Germination Studies On The *Dichanthium annulatum* Complex

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U.S.D.A.

Technical Bulletin T-90
March, 1961

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Introductions of *Dichanthium annulatum* (Forsk.) Stapf representing its entire range of distribution are being studied at the Oklahoma Agricultural Experiment Station at Stillwater, Celarier and Harlan (4). This complex is widespread throughout East and South Africa and adjacent islands. It occurs sparsely in West Africa and is found in Saudi Arabia, Israel, Lebanon, Syria, Iran, and Pakistan, and throughout India, Ceylon, the Indo-Malaya area and China. A few varieties are found in Australia and on some of the Pacific Islands. The complex includes three types: Tropical, Mediterranean, and South African.

A large number of very productive types selected from the material show promise of being valuable pasture and range grasses. Some of these selections are being tested for adaptation over a wide area in the Southern half of the United States.

Little or no information is available on the germination requirement of the *D. annulatum* complex; therefore, germination studies were initiated in conjunction with the Old World bluestem breeding program.

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The research reported herein was done under Oklahoma Station Project 784.

METHODS AND MATERIALS

The germination requirements of the *Dichanthium annulatum* complex were surveyed in 1957 and 1959, when six accessions were studied. Since this material is primarily tropical, the lack of winter hardiness makes it difficult to study the same accession each year. As shown in Table 1, the data presented were obtained from 10 different accessions. Two of these, A-3182 and A-3242, were studied during both years.

Table 1.—Accessions of *Dichanthium annulatum* studied, 1957 and 1959.

Accessions 1957	1959	Source	Type
3242	3242	Calcutta, India	Tropical
1526	—	South Texas (Introduced)	Tropical
5302	—	Karnal, India	Tropical
5411	—	Delhi, India	Tropical
3182	3182	Israel	Mediterranean
4830	—	Saudi Arabia	Mediterranean
—	6577	Delhi, India	Tropical
—	6192	Burma	Tropical
—	4099	Punjab, India	Tropical
—	5398	Karnal, India	Tropical

To be certain that pure seed units were being used, the caryopses used in the germination studies conducted in 1957 were extracted by hand. In 1959, only the rough pure seed unit was used except for one treatment. The seed units were carefully selected to be sure that sound caropyses were actually present. The caryopses also were examined carefully, and only those which were apparently perfect and contained unbroken grain were used. Ergotized grain is not as common in this group as in *Bothriochloa ischaemum* (L.) Keng (1).

One hundred seeds or seed units were used in 1957 for each replicate of each treatment within each accession. In 1959, 50 seed units were used. The preparation and procedure of study used were essentially the same as used in the *Bothriochloa ischaemum* studies conducted by Ahring and Harlan (1) and based on germinator uniformity trials conducted by Ahring, *et al.,* (2).

Three Stults germinators, one senior model with dual chambers and two junior models were used. Germination environments were set as follows for both years:

Constant 20° C.	16 hours dark and 8 hours light
Constant 30° C.	16 hours dark and 8 hours light
Alternate	16 hours dark at 20° and 8 hours light at 30° C.
Alternate	16 hours .dark at 20° and 8 hours light at 35° C.

However, in 1957 after a 50-day period of study, test samples were transferred to the following environments.

Constant 20° to 20-30° C. alternate
Constant 30° to 20-35° C. alternate
Alternate 20-30° to 20° C. constant
Alternate 20-35° to 30° C. constant

The following treatments were used in 1957 and 1959 as indicated.

1957	1959
1. Caryopses; H_2O moistened substrate	1. Rough seed unit; H_2O moistened substrate
2. Caryopses; 0.2% KNO_3 moistened substrate	2. Rough seed unit; pre-chilled 5 days
3. Caryopses; pre-chilled 14 days	3. Rough seed unit; pre-chilled 14 days
4. Caryopses; pre-chilled 5 days	4. Caryopses; H_2O moistened substrate
	5. Rough seed unit; dried 12 hours at 45-50° C.
	6. Rough seed unit; dried 24 hours at 45-50° C.

Pre-germination treatments were arranged so that all treatments used entered the germinators on the same day. Samples were counted at about 7-day intervals for 64 days in 1957 and for 28 days in 1959.

RESULTS AND DISCUSSION

Preliminary Studies

Preliminary studies were conducted to measure the effect of a dilute solution (KNO_3) and of environment, whether alternating or constant, on the germination of freshly harvested seed of *Dichanthium annulatum*. These tests on dead-ripe seed harvested 14 to 30 days earlier showed a definite variety $\times$ environment interaction. Tropical, Mediterranean, and South African types, in general, germinate very slowly in the 20° C.

constant and 20-30° alternate environments, requiring an average of 28 to 60 days before the initiation of germination. This compares to an average of 33 to 72 percent germination at the end of 14 to 38 days at 25-35° alternate and constant 30° temperature.

Seed of a South African type, A-4080, required 28 days before starting to germinate and 64 days to attain an average germination of 88 percent at constant 20°C. In contrast, only 21 days were required in a 30° constant environment for seed of this type to reach the same germination level. Germination was slow in environments 20-30° and 25-35° alternate. The addition of 0.2% KNO_3 as a moistening agent stimulated seed germination in the constant 20° and 20-35° alternate, but the level of germination was not as great as that obtained in the 30° constant with the substrate moistened with water.

Two tropical types, A-2564 and A-5411, responded similarly. An average of 28 days was required for A-2564 to initiate seed germination and 50 days for it to attain an average of 77 percent in a 20°C. constant environment. In the 30° constant only 14 days were needed to obtain an average germination of 95 percent; whereas, 6 and 63 percent germination were obtained in 38 days in the alternate 20-30° and 20-35° environments respectively.

The start of seed germination of A-5411 compared to A-2564 was slow requiring 40 days in the 20°C., but the level of seed germination at the end of 50 days was the same for both accessions. In the 30° constant environment 14 days were required by A-2564 to attain an average germination of 95 percent while, 38 days were required by A-5411. The average germination of A-5411 in the alternate 20-30° and 20-35° of 63 and 96 percent, respectively, was much higher in comparison than A-2654 for the same period. The addition of KNO_3 as a substrate-moistening agent stimulated germination of both accessions in the 20-30° alternate environment but, not enough to obtain the level found in the constant 30° and the 25-35° environment for the same period.

A Mediterranean type, A-3903, germinated very well in three of the four environments tested. At the end of 14 days averages of 2, 97, 86, and 94 percent germination were obtained at 20°, 30°, 20-30°, and 25-35°C, respectively. Potassium nitrate was no more effective than water as a moistening agent in increasing the germination.

In India, seed dormancy of *D. annulatum,* as measured by Oke (5), was found to last 3 to 4 months. However, information obtained from these preliminary studies suggests that seed

dormancy is not a problem in freshly harvested seed of *D. annulatum,* germinated in an optimum environment.

Effect of Environment

Significant differences existed between germination environments, accessions, and treatments within environments.

Table 2 shows the depressing effect of constant 20°C. environment on the germination of *D. annulatum* regardless of the pre-germination treatment.

The 20°C. constant was consistently the least desirable on the basis of average germination, and by comparing the check treatments within environments by accessions at the 21-day and 50-day count intervals. Only the Mediterranean type *D. annulatum* A-3182 and A-4830 and the tropical type A-5411 receiving a 0.2% KNO_3 treatment germinated well.

At the end of a 21-day test the germination of the check treatments in the alternate 20-30°C. and 20-35° as compared to the 20° and 30° environments was generally greater in the constant 30°. The accessions, except tropical type 5302 and a Mediterranean type 3182, germinated best on an average at constant 30°. The level of germination of these two accessions was about the same at 30° as at 20-35°. Prolonging the study to 50 days before transferring to other environments has little effect on increasing the germination over that already attained at the end of 21 days at 20° and 30° constant. The reverse was true in the alternate environments. The average germination of all accessions except 3182 and 3242 at 20-30° and 20-35°, respectively, at the end of the 50-day test interval was significantly more than at the 21-day interval.

Effect of Treatment

Comparison of the average germination by treatment at the 21-day and 50-day counts shows the ineffectiveness of KNO_3 in stimulating germination. The addition of KNO_3 was beneficial at only constant 20°C. The effect of adding KNO_3 was unfavorable on an average except on two Mediterranean types, 3182 and 4830.

In each case, pre-chilling the seed at 5-10°C. for 14 days seemed to suppress the germination regardless of the environment. The germination performance of seed pre-chilled 5 days was best at 30° and 20-35°; however, in general, pre-chilling for 5 days had little or no effect.

Table 2.—Average percent normal seedlings found at the 21-day and 50-day count intervals in each of six *D. annulatum* accessions in 1957 receiving 4 pre-germination treatments within each of 4 environments, and the effect of an environmental transfer.

Accession No.	Trt.[1]	21-day germination				50-day germination				Temperature Change 14 day period			
		20°C	30°C	20-30°C	20-35°C	20°C	30°C	20-30°C	20-35°C	20° to 20-30°C	30° to 20-35°C	20-30° 20°C	20-35° to 30°C
Tropical													
3242	1	18	77	62	49	24	79	65	74	+44	+1	+3	+ 7
	2	32	71	60	62	40	72	65	65	+39	0	0	+ 3
	3	13	56	48	46	17	57	50	51	+29	0	+2	+ 1
	4	29	87	75	64	30	87	76	86	+56	+2	+3	+ 1
1526	1	33	69	63	55	40	74	73	73	+20	0	0	+ 1
	2	42	66	49	55	47	66	57	61	+ 8	0	0	+ 2
	3	18	37	37	33	21	37	39	34	+13	+1	+1	+ 1
	4	36	59	48	55	37	63	57	66	+19	+1	0	+ 1
5302	1	12	59	36	59	19	66	67	72	+49	+8	0	+ 8
	2	16	65	53	52	21	65	61	57	+26	0	0	0
	3	9	50	32	40	10	54	40	46	+28	+1	0	0
	4	24	60	58	54	28	68	76	68	+38	+5	0	+ 1
5411	1	36	59	51	51	49	61	61	64	+10	+3	+1	0
	2	52	58	66	52	75	58	71	54	+ 9	0	0	0
	3	27	65	61	57	39	66	64	61	+15	0	0	0
	4	37	55	55	78	43	56	59	86	+15	0	0	0
Mediterranean													
3182	1	17	68	76	72	26	74	81	77	+42	+3	0	+ 1
	2	73	76	72	65	76	77	75	65	+ 9	0	0	0
	3	49	67	72	65	52	68	73	66	+15	+5	0	0
	4	67	89	92	84	70	91	94	87	+20	0	0	+ 1
4830	1	39	86	68	67	43	94	87	81	+44	+2	+2	+ 8
	2	70	89	86	82	73	90	89	88	+15	0	0	+ 1
	3	43	65	56	47	45	66	64	56	+21	0	+5	+13
	4	42	88	76	67	44	89	88	81	+46	+3	0	+ 7
Overall average by treatment	1	26	69	58	58	33	74	72	73	+34	+3	+1	+ 6
	2	47	71	64	61	54	71	70	65	+17	0	0	+ 1
	3	26	56	51	48	30	58	55	52	+20	+1	+1	+ 1
	4	39	73	67	67	42	76	75	79	+32	+2	+1	+ 2
Average of environment regardless of treatment		34	67	60	58	40	70	68	67	+26	+1	+1	+ 3

[1]Treatments: (1) check (caryopses); (2) substrate mositened with a 0.2% KNO_3 solution; (3) pre-chilled on moist substrate for 14 days at 5-10°C; (4) pre-chilled for 5 days at 5-10°C.

Effect of Temperature Change

Changing the treatments of each accession from one environment to another after the 50-day test interval made little difference in the total average germination at 30°C., 20-30°, and 20-35° in 1957. However, changing the test in the 20° constant to an alternate 20-30° environment for 14 days significantly increased the average germination from 33 to 68 percent (see Table 2). This indicates that the number of viable firm or sound seed remaining at the end of the 50-day test period was unaffected by the environment and that this environment alone is of no value in the germination of *D. annulatum.*

The largest increase in average percent germination resulting from temperature transfer was consistently found in the check and 5-day prechill treatments. By comparing the average percent germination obtained after temperature change, it appears that the constant 20°C. environment in general has an adverse effect on the total germination. Apparently, imbibed seeds are harmed by holding in an unfavorable environment for prolonged periods. The only exceptions were where 0.2% KNO_3 was used as a moistening agent on the tropical types A-3242 and A-5411. The total average percent germination of accession 5411 receiving this treatment was best in the constant 20° environment and not significantly different from the best germination treatment on accession 3242. The overall performance of the Mediterranean types was as high in the constant 20° changed to 20-30° as in the other environments and temperature changes tested.

Results of tests made at the constant 30°C. environment for the entire 64-day test period show that little or no advantage is gained by testing *D. annulatum* over 21 to 28 days or by transfer to a new environment.

Using the information obtained in 1957, Studies conducted in 1959 were terminated at the end of 28 days. Although different treatments were used, the average germination of each accession by environment (Table 3), showed that different *D. annulatum* accessions respond differently to different temperatures. The most favorable environments in order of rank were 30°, 20-30°, 20-35°, and 20°C. As in the study conducted in 1957, tropical types germinate best in a constant 30° environment, whereas Mediterranean types seem to germinate well at all temperatures except constant 20°.

Effect of Treatment by Environment

Omitting KNO_3 as a germination treatment and adding treatments 4, 5, and 6 (Table 4) and using rough seed units dried 12 and 24 hours,

Table 3.—The average percent germination, regardless of treatment, of 6 accessions of *D. annulatum*; by environments, 1959.

Accessions	Type	Constant		Alternate	
		30°	20°	20-30°	20-35°
3242	Tropical	62.0	8.4	44.0	31.2
6577	Tropical	70.4	26.4	59.2	53.6
6192	Tropical	55.6	10.4	46.4	34.8
4099	Tropical	55.6	26.8	52.8	59.2
5398	Tropical	14.0	7.6	9.6	10.4
3182	Mediterranean	63.2	28.8	61.6	59.6
Environment average		53.5	18.1	45.6	41.5

respectively, at 45 to 50°C., it was found that drying the seed for 24 hours before placing on a moist substrate was the most significant pre-germination treatment regardless of environment. At constant 30° the average germination of the hand-extracted caryopses was greater than that of the rough seed unit. In the other environments, however, the germination of the rough seed unit was generally higher than the caryopses. Pre-chilling the seed for 14 days on a moist substrate at 5-10° did not show the suppressing effect on germination evident in 1957. At 20-35°, pre-chilling the seed of *D. annulatum* for 14 days before study did not increase the germination except that of A-6192 over the level obtained by the check treatments.

These studies unintentionally included more tropical than Mediterranean and South African types; therefore, any conclusions reported here should pertain mainly to the germination characteristics of the tropical type of *D. annulatum*. More detailed studies are now in progress to compare the germination requirements of the three different types.

From the data presented here it is apparent that freshly harvested seed of the tropical *D. annulatum* has a temperature requirement of constant 30°C. for maximum germination. None of the standard pre-germination treatments used in commercial laboratories and recommended for a number of other grasses in the Association of Official Seed Analysts rules for seed testing (3) are of benefit in the germination of this species. It was evident, however, that germinating the rough pure seed unit and the extracted grain on a water-moistened substrate in a 30°C. constant environment is not enough to promote maximum germination. Drying the seed at 45-50° for 24 hours before placement on a

Table 4.—Average percent germination by treatment within each environment for 6 accessions of *D. annulatum* studied in 1959.

Accession	Type	Checks		Pre-chilled		Dried	
		Rough	Caryopses	5 days	14 days	12 hours	24 hours
		1	4	2	3	5	6
			30°C.				
3242	Tropical	52.0	73.2	52.0	56.0	53.2	85.2
6577	Tropical	68.0	73.2	66.4	70.4	65.4	80.0
6192	Tropical	50.4	49.2	52.0	45.2	54.4	82.4
4099	Tropical	56.0	48.0	49.2	62.4	36.0	82.4
3182	Mediter.	69.2	60.0	56.0	54.4	61.2	78.4
5398	Tropical	9.2	10.4	5.2	10.4	16.0	25.2
			20-30°C.				
3242	Tropical	48.0	13.2	49.2	34.4	36.0	70.4
6577	Tropical	62.4	38.4	69.2	53.2	46.4	84.0
6192	Tropical	30.4	44.0	37.2	46.4	40.0	80.0
4099	Tropical	53.2	38.4	52.0	58.4	36.0	78.4
3182	Mediter.	64.0	50.4	61.2	48.0	66.4	80.0
5398	Tropical	9.2	9.2	5.2	6.4	4.0	22.4
			20°C.				
3242	Tropical	3.2	6.4	5.2	2.4	13.2	16.0
6577	Tropical	24.0	16.0	17.2	16.0	34.4	50.4
6192	Tropical	3.2	14.4	6.4	2.4	13.2	21.2
4099	Tropical	33.2	29.2	21.2	20.0	16.0	41.2
3182	Mediter.	29.2	26.4	21.2	20.0	17.2	57.2
5398	Tropical	4.0	16.0	0.0	1.2	20.0	5.2
			20-35°C				
3242	Tropical	29.2	21.2	30.4	32.0	16.0	60.0
6577	Tropical	48.0	46.4	58.4	49.2	42.4	76.0
6192	Tropical	21.2	29.2	26.4	34.4	28.0	69.2
4099	Tropical	65.2	58.4	60.0	58.4	42.4	76.0
3182	Mediter.	53.2	48.0	56.0	58.4	61.2	77.2
5398	Tropical	10.4	10.4	5.2	6.4	10.4	20.0

moist substrate in a 30° constant environment was the best method of germination studied.

It appears from the accessions used in this study that the only difference between the Mediterranean and tropical types is in their reaction to the different germination environments. Mediterranean types seem to germinate well in 30° constant, 20-30°, and 20-35°C. alternate environments and respond to 24 hours drying at 45-50° and a 5-day prechill at 5-10° pre-germination treatment.

The South African types appear from preliminary studies to respond to environments similar to the tropical *D. annulatum*.

Polyembryony

Before the temperature change (Table 5) the occurrence of more than one functional embryo was noted only occasionally in the *D. annulatum* accessions studied in 1957. From a total of 38,400 seed units, 4 treatments and 4 replications containing 100 seeds within each of the 4 environments, only 5 twins were obtained at the end of a 50-day test period. Three of the 5 twins were found in the constant 20°C. environment and two in the alternate 20-30°. Four of the total belong to accession 4830 and the other twin occurred in accession 1526 within the constant 20° environment.

An increase in number of more than one functional embryo was found by transferring each accession to a different environment at the end of a 50-day test period. A total of 60 twins was observed 14 days after the temperature change. Forty-two of these were found in the constant 20°C. changed from the 20-30°, whereas only one twin was

Table 5.—Total number of twins produced, by treatment and accessions, after temperature change; 1957.

Accession	Trt.	Environments				Total
		20/20-30°	30/20-35°	20-30/20°	20-35/30°	
3242	1	–	–	–	–	
	2	–	–	–	–	
	3	–	–	3	–	
	4	–	–	7	–	10
1526	1	–	–	–	–	
	2	–	–	–	–	
	3	–	–	–	–	
	4	–	–	–	–	0
5302	1	–	–	3	1	
	2	–	–	–	–	
	3	–	–	2	1	
	4	–	1	1	1	10
5411	1	–	–	–	–	
	2	–	–	2	–	
	3	–	–	1	–	
	4	–	–	–	–	3
3182	1	–	6	–	–	
	2	–	–	–	–	
	3	–	–	–	–	
	4	–	–	–	–	6
4830	1	–	1	4	1	
	2	–	2	6	–	
	3	–	–	8	–	
	4	1	1	6	2	32

found in the 20-30° changed from the 20° constant environment. At the same time, 11 twins were found in the 20-35° changed from the 30° constant and only 6 were found by changing from a 20-35° to a 30° constant.

Regardless of environment, 32, 10, 10, 6, 3, and 0 twins were found in accessions 4830, 5302, 3242, 3182 and 1526, respectively, following transfer.

The studies in 1959 verified that the occurrence of more than one functional embryo is more frequent when a change of environments is made.

The number of abnormal seedlings found varied with the pre-germination treatment and environment. Although the data are not shown, treatments producing the largest number of abnormal seedlings in the constant 20° and 30°C. environments were the 5-day pre-chill and KNO_3 treatments respectively, whereas in the alternate environments the largest number of abnormals was found in the check and pre-chill treatments.

The largest number of firm or sound seed remaining at the end of the test period was found in the constant environments when KNO_3 was used as a moistening agent. In the alternate environments, however, the check treatments contained the highest number of remaining firm seed.

SUMMARY

The data presented indicate that the most favorable environments for germinating *Dichanthium annulatum,* in order of rank, were the 30°, 20-30°, 20-35°, and 20°C.

Freshly harvested seed of the tropical type *D. annulatum* seems to require constant 30° for maximum germination. The only difference between Mediterranean types studied and the tropical types was that the Mediterranean types germinate well in a 30° constant, 20-30°, and 20-35° alternate environments. The South African types appear to respond to environments similar to the tropical types.

The information obtained from these studies indicates that seed dormancy is not a problem in freshly harvested seed of *D. annulatum* if an optimum germination environment is used.

Pre-germination treatments except drying the seed at 45-50°C. for 24 hours before placing on a moist substrate had little or no effect on germination.

The occurrence of more than one functional embryo varies with accession and environment. Twin seedlings were more frequently found in the Mediterranean than in the tropical types. Except in A-1526, a marked increase in number of twin seedlings was found by changing each accession from their initial environment to another. Changing the accessions from alternate 20-30° to constant 20° was most successful in promoting germination of more than one functional embryo.

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