Pollen–Pistil Interactions Result in Reproductive Isolation between *Sorghum bicolor* and Divergent *Sorghum* Species

George L. Hodnett, Byron L. Burson, William L. Rooney, Sally L. Dillon, and H. James Price*

**ABSTRACT**

*Sorghum [Sorghum bicolor (L.) Moench]* breeders have long recognized the importance of exotic germplasm and noncultivated sorghum races as sources of valuable genes for genetic improvement. The genus *Sorghum* consists of 25 species classified as five sections: *Eu-sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum*, and *Stiposorghum*. Species outside the *Eu-sorghum* section are sources of important genes for sorghum improvement, including those for insect and disease resistance, but these have not been used because of the failure of these species to cross with sorghum. An understanding of the biological nature of the incompatibility system(s) that prevent hybridization and/or seed development is necessary for the successful hybridization and introgression between sorghum and divergent *Sorghum* species. The objectives of this study were to determine the reason(s) for reproductive isolation between *Sorghum* species. The current study utilized 14 alien *Sorghum* species and established that pollen–pistil incompatibilities are the primary reasons that hybrids with sorghum are not obtained. The alien pollen tubes showed major inhibition of growth in sorghum pistils and seldom grew beyond the stigma. Pollen tubes of only three species grew into the ovary of sorghum. Fertilization and subsequent embryo development were not common. Seeds with developing embryos aborted before maturation, apparently because of breakdown of the endosperm.

**S**orghum is a major cereal crop of marginal rainfall areas of the tropics, with selected varieties widely grown in temperate regions. Recent annual grain sorghum yields have exceeded 61 000 000 Mg worldwide, with 13 207 000 Mg produced in the USA (Smith, 2000). Twenty-five species form the genus *Sorghum* (Lazarides et al., 1991) which consists of five subgenera or sections, *Eu-sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum*, and *Stiposorghum* (Garber, 1950; deWet, 1978). Members of the *Eu-sorghum* section have a natural range through Africa and southern Asia. The cultivated sorghum (*S. bicolor*) and its subspecies *dummondi* and *arundinaceum*, as well as the wild species *S. alnum* Parodi, *S. propinquum* (Kunth) Hitchc., and Johnsongrass [*S. halepense* (L.) Pers.] are in the *Eu-sorghum* section (deWet, 1978). *Chaetosorghum* and *Heterosorghum* are monotypic sections that are native to the Australo-Pacific region; whereas, the *Para-sorghum* section consists of seven Asian, Australian, and central American species (Lazarides et al., 1991). Ten species that occur in northern Australia comprise the *Stiposorghum* section (Lazarides et al., 1991).

*Sorghum* breeders have long recognized the importance of exotic germplasm (Duncan et al., 1991). Noncultivated sorghum races have been extensively used as sources of genes for sorghum improvement (Rosenow and Dahlberg, 2000). However, no species outside the *eu-sorghum* section have been utilized because of strong reproductive barriers (Garber, 1950; Schertz and Dalton, 1980; Doggett, 1988). Resistance to major insects and diseases, for example, midge [Stenodiplosis (Contarinia) sorghicola (Coquillett)] and downy mildew [caused by *Peronosclerospora sorghi* (Weston and Uppal) Shaw], that attack sorghum has been found in species of the *Chaetosorghum*, *Heterosorghum*, *Para-sorghum*, and *Stiposorghum* sections (Franzmann and Hardy, 1996; Sharma and Franzmann, 2001; Kamala et al., 2002). These species are potential sources of resistance genes (Hacker et al., 1992).

A prerequisite for using wild species as germplasm is successful hybridization and backcrossing. It is apparent that the production of hybrids between sorghum and diverse *Sorghum* species will require an understanding of the biological nature of the incompatibility system(s) that prevent hybridization and/or seed development. Reproductive barriers occur at both the prezygotic or postzygotic levels. Prezygotic mechanisms may involve the failure of pollen germination, pollen tube growth, and/or fertilization. Postzygotic mechanisms include embryo lethality due to genotypic interactions or embryo death following abortion of the endosperm. There is little reported in the literature characterizing reproductive barriers between sorghum and wild *Sorghum* species. Sun et al. (1991) studied pollen tube growth in reciprocal pollinations between sorghum and *S. versicolor* Anderss. They determined that the primary reason for reproductive isolation in reciprocal crosses between the two species was the inhibition of pollen tube growth.

If hybrids can be produced between sorghum and wild species outside the *Eu-sorghum* section, additional barriers to introgression may occur. The base chromosome number of species in the *Para-sorghum* and *Stiposorghum* sections is *x* = 5; whereas, in the *Eu-sorghum* section, including sorghum, it is *x* = 10. Species belonging to the *Para-sorghum* and *Stiposorghum* sections have larger chromosomes than those of sorghum, and their genome size is up to 2.5 times larger than sorghum (Price et al., 2005). Hybrids between sorghum and species of the *Para-sorghum* and *Stiposorghum* sections would likely be sterile because of reduced or lack of chromosome

**Abbreviations:** EBN, endosperm balance number.

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pairing and irregular chromosome segregation during meiosis involving members of the $x = 5$ and $x = 10$ genomes. The monotypic sections Heterosorghum (S. laxiflorum Bailey) and Chaetosorghum (S. macrospernum Garber) are more closely related to Eu-sorghum, based on morphology (Garber, 1950), phylogenetic affinity (Dillon et al., 2004), karyotype (Wu, 1990, 1993), and genome size (Price et al., 2005). Although they are apparently polyploid ($2n = 40$), the karyotypic similarities suggest that chromosome pairing would be more likely during meiosis in hybrids between sorghum and both S. laxiflorum and S. macrospernum than in hybrids between sorghum and species with a base number of $x = 5$. Therefore, S. laxiflorum and S. macrospernum may be the most promising for introgression into sorghum.

The objectives of this research were to observe pollen germination and tube growth of divergent Sorghum species in sorghum pistils to determine if pistil–pollen interactions contribute to reproductive isolation between sorghum and species with a base number of $x = 5$ and $x = 10$.

**MATERIALS AND METHODS**

**Plant Materials**

Accession, herbarium voucher, and chromosome numbers of the 14 wild Sorghum species are listed in Table 1. The cultivated sorghum used was a paired inbred line (BTx623 and ATx623) with subtropical adaptation that is widely used as the female parent in the production of commercial hybrids. The ATx623 plants are male sterile; whereas, the BTx623 plants are male fertile. All plants were grown in greenhouses at College Station, TX, and were maintained at a temperature from 20 to 33°C. The plants flowered from January through May without any supplemental light.

**Pollenization Techniques**

To determine if pollen–pistil interactions contribute to the reproductive isolation between sorghum and Sorghum species classified in sections other than Eu-sorghum, pollen from one plant of each accession of 14 different Sorghum species (Table 1) was transferred to receptive sorghum stigmas. Pollen was collected at anthesis and brushed onto stigmas of the cytoplasmic male-sterile sorghum line, ATx623. To prevent contamination, the male-sterile sorghum plants were grown in a different greenhouse from the male-fertile plants.

**Fixation and Storage of Inflorescences**

Inflorescences were collected at intervals after pollination and fixed in either FAA (18:1:1, 70% ethanol/glacial acetic acid/formaldehyde) or 3:1, 95% ethanol/glacial acetic acid for 12 to 18 h. Initially FAA was used but slight discoloration of the pistils occurred. Discoloration did not occur in tissues fixed in 3:1, 95% ethanol/glacial acetic acid and, therefore, it was the preferred fixative. After fixation, the pistils were excised from the inflorescences and stored in 70% ethanol at $-20$°C until examined.

**Quantification of Pollen Germination and Pollen Tube Growth**

Pistils were processed using a slightly modified version of the protocol described by Kho and Baer (1968). Pistils were cleared and softened in $0.8 \ M$ NaOH overnight, stained with 0.025% (w/v) aniline blue in 0.1 $M$ K$_3$PO$_4$, for approximately 30 min, and mounted on microscope slides in 50% 0.1 $M$ K$_3$PO$_4$ and 50% glycerol. The slides were kept in the dark until observed with a Zeiss Universal II microscope (Carl Zeiss Inc., Gottingen, Germany). Callose, a β-1, 3-polyglucan, occurs in pollen tubes, and when exposed to aniline blue stain, it fluoresces under 350- to 400-nm light (Martin, 1959; Dumas and Knox, 1983). Fluorescence was induced using 390- to 420-nm light filtered from a mercury lamp with a 450-nm emission filter. Images were captured with an Optronics VI-470 system (Optronics Inc., Goleta, CA) and digitally stored and processed with Optimas (v. 6.1) image analysis software (Optimas Corp., Bothell, WA).

For a control, pollen from the male-fertile sorghum line, BTx623, was transferred onto stigmas of its cytoplasmic male-sterile counterpart, ATx623. Pollen germination and pollen tube growth were observed at 45 min and 1 h following pollination. Germination of alien pollen and the extent of tube growth

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**Table 1. Fifteen Sorghum species used in this study.**

<table>
<thead>
<tr>
<th>Section</th>
<th>Species</th>
<th>Accession number</th>
<th>Herbarium voucher number</th>
<th>$2n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaetosorghum</td>
<td>S. macrosperrum Garber</td>
<td>302367</td>
<td>DNA C867</td>
<td>40</td>
</tr>
<tr>
<td>Para-sorghum</td>
<td>S. leioladum (Hack.) C. E. Hubb</td>
<td>300184</td>
<td>DNA D015602</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. matarankense Garber &amp; Snyder</td>
<td>302517</td>
<td>BRI AQ773676</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. nitidum (Vahl.) Pers.</td>
<td>302542</td>
<td>BRI AQ740677</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>S. purpureo-sericem (A. Rich.) Aschers &amp; Schweinf.</td>
<td>318068</td>
<td>IS 18945</td>
<td>10</td>
</tr>
<tr>
<td>Stiposorghum</td>
<td>S. amplum Lazarides</td>
<td>302455</td>
<td>CANB 480260</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>S. angustus S. T. Blake</td>
<td>302605</td>
<td>BRI AQ585981</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. brachypodium Lazarides</td>
<td>302480</td>
<td>CANB 480297</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. bulbosum Lazarides</td>
<td>302482</td>
<td>JC 2129</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. ecarinatum Lazarides</td>
<td>302648</td>
<td>DNA D129449</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. interjectum Lazarides</td>
<td>302445</td>
<td>JC 2087</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>S. intras F. Muell. ex Benth.</td>
<td>302390</td>
<td>BRI AQ773629</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. intras F. Muell. ex Benth.</td>
<td>302476</td>
<td>BRI AQ773632</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. plumosum (R. Br.) F. Beauv.</td>
<td>302635</td>
<td>DNA D129468</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>S. timoreense (Kunth) Buser</td>
<td>302494</td>
<td>DNA C869</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. timoreense (Kunth) Buser</td>
<td>302632</td>
<td>DNA D129471</td>
<td>10</td>
</tr>
<tr>
<td>Eu-sorghum</td>
<td>S. bicolor (L.) Moench</td>
<td>ATx623</td>
<td>Male fertile</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Cytoplasmic male sterile</td>
<td>ATx623</td>
<td>Male fertile</td>
<td>20</td>
</tr>
</tbody>
</table>

‡ AusTRCF number, Australian Tropical Crops and Forages Collection, Queensland Department of Primary Industries and Fisheries.

§ CANB, Australian National Herbarium, Canberra, ACT Australia; BRI, Queensland Herbarium, Mt. Coot-tha, QLD Australia; DNA, Northern Territory Herbarium, Darwin, NT Australia; JC, Jeff Corfield Collection, Townsville, QLD Australia; IS, ICRI SAT.

† Price et al., 2005.
on sorghum pistils were initially recorded for pollinations involving all species at 24 h after pollination. Statistical analysis of pollen germination and pollen tube growth into the stigma branches, stigma axis, style, and ovary was completed in a completely randomized design using data from individual pistils for replication. Because different numbers of pistils were evaluated for each species, a general linear model was used and differences among means were detected using a Fisher protected LSD (Steel and Torrie, 1980). A subset consisting of *S. angustum* S.T. Blake, *S. ecarinatum* Lazarides, *S. macrospermum*, *S. matarankense* Garber & Snyder, and *S. purpureo-sericeum* (A. Rich.) Aschers & Schweinf., was used to further characterize the progress of pollen tube growth at selected earlier intervals between 2 and 12 h. These were chosen because pollen tubes of these five species had grown into the style or ovary of *S. bicolor* by 24 h.

Detection of Fertilization and Embryo Development

Pollen from *S. ecarinatum*, *S. macrospermum*, and *S. matarankense* were individually dusted on stigmas of male-sterile sorghum ATx623. On Day 15 post-pollination, florets were dissected and examined for embryo formation using a dissecting microscope.

RESULTS

Pollen Germination and Pollen Tube Growth in *S. bicolor*

More than 98% of the *S. bicolor* BTx623 pollen germinated when placed on stigmas of ATx623. The pollen tubes rapidly entered the stigma branches and within 45 min post-pollination they had grown into the stigma branches, axis of the stigma, and the style, respectively, but not into the ovary. Pollen tubes had grown through the ovary within 1 h after pollination (Table 2). Figures 1A and 1B show pollen tubes of BTx623 in the stigmas and ovary, respectively, of ATx623 at 1 h after pollination.

Table 2. Pollen germination and tube growth in *Sorghum bicolor* (ATx623) pistils following pollination with alien *Sorghum* species.

<table>
<thead>
<tr>
<th>Pollen source</th>
<th>Time after pollination</th>
<th>Pistils observed</th>
<th>Pollen grains observed</th>
<th>Pollen germination</th>
<th>Stigma branches</th>
<th>Stigma axis</th>
<th>Style</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. bicolor</em> (control)†</td>
<td>1</td>
<td>15</td>
<td>643</td>
<td>98.2</td>
<td>80.3</td>
<td>56.0</td>
<td>13.1</td>
<td>8.1</td>
</tr>
<tr>
<td><em>S. purpureo-sericeum</em></td>
<td>24</td>
<td>12</td>
<td>1595</td>
<td>94.8</td>
<td>13.0</td>
<td>4.6</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. macropermum</em></td>
<td>24</td>
<td>16</td>
<td>1235</td>
<td>92.3</td>
<td>40.9</td>
<td>16.4</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td><em>S. angustum</em></td>
<td>24</td>
<td>22</td>
<td>1283</td>
<td>89.1</td>
<td>50.8</td>
<td>5.8</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. ecarinatum</em></td>
<td>24</td>
<td>12</td>
<td>1830</td>
<td>88.5</td>
<td>39.2</td>
<td>5.6</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. timorense</em> (302632)</td>
<td>24</td>
<td>17</td>
<td>1065</td>
<td>81.2</td>
<td>28.0</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. matarankense</em></td>
<td>24</td>
<td>25</td>
<td>2726</td>
<td>81.0</td>
<td>46.8</td>
<td>13.2</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td><em>S. brachypodum</em></td>
<td>24</td>
<td>18</td>
<td>1045</td>
<td>74.5</td>
<td>35.4</td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. plumosum</em></td>
<td>24</td>
<td>41</td>
<td>1426</td>
<td>73.8</td>
<td>18.9</td>
<td>1.3</td>
<td>0.2</td>
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</tr>
<tr>
<td><em>S. intrans</em> (302390)</td>
<td>24</td>
<td>29</td>
<td>1394</td>
<td>71.0</td>
<td>24.1</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. nitidum</em></td>
<td>24</td>
<td>38</td>
<td>762</td>
<td>71.0</td>
<td>13.3</td>
<td>0.1</td>
<td>0.0</td>
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<tr>
<td><em>S. intrans</em> (302476)</td>
<td>24</td>
<td>15</td>
<td>1974</td>
<td>76.9</td>
<td>23.7</td>
<td>1.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. timorense</em> (302494)</td>
<td>24</td>
<td>15</td>
<td>1082</td>
<td>66.2</td>
<td>5.1</td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td><em>S. amplus</em></td>
<td>24</td>
<td>18</td>
<td>1488</td>
<td>65.9</td>
<td>12.0</td>
<td>0.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>S. interjectum</em></td>
<td>24</td>
<td>18</td>
<td>1315</td>
<td>65.8</td>
<td>29.3</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td><em>S. bulbosum</em></td>
<td>24</td>
<td>14</td>
<td>1428</td>
<td>52.2</td>
<td>31.9</td>
<td>0.2</td>
<td>0.0</td>
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</tr>
<tr>
<td><em>S. leiocladum</em></td>
<td>24</td>
<td>5</td>
<td>148</td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>71.5</td>
<td>27.9</td>
<td>1.3</td>
<td>0.4</td>
<td>6.2</td>
</tr>
</tbody>
</table>

† *Sorghum bicolor* (ATx623) × *S. bicolor* (BTx623) was used as a control and the pollen tubes had grown into and through the ovary by 1 h post-pollination.
the style in a crooked manner (Fig. 1E, 1G). In addition to growth inhibition and crooked growth paths, several other aberrant forms of alien pollen tube growth were observed in sorghum pistils that were not observed in the control. These included: (i) pollen tube growth in a convoluted fashion without entering a stigma branch (Fig. 1D); (ii) tubes growing parallel to the stigma branch without entering the stigma (Fig. 1H); (iii) tubes growing out of and then re-entering the stigma branch (Fig. 1I); (iv) tubes that grew toward the apex of the stigma (Fig. 1J); (v) swelling of the tip of the tube (Fig. 1K); and (vi) tube growth terminating at callose in the stigma axis (Fig. 1L). For *S. amplum*, *S. angustum*, *S. brachypodum* Laza-rides, *S. intrans* F. Muell. ex Benth., *S. nitidum* (Vahl.)
Pollen source & Time after pollination & Pistils observed & Pollen grains observed & Pollen germination & Stigma branches & Stigma axis & Style & Ovary & Pollen tube growth to the:
\hline
S. angustum & 2 & 17 & 1388 & 93.3 ± 1.8 & 17.1 ± 1.9 & 2.3 ± 0.5 & 0.4 ± 0.1 & 0.0 ± 0.0
& 4 & 15 & 914 & 93.7 ± 1.2 & 57.2 ± 2.4 & 9.3 ± 1.5 & 0.1 ± 0.1 & 0.0 ± 0.0
& 6 & 18 & 695 & 92.4 ± 1.9 & 24.6 ± 2.6 & 2.0 ± 0.5 & 0.0 ± 0.0 & 0.0 ± 0.0
& 12 & 17 & 969 & 95.4 ± 1.4 & 29.0 ± 6.1 & 0.5 ± 0.3 & 0.0 ± 0.0 & 0.0 ± 0.0
& 24 & 22 & 1283 & 89.1 ± 2.2 & 50.8 ± 4.7 & 5.8 ± 1.4 & 0.1 ± 0.1 & 0.0 ± 0.0
\hline
S. ecarinatum & 2 & 15 & 2327 & 98.7 ± 0.3 & 68.6 ± 2.6 & 16.7 ± 2.2 & 1.5 ± 0.6 & 0.1 ± 0.1
& 4 & 17 & 3022 & 96.6 ± 0.6 & 49.5 ± 2.1 & 11.0 ± 1.6 & 0.8 ± 0.4 & 0.1 ± 0.1
& 6 & 17 & 2107 & 92.0 ± 3.4 & 54.3 ± 4.9 & 16.9 ± 2.0 & 2.1 ± 0.4 & 0.2 ± 0.1
& 12 & 16 & 1615 & 90.3 ± 0.9 & 51.0 ± 2.1 & 10.7 ± 1.9 & 2.1 ± 0.1 & 0.0 ± 0.1
& 24 & 12 & 1830 & 88.5 ± 1.8 & 39.2 ± 2.3 & 5.6 ± 0.6 & 1.2 ± 0.3 & 0.1 ± 0.1
\hline
S. macrosporum & 2 & 16 & 794 & 91.1 ± 3.2 & 30.6 ± 3.0 & 13.0 ± 1.6 & 1.3 ± 0.7 & 0.1 ± 0.1
& 4 & 16 & 869 & 94.1 ± 1.9 & 26.2 ± 2.8 & 7.9 ± 0.7 & 1.3 ± 0.4 & 0.4 ± 0.1
& 6 & 19 & 1614 & 96.3 ± 0.6 & 38.0 ± 3.1 & 9.8 ± 1.8 & 1.1 ± 0.3 & 0.3 ± 0.1
& 12 & 26 & 2075 & 94.2 ± 0.8 & 36.1 ± 3.8 & 9.5 ± 1.0 & 1.5 ± 0.3 & 0.4 ± 0.1
& 24 & 16 & 1235 & 92.3 ± 1.7 & 40.9 ± 4.3 & 16.4 ± 2.9 & 1.9 ± 0.6 & 0.3 ± 0.2
\hline
S. matarankense & 2 & 17 & 3489 & 94.3 ± 0.7 & 63.7 ± 2.3 & 26.3 ± 1.9 & 1.8 ± 0.5 & 0.2 ± 0.1
& 4 & 20 & 3586 & 94.6 ± 0.5 & 61.0 ± 1.5 & 20.9 ± 2.2 & 3.0 ± 0.5 & 0.2 ± 0.1
& 6 & 16 & 3743 & 93.4 ± 1.7 & 52.5 ± 2.7 & 12.7 ± 1.6 & 2.3 ± 0.3 & 0.1 ± 0.1
& 12 & 18 & 1914 & 94.2 ± 1.1 & 62.5 ± 2.7 & 24.9 ± 2.1 & 4.7 ± 0.7 & 0.3 ± 0.2
& 24 & 25 & 2726 & 81.0 ± 2.0 & 46.8 ± 2.7 & 13.2 ± 2.6 & 3.5 ± 2.7 & 0.5 ± 0.2
\hline
S. purpureo-sericeum & 2 & 13 & 901 & 94.4 ± 0.7 & 33.6 ± 1.9 & 6.9 ± 1.0 & 1.7 ± 0.4 & 0.0 ± 0.0
& 4 & 16 & 1003 & 95.9 ± 1.1 & 41.1 ± 2.0 & 2.4 ± 0.6 & 0.2 ± 0.1 & 0.0 ± 0.0
& 6 & 17 & 1093 & 91.6 ± 1.3 & 34.4 ± 3.0 & 6.3 ± 1.5 & 0.1 ± 0.1 & 0.0 ± 0.0
& 24 & 12 & 1593 & 94.8 ± 1.5 & 13.0 ± 2.1 & 4.6 ± 1.3 & 0.9 ± 0.4 & 0.0 ± 0.0
\hline
S. bicolor (control) & 1 & 16 & 643 & 98.2 ± 0.7 & 80.3 ± 2.9 & 56.0 ± 3.6 & 13.1 ± 2.4 & 12.1 ± 3.1
\hline
\end{tabular}

† Mean ± standard error of the mean.

Detection of Fertilization and Embryo Development

The frequency of fertilization, as measured by embryo formation, was determined for the three species in Table 3 that had pollen tubes penetrating the sorghum ovary. Sorghum florets that had been pollinated with S. ecarinatum, S. macrosporum, and S. matarankense pollen were dissected from inflorescences 15 d after pollination. From these pollinations, the frequency of seed with immature embryos was 10/1119, 1/1237, and 13/533 for S. ecarinatum, S. macrosporum, and S. matarankense, respectively. However, the endosperm aborted in all hybrid seed produced, which resulted in seed failure.

DISCUSSION

Interspecific hybridization and introgression is a means to tap genes of agronomic importance for crop improvement programs. However, reproductive isolation barriers often exist between divergent relatives and crop species that render introgression difficult. These barriers may affect any part of the plant’s reproductive cycle including lack of fertilization, endosperm failure, embryo abortion, seedling lethality, hybrid sterility, and hybrid breakdown.

A very common prezygotic reproductive barrier results from pollen–pistil incompatibility, where growth of pollen tubes from one species is inhibited in the stigma of another species. There is currently considerable interest regarding the physiology and molecular biology of pollination and fertilization in plants (Franklin-Tong, 2002; Lord, 2003). The poorly understood events starting at pollination and terminating at fertilization involve complex and harmonious interactions between the microgametophyte and the pistil. Signaling occurs between the microgametophyte and the cells and the extracellular matrix of the pistil. Pollen tubes are guided to the micropyle by signals originating in the style and embryo sac (Lord and Russell, 2002). Adverse pistil–pollen interactions that include inhibition of tube growth follow-

Table 3. Pollen germination and tube growth of five alien Sorghum species in sorghum ATx623 pistils at progressive intervals post-pollination.
ing interspecific pollinations involving sorghum conceptually may be viewed as the consequence of inharmonic genetic interactions due to genetic divergence among the species.

When fertilization and embryo development do occur in sorghum interspecific crosses, the seed abort due to early breakdown of the endosperm. Endosperm breakdown in interspecific crosses is a common form of post-zygotic reproductive isolation. Whether or not endosperm develops normally in seed from interplody interspecific and interspecific crosses has been proposed to be due to the endosperm balance number (EBN) which determines the effective ploidy in the endosperm of each species (Johnston et al., 1980). This hypothesis is that either the maternal/paternal genome ratio or the EBN must be in a 2:1 maternal/paternal ratio for successful endosperm development.

In addition to inhibition of pollen tube growth, several types of aberrations were observed in alien tubes growing in sorghum pistils that were not observed in the control. Alien pollen tubes typically displayed a crooked growth path through sorghum pistils. Irregular pollen tube growth was observed in ovules of blue panicgrass (Panicum antidotale Retz.) when pollinated with Klein-grass (Panicum coloratum L.) pollen (Burson and Young, 1983). Kleingrass pollen tubes became disoriented in the ovaries and grew in a random manner that prevented the tubes from entering the micropyle. A common growth-form aberration of alien pollen tubes in sorghum pistils was the enlargement or swelling of tips. This phenomenon has been observed for other species. In crosses between wheat (Triticum aestivum L.) and rye (Secale cereale L.), swollen rye pollen tubes have been reported in wheat pistils (Lange and Wojciechowska, 1976; Jalani and Moss, 1980). Sorghum pollen tubes with swollen tips and a twisted growth pattern were observed in pearl millet [Pennisetum glaucum (L.) R. Br.] styles (Heslop-Harrison, 2000). In the sorghum pistils of the current study, a common observation was the presence of callose in the stigma axis. During normal growth of a grass pollen tube, callose may form near the pollen tube and block plasmodesma connections between cells (Heslop-Harrison and Heslop-Harrison, 1981). Callose accumulation in the stigma, in response to pollen from related species, is a common phenomenon (Dumas and Knox, 1983). The accumulation of callose in sorghum stigmas, in response to alien pollen tubes, is apparently a manifestation of inharmonious gene interaction between pollen and the pistil.

One approach to increase the frequency of interspecific hybridization is to discover genes that eliminate or reduce the factor(s) that cause reproductive isolation. In wheat, duplicate crossability genes Kr1 and Kr2 influenced interspecific crossability (Riley and Chapman, 1967). In the crosses, wheat × rye and wheat × bulbous barley (Hordeum bulbosum L.), the dominant alleles retarded and inhibited pollen tube growth at the base of the wheat style and in the ovary wall (Lange and Wojciechowska, 1976; Snape et al., 1979; Jalani and Moss, 1980). Genes exist in bulbous barley that override the action of the Kr1 allele, and allow the barley pollen tubes to grow into the wheat pistils (Sitch and Snape, 1986).

In sorghum, variation exists among genotypes that influences pollen–pistil incompatibilities for at least one interspecific cross. Sun et al. (1991) reported that the growth of S. versicolor pollen tubes into sorghum pistils was influenced by the genotype of the sorghum line used. Of the three genotypes used, KS36A, KS5A, and ATx623, the S. versicolor pollen tubes grew further into ATx623 pistils than into those of the other two genotypes. However, successful hybridization was not achieved. Nonetheless, screening divergent sorghum lines may result in the discovery of genes that allow interspecific hybridization in sorghum.

In conclusion, the primary reason why interspecific hybridization does not occur in sorghum is that growth of alien pollen tubes is inhibited in sorghum pistils. For three species where limited fertilization and embryo formation occurred, the endosperm in immature seed aborted and no viable seed were produced. Thus pollen–pistil interactions and post-fertilization events are the reasons why hybrids have not been produced. Potential utilization of interspecific hybrids in sorghum breeding programs will require overcoming the inhibition of alien pollen tube growth and the rescue of hybrid embryos before the seed aborts. In vitro culture, a commonly used method to grow rescued embryos from aborted seeds following interspecific hybridization (Sharma, 1999), may be an approach to obtain interspecific sorghum hybrids. Additional obstacles to introgression may include hybrid sterility due to differences in chromosome number or the lack of homology between the genomes in the hybrid.

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