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Observations on the Behavior, Genetics, and Cytology of Two South African *Digitaria valida* Stent Accessions in Puerto Rico

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INTRODUCTION

Digitaria is a Panicean genus with mainly Tropical distribution. Several species native to South Africa have proved to be of economical importance in their home country (Chippendall (6)² 1959). Species with aggressive and stoloniferous growth habits have been used with success as forage plants and in combatting erosion. One of such South African grasses, *Digitaria decumbens* Stent, has become, since its introduction to the U.S. in 1935, one of the favorite pasture plants in South Florida and many regions of Tropical America, including Puerto Rico (1,18,21).

Pangolagrass, as this grass is commonly known, is practically sterile—so far only one seed is reported to have germinated (14), resulting in a plant with 27 chromosomes and a highly abortive micro- and megasporogenesis (25). Although the species may have different ecotypes in its country of origin, the introductions, consisting normally of restricted samples, contain little or no such natural variation. The exclusively vegetative mode of reproduction of the introduced ecotype limits genetic variation. It also blocks breeding by crossing.

As pangolagrass, despite of its great merits in Puerto Rico, is susceptible to certain natural hazards like pest insects and virosis (14,21), it would be desirable to find or to develop other *Digitaria* forms that would meet even more strict requirements than *Digitaria decumbens*. Thus numerous accessions of over 20 South African *Digitaria* species have been recently introduced to this Station, to study their performance and their suitability for crossbreeding. One of the most promising species is *Digitaria valida* Stent, a grass closely related to *D. decumbens* and to *D. pentzii* Stent. According to Chippendall (6), both *D. decumbens* and *D. valida* are only forms of *D. pentzii*.

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² Italic numbers in parentheses refer to Literature Cited, pp. 284-5.

An analysis of the taxonomic characters and breeding behavior of a *D. valida* strain, introduced via Cuba, was made by Sotomayor-Ríos *et al.* (26). They counted $2n = 42$ chromosomes, which differs from earlier determinations $2n = 24$ (30), $2n = 30$ (27), and $2n = 36$ (28). Microsporogenesis was irregular, with uni- and multivalents, anaphasic laggards, and micronuclei; perfect stainability was encountered in only 2.3 percent of pollen. Megaspore mother cell and megaspore tended to degenerate, and normal megasporogenesis was seldom seen. Karyopsis occurred in 1.5 percent of florets, but no germination of seeds was observed.

Since the above study was made, 11 South African *Digitaria valida* accessions obtained from the New Crops Research Branch, U.S. Department of Agriculture, have been maintained here for cytological and reproduction studies. Observations made on these plants are presented here, with a special reference to the two most promising accessions.

MATERIALS AND METHODS

The accessions under study were numbered 1950C and 1953A, corresponding to USDA Plant Introduction Nos. 209177 and 209372, respectively. Both are from the Transvaal, 1950C originating from Krüger National Park, 1953A from an unnamed locality between Zeerust and Ottoo-hoop. Both localities are approximately at lat. 29° S., that is, about 7° further from the equator than Puerto Rico.

Most observations were made from potted plants maintained either in a greenhouse or outside.

THE DEVICE USED FOR PROLONGATION OF DAYLIGHT³

Four 115-v 200-w bulbs, provided with 1-ft. 6-in. white reflectors, were installed in a greenhouse, in rows of two, 6 ft. 4 in. above ground, the distance between rows being 2 feet, and between lamps in each row, 6 feet. The intense light covered about 6×8 feet of the ground, which is a sufficient space for 18 to 20 plants. An electric timer-clock was coupled in the circuit for determination of the hours of extra illumination.

MEIOSIS IN POLLEN MOTHER CELL (PMC)

The waxy glumes efficiently protect the closed flowerbud against fixatives and stains. We are not sure that fixing such flowerbuds under low pressure (aspiration) is enough to bring sufficient fixative into the bud. We therefore preferred to cut the tips of the flowerbuds away with scissors. A good fixation and coloring is thus obtained under normal pressure. Although the method is laborious, it pays for itself completely later, when the

³ In planning and installing this device, valuable help was obtained from Dr. M. A. Tió, Phytobiologist of this Station.

stained anthers can be simply removed out of each bud by squeezing with forceps.

In *Digitaria valida* spikes the topmost flowers are solitary, the lower ones occurring in twos. The lower of the two is supposed to be sterile in *D. decumbens* (25). For this reason we did not study these flowers.

The PMC divisions occur in spikes where the uppermost protective leaf has turned at a right angle with the stem. Spikes at this phase were collected between 8 and 11 a.m. The top $1\frac{1}{2}$ -2 inches of each spikelet were removed and fixed in Östergren's (20) fixative (without 2, 4-dinitrophenol), originally recommended by him for technically difficult root-tip chromosomes of Gramineae. It is useful to maintain the buds in the rachis, because their sequence helps to locate the proper meiotic stage. After having been kept overnight in the fixative, the rachis with buds were transferred to Dyer's (10), lactopropionic orcein for 1 day or more, then anthers were squeezed out of the buds and squashed on albuminized slides. The anthers in proper stages stain yellowish-red and are somewhat translucent, whereas younger and older anthers stain deep red. As PMC are not easily separable from the somatic tissue, the anthers were squashed as a whole. A simple wooden press⁴ which applies a pressure of 150 to 250 kg./cm.² on the cover slip, helps in flattening the tissue.

Pollen stainability (starch content) was determined with a mixture of cotton blue, lactophenol, and Turtox CMC-10 (7). For determination of pollen germination, grains were collected early in the morning. The methods give by Narasimhan (17), Conger (8), Brewbaker (3), and Eskilsson (11), and numerous modifications of these methods, were tried. All attempts failed.

OBSERVATIONS

GENERAL OBSERVATIONS ON THE GROWTH HABITS OF THE 11 ACCESSIONS TO WHICH 1950C AND 1953A BELONG

This sample of 11 accessions was obtained in seed form, and sown first in seedbeds in the greenhouse and then transplanted to open field plots. Later, selected plants were planted in pots and kept in a greenhouse since the winter 1961. The natural lengthening of daylight in the spring induced a profuse flowering in most of the accessions during the following June. In the spring of 1962, very few flowers developed, although the plants had been divided and replanted in new soil. It seems that only the relatively young stage, after germination of the seed, responded well to the Puerto Rican spring conditions to which they were subjected in the greenhouse.

⁴ Developed by Dr. Veikko Sorsa, Institute of Genetics, University of Helsinki, Finland.

Because we needed information on the normality *vs.* abnormality of the meiosis and embryosac development, we could not rely on scanty, probably crippled, flower material produced under apparently adverse conditions. We needed vigorously flowering plants where the reproductive processes would develop as optimally as possible.

To find a solution for this need, the plants were divided and replanted in identical pots containing the same soil. Three groups were formed, each of them containing the complete set of 11 accessions. One group was located on tables outside the greenhouse, with no overshadow, but under regular daily watering. The second group was placed in the greenhouse under the artificial illumination device, as described in Materials and Methods (p. 270). Three hours of extra illumination, from 3:00 to 6:00 a.m., were given to the plants. The third group was maintained in the greenhouse not far from the second group, but outside of the direct artificial illumination. In addition, the four accessions that had not flowered in the first and second spring, were also planted in plots in Corozal Substation (elevation 410 ft., yearly rainfall 76.84 inches, medium temperature of the year 75.8°F.; as compared with 124 ft., 76.58 inches, and 77.0°F. of Río Piedras, respectively). This division of the material was made in November 1962.

Within half a year we had obtained information sufficient to know how to proceed. Of the Corozal plants, two flowered, one did not, and one died. Some of the plants placed on tables outside the greenhouse flowered, but most of them started developing runners and did not flower. The sample placed in the greenhouse outside of the artificial illumination behaved as did the sample of the foregoing year: just a few accessions developed a few spikes. The plants placed in the artificial illumination device grew vigorously, forming erect abundant leaves, and profuse, strong flowers, and few or no runners. After this experience, we maintained the light treatment unaltered, and with it we have been able to induce flowering at all seasons of the year and in all but one of the accessions.

THE RESPONSE OF 1950C AND 1953A TO THE ENVIRONMENT AND THEIR REPRODUCTIVE BEHAVIOR

The first two accessions that responded to prolonged illumination in late winter in 1963 were the two plants labeled 1950C and 1953A. They formed a very strong, high, erect growth, and abundant, erect spikes, and no runners. Flowering started 3 weeks earlier than in the other accessions. Thus, first there were no other flowering species of *Digitaria* in the greenhouse than the plants 1950C and 1953A, placed close together. The possibility of open cross-pollination was practically excluded, and the seed formed in these plants was expected to be the product of either self-pollination or apomixis,

or of crossing between the two accessions. We therefore decided to collect and sow the seed to analyze the probable progeny.

No karyopsis occurred in the vast majority of flowers. The total yield of empty and full seeds was sown on March 19, 1963, without trying to count the percentage of full seed. Thus 22 daughter plants of the 1950C and 72 of the 1953A were obtained. The young plants were individually planted in pots and placed on tables outside the greenhouse, in full sun and under regular watering. Soon many of them showed notably aberrant types from the mother type. Both progenies were classified according to their growth characteristics in three groups:

<i>Progeny of:</i>	<i>Maternal type</i>	<i>Intermediate type</i>	<i>Extreme aberrant type</i>	<i>Total</i>
1950C	7	11	4	22
1953A	6	26	40	72
	(6)	(11)	(10)	(27)

The progeny of 1953A was reduced by selecting the most representative plants in each group (numbers in brackets above).

Table 1 shows the status of flowering and runner development July 1, when the plants were 14 weeks old, and on September 1. The mother-type plants flowered early and grew erect. The runners appeared late, and were few in number in most cases. Most of the extreme aberrant plants did not flower at all. The few flowers that appeared were also early. Formation of runners was early and abundant. In the intermediate type of plants, flowering and runner formation started more or less simultaneously, but here also the vigorous runner growth continued after the flowering had ceased. Figure 1 shows a typical extreme aberrant and a typical mother-type plant.

In September the pots were filled, the brittle runners so tangled, and the growth conditions so hampered that the observations were discontinued. The three most representative plants of each type were selected in each progeny for future observations. The rest of the plants were discarded.

Seed was collected from five plants each of the maternal and the intermediate types. The extreme aberrant plants did not produce seed. This time, the meticulous labor of selecting the full seed from the empty ones was accomplished. The data obtained show that the karyopsis occurs in few flowers, and the germination of the full seed is very low (table 2).

Figure 2,A shows the whole progeny 2 months after sowing: 1 descendant of an intermediate-type 1953A strain, and 16 descendants of the 1950C strain. In this early phase the plants started showing differences in growth type and vigor. They were planted in individual pots, using again the same soil, and were placed this time in the greenhouse, but without artificial il-

lumination. Although most of the plants grew erect, there were exceptions. Figure 2,B shows two decumbent plants on March 1, 1964. They look quite alike, but the left one developed runners, the right one lodging flowers. Figure 3,A shows the very extreme types: One erect, early, and well-flowering, just as the original mother type, the other cretinique, weak, with lodg-

TABLE 1.—*Measures of some flowering and growth characteristics in the progenies of the accessions 1950C and 1953A¹*

Progeny types	Number of plants	Mean number of spikes		Mean difference	Standard error of the difference
		July 1	Sept. 1		
1950C-PM	7	6.29	0.14	6.15**	1.2936
1950C-PI	11	5.36	.36	5.00**	1.5406
1950C-PE	4	.50	.00	.50NS	.2887
1953A-PM	6	4.17	.00	4.17**	.7031
1953A-PI	12	3.42	.50	2.92**	.7483
1953A-PE	10	.50	.00	.50NS	.3073
<i>Mean number of runners</i>					
1950C-PM	7	0.43	2.86	2.43*	0.9583
1950C-PI	11	6.55	20.29	13.74**	3.4276
1950C-PE	4	8.00	15.25	7.25NS	3.8160
1953A-PM	6	.50	3.50	3.00NS	2.0896
1953A-PI	12	6.50	10.33	3.83NS	2.3252
1953A-PE	10	14.30	19.20	4.90NS	2.6112
<i>Mean length of runners²</i>					
1950C-PM	7	5.36	36.36	31.00**	9.6074
1950C-PI	11	50.00	72.43	22.43**	6.8703
1950C-PE	4	60.75	62.50	1.75NS	8.8045

¹ PM = mother type, PI = intermediate type, PE = extreme aberrant type.

² Not measurable for 1953A plants, due to fragmentation.

ing flowerstalks. All these differences were more pronounced in the first 2 months of life.

In order to estimate the extent of the environmental influence on the growth type, we kept one set of the spared first filial generation plants outside the greenhouse on tables, and another set in the greenhouse under our illumination device. Again, the same size of pots and the same soil were used for all plants. The results were impressive. As seen in figure 3,B, the same plant grew erect, flowered abundantly, and had no runners when kept below the device, but turned completely flowerless and stoloniferous when



FIG. 1.—An extreme aberrant (left) and a mother-type plant from the progeny of 1950C, 15 weeks after sowing.

TABLE 2.—Seed set and germination percentage of progenies of the accessions 1950C and 1953A, and the germination of the seed

Plant	Total flowers	Seeds	Seed set	Seeds planted	Seeds germinated	Percentage germination
	<i>Number</i>	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Number</i>	
1953A:						
PM- 1	85	—	0	—	—	—
PM- 3	1,028	37	3.59	35	—	—
PI- 9	578	16	2.78	16	—	—
PI- 11	1,094	59	5.39	57	1	1.75
1950C:						
PM- 3	1,293	150	11.60	150	3	2.00
PM- 5	365	16	4.38	16	—	—
PM- 7	604	76	12.58	76	5	6.58
PI- 3	846	30	3.53	30	5	16.66
PI- 10	945	67	7.09	67	2	2.96
PI- 11	234	37	15.81	32	1	3.12

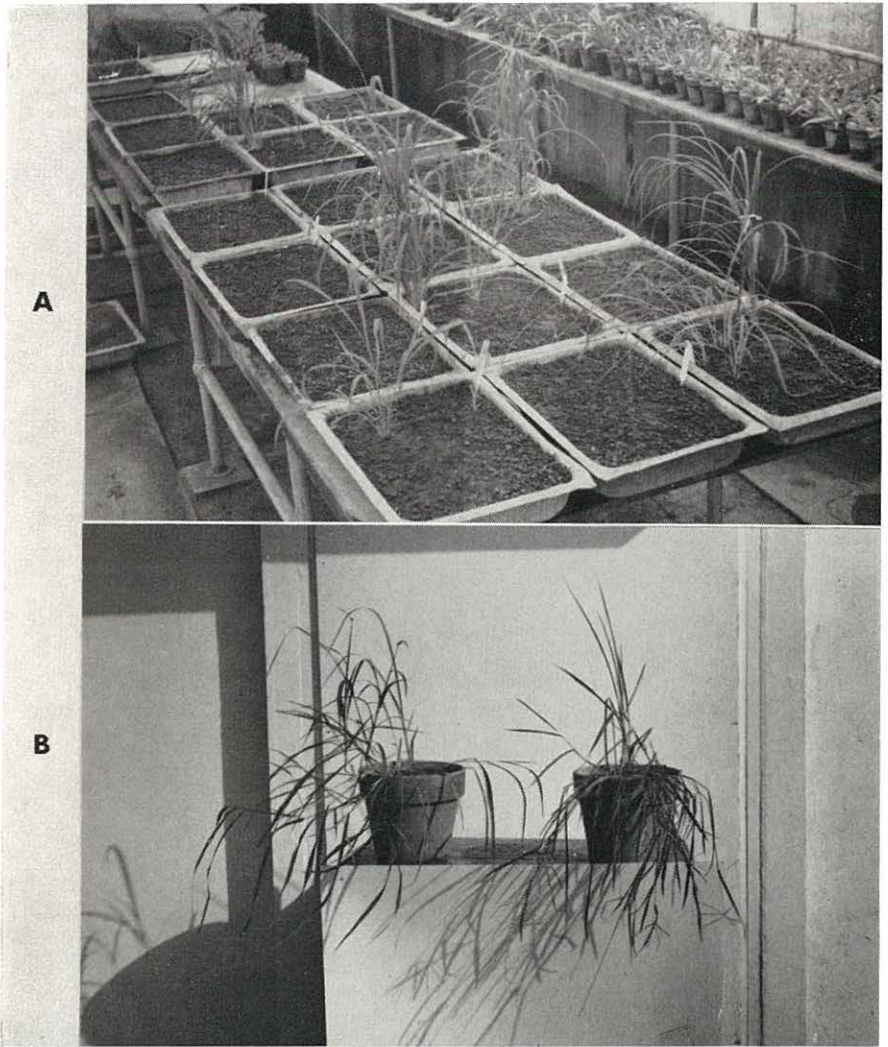


FIG. 2.—A, Total seed obtained from open pollination of the progeny of 1953A resulted in 1 plant (upper group of trays), whereas the progeny of 1950C produced 16 plants (lower group of trays). B, Two decumbent 4-month-old plants of the 1950C strain. The left one starts developing runners; the right one, lodging flowers.

kept under our outside conditions. This extreme difference was encountered among the intermediate types, which showed their ambivalence already in the early age when they were on the tables. However, the same tendency was noted in the mother type and extreme aberrant plants also.

Summing up: Karyopsis occurred in a few flowers of the accessions 1950C and 1953A, and the seed thus formed had a low germination percentage. Progenies of both accessions, kept in identical environment, showed notable morphological variation as compared with the mother plants. This suggests that the reproduction was not by apomixis, but instead, a genetic recombination must have taken place.

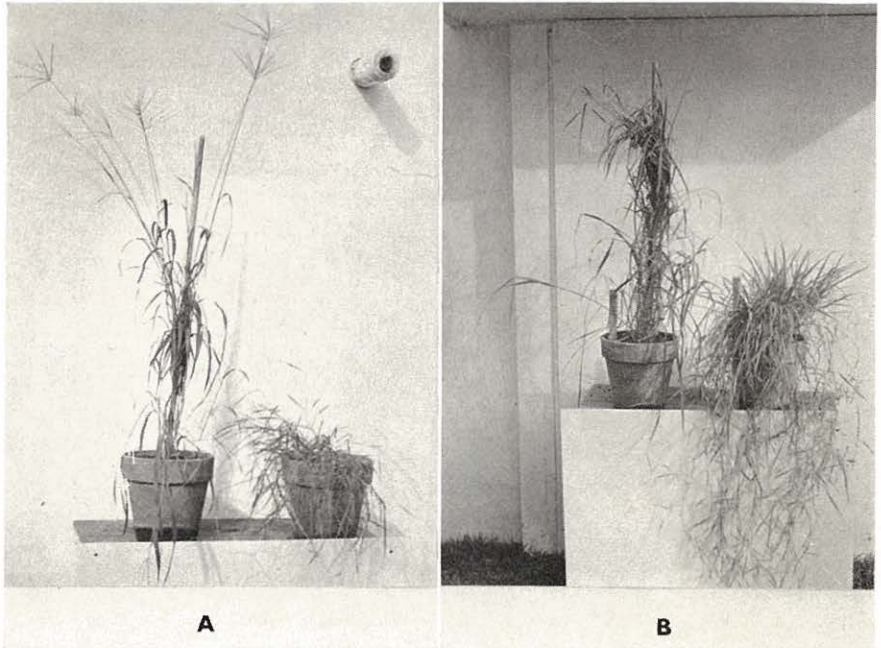


FIG. 3.—A, Extreme variants of second progeny of the 1950C strain at the age of 4 months; B, a plant of the first progeny of 1953A, divided in two and kept in prolonged daylight (left) and outside (right).

The characteristics of the progenies were very heavily influenced by the age and the environment. They were clearest in juvenile plants, 2 to 4 months after germination of the seed. Radical changes of environment, especially an additional light, were capable of producing drastic changes in these characteristics.

CYTOLOGICAL OBSERVATIONS

A study of male meiosis and pollen fertility, and of embryosac development, was made in order to understand the low fertility, and the reproductive behavior of the two accessions.

PMC meiosis: 1950C has 36 metacentric chromosomes which form 18 bivalents in the pollen mother cells. In addition, there are 1 to 4 accessory chromosomes, which either remain univalents, in which case they may be able to divide equationally in the first metaphase, or pair to form 1 to 2 bivalents (figs. 4 and 5). Their terminalization of chiasmata is usually not

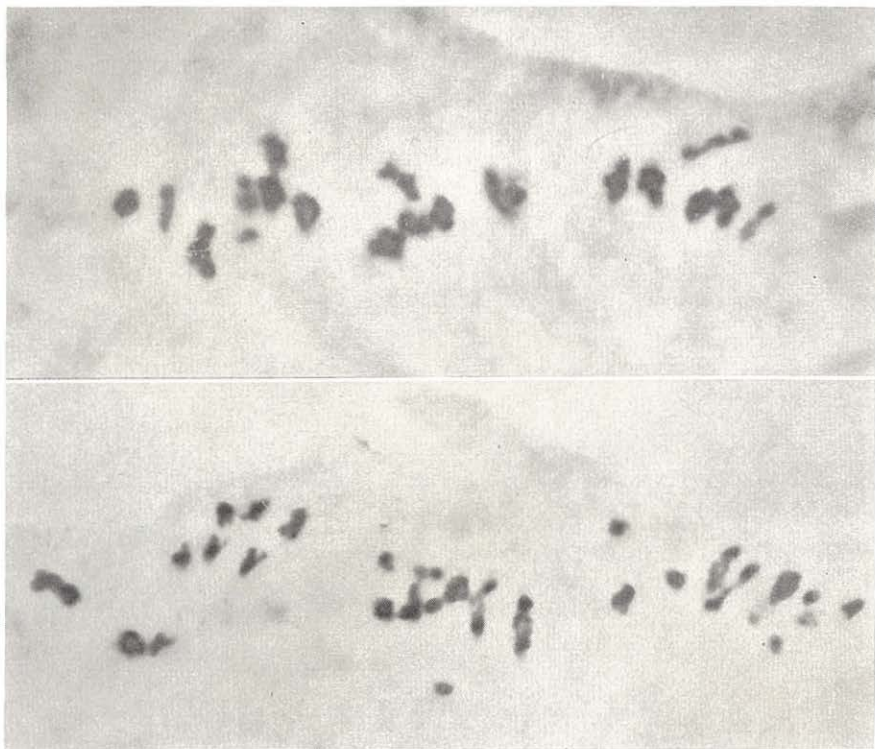


FIG. 4.—Accession 1950C: first division of PMC. The upper group shows a young metaphase with $19^{II} + I^I$, presumably all bivalents showing unterminalized chiasmata are cross-bivalents. The lower group shows a just-beginning first anaphase. Magnification $2400\times$.

completed until the metaphase plate I has been formed. Owing to the unterminalized ends (late cross figures), the bivalents may sometimes look like trivalents, but the following anaphase never revealed a true trivalent. Ring bivalents were not encountered. Thus only one of the arms of each chromosome pair is involved in chiasma formation.

In the 1953A, the chromosome number is 36, and 18 bivalents are formed (fig. 6).

These findings suggest that the pollen fertility should be good. Indeed, 77 percent of 1950C pollen and 94 percent of 1953A pollen showed good colorability. It was felt desirable to check the pollen germination also, in

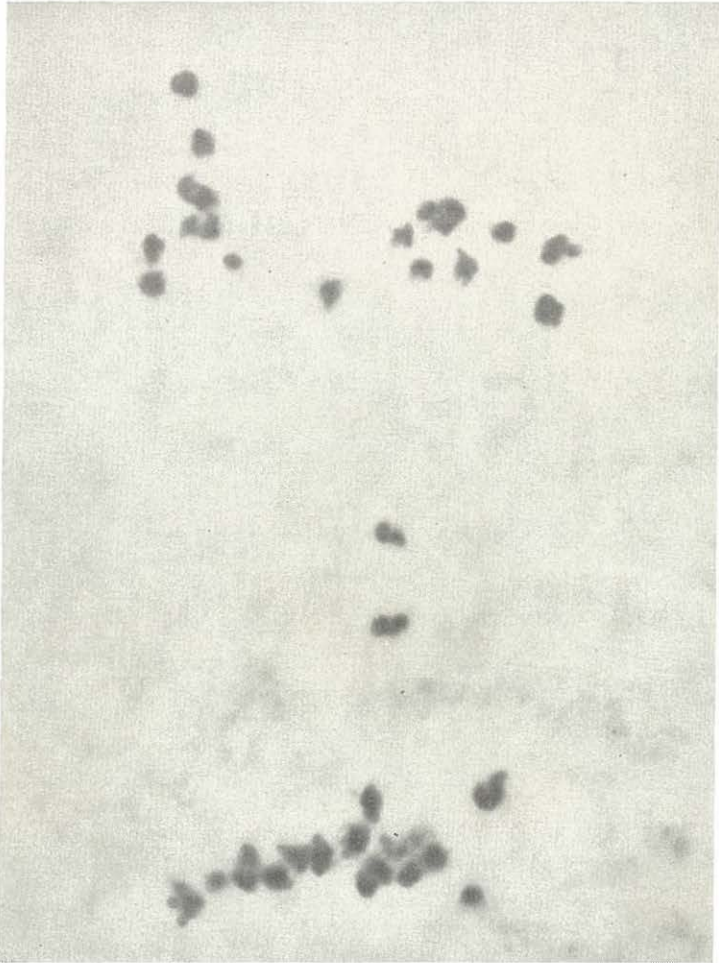


FIG. 5.—Accession 1950C: late first anaphase showing 18 + 18 chromosomes and a late-dividing extra chromosome. Magnification 2700 \times .

order to be more sure that the low fertility is not on the male side. Despite the many methods and their variations tested (see Materials and Methods p. 270), no germination was observed. We cannot say whether this negative results from pollen sterility, or from very specific germination requirements that our attempts did not meet.

The following tabulation presents the 7 best microsporogenesis performers of our 11 accessions, including the 1950C and 1953A:

<i>Accession</i>	<i>Pollen stainability percentage</i>	<i>Mode of pairing</i>
1945B	14	18 ¹¹
1945C	38	18 ¹¹ ; 17 ¹¹ + 2 ¹
1948A	—	18 ¹¹
1948B	70	18 ¹¹ + 1-2 ¹ ; 19 ¹¹
1949C	53	9 ¹¹
1950C	77	18 ¹¹ + 1-4 ¹ ; 19 ¹¹ + 1-2 ¹
1953A	94	18 ¹¹

Embryosac development: In both 1950C and 1953A, a normal, reduced



FIG. 6.—Accession 1953A: 2 PMCs in first metaphase showing 18 bivalents. All bivalents are rod-formed, and many of them still show unterminalized chiasmata. Magnification 1500 \times .

embryosac was encountered in about half of the studied flowers. It contained an egg, two synergids, two polar nuclei, and six to eight antipods. In the other half of the material, a variable degree of development of the apomictic sac was found, but never a fully formed apomictic sac. A regular formation of the apomictic embryosac seems wholly suppressed in these two accessions.

The embryosac development of 1950C and 1953A is described in more detail in a paper of the junior author (22).

DISCUSSION AND CONCLUSIONS

RESPONSE TO ENVIRONMENT

Research of the last four decades has discovered much about the physiology of flowering. The plants seem to be capable of measuring the length of the days, using a photosensitive pigment of their leaves. When the specific, inductive day length is measured, a hormone released by the pigment causes formation of flowers (23). In addition to the photoperiodism, numerous other environmental factors are involved, the temperature and humidity being most important of them.

It is obvious that in each wild ecotype those genotypes that adapt themselves best to the local environment, including photoperiod, temperature, and rain relations, have been favored by evolution. Transferred from their wild habitats, such wild plants may react to their new environments in diverse ways, of which the inhibition of flowering is one.

When we started the present study, our only aim was to check normality *vs.* abnormality of the meiosis and the embryosac development in *Digitaria valida*. However, we were soon forced to start experimenting with light in order to induce and improve flowering in it. Our purpose was thus not to study photoperiodism, and we did not plan experiments in that field. Some results of this obligatory experimenting emphasize, however, the necessity of taking into account the photoperiodism and other environmental flowering factors in evaluating the performance of an introduced accession, including its meiosis and embryosac development.

First, we made several observations that suggest that young plants of *Digitaria valida* are less influenced by environment than the older ones. Thus we obtained a good flowering in normal day-length in the first spring after having sown the introduced seed, but not more in the following springs when the plants continued living under the same conditions. In the two successive progenies of 1950C and 1953A the morphological differences between plants were best recognizable in the first 5 to 10 weeks after germination. It seems that *Digitaria valida* has a similar juvenile stage, little influenced by environment, as reported in many other plants, including grasses (5,9). In analyzing the progenies it is therefore important to observe the first weeks of the plants, because many differences between them may diminish later.

Secondly, we observed that a plant that grows laterally outdoors, forming runners and no flowers, can be induced to profuse flowering by an artificial prolongation of the daylight. For the cytological work and crossing this is just as handy as it has proved to be for commercial gardeners interested in flower production only: it makes flower induction possible at desired times.

Thirdly, an important question arises: To what extent is the course of meiosis and embryosac development a function of the environmental factors controlling flowering? We know that the temperature controls, to some extent, formation of chiasmata and thus the genetic recombination, and occurrence of univalents. More pronounced deviations from the optimal temperature can stop cell divisions, and drought may have a similar effect. To our knowledge, no data are available on the relation of meiosis and embryosac development to photoperiodism, but it is already known that for a plant there are sub- and supraoptimal photoperiods, in which case the flowers are induced slowly. Also, an insufficient exposition to the optimal photoperiod tends to induct rudimentary flower parts (1).

In his review, Heslop-Harrison (12) cited an extensive literature on the influence of photoperiodism on the expression of sex in plants. Generally photoperiodic treatment which causes early and heavy flowering also promotes female sex expression as against male. In cases where pollen fertility was checked, it was also affected.

Such a relationship between environmental flowering factors and internal maturation processes in the flowers would have two practical consequences in studying introduced plants. First, comparison of meiosis and embryosac development should be made only between plants kept in optimal flowering condition, *i.e.* each one, which does not mean all under the same condition! Secondly, offering of optimal flowering conditions would probably improve meiosis or embryosac development in critical, near-sterile cases, and thus make crossing experiments possible.

CHROMOSOME RELATIONS

With earlier determinations, we have now from *Digitaria valida* the following series of somatic chromosome numbers: 18, 24, 30, 36, 42. In the closely related *D. decumbens*, 30 is apparently the common somatic number, 27 having been encountered in one clone arisen from one seed (14,25). *D. pentzii* again, has either 18 (24), 36 (4), or 54 (15) somatic chromosomes. Apart from the probably sporadic variant 27, all these numbers are divisible by 6, which seems to favor the interesting suggestion of Sotomayor-Ríos *et al.* (26), that 6 could also be a basic number for *Digitaria*. However, a lack of somatic (diploid) numbers 12 in the whole tribe Paniceae leaves the matter uncertain.

The seven best microsporogenesis performers of our present sample of 11 accessions are best explained as belonging to the basic No. 9 series (tabulation on p. 280). Thus one (1949C) would be diploid, the others tetraploid. Extra chromosomes, encountered usually in a univalent situation in the PMC of accessions 1945C, 1948B, and 1950C, are apparently accessory chromosomes. Their ability of equational division and of sometimes

forming bivalents, renders them capable of persisting in the strains even when the reproduction is sexual. Although the accessories are considered subinert, and therefore tolerable in varying numbers, it is interesting to know that their few genetic effects often concern the fertility and the vigor of the plants. In most cases the effect has been detrimental, although opposite cases are known also (16). By coincidence or not, our accession 1953A, without accessories, appears more fertile than the 1950C which has some of them.

The main reason for the successful PMC meiosis in 1950C and 1953A is, however, the quite correct pairing. Despite the polyploid nature of the karyotype, no multivalents are formed. It could be that the diploid phase preceding tetraploidization has been hybrid in character, 1950C and 1953A being thus like allotetraploids. It is also possible that the chiasma frequency is not high enough to favor multivalent formation. Despite metacentricity of the chromosomes, we have never encountered a ring bivalent in *Digitaria valida*. Probably only one chiasma is formed per bivalent.

Hirayoshi and Yasue (13) have also described a normal PMC meiosis in two polyploid *Digitarias*, *D. ischaemum* ($2n=36$) Muhl. and *D. ciliaris* Pers. ($2n=54$). According to their figures, these species regularly form ring bivalents. Alloploidy was assumed by the authors. Later Yasue (29) was able to make the crossing *ciliaris* \times *ischaemum*, which resulted in a quite fertile (66 percent) seed, and a progeny severely affected by lack of chromosome pairing in PMC.

We think that the evidence of sexuality in the accessions 1950C and 1953A is sufficient for trying them in crossing experiments. The poor seeding and low germination of the seed are limitations, but they are partly compensated by the low incidence of apomixis.

SUMMARY

A remarkable improvement of inflorescence was induced in a sample of 11 *Digitaria valida* Stent accessions by prolongation of the daylight to approximately 16 hours. Two accessions, labeled 1950C and 1953A, showed the best reproductive performance under these conditions. Both developed some viable seed, and despite their poor germination, a progeny was obtained from both. Types aberrant from the mother type grew in both progenies. Obviously, a genetic recombination had taken place, although it remains uncertain, whether it was by crossing or by automixis.

A cytological study revealed a high pollen stainability and an almost regular PMC meiosis in the parent plants. 1950C has extra chromosomes, apparently accessories with little genetic effects. An analysis of the embryo-sac development revealed a normal sac in about 50 percent of the cases and a suppression of the apomictic sac in others.

It is concluded that the accessions 1950C and 1953A can be used in crossing experiments.

RESUMEN

Se indujo un notable mejoramiento en la inflorescencia de 11 accesiones de *Digitaria valida* Stent, prolongándose el fotoperíodo a 16 horas diarias aproximadamente. Dos de las accesiones, designadas como 1950C y 1953A, registraron el mayor mejoramiento en el proceso reproductivo bajo estas condiciones. Ambas accesiones produjeron alguna semilla viable, y a pesar de que no germinaron bien se obtuvo progenie de ambas. Ambas progenies produjeron formas aberrantes del tipo materno. Es obvio que hubo una recombinación genética, aunque no se sabe con certeza si ésta se debió a cruzamiento o a automixis.

Estudios citológicos revelan un alto porcentaje de polen teñible y una meiosis regular en CMP del material paterno. La accesión 1950C posee cromosomas adicionales, aparentemente accesorios, con pocos efectos genéticos. Un análisis del desarrollo del saco embrionario reveló que el saco era normal en cerca de la mitad de los casos y que hubo una supresión del saco apomético en otros.

Se concluye que las accesiones 1950C y 1953A pueden usarse en experimentos de cruzamiento.

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