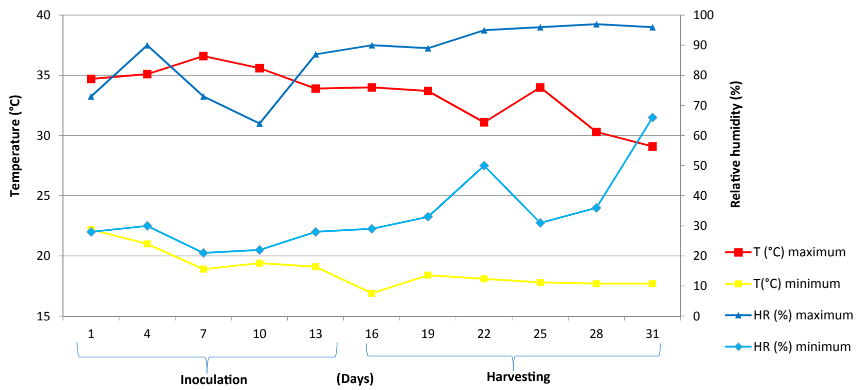

Fig. 1. Temperature and relative humidity (maximum and minimum) during the production cycle (May 2015) of ten selected strains of *Ustilago maydis* at San Andres de Nicolás Bravo, Mexico.

Table 3
Phenotypic and phenological characteristics of corn hybrid 'Tornado XR' ™, during the production cycle[a] of selected strains of *Ustilago maydis*.

Phenology event	Days after planting	
	Start	End
Tasseling and detasseling	85 ± 0.1	104 ± 0.2
Silking and inoculation time	90 ± 0.1	104 ± 0.2
Harvesting	107 ± 0.2	122 ± 0.1
	Days after inoculation	
Time for maturation of galls	20 ± 0.4	
Phenotypic characteristics (cm)		
Plant size	198 ± 31.4	
Ear insertion	96 ± 9.1	
Infected ear size	24 ± 1.3	

[a] Tests performed at San Andrés de Nicolás Bravo, México.

incidence, 44 % weighted severity, 80 g/plant of weighted yield) as can be seen in Figs. 2–4. By contrast, the multi-teliosporic strain, EM2, did not exhibit any advantages in production variables (Figs. 2–5). Most strains did not show significant statistical differences in terms of quality parameters, such as husk protection (average 3.8) and gall size (average 2.5). However, the wild-type strain Pu2-17 × Pu2-23 displayed better quality characteristics (husk protection: 4.3; gall size: 3.7) [Table 4].

3.4. Sensory evaluation of edible galls, "cuitlacoche"

Variability was recorded in the sensory space generated by seven flavor attributes (acid, bitter, astringent, sweet, maize, earthy, umami) of galls produced by ten *U. maydis* strains. As shown in Fig. 6, a 77.94 % variation was obtained for all data in two principal axes of the Generalized Procrustes Analysis (GPA), indicating a good consensus configuration of taste attributes. It was observed that all wild-type strains (EM1-6 × EM1-10, EM4-10 × EM2-4, EM2 multi-teliosporic, Pu1-6 × Pu1-16, Pu2-17 × Pu2-23), corresponding to numbers 4 to 8 in Table 2, are inversely correlated with axis one. The wild-type strains from State of Mexico (EM1-6 × EM1-10, EM4-10 × EM2-4, EM2 multi-teliosporic) had a dominant flavor of umami, and were positively correlated to axis two. The strains Pu1-6 × Pu1-16 and Pu2-17 × Pu2-23 from the State of Puebla showed sweetness and maize flavor, inversely correlating to axis two. In the

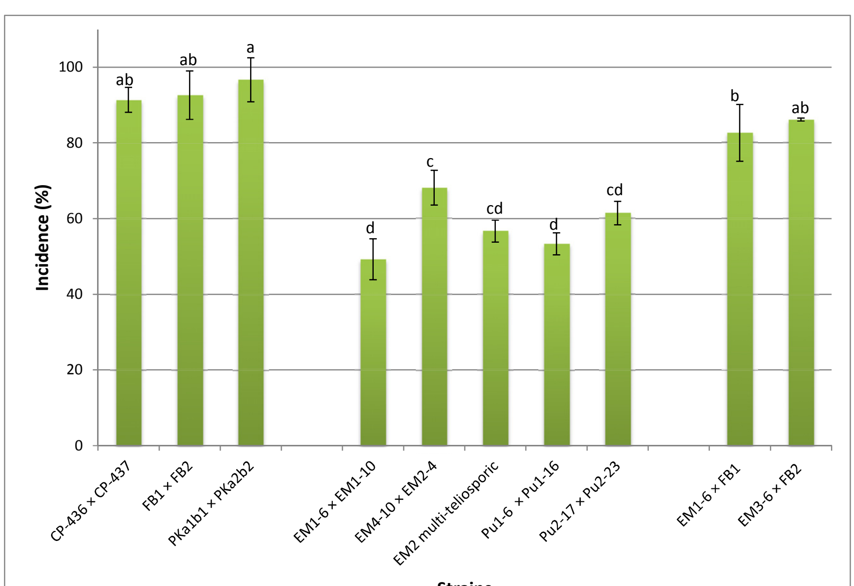

Fig. 2. Incidence of infection in 'Tornado XR' ™ corn ears inoculated with selected strains of *Ustilago maydis*. Different letters indicate statistically significant differences (Tukey, $p < 0.01$).

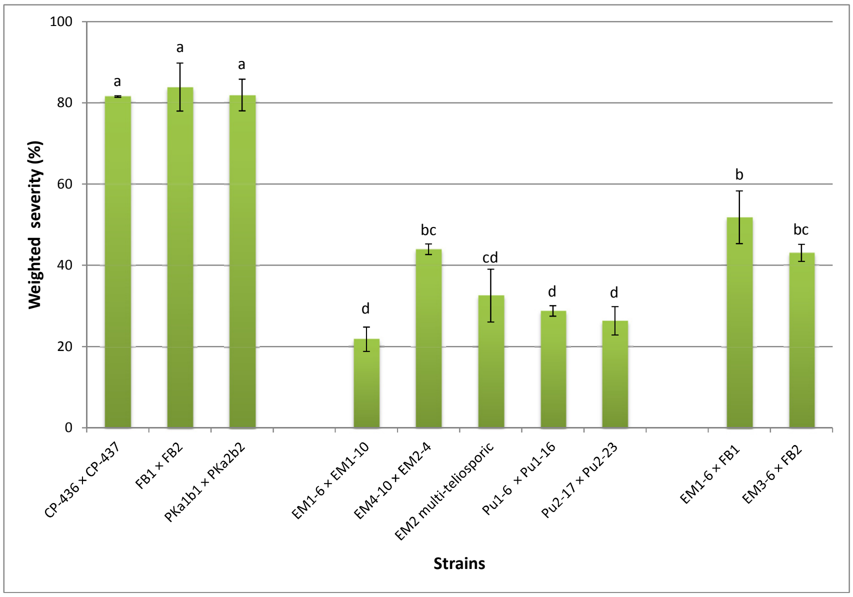

Fig. 3. Weighted severity of infection on 'Tornado XR' ™ corn ears inoculated with selected strains of *Ustilago maydis*. Different letters indicate statistically significant differences (Tukey, $p < 0.01$).

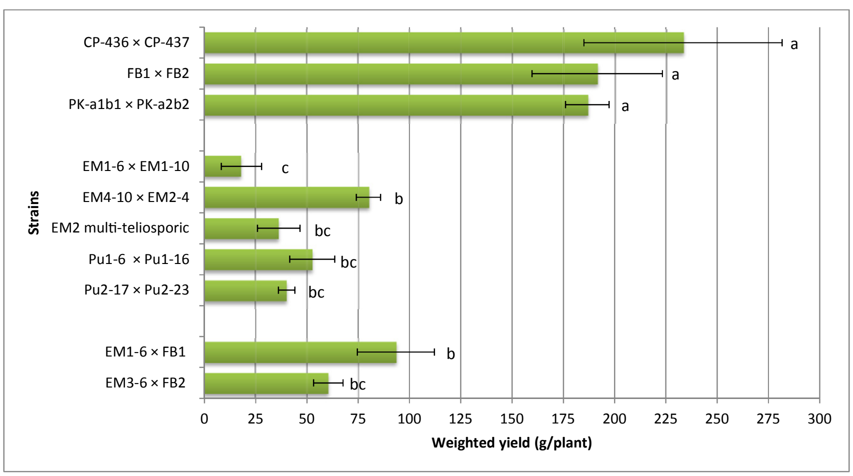

Fig. 4. Weighted yield (g/plant) of galls in 'Tornado XR' ™ corn ears inoculated with selected strains of *Ustilago maydis*. Different letters indicate statistically significant differences (Tukey, $p < 0.01$).

group of strains positively correlate to axis one, the tester strain PK-*a1 b1* × PK-*a2 b2* (number 3 in Table 2) was predominantly acid, astringent and bitter in flavor. The hybrids EM1-6 × FB1 and EM3-6 × FB2 (numbers 9–10, respectively, in Table 2) showed good sensory characteristics; earthy flavor similar to that of the tester strain, FB1 × FB2 (number 2, in Table 2). The tester strain CP-436 × CP-437 (number 1, in Table 2) turned out to be further away from all the flavor terms, in comparison with the rest of strains (Fig. 6).

4. Discussion

Castañeda de León and Leal Lara (2012) reported that high relative humidity (>70 %) and moderate to high environmental temperatures (18°C–35 °C), are favorable conditions for the controlled production of "cuitlacoche". In general, these conditions were met in this study at the locality of San Andrés de Nicolás Bravo, State of Mexico. *U. maydis* strains were capable of infecting and developing on maize ears for the controlled production of "cuitlacoche". Tester, wild-type and hybrid strains studied showed significant differences in virulence. Greater yields were recorder in tester strains. Although several authors (Christensen, 1963; Thakur et al., 1989) had proposed differing virulence in *U. maydis* isolates, Pope and McCarter (1992a) generate experimental evidence of such variation. They used 15 strains of *U. maydis*, and found incidences and severities above 90 % in nine of them. Later, du Toit and Pataky (1999) reported incidences of 100 % using the strain 521 × 518. Similar results were recorded in this study using all tester strains, which showed a high degree of incidence (>90 %), severity (>80 %) and productivity (>12 t/ha). Other studies, in which different compatible haploid isolates were tested evaluating plant characteristics, inoculum densities, fecundation periods, maize development stages and inoculation methods (du Toit and Pataky, 1999; Garibaldi, 2003), reported lower productivities than that obtained using tester and hybrid strains in this study. Cota Navarro (2004) inoculated eight strains, sixteen compatible wild haploid isolates,

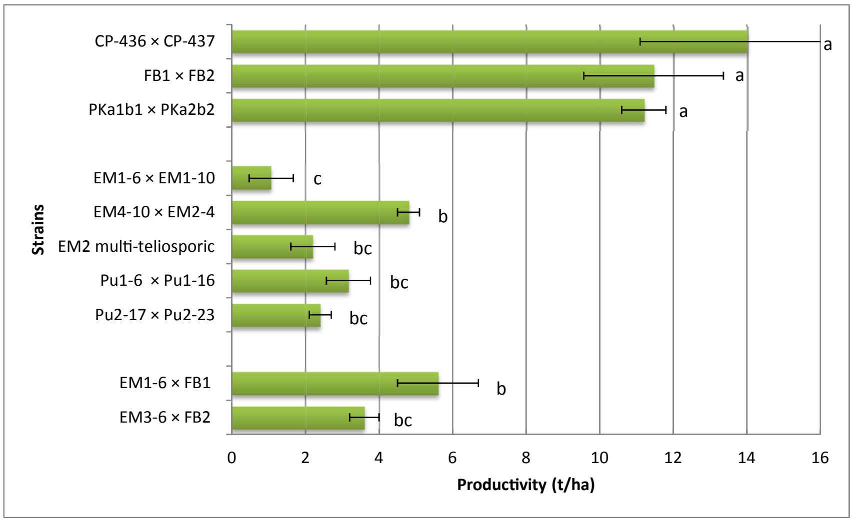

Fig. 5. Productivity (t/ha) of galls in 'Tornado XR' ™ corn ears inoculated with selected strains of *Ustilago maydis*. Different letters indicate statistically significant differences (Tukey, $p < 0.01$).

Table 4
Ustilago maydis strains with highest and lowest values for husk protection and gall quality on 'Tornado XR' ™ corn ears.

Pairings	Husk protection (scale: 1–5)	Gall quality (scale: 1–5)
Higher quality		
P2-17 × P2-23	4.3 ± 0.1[a]	3.7 ± 0.4[a]
EM1-6 × EM1-10	4.2 ± 0.4[a]	2.1 ± 0.4[bc]
EM1-6 × FB1	4.1 ± 0.2[ab]	2.9 ± 0.3[ab]
Lower quality		
FB1 × FB2	3.3 ± 0.2[b]	2.3 ± 0.1[bc]
PKa1b1 × PKa2b2	3.6 ± 0.1[ab]	2.0 ± 0.2[bc]

[abc]Different letters in a column indicate statistically significant differences (Tukey, $p < 0.01$).

and two solopathogenic strains from north of Mexico on a maize hybrid (7573™). Incidence of 56 %, maximum severity of 70 %, and a productivity of 2 t/ha were reported using the strain $A_4 \times A_{10}$. This is similar to the average incidence (58 %) and productivity (2.7 t/ha) recorder for EM and Pu wild-type strains in this study. These native strains of *U. maydis* showed good productivity, quality and sensory characteristics. The wide range of variation observed in various haploid isolates, particularly associated to infectivity and production, is multifactorial, and probably as a result of susceptibility and resistance of maize varieties, inoculum quality, inoculation technique, and the pollination index, among other factors. Experimental results, however, indicated that the degree of virulence of *U. maydis* strains is one of the main factors involved in such variation. Testing strains for selection of highly infective and productive compatible haploid isolates is accordingly important for the controlled production of "cuitlacoche".

Multi-teliosporic strains have frequently been used to infect maize varieties to evaluate development of the pathosystem and production of galls. In this study, the inoculation of the wild-type multi-teliosporic strain (EM2) tested for virulence was ineffective,

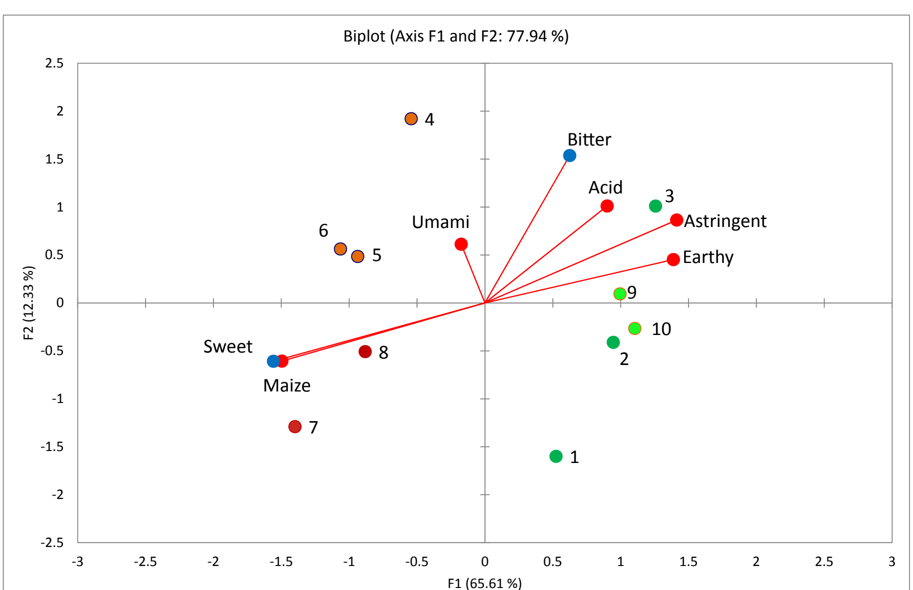

Fig. 6. Sensory space of taste attributes from edible galls produced by selected strains of *Ustilago maydis* (strains number according to Table 2).

as the incidence (57 %), weighted yield (36 g/plant) and productivity (2 t/ha) were lower than those recorded in tester and hybrid strains, as well as in several wild-type isolates. These results were similar to those previously reported for multi-teliosporic strains (Martínez-Martínez et al., 2000; Madrigal-Rodríguez et al., 2010; Zamani et al., 2011; Hassan et al., 2013). Christensen (1963) highlighted this point indicating that the inoculation of various biotypes of *U. maydis* can cause a decrease in severity. Teliospores are diploids and, when meiosis takes place during germination, a wide range of recombinants is generated leading to a change of genotype in the population every new generation. Therefore, the inoculations of maize with multi-teliosporic strains give rise to dikaryons having diverse genetic composition and virulence capacity.

Hybrids between EM wild-type haploid isolates and FB tester strain, EM1-6 × FB1 and EM3-6 × FB2, were studied showing that the degree of virulence increased considerably (at least 26.6 %) in comparison with wild-type haploid isolates. In a similar work, Thakur et al. (1989) tested the infection of maize ears inoculated with isolates of *U. maydis* from different locations in the U.S.A., reporting good results combining isolates from Raleigh (NC), Newfield (NY) and from Madison (WI). Production of galls by hybrids in this study demonstrated that was considerably increased in comparison to the wild-type haploid isolates. In the case of hybrid EM1-6 × FB1, incidence increased from 49 % to 83 %, weighted severity from 22 % to 52 %, weighted yield from 18 g/plant to 93 g/plant, and productivity from 1 t/ha to 6 t/ha. The generation of *U. maydis* hybrids was a good strategy for developing more efficient and productive strains. This strategy has also been useful in other edible mushrooms, such as *Pleurotus* spp. and *Lentinula* spp. Hybrids showing enhanced characteristics (*e.g.* increased biological efficiency, tolerance to stressing physical factors and diseases, improved productive and commercial features) have been generated combining strains from different origin (Arias et al., 2000; Silva et al., 2005; Leal-Lara et al., 2016).

Although “cuitlacoche” is a food product highly demanded and appreciated in Mexican gastronomy, there is a paucity of reports about its sensory attributes. Pataky and Chandler (2003) provided galls to a chef for evaluating their flavor; which were obtained from two maize lines, sweet maize and a male-sterile, at two harvesting times (12 and 17 d post-inoculation). Galls were subjectively evaluated considering color, texture, and flavor. The taste of galls produced in male-sterile maize was found to be slightly different from those coming from sweet maize, despite that both flavors were considered “acceptable”. Flavor attributes, particularly more sweetness and less bitterness, have become important in consumer preferences about “cuitlacoche” produced by the controlled inoculation of maize plants (Castañeda de León et al., 2016). A wide variation was recorded for sensory attributes of “cuitlacoche” produced after inoculation of the maize hybrid Tornado XR™ with ten *U. maydis* strains tested in this study. The high value of the consensus configurations (77.94 %) in the sensory space of taste attributes indicated low experimental variation, allowing specific sensory terms for each *U. maydis* isolate. The tester strain PK-*a1 b1* × PK-*a2 b2* showed a predominantly acid, astringent and bitter flavor. These flavors together generate low acceptance by the consumer. This strain has also been used in other experiments showing variability in the intensity of bitterness, depending on the inoculated maize hybrid (Castañeda de León et al., 2016). Although wild-type strains originated from different geographical regions (States of Mexico and Puebla), all of them showed less bitterness and acidity, and high sweet intensity, taste of maize and umami, which are flavor attributes preferred by consumers. These flavors are also present in galls developed by natural infection. Thus, wild-type isolates can be selected to generate improved hybrids that predominantly show suitable commercial characteristics. In the case of galls produced by hybrid strains between EM wild-type and FB tester strain, EM1-6 × FB1 and EM3-6 × FB2, the earthy flavor was predominant, as it was in galls from the tester strain FB1 × FB2. The earthy flavor in low to medium intensity is a characteristic expected by the Mexican consumer of cuitlacoche.

5. Conclusions

This study showed the importance of selecting wild-type haploid genotypes to develop improved hybrids of *U. maydis* from the wide diversity of native genetic resources found in Mexico. The production of improved *U. maydis* strains for the commercial production of maize smut galls, “cuitlacoche”, for human consumption, should focus not only on higher yields (t/ha) and better product quality (husk protection, galls size), but also on their commercial flavor characteristics according to consumer preferences and perceptions.

Acknowledgments

We thank the National Council of Science and Technology (CONACYT) for their support for this research through the PhD scholarship No 49140.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.04.003.

References

Arias, A., Ramírez, R., Leal, H., 2000. Cultivation of *Pleurotus ostreatus* hybrids resistant to 2DG obtained by pairings of neohaplonts from selected dikaryons. In: Van Griensven (Ed.), Proceeding of the 15th International Congress on the Science and Cultivation of Edible Fungi, vol. I, pp. 305–309.
Banuett, F., Herskowitz, I., 1989. Different *a* alleles of *Ustilago maydis* are necessary for maintenance of filamentous growth but not for meiosis. Proc. Natl. Acad. Sci. U.S.A. 86, 5878–5882.
Bassetti, F., Westgate, M.E., 1993a. Emergence, elongation, and senescence of maize silks. Crop Sci. 3, 271–275.
Bassetti, F., Westgate, M.E., 1993b. Senescence and receptivity of maize silks. Crop Sci. 33, 275–278.
Castañeda de León, V.T., Leal Lara, H., 2012. Logros y desafíos de la producción masiva de “cuitlacoche” *Ustilago maydis* en México. In: Sánchez, J.E., Mata, G. (Eds.), Hongos comestibles y medicinales en Iberoamérica. El Colegio de la Frontera Sur, Mexico, pp. 193–206.
Castañeda de León, V.T., Martínez Carrera, D., Morales Almora, P., Sobal Cruz, M., Gil Muñoz, A., Leal Lara, H., 2016. El cuitlacoche, fruto de la interacción *Ustilago maydis* - maíz, una aportación de México al mundo, en el sistema agro-alimentario microbiano. In: Martínez-Carrera, D., Ramírez Juárez, J. (Eds.), Ciencia, Tecnología e Innovación en el Sistema Agroalimentario de México. Editorial del Colegio de Posgraduados AMC-CONACYT-UPAEP-IMINAP, San Luis Huexotla. Texcoco Mexico, p. 856.
Christensen, J.J., 1963. Corn Smut Caused by *Ustilago maydis*. Monograph 2. American Phytopathol. Soc., St. Paul Minnesota.
Cota Navarro, C.B., 2004. Evaluación de cepas locales de *Ustilago maydis* en maíz para la producción de huitlacoche en el Valle del yaqui. Dissertation. Instituto Tecnológico de Sonora. Cd. Obregón Sonora.
Dairou, V., Sieffermann, J.M., 2002. A comparison of 14 jams characterized by conventional profile and a quick original method, the flash profile. J. Food Sci. 67 (2), 826–834.
Dijksterhuis, G., 1996. Procrustes Analysis in Sensory Research. Elsevier, Netherlands, pp. 185–219.
du Toit, L.J., Pataky, J.K., 1999. Effects of silk maturity and pollination on infection of maize ears by *Ustilago maydis*. Plant Dis. 83, 621–626.
Garibaldi, J.M., 2003. Fertilización nitrogenada y densidad de plantas de maíz (Zea mays) L. con fertigación y acolchado plástico en la producción de huitlacoche Ustilago maydis (D. C.) Corda, en el valle del Yaqui. Dissertation. Instituto Tecnológico de Sonora. Cd. Obregón Sonora.
Hassan, W.A., Yousif, A., Saido, K., 2013. Productivity and susceptibility of corn hybrids accompanied with artificial inoculum of common smut *Ustilago maydis*. Int. J. Pure Appl. Sci. Technol. 18, 52–58.
Holliday, R., 1974. Ustilago maydis. In: King, R.C. (Ed.), Handbook of Genetics. Plenum, New York, pp. 575–595.

Leal Lara, H., Adaya González, J., Curiel Pérez, L., Marroquín Corona, C., Morales Olivares, C., Sánchez Hernández, A., Sánchez Marín, E., Segura Moctezuma, S., Castañeda de León, V., Valencia Del Toro, G., 2016. Aportaciones de investigaciones básicas y aplicadas para impulsar la producción de hongos comestibles en México. In: Martínez-Carrera, D., Ramírez Juárez, J. (Eds.), Ciencia, Tecnología e Innovación en el Sistema Agroalimentario de México. Editorial del Colegio de Posgraduados AMC-CONACYT-UPAEP-IMINAP, San Luis Huexotla. Texcoco Mexico, p. 856.

Madrigal-Rodríguez, J., Villanueva-Verduzco, C., Sahagún-Castellanos, J., Acosta, M., Martínez, L., Espinosa, T., 2010. Ensayos de producción de huitlacoche (*Ustilago maydis* Cda.) hidropónico en invernadero. Rev. Chapingo Ser. Hortic. 16, 177–182.

Martínez-Martínez, L., Villanueva-Verduzco, C., Sahagún-Castellanos, J., 2000. Susceptibilidad y resistencia del maíz al hongo comestible huitlacoche (*Ustilago maydis* Cda.) mejorando su resistencia. Rev. Chapingo Ser. Hortic. 6, 241–255.

Pataky, J.K., 1991. Production of cuitlacoche [*Ustilago maydis* (DC.) Corda] on sweet corn. Hort. Sci. 26, 1374–1377.

Pataky, J.K., Nankam, C., Kerns, M., 1995. Evaluation of a silk-inoculation technique to differentiate reactions of sweet corn hybrids to common smut. Phytopathology 35, 1323–1328.

Pataky, J.K., Chandler, M.A., 2003. Production of huitlacoche, *Ustilago maydis*: timing inoculation and controlling pollination. Mycologia 95, 1261–1270.

Pataky, J.K., Richter, P.M., 2007. Silk abscission in two sweet corn (*Zea mays* L.) hybrids that differ in susceptibility to common smut infection of ears. Hortscience 42 (6), 1409–1412.

Pope, D.D., McCarter, S.M., 1992a. Evaluation of inoculation methods for inducing common smut of corn ears. Phytopathology 82, 950–955.

Pope, D.D., McCarter, S.M., 1992b. Smut incidence and severity after inoculating developing corn ears with *U. maydis* using different methods. Phytopathology 82, 500–514.

SAS, 2007. Statistical Analysis System Institute, Cary, North Carolina.

Silva, E.M., Machuca, A., Milagres, M., 2005. Evaluating the growth and enzyme production from *Lentinula edodes* strains. Process Biochem. 40, 161–164.

Snetselaar, K.M., Carfioli, M.A., Cordisco, K.M., 2001. Pollination can protect maize ovaries from infection by *Ustilago maydis*, the corn smut fungus. Can. J. Bot. 79, 1390–1399.

Thakur, R.P., Leonard, K.J., Pataky, J.K., 1989. Smut gall development in adult corn plants inoculated with *Ustilago maydis*. Plant Dis. 73, 921–925.

Zamani, M., Rahjoo, V., Parchamian, M., 2011. Evaluation of the reaction of early maturing maize genotypes to common smut using artificial inoculation. Proc. Nat. Sci. Matica Srpska J. Nat. Sci. 121, 71–77.

Zimmerman, S.A., Pataky, J.K., 1992. Inoculation techniques to produce galls of common smut on ears of sweet corn. Phytopathology 82, 995.