STUDY OF THE FREQUENCY OF SEED SETTING AND EARLY EMBRYOGENESIS IN THE INTERSPECIFIC CROSS SORGHUM VULGARE (PERS.) X SORGHUM HALEPENSE (L.) PERS.

by

YACHARAPPA CHIDAMBER PANCHAL

B. Sc., (Agri.), Hon's., University of Karnatak
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INTRODUCTION

The ability of the parents to produce a viable hybrid is one of the first requirements in successful plant breeding. This requirement is often difficult to meet in interspecific crosses. The present work deals with the frequency of seed set and with seed development in the interspecific cross, Sorghum vulgare Pers. x Sorghum halepense (L.) Pers.

Karper and Chisholm (37) reported that, Sorghum versicolor J.N.Anderss. has 10, S.vulgare 20, and S.halepense 40 chromosomes. Snowden (58) reported that there are seventeen wild sorghums which have the same chromosome number (2n = 20) as the cultivated sorghums and which can be crossed readily with them to produce fertile hybrids. Celarier (21) added the species Sorghum stapfii Fischer to this list. Of these species, S.vulgare var. sudanense Stapf. has been used with success in forage crop breeding.

S.halepense, popularly known as johnsongrass, is a perennial form with rhizomes. It is native to Africa and Southeast Asia and for many years has grown wild in the Mediterranean region of Europe and the Southern United States of America (1, 20). All rhizomatous species of sorghums have forty chromosomes except Sorghum propinquum (Kunth) Hitch. which has twenty chromosomes (20).

Karper and Chisholm (37) attempted to cross S.versicolor (n = 5) with both S.vulgare var. sudanense (n = 10) and S.virgatum (n = 10), and also with S.halepense (n = 20), but

were unsuccessful. A total of 217 S.vulgare var. sudanense flowers was emasculated and pollinated on three consecutive mornings with S.halepense, and 53 reciprocal crosses were attempted. They obtained only one hybrid seed.

Since new methods of bulk emasculation with hot water have been developed by Stephens and Quinby (61), and since the development of male-sterile varieties (63), species hybrids in sorghums should more easily be produced, as many more ovules can now be pollinated.

Attempts to cross S.vulgare with S.halepense have been made by several investigators (7, 19, 20, 28, 30, 32, 43, 53). Success was obtained when S.vulgare was used as the pistillate parent. The F_1 from these crosses had only partial seed set and showed a high degree of sterility. Hadley (32) suggested that the frequency of hybrids resulting from "unreduced eggs" was higher when the male-sterile stocks were used as female parents. It is not yet known, however, whether the diploid eggs were the result of nonreduction in the meiotic process or were reduced eggs that became doubled during delay in fertilization.

Casady (18) reported that failure of attempted 2n-by-4n crosses was due, not to the failure of fertilization, but to breakdown of the post-fertilization process resulting probably from the difference in chromosome number. The partial sterility among the hybrids of kafir and milo was concluded by Karper and Chisholm (37) to be due to morphological or physical factors

rather than chromosomal irregularities.

REVIEW OF LITERATURE

Cross Incompatibility

Brink and Cooper (14) found in several species that collapse of the immature seed is a frequent cause of sterility following interspecific matings in flowering plants. Plants belonging to the same species cross readily while those belonging to different species are difficult to cross if they can be crossed at all. Garber (29) and Endrizzi (28) were unable to effect crosses between members of Para-sorghum and Eu-sorghum. In the genus Sorghum several species do not cross readily.

Ayyangar (4) and Garber (30) have reported cross incompatibility even among species which have similar characteristics and possess the same number of chromosomes. Celarier (21) and Bhutany (9) reported that plants of johnsongrass from different regions differ in their ability to cross with the cultivated sorghums. Cross incompatibility has been reported among species of several plant genera e.g., Nicotiana (26), Trifolium (2), Oenothera (45), and Zea (55).

One of the simplest incompatibility mechanisms is that reported by East and Mangelsdorf (27) in Nicotiana sanderae. Hort., which has since been referred to as the Nicotiana type of incompatibility, personate incompatibility, or incompatibility due to oppositional factors. Incompatibility in this system is determined by a multiple allelic series of S genes at one locus.

In diploids there is one S gene in the pollen and two in the somatic tissue of the style which the pollen tube must traverse. Pollen tube establishment or growth is inhibited in styles containing an allele in common with that of the pollen. The cause of cross sterility among the different varieties of popcorn reported by Brunson and Smith (16) has also been considered by Schwartz (55) to be genic.

Hybrid Inviability

Stebbins (60) has dealt with the inviability of species hybrids in detail and has classified causes of hybrid inviability into three main categories:

1. General incompatibility between the parental chromosomes and genes.
2. Incompatibility involving cytoplasmic and plastid differences.
3. Incompatibility between the embryo and surrounding tissues.

Sears (54) reported a gene in Triticum monococcum L. which has a dominant lethal effect when combined with a genome from Aegilops umbellulata Zhuk. Kostoff (40) reported that hybrids between Nicotiana glauca R. Gr. and Nicotiana rustica L. died as embryos whereas, hybrids from reciprocal crosses were quite viable. Kehr and Smith (38) concluded that disturbed growth conditions in interspecific crosses in Nicotiana are due to an upset in the harmonal system resulting from gene interaction.

Stebbins (60) concluded that the genetic heterogeneity of species, in respect to factors affecting the viability and fertility of interspecific hybrids, was a general phenomenon.

Hybrid Sterility

Dobzhansky (25) and Stebbins (60) have reviewed extensively the available literature on hybrid sterility both in animals and plants. According to these authors sterility could be the result of unbalanced genic recombinations or of structural or numerical differences in the chromosomes. Sterility in interspecific hybrids is a common factor, but its mode and extent varies considerably with different groups of organisms. The degree of fertility depends upon the genetic harmony of the species concerned. Disharmonic genetic recombinations may interfere with the normal reproductive process at any stage from early meiosis to the development of the embryo.

Varying degrees of sterility have been observed in F_1 and subsequent generations despite normal meiosis. Oka (51) reported degrees of sterility in rice ranging from one percent to one hundred percent in different cross combinations of the types belonging to the subspecies indica and japonica. He attributed sterility in these types to genetic factors which were found to be linked with such morphological characters as glutinous pollen and apiculus. Increased vigor was also found to be associated with hybrid fertility.

Weak segregates with high sterility among the progeny of

fertile interspecific hybrids have been reported in several different groups of animals and plants. Stebbins provided a long list of evidences on this subject. The phenomenon has been termed "Hybrid breakdown" by Dobzansky (25). Weak and poor segregates have also been reported by Kidd (39) in crosses among different types of sorghums.

Vinall (67) obtained only three seeds in numerous attempts to cross Sorghum halepense with other common cultivated sorghums. Mikhailoski (49) reported considerable success in hybridizing S.vulgare and S.halepense, but crosses using S.vulgare as the female parent were successful. Most of the hybrids had rhizomes and a shattering rachis. The size of the grain was intermediate between that of the parents. Bennet and Hogg (7) reported a natural hybrid between johnsongrass and Honey sorgho. The F_1 was quite fertile. The F_2 segregated widely through the complete range of intermediate types. F_2 plants were fertile and generally vigorous. Forty percent of the plants had vigorous crowns and rhizomes and survived the winter. In some controlled crosses these authors obtained a seed set as high as eighty-eight percent, but the seeds aborted early in development. Randolph (52) reported no difficulty in obtaining hybrid seeds in an attempt to hybridize S.halepense and colchicine-induced, autotetraploid S.vulgare var. sudanese.

Casady (20) reported no difficulty in obtaining viable fertile seeds in crosses between autotetraploid sudangrass (n = 20) and (johnsongrass x 4n sudangrass). The frequency of

hybridization was considerably higher when the autotetraploid sudangrass was used as the pistillate parent. Casady (19) also made some crosses with diploid (n = 10) sudangrass and (johnsongrass x 4n sudangrass) but failed to obtain matured seeds. However, 28.40 percent of the emasculated florets produced aborted seeds indicating that cross incompatibility was due to a breakdown of post-fertilization processes rather than to a failure of fertilization.

Brink and Cooper (15) stated that seed abortion due to endosperm breakdown is probably the most effective barrier to hybridization between diploids and their tetraploid derivatives. In their study of Lycopersicum pimpinellifolium R. Gr. they found that 4n-by-4n matings gave endosperm development similar to 2n-by-2n matings, but 2n-by-4n and 4n-by-2n matings resulted in slow growth and eventual collapse of the endosperm.

Cooper and Brink (23) attributed the cause of abortion of hybrid seeds of Nicotiana rustica x Nicotiana glutinosa L. to hyperplastic growth of the nucellus which completely occluded the endosperm by overgrowing the chalazal end. The authors observed that there was 95.00 percent fertility of the ovules, but that abortion occurred at various stages ranging from six to thirteen days after pollination.

Brink and Cooper (15) made the following statements concerning the role of embryo and endosperm in the life history of the angiosperms:

1. The embryo embodies the line of descent and is therefore the principal component of the seed, but the

conditions essential for growth and differentiation of the zygote are not present in the angiosperms at the time of fertilization.

2. The significance of the endosperm lies mainly in the fact that it plays a major role in the development and maintenance of the medium suitable for the growth of the embryo.

3. In the normal 2n-by-2n matings the chromosome ratio among the embryo, endosperm, and maternal tissue is 2 : 3 : 2, and any change in this ratio may result in the breakdown of the endosperm with the ensuing death of the embryo.

Lee and Cooper (44) observed the breakdown of the endosperm in interspecific crosses in Solanum species, and according to these authors, the cells in the inner layers of the integument breakdown and form a ring of decomposing tissues which does not stain. Vinall and Getty (66) postulated that the failure of hybridization of S.vulgare and S.halepense was due to an antagonism or unfavorable reaction between the reproductive organs of the two species.

According to Boyes and Thompson (11) the development of the fertilized ovule into a matured seed depends upon whether the genotype of the endosperm possesses certain functional properties relative to those of the associated maternal genotype. Blakeslee (10) observed that in the cross, Datura stramonium and Datura metel, the embryo and endosperm disintegrate from six to fifteen days after pollination. He also noted that these structures in the hybrid seeds develop slowly and that the epithelial cells which surround the embryo sac become enormous in size and upset the normal nutritional process.

Laibach (43) cultured fourteen-day-old embryos from crosses between Linum austricum Touy. x Linum perenne L. var. Lewisii and brought them to normal maturity, thus showing the lack of viability of the hybrid seeds was not due to genetic lethality as believed by some investigators.

Skirm (56) successfully grew interspecific hybrids of Prunus on nutrient agar, whereas under normal conditions, there was usually abortion of the embryo at an early stage of development. Seventy-six interspecific crosses with a two-year record of viability were successfully grown by embryo culture. Blakeslee and Satina (10) grew species hybrids of Datura stramonium by embryo culture on Van Overbeek's medium. Bhutany (9) also grew the hybrids of S.vulgare and S.halepense on embryo cultures.

Endrizzi (28), Hadley (32, 33), Merwine (48), and Bhatti (8) obtained tetraploids easily by pollinating many spikelets of male-sterile, diploid sorghums with pollen from the tetraploid species S.halepense and S.almum Parodi (2n = 40). Hadley (32) studied four hybrids obtained by pollinating the diploid grain sorghum, Blackhull Kafir, with S.halepense. Three of these were assumed to be the result of a union of unreduced eggs of the diploid female and normal gametes from the tetraploid male. Hadley noted only two chromosome numbers among the hybrids. Apparently, where the female parent was a male-fertile type emasculated by hot water, the predominant chromosome number was thirty. If all hybrids of this type are considered a single

group, the ratio becomes 24 : 2 for 30- and 40-chromosome complements respectively. On the other hand, where either genetic (ms_2 ms_2) or cytoplasmic male-sterile was used as female parent, a higher number of 40-chromosome hybrids resulted, the ratio being 7 : 21 for 30- and 40-chromosome complements respectively. Similar results have been obtained by Endrizzi (28), Bhutany (9), and Bhatti (8). In addition, Endrizzi (28) obtained several hybrids of Sorghum vulgare and S.halepense having 30, 40, and 39 chromosomes.

It is not clear whether the diploid eggs were the result of non-reduction in the meiotic process or were reduced eggs that became doubled during delay in fertilization (33). Hadley (33) assumed that male-sterile plants produce a higher frequency of unreduced eggs than do hot-water, male-fertile plants. Bhutany (1) also found chromosomal behavior of this type in his experiments. Hadley proposes that, on a genome basis, hybrids with 30 chromosomes are two thirds johnsongrass and one third S.vulgare while those with 40 are half johnsongrass and half S.vulgare. Both types of hybrids had open heads, but the heads of plants with 30 chromosomes were more lax and finer than those with 40 chromosomes.

Hadley (32) observed in his studies that every one of the 40- chromosome plants had rhizomes but that the degree of expression was very low with some individuals. The 40-chromosome hybrids did not withstand freezing conditions in the field. All 30-chromosome hybrids were highly sterile, whether self-

pollinated or open-pollinated. After repeated pollination by sudangrass, the 30-chromosome hybrids produced seeds, indicating that such hybrids produce a low frequency of eggs with sufficient genetic balance to yield viable zygotes. Plants with more than 40 chromosomes were not as strongly rhizomatous as johnsongrass although some were excellent in forage production. A 50-chromosome plant was particularly weak.

Hadley noticed two types of 40-chromosome plants, those which were completely male-sterile but highly female-fertile, and those which were highly self-sterile. Plants of the former group were typical male-steriles. They had florets with rather small, narrow anthers which did not dehisce. These anthers were not as rudimentary as those of either ms_2 / ms_2 kafir plants or cytoplasmic, male-sterile kafir plants. Seed set under either self- or open-pollination was estimated at 75 percent or more on all of these hybrids.

Hadley and Mahan (33) obtained plants having 21, 22, 30, 33, and 43 chromosomes from backcross progenies of a 30-chromosome hybrid of S.vulgare and S.halepense. S.vulgare was used in the backcross. Fertility as estimated by pollen counts, seed set, and germination was generally low and showed great variation from plant to plant. Seeds from self-pollinated plants having segments of johnsongrass chromosomes, particularly plants carrying genes for rhizome formation, germinated poorly and included many chlorophyll aberrants.

Fertilization and Post-fertilization Processes

Ayyangar et al (4, 5), Stephens and Quinby (62), and Artschwager and McGuire (3) have extensively reviewed the literature on reproduction in sorghums. Sorghums, like most angiosperms, have a monosporic, eight-nucleate embryo sac the "Normal" or "Poligonum" type (47). It is formed by three divisions of the functioning megaspore. Of the eight nuclei arising in this manner, those at the micropylar end give rise to the egg and the two synergids. Those at the chalazal end give rise to the antipodal cells, and the remaining two, one from each pole, fuse in the center to form a secondary nucleus. In the male cycle pollen grains are formed from the pollen mother cells which undergo meiosis in the normal manner. Tetrads are formed after cytokinesis and following furrowing. Soon after liberation the pollen grains become round, increase in size, and form intine and extine layers. They are usually trinucleate with haploid chromosome complements.

Fertilization. Pollen retains vitality for five to ten hours depending upon environmental conditions (3, 4, 5, 62). The stigma is sometimes receptive before blooming and several days after. Pollen tubes grow down the stylar canal into the cavity of the ovary, but one or two apparently penetrate the micropyle and discharge the sperm nuclei. Fusion of the polar nuclei occurs simultaneously with the fertilization of the egg nucleus. Stephens and Quinby (62) and Artschwager (3) reported the time factor for fertilization to be about two hours, and the length

of the rest period about four hours or double the time it takes for the pollen tube to traverse the length of the stigma and discharge the sperm nuclei into the embryo sac.

Post-fertilization development. The newly fertilized egg contains two nuclei, one larger than the other. Fusion of the two nuclei occurs shortly after fertilization, only one being present when the primary endosperm nucleus divides. The zygote and the primary endosperm nucleus do not divide immediately after fertilization. They undergo a rest period, which is short for the endosperm nucleus but of considerable duration for the zygote. During this rest period general growth processes in the embryo sac and elsewhere in the ovule continue. The filiform apparatus resulting from degeneration of the synergids extends fanlike above the micropyle even after fertilization. The life of the antipodal cells does not terminate with fertilization. While their nuclei degenerate, the antipodals continue to enlarge and their walls thicken appreciably. They retain their position near the chalazal end of the embryo sac, and their remnants can be seen until five days after fertilization (3, 4, 5, 62).

The primary endosperm nucleus is usually situated above the egg but often assumes a lateral position just before it divides. Before the rest period of the egg comes to an end, four to six endosperm nuclei surrounding a large vacuole are present. Wall formation takes place, proceeding toward the center until the whole sac is completely filled and the nucellus

is displaced. At the base of the ovule, the endosperm cells are small, elongated and angular. Deposition of starch begins on the sixth day, and the outermost cells start forming the aleurone layer approximately the ninth day after anthesis (3).

The fertilized egg divides transversely into two cells. Wall formation in early embryogeny is basipetal in succession for sorghum as for other grasses. The suspensor pushes the embryo away from the micropyle and persists until the seventh day. Later it is absorbed by the endosperm. Growth of the embryo is first symmetrical. The young embryo is filiform, later club shaped, and by the sixth day lozenge shaped. It is continuous with the suspensor. After the sixth day differentiation of the scutellum, coleoptile, and coleorhiza begins. The embryo is completely formed by the twelfth day (3).

Origin of Johnsongrass (Sorghum halepense (L) Pers.)

Casady and Anderson (19) presented evidence suggesting that S.halepense arose as an autotetraploid or as a hybrid of two closely related species. Longley (46) suggested that it could have arisen as an autotetraploid from a 10-chromosome annual form.

On the other hand, Huskins and Smith (35) Daura and Stebbins (24), Hadley (32), Merwine (48), Krishnaswami, and Raman (41) concluded that the species arose as an allopolyploid. Hadley (32) concluded that S.halepense originated as the result of crossing between two diploid species, one of which was

Sorghum vulgare, followed by chromosome doubling.
Thus,

AABB X AACC ——— AABC ——— 2 (AABC).

Endrizzi (28) and Celarier (20) supported Hadley's findings.

Celarier (20) further proposed that, S.halepense arose as a 40-chromosome species in Southeast Asia as the result of chromosome doubling of a hybrid between Sorghum propinquum, 2n = 20, (the only 20-chromosome species that has rhizomes) and some other 20-chromosome species of the subsection Arundinacea. Bhatti et al (8) suggested that S.halepense originated as a segmental allopolyploid from natural crosses of S.vulgare and S.virgatum in the Mediterranean region of Africa.

MATERIALS AND METHODS

Cultural Methods

Three cytoplasmic male-sterile varieties viz., Martin, Combine Kafir-60, and Redlan were used as female parents. Seeds were sown in six-inch clay pots on December 13, 1960. Fifty pots were sown for each variety, and the seedlings were raised in the greenhouse. Stands were thinned 14 days and 22 days after seeding. After the second thinning only one seedling was left in each pot.

Johnsongrass (S.halepense) was utilized as the male parent. Fifty vegetatively propagated plants were raised in six-inch pots.

As soon as panicles of male-sterile varieties reached the flag leaf stage they were covered with paper bags to prevent outcrossing. Similarly, panicles of johnsongrass were bagged for collection of pollen. Pollen collected in the bags was used for pollinating the cytoplasmic, male-sterile plants between 11 a.m. and 12 noon. The plants in which embryological studies were to be made were pollinated only once, when the complete head was in full bloom. A panicle took four to six days to complete flowering. The plants in which frequency of seed set was to be studied were pollinated twice in consecutive days. After pollination the heads were covered with pollinator bags.

Since seed set percentage was very low, pollination was continued on the secondary tillers till the end of July 1961. The frequency of seed set was calculated on the basis of the number of heads and spikelets pollinated, the number of fully developed seeds, and the visible shrivelled seeds (Fig. 7). The data are presented in the tables 1, 2, and 3.

Embryological Methods

Five plants in each variety were selected for embryological studies. After pollination 20 to 25 spikelets were collected and fixed in Carnoy's solution:

95% Ethyl alcohol	6 Parts
Glacial acetic acid	3 Parts
Chloroform	1 Part

Collections were made at 24 hour intervals for seven days.

The spikelets were transferred from Carnoy's solution to 70% ethyl alcohol, and the material was stored at 5°C. Subsequent procedure was as follows:

30% Ethyl alcohol		12 hours
50% " "	+ Eosin	12 "
60% " "		12 "
70% " "		12 "
90% " "		12 "
100% " "		12 "
75% " "	+ 25% Xylol	12 "
50% " "	+ 50% "	12 "
25% " "	+ 75% "	12 "
100% Xylol		12 "

Three to four changes in paraffin were made until there was no odor of xylol. Tissue mat with a melting point of 56°C. was used for final embedding. Embedding was accomplished through a technique described by Gray (32).

Sections 10 to 15 mm. in thickness were prepared by means of a rotary microtome. They were affixed to slides by means of Mayer's adhesive which was prepared as follows:

Fresh egg white	50 ml.
Glycerin	50 ml.
Sodium salicylate	1 gm.

Staining with Safranin O and fast green was done according to the following schedule described, with slight modifications, by Barnett (6):

1. Xylene .. .. .. 5 minutes
2. Xylene .. .. .. 5 "
3. Ethyl alcohol (absolute) .. 5 "
4. 70% Ethyl alcohol .. .. 5 "
5. 30% " " .. .. 5 "
6. Distilled water .. .. 5 "
7. Safranin O dye solution .. Time varies (30–40 minutes)

(Dissolve 5 gram dye in 200 ml. Methyl Cellosolve, then add 100 cc. 95% Ethyl alcohol and 100 cc. distilled water, 4 gram sodium acetate and 8 cc. Formaldehyde)

8. Distilled water .. .. Approx. 5 minutes
9. Distilled water .. .. Approx. 5 minutes
10. (Next two steps were used when the stain was dark enough in the safranin solution.)
    a. 95% Ethyl alcohol containing 0.25% picric acid for 1 second.
    b. 95% Ethyl alcohol containing 10% Ammonium hydroxide, 4 drops per 100 cc. for 2 seconds.
11. 50% Ethyl alcohol .. .. Approx. 5 minutes
12. 95% Ethyl alcohol .. .. Approx. 5 minutes
13. 100% Ethyl alcohol .. .. Approx. 5 minutes
14. 100% Ethyl alcohol .. .. Approx. 5 minutes
15. Fast-green dye solution .. 20 to 30 seconds (time varies)

(Dissolve 2 gm. dye in 100 cc. absolute alcohol and

100 cc Methyl-cellosolve. Mix 2 parts of this solution with one part of a solution of 25 parts absolute alcohol and 75 parts clove oil.)

16. Pure clove oil .. .. 10 seconds
17. Clearing mix .. .. 20 seconds
(2 parts of clove oil, 1 part of absolute alcohol and 1 part of xylol.)
18. Xylene .. .. .. 5 minutes
19. Xylene (2 changes.) .. .. 5 minutes each
20. Mount in Canada Balsam ..

Cytological Methods

Whole inflorescences of the hybrid plants were fixed in Carnoy's solution for 24 hours and stored in 70% ethyl alcohol. The material was collected between 10 a.m. and 11 a.m. Cytological studies were made according to a smear technique described by Johannsen (36).

Photomicrographs were taken by means of a Kodak Pony camera mounted on a triocular Spencer Microstat Microscope. The films used were Adox. K. B. 14. 35mm.

RESULTS AND DISCUSSION

Data on the frequency of seed set, and frequency of fertilization, and establishment of offsprings of S.vulgare x S.halepense are presented in tables 1 to 5. All three varieties of S.vulgare exhibited low seed set (33.30 percent) in crosses with

S.halepense. 96.21 percent of the visible seeds produced were shrivelled.

The study of six-day ovaries showed that the average percentage of stimulated (and presumably fertilized) ovaries was 81.66. Apparently 40.77 percent of the fertilized ovaries failed to produce visible seeds (tables 4 and 6).

In crosses between S.vulgare and S.halepense Hadley (32), Endrizzi (28), and Bhatti et al (8) obtained mature, viable seeds in most of the S.vulgare spikelets which were male-sterile, the percentage being higher in cytoplasmic, male-sterile stocks than in genetic, male-sterile stocks (32). Karper and Chisholm (37), and Casady and Anderson (20) failed to obtain well-developed hybrid seeds in emasculated spikelets. The latter workers observed that 28.40 percent of the emasculated spikelets produced aborted seeds. Higher seed set percentages, through the use of male-sterile stocks, have been reported by several workers (8, 9, 22, 27, 32). Hadley (32) reported that the frequency of seed set (assumed to be due to unreduced eggs) was 71.40 percent and 80.00 percent with genetic and cytoplasmic, male-sterile stocks respectively. But so far no one has studied the frequency on a large scale in the greenhouse or in the field. Hadley (32), Bhutany (9), and Celarier (20) noted that S.halepense plants from different regions differed in their compatibility with S.vulgare. It appears that the S.halepense plants in the present study were of the less compatible type.

Table 1. Frequency of seed set in S.vulgare (Var. Martin) x S.halepense.

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
1	2	198	66	1
2	1	76	22	1
3	3	208	58	2
4	2	192	39	0
5	3	150	55	2
6	2	125	34	4
7	2	145	42	0
8	2	159	39	0
9	1	303	93	4
10	1	116	42	1
11	3	105	39	3
12	3	150	70	2
13	6	202	42	5
14	2	110	39	1
15	2	250	90	2
16	2	110	29	3
17	3	140	32	1
18	1	121	36	0
19	2	134	41	0
20	1	98	28	0
21	4	145	38	1
22	1	96	27	0

Table 1 (cont.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
23	3	205	68	10
24	2	105	33	1
25	1	75	18	1
26	2	138	22	2
27	1	180	38	1
28	2	165	90	13
29	2	110	80	12
30	2	109	56	0
31	2	138	42	4
32	1	128	32	0
33	3	149	45	2
34	1	99	32	0
35	2	215	94	0
36	2	128	39	0
37	1	80	35	0
38	2	105	27	0
39	2	98	32	0
40	2	110	50	0
41	1	132	39	0
42	2	118	68	3
43	3	140	38	0
44	3	124	49	0
45	4	315	101	2

Table 1 (concl.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
46	2	75	22	1
47	2	110	40	0
48	2	98	32	0
49	3	130	39	5
50	2	115	29	0
Grand Total	102	7026	2191	92

Table 2. Frequency of seed set in *S. vulgare* (Var. Combine Kafir-60) x *S. halepense*.

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
1	3	115	45	5
2	2	128	62	0
3	1	78	22	1
4	4	112	52	8
5	2	105	45	2
6	1	92	34	0
7	2	116	45	0
8	1	101	40	0
9	3	96	32	4
10	2	119	43	0
11	1	128	37	3
12	1	142	45	4
13	2	90	34	2
14	2	115	48	5
15	1	109	39	0
16	1	96	28	0
17	2	127	45	0
18	1	88	29	0
19	2	110	38	0
20	2	125	43	0
21	1	93	31	0
22	1	115	45	1

Table 2 (cont.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
23	2	98	33	0
24	2	123	48	0
25	2	117	38	0
26	2	78	29	2
27	2	98	34	2
28	1	103	36	0
29	1	95	38	6
30	1	109	44	0
31	1	136	37	0
32	2	129	31	0
33	2	135	47	0
34	1	93	32	0
35	1	78	23	2
36	1	93	34	0
37	1	116	37	3
38	2	122	43	0
39	1	96	32	0
40	1	110	43	5
41	1	78	23	0
42	1	74	31	0
43	2	96	32	3
44	1	98	36	0
45	3	150	62	11

Table 2 (concl.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
46	1	103	33	0
47	2	145	49	6
48	1	94	27	0
49	1	103	32	0
50	1	78	21	0
Grand Total	77	5348	1887	75

Table 3. Frequency of seed set in *S.vulgare* (Var. Redlan) x *S.halepense*.

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
1	3	85	21	3
2	2	106	34	0
3	4	204	59	0
4	1	96	24	0
5	2	125	39	1
6	1	92	24	0
7	2	60	22	2
8	2	96	28	2
9	4	175	53	0
10	2	132	39	0
11	1	75	21	0
12	1	92	28	0
13	2	102	32	0
14	1	91	30	0
15	1	79	23	0
16	2	116	27	0
17	2	96	22	0
18	3	123	31	0
19	2	104	27	0
20	1	76	31	0
21	2	98	32	0
22	3	127	29	0

Table 3 (cont.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
23	1	78	22	0
24	1	81	24	0
25	2	97	32	0
26	1	87	27	0
27	1	92	31	0
28	2	99	26	1
29	1	86	23	0
30	1	89	31	0
31	2	72	19	4
32	1	78	23	0
33	2	68	15	1
34	1	94	29	1
35	1	74	23	0
36	2	88	27	0
37	1	134	38	3
38	2	106	25	0
39	1	110	25	2
40	2	140	60	14
41	2	131	39	0
42	2	120	44	1
43	1	105	27	1
44	1	95	24	3
45	1	180	55	4

Table 3 (concl.)

Plant Number	Number of heads pollinated	Number of spikelets pollinated	Number of shrivelled seeds	Number of fully developed seeds
46	2	116	37	0
47	2	55	15	1
48	2	125	34	3
49	1	78	22	0
50	1	96	35	0
Grand Total	83	4924	1508	47

Table 4. Summary of the frequency of seed set in S.vulgare x S.halepense cross.

S.vulgare varieties	No. of heads pollinated	No. of spikelets pollinated	No. of shrivelled seeds	No. of developed seeds	Percentage of visible seed set	Percentage of seeds fully developed
Martin	102	7026	2191	92	32.37	1.42
Combine Kafir-60	77	5348	1887	75	36.38	1.40
Redlan	83	4924	1508	47	31.17	0.96
	262	17,298	5,586	214	33.30	1.26

Table 5. Germination, establishment and hybrid plants. (S.vulgare x S.halepense)

S.vulgare varieties	Number of seeds sown	Number of seeds germinated	Number of plants survived	Number of hybrid plants
Martin	92	92	19	11
Combine Kafir-60	74	74	67	0
Redlan	47	46	32	1
Total	213	212	118	12

Table 6. Percentage of fertilized ovaries after 6 days after pollination in S.vulgare x S.halepense.

S.vulgare varieties	Number of spikelets observed	Number of fertilized ovaries	Percentage of spikelets fertilized
Martin	20	16	80
Combine Kafir-60	20	18	90
Redlan	20	15	75
Total	60	49	81.66

EXPLANATION OF PLATE I

Fig. 1. Rhizomes of *S.halepense*.

Fig. 2. Rhizomes in the hybrid plants. (*S.vulgare* x *S.halepense*).

Fig. 3. F_1 hybrid plants as compared to the *S.vulgare* plant (one to the left).

Fig. 4. Panicle of *S.halepense*.

Figs. 5 and 6. Panicles of the hybrid.

Fig. 7. a. Seeds of *S.halepense*.

b. Seeds of *S.vulgare*.

c. Shrivelled seeds of the cross. (*S.vulgare* x *S.halepense*).

PLATE I

1

2

3

4

5

6

a b c

7

Embryogenesis

Histological changes occurring in ovule, the endosperm, and the embryo during the seven days following fertilization are indicated in tables 7 to 9.

Ovule development. The length of the ovule on the first day after fertilization was 695 microns, whereas that of the normal S.vulgare was 1230 microns. In crosses within S.vulgare ovule length doubles each day for the first four days. In crosses between S.vulgare and S.halepense the ovules increase in length at about half this rate. On the seventh day, the ovule of the female parent was 1676 microns in length compared to 2700 microns for normal ovules.

Endosperm development. The rate of development of the hybrid endosperm is indicated in table 7. Figures 1 to 9, PLATE II, indicate the nature and rate of development from the first day to the seventh day after pollination. Figure 2, PLATE II, shows the fusion of the polar nuclei just above the egg nucleus. The endosperm cells were small and compact with very little vacuolization. As the normal endosperm advanced in age its cells became large and isodiametric and developed larger vacuoles. By the seventh day it filled most of the ovule pushing aside the nucellus. In the cross, however, it has grown to only two thirds of the ovule. Moreover, it was still in the developing phase whereas, the normal endosperm had entered upon the phase of differentiation. Slow development of the endosperm appeared to be the first visible abnormality in interspecific crosses.

Table 7. The rate of development of the ovule in S.vulgare x S.halepense cross and S.vulgare.

Cell No.	1-day		2-days		3-days		4-days		5-days		6-days		7-days	
	L*	W**	L	W	L	W	L	W	L	W	L	W	L	W
	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ	μ
1	820	672	888	664	1048	656	1160	800	1296	1024	1216	1056	1696	1216
2	882	648	848	656	1088	720	1320	840	1200	784	1652	952	1512	1040
3	736	592	808	640	1120	712	1216	1008	1168	816	1424	896	1880	944
4	602	552	736	544	1048	688	1136	976	1304	776	1344	1000	1760	1056
5	664	584	720	584	1024	736	1224	864	1362	704	1272	832	1640	1048
6	696	496	646	648	1160	920	1320	1120	1216	768	1384	1040	1600	1104
7	584	544	686	664	1000	744	1206	1104	1312	560	1312	1088	1512	1312
8	656	624	800	624	1024	696	1232	1032	1280	648	1368	1048	1872	1245
9	664	600	808	592	920	784	1136	1040	1376	784	1296	944	1660	1376
10	680	512	816	562	912	656	1240	992	1348	800	1368	1032	1632	1016
To.	6954	5824	7756	6148	10344	7312	12190	9776	12898	7744	13636	7888	16764	11357
Av.	695	582	775	615	1034	731	1219	977	1290	774	1363	988	1676	1135
S.vulgare 3/	1230	-	-	-	2040	-	2300	-	2580	-	2600	-	2700	-

*L = Length
**W = Width

3/ Data from Artschwager and McGuire (3).

Table 8. The rate of development of the Endosperm in S.vulgare x S.halepense and S.vulgare.

Cell No.	1-day		2-days		3-days		4-days		5-days		6-days		7-days	
	L*	W**	L	W	L	W	L	W	L	W	L	W	L	W
1			180	112	512	328	600	408	716	528	920	622	860	816
2			128	108	464	304	708	432	800	640	960	754	988	824
3			192	112	432	320	586	416	760	572	892	588	1112	856
4			192	145	400	328	612	392	808	560	864	612	1200	912
5			162	128	464	336	646	448	816	496	822	716	1156	1002
6			162	112	506	384	608	452	754	528	904	614	1088	836
7			168	128	600	400	656	376	770	512	800	608	968	908
8			112	148	576	420	640	428	800	552	856	645	1148	1008
9			120	112	592	384	632	412	824	567	912	770	1312	960
10			128	112	460	376	608	476	808	614	808	812	1216	980
Total			1544	1217	5006	3580	6296	4240	7856	5569	8738	6741	11048	9102
Average			154	123	501	358	630	424	785	557	874	674	1105	910
S.vulgare 3/ 410			-	-	1212	-	1640	-	2340	-	2350	-	2400	-

*L = Length
**W = Width

3/ Data from Artschwager and McGuire (3).

Brink and Cooper (15) discussed in detail the development of the young seed after fertilization. They reported that the development of the seed is dependent upon the maintenance of the rapid growth of the endosperm, which requires a delicate "physiological balance" between the endosperm and the adjacent maternal tissue. They further suggested that the incompatibility arises when the ratio of the chromosome numbers of the maternal tissue and the endosperm varies in either direction.

In the present cross between S.vulgare (2n = 20) and S.halepense (2n = 40) the chromosome-number ratio is not in accord with the 2 : 3 : 2 ratio formulated by Muntzing (50). He observed that shrivelled seed from autotetraploid rye gave more aneuploid plants than did well developed seed and concluded that the slightest deviation from the 2 : 3 : 2 ratio (resulting in this case from the aneuploid gametes) can result in endosperm breakdown.

The present study of embryo development was limited to seven days after pollination. From the results, it would seem that the rate of development of the endosperm, the embryo, and the ovule is reduced in the cross. The exact reason of retarded endosperm development is not yet clear, especially in the case of interspecific crosses. All other visible abnormalities accompanying seed failure seem to be secondary to this phenomenon.

Casady (18) observed that in 2n-by-4n crosses seed development appeared to progress normally until approximately the

fifteenth day after pollination but that shortly thereafter the young seed began to show shrivelling and discoloration, a condition which continued until the seed was entirely shrivelled and discolored. In the present study, although 81.66 percent of the ovules were fertilized, only 33.30 percent developed to the visible-seed stage.

Cooper and Brink (23), who studied seed development in Nicotiana crosses, stated that the direct cause of failure in the hybrid seed appeared to be the starvation of the endosperm through a more or less complete obstruction of the immediate line of supply by the overgrown (hyperplastic) nucellus. They postulated that the hyperplastic nucellus acts as a barrier to the inward movement of materials and diverts to its own use the food which would otherwise pass to the endosperm and to the embryo. They referred to this phenomenon as "somatoplastic sterility". It is essentially a malnutritional phenomenon associated with the genotypic diversity of tissues within the seed. Kraus, Bradbury and Blakeslee, as quoted by Cooper and Brink (23), found the same type of disturbance in the nutritive process in orchard fruits, sour cherries, and Datura species respectively.

Haberlandt, as quoted by Maheshwari (47), stated that the proper conversion of starch in the endosperm to the soluble form to be used by the embryo is the function of the enzyme diastase which is present in the aleurone layer. The hyperplastic growth of the nucellus may be due to the failure of the enzyme or other

inhibitary factors. The nucellus overgrows at the expense of the food of the embryo. A study of this relationship may explain the failure of the endosperm following interspecific crosses.

Embryo development. Data on the rate of the development of the embryo is presented in table 9. Figure 5, PLATE II, shows the egg nucleus fused with the sperm nucleus. Details of the stages of embryo development could not be recorded because of the difficulty in getting sections at the right stage. Growth of the embryo is slow as in case of that of the endosperm probably because of the difference between the chromosome numbers of the sperm and egg nuclei. The rate of development of the proembryo is less than that of the endosperm, because the embryo undergoes a much longer rest period than does the endosperm (3).

The antipodals did not degenerate soon after fertilization as did the synergids. The nuclei of the antipodals degenerated, but the antipodals went on enlarging with the development of thick walls. They retained their position near the chalazal end, Fig. 8, PLATE II. Remnants of the antipodals were observed in the ovules five days after fertilization.

Table 9. Rate of development of the embryo in S.vulgare x S.halepense and S.vulgare.

S.vulgare x S.halepense			S.vulgare 3/		
Age	Length	Width	Length	Width	Form
	μ	μ	μ	μ	
1 day	26	18*	38	23	2 celled
2 days	31	22**	-	-	filiform
3 days	42	28**	70	38	ligulate
4 days	64	40**	92	69	club shape
5 days	94	53*	105	85	-do-
6 days	132	62**	164	85	-do-
7 days	198	82*	420	140	-do-

*Measurements are of single embryo.
**Average measurements.
3/Data from Artschwager and McGuire (3).

EXPLANATION OF PLATE II

Fig. 1. 1 day old ovule with primary endosperm nucleus and egg nucleus present. (160 x)

Fig. 2. 1 day old embryo and endosperm.

Fig. 3. -do- enlarged.

Fig. 4. 2 day old endosperm.

Fig. 5. 3 day old endosperm.

Fig. 6. 4 day old endosperm.

Fig. 7. 5 day old endosperm.

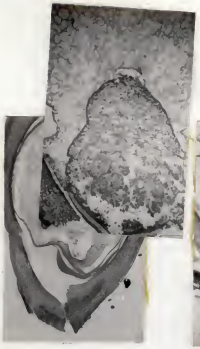
Fig. 8. 6 day old endosperm.

Fig. 9. 7 day old endosperm.

Figs. 1, 2, 4, 5, 6, 7, 8, and 9 160 x

Fig. 3. 775 x

PLATE II



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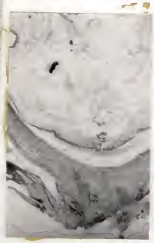

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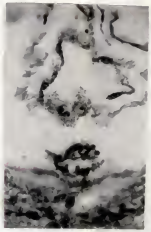

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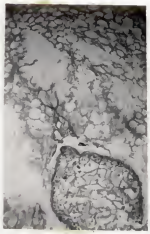

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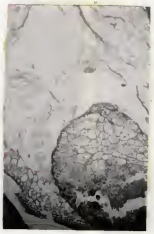

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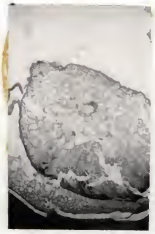

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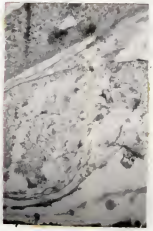

9

F_1 Hybrids

There were eleven hybrid plants from Martin x S.halepense, one hybrid from Redlan x S.halepense, and none from Combine Kafir-60 x S.halepense. It is surprising that Combine Kafir-60, which is known to cross easily with other sorghum lines, did not produce a single hybrid (28, 32). All hybrid plants had leaves with white midrib.

All the hybrids were highly sterile triploids (n = 30). Triploid plants obtained from diploid-by-tetraploid crosses have been studied by several workers (28, 32, 37, 39). The literature contains reports of both sterility and fertility in triploid hybrids. According to Stebbins et al (59) the triploid hybrids, Agropyron inerme Scriben. and Smith Rydb. x Elymus glacus Buckl and A. parishii Scribn. and Smith x A. spicatum, Pursh. Scribn. and Smith produced completely aborted pollen and failed to set seed. In the present study all F_1 plants apparently originated from the union of normal gametes of the parent species (2n x 4n ---3n).

At meiosis the chromosomes of a triploid may associate as trivalents, bivalents and univalents. At any rate, gametes with unbalanced chromosome numbers are produced by triploids. Characteristically, many triploids are quite sterile as a result of unbalanced gametes and unbalanced physiological and genotypic constitutions.

Hadley (32), however, used a diploid, cytoplasmic, male-sterile sorghum as a female parent in a cross with S.halepense

(from Israel). He obtained mostly triploids in the F_1. He also noticed that 30-chromosome hybrids were very irregular in pairing, showed many laggards and bridges, and set no seed when self-pollinated. He postulated that the 30-chromosome hybrid could be represented by the formula 2 (AB) (AC) and that its mode of pairing was 5-trivalents, 5-bivalents, and 5-univalents. He further assumed that genomes B and C were similar, though not identical, a situation which would explain the occurrence of more than 5-trivalents. He hypothesized that cryptic structural differences caused sterility in the hybrids. Hadley assumed that a 30-chromosome hybrid has two genomes of S.halepense and one genome of S.vulgare. He obtained seeds from 30-chromosome F_1 plants by backcrossing to 20-chromosome plants, thus supporting the above assumption.

Rhizome development was observed in all F_1 plants but varied in degree. (Figs. 1 and 2, PLATE I)

SUMMARY

1. The cultivated sorghums of greater economic importance consist of two species: Sorghum vulgare Pers., which has 20 chromosomes, and Sorghum halepense (L) Pers. (johnsongrass), which has 40 chromosomes.

2. Cytoplasmic, male-sterile lines of three S.vulgare varieties viz., Martin, Combine Kafir-60, and Redlan were used as female parents in crosses with S.halepense.

3. The frequency of seed abortion was high. Although

81.66 percent of the pollinated spikelets were fertilized, only 33.30 percent produced visible seeds and only 1.26 percent produced fully developed seeds. Of 118 offspring established only twelve were hybrids.

4. All the hybrids were highly sterile triploids (n = 30). Hybrid vigor was well expressed in tiller development, height, leaf width, and stem thickness. Rhizome development varied with individual plants, but was not so pronounced in any hybrid as in the male parent.

5. Early embryogenesis was studied till the seventh day after pollination. Results indicated that the embryo and the endosperm developed more slowly in the interspecific cross than crosses within S.vulgare.

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LITERATURE CITED

1. Anderson, L. E.
Johnsongrass in Kansas, Agri. Experiment Sta. Cir. No. 380. 1960.

2. Atwood, S. S. and Brewbaker, J. L.
Multiple oppositional allels in autoploid cloves. Res. Genetic Soc. Amer. 19:87. 1950.

3. Artschwager, Earnst and McGuire, Ruth.
Cytology of reproduction in Sorghum vulgare. Journal Agri. Res. Vol. 78 No. 12:659 - 673. 1949.

4. Ayyangar, G. N. R. and Rao, V. P.
Studies in sorghum: I. Anthesis and pollination. Indian Jour. Agri. Sci. 1:445 - 454. 1931.

5. Ayyangar, G. N. R. and Rao, V. P.
Studies in sorghum - the great millet. III. Anther, pollen and stigma. Indian Jour. Agri. Sci. 6:1299 - 1322. 1936.

6. Barnett, F. L.
Cytogenetic studies of interspecific hybrids in the Bromopsis section of Bromus. Ph. D. Dis. Pennsylvania State University, 1956.

7. Bennet, H. W. and Hogg.
The F_2 and F_3 generations of sorghum x johnsongrass hybrid. Proc. Assoc. Soc. Agri. workers 43rd convention. 84 - 85. 1942.

8. Bhatti, A. G., Endrizzi, J. E., and Reeves, R. G.
Origin of johnsongrass. Jour. Heredity. Vol. 51 No. 3, 106 - 110. 1960.

9. Bhutany, W. T.
Chromosome behavior of five sorghum species and the use of embryo culture in growing their interspecific hybrids. Unpublished M. S. thesis A & M College, Texas. 1955.

10. Blakeslee, L. F. and Satina sophie.
New hybrids from incompatible crosses in Datura through culture of excised embryos on malt media. Sci. 99:331 - 334. 1944.

11. Boyes, J. W. and Thompson, W. P.
The development of the endosperm and embryo in reciprocal interspecific crosses in cereales. Jour. Genetics, 34:203 - 227. 1937.

12. Brown, Meta Suche.
Haploid plants in sorghum. Jour. Heredity. 34:163 - 166. 1943.

13. Brink, R. A., Cooper, D. C., and Asherman, L. E.
A hybrid between Hordeum jubatum x Secale cereale reared from an artificially cultivated embryos. Jour. Heredity. 35:67 - 75. 1944.

14. Brink, R. A., and Cooper, D. C.
Seed collapse- following mating between diploid and tetraploid races of Lycopersicum pimpinellifolium. Genetics. Vol. 30, 376 - 401. 1945.

15. Brink, R. A. and Cooper, D. C.
The endosperm in seed development. Botanical Review. 13. 1947.

16. Brunson, A. M. and Smith, G. M.
Hybrid Popcorn. Agro. Journal. 37:176 - 183. 1945.

17. Chin, T. C.
The cytology of polyploidy sorghum. Amer. Jour. Bot. 33:611 - 614. 1946.

18. Casady, A. J.
Hybridization, cytological and inheritance studies of a sorghum cross- autctetraploid sudangrass x (johnsongrass x 4n sudangrass). Master's thesis, Kansas State University, 1950.

19. Casady, A. J., and Anderson, K. L.
Hybridization, cytological, and inheritance studies of a sorghum cross- autotetraploid sudangrass x (johnsongrass x 4n sudangrass). Agro. Jour. 44:189 - 193. 1952.

20. Celarier, Robert P.
Cytotaxonomic notes on the subsection Halepensia of the genus sorghum. Bull Tor. Bot. Club 85:49 - 62. 1958.

21. Celarier, Robert P.
Cytotaxonomy of the Andropogoneae. III. Sub-tribe Sorgheae, Genus sorghum. Cytologia. Vol. 23, No. 4: 395 - 418. 1959.

22. Celarier, Robert P.
Tertiary Butyl alcohol dehydration of chromosome smears. Stain technology. Vol. 31, No. 4:155 - 157. 1956.

23. Cooper, D. C. and Brink, R. A.
Somatoplastic sterility as a cause of seed failure after interspecific hybridization. Genetics. Vol. 25. 593 - 617. 1940.

24. Daura, B. N. and Stebbins, G. L., Jr.
A polyhaploid obtained from a hybrid derivatives of Sorghum halepense x Sorghum vulgare. Var. sudanese. Genetics. 37:369 - 374. 1952.

25. Dobzansky, T.
Genetics and origin of species. 3rd Ed. Columbia University Press, New York. 1951.

26. East, E. M. and Yarnell, S. H.
Studies on self-sterility. Genetics. 14:455 - 487. 1929.

27. East and Mangelsdorf, A. J.
Distribution of self-sterility in the flowering plants. Proc. Amer. Phil. Soc. 82:449 - 518. 1940.

28. Endrizzi, John E.
Cytological studies of some species and hybrids in the Eu-sorghums. Botanical Gaz. Vol. 119. No. 1 - 10. 1957.

29. Garber, E. D.
A cytological study of the genus sorghum. Subsections Para-sorghum and Eu-sorghum. Amer. Nat. 78:89 - 94. 1944.

30. Garber, E. D.
Cytotaxonomic studies in the genus sorghum. University Calif. Pub. Bot. 23:283 - 362. 1950.

31. Gray, Peter.
Hand book of Basic Microtechnique. McGraw-Hill Book Company, New York. 1958.

32. Hadley, H. H.
Cytological relationships between Sorghum vulgare and S.halepense. Agro. Jour. 45:139 - 143. 1953.

33. Hadley, H. H. and Mahan, J. L.
The cytogenetic behavior of the progeny from a back-cross (S.vulgare x S.halepense x S.vulgare). Agro. Jour. 48:102 - 106. 1956.

34. Hadley, H. H.
Chromosome, fertility and rhizome expression of hybrids between grain sorghum and johnsongrass. Agro. Jour. 50:278 - 282. 1958.

35. Huskin, C. L. and Smith, S. G.
A cytological study of the genus sorghum. Pers. II. The meiotic chromosomes. Jour. Genetics. 28:387 - 395. 1934.

36. Johannsen, D. A.
Plant Microtechnique. McGraw-Hill Book Company, New York. 1940.

37. Karper, R. E. and Chisholm, A. T.
Chromosome numbers in sorghum. Amer. Jour. Bot. Vol. 23 No. 5, 369 - 374. 1936.

38. Kehr, A. E. and Smith, H. H.
Genetic tumors in Nicotiana hybrids abnormal and pathological plant growth. Brookhaven Symposia in Biol. 6, 55 - 78. 1954.

39. Kid, H. J.
Haploid and triploid sorghum. Jour. Heredity. 43:204 - 225. 1952.

40. Kostoff, D.
Ontogeny, genetics and cytology of Nicotiana Hybrids. Genetics. 12:133 - 139. 1960.

41. Krishnaswami, N., Raman, V. S., and Chandrasekharan, P.
An interspecific hybrid of grain sorghum and johnsongrass. Current Science. 25:195 - 197. 1956.

42. Keimé, W. F.
Interspecific hybridization in Trifolium lotus, utilizing embryo culture technique. Agro. Jour. 46:601 - 612. 1953.

43. Laibaich.
Ectogenesis in plants. Methods and genetic possibilities of propagating embryos otherwise dying in the seed. Jour. Heredity. 20:200 - 205. 1929.

44. Lee, John H. and Cooper, D. C.
Seed development following hybridization between diploid solanum species from Mexico, Central and South America. Amer. Jour. Bot. Vol. 45:No. 22. 1958.

45. Lewis, D.
Incompatibility in flowering plants. Bio. Review, Cambridge Phil. Soc. 24:472 - 496. 1949.

46. Longley, A. E.
Chromosomes in grass sorghums. Jour. Agri. Res. 44: 317 - 321. 1932.

47. Maheshwari, P.
An introduction to the embryology of Angiosperms. McGraw-Hill Book Company, New York. 1950.

48. Merwine, N. C.
A cytogenetic study of a hybrid in the genus sorghum: S.vulgare Pers. x S.halepense (L.) Pers. Ph. D. Dissertation, Miss. State College. 1957.

49. Mikhailovskii, V. A.
A new cultivated plant-perennial sorghum. (Selktsiua i semenovodstove) breeding and seed growing. No. 7:39 - 40. from plant breeding abstract No. 446 10: 113. 1940.

50. Muntzing, A.
Aneuploidy and seed shrivelling in tetraploid rye. Hereditas. 29. 1943.

51. Oka, H.
Studies in tetraploid Rice, VI. Fertility variation and segregation ratios in tetraploid hybrids of rice, Oryza sativa (L) Cytologia. 20:256 - 266. 1955.

52. Randolph, L. F. and Cox, L. G.
Factors influencing germination of the iris seed and relation of inhibitary substances to embryo dormancy. Proc. Amer. Soc. 43:284 - 300. 1943.

53. Raman, V. S. and Krishnaswami, N. A.
A chromosomal chimera in S.halepense. (Linn). Indian Jour. Agri. Sci. 25:47 - 50. 1955.

54. Sears, E. R.
Inviability of inter-generic hybrids involving Triticum monococcum and T. aegilopiodes. Genetics. 29:113 - 127. 1944.

55. Schwartz, D.
The analysis of a case of sterility in maize. Proc. Nat. Acad. Sci. 396:719 - 724. 1950.

56. Skirm, G. W.
Embryoculture as an aid to plant breeding. Jour. Heredity. 33:211 - 215. 1942.

57. Snowden, J. D.
The cultivated races of sorghum. Aldlard and Son Ltd. London. 1936.

58. Snowden, J. D.
The wild fodder sorghums of the section Eu-sorghums. Jour. Linn. Soc. of London. 55:191 - 260. 1955.

59. Stebbins, G. L. and Singh, R. A.
Artificial and natural hybrids in Gramineae tribe Hordeae. IV. Two triploid hybrids of Agropyron and Elymus. Amer. Jour. Bot. 37:388 - 393. 1950.

60. Stebbins, G. L.
The inviability, weakness and sterility in interspecific hybrids. Advances in genetics. 9:147 - 215, 1958.

61. Stephens, J. C. and Quinby, J. R.
Bulk emasculation of sorghum flowers. Jour. Amer. Soc. Agronomy 25:233 - 234. 1933.

62. Stephens, J. C. and Quinby, J. R.
Anthesis, pollination and fertilization in sorghum. Jour. Agri. Res. 49:123 - 136. 1934.

63. Stephens, J. C.
Male sterility in sorghum, its possible utilization in production of hybrid seed. Jour. Amer. Soc. Agro. 29:690 - 696. 1937.

64. Stephens, J. C. and Holland, R. F.
Cytoplasmic-male sterility for hybrid sorghum for seed production. Agro. Jour. 46:20 - 24. 1954.

65. Thompson, W. P.
The cause of hybrid sterility in hybrids of sorghum and johnsongrass. Mem. Hort. Soc. New York. 3:75 - 77. 1926.

66. Vinall, H. N. and Getty, R. E.
Sudangrass and related plants. U. S. D. A. Bull. 981. 1921.

67. Vinall, H. N.
Partial sterility in hybrids of sorghum and johnsongrass. Memo. Hort. Soc. New York. 3:75 - 77. 1926.

STUDY OF THE FREQUENCY OF SEED SETTING AND EARLY EMBRYOGENESIS IN THE INTERSPECIFIC CROSS SORGHUM VULGARE PERS. X SORGHUM HALEPENSE (L.) PERS.

by

YACHARAPPA CHIDAMBER PANCHAL

B. Sc., (Agri.), Hon's., University of Karnatak
Mysore, India, 1951

AN ABSTRACT OF A MASTER'S THESIS

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The cultivated sorghums of greatest economic importance consist of two species, Sorghum vulgare Pers., which has 20 chromosomes, and Sorghum halepense (L.) Pers., which has 40 chromosomes. In sorghums, as in other flowering plants, collapse of the immature seed is a frequent cause of sterility following interspecific matings. The present study deals with crosses between Sorghum halepense and three cytoplasmic, male-sterile varieties of Sorghum vulgare. Study of early embryogenesis is also undertaken.

Of 17,298 cytoplasmic, male-sterile spikelets dusted with pollen from Sorghum halepense, 5,586 (33.30 percent) produced visible seeds. Only 214 (1.26 percent) developed normal seeds. Microscopic study indicated that 81.66 percent of the spikelets were fertilized under greenhouse conditions. It indicated that the remaining 48.36 percent in which fertilization had taken place had been aborted before visible seeds were formed.

Though germination of the seeds was nearly one hundred percent, establishment of the seedlings was low. Of 214 seeds germinated, 118 were established. Only twelve hybrid plants were obtained. Eleven of these were from Martin x S.halepense, and one was from Redlan x S.halepense.

All hybrid plants were triploid (2n = 30) and highly sterile. They were tall and leafy and exhibited white midribs, profuse tillering, and varying degrees of rhizome development. They flowered a week earlier than the S.vulgare plants.

In interspecific crosses the rates of development of the

ovule, endosperm, and embryo were approximately half the rates in interspecific matings. The endosperm cells were compact, small, and much less vacuolated than those of normal endosperm. They did not fill most of the central cavity of the ovule by the seventh day after pollination as is the case with normal endosperm cells.

The first visible abnormality following the interspecific cross was the slow development of the endosperm and its failure to differentiate at the normal rate. This failure may have been due to a disturbance in a delicate "developmental or physiological balance" among the embryo, endosperm, and the maternal tissue. It may also have been due to a hormonal effect or to factors inhibiting the normal enzymatic activities in the developing seed.