Article

Sexual Compatibility Types in F₁ Progenies of Sclerospora graminicola, the Causal Agent of Pearl Millet Downy Mildew

Chandramani Raj 1,2* and Rajan Sharma 1,*

1 International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502324, Telangana, India; chandramani.raj@icar.gov.in
2 ICAR-Indian Institute of Sugarcane Research, Raebareli Road, Lucknow 226002, Uttar Pradesh, India
* Correspondence: r.sharma@cgiar.org

Abstract: Sclerospora graminicola is primarily heterothallic in nature with two distinct mating types (G₁ and G₂); however, homothallism does exist in the pathogen populations. In this study, a cross was made between two self-sterile isolates (Sg 019, Mat₁-2, G₂ × Sg 445-1, Mat₁-1, G₁) of S. graminicola and a total of 39 F₁ progenies were established. The study on sexual compatibility types in F₁ progenies was conducted by crossing each F₁ progeny with both the parents (Sg 445-1, Mat₁-1, G₁; and Sg 019, Mat₁-2, G₂). The results revealed the presence of four sexual compatibility types, viz. G₁G₁, G₁G₂, G₂G₂ and G₀ (neuter) in the progenies. The G₁G₂ progenies that produced oospores with both the parents were found as self-fertile (homothallic) and self-sterile (heterothallic) types. Similarly, self-fertile parental type G₁ and G₂ progenies were designated as secondary homothallism, whereas self-sterile parental type G₁ and G₂ progenies were of heterothallic type. The result of the present study revealed Mendelian segregation of mating type locus in S. graminicola which indicates that sexual reproduction plays an important role in the evolution of new genetic recombinants in the pathogen. The study also helps in understanding the genetic structure of S. graminicola populations and potential for possible evolution of new virulences in the pathogen.

Keywords: mating types; homothallism; heterothallism; secondary homothallism; neuter

1. Introduction

Pearl millet [Pennisetum glaucum (L.) R. Br.] is a choice crop of more than 90 million people cultivated on approximately 27 million hectares in the arid and semi-arid tropics of the world [1]. In India, mainly the states of Rajasthan, Gujarat, Haryana, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh produce 8.74 million tons of pearl millet. The crop is cultivated on 7.20 million hectares with a productivity of 1214 kg ha⁻¹ [2]. Although average productivity of pearl millet in India has increased since the 1950s (305 kg ha⁻¹) [3], it has also witnessed the devastating crop losses of up to 80% at periodic intervals caused by the downy mildew (DM) pathogen, Sclerospora graminicola [(Sacc). Schroet] [4]. The corresponding changes in the population structure of the pathogen over a period of time have played a key role in the destruction of the crop. The reason behind the evolution of new pathotype/s has been attributed to extreme selection pressure from the host along with sexual reproduction in S. graminicola populations [5].

The oospores formation in S. graminicola has been reported either through heterothallism, in which two self-sterile isolates having distinct sexual compatibility types, G₁ and G₂, fuse together [6,7], or through secondary homothallism in self-fertile isolates that contain the determinant of both compatibility types [8]. In general, one isolate produces functional antheridia and the other isolate forms oogonia during a reciprocal crossing between two self-sterile isolates and the evidence of relative sexuality within isolates determines the contribution of antheridia and oogonia by each parent [9]. However, the presence of
multiple compatibility types has been reported in other oomycetes. Four compatibility types (A₁, A₂, A₁A₂ and neuter) have been observed in the F₁ progenies of the crosses derived from two distinct mating type isolates (A₁ × A₂) of Phytophthora spp. [10,11]. The production of oospores in one mating type (G₂) of S. graminicola isolate without fusion with any mating type [6] and no formation of oospores in isolate Sg 110-2 with any one of the designated mating types (G₁ and G₂) [12] indicated the presence of multiple compatibility types in S. graminicola [6]. Therefore, this study was planned to investigate the occurrence of self-sterile, self-fertile and neuter (sterile) isolates in S. graminicola to ascertain the multiple sexual compatibility types within the pathogen.

2. Materials and Methods

2.1. Collection and Maintenance of Isolates

A total of 52 isolates of S. graminicola were collected from different pearl millet growing areas of India during 1992 to 2012 (Table 1). The single zoospore isolates of each collection were established [12] and were maintained separately either on their original host or on another susceptible host in the isolation polyacrylic chambers (60 cm × 45 cm × 45 cm) in the glasshouse at ICRISAT, India.

Table 1. Sources of Sclerospora graminicola isolates collected from different pearl millet growing states of India.

| Identity | Location         | State     | Year | Maintenance Host |
|----------|------------------|-----------|------|------------------|
| Sg 018   | Patancheru       | Telangana | 1992 | 7042 S           |
| Sg 019   | Patancheru       | Telangana | 1992 | 7042 S           |
| Sg 021   | Ahmednagar       | Maharashtra | 1993 | 7042 S           |
| Sg 048   | Mysore           | Karnataka | 1994 | 852 B            |
| Sg 139   | Jodhpur          | Rajasthan | 1997 | Nokha Local      |
| Sg 150   | Jala             | Maharashtra | 1997 | 834 B            |
| Sg 151   | Durgapur         | Rajasthan | 1997 | 834 B            |
| Sg 153   | Patancheru       | Telangana | 1997 | 843 B            |
| Sg 200   | Jamnagar         | Gujarat   | 1998 | ICMP 451         |
| Sg 212   | Durgapur         | Rajasthan | 1998 | ICMP 451         |
| Sg 298   | IARI             | New Delhi | 1999 | W 504-1-1        |
| Sg 334   | Bhiwani          | Haryana   | 2001 | 7042 S           |
| Sg 384   | Barmer           | Rajasthan | 2003 | ICMP 451         |
| Sg 409   | Patancheru       | Telangana | 2004 | PMB 11571-2      |
| Sg 431   | Patancheru       | Telangana | 2005 | 7042 S           |
| Sg 445   | Banaskantha      | Gujarat   | 2005 | Pioneer 7777     |
| Sg 457   | Sujnapur, Jaipur | Rajasthan | 2006 | ICMP 451         |
| Sg 492   | Iglas            | Uttar Pradesh | 2007 | ICMP 451         |
| Sg 510   | Badaun           | Uttar Pradesh | 2008 | 7042 S           |
| Sg 519   | Rewari           | Haryana   | 2009 | 7042 S           |
| Sg 520   | Bhiwani          | Haryana   | 2009 | 7042 S           |
| Sg 521   | Rewari           | Haryana   | 2009 | 7042 S           |
| Sg 526   | Jodhpur          | Rajasthan | 2009 | 7042 S           |
| Sg 528   | CAZRI, Jodhpur   | Rajasthan | 2009 | 7042 S           |
| Sg 529   | CAZRI, Jodhpur   | Rajasthan | 2009 | 7042 S           |
| Sg 530   | Karodi, Aurangabad | Maharashtra | 2009 | 7042 S           |
| Sg 531   | Nashik           | Maharashtra | 2009 | 7042 S           |
| Sg 532   | Srirampur, Ahmednagar | Maharashtra | 2009 | 7042 S           |
| Sg 533   | Newasa, Ahmednagar | Maharashtra | 2009 | 7042 S           |
| Sg 535   | Gangapur, Aurangabad | Maharashtra | 2009 | 7042 S           |
| Sg 540   | Jambal, Aurangabad | Maharashtra | 2010 | 7042 S           |
| Sg 541   | Pimpalgaon, Aurangabad | Maharashtra | 2010 | 7042 S           |
| Sg 542   | Aurangabad       | Maharashtra | 2010 | 7042 S           |
| Sg 543   | Aurangabad       | Maharashtra | 2010 | 7042 S           |
| Sg 544   | Aurangabad       | Maharashtra | 2010 | 7042 S           |
| Sg 545   | Aurangabad       | Maharashtra | 2010 | 7042 S           |
Table 1. Cont.

| Identity | Location           | State          | Year  | Maintenance Host |
|----------|--------------------|----------------|-------|------------------|
| Sg 546   | Tanda, Aurangabad  | Maharashtra    | 2010  | 7042 S           |
| Sg 547   | Jalna              | Maharashtra    | 2010  | 7042 S           |
| Sg 548   | Dakkalgao, Jalna   | Maharashtra    | 2010  | 7042 S           |
| Sg 549   | Hothnur, Aurangabad| Maharashtra    | 2010  | 7042 S           |
| Sg 550   | Kannad, Aurangabad | Maharashtra    | 2010  | 7042 S           |
| Sg 551   | Chalisgaon, Jalgaon| Maharashtra    | 2010  | 7042 S           |
| Sg 552   | Sindkheda, Dhule   | Maharashtra    | 2010  | 7042 S           |
| Sg 553   | Dondaicha, Dhule   | Maharashtra    | 2010  | 7042 S           |
| Sg 554   | Indave, Dhule      | Maharashtra    | 2010  | 7042 S           |
| Sg 555   | NARP, Aurangabad   | Maharashtra    | 2010  | 7042 S           |
| Sg 556   | Kothigaon, Banaskantha | Gujarat | 2010  | 7042 S           |
| Sg 557   | Lodhnoor, Banaskantha | Gujarat | 2010  | 7042 S           |
| Sg 558   | Gagana, Banaskantha | Gujarat | 2010  | 7042 S           |
| Sg 559   | Jamdi, Banaskantha | Gujarat        | 2010  | 7042 S           |
| Sg 560   | SK Nagar, Banaskantha | Gujarat | 2010  | 7042 S           |
| Sg 561   | IARI               | New Delhi      | 2010  | ICMP 451         |

2.2. Identification of Self-Sterile or Self-Fertile Isolates

To identify the homothallic or heterothallic isolates, the single zoospore isolates-infected plants were allowed to mature for formation of oospores in separate isolation chambers. Necrotic leaf pieces from 2-month-old seedlings infected with each isolate were collected in brown paper bags, cut into 1-centimeter-long pieces, dried under shade and stored at room temperature (25 ± 2°C) until further observation. The small leaf pieces were surface sterilized with NaOCl (2%) and washed thoroughly with sterilized distilled water. These leaf pieces were cleared by incubating them at 40°C in NaOH (5%) for 12 to 16 h. Cleared leaf pieces were rinsed in distilled water and observed under a microscope using a 10× objective for the presence of oospores. Isolates which did not show oospore formation were selected as self-sterile isolates for further studies.

2.3. Selection of Highly Virulent Self-Sterile Isolate

The sporangial inocula of all the self-sterile heterothallic isolates were raised on seedlings of a highly susceptible genotype 7042 S in isolation chambers in the glasshouse. The sporangia from sporulating leaves were harvested in ice-cold distilled sterile water and spore concentration was adjusted to 1 × 10^6 spores mL^−1. Pot-grown seedlings of the pearl millet differential lines P 7-4, P 310-17, 700651, 7042 R, IP 18292, IP 18293 and 852 B and two known downy mildew (DM) susceptible lines—ICMP 451 and 7042 S—were spray-inoculated at coleoptile stage using an atomizer. The inoculated seedlings were incubated at 20°C with >90% Relative Humidity (RH) for 20 h, and then transferred to greenhouse benches at 25 ± 2°C and >90% RH for disease development for the next 2 weeks. DM incidence was recorded 14 days after inoculation as percentage of infected plants. The isolates with ≤10% disease incidence were considered avirulent and those with >50% disease incidence as virulent on the specific genotype.

2.4. Confirmations of Mating Type of Virulent Test Isolate (Sg 445-1)

The reference isolates Sg 018 (Mat-1, G1) and Sg 019 (Mat-2, G2) and test isolate Sg 445-1 (single zoospore selection from Sg 445) of S. graminicola were maintained separately on 7042 S. To detect the mating type of the test isolate, Sg 445-1 was crossed with both the reference mating type isolates (Sg 018 × Sg 445-1; and Sg 019 × Sg 445-1). Sporangial inoculum of each isolate (1 × 10^6 sporangia mL^−1) was prepared individually in ice-cold distilled sterile water. Sporangial suspensions of Sg 018 and Sg 445-1, and Sg 019 and Sg 445-1 were mixed in equal proportion (1:1) and spray inoculated on the highly susceptible pearl millet line 7042 S separately. The inoculated seedlings were incubated and transferred to isolation chambers. The infected seedlings were grown in the isolation chambers and allowed to mature. The necrotic tissues from these infected seedlings (>2 months old) were observed for oospore formation.
2.5. Establishment of F₁ Progenies from Oospores Generated from Sg 019 × Sg 445-1 Crosses

To generate progenies from F₁ oospores (Sg 019 × Sg 445-1), infected leaf samples with oospores were dried in the shade, ground and strained to make a fine powder. Oospores were checked again for their presence in the matured leaf powder. Sterilized potting mixture (soil, sand, and farmyard manure in a ratio of 3:2:2 by volume) was infested with oospore inoculum (20–25 g) and the pots (15 cm diameter) containing the infested mixture were sown with a susceptible genotype 7042 S (25 seeds per pot). Each pot was covered with a polythene bag and incubated at 40 °C for 3–4 days for rapid seed germination. Pots were transferred to isolation chambers in a glasshouse at 25 ± 2 °C to avoid any cross contamination from other isolates. Pots were watered adequately every day and observed regularly for DM symptoms on the seedlings. When the first infected seedling in a pot was noticed, it was removed from the pot and was transplanted into another pot containing sterilized soil and shifted to an isolation chamber. Sporangia from each seedling were maintained separately on 7042 S as an individual F₁-progeny in isolation chambers at 25 ± 2 °C in the glasshouse. A total of 39 F₁ progenies were established to determine sexual compatibility types in S. graminicola. Since infected seedlings occurred infrequently and rarely, each infected seedling was assumed to have infection from a single oospore.

2.6. Identification of Sexual Compatibility Types and Self-Sterile/Fertile Nature of F₁ Progenies

To detect sexual compatibility types of F₁ progenies, all the 39 F₁ progenies derived from the cross Sg 019 × Sg 445-1 were crossed with both the parents (Sg 445-1, Mat-1, G₁; and Sg 019, Mat-2, G₂) separately. Sporangial inoculum (1 × 10⁶ sporangia mL⁻¹) of each of the F₁ progenies and both the parents was prepared separately in ice-cold distilled sterile water, mixed in equal proportion (1:1) and spray inoculated on the highly susceptible pearl millet line 7042 S separately. The inoculated seedlings were incubated, transferred to isolation chambers and the infected seedlings were allowed to mature for production of oospores. In addition, to identifying the self-sterile or self-fertile nature of F₁ progenies, the single-zooospore infected plants were allowed to mature in separate isolation chambers and observed for the presence of oospores.

3. Results

3.1. Selection of Self-Sterile Heterothallic Isolates

The 60-day-old, infected leaves of 52 single-zooospore isolates of S. graminicola were checked for presence of oospores. No oospores were detected in 33 isolates, whereas oospores were formed by the remaining 19 isolates (Table 2). Isolates without oospores formation were designated as self-sterile or heterothallic while those producing oospores were designated as self-fertile or homothallic. Thus, a total of 33 heterothallic isolates were selected and the 19 homothallic isolates were excluded from the further studies.

3.2. Selection of Highly Virulent Self-Sterile Isolate

All the 33 self-sterile heterothallic isolates including reference mating type isolates Sg 018 (Mat-1/G₁) and Sg 019 (Mat-2/G₂) were screened on seven host differentials (P 7-4, P 310-17, 700651, 7042 R, IP 18292, IP 18293 and 852 B) and the two known DM susceptible lines (ICMP 451 and 7042 S). The screening identified Sg 445-1 as the most virulent isolate and Sg 018 and Sg 019, the two reference mating type isolates, as avirulent on specific genotypes; hence, they were selected for the crossing and generation of F₁ progenies (Table 3).

3.3. Confirmations of Mating Type of Virulent Test Isolate (Sg 445-1)

The cross between virulent test isolate Sg 445-1 with both the reference mating types Sg 018, Mat-1, G₁ and Sg 019 Mat-2, G₂ isolates (Sg 018 × Sg 445-1 and Sg 019 × Sg 445-1) yielded oospore production in the cross Sg 019 × Sg 445-1, whereas no oospore formations were recorded in Sg 018 × Sg 445-1. This indicated Mat-1/G₁ mating type of Sg 445-1.
Thus, two parents Sg 019 (avirulent) and Sg 445-1 (virulent) of different mating types were selected for crossing and generation of 39 F₁ progenies.

Table 2. Observation on oospore formation in 52 selfed Sclerospora graminicola isolates.

| S.No. | Isolate No. | Oospore Formation | S.N. | Isolate No. | Oospore Formation |
|-------|-------------|-------------------|------|-------------|-------------------|
|       |             | No Oospore | Oospores |       | No Oospore | Oospores |
| 1     | Sg 018      | ✓         |          | 28   | Sg 532    | ✓         |
| 2     | Sg 019      | ✓         |          | 29   | Sg 533    | ✓         |
| 3     | Sg 021      | ✓         | ✓        | 30   | Sg 535    | ✓         |
| 4     | Sg 048      | ✓         |          | 31   | Sg 540    | ✓         |
| 5     | Sg 139      | ✓         |          | 32   | Sg 541    | ✓         |
| 6     | Sg 150      | ✓         |          | 33   | Sg 542    | ✓         |
| 7     | Sg 151      | ✓         |          | 34   | Sg 543    | ✓         |
| 8     | Sg 153      | ✓         | ✓        | 35   | Sg 544    | ✓         |
| 9     | Sg 200      | ✓         |          | 36   | Sg 545    | ✓         |
| 10    | Sg 212      | ✓         |          | 37   | Sg 546    | ✓         |
| 11    | Sg 298      | ✓         |          | 38   | Sg 547    | ✓         |
| 12    | Sg 334      | ✓         | ✓        | 39   | Sg 548    | ✓         |
| 13    | Sg 384      | ✓         | ✓        | 40   | Sg 549    | ✓         |
| 14    | Sg 409      | ✓         | ✓        | 41   | Sg 550    | ✓         |
| 15    | Sg 431      | ✓         |          | 42   | Sg 551    | ✓         |
| 16    | Sg 445      | ✓         |          | 43   | Sg 552    | ✓         |
| 17    | Sg 457      | ✓         |          | 44   | Sg 553    | ✓         |
| 18    | Sg 492      | ✓         |          | 45   | Sg 554    | ✓         |
| 19    | Sg 510      | ✓         |          | 46   | Sg 555    | ✓         |
| 20    | Sg 519      | ✓         |          | 47   | Sg 556    | ✓         |
| 21    | Sg 520      | ✓         |          | 48   | Sg 557    | ✓         |
| 22    | Sg 521      | ✓         |          | 49   | Sg 558    | ✓         |
| 23    | Sg 526      | ✓         |          | 50   | Sg 559    | ✓         |
| 24    | Sg 528      | ✓         |          | 51   | Sg 560    | ✓         |
| 25    | Sg 529      | ✓         |          | 52   | Sg 561    | ✓         |
| 26    | Sg 530      | ✓         |          | 53   | Sg 562    | ✓         |
| 27    | Sg 531      | ✓         |          |      |          | ✓         |

Table 3. Differential reaction of the isolates selected for developing F₁ progenies.

| Pathotype | Mating Type | Percent Disease Incidence on Host Differential Lines |
|-----------|-------------|--------------------------------------------------|
|           |             | 700651 | 7042 R | 7042 S | 852 B | ICMP451 | IP18292 | IP18293 | P310-17 | P7-4 |
| Sg 018    | Mat-1       | 4     | 47    | 97     | 0     | 94      | 96      | 4       | 0       | 8    |
| Sg 019    | Mat-2       | 0     | 38    | 95     | 0     | 91      | 98      | 0       | 0       | 3    |
| Sg 445    | ?           | 53    | 75    | 100    | 100   | 100     | 100     | 80      | 46      | 63   | 86   |

3.4. Identification of Sexual Compatibility Types and Self-Sterile/Fertile Nature of F₁ Progenies

A total of 39 F₁ progenies were derived from the cross of Sg 019 Mat-2, G₂ × Sg 445-1 Mat-1, G₁. In contrast to the distinct mating types of the parents (G₁ and G₂), progenies were of four compatibility types viz. G₁, G₂, G₁G₂ and G₀ (neuter) (Table 4). Of 39 F₁ progenies, four belonged to G₁, 13 to G₂, 21 G₁G₂ and one to neuter categories (Tables 4 and 5). Further, the self-fertile or self-sterile nature of all the 39 F₁ progenies was evaluated on the basis of production of oospores. Among 21 G₁G₂ progenies, 19 supported self-production of oospores while 2 were free of any oospores in the matured leaves. Out of four G₁ progenies, oospores were observed in three progenies and one was recorded as a non-oospore producer when selfed. Of the 13 G₂ progenies, 7 supported self-production of oospores whereas no oospore formation was observed in the matured leaves infected with the remaining 6 F₁ progenies.
Table 4. Determination of sexual compatibility types of F₁ progenies based on oospores formation with Sg 445, Mat-1 (G₁) and Sg 019, Mat-2 (G₂).

| Population | Oospore Formation with Sg 445-1 (G₁) | Mating Type of Population | Self-Fertile/Sterile Remarks |
|------------|-------------------------------------|---------------------------|-----------------------------|
| P₁         | N                                   | Y                         | G₁                         | N                           | Heterothallic              |
| P₅         | Y                                   | N                         | G₂                         | N                           | Heterothallic              |
| P₆         | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₇         | Y                                   | N                         | G₂                         | N                           | Heterothallic              |
| P₈         | Y                                   | N                         | G₂                         | N                           | Heterothallic              |
| P₁₀        | N                                   | Y                         | G₁                         | Y                           | Secondary homothallic      |
| P₁₁        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₁₂        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₁₄        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₁₈        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₁₉        | Y                                   | N                         | G₂                         | N                           | Heterothallic              |
| P₂₀        | N                                   | Y                         | G₁                         | Y                           | Secondary homothallic      |
| P₂₁        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₂₂        | Y                                   | N                         | G₂                         | N                           | Heterothallic              |
| P₂₃        | Y                                   | Y                         | G₁ G₂                      | N                           | Heterothallic              |
| P₂₄        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₂₅        | Y                                   | Y                         | G₁ G₂                      | N                           | Heterothallic              |
| P₂₆        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₂₇        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₂₈        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₂₉        | Y                                   | Y                         | G₁ G₂                      | N                           | Heterothallic              |
| P₃₀        | N                                   | N                         | Neutral                    | Y                           | Neuter                     |
| P₃₁        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₃₂        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₃₃        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₃₄        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₃₅        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₃₆        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₃₇        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₃₈        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₃₉        | N                                   | Y                         | G₁                         | Y                           | Secondary homothallic      |
| P₄₀        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₁        | Y                                   | N                         | G₂                         | Y                           | Secondary homothallic      |
| P₄₂        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₃        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₄        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₅        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₆        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |
| P₄₇        | Y                                   | Y                         | G₁ G₂                      | Y                           | Homothallic                |

N = no oospore, Y = oospores formed.

One unique neuter (G₀) progeny was recorded as a non-oospore former, which was neither self-fertile nor produced oospore by crossing with any of the two parents. The F₁ progenies which produced oospore by crossing with both the parents were designated as G₁G₂. Both self-sterile and self-fertile progenies were observed among G₁G₂s. In S. graminicola, it is reported that oospore formation is very low when isolates are selfed, whereas the number of oospores formed is quite high when the isolates of different mating types are crossed [6,12]. Similar observations were made in the present study. In the case of selfed G₁G₂ F₁s, about 10 oospores were observed per leaf piece (1 cm²), whereas ~100-300 oospores were found when they were crossed with either of the parents. Thus, the 19 self-fertile (G₁G₂) progenies, which showed production of oospores with both parents, were designated as homothallic, while two self-sterile (G₁G₂) progenies were designated as heterothallic type (Table 5). Similarly, the self-fertile parental type G₁ and G₂ proge-
nies were denoted as secondary homothallic whereas self-sterile parental type G1 and G2 progenies were of heterothallic type.

Table 5. Summary of determination of sexual compatibility types of F1 populations based on oospore formation with Sg 445, Mat-1(G1) and Sg 019, Mat-2 (G2).

| No. of Progenies | Self-Fertile | Oospore Formation | Compatibility Types | Remarks |
|------------------|--------------|-------------------|---------------------|---------|
|                  |              | Sg 445-1           | Sg 019              |         |
| 19               | Y            | Y                  | Y                   | G1 G2   | Homothallic |
| 2                | N            | Y                  | Y                   | G1 G2   | Heterothallic |
| 3                | Y            | N                  | Y                   | G1      | Secondary homothallic |
| 1                | N            | N                  | Y                   | G1      | Heterothallic |
| 6                | N            | Y                  | N                   | G2      | Heterothallic |
| 7                | Y            | Y                  | N                   | G0      | Neuter |
| 1                | N            | N                  | N                   | G0      | Neuter |

N = no oospore, Y = oospores formed.

4. Discussion

The oospore formation in plant pathogenic oomycetes depends on the presence of two sexual compatibility types or their determinants [13–17]. In S. graminicola, two types of mating/compatibility types, viz. G1 and G2, have been proposed earlier [6,7,12] which are responsible for sexual reproduction between two self-sterile isolates, and within self-fertile isolates. Since sexual reproduction is dependent upon both compatibility types, it is speculated that the self-fertile isolates contain both compatibility types in the same seedling. The earlier studies [6,7,12] also reported self-fertile isolates and placed these isolates in G2 mating types tentatively and suggested that determination of sexual compatibility type in S. graminicola is likely to be complex and the nomenclature of G1/G2 compatibility types may not necessarily imply their distribution in a population. In addition, the neuter (sterile) type of S. graminicola isolate (Sg 110-2) was also observed [12], which failed to produce oospores with any of the parent isolates and was also placed under G1/G2 compatibility types.

Since vegetative structures of oomycetes exist in diploidy level, the mating type alleles have been reported to be controlled by a single mating type locus in Phytophthora spp. [11,18,19] due to equal numbers of A1 or A2 types in the progenies. However, skewed numbers of one or the other mating types have also been reported [10,20–22]. Although normal Mendelian segregation of alleles expects four different combinations of alleles for a given locus in the progenies of heterozygous parents, inheritance of mating type alleles of a single locus has been explained in three different ways to explicate the almost equal ratios of A1 and A2 progenies in Phytophthora spp. [11,18,20].

In the first model, one mating type is represented by heterozygous (A/a) condition and the other in homozygous (a/a) condition at the mating type locus in Phytophthora spp. [11,18,19] due to equal numbers of A1 or A2 types in the progenies. However, inconsistent ratios in the progenies of heterozygous (A/a) and homozygous (a/a) parents have been reported in contrast to this model [15,22,23]. The second model suggests the presence of balanced lethal loci due to survival of only two genotypes A1 (M1/Mn) and A2 (M2/Mn) instead of the four different genotypes (M1/Mn, M2/Mn, M1/M2 or M2Mn) in the progenies of A1 (M1/Mn) and A2 (M2/Mn) mating type parents in Phytophthora infestans [18]. The third model, a hybrid of the earlier two, explains the existence of ambiguous A1-A2 genotype in P. parasitica, which was consistent with the first model in which the A1 mating type was represented by heterozygous (M1A/MnA) and A2 homozygous (M1A/M1A) conditions for the alleles at the mating-type locus [11]. In contrary to all three models, the present study revealed four different compatibility types (4G1, 13G2, 21G1G2 and one G0, neuter) in 39 F1 progenies from the cross of two distinct self-sterile heterothallic parents (Sg 445-1 Mat-1, G1 × Sg 019 Mat-2, G2) that indicated normal Mendelian segregation of mating types (Table 6) in S. graminicola. In the earlier studies [6,12], four different compatibility
types were also noticed in *S. graminicola* though all the progenies were accommodated in G1/G2 compatibility types either due to skewed distribution of mating types or lack of nomenclature in *S. graminicola*. The discussed three models were found inadequate to explain the usual segregation in *S. graminicola* and unequal ratio of G1:G2 along with ambiguous G1G2 sexual compatibility types. Therefore, an alternative scheme for mating-type determination was considered and the segregation could be speculated due to presence of mating type alleles in heterozygous state in both parents [G1g1 (Mat-1) for G1 and G2g2 (Mat-2) for G2] at the same locus. In *Phytophthora*, isolates forming oospores only with the A1 or A2 testers are designated as A2 and A1, respectively, whereas the isolates which can form oospores with both A1 and A2 testers are designated as A1A2 and those that fail to form oospores are designated as A0 (sterile or neuter) [24] which supports the results of this study.

Table 6. Mendelian segregation of sexual compatibility types in two distinct self-sterile heterothallic parents (Sg 445-1, Mat-1, G1 × Sg 019, Mat-2, G2) of *Sclerospora graminicola*.

| G1g1 (Mat-1) × G2g2 (Mat-2) |
|-----------------------------|
| ↓                           |
| G1                          |
| G2                          |
| (Mat-1)                     |
| G1 g2 (Mat-2)               |
| G1 g2 (Mat-1)               |
| g1                          |
| g2                          |
| (Mat-2)                     |
| (G0, Neuter)                |

The mating system plays an important role in the evolution of plant pathogens during strong selection pressure from the resistant host or chemical control measures or harsh environmental conditions [25,26]. In oomycetes, the predominant co-existence of two mating types (G1 and G2 or A1 and A2) [6,7,11,12,18,19] and generation of multiple compatibility types (A1, A2, A1A2 and neuter) in the F1 progenies upon sexual reproduction between two distinct mating types (A1 × A2) [10,11] might provide advantage to pathogens during unfavorable conditions. *Sclerospora graminicola* has a high outcrossing capacity which renders the pathogen to evolve into new pathotype/s upon selection pressure and helps in adaptation to different ecosystems [12]. Therefore, effective management of downy mildew pathogen in pearl millet would be targeted towards understanding the change in population structure, particularly virulence pattern, and its utilization in resistance-breeding programs for the development of resistant cultivars.

**Author Contributions:** Conceptualization, R.S.; methodology, R.S. and C.R.; formal analysis, C.R.; investigation, C.R.; resources, R.S.; writing—original draft preparation, C.R.; writing—review and editing, R.S.; supervision, R.S.; project administration, R.S.; funding acquisition, R.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the CGIAR Research Program on Grain Legumes and Dryland Cereals (CRP-GLDC) and the Pearl Millet Hybrid Parents Research Consortium (PMHPRC).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data available at [http://dataverse.icrisat.org/privateurl.xhtml?token=f0c65f21-6a85-4b3f-901d-1803703d6311](http://dataverse.icrisat.org/privateurl.xhtml?token=f0c65f21-6a85-4b3f-901d-1803703d6311), accessed on 18 March 2022.

**Acknowledgments:** We thank P. Jaganmohan Rao for his help in establishing single zoospore isolates and greenhouse screenings.

**Conflicts of Interest:** The authors declare no conflict of interest.
References

1. Gupta, S.K.; Patil, K.S.; Rathore, A.; Yadav, D.V.; Sharma, L.D.; Mungra, K.D.; Patil, H.T.; Kumar, R.; Chaudhary, V.; Das, R.R.; et al. Identification of heterotic groups in South-Asian-bred hybrid parents of pearl millet. Theor. Appl. Genet. 2020, 133, 873–888. [CrossRef] [PubMed]

2. Chelputri, D.; Sharma, R.; Durga, K.K.; Katiyar, P.; Mahendrakar, M.D.; Singh, R.B.; Yadav, R.S.; Gupta, R.; Srivastava, R.K. Mapping quantitative trait loci (QTLs) associated with resistance to major pathotype-isolates of pearl millet downy mildew pathogen. Eur. J. Plant Pathol. 2019, 154, 983–994. [CrossRef]

3. Yadav, O.P.; Rai, K.N. Genetic improvement of pearl millet in India. Agric. Res. 2013, 2, 275–292. [CrossRef]

4. Sharma, R.; Upadhyaya, H.D.; Sharma, S.; Gate, V.L.; Raj, C. New sources of resistance to multiple pathotypes of *Sclerospora graminicola* in the pearl millet mini core germplasm collection. Crop Sci. 2015, 55, 1619–1628. [CrossRef]

5. Pushpavathi, B.; Thakur, R.P.; Rao, K.C. Inheritance of avirulence in *Sclerospora graminicola*, the pearl millet downy mildew pathogen. Plant Pathol. 2006, 5, 54–59. [CrossRef]

6. Michelmore, R.W.; Pawar, M.N.; Williams, R.J. Heterothallism in *Sclerospora graminicola*. Phytopathology 1982, 72, 1368–1372. [CrossRef]

7. Idris, M.O.; Ball, S.L. Inter-and intracontinental sexual compatibility in *Sclerospora graminicola*. Plant Pathol. 1984, 33, 219–223. [CrossRef]

8. Michelmore, R.W.; Ingram, D.S. Secondary homothallism in *Bremia lactucae*. Trans. Brit. Mycol. Soc. 1982, 78, 1–9. [CrossRef]

9. Galindo, A.; Gallegly, M.E. The nature of sexuality in *Phytophthora infestans*. Phytopathology 1960, 50, 123–128.

10. Khaki, I.A.; Shaw, D.S. The inheritance of drug resistance and compatibility type in *Phytophthora infestans*. Trans. Brit. Mycol. Soc. 1967, 50, 1–9. [CrossRef]

11. Fabritius, A.L.; Judelson, H.S. Mating-type loci segregate aberrantly in *Phytophthora infestans* but normally in *Phytophthora parasitica*: Implications for models of mating-type determination. Curr. Genet. 1997, 32, 60–65. [CrossRef] [PubMed]

12. Pushpavathi, B.; Thakur, R.P.; Rao, K.C. Fertility and mating type frequency in Indian isolates of *Sclerospora graminicola*, the downy mildew pathogen of pearl millet. Plant Dis. 2006, 90, 211–214. [CrossRef] [PubMed]

13. Bishop, H. A study of sexuality in *Saprolegnia reinischii*. Mycologia 1940, 32, 505–529. [CrossRef]

14. Papa, K.E.; Campbell, W.A.; Hendrix, F.F., Jr. Sexuality in *Pythium sylvaticum*: Heterothallism. Mycologia 1967, 59, 589–595. [CrossRef]

15. Gallegly, M.E. Genetics of pathogenicity of *Phytophthora infestans*. Annu. Rev. Phytopathol. 1968, 6, 375–396. [CrossRef]

16. Michelmore, R.W.; Sansome, E.R. Cytological studies of heterothallism and secondary homothallism in *Bremia lactucae*. Trans. Brit. Mycol. Soc. 1982, 79, 291–297. [CrossRef]

17. Brasier, C.M. Evolutionary biology of Phytophthora. Part I. Genetic system, sexuality and the generation of variation. Annu. Rev. Phytopathol. 1992, 30, 153–171. [CrossRef]

18. Judelson, H.S.; Spielman, L.J.; Shattock, R.C. Genetic mapping and non-Mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. Genetics 1995, 141, 503–512. [CrossRef]

19. Judelson, H.S. Genetic and physical variability at the mating type locus of the oomycete, *Phytophthora infestans*. Genetics 1996, 144, 1005–1013. [CrossRef]

20. Gallegly, M.E. Genetics of Phytophthora. Phytopathology 1970, 60, 1135–1141. [CrossRef]

21. Timmer, L.W.; Castro, J.; Erwin, D.C.; Belser, W.L.; Zentmyer, G.A. Genetic evidence for zygotic meiosis in *Phytophthora capsici*. Am. J. Bot. 1970, 57, 1211–1218. [CrossRef]

22. Shattock, R.C.; Tookey, P.W.; Fry, W.E. Genetics of *Phytophthora infestans*: Characterization of single-oospore cultures from A1 isolates induced to self by intraspecific stimulation. Phytopathology 1986, 76, 407–410. [CrossRef]

23. Spielman, L.J.; Sveigard, J.A.; Shattock, R.C.; Fry, W.E. The genetics of *Phytophthora infestans*: Segregation of allozyme markers in *F*₂ and backcross progeny and the inheritance of virulence against potato resistance genes *R*₂ and *R*₄ in *F*₁ progeny. Exp. Mycol. 1990, 14, 57–69. [CrossRef]

24. Ho, H.H. The Taxonomy and Biology of Phytophthora and Pythium. J Bacteriol Mycol Open Access 2018, 6, 174. [CrossRef]

25. Francis, D.M.; Clair, D.A.S. Population genetics of *Pythium ultimum*. Phytopathology 1997, 87, 454–461. [CrossRef]

26. Billiard, S.; López-Villavicencio, M.; Hood, M.E.; Giraud, T. Sex, outcrossing and mating types: Unsolved questions in fungi and beyond. J. Evol. Biol. 2012, 25, 1020–1038. [CrossRef]