CROSS INCOMPATIBILITY BETWEEN *SORGHUM BICOLOR* AND *S. VERSICOLOR*

by

YI SUN
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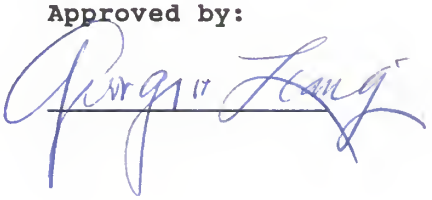

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ACKNOWLEDGMENTS

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CROSS INCOMPATIBILITY BETWEEN SORGHUM BICOLOR and S. VERSICOLOR

INTRODUCTION

The genus Sorghum has been subdivided into five sections: Sorghum, Stiposorghum, Para-Sorghum, Chaetosorghum, and Heterosorghum. It has been proposed that the origin of cultivated sorghum [S. bicolor L. (Moench), 2n=20] lies within section Sorghum (Doggett, 1976; de Wet, 1978). To improve cultivated sorghum, the fifth most important crop in the world, we need to explore and utilize gene pools existing in its related species.

S. versicolor is one of such species for providing desirable exotic genes. S. versicolor [J. N. Anderess, (2n=10)] belongs to section Para-Sorghum under genus Sorghum (Snowden, 1935). It originated from southeastern Africa, and is a self-fertilized annual species characterized by bearded nodes and simple panicles. S. versicolor plants have trichomes on the abaxial surface of leaves, which is considered a character associated with aphid resistance as well as drought tolerance. The average chromosome length of S. versicolor at somatic metaphase is much longer than that of S. bicolor (Garber, 1950; Gu et al. 1984). The restriction pattern of S. versicolor mitochondrial DNA (mtDNA) is distinctly different from those of S. bicolor and

S. halepense genotypes (Lee and Liang, 1989). Attempts to cross S. versicolor with either S. bicolor or other species within Para-Sorghum have been unsuccessful (Garber, 1950; Wu, 1982; Suksayretrup and Liang, 1989).

Cross incompatibility may occur during pollen germination, pollen tube growth, fertilization, and seed development. Observing the process of pollen tube growth has been made possible by the fluorescent technique of Martin (1959), which facilitates incompatibility studies by tracing callose deposition during pollen tube growth in the style and embryo sac.

It has been suggested that certain contact-guidance and chemotropism reactions control the orientation of pollen tubes in stigmas and styles (Rosen, 1971b). Some phytohormones such as gibberellic acid (GA_3) and other factors such as Ca^{++} and boron have increased the percentage of in vivo and in vitro germination of pollen and regulated pollen tube growth in some incompatible pollinations (Rosen 1971b; Stanley and Liskens 1974; Polito, 1983). Physiological age of the style had a significant effect on the growth of pollen tubes in detached styles of Lilium longiflorum (Townsend, 1971). Since little work has been done with Sorghum, our study was designed to observe pollen germination and pollen tube growth in reciprocal crosses between S. bicolor and S. versicolor in an attempt to locate barrier(s) of cross incompatibility and to reveal the

effects of genotype, GA_3, and physiological age of florets on cross incompatibility.

MATERIALS AND METHODS

Male sterile lines of S. bicolor, KS 5A, KS 36A, and TX 623A, were used as maternal parents in crosses with S. versicolor, Combine Kafir 60B, TX 623B, and Xin White were used as pollen parents in crosses with S. versicolor. Plants of S. versicolor with or without emasculation were used as maternal or paternal parents in crosses with S. bicolor. Seeds of S. versicolor were kindly provided by Dr. T. P. Wu of the Botanic Institute , National Academy of Sciences, Taiwan, China. Plants were grown in a green house during June, 1988 through August, 1989.

Experiment 1. Rate of pollen tube growth .

Florets of KS 5A were pollinated with pollen from S. versicolor, and emasculated S. versicolor florets were pollinated with pollen from Xin White. The pollinated florets were fixed at 10, 30 min, and 1, 2, 3, 6, and 12 h after pollination. Selfed florets from S. versicolor and S. bicolor (TX 623A X TX 623B) were also fixed at the same time and used as checks. Twenty florets were taken for each treatment. Temperature at the time of pollination was 28 ± 2^0 C.

Experiment 2. Effects of genotype and physiological age on the pollen germination and pollen tube growth.

Days after anthesis for the above three A lines of S. bicolor and emasculated florets of S. versicolor were recorded as physiological age of florets. The day of flowering was recorded as zero day after anthesis, so on up to the eighth day after anthesis. Plants were pollinated by brushing fresh pollen onto stigmas for those florets of S. bicolor at the physiological age of 0-8 days. Crosses with S. versicolor as the maternal parent were made only on florets at 0, 3, and 6 days after anthesis. Pollen donors for these crosses were the male fertile lines above. Fixation was made 6 h after pollination. Ten florets were taken from each treatment. Temperature at the time of pollination was 30 ± 3^0 C.

Experiment 3. Gibberellic acid (GA_3) treatment.

GA_3 at a concentration of 75 ppm was sprayed onto stigmas 3 days before pollination and continued at 24-h intervals until one day after pollination. Other treatments were the same as in the experiment two, and the observations of the experiment two were used as untreated checks for this experiment.

Experiment 4. Stigma-excision and pollination.

Stigmas of S. bicolor (TX 623A) were excised on the day of anthesis. Pollen from S. versicolor and S. bicolor (TX 623B) was brushed onto the styles separately. The pollinated

florets were fixed at 1 and 6 h after pollination. Thirty florets were taken for each treatment. Temperature at pollination time was 30 ± 3^{0} C.

Fixing and staining. Pollinated florets were fixed in FAA fixing solution (formalin : 80% ethanol : glacial acetic acid = 1 : 8 : 1) for 24 h or more. After rinsing in tap water twice, the tissues were cleared and softened in saturated NaOH aqueous solution (9 h for S. bicolor and 6 h for S. versicolor). The softened florets were gently washed with tap water three times, then stained in 0.1% aniline blue in 0.1 N K_3PO_4 aqueous solution for 4 h or more.

Microscopic observations. Florets were mounted in several drops of staining solution on glass slides, and glumes were removed with needles under a dissecting microscope. The entire pistil was squeezed by tapping cover slips gently with needles. Observations were made on a Carl Zeiss microscope with fluorescence filter attachment employing a 50W mercury lamp, exciter filter BG-12, reflector 460 and barrier filter 53. Photographs were taken with Technical Pan films (ASA 100). Pollen tube length was measured with a micrometer. The longest pollen tube in each floret was considered as the pollen tube length for that floret.

RESULTS

Rate of pollen germination and pollen tube growth following interspecific reciprocal pollinations.

For the crosses between S. bicolor and S. versicolor, pollen germination was noted in 30 minutes after pollination. Although most of the pollen tube growth was limited mainly to the stigma areas in the reciprocal crosses, pollen tubes of S. versicolor on stigmas of S. bicolor grew faster and longer than those of S. bicolor on stigmas of S. versicolor (Table 1; Fig. 1). On about one third of the florets, pollen tubes grew into styles, and a few reached ovaries and approached the micropilar end. Pollen tube length varied significantly among hours and crosses. The interaction between hours and crosses, was also significant (Table 2). Most of the pollen tube growth in the wide crosses ceased at around 6 h after pollination (Table 1; Fig. 1). Thus the difference in the length between observations made at 6 h and 12 h after pollination was not significant. An examination of the tube length of reciprocal crosses at various hours individually showed that pollen tube growth rates for the crosses of S. bicolor X S. versicolor and S. versicolor X S. bicolor during the first hour of pollination were nonsignificant. From the second hour on, the differences in tube length became more conspicuous. At hour 6, pollen tube length in the cross of

S. bicolor X S. versicolor were significantly longer than those in the cross of S. versicolor X S. bicolor. (Table 1; Fig. 1).

When S. bicolor and S. versicolor were selfed, pollen germination was observed 10 min after pollination (Fig. 4), and pollen tubes grew rapidly between the first and third hours. By the end of the third hour, pollen tubes were observed at the micropylar end of 70% of the florets for S. bicolor and 80% of the florets for S. versicolor. Pollen tubes from the self pollen of S. versicolor grew faster than those from S. bicolor and the wide crosses (Table 1; Fig. 1). The slopes depicting pollen tube growth rates against time were small and stationary in the wide crosses, whereas those in the selfs showed clearly a lag phase and a exponential phase which was followed by deceleration and stationary phases.

Effects of genotype and physiological age on pollen tube growth in the wide crosses.

Genotype and physiological age had significant effects on pollen tube growth for the crosses using S. bicolor as maternal parents (Table 3). There was a significant interaction between genotype and physiological age, i.e., effect of genotypes varied depending on physiological age. When plants of S. versicolor were used as maternal parents, however, differences among genotypes of S. bicolor as pollen

source were not significantly different (Tables 4 and 5). Likewise, different physiological ages for florets of S. versicolor did not result in significant difference in growth rates for pollen tubes from S. bicolor (Tables 4 and 6); also the interaction between genotypes and physiological age was not significant.

Differences among genotypes of S. bicolor in permitting the pollen tubes of S. versicolor to grow were significant. TX 623A was the most permissive one, followed by KS 5A, and KS 36A was the least permissive genotype to the pollen of S. versicolor (Table 7; Fig. 2).

Pollination made on florets of S. bicolor of different physiological ages produced significantly different results. Pollination made on days 3 and 4 after flowering resulted in the longest pollen tube extension, whereas day 8 resulted in nearly zero pollen tube growth implying the total loss of pollen acceptability (Table 8; Fig. 2).

Differences in pollen tube growth occurred mainly on days 2-7 except the day 5. On days 0, 1, and 8, there was little difference among the genotypes in permitting pollen tubes to grow (Table 9), suggesting that mechanism(s) involving pollen-stigma interaction is important during a well-defined period.

Effect of GA_3.

GA_3 had little effect in regulating pollen tube growth

physiological age, were not significant, nor was the three-way interaction involving GA_3, genotype, and physiological age (Tables 3 and 10; Fig. 3).

Style pollination.

Pollen germination was not observed on the stigma-excised styles which were pollinated with the pollen from S. versicolor. In the pollination between TX 623A and TX 623B, pollen germination did not occur on the styles at 1 h after pollination. Germination was observed, however, on 5 styles (which acounted for 1/6 of the observed styles) that were fixed at 6 h after pollination (Fig. 5), and 2 of them had pollen tubes in ovaries (Fig. 6).

Some abnormal pollen tube growth was observed in the wide crosses, including disoriented growing pollen tubes (Figs. 7 and 8), enlarged pollen tube tips, and curled tubes (Fig. 9). In styles of selfed plants, pollen tubes could be observed in bundles with weak reflection of fluorescence (Fig. 10). In styles of wide-crosses, only 1, 2, at most 3 pollen tubes characterized by having heavy deposition of callose (Fig. 11) were observed.

DISCUSSION

For wide crosses between S. bicolor and S. versicolor, pollen germinated on more than 90% of the stigmas of the maternal plants, implying some compatibility between the two

species. Pollen tubes of S. bicolor were confined mainly to the stigmas of S. versicolor, while pollen tubes of S. versicolor grew into styles of S. bicolor. The differences in the pollen tube length may indicate the degrees of acceptability by maternal plants. Pollen tube growth may also indicate vitality of pollen from the paternal plants, i.e., pollen of S. versicolor appears more robust and less sensitive to the conditions in which germination and tube growth occur.

Different genotypes of S. bicolor had significant differences in acceptability to pollen tube growth of S. versicolor. We could select for more permissive genotypes to make wide crosses to increase the chance of success. A few pollen tubes of S. versicolor were observed near the micropylar end of the ovules of S. bicolor, but no evidence of fertilization was found. Sangduen and Hanna (1984) reported that successful crosses between 40 chromosome S. bicolor and S. halepense (2n=40) were achieved due to the normal chromosome ratio between embryo and endosperm which is 2 : 3. So it may be practical to double the chromosome number of S. versicolor before making the wide crosses. As paternal parents, different genotypes of S. bicolor did not differentially affect pollen tube growth in wide crosses. Pollen tubes of S. bicolor rarely grew beyond stigma areas of S. versicolor. Only two pollen tubes of S. bicolor were

found in the styles of S. versicolor in more than two hundred observations. It is possible that either inhibiting factors were stronger in stigmas and styles of S. versicolor, or that pollen from S. bicolor were less robust than those of S. versicolor.

Physiological age of florets of S. bicolor had a significant effect on growth of pollen tubes of S. versicolor. Differences due to physiological age may be attributable to the combination of stigma acceptability and its immune system which detects the presence of foreign pollen. Selecting florets at the most acceptable age for wide crosses should provide the most desirable results. Florets of S. versicolor with different physiological ages did not play important role in pollen tube growth in the wide crosses.

GA_3 has very little effect in inducing pollen germination and pollen tube growth in crosses between S. bicolor and S. versicolor. It may have some effect in inducing foreign pollen to germinate in certain entirely incompatible wide crosses (Niu et al. 1986). However, if pollen does germinate and a pollen tube grows to a certain extent, GA_3 has little effect in promoting pollen tube growth.

Selfed pollen from S. bicolor germinated on styles with excised stigmas, but germination was slower than selfed pollen on stigmas. This suggested that chemical substances

that stimulate pollen germination also exist in styles at a lower concentration compared with those on stigma surfaces. The less concentrated chemicals apparently failed to induce pollen germination for S. versicolor.

Deformed pollen tube growth seems to be a common phenomenon in wide crosses where pollen germination occurs. Similar observations were found by Sangduen et al. (1983) in their interspecific studies of Medicago species, by Boyle and Stimart (1986) in their study on cross incompatibility between Zinnia elegans and Z. angustifolia, and by Ascher (1986) in his interspecific studies. Disoriented pollen tube growth in the wide crosses may imply that stigmas had no or very little chemotropic activity, which is required to guide the normal orientation of the pollen tubes. Lack of chemotropic activity may have caused abnormal growth of pollen tubes in the wide crosses. Pollen tubes usually obtain nourishment from maternal styles in compatible crosses so that the tube growth can transfer from autotropic to heterotropic mode (Rosen, 1971a; Stanley, 1971), but for incompatible crosses pollen tubes may receive little nourishment from the styles. Lack of essential nourishment may have stimulated more callose deposition in wide crosses.

Fairly high coefficients of variation were obtained in the study (24.1 for exp. 1, and 92.1 for both exp. 2 and 3).

The reason for this high variation might be that pollen tube growth in the wide crosses was influenced greatly by certain random factors including the change of temperature, humidity, light intensity, growing conditions of maternal plants, and genotype or vitality of pollen. The effect of random factors on pollen tube growth in incompatible pollination was also discussed by Weisman (1986) in his study on self incompatibility of sugar beet.

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Table 1. Rate of pollen tube growth following reciprocal pollinations of *S. bicolor* and *S. versicolor*

Pollination	Time				
	1h	2h	3h	6h	12h
			(μm)		
Selfed *S. versicolor*	646.0[a]	4025.0[a]	4765.0[a]	4900.0[a]	4900.0[a]
Selfed *S. bicolor*[2]	412.5[ab]	3110.0[b]	3524.5[b]	4400.0[b]	4400.0[b]
S. bicolor x *S. versicolor*[3]	190.7[bc]	393.3[c]	506.0[c]	750.7[c]	712.7[c]
S. versicolor x *S. bicolor*[4]	47.0[c]	91.0[c]	201.5[c]	229.5[d]	171.0[d]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Multiple Range test.

2) The genotypes were TX 623A X TX 623B.

3) The female parent for *S. bicolor* was KS 5A.

4) The male parent for *S. bicolor* was Xin White.

Table 2. Analysis of variance for rate of pollen tube growth following interspecific pollinations

Source of variation	df	SS	MS
Hour	4	277266686.5	69316671.6**
Cross	3	1042831028.8	347610342.9**
Hour x cross	12	209524407.5	17460367.2**
Error	380	80997665.0	213151.8
Total	399	1610619787.8	

** Significant at P=0.01.

Table 3. Analysis of variance for S. versicolor pollen tube length on stigmas of three male sterile lines of S. bicolor at various physiological ages with GA_3 treatment

Source of variation	df	SS	MS
GA_3	1	69632.3	69632.3^{NS}
Genotype	2	15330157.0	7665078.5**
Physi. age	8	114092510.8	14261563.8**
Geno. x Physi. age	16	17216537.8	1076033.6**
GA_3 x Geno.	2	32119.6	16059.8^{NS}
GA_3 x Physi. age	8	321522.8	40190.3^{NS}
GA_3 x Physi. age x Geno.	16	1002056.5	62628.5^{NS}
Error	502	143606286.1	286068.3
Total	539	290668766.4	

** Significant at P=0.01; NS, not significant.

Table 4. Analysis of variance for S. bicolor pollen tube length on stigmas of S. versicolor at various physiological ages

Source of variation	df	SS	MS
Genotype	2	183848.9	91924.5^{NS}
Physio. age	2	72082.2	36041.1^{NS}
Geno. x Physio. age	4	19704.4	4926.1^{NS}
Error	81	3708880.0	45788.6
Total	89	3984515.6	

NS, not significant.

Table 5. Pollen tube length of three genotypes of S. bicolor on stigmas of S. versicolor

Genotype	Number observed	Pollen tube length (μm)
Tx 623B	30	206.0[a]
Xin White	30	125.3[a]
CK 60B	30	100.0[a]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Multiple Range test.

Table 6. Pollen tube length of S. bicolor on stigmas of S. versicolor at various physiological ages

Days after flowering	Number observed	Pollen tube length (μm)
0	30	183.7[a]
3	30	126.7[a]
6	30	121.0[a]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Mutiple Range test.

Table 7. Pollen tube length of S. versicolor in florets of three male sterile lines of S. bicolor

Entry	Number observed	Tube length (μm)
TX 623A	180	977.2[a]
KS 5A	180	860.4[b]
KS 36A	180	576.0[c]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Multiple Range test.

Table 8. Pollen tube length of S. versicolor in florets of S. bicolor at various physiological ages

Days after flowering	Number observed	Tube length (μm)
3	60	1415.5[a]
4	60	1323.0[ab]
5	60	1165.2[b]
2	60	1132.3[b]
6	60	742.2[c]
0	60	605.5[c]
1	60	589.3[c]
7	60	261.6[d]
8	60	6.2[e]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Multiple Range test.

Table 9. Pollen tube length of S. versicolor in florets of three male sterile lines of S. bicolor at various physiological ages

Entry	Days after flowering 0	1	2	3	4	5	6	7	8
	(μm)								
TX 623A	487.5[a]	534.0[a]	1657.0[a]	1857.0[ab]	1492.5[ab]	1213.5[a]	870.5[a]	670.5[a]	12.5[a]
KS 5A	751.0[a]	696.5[a]	1128.0[b]	1534.0[b]	1397.0[bc]	1244.0[a]	914.0[a]	72.9[b]	6.0[a]
KS 36A	575.0[a]	537.5[a]	612.0[c]	855.5[c]	1079.5[c]	1038.0[a]	442.0[b]	41.5[b]	0.0[a]

1) Means within a column followed by the same letter are not significantly different at 5% level of Duncan's Multiple Range test.

Table 10. Effect of GA_3 on pollen tube growth

Treatment	Number observed	Pollen tube length (μm)
GA_3	270	815.9[a]
Control	270	793.4[a]

1) Same letter indicates nonsignificance at 5% level of Duncan's Multiple Range test.

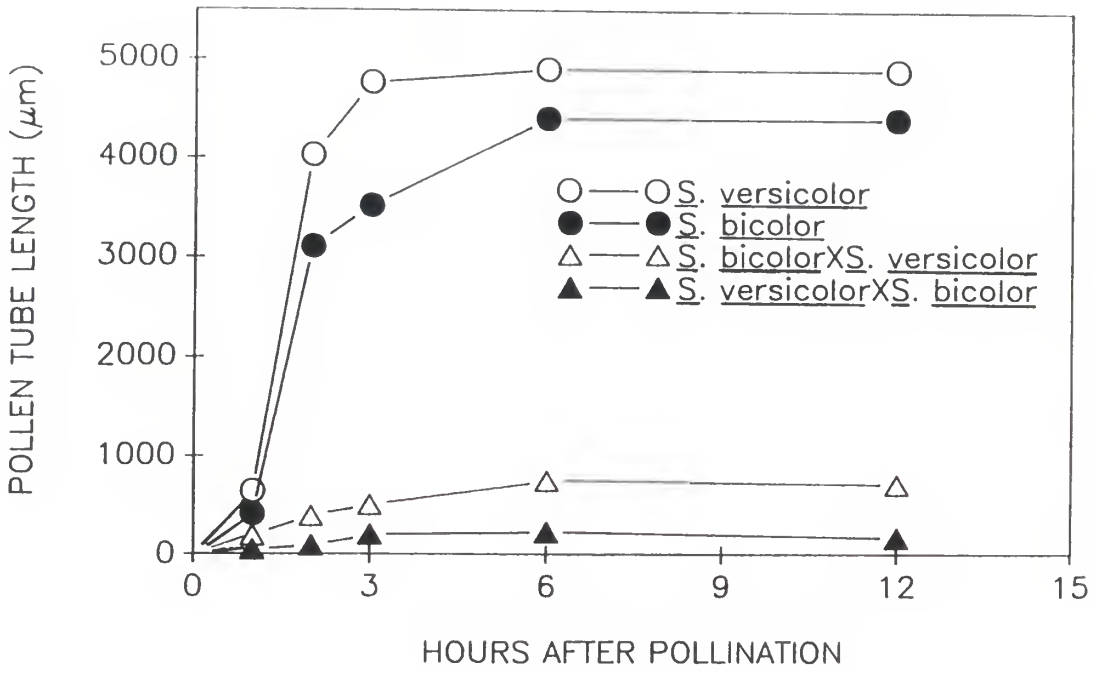

Fig. 1. Rate of pollen tube growth following reciprocal pollinations of *S. bicolor* and *S. versicolor* and two self pollinated checks.

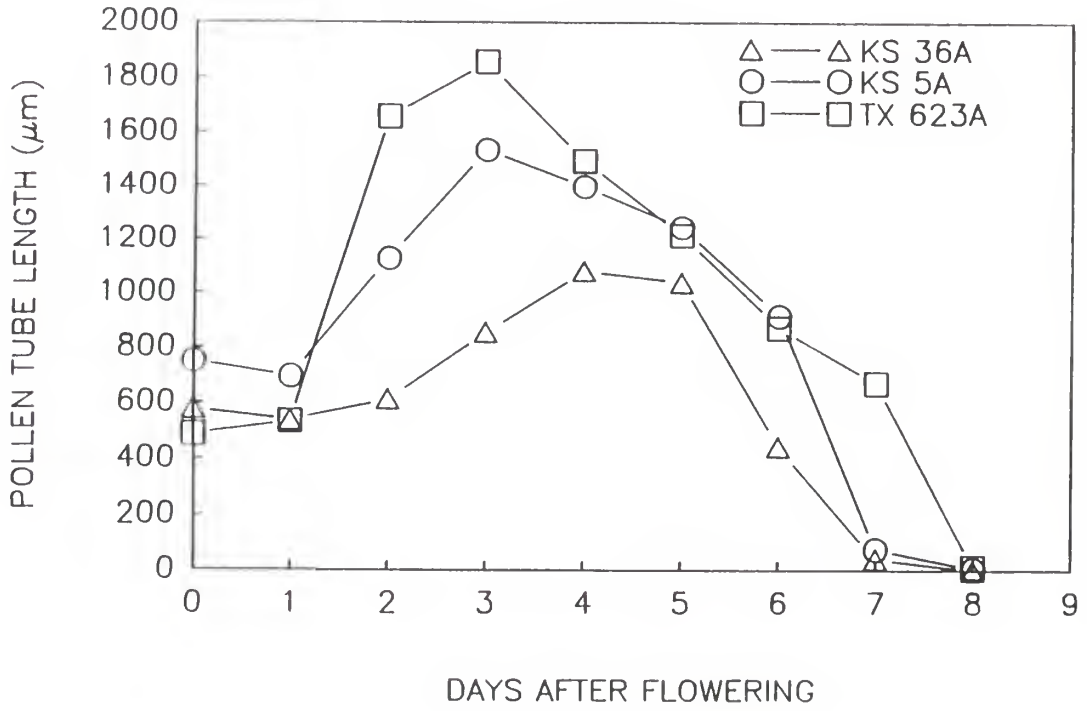

Fig. 2. Pollen tube length of S. versicolor in florets of three male sterile lines of S. bicolor at various physiological ages.

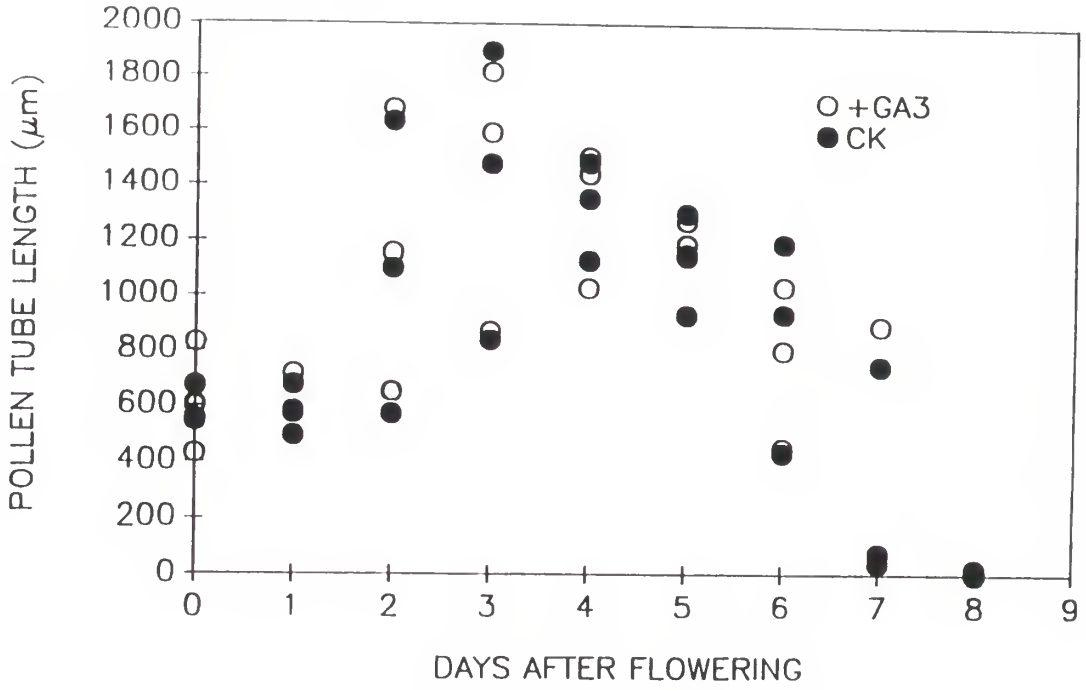

Fig. 3. The effect of GA_3 on pollen tube growth in reciprocal pollinations of S. bicolor and S. versicolor.

Fig. 4. Germinating pollen tubes of S. bicolor on the stigmas of S. bicolor.

Fig. 5. Germinating pollen tube of S. bicolor on the stigma-excised style of S. bicolor.

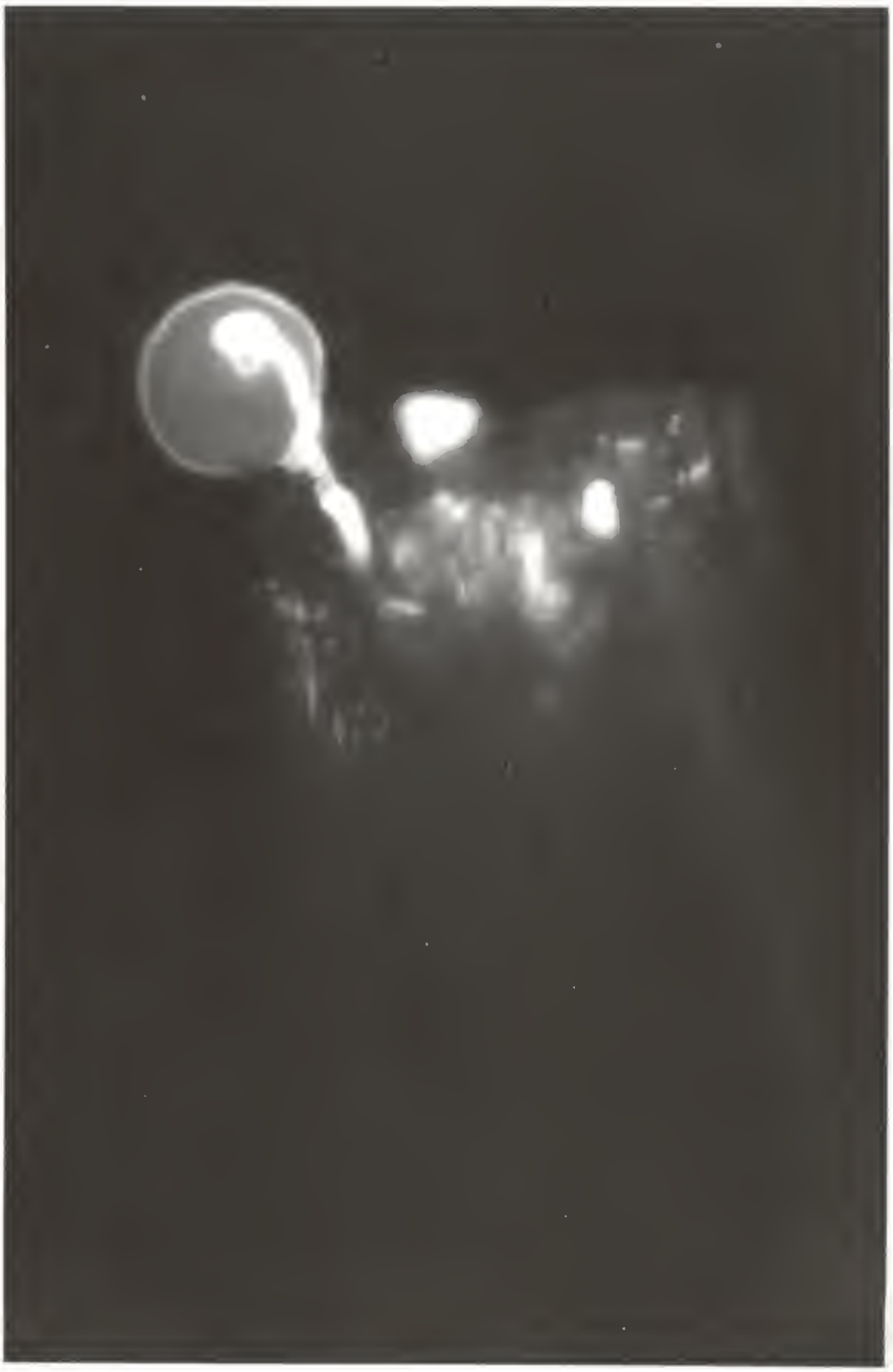

Fig. 6. Pollen tube of S. bicolor in the stigma-excised style and ovary of S. bicolor.

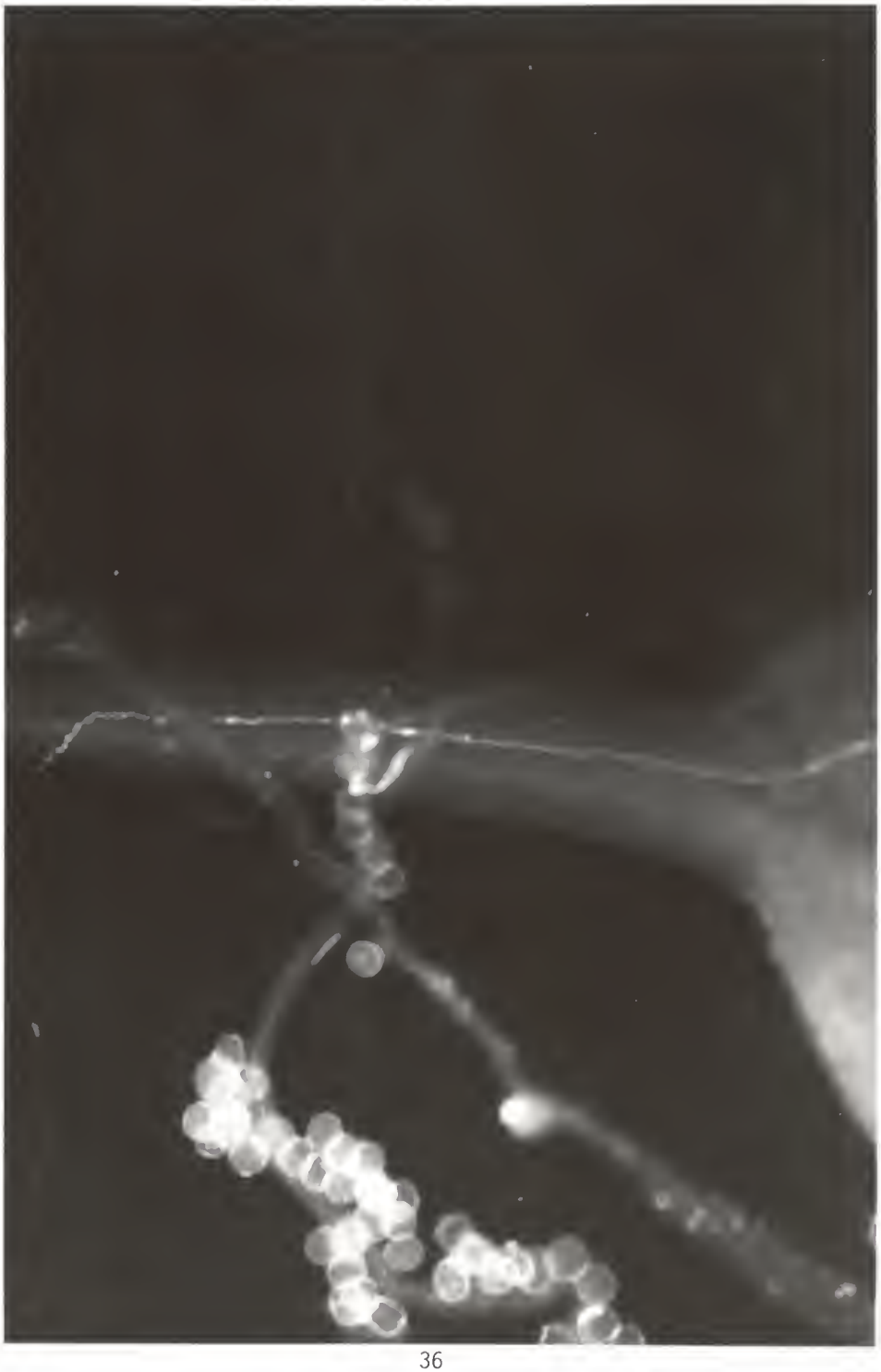

Fig. 7. Inversely growing pollen tube of S. versicolor on the stigmas of S. bicolor.

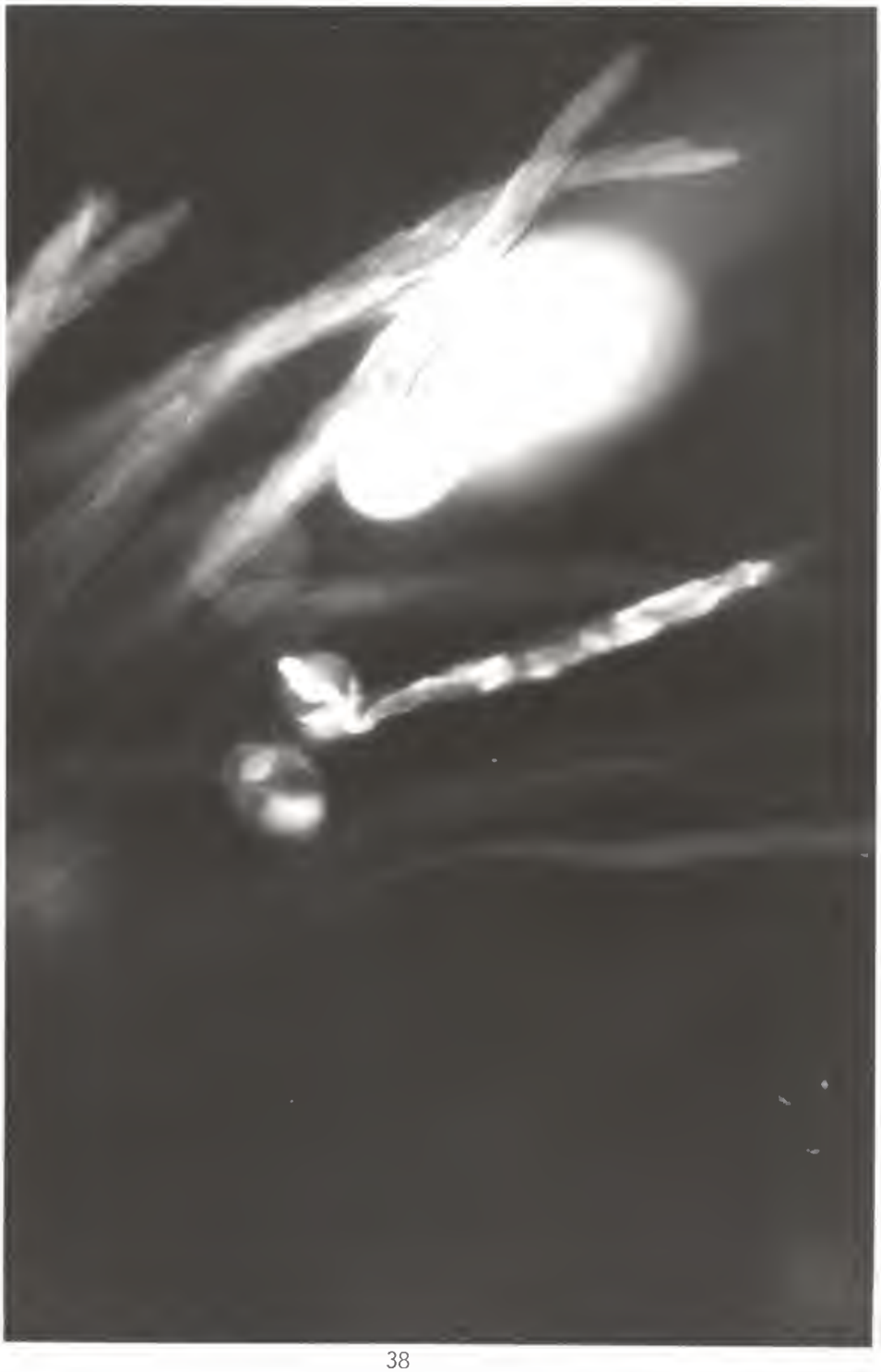

Fig. 8. Inversely growing pollen tube of S. versicolor on the stigmas of S. bicolor.

Fig. 9. Curled pollen tubes of S. versicolor on the stigmas of S. bicolor.

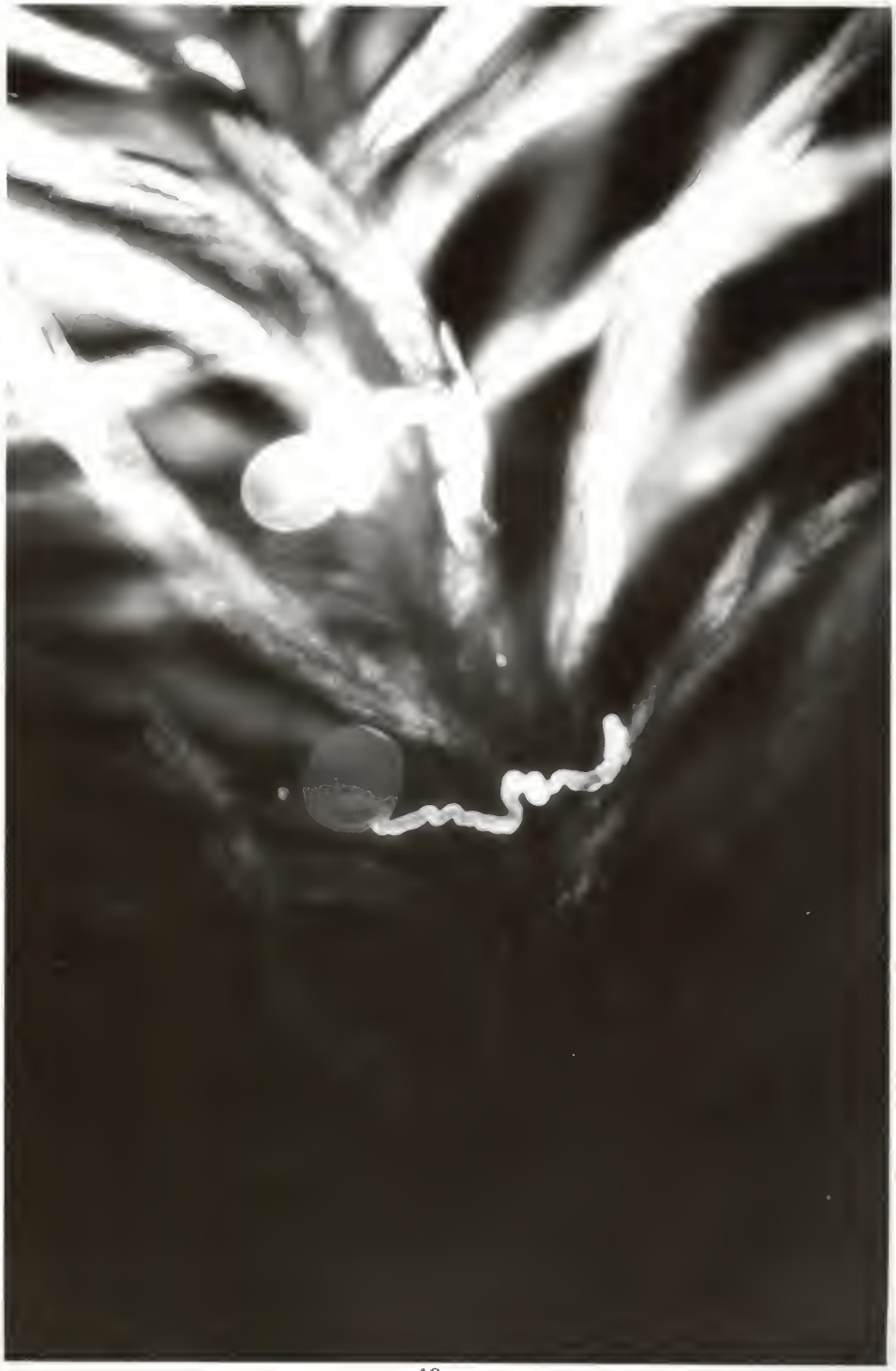

Fig. 10. Pollen tubes of S. bicolor in the style and ovary of S. bicolor.

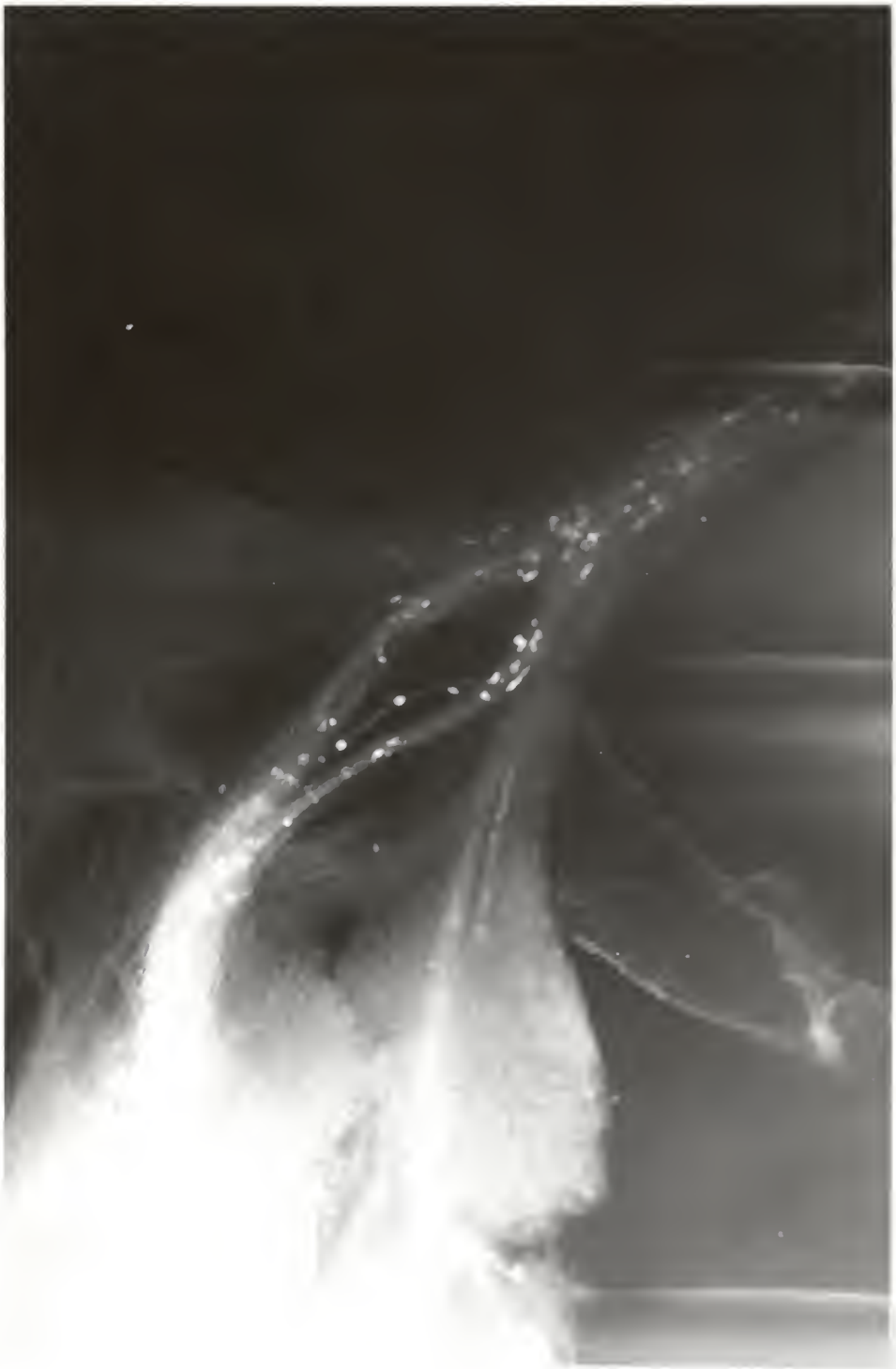

Fig. 11. Pollen tube of S. versicolor in the style of S. bicolor.

CROSS INCOMPATIBILITY BETWEEN *SORGHUM BICOLOR* AND *S. VERSICOLOR*

by

YI SUN

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ABSTRACT

To explore widely related gene pools for cultivated sorghum and to study the phylogenic relations within the genus Sorghum, various methods were attempted to achieve hybridization between Sorghum bicolor (2n=20) and S. versicolor (2n=10), including reciprocal crosses, style pollination, hormonal treatment, etc. Experiments were conducted under green house conditions. Selfed parental plants (or A lines X B lines in case of S. bicolor) were used as checks in the study. A fluorescent microscope was used to detect pollen germination on stigmas and to measure pollen-tube growth in styles. Pollen germinated on stigma surfaces in the reciprocal pollinations between the two species. A few pollen tubes of S. versicolor grew into styles and approached ovules of S. bicolor. In contrast, only two pollen tubes of S. bicolor were found in styles of S. versicolor. No zygote was found in either cross pollination. Gibberellic acid (75 ppm) had little effect on pollen germination and pollen tube growth. Used as female parents, there were significant differences among male sterile lines of S. bicolor in allowing pollen-tube growth in styles for pollen from S. versicolor, but the differences were not significant among genotypes of S. bicolor when they were used as pollen sources. Physiological age (days after

anthesis) of florets also had an impact on the pollen-tube growth. After stigma excision, styles of S. bicolor allowed their own pollen to germinate, but not foreign pollen from S. versicolor. Results implied that the initial barrier of cross incompatibility between these two species exists in stigmas and styles, and the degree of incompatibility varied between reciprocal crosses.