

HYBRIDIZATION IN PANICUM VIRGATUM L.

by

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INTRODUCTION

Switchgrass, Panicum virgatum L. is a perennial, warm-season grass which is native to a large area of North America. It has been used for hay and pasture for some time, but its most extensive use at present is probably soil conservation. For this it is well adapted because of its strong vigorous seedlings and adaptation to a wide range of soils and climates. Little work has been done on the genetics of switchgrass. As more work is done toward the development of superior strains, it becomes increasingly desirable to understand the breeding behavior of the species. The purpose of this study was to examine the mode of reproduction of selected clones of switchgrass. It was also desired to determine whether the upland and lowland types would readily cross.

REVIEW OF LITERATURE

P. virgatum is a widespread and highly variable species. Hitchcock (1951) describes its range as extending from Nova Scotia and Ontario to Montana, south to Florida, and Central America. He describes only two botanical varieties, P. virgatum var. spissum Linder. and var. cubense Griseb., both being in the eastern part of the range. Hughes, et al. (1952) stated, "Two native types are recognized, the lowland and the upland types. The lowland type is much taller and coarser stemmed than the upland type".

Church (1929) reported a basic chromosome number of 9 in the genus Panicum. This was later substantiated by Burton (1942), in a cytological study in the tribe Paniceae, and by Brown (1948).

A polyploid condition in switchgrass was reported by Church (1940). Plants from Oklahoma were mainly tetraploids ($2n=36$), but a few noticeably different plants, that were smaller and very pilose in contrast to the others, proved to be octoploids ($2n=72$). Progeny similar to the Oklahoma octoploid but not as pilose were obtained from a Kansas octoploid. In the same paper Church reported a chromosome number of 36 for P. virgatum var. spissum Linder, which was collected in Massachusetts. Brown (1948) reported a chromosome number of $2n=36$ for P. virgatum var. subense Griseb.

According to Church (1940) P. virgatum var. spissum was quite glabrous, had short spikelets, and was intermediate in height to the western tetraploids and octoploids. Its nuclear complement included five or six pairs of chromosomes decidedly smaller than any of those observed in the western tetraploid. Church concluded from the comparative studies of eastern and western forms of switchgrass that a form of autopolyploidy was present.

In a cytological study of 11 Panicum species, Burton (1942) found a polyploid series and reported a switchgrass plant from Gainesville, Florida, to be $2n=72$. Nielsen (1944) reported a polyploid series of 18, 36, 54, 72, 90 and 108 in switchgrass.

These plants came from Wisconsin and Montana, south to Arkansas and Arizona.

Aneuploids in switchgrass have been reported by Brown (1948), and Carver (1957). Brown, in a study of plants from southeastern United States, reported $2n$ chromosome numbers of 21, 25, 30 and 32. It appeared that some of the chromosomes were fragments. Carver, in a study of plants from Texas, Oklahoma, and Kansas, reported aneuploids with $2n$ chromosome numbers of 68, 70, 76 and 78 as well as euploids with $2n$ numbers of 36, 54, and 72.

Cornelius and Johnston (1941) noted a great variability in rust resistance and other characters. From the 59 isolates which Nielsen (1944) studied, there did not appear to be any relationship between region and chromosome number. There was a wide morphological range, but the variation was slightly greater among than within chromosome races.

In a study of the native switchgrass populations of Nebraska, Eberhart and Newell (1959) found that by observation the plants could be divided into two general types. Those plants from the northern and western areas were semi-decumbent, relatively fine stemmed, usually blue-green in color, and were more rhizomatous than the ones from the southeastern areas which were taller, erect, coarse stemmed, and light green. Large phenotypic variations among plants within strains were observed, but seedling vigor and number of culms per plant in the first year's growth were highly heritable.

McMillan (1956) observed a difference of 34 days in the blooming date of switchgrass from west to east in Nebraska. No correlation was found between flowering date and habitat for plants from the North Platte area. Several different types from the western areas had the same blooming date. McMillan suggested that a combination of late spring frosts, short wet seasons, early dry seasons, and early fall frosts in the western sites could have favored individuals of rapid growth and early flowering in different populations.

In a cytogeographical study of P. virgatum from Manitoba and eastern Montana to Texas, McMillan and Weiler (1959) noted that plants from different areas differed in time of blooming. There was a tendency of the lower ploidy plants to reach initial anthesis earlier than the higher ploidy types within certain population samples. It was found that $2n=36$ was more northern and western in distribution than $2n=72$, with the tetraploid plants being shorter in the more northern areas. They noted that populations with a preponderance of 72-chromosome types were chiefly in Kansas. The apparent disagreement between Church (1940) and McMillan and Weiler (1959) as to whether the 36-chromosome plants are predominant in the northern or southern areas suggests that two different tetraploid populations may be involved.

In the study by McMillan and Weiler, it was observed that the $2n=54$ is less common and is always associated with one or both of the other two numbers (36 or 72), usually with both.

They suggested that 54 is a secondarily derived number, possibly of hybrid origin.

Rogler (1943) found that as the origin becomes more southern the ability to survive low temperatures decreases. In a study covering about the same area, Nielsen (1947) found no correlation between polyploidy and winterhardiness. Jones and Newell (1946) observed that temperatures below 72° F inhibited blooming. They found the average pollen dispersal period per inflorescence was 12 days with anthesis occurring from 10 a.m. to 2 p.m. In a study involving 40 grasses, Jones and Newell (1948) concluded that P. virgatum was a relatively light producer of pollen.

Switchgrass has been considered a cross-pollinated species although the possibility of some apomixis has been recognized. According to Brown and Emery (1958) the Gramineae family is characterized by a rather high frequency of apomixis. In their study of 153 species in the subfamily Panicoideae, apomixis was found in 43 or about 28 percent. From their study they concluded that the North American species, P. virgatum, is probably both sexual and aposporous. Two plants from Texas appeared to be normal and sexual, but another from Texas and one from Rhode Island had embryo sacs which, though difficult to characterize, appeared to be four-nucleate. Apomixis is suggested by apospory, but is not proven unless parthenogenesis or apogamy follows.

In a cytological study Carver (1957) observed that all

lowland plants had 36 chromosomes. They were quite regular at meiosis and produced pollen of high stainability. Chromosome numbers of 54, 72, 68, 70, 76 and 78 were reported for the upland type. This type proved to be quite variable both in meiotic behavior and pollen stainability.

Means (1958) made a morphological study involving upland and lowland types. He found that leaf length, leaf width, panicle length, height of leaf tips, and number of tillers per plant were highly correlated with one another. In contrast, internode diameter was not correlated with any other character. He concluded that the lack of correlation between internode diameter and any of the other characters indicated that selection for fineness of culms need not result in adverse changes in other characters contributing to forage value.

MATERIALS AND METHODS

Plant Materials

Sixteen parent plants constituted the basis of this study. They originally were part of a preliminary evaluation nursery at the Kansas Agricultural Experiment Station. In Table 1 these plants are grouped according to morphological type with their sources and chromosome numbers as previously determined by Carver (1957).

Table 1. Chromosome numbers and sources of plants.

Code No.	Kansas State No.	Chromosome No.	Source
<u>Lowland</u>			
2	51047-1	36	Treese, Kans.
3	51047-3-2	36	Treese, Kans.
4	51048-1-2	36	Treese, Kans.
5	51049-1-2	36	Treese, Kans.
6	51050-1	36	Treese, Kans.
<u>Upland</u>			
7	51034-2	72	Welda, Kans.
8	51083-1	72	Cushing, Okla.
9	51086-1	72	Perkins, Okla.
10	51087-1	54	Perkins, Okla.
11	51090-2	70	Bristow, Okla.
12	51096-1	72	Purcell, Okla.
13	51116-1	72	Kinsley, Kans.
14	51135-1	72	Bonham, Texas
15	51232-1-2	68	Manitou, Okla.
16	51235-1	78	Fredrick, Okla.
17	2130-19	76	Okla. Selection

For future reference, voucher specimens of these plants were placed in the herbarium at Kansas State University (KSU). Descriptions of the distinguishing features of these parent plants are given below.

All of the lowland plants were glabrous and blue-green in color. The most readily visible distinguishing feature was stigma color. Numbers 2, 4 and 6 had purple stigmas as shown in Fig. 1, Plate I, while number 5 had white stigmas with purple centers (Fig. 4, Plate I) and plant 3 had white stigmas (Fig. 5, Plate I). Plants 3 and 5 were shorter and finer-stemmed than the other lowlands, and number 3 appeared to have shorter,

narrower leaves than the others.

Upland plants were more variable, and individuals could be identified by their vegetative characteristics.

Plant 7. A coarse-stemmed, green plant with longer-than-average leaves which had pubescence over the entire upper side of the blade.

Plant 8. A short, blue-green plant which had short leaves and pubescence extending one to one and one-half inches from the base of the blade. The anthers were yellow-orange in contrast to the normal orange color.

Plant 9. A blue-green plant which had short and narrow leaves. Pubescence did not extend as far from the base of the blade as on number 8.

Plant 10. A coarse-stemmed, green plant with sparse pubescence extending approximately one inch from the base of the blade. It did not appear to be as vigorous as the other plants, in either top growth or root development. Usually only three or four culms were produced when the plant was grown in a six-inch clay pot.

Plant 11. A coarse-stemmed, green plant with the upper surface of the leaf blades appearing slightly blue. The leaf blades were wider and longer than those of most other upland plants with sparse pubescence extending two inches from the base primarily along the midrib.

Plant 12. A fine-stemmed plant with a dark blue-green color. The leaf blades were long and narrow and often exhibited

purple streaks, especially toward the tips. Pubescence extended three-quarters of an inch from the base of the blade.

Plant 13. This plant has a slight blue-green color and leaf pubescence extending about one-half an inch from the base of the blade.

Plant 14. A coarse-stemmed, broad-leaved, green plant with pubescence extending about two inches from the base of the leaf blade.

Plant 15. This plant was short, fine stemmed, and blue-green in color. It has short, relatively narrow leaves. Very little pubescence was present, extending usually one-quarter of an inch, but sometimes up to one-half an inch, from the base of the leaf blade.

Plant 16. This plant was slightly blue-green in color and had narrow leaves. Pubescence was similar to that on number 15.

Plant 17. This was a green, coarse-stemmed, long-leaved plant. Leaf-blade pubescence was sparse and extended one to three inches along the midrib.

Cultural Practices

Parent plants were transplanted into five-gallon cans early in 1961. Each parent plant was usually broken into two or three propagules which were established in separate cans. For additional study some plants were grown in six-inch clay pots. Excellent growth was obtained on all plants by mid-summer when they began flowering. All plants were clipped in the fall after

the seed was harvested.

After some regrowth had occurred, the plants were subjected to the equivalent of a long-day photoperiod by lighting from 12 p.m. to 1 a.m. When blooming began again several plants were isolated for self-fertility studies.

The progenies were grown in three-inch clay pots, with only one plant per pot. These plants were grown on a sand bench to allow further development of the root systems. Since a large portion of the root system was in the sand, fertilizer was used to aid their growth. All progenies remained in the pots until examined for distinguishing features.

Crossing and Selfing Procedures

Most crosses and self pollinations were attempted by distance isolation of the plants involved. Only a few plants were bagged. Individuals to be selfed or crossed were located in separate greenhouses when possible, but due to a lack of isolated areas several plants were sometimes put in the same area. Therefore, a plant was sometimes considered isolated when separated by as little as 15 feet from another plant. Since switchgrass pollen is disseminated by wind such isolations could not be considered absolute. It was impossible to exclude all wind movement, and for this reason a record of all plants within each greenhouse was kept.

Emasculation by means of tweezers was attempted on several plants, but almost always resulted in severe damage to the floret.

This trouble was attributed primarily to difficulty in entering the floret which in turn resulted from the indurate nature of the lemma and palea.

In the selection of plants for crossing an attempt was made to combine individuals which differed phenotypically to the extent that intermediate hybrids could be readily distinguished. To eliminate the possibility that some florets could have been pollinated by an unknown source before being isolated, all spikelets which had bloomed prior to the time of isolation were removed.

During the spring and early summer of 1962, single plants were isolated in separate greenhouses to determine seed set under self pollination. Panicles of these plants were vigorously shaken each day to enhance pollen dispersal. Plants which were not isolated were used as open-pollination checks. These plants received some agitation but not the vigorous shaking received by the self-pollinated plants. At the time, these plants provided the best comparisons as to amount of seed set under these conditions.

Seed Harvest and Germination

Panicles were harvested after the glumes were brown, and usually after the rest of the inflorescence had lost its green color. Panicles were hand stripped, and the seed was cleaned by means of a rubbing board and seed blower. In seed germination the procedure outlined in the Rules for Testing Seeds (1960), as

adopted by the Association of Official Seed Analysts, was followed as nearly as possible. Seed was placed on filter paper in petri dishes, and prechilled at 5°C for two weeks. A solution of 0.2% potassium nitrate was used to keep the filter paper moist. During the early stages of the experiment a small amount of Captan was used to control fungi. Later, control was improved by autoclaving the filter paper in the petri dishes and by using a potassium nitrate solution containing 0.5% of chlorox. The germinator was lighted by means of two 60-watt bulbs and was maintained at 15°C for eight hours followed by 30°C for 16 hours.

As the seeds germinated, they were transplanted to three-inch clay pots, six seedlings per pot. These were maintained in a greenhouse at 80-85°F. When these seedlings were approximately two inches high they were transplanted to three-inch clay pots, one per pot.

A different procedure was followed for plants harvested in 1962. All spikelets were counted and hand stripped. The seeds were then removed by hand rubbing. This was done to insure accurate counts and to be sure that no seed was damaged.

Pollen Counts

In an attempt to determine if there was a difference in the proportion of normal pollen produced, parts of panicles with mature pollen were collected and fixed for 24 hours in a Carnoy's solution consisting of:

6 parts 95 percent ethanol,
 3 parts chloroform,
 1 part glacial acetic acid.

Fixed material was stored in 70 percent ethanol at 50°C until examined. Pollen to be examined was obtained from the three anthers of the more mature floret in a spikelet. The pollen was then evaluated upon its stainability in the following solution:

potassium iodide 1 gm.,
 iodine 1 gm.,
 ethanol 100 ml.

Pollen grains were classified A, B, or C depending upon their individual staining. Grains representative of each class may be described as follows:

Class A: Non-shrivelled grains with 90 percent or more of their contents intensely and uniformly stained,

Class B: Grains having some stain but considered abnormal due to shrivelling, mottling, partial staining (less than 90 percent), and/or low stain intensity,

Class C: Grains exhibiting no stain.

For each plant 500 pollen grains were examined.

Photographs

Photographs are enlargements made from color slides.

Original pictures were taken with a 35 mm. Exakta VX camera equipped with extension tubes. Final magnification was approximately 5X for all figures.

Stigma Color Classification

Five classes of stigma color were recognized. Descriptions and photographs of these classes are on pages 18 and 19. Classification was made within two days of stigma emergence.

EXPERIMENTAL RESULTS

Self- and Open-Pollination Seed Set

Data on self- and open-pollination seed set are summarized in Table 2. Seed set under open pollination was not especially high, but this may be accounted for by environmental conditions. Results of the Mann-Whitney U-test (Siegel, 1956) showed a significant reduction in seed set following self pollination of lowland plants ($P=0.050$). Reduction of seed set following self pollination of upland plants was only weakly significant ($P=0.071$). When both types were combined, reduction in seed set following self pollination was significant ($P=0.024$). The high P value obtained for upland plants can be largely attributed to the high self-pollination seed set by plant 7. If an open-pollination check had been available, it might have offset to some extent the high percentage seed set obtained under self pollination.

Pregeny Results

Results of attempted crosses and self pollinations made in 1961 are shown in Table 5. All seeds which germinated appeared normal, but some seeds which did not germinate had dark embryos. All seedlings appeared normal with no obvious differences in

Table 2. Self- and open-pollination seed set.

<u>Self pollination</u>			:	<u>Open pollination</u>			
<u>Plant</u>	<u>Spikelets*</u>	<u>Seeds</u>	<u>Percent</u>	:	<u>Spikelets</u>	<u>Seeds</u>	<u>Percent</u>
<u>Lowland</u>							
2	5058	13	0.26	:	4226	481	11.38
	1440	3	0.21	:			
	1157	0		:			
	634	5	0.08	:			
	<u>8259</u>	<u>21</u>	<u>0.25</u>	:	<u>4226</u>	<u>481</u>	<u>11.38</u>
3	783	5	0.64	:	611	163	26.68
	638	4	0.63	:	567	50	8.82
	597	2	0.34	:	564	130	23.05
	<u>2018</u>	<u>11</u>	<u>0.55</u>	:	<u>1742</u>	<u>343</u>	<u>19.69</u>
4	1373	20	1.46	:	3734	745	19.95
	969	3	0.31	:	2822	1170	41.46
	769	2	0.26	:			
	678	8	1.18	:			
	568	2	0.35	:			
	337	1	0.30	:			
	<u>4694</u>	<u>36</u>	<u>0.77</u>	:	<u>6556</u>	<u>1915</u>	<u>29.21</u>
<u>Upland</u>							
7	496	67	13.51	:			
	404	54	13.37	:			
	392	70	17.86	:			
	356	62	17.42	:			
	270	38	14.07	:			
	209	22	10.53	:			
	<u>2127</u>	<u>313</u>	<u>14.72</u>	:	-	-	-
11	755	22	2.91	:			
	685	12	1.75	:			
	581	9	1.55	:			
	533	5	0.94	:			
	511	8	1.57	:			
	422	6	1.42	:			
	<u>3487</u>	<u>62</u>	<u>1.78</u>	:	-	-	-

Table 2. (cont.)

Plant	Self pollination			:	Open pollination		
	Spikelets*	Seeds	Percent		Spikelets	Seeds	Percent
12	605	4	0.66	:	375	5	1.33
	595	1	0.17	:	193	4	2.07
	447	1	0.22	:	104	2	1.92
	311	0		:	80	7	8.75
	260	1	0.39	:			
	244	5	2.05	:			
	187	5	2.67	:			
	185	1	0.35	:			
	180	2	1.11	:			
	176	0		:			
	129	4	3.10	:			
	121	1	0.83	:			
	119	1	0.84	:			
	108	2	1.85	:			
	107	4	3.74	:			
	106	1	0.94	:			
	72	1	1.39	:			
	71	0		:			
	59	0		:			
	57	1	1.75	:			
	38	0		:			
	30	0		:			
	<u>4207</u>	<u>35</u>	0.83	:	<u>752</u>	<u>18</u>	2.39
14	561	1	0.18	:	491	32	6.52
	496	0		:	296	11	3.72
	475	0		:	197	0	
	300	0		:	156	3	1.92
	239	0		:	123	0	
				:	<u>121</u>	<u>6</u>	4.96
	<u>2071</u>	<u>1</u>	0.05	:	<u>1384</u>	<u>52</u>	3.76
15	257	1		:	299	118	39.47
	253	4	1.58	:	285	110	38.60
	243	15	6.17	:	244	68	27.87
	200	2	1.00	:	225	92	40.89
	197	1	0.51	:	195	53	27.18
	177	0		:			
	176	0		:			
	174	1	0.58	:			
	159	0		:			
	158	1	0.63	:			
	139	0		:			
	130	0		:			
	<u>102</u>	<u>1</u>	0.98	:			

Table 2. (concl.)

				Self pollination	:	Open pollination		
Plant:	Spikelets*	Seeds	Percent			Spikelets	Seeds	Percent
15 (cont.)	101	1	0.99	:				
	94	0		:				
	89	0		:				
	88	0		:				
	77	0		:				
	62	0		:				
	60	0		:				
	58	1	1.72	:				
	58	1	1.72	:				
	52	0		:				
	46	0		:				
	46	0		:				
	45	0		:				
	41	0		:				
	39	0		:				
	29	0		:				
	27	0		:				
	21	0		:				
	<u>3430</u>	<u>25</u>	0.73	:	<u>1248</u>	<u>441</u>	35.34	

* Each line is one panicle. The total number of spikelets and seeds with the corresponding percentage is then given for each plant.

vigor.

Descriptions of the progenies are given below.

Self-Pollinated Lowland.

Plant 3. Three offspring flowered twice and could not be distinguished from the parent.

Plant 4. The single offspring could not be distinguished from the parent plant.

Lowland x Lowland.

Crosses 3x4 and 4x3. Stigma colors of the progenies were divided into classes described on page 18. Corresponding

EXPLANATION OF PLATE I

- Fig. 1. Stigmas which were entirely purple, Class I.
- Fig. 2. Stigmas purple, except for white fringe around the outer edge, Class II.
- Fig. 3. Purple-centered stigmas with remainder of stigmas white, Class III.
- Fig. 4. Stigmas with only a trace of purple down the center, Class IV.
- Fig. 5. Stigmas entirely white, Class V.

PLATE I



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5

photographs illustrate each type. The distribution of stigma color is summarized in Table 3.

Table 3. Stigma color.

Cross	Class I	Class II	Class III	Class IV	Class V	Total
3x4	45	94	40	3	1	183
4x3	52	75	34	7	0	168
Total	97	169	74	10	1	

According to the Kolmogorov-Smirnov test (Siegel, 1956), the reciprocal crosses yielded essentially the same distribution of stigma colors in the F_1 . Some stigmas were difficult to classify, especially those in Classes II and III. Late-blooming progeny from the 4x3 cross had more light-colored stigmas than those which had bloomed earlier.

Cross 6x26. This was considered a lowland x lowland cross (even though 26 was obtained from an attempted lowland x upland cross) since 26 showed no signs of being intermediate and was considered a lowland. The 15 progeny from this cross also produced a range in stigma color. Stigmas varied in color from Class II through IV with approximately equal numbers in each class.

Self-Pollinated Upland.

Plant 7. The six offspring looked like the parent.

Plant 8. The offspring could not be distinguished on the basis of color or leaf pubescence. All 11 looked like the parent plant.

Plant 9. These 62 progeny were all blue-green, but there was some variation in the leaf pubescence. On the parent,

pubescence extended from one-half of an inch to one inch from the base of the leaf blade. Eleven progeny were like this, but 51 had pubescence extending only one-quarter of an inch from the base of the blade.

Plant 11. Of the 40 progeny 22 looked like the parent plant, but three had more pubescence on the leaf blades while 14 had less. One plant was blue-green with narrow leaves and had leaf blade pubescence extending one-quarter to one-half an inch from the base.

Plant 15. Of the 329 offspring, 288 looked like the parent plant, but 39 had more leaf blade pubescence. In addition, one plant had pubescence extending two to three inches up the leaf blades while another had light-colored stigmas. Considerable variation in rhizome development was also noted.

Plant 16. Both offspring looked like the parent plant.

Plant 17. The single offspring was like the parent plant.

Upland x Upland.

Cross 10x7. All 42 progeny had few stems as did the female parent. With respect to the pattern of leaf blade pubescence four classes of offspring were distinguished. Four plants resembled the maternal parent while five were like the paternal parent. Of the other plants, eight had three inches of pubescence, primarily along the midrib while in 25 pubescence extended about five inches from the base of the blade.

Cross 11x16. In general all 22 progeny resembled the female parent in color but were more like the male parent in leaf width.

Pubescence on eight progeny was similar to that of the female parent, but 14 had only sparse pubescence which extended a maximum of one-quarter of an inch from the base of the leaf blade.

Cross 16x11. Both offspring resembled the female parent, but one had wider leaves than the other.

Cross 13x16. Of the 33 progeny 2 were green while another had a bluish color like that of the parents. Pubescence of 22 progeny resembled that of the parents, but on 11 others it extended four to six inches from the base of the leaf blade.

Upland x Lowland. The most important feature of these progenies was that all were of the upland type.

Cross 12x3. Of the 43 progeny examined, 37 looked like the female parent. The others differed in amount of pubescence on the leaf blades. One progeny had just a trace of pubescence, two had about half an inch, and three had pubescence extending three inches from the base of the blade.

Cross 12x4. All three offspring looked like the female parent.

Cross 15x2. Of the 112 progeny 61 looked like the female parent. Pubescence extended one-half to three-quarters of an inch from the base of the leaf blade on 33 other progeny. On 18 others it extended over one inch from the base of the blade. Two progeny were considerably coarser stemmed than the others. Stigma color was purple in all plants but varied in shade.

Cross 17x27. Sixteen of the 17 progeny resembled the female parent. One possessed long, dense pubescence which extended four

inches from the base of the leaf blade.

Lowland x Upland. All these progenies were of the lowland type.

Cross 2x15. Some variation in stigma color was present, but most stigmas were purple. Five plants were in Class I with respect to stigma color while 16 were in Class II. In addition, three were in Class III, and two were in Class IV.

Crosses 3x12, 3x15, 4x12, and 4x14. These progenies could not be distinguished from their respective maternal parents.

Open Pollination. The only open-pollination progeny studied was that of plant 29. Plant 29 was a self-pollination offspring of number 3 and, like 3, possessed white stigmas. The 13 open-pollination progeny of this plant exhibited a range of stigma color similar to that noted in the 3x4 and 4x3 crosses. Two were in Class I with respect to stigma color, five were in Class II, four in Class III, and two in Class IV. No offspring possessed completely white stigmas.

Pollen Stainability

Results of pollen-grain counts are shown in Table 4. All plants produced high percentages of well-stained pollen with no consistent differences among plants noted.

Pollen collections were made from late February through June, and stainability seemed to some extent, to be associated with date of collection. Samples collected in late February exhibited relatively low stainability. Percentage stainability

seemed to increase as the season progressed until late April after which there was some suggestion of a decline.

Table 4. Pollen stainability.

Plant no. or cross*	:	Number of grains classified as		
		A	B	C
2	:	488	12	0
3	:	499	0	1
4	:	484	16	0
8	:	487	7	6
9	:	498	2	0
10	:	490	9	1
11	:	495	5	0
12	:	483	15	2
13	:	483	17	0
17	:	497	3	0
6x26	:	492	1	7
10x7-1	:	487	13	0
10x7-2	:	492	9	0
10x7-3	:	496	4	0
10x7-4	:	484	16	0
10x7-5	:	490	4	6
11x16-1	:	495	5	0
11x16-2	:	496	4	0
11x16-3	:	492	8	0
11x16-4	:	490	10	0
13x16-1	:	490	10	0
13x16-2	:	484	16	0
15x2-1	:	484	10	6
15x2-2	:	491	9	0
15x2-3	:	495	5	0
15(x)	:	495	5	0

* Additional numbers after a cross indicate a specific offspring.

Basal Color

When the seedlings were four to six inches high, a wide variation in basal color was noted. This color ranged from green, through brown, to reddish purple. In lowland plants it was green to light purple while in upland plants it varied from light purple to an intense reddish purple. Although there was some overlapping most of the upland seedlings were sufficiently

darker in basal color that they could be distinguished from lowland individuals. Progeny from a given cross or self pollination were generally uniform with respect to this trait.

DISCUSSION

Self Compatability

With the exception of number 7, all plants appeared to be highly self sterile. This is not surprising since switchgrass is considered a cross-pollinated species. The number of progenies obtained by attempted crosses and self pollinations made in 1961 also indicated self sterility. Considerably fewer progenies were usually obtained from attempted self pollinations.

Since only one plant was used per isolation in the self-pollination seed set study it is possible that the supply of pollen was inadequate. It may therefore be possible to obtain higher self-pollination seed set by providing larger amounts of pollen or better dispersal of available pollen.

Self-sterility appeared to be more pronounced in the lowland type although most upland plants were also quite self-sterile. The 1962 seed set data indicated considerable variation in self fertility among upland plants. The number of progeny obtained from attempted self pollinations and from upland-by-lowland crosses also indicated great variability in self fertility. Although leaf blade pubescence varied, most progeny from an attempted self or cross pollination appeared rather uniform with leaf-blade pubescence being the only trait which

was used in most comparisons.

Gross Compatability

Gross compatability among lowland plants is discussed under stigma color while compatability between upland and lowland types and among upland plants is dealt with below.

Between Upland and Lowland. Results of attempted crosses between upland and lowland plants indicated a high level of cross incompatability. All progenies of attempted crosses were of the same type as the maternal parent and presumably the result of self fertilization.

Occurrence of 54-chromosome plants in nature suggests that crossing between 36- and 72-chromosome individuals does occur, but the 36-chromosome plants involved could be upland rather than lowland. Prior studies have suggested the existence of 36-chromosome upland plants, especially in northern areas. The 54-chromosome plant involved in this study fitted the description of the upland type in every way so that its morphological appearance suggested that both of its parents were upland. However, a hybrid between a 72-chromosome upland plant and a 36-chromosome lowland plant might be expected to resemble the upland parent rather closely since two-thirds of its chromosome complement would have been derived from the octoploid parent.

It is possible that in attempted upland-by-lowland crosses there was cross fertilization followed by a breakdown in the development of the embryo. The 54-chromosome plants which occur

in nature could result from occasional crosses in which this breakdown does not take place. This would explain why the 54-chromosome plants are not as common as the 36- and 72-chromosome individuals.

The complete bivalent pairing observed in tetraploid plants by Carver (1957) is an indication, but not proof, of allopolyploid origin (Riley, 1949). According to Stebbins (1958), in crosses between related allopolyploids having different degrees of polyploidy, the endosperm and embryo always develop more satisfactorily when the higher polyploid is the ovulate parent. If this is the case in switchgrass, then crosses between 36- and 72-chromosome plants would be more likely to succeed when the octoploid individuals were used as maternal parents.

If upland and lowland types do not cross, certified seed production would be greatly simplified since these two types could be treated as separate crops. This would mean they would need to be separated only enough to prevent mechanical mixing of the seed. This is in sharp contrast to the wide isolation distances required for cross pollinating varieties of the same species.

Since the upland and lowland types do not appear to cross, the question arises whether they are distinct species or subspecies. Cross incompatibility provides some basis for considering them separate species, but instances in which closely related individuals fail to cross are common. The difference in chromosome number could account for such incompatibility.

On the other hand, the two types differ considerably in their morphology, and a combination of morphological difference and cross incompatibility constitutes more substantial evidence that different species or subspecies are involved. Such a conclusion would be hazardous, however, until the morphological differences have been thoroughly studied.

Among Upland Plants. It was difficult to determine whether progeny obtained from attempted upland crosses were actually hybrids or simply the result of self fertilization. This was primarily because of the absence of a distinguishing feature which would provide such information. Since progenies were observed while still immature, it was difficult to determine how much variation within a group of progeny was genetic and how much could be attributed to environmental conditions.

The attempted cross, 10x7, seemed to provide the best evidence of crossing. Most of these progeny had leaf-blade pubescence similar to that of the maternal parent, but a few had pubescence over the entire upper surface of the leaf blade as did the paternal parent. It is possible that this was simply segregation of self-pollination progeny of plant 10. This appears doubtful, however, because of the small amount of self fertilization which appears to occur in this species. Although no chromosome counts were made, pollen stainability was as good as that obtained from any other group of plants. Since this was supposed to be a 54-by-72 chromosome cross, it would seem that the resulting progeny should be meiotically irregular and hence incapable of producing a high percentage of good pollen. It

therefore seems that either the 54-chromosome number was an inaccurate count or that some unusual mode of reproduction was involved.

Progenies obtained from other attempted crosses between upland plants seemed to exhibit enough variability to eliminate the possibility of a high level of apomixis. Since cross pollination generally yielded higher seed set than self pollination it is reasonable to assume that cross pollination is necessary for maximum seed set. The limited amount of information available indicates morphological variability within different chromosome races of the upland type. It is quite possible that the mode of reproduction also varies.

Upland switchgrass features a number of conditions which usually occur in apomictic species. It is a high polyploid and a perennial, and it possesses a wide range of chromosome numbers. In addition, Jones and Newell (1948), found it to be a light producer of pollen.

At present the mode of reproduction is not known, but evidence indicates that upland plants reproduce both sexually and asexually. If the plants are at least partially apomictic pseudogamy may be involved. The high percentage of good pollen which was produced, and indications of a higher seed set when pollen from other plants was available, suggest some pseudogamy.

Stigma Color

Stigma color was used extensively in the study of lowland

plants due to the variation in this trait in both parents and progeny. Most extensive use was made in crosses 3x4 and 4x3. As previously mentioned, stigmas of plant 3 were white while those of number 4 were purple. Distribution of the progeny, with respect to the five stigma-color classes, was essentially the same for the reciprocal crosses. This strongly suggests that self fertilization and apomixis play rather negligible roles in the reproduction of these plants. Cross-pollination seed set far above that expected under self pollination also suggests cross fertilization as the major mode of reproduction in the lowland type.

An interesting feature was that all self-pollination progeny of plants 3 and 4 appeared identical to their respective parents. It is possible that these plants were homozygous for genes controlling stigma color, but such homozygosity in both parents would be unlikely in a cross-breeding population unless one of the alleles occurred in a very low frequency. On the other hand, the number of self-pollination progeny studied may have simply been too low to demonstrate segregation. It is also possible that these plants are facultative apomicts and that they reproduce asexually when cross fertilization is not permitted.

It is interesting to speculate upon the mode of inheritance of stigma color in these crosses. Since the lowland plants exhibit bivalent meiotic pairing, (Carver, 1957), it seems unlikely that tetrasomic inheritance is involved. This cannot be ruled out completely, however, since Lotus corniculatus has bivalent pairing but was found by Dawson (1941) to exhibit

tetrasomic inheritance. A dosage effect involving at least two loci is also possible.

Pollen Stainability

The fact that the proportion of well-stained pollen varied with time of year emphasizes the importance of the environment in such studies. Results of studies concerning the effect of environment upon meiotic behavior have been summarized by Carnahan and Hill (1961). They reported that as temperature increased to 36°C, meiotic irregularity of Dactylis glomerata and Secale cereale also increased. In the present study the decline in proportions of well-stained pollen during summer months may have been a high-temperature effect. The poor pollen samples collected during the winter may also have resulted from unfavorable temperatures. If temperatures below 72°F inhibit blooming, as noted by Jones and Newell (1946), it is possible that poor pollen would be produced under marginal temperatures that are high enough to permit some flowering.

There may have been a tendency to select anthers containing above-average proportions of good pollen since slides showing excessive proportions of poorly stained pollen were frequently discarded on the assumption that the anthers involved were not at the appropriate stage of maturity. It is probable therefore, that recorded counts indicate maximum rather than average production of good pollen.

Pollen counts made in this study differ considerably from

those obtained on the same plants by Carver (1957). Carver, who made field collections, found a range of 99 to 65 percent for Class A pollen while in this study it varied from 99.8 to 96.6 percent. The discrepancy between the two observations and the small amount of variability obtained in this study suggest that the amount of well-stained pollen may be rather uniform under optimum conditions.

Basal Color

The wide range of basal color may be a manifestation of dosage effect since the darker colors were observed in the higher ploidy plants. From the limited observations made in this study it would seem that this difference in basal color could be used to distinguish upland and lowland types in the seedling stages. Before basal color is used as means of seedling identification, however, seedlings from a larger number of upland and lowland sources should be studied.

SUMMARY

Sixteen parent plants from seed collected in Kansas, Oklahoma, and Texas constituted the basis of this study. It was desired to study the mode of reproduction in P. virgatum and to investigate the cross compatability of upland and lowland types. The entire study was conducted under greenhouse conditions. Crosses and self pollinations were attempted by distance isolation. Seed set for fertility comparisons was determined by

hand threshing and counting.

Lowland plants were highly self sterile. Upland plants were generally self sterile, but were more variable in this respect than lowland individuals.

Observations on the inheritance of stigma color in lowland crosses indicated that reproduction in this type was mainly sexual. Evidence of sexuality in upland crosses was also obtained, but the possibility of a moderate level of apomixis was not eliminated.

Attempts to cross upland and lowland plants failed to produce any offspring with readily recognizable hybrid traits. A high level of cross incompatibility between upland and lowland types was consequently suggested.

Pollen stainability results indicated that all plants probably produce equally good pollen under optimum conditions. Collections of pollen made when temperatures were high or during the winter had a much higher proportion of poorly-stained pollen than those collected during the spring.

A wide range of basal color was noted in seedlings, with the darker colors present in the higher-ploidy plants. It seemed that this trait could be used to distinguish upland and lowland seedlings.

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APPENDIX

Table 5. Attempted crosses and self pollinations.

Code no.:	Self pollination or cross	No. seeds germinated:	Comments*
<u>Lowland Self Pollination</u>			
50	2	-	1 panicle bagged (400)
	3	3	bagged, progeny 21, 29, and 30
51	4	1	5 panicles
52	5	-	
53	6	-	1 panicle bagged (800)
43	21	-	no seed
65	22	-	1 panicle bagged (300)
66	23	-	1 panicle bagged (400)
44	26	-	no seed
45	27	-	no seed 1 panicle
	27	-	no seed 1 panicle
	29	-	no seed 1 panicle
<u>Lowland x Lowland</u>			
72	3x4	241	more seed 27 panicles (8100)
73	4x3	227	more seed 20 panicles
78	6x26	25	several panicles, 1 harvested
79	26x6	-	several panicles, 3 harvested
86	20x28	-	1 panicle bagged (300)
87	28x20	-	1 panicle bagged (300)
<u>Upland Self Pollination</u>			
40	7	12	
49	7	-	1 panicle bagged (300)
54	8	14	5 panicles
55	8	2	1 panicle bagged (350)
56	9	9	many panicles, 1 harvested
57	9	91	many panicles
58	11	78	18 panicles (3000) more seed
59	15	54	many panicles, 1 harvested (more seed)
60	15	-	9 panicles bagged (900)
61	15	394	many panicles
41	16	2	
42	17	3	
62	17	-	1 panicle bagged

Table 5. (concl.)

Code : Self pollination : No. seeds :			
no. :	or cross	: germinated :	Comments*
<u>Upland x Upland</u>			
80	9x17	-	
81	10x7	75	more seed, 2 or 3 panicles
82	11x16	26	several panicles, 1 harvested
83	16x11	6	many panicles, 1 harvested
84	11x12	1	1 panicle each, bagged (300)
85	13x16	61	many panicles, more seed
<u>Upland x Lowland</u>			
75	12x3	59	
77	12x4	11	20 panicles, more seed
71	14x2	2	many panicles, more seed
69	15x2	154	many panicles, more seed
46	17x27	40	
<u>Lowland x Upland</u>			
67	2x11	-	1 panicle each bagged (400)
70	2x14	-	23 panicles
68	2x15	52	many panicles, more seed
74	3x12	14	25 panicles, more seed
	3x15	1	bagged, progeny 28
76	4x12	20	15 panicles, more seed
	4x14	7	bagged, progeny 20,22,23,24, 25,26 and 27
88	23x8	-	1 panicle harvested (200)
<u>Open Pollination</u>			
90	29	18	several panicles, more seed

* Numbers in parenthesis indicate approximate number of spikelets harvested.

HYBRIDIZATION IN PANICUM VIRGATUM L.

by

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The mode of reproduction in Panicum virgatum and the cross compatability of upland and lowland types of this species were investigated. Sixteen parent plants from seed collected in Kansas, Oklahoma, and Texas constituted the basis of the study. The entire investigation was conducted in greenhouses. Attempted crosses and self pollinations were made by distance isolation or separation in different greenhouses. Seed set in fertility comparisons was determined by hand threshing and counting.

Lowland plants were highly self sterile, yielding a seed set of generally less than one percent when self pollinated. Upland plants were generally self sterile, but were more variable in this respect than lowland plants.

Observations on the inheritance of stigma color in lowland crosses indicated that reproduction in this type is mainly sexual. Evidence of sexuality in upland crosses was also obtained, but the possibility of a moderate level of apomixis was not eliminated.

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