Evaluation of sugarcane smut resistance in wild sugarcane (Saccharum spontaneum L.) accessions collected in Japan

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ABSTRACT
Sugarcane smut, caused by Sporisorium scitamineum, is one of the most important sugarcane diseases in Japan. Wild sugarcane, Saccharum spontaneum, is known to be a key breeding material to obtain high-yielding clones. In this study, we sought to identify Japanese wild sugarcane accessions with high resistance to smut. Thirty wild sugarcanes and three sugarcane cultivars were tested by the pinprick method. The results of the inoculation tests aided in identifying wild sugarcanes with high resistance to smut disease, namely JW90, Iriomote8, and Iriomote15. After screening the germplasm, progeny distribution of smut resistance from the inoculation test and dry matter productivity in the smut disease-free field were compared. The highly resistant wild sugarcane accession had a much better impact on progeny distribution of smut resistance compared with the susceptible accession. No relationship was found between smut resistance and dry matter productivity in both populations.

Modern sugarcane cultivars (Saccharum spp. hybrids) are derived from interspecific crossing between Saccharum officinarum and its relatives (Daniels & Roach, 1987). In particular, the wild sugarcane Saccharum spontaneum is known to be a key antecedent with outstanding characteristics such as tolerance to environmental stresses and ratooning ability. Thus, interspecific hybrids with high biomass productivity can be obtained by crossing with wild sugarcanes (Nagarajan, Alarmelu, & Shanthi, 2000; Roach, 1977; Wang et al., 2008).

In Japan, sugarcane breeders have tried to develop not only cultivars for sugar mills but also high-yielding cultivars for forage use through interspecific crossing. These efforts have led to the development of two cultivars for forage use by using an overseas wild sugarcane, Glagah Kloet (Sakaigaichi & Terajima, 2008; Sakaigaichi et al., 2014). However, these breeding programs might not necessarily be considered successful because most of the breeding lines crossed with Glagah Kloet are susceptible to smut disease.

Sugarcane smut, caused by Sporisorium scitamineum (previously called Ustilago scitaminea), is one of the most important sugarcane diseases in Japan and other sugarcane-growing countries. A typical symptom of this disease is the development of a whip-like sorus from the top of the infected stalks, and the spores of the exposed sorus are spread by wind and rain (Comstock, 2000). The best control practice is the use of resistant cultivars (Yamauchi, 1989; Comstock, 2000; Croft & Braithwaite, 2006). The use of cultivars susceptible to smut disease, such as NCo310 and Ni9, caused yield losses in Japan.

Wild sugarcanes grow naturally from Asia to parts of Oceania and Africa (Panje & Babu, 1960). Nagatomi et al. (1984), Hanada et al. (1991), and Shimoda et al. (2000) have indicated natural habitats of wild sugarcane in Japan. Wild sugarcane accessions collected in Japan have been conserved as genetic resources. Therefore, Japanese wild sugarcanes with high resistance to smut might exist and contribute to the improvement of current interspecific breeding programs. Despite their importance, limited scientific information exists on this aspect.

In this study, we first sought to identify Japanese wild sugarcanes having high resistance to smut. After screening the germplasm, we validated the effect of smut-resistant wild sugarcane as breeding material by comparing two populations – one derived from a highly resistant wild sugarcane accession (previously called Ustilago scitaminea) and another derived from a highly resistant wild sugarcane accession (previously called Ustilago scitaminea).
smut-resistant wild sugarcane and the other from a susceptible one – in terms of smut resistance and dry matter productivity.

Materials and methods

Experiment 1: evaluation of smut resistance of Japanese wild sugarcanes

This study was conducted at the NARO Kyushu Okinawa Agricultural Research Center in Nishinoomote, Kagoshima Prefecture, Japan (30°44′ N, 131°04′ E).

Thirty Japanese wild sugarcane accessions conserved as a sub-bank of the NARO Genebank Project were tested in Exp. 1. The collection sites and accession numbers of these wild sugarcanes are shown in Table 1 in accordance with the NARO Genebank database. All the wild sugarcanes used in this study were indigenous to Nansei Islands, where most of Japan’s sugarcane is grown. Of the 30 wild sugarcanes tested, 14 were from Okinawa Prefecture and 16 were from the Amami islands of Kagoshima Prefecture. The resistant cultivar NiF8 and the susceptible cultivars NCo310 and Ni9 were also studied for comparison.

Wild sugarcanes were conserved as ratoon crops by cutting the shoots every year. Single-bud stem cuttings (n = 60) were prepared from the stalks of wild sugarcanes for inoculation tests. When the number was lower than 60, we prepared as many stem cuttings as possible. Single-bud stem cuttings (n = 80) were prepared from NiF8, Ni9, and NCo310.

In Japan, only one race of the smut fungus appears to exist (Yamauchi, 1989). The teliospores used in the present study were obtained from the Okinawa Prefectural Agricultural Research Center. They were collected from the sori of naturally infected stalks of Ni9 in Okinawa Island. Inoculation was carried out by the pinprick method, as described in Yamauchi (1989).

A concentration of \(10^8\) ml\(^{-1}\) teliospores was applied around the injured points. After inoculation, the plants were kept under high humidity for over 3 days. The plants were transplanted into plastic containers filled with sterilized soil and grown in a greenhouse. The number of infected plants was counted at least once a

Table 1. Smut resistance of Japanese wild sugarcanes.

| Collection site | Accession name | JP number | 2004 autumn | 2005 spring | 2006 autumn | 2007 spring | 2008 autumn | 2014 spring | Average | Smut group |
|----------------|----------------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|---------|------------|
| Okinawa Prefecture | Miyakojima Is. | JW69 | 174,473 | 6.7 | 4.7 | 17.0 | 9.4 | 4 | 3 |
| | Miyakojima Is. | JW70 | 152,907 | 4.3 | 7.1 | 5.0 | 5.5 | 3 | 1 |
| | Miyakojima Is. | JW90 | 174,477 | 0.0 | 0.0 | 0.0 | 0.0 | 1 |
| | Iriomote Is. | JW195 | 174,481 | 12.5 | 26.3 | 19.4 | |
| | Iriomote Is. | JW259 | 174,484 | 7.3 | 8.6 | 21.0 | 12.3 | 4 |
| | Iriomote Is. | JW289 | 174,487 | 31.8 | 25.0 | | 28.4 | 5 |
| | Iriomote Is. | JW331 | 174,488 | 38.2 | 36.8 | | 37.5 | 6 |
| | Iriomote Is. | Iriomote8 | 175,508 | 0.0 | 0.0 | 0.0 | 1 |
| | Iriomote Is. | Iriomote10 | 175,510 | 12.5 | 2.1 | 5.0 | 6.5 | 3 |
| | Iriomote Is. | Iriomote15 | 175,515 | 0.0 | 0.0 | | 0.0 | 1 |
| | Iriomote Is. | Iriomote27 | 175,527 | 38.1 | 23.8 | | 31.0 | 6 |
| | Iriomote Is. | Iriomote28 | 175,528 | 0.0 | 0.0 | 0.0 | 0.0 | 1 |
| | Iriomote Is. | Iriomote31 | 175,531 | 38.7 | 45.0 | 19.0 | 34.2 | 6 |
| | Iriomote Is. | Iriomote37 | 175,537 | 67.7 | 23.5 | 33.0 | 41.4 | 6 |
| Kagoshima Prefecture | Tokunoshima Is. | T1 | 172,000 | 61.4 | 83.0 | 72.2 | 7 |
| | Tokunoshima Is. | T10 | 172,004 | 0.0 | 2.9 | 1.5 | 2 |
| | Tokunoshima Is. | T16 | 172,006 | 0.0 | 0.0 | 0.0 | 1 |
| | Tokunoshima Is. | T19 | 172,007 | 55.9 | 68.8 | 64.8 | 63.2 | 7 |
| | Kikai Is. | K4 | 171,997 | 32.0 | 29.0 | 30.5 | 6 |
| | Kikai Is. | K5 | 171,998 | 31.6 | 34.6 | 33.1 | 6 |
| | Tokunoshima Is. | 99JW-9 | 232,222 | 5.5 | 0.0 | 2.7 | 3 |
| | Tokunoshima Is. | 99JW-10 | 232,223 | 4.3 | 8.7 | 6.5 | 3 |
| | Amamioshiro Is. | 99JW-14 | 232,227 | 46.6 | 22.9 | 34.7 | 6 |
| | Amamioshiro Is. | 99JW-15 | 232,228 | 6.1 | 9.0 | 5.0 | 3 |
| | Amamioshiro Is. | 99JW-17 | 232,230 | 39.2 | 27.3 | 33.2 | 6 |
| | Amamioshiro Is. | 99JW-18 | 232,231 | 0.0 | 8.0 | 2.7 | 2 |
| | Amamioshiro Is. | 99JW-19 | 232,232 | 4.2 | 0.0 | 6.0 | 3.4 | 2 |
| | Kikai Is. | 99JW-20 | 232,233 | 12.1 | 2.3 | 4.8 | 3 |
| | Kikai Is. | 99JW-23 | 232,236 | 6.7 | 4.9 | 13.0 | 8.2 | 3 |
| | Kikai Is. | 99JW-26 | 232,239 | 10.9 | 5.1 | 0.0 | 5.3 | 3 |
| Cultivars | NiF8 | 172,051 | 16.4 | 11.6 | 15.2 | 5.7 | 23.6 | 7.0 | 2.0 | 1.5 | 10.4 | 4 |
| | NCo310 | 171,861 | 64.7 | 70.9 | 50.0 | 62.5 | 87.7 | 72.0 | 35.6 | 63.4 | 7 |
| | Ni9 | 172,865 | 66.2 | 54.7 | 73.8 | 58.1 | 82.1 | 49.1 | 64.0 | 7 |

JP numbers indicate the serial numbers used for managing germplasm at the NARO Genebank.

Smut groups were classified on the basis of smut infection percentage by model selection using stepwise regression analysis (minimum AICc, 2877.09).
month for approximately 11 months. We judged the plants as infected when the whip-like sori emerged. Any infected plants were removed from the containers. Infection percentage was calculated by the following formula.

\[
\text{Infection percentage (\%)} = \left(\frac{\text{infected plants}}{\text{total germinated plants}}\right) \times 100
\]

Inoculation tests were conducted eight times from 2004 to 2015. Inoculation was carried out in the spring season (May to June) or autumn season (October to November). Each wild sugarcane accession was tested repeatedly twice or thrice. Only NiF8 was examined every time, and NCo310 and Ni9 were tested seven times and six times, respectively.

**Experiment 2: smut resistance and dry matter productivity of two populations derived from resistant and susceptible wild sugarcanes**

In Exp. 1, we focused on screening Japanese wild sugarcanes for smut resistance. In Exp. 2, we validated the effect of resistant wild sugarcanes on the distribution of smut resistance from inoculation test and dry matter productivity from field test by comparing the two populations, one derived from a highly resistant wild sugarcane and the other from a susceptible one.

According to Exp.1., Iriomote15 and Iriomote37 were chosen as highly resistant and susceptible wild sugarcane accessions, respectively. These two wild sugarcanes were collected from the same island called ‘Iriomote Island’ (Hanada et al., 1991). According to the NARO Genebank database and our visual-observation, the stem length and stem diameter of Iriomote15 and Iriomote37 are almost the same. Twenty-two breeding lines derived from NiF8 and Iriomote15 were tested. Twenty other breeding lines derived from NiF8 and Iriomote37 were also tested. These clones were grown from 2009 to 2012 in the field of NARO/KARC where no smut disease was observed even though the susceptible cultivars such as Ni9 and NCo310 are grown.

To establish the plot, single-bud stem cuttings of these progenies were planted on 16 April 2009 at a planting density of 6.36 buds m⁻², with 110 cm between rows. The area of each plot was 1.65 m², with a 1.5 m row in each plot without replication. For each crop, chemical fertilizer was applied as a basal dressing (7.2g N m⁻², 12.0g P₂O₅ m⁻², and 6.0 g K₂O m⁻²) and as a topdressing (9.0g N m⁻², 0.0 g P₂O₅ m⁻², and 9.0 g K₂O m⁻²).

Each progeny was harvested on 20 October 2009 as a plant crop; 27 September 2010, as the 1st ratoon crop; and 22 September 2011, as the 2nd ratoon crop. Single-bud stem cuttings (n = 100) were prepared from the stalks of wild sugarcanes for inoculation tests. When the number was lower than 100, we prepared as many stem cuttings as possible. The inoculation method was identical to that described in Exp. 1. Inoculation tests were repeated three times from 2009 to 2012. Inoculation in the tests was carried out in autumn (October to November).

The shoot dry matter yields per year were also compared in the 1st and 2nd ratoon crops as follows. At harvest, stem number was defined as the number of tillers. Meanwhile, to measure stem dry weight, we sampled three stems that exhibited healthy and typical growth from a row, chopped them into small pieces, and dried them at 80°C for 48 h. Shoot dry matter yield was then calculated as the product of stem number and single stem dry weight.

**Statistical analysis**

In Exp.1, Japanese wild sugarcanes and cultivars were classified on the basis of the results of the smut inoculation test by model selection using stepwise regression analysis (JMP ver. 11.2, SAS Institute). In this model, we assessed the effect of germplasm, tested year, and season on the number of infected or non-infected plants in inoculation test. When the corrected Akaike’s information criterion (AICc) was minimum, the group composition was determined.

In Exp.2, statistical analysis was conducted using the software package (SPSS ver. 21.0, IBM). The median values of smut infection percentage and shoot dry matter yields of the two different populations were analyzed by a nonparametric test, Mann–Whitney U-test.

**Results and discussion**

**Experiment 1: evaluation of smut resistance of Japanese wild sugarcanes**

The resistant cultivar NiF8 and the susceptible cultivars NCo310 and Ni9 were used for comparison in this study. The average infection percentages of NiF8, NCo310, and Ni9 were 10.4%, 63.4%, and 64.0%, respectively (Table1). Smut resistance of Japanese wild sugarcanes indicated a considerable variation, and the average infection percentages ranged from 0.0% (JW90, Iriomote8, Iriomote15, Iriomote28, and T16) to 63.2% (T19) and 72.2% (T1) (Table1). Alexander, Rao, Mohanraj, Prakasam, and Padmanaban (1991) and Burner, Grisham, and Legendre (1993) investigated the smut resistance of wild sugarcanes from India, Indonesia, and Philippines. The present...
This study is the first to identify Japanese wild sugarcanes with high resistance to smut disease.

Thirty Japanese wild sugarcanes and three cultivars were classified into seven groups by model selection, as shown in Table 1 (minimum AICc, 2877.09). The highly resistant group, group 1, contained JW90, Iriomote8, Iriomote15, Iriomote28, and T16. A resistant cultivar, NiF8, was classified into the moderately resistant group, group 4. The susceptible cultivars NCo310 and Ni9 were classified into the highly susceptible group, group 7, and T1 and T19 were in the same group. Large variations in smut resistance were found among the wild sugarcanes collected from the same islands of Iriomote and Tokunoshima, although their natural habitats are closely located (Table 1).

The barriers to infection of sugarcane smut depend on bud morphology as well as host physiology (Dean, 1982). The popular inoculation method for screening resistant clones is to dip stem cuttings in a suspension of Sporisorium scitaminea. In the present study, the inoculation test was carried out by the pinprick method, in which smut teliospores were injected into the apical meristems. Although we have no scientific data on this topic, not bud morphology but host physiology might cause the difference to smut disease among Japanese wild sugarcanes.

**Experiment 2: smut resistance and dry matter productivity of two populations derived from resistant and susceptible wild sugarcanes**

Smut infection percentage is shown by the average of three replications from the plant crop to the 2nd ratoon crop (Figure 1). The population derived from NiF8 and Iriomote15 demonstrated variation in smut resistance. The infection percentage ranged from 0.4% to 76.6%, and the median value was 13.0%. Smut infection percentage of the other population derived from NiF8 and Iriomote37 ranged from 16.2% to 86.0%, with median value of 48.0%. The difference between the two median values was significant at the 1% level of probability, as calculated by the Mann–Whitney U-test.

As clearly shown in Figure 1, the highly resistant wild sugarcane had a much better impact on its progeny’s distribution of smut resistance when compared with the susceptible wild sugarcane. In sugarcane cultivars and breeding lines, it is known that the use of resistant parents enhance the percentage of resistant progenies (Chao, Hoy, Saxton, & Martin, 1990; Wu, Heindz, & Meyer, 1983). To our knowledge, the present study is the first to experimentally demonstrate the importance of wild sugarcane as breeding material of smut resistance.

Shoot dry matter yield was also investigated in the field where no smut disease was observed. The yield is indicated by the average of two replications from the 1st ratoon crop to the 2nd ratoon crop (Figure 2). Shoot dry matter yields of the population from NiF8 and Iriomote15 ranged from 1.57 kg m⁻² yr⁻¹ to 4.93 kg m⁻² yr⁻¹, with a median value of 3.13 kg m⁻² yr⁻¹. Shoot dry matter yield of the population derived from NiF8 and Iriomote37 ranged from 1.81 kg m⁻² yr⁻¹ to 5.70 kg m⁻² yr⁻¹, with a median value of 3.31 kg m⁻² yr⁻¹. The difference between the two median values was not significant as calculated by the Mann-Whitney U-test. Wu et al. (1983) indicated that there was no correlation...
between smut grade and stalk diameter, stalk number, or plant volume from the progenies of diallel crosses by using clones for sugar mills. In the present study, there was no relationship between smut resistance from inoculation test and dry matter productivity in the field in both populations (Figure 3). Our results are in agreement with those of Wu et al. (1983), indicating that it is possible to breed lines with both high smut resistance and high yield productivity.

As mentioned above, the wild sugarcane is known to have outstanding characteristics, and interspecific hybrids with high biomass productivity can be obtained by crossing with wild sugarcanes (Nagarajan et al., 2000; Roach, 1977; Wang et al., 2008). As described in Yamauchi (1989), Japan has only one race of the smut fungus so far. Taken together, our findings on the identification of Japanese wild sugarcanes with high resistance to smut disease contribute to the improvement of sugarcane breeding program in Japan.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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