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A New Method to Obtain Infective *Ustilago maydis* Binucleate Conidia for Corn Smut Production

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Abstract: The fungus *Ustilago maydis* produces galls or tumors on corn ears called corn smut or huitlacoche. Used for human consumption in several countries for its nutritional and sensory traits, huitlacoche is considered a delicacy in Mexican cuisine and has a significant economic value. Hybrid *U. maydis* strains are regularly used for the large-scale production of huitlacoche; however, depending on the genetic characteristics of the parent strains, the pathogenicity and infection rate of hybrid fungi are often suboptimal due to compatibility issues between different strains. Using double-loaded organisms is common in agriculture to improve product characteristics, performance, and shelf-life. A methodology to obtain unicellular *U. maydis* strains with a double genetic load (n + n) capable of producing galls on corn ears without mating (hybridization) is reported herein. This methodology resulted in 206 *U. maydis* isolates. Screening showed that 147 corn plants (>70%) underwent infection and gall production. Of the 147 gall-producing *U. maydis* strains, those with the highest field performance were selected. Three strains, Um-UAEMor-78 (yielding 21.65 ton/ha), Um-UAEMor-120 (22.31 ton/ha), and Um-UAEMor-187 (22.99 ton/ha), showed higher yields than the control strain, CP-436(a1b1) × CP-437(a2b2) (17.80 ton/ha). A specific methodology to obtain unicellular *U. maydis* strains with a double genetic load capable of infecting baby corn ears and forming galls is described for the first time, providing a novel alternative for producing huitlacoche and helping to improve the yields and morphological traits of galls.

Keywords: *Ustilago maydis*; huitlacoche; corn crop; productivity; dikaryotic strain

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1. Introduction

Corn smut or huitlacoche, the result of an infection by the fungus *Ustilago maydis*, often observed on corn crops, has been a delicacy in Mexico since pre-Columbian times, especially in Central and Southern Mexico, as well as in countries with a significant Mexican population, such as the USA. This led to a great demand in domestic and international markets.

Today, specific *U. maydis* strains are selected in laboratory and field tests to favor higher huitlacoche yields and used to produce genetically compatible hybrids capable of infecting corn ears and forming galls. However, the shelf-life of the product is usually shorter due to the accelerated metabolism of hybrids, resulting in economic losses for distributors and consumers.

The mating processes of *U. maydis* are complex. When the fungus reproduces sexually, genetic variability is observed in each cross. The resulting genetic traits, along with the agronomic practices, heavily influence yield, infectivity, and infection severity. Mating in *U. maydis* is tetrapolar after meiosis, generating four genetically different basidiospores. Two genes, loci a and b, play a role in fungal pathogenicity. Two alleles have been reported

for locus a, involved in cell–cell recognition [1]. Locus b, which regulates the stages of sexual development [2–4], has multiple alleles. The union of loci a and b is required for pathogenesis, but heterozygosity in a or b does not block mating reaction [5]. The heterothallic character of this fungus produces new biotypes by sexual mating and hybridization, generating haploid strains with different degrees of pathogenicity. On the other hand, no asexual reproduction mechanism is known for this species, although the possibility of reproduction by heterokaryotic biotypes, a general reproduction method for fungi, cannot be ruled out. The natural generation of spores with a double genetic load, a widespread phenomenon in fungi, has not been widely addressed or studied.

Hybrids of compatible parent strains are used today for huitlacoche production in open-sky or greenhouse conditions, with yields ranging from 7 to 14 ton/ha. An analysis on the susceptibility of native corn plants and the selection of improved hybrids demonstrated that each host exhibits a certain susceptibility to *U. maydis* infection.

The demand for huitlacoche and other edible fungi is growing in Mexico due to their nutrimental content and/or potential therapeutic activity. Fungi are rich in proteins, fiber, and complex carbohydrates, including alpha- and beta-glucans; these compounds have been attributed antioxidant, hypocholesterolemic, immunomodulatory, anticancer, anti-inflammatory, antibacterial, and antidiabetic effects. This work is aimed at establishing a methodology to isolate strains with a double genetic load capable of infecting corn ears and producing galls without mating, helping to improve yields in huitlacoche production.

2. Material and Methods

2.1. Biological Material

Commercial haploid CP-436 and CP-437 strains were donated by the Edible, Functional, and Medicinal Fungus Biotechnology Center (CB-HCFM), Puebla campus of the College of Postgraduates, Mexico. The strains were maintained on PDA culture medium and resected every two months until use.

2.2. Isolation of Haploid Sporidia and Dikaryotic Conidia

Teliospores were obtained from a previously established huitlacoche bay crop using the 436 × 437 hybrid. For the isolation of haploid teliospores, fully developed mature gall tissues with a dark grey appearance were used, with the tissues being collected 30 days after inoculation in a corn plant. The galls were washed with 1% sodium hypochlorite solution (*v*/*v*). Once cleaned, 1 g of tissue was placed in 1.5 mL Eppendorf tubes and stirred in a vortex for 1 min. Serial dilutions up to 10^{-4} were made in sterile distilled water; 10 µL of each dilution was deposited in Petri dishes with PDA culture medium (Difco, BD, Franklin Lakes, NJ, USA) and incubated at 28 °C for 84 h to obtain pure unicellular cultures.

To isolate dikaryotic conidia, immature gall tissue with a whitish appearance was used, collecting it 10 days after inoculation in the corn plant. Gall tissue (telium) contains binucleate cells, young heterokaryotic teliospores (n + n), and mature dikaryon teliospores (2n) before karyogamy to complete the fungus life cycle. A sample was taken from immature galls with a sterile bacteriological loop, striated on a Petri dish with PDA culture medium, and left to incubate for 120 h until yeast-like colony growth was observed. A typical yeast morphology was confirmed under a microscope. In total, 206 colonies or strains were isolated with this methodology. The colony was striated again to obtain unique colonies from a basidiospore, which were grown and stored for use. Inoculums from each sporidium were obtained by taking one colony-forming unit (UFC) and culturing it in 2% malt extract liquid medium under constant agitation at 28 °C for 48 h before use.

2.3. Morphological Characterization of Haploid Strains and Dikaryotic Conidia

The morphological traits of *U. maydis* colonies after the fuzz phenotype reaction [5] were used for selecting dikaryotic strains. *U. maydis* strains were cultured in PDA medium (Difco) added with activated carbon (Hycel Chemicals, Zapopan, Mexico), observing a contrast in colony morphology. Dikaryotic strains grow as white colonies with a cottony

appearance; under a microscope, filamentous growth is observed. Meanwhile, haploid strains form colonies with a creamy, non-filamentous morphology; under a microscope, yeast-like growth is observed.

2.4. U. maydis Inoculation in Corn Plants

For corn plant infection, inoculums were prepared in 50 mL of sterile diastase-free malt extract liquid medium. The medium was inoculated by taking a loop of cell growth in solid culture medium and incubating under constant agitation at 150 revolutions per minute (rpm) for 48 h at 25 ± 2 °C. Inoculum quality was monitored by observation under a microscope to detect contamination (bacteria and/or filamentous fungi). The culture was diluted twice (1:2) in sterile distilled water to a concentration of 10^6 UFC/mL in the final inoculum.

Corn plants were inoculated following a methodology developed at the Mycology Laboratory of the Autonomous University of the State of Morelos, using hybrid Asgrow A-7573 corn plants that maintained a baby corn ear appearance after 6–8 days of growth (silky shoots or early phenological stage R1). Two equidistant points in the mid-section of a baby corn ear were injected with 0.5 mL of inoculum (1 mL per corn ear) using a 2 mL ECO-MATIC semi-automatic syringe (HSW, Tuttlingen, Germany), ensuring that the inoculum was homogeneously distributed on the corn ear.

2.5. Analysis of Severity Degree, Infection Rate, Yield, and Production

Severity degree (SEV_i) was evaluated as the proportion of corn ear covered with the fungal galls [6]. Five severity categories (G0–G4) were defined for the development of galls on the corn. SEV_1 = no presence of galls (G0); $SEV_2 \geq$ 0–25 galls covering $\leq 1/4$ of the length of corn ear (G1), $SEV_3 \geq$ 25–50 galls covering $> 1/4$ but $\leq 1/2$ of the length of corn ear (G2); $SEV_4 \geq$ 50–75 galls covering $> 1/2$ but $\leq 3/4$ of the length of corn ear (G3); and $SEV_5 \geq$ 75–100 covering $> 3/4$ of the length of corn ear (G4). Incidence percentage (PI) was calculated by dividing the count of infected corn ears in each severity category and experimental unit by the total number of inoculated corn and multiplied by 100 [7]. Potential huitlacoche yields per hectare were calculated by multiplying infection rates by plant density per hectare and by the weight (g) obtained from each corn ear and dividing by one thousand. ISE = severity index; ISE = [(SEV_1 0) + (SEV_2 0.25) + (SEV_3 0.50) + (SEV_4 0.75) + (SEV_5 1.0) total infected corn ears] × 100.

2.6. Data Analysis

Data were analyzed by an analysis of variance (ANOVA) in a randomized block design with a risk $\alpha = 0.5$, followed by a Tukey's test to assess differences between groups. Field tests were performed on 25 plants per strain, each in triplicate, using 200 haploid and 200 dikaryotic strains. All analyses were performed using the SAS 9.0 statistical package for Windows.

3. Results

3.1. Isolation of Haploid and Dikaryotic Sporidia

When the fuzz test was performed, sporidia obtained from mature teliospores showed either a smooth or a rough colonial morphology, with no presence of promycelium (Figure 1B,D). In contrast, sporidia from immature teliospores obtained by the methodology herein developed showed one of two morphological traits, cotton-like colonies with the presence of promycelium, or smooth colonies with no promycelium. Using this methodology, 206 colonies were isolated from immature galls. The colonies were characterized by color, the presence or absence of promycelium, and a smooth or rough appearance (Figure 1). Of the 206 colonies obtained from immature galls, 138 showed the presence of promycelium, and 67 showed a creamy appearance, with no promycelium. A phenotype marked by the presence of promycelium and a cottony appearance is frequently observed

in the fuzz test when crossing genetically compatible sporidia capable of infecting corn plants and forming galls.

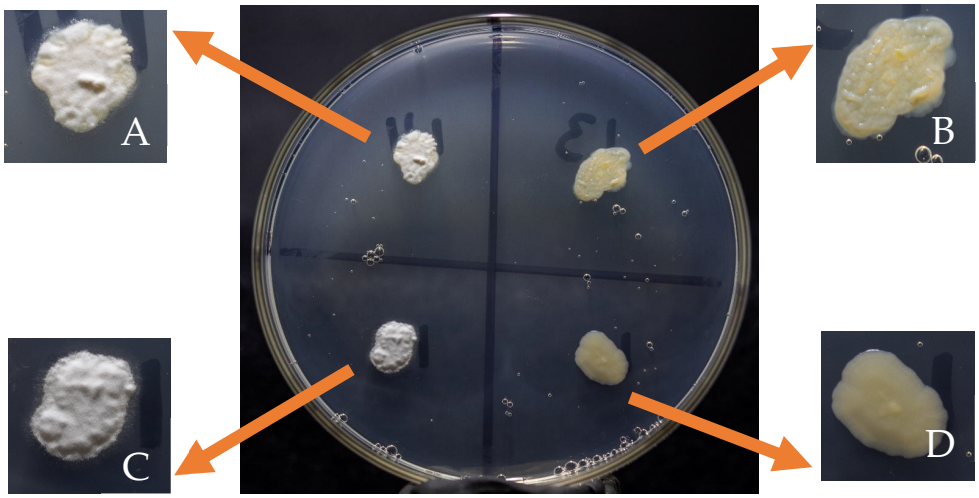

Figure 1. Morphological aspects. Different morphologies of *U. maydis* colonies in the fuzz test are shown. (**A**). Presence of promycelium and a rough appearance. (**B**). Absence of promycelium, a rough appearance, and a cream color. (**C**). Presence of promycelium and a rough appearance. (**D**). No promycelium, smooth appearance, and a cream color.

3.2. Preliminary Analysis of Strains from Mature or Immature Galls in Corn Crops

U. maydis strains obtained from mature or immature galls were tested on corn ears. None of the 200 strains obtained from mature galls were able to produce new galls in corn ears (four replicates). Strains from immature galls caused a successful infection, albeit with varying effectiveness and severity, and produced galls with differing morphological traits. Of the 206 strains isolated, 147 were able to produce galls in baby corn ears. Figure 2. The characteristics of these experimental infections, including gall size, degree of severity, and gall coloration and appearance (white, light or dark gray, or white with gray spots) are shown in Table 1.

Table 1. Characteristics of infection in corn by 206 U. maydis strains from immature galls.

Gall Appearance	ISE	SEV_i (UM-UAEM)				
		G0	G1	G2	G3	G4
Small–white	25		7			
Small–grey	25		17			
Large–white	25					
Large–grey	25				57	64, 65, 69, 72, 73, 75, 99
Small–spotted	25					
Small–white	50					
Small–grey	50		169	2, 58	198	50, 160
Large–white	50					86, 187
Large–grey	50					5, 21, 51, 62, 67, 83, 114, 132, 155, 170, 175
Small–spotted	50			19		
Large–spotted	50					186
Small–white	75		80		40	41
Small–grey	75		47		25, 26, 35, 74	1, 4, 31, 39, 61, 68, 70
Large–white	75					
Large–grey	75			36, 43, 45, 48, 95	28, 30, 37, 42, 89	6, 91, 94, 100
Small–spotted	75					14, 18, 23, 76, 77
Small–white	100		3			10, 34, 46,134,135,161

Table 1. *Cont.*

Gall Appearance	ISE	SEV_i (UM-UAEM)				
		G0	G1	G2	G3	G4
Small–grey	100		27	53, 201	13, 20, 199	8, 11, 12, 15, 28, 55, 59, 79, 81, 90, 93, 102, 158, 163, 200
Large–white	100					133
Large–grey	100			96, 202	104, 110, 111, 151	29, 33, 78, 92, 105, 106, 108, 109, 112, 113, 117, 119, 120, 123, 124, 126, 128, 130, 131, 137, 138, 139, 142, 147, 152, 156, 159, 162, 167, 176, 177, 182, 183, 184, 185, 191
Small–spotted	100			38	32, 103, 116	22, 84, 85, 165
Large–spotted	100					118, 141

G0–G4 = severity degree. ISE = severity index. Um-UAEMor-N = Um: *Ustilago maydis*, UAEMor: Universidad Autónoma del Estado de Morelos, N: strain number.

Figure 2. Baby corn ears infected with *U. maydis* strains isolated from white galls. (**A**). UAEMor_Um-78; (**B**). UAEMor_Um-85; (**C**). UAEMor_Um-62; (**D**). UAEMor_Um-68; (**E**). UAEMor_Um-95; and (**F**). UAEMor_Um-5. $\alpha = 0.05$.

According to the preliminary analysis, 67 of the 206 strains produced small galls (≤1.5 cm), and 80 produced large galls (>1.5 cm). This cut-off value was selected considering the size scale used by commercial huitlacoche producers in Mexico. All small galls were light grey in color with dark spots and had a hard consistency due to the poor development of galls, with a predominance of filamentous fungal tissue and a poor development of mature teliospores (Figure 3A). On the other hand, the large galls were light gray in color with no dark spots; these galls showed a regular, firm consistency, the presence of abundant mature teliospores, and complete development, with appropriate characteristics for commercialization (Figure 3B).

(**A**) (**B**)

Figure 3. Representative images of galls produced by *U. maydis* strains. Gall size and coloration is shown. (**A**). Baby corn ear with small galls (≤1.5 cm) with a spotted gray surface. (**B**). Baby corn ear with large galls (>1.5 cm) and a regular, gray-colored surface.

High infection rates in the baby corn ears were observed in the corn crop test. Interestingly, different *U. maydis* strains showed different infective capacities and levels of gall quality. First-class gall quality was observed in 23 out of the 206 strains studied, with a severity degree of 100%, a voluminous development of infected baby corn ears, large galls, moderate gall stiffness, and a dark gray color. In the 206 strains, the mean infection rate was 71%, and the mean severity degree was G4 (Table 2). Samples of all isolated strains were kept as valuable resources to develop novel products and characteristics for the market.

Table 2. Analysis of 206 *U. maydis* strains tested in corn crops.

Strains Inoculated	Baby Corn Ears Infected	Infection Count	Infection Rate	Severity Degree
206	824	71.35%	71.3%	G4

Average infection count, infection rate, and severity degree are shown. *n* = 206 strains, 4 replicates per strain.

3.3. Evaluation of 23 U. maydis Dikaryotic Strains Capable of Producing Galls in Baby Corn Ears

The 23 *U. maydis* strains that produced large galls, a high severity degree, and high yields were tested again in the field, inoculating 25 plants per strain, with three replicates. The selected strains are listed in Table 3.

Table 3. Evaluation of 23 selected strains and the commercial strain CP 436 × 437.

Selected Strains	Average Gall Weight (g)	Infection Rate (%)	Severity Degree	Yield per Plant Inoculated (th^{-1})	Yield per Hectare, RPH (th^{-1})
Um-UAEMor-6	119.73 ± 44.11 ^{HFGE}	100	G4	5.98 ± 2.32	7.18
Um-UAEMor-8	137.18 ± 59.21 ^{DFE}	100	G4	8.06 ± 3.71	8.23
Um-UAEMor-11	151.07 ± 54.18 ^{DCE}	100	G4	10.79 ± 4.01	9.06
Um-UAEMor-23	157.5 ± 40.04 ^{DCE}	100	G4	9.84 ± 2.50	9.45
Um-UAEMor-24	125.78 ± 37.00 ^{DFGE}	100	G4	6.62 ± 1.93	7.54
Um-UAEMor-28	145 ± 70.54 ^{DFCE}	100	G4	9.06 ± 4.40	8.70
Um-UAEMor-31	156.5 ± 40.33 ^{DCE}	100	G4	7.82 ± 2.05	9.39
Um-UAEMor-34	145.29 ± 56.65 ^{DFCE}	100	G4	8.07 ± 3.72	8.71
Um-UAEMor-39	175.55 ± 75.03 ^{DC}	100	G4	9.75 ± 3.94	10.53
Um-UAEMor-59	153.52 ± 49.31 ^{DCE}	100	G4	9.03 ± 2.86	9.21
Um-UAEMor-61	78.46 ± 34.32 ^{HGI}	100	G4	6.03 ± 2.74	4.70
Um-UAEMor-68	73.57 ± 30.02 ^{HI}	100	G4	5.25 ± 2.14	4.41
Um-UAEMor-78 *	360.83 ± 107.49 ^{A}	100	G4	20.04 ± 4.71 *	21.65
Um-UAEMor-79	85.78 ± 50.00 ^{HGI}	100	G4	4.28 ± 2.58	5.14
Um-UAEMor-91	75.16 ± 35.46 ^{HGI}	75	G3	3.75 ± 1.93	4.51
Um-UAEMor-92	100 ± 20.25 ^{HFGI}	100	G4	7.14 ± 2.30	4.20
Um-UAEMor-93	189.73 ± 72.66 ^{C}	100	G4	9.98 ± 3.52	11.38
Um-UAEMor-94	73.57 ± 46.15 ^{I}	100	G4	5.25 ± 2.41	4.41
Um-UAEMor-120 *	371.86 ± 119.03 ^{A}	100	G4	18.59 ± 2.09 *	22.31
Um-UAEMor-138	121.25 ± 69.02 ^{HFGE}	100	G4	6.06 ± 3.10	7.27
Um-UAEMor-155	85.78 ± 50.28 ^{HGI}	100	G4	4.51 ± 2.67	5.14
Um-UAEMor-185	85 ± 57.17 ^{HGI}	100	G4	5.00 ± 3.27	5.10
Um-UAEMor-187 *	383.25 ± 137.17 ^{A}	100	G4	19.16 ± 6.49 *	22.99
CP-436 X CP-437	296.75 ± 107.49 ^{B}	100	G4	14.83 ± 3.57 *	17.80

Mean shelled gall weight ± standard deviation, infection rate, severity degree, yield per inoculated plant, and yield per hectare. RPH = yield per hectare, RPH = [(PI × Sowing density) × (GMI)]/1000. Different letters indicate statistically significant differences. $n = 25$. $P = 0.001$. An analysis of variance (ANOVA) was performed in a randomized block design. A risk of $\alpha = 0.5$ was selected. * Strains with gall weight higher than the control strain.

Statistically significant differences in fresh weight yield for shelled galls were found between the strains studied herein. A mean fresh weight (PW) yield of 383.25 ± 137.17 g, 360.83 ± 107.49 g, and 371.86 ± 119.03 g was determined for the strains Um-UAEMor-187, Um-UAEMor-Mor-78, and Um-UAEMor-120, respectively. Meanwhile, the PW yield for the control strain (CP-436 × CP-437) was 296.75 ± 107.49 g. Significant differences were found between the strains under study with respect to the control. The strains Um-UAEMor-187, Um-UAEMor-78, and Um-UAEMor-120 produced the best mean yields. The severity was 100% for all strains except Um-UAEMor-91, which showed a severity degree of G3, which corresponds to 75% (Table 3).

Under visual observation, significant differences were found in 3 strains with respect to the other 20 strains selected for field testing. The strains Um-UAEMor-187 (Figure 4A), Um-UAEMor-78 (Figure 4B), and Um-UAEMor-120 (Figure 4C) produced galls with a uniform appearance, large size, and dark grey color. The commercial strain CP-436 × CP-437 (Figure 4D) showed similar characteristics. Thus, the strains under study show promising traits for commercial use on a large scale.

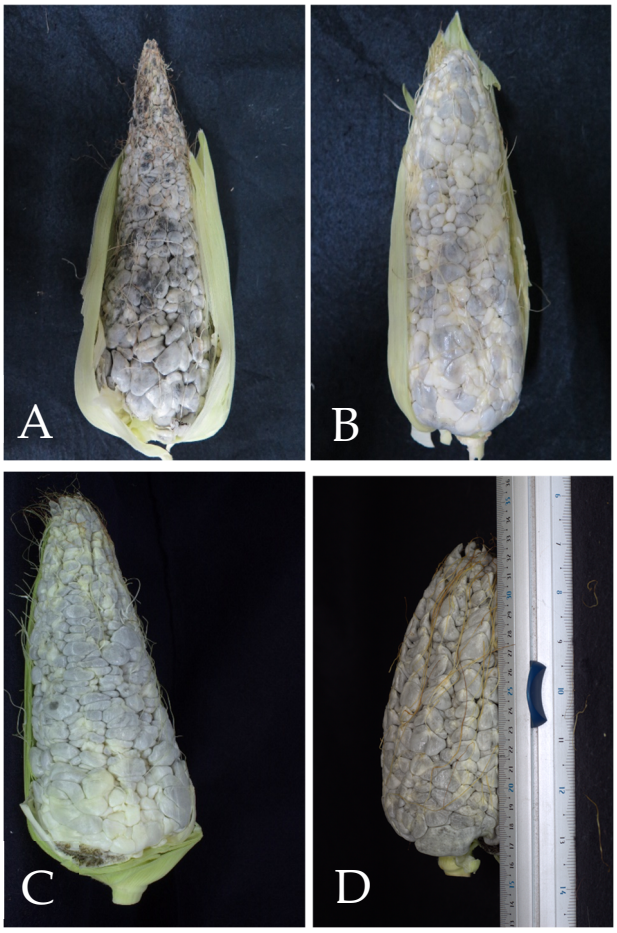

Figure 4. Baby corn ears infected with the highest-yielding *U. maydis* strains in the field. (**A**). Um-UAEMor-120; (**B**). Um-UAEMor-798; (**C**). Um-UAEMor-187; and (**D**). Commercial strain CP-436 × CP-437. Corn ears infected with the strain Um-UAEMor-78 showed galls with a uniform appearance and mostly gray color, with some white galls. Corn ears infected with the strain Um-UAEMor-120 showed a severity degree of 100%, with small, dark gray-colored galls. Corn ears infected with the strain Um-UAEMor-187 showed gray-colored galls that were heterogeneous in size.

4. Discussion

Following a previously reported methodology [5], placing compatible *U. maydis* strains on PDA culture medium supplemented with activated carbon allowed us to observe the Fuz+ phenotype, characterized by the presence of promycelium. This phenotype, which was observed in 138 of the 206 strains isolated, could indicate the presence of dikaryotic strains able to infect baby corn ears, as this character is only expressed when loci a and b, which control the *U. maydis* life cycle, are present. Thus, the Fuz+ phenotype is clearly linked to the capacity of inducing tumors and producing teliospores that undergo meiosis. This methodology allowed us to isolate strains with good traits for commercial huitlacoche production, which could also be a valuable tool to develop genetic resources for biotechnological research, as they are susceptible to domestication and application for mass production. In contrast to hybrid-based technology for the isolation of strains capable of producing huitlacoche, this methodology is effective for obtaining dikaryotic strains with high infectivity, which could help to improve yields in the production of commercial hybrid strains. In addition, the dikaryotic character of the strains improves their long-term genetic stability, increasing the infection rates and yields in industrial-scale production.

Along with the pathogenicity of the fungus, the commercial production of huitlacoche depends on biotic factors such as the stage of development of the corn plant and the genetic susceptibility of the seed [8–10]. To consider these factors, data from previous com-

mercial crops were gathered, and a corn variety currently used by huitlacoche producers was selected.

Yields varied depending on the *U. maydis* strain used. The strains Um-UAEMor-187, Um-UAEMor-78, and Um-UAEMor-120 produced interesting yields of 22.99, 21.65, and 22.31 ton/ha, respectively, with a density of 60,000 plants per hectare. For comparison, the control strain produced a yield of 17.80 ton/ha, a result significantly lower with respect to the dikaryotic strains resulting from this research. The methodology to obtain strains capable of infecting corn plants with a severity degree of G4 was standardized to prevent the effect of variation in factors such as the inoculation method. Valdez et al. [11] and Salazar et al. [12] reported yields of up to 14.1 ton/ha with native corn using artificial inoculation and maintaining the production under controlled conditions. Similar results were reported by Aguayo et al. and Martínez et al. [6,13], with yields of 9.11, 8.42, 8.20, and 8.00 ton/ha, respectively, working with 300 families based on a sample of 19 plants inoculated to a density of 60,000 plants/ha. On the other hand, Pataky and Chandler [14] reported a yield of 7.86 ton/ha.

With respect to the severity index (ISE), Madrigal et al. [7] reported an ISE value of 90% in the hybrid H-58 corn strain in a density of 62,500 plants/ha. Garcilazo [15] reported infection rates of 75% with hybrid corn varieties. In this study, ISE ranged from 75 to 100%, with significant differences with respect to the control strain, in a density of 60,000 plants/ha. In other words, the strains isolated in this study are highly infective. Calderon [16] reported that the ISE value determined in each strain not only depends on the corn variety or color but also on the virulence of the *U. maydis* strain used. Our results suggest that, in addition to the virulence of the strain, the inoculation technique plays a major role as a non-automated, not fully controlled parameter and a source of variation in ISE values.

Incidence percentage (PI) values of 100% were determined for the strains Um-UAEMor-187, Um-UAEMor-78, and Um-UAEMor-120, as well as for the control strain. This indicates that the dikaryotic strains under study show a promising infective potential. Garcilazo [15] reported PI values of 79–88.70%. Salazar et al. reported higher PI values when inoculating Cobra hybrid (85.83%), Oso (84.50%), A7573 (78.52%), criollo R12 (85.00%), B7 (83.37%), and B3 (73.05%). Valdez et al. (2009) evaluated the production of huitlacoche in 15 varieties and reported IP values ranging from 30.90 to 92%, similar to the values reported herein.

Dikaryotic strains are used in pharmaceutical, agricultural, and environmental remediation settings to improve production yields, as they grow faster than their parent strains. These strains could improve the phenotypic qualities of huitlacoche as well [17].

5. Conclusions

The obtention of *U. maydis* strains capable of forming galls in corn ears without the mating of homokaryotic strains is reported herein. A methodology was generated to isolate strains with a double genetic load that could potentially be useful for huitlacoche production by interrupting the life cycle of *U. maydis*. This methodology could be used to generate valuable genetic resources for scientific research and help huitlacoche producers to achieve a significant social impact. Over 20 strains were selected based on gall characteristics, yield, severity degree, and infection index. Three strains showed potential for commercial use and mass production in the field, producing galls with desirable appearances, sizes, colors, and textures, along with high yields and a high infective capacity. These improved strains could prove to be valuable to produce huitlacoche with better characteristics than the strains currently used.

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