STUDIES ON SOME RANGE GRASSES OF PAKISTAN

by

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Summary. *Three species of genus Cymbopogon, viz C. jwarancusa (Jones) Schult., C. parkeri Stapf and C. olivieri (Boiss.) Bor were vegetatively propagated in the grass nursery of Botany Department, University of Karachi. Various stages of their life cycle were studied under field and laboratory conditions. Inflorescence development period, anthesis time, number of sterile and normal florets per inflorescence were determined, and pollen grain fertility, pollen grain germination and viability, and seed germination tests were carried out. Mean size, shape and weight of seed and % seed set were also determined.*

Eleven species of various grasses including the above three were also studied for the establishment of relationship between TTC reactivity and routine germination test. The two were found highly correlated.

Introduction. The genus *Cymbopogon* Spreng, includes perennial tufted and aromatic grasses which are confined to the tropics and are fairly well distributed in Pakistan with five species in the plains and hilly tracts. Members of this genus yield fodder and aromatic compounds. The present paper deals with the life cycle and behaviour of three species of *Cymbopogon* Spreng, and with the relationship between TTC reactivity and routine germination test for 11 species of grasses, including the above.[3] TTC salt is colourless, highly sensitive to light and an oxidation—reduction indicator, and forms non-diffusable red formazan upon reduction in the living tissue only. The narcotic cells fail to take carmine colouration with TTC salt.

Material and methods. The experiments were carried out at grass nursery of Botany Department, Karachi University, from Jan. 1975 to May 1976. The following observations were recorded:

(1) Rate of inflorescence development, flowering period and anthesis time, observed daily under field conditions.

(2) % sterile florets in an inflorescence: Five inflorescences were observed in each study plant, the total number of male, bisexual and morphological abnormal florets were counted.

(3) Pollen fertility: By staining with acetocarmine, acid fuchsin, cotton blue, eosin yellow and Iodine solution.

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(4) Pollen grain germination: 5 percent Pfahler's (1967) media and stigmatic juice plus tap water was used. Slides were kept in a moist chamber and allowed to incubate in dark for 3 to 5 hours at room and fixed temperature, according to Cook and Walden 1967.

(5) Mean size, shape and weight of the seeds and percentage of seeds in each open pollinated plant.

(6) Seed viability and germination: Seed viability was tested according to the method of Singh and Mehdi (1965), and seed germination according to Shahzadi and Qadir (1969).

For studying the relationship between TTC reactivity and routine germination test one hundred physically normal seeds for each of 11 species of grasses were soaked in water for 5 hours. The soaked seeds were immersed in 1% aqueous TTC solution (Lakon 1948) and placed in a dark chamber for about 24 hours. Then the seeds were washed with lactophenol solution and each seed dissected longitudinally through the embryo with a sharp razor. The cut seeds were then examined under bionocular microscope and classified as viable or nonviable on the basis of staining of the critical regions of the embryo.

The same number and quality of seeds for each species were allowed to germinate on moist filter paper in patridishes at room temperature. Germination, taken as emergence of radicles, was recorded at 24 hours interval for seven days.

Results. *Flowering.* In field conditions the sprouting and development of inflorescence takes place throughout the year, though the period for maximum development of the inflorescence depends on climatic conditions:

Average data for study plants

		Nov-Feb		March-May		June-October	
		Min.	Max.	Min.	Max.	Min.	Max.
1.	Inflorescence development in days	28	36	21	28	18	24
2.	Flowering in days	8	15	12	22	8	27
3.	Maximum anthesis time (a.m.)	7.30	8.30	6.30	7.30	6.15	8.15

Percent of sterile florets. The percentage of sterile florets and percentage of florets to seed set are determined on morphological basis. Data given below indicate that more than 50% of the florets are sterile, barren or abnormal.

Mean percentage sterility on morphological basis

Number of replicates	Species	Total florets	O	B	A	O	B	A	Ste %	Nor %
5	Cym. jwa.	6318	2662	169	376	2772	225	114	56	44
5	Cym. park.	5185	2214	275	367	1722	349	256	67	33
5	Cym. olivi.	6308	3192	252	308	2388	96	72	62	38

(B = Barren, A = Abnormal, Ste = Sterile, Nor = Normal)

Pollen grains. The pollen grains generally fall into three categories, big, medium and small, and spherical, round and shrunken. The formation of shrunken pollen grains may be due to some abnormal phase of meiosis. The size of the pollen grains may be regulated by climatic factors, (Heimo 1969).

Pollen fertility and viability was tested with Acetocarmine, acid fuchsin, cotton blue, eosin yellow and Iodine solution. The sterile or nonviable pollen grains, failed to stain. The mean percentages, of stained pollen grains are given below:

Reagent	C. jawarancusa	C. parkeri	C. olivieri
	(% of stained pollen grains)		
Acetocarmine	76	84	88
Acid fuchsin	89	76	91
Cotton blue	88	69	89
Eosin yellow	80	79	86
Iodine	92	81	89

The mean germination percentages of pollen grains are given below:

Species	Stigmatic, at room temperature	Pfahler's solution (0.5%) at room temperature	Juice + tap Water at incubator
	20-25°C	20-25°C	32°C
Cym. jwa.	15	29	22
Cym. park.	10	30	31
Cym. olivi.	19	22	20

No correlation is found between pollen fertility and pollen germination test.

Seed setting. Since more than 50 percent of florets are male or sterile, therefore, percentage of seed set is very low. After fertilization, the approximate period required for seed formation is 75 to 80 days in November to February, 55-70 in March to May and 40-50 days in June to October.

The Mean percentage of seedset, shape size and weight of the seeds is given below:

Species	Seedset, %	Shape	Breadth (mm)	Length (mm)	Weight/seed (in gram)
Cym. jwa.	32	Oval	1.9—1.25	2.2—2.5	.00035
Cym. park.	20	Lanceolate	0.9—1.25	2.5—3.0	.00058
Cym. olivi.	15	Oval	0.8—1.25	2.1—2.7	.00047

Seed germination test and its comparison with TTC staining

The results of seed germination are compared below with results of TTC staining:

Name	Max. days for total germination	Rate of germination days.	% germination in first week	Total germination in max. days	Mean % of stained seed in 2-3-5-TTC
Cym. jwa.	14	6	99	100	100
Cym. park.	14	7	71	81	95
Cym. olivi.	25	2	56	64	69

The highest percentage of seed germination is recorded in the first week and declines later on with time. Different species require different period for complete germination. TTC staining gives about the same results as germination test.

TTC reactivity. The results of TTC reactivity and routine germination test are given below for 11 species:

Species	Viability in TTC solution %	Viability by germination test %
Apluda mutica L.	83	80
Bothriochloa ischaemum (L.) Keng	80	81
Chrysopogon serrulatus Trin.	85	81
Chrysopogon serrulatus Trin.	80	78
Cymbopogon caesius (Nees) Stapf	96	91
Cymbopogon jwarancusa (Jones) Schult.	83	80
Cymbopogon jwarancusa (Jones) Schult.	96	95
Cymbopogon jwarancusa (Jones) Schult.	96	94
Cymbopogon martini (Roxb.) Wats.	89	81
Cymbopogon parkeri stapf	95	92
Cymbopogon parkeri stapf	90	89
Cymbopogon olivieri (Boiss.) Bor	80	72
Dichanthium annualtum (Forssk.) Stapf	100	100
Hyparrhenia hirta (L.) Stapf	76	71
Hyparrhenia hirta (L.) Stapf	86	83
Hyparrhenia hirta (L.) Stapf	75	70
Themeda anathera	23	20
T. anathera	50	49
T. anathera	76	73
T. anathera	83	81

General discussion and conclusions. The study of inflorescence development period, flowering period and anthesis time indicate that June to October are the best months for their growth. In these months climatic factors are suitable, therefore plants normally spread very rapidly by means of vegetative growth as well as reproductive. The sterility test of florets on morphological basis, suggested that a good number of florets are male or barren and percentage of florets which are capable for seedset, is low, therefore the probability of seedset in an inflorescence is lower than 50 percent. In normal phase seed formation is based on fertilization and for this phenomenon pollen grains must be fertile and viable, therefore pollen staining test by means of various reagents, show highest fertility and viability. One way analysis of variance shows significant, difference among the various staining reagents. Higher percentage of fertility result in higher percent of pollen germination, which in its turn is expected to give higher percentage of seedset, but table 4 shows negative results. Pollen grain germination tests do not relate with pollen-grain fertility tests. To be more close with the natural condition the stigmatic juice plus tap water was used, but there may be several reasons, stigmatic juice plus tap water was not substitute of natural condition, it is difficult to say either substrate used in the test was the best possible or whether it is satisfactory, therefore it is suggested that stigmatic juice plus tap water failed to provide proper supply of the nutrients both in terms of quality and quantity. It is also found that proper temperature is also necessary for pollen grain germination. As already given, the germination is increased when experiment is carried out at 32°C incubation. It is also obtained from the same table that when pollen grains are cultured in Pfhler's (1967) medium percentage of pollen germination increased, but it is still not correlated with fertility. It may be possible that there are some unknown factors which hinder germination, even in the case when staining test give no clear indication of sterility.

The above study may provide a good understanding of behaviour of study grasses, but what are the factors which culminate the seedset are yet to be investigated. However, this information will perform a quite sufficient radical base for the future investigations.

During the TTC study a range of coloration was observed under microscope in the critical regions of the embryos. To avoid probability of error those seeds were considered of high germinability in which the greater part of the embryo has been deeply stained. In all cases there was a high correspondence between TTC and routine germination test. This is in accordance with the results obtained by Lakon (1948), Potrer, Durrel and Romn (1966), Chinki and Ching (1965) and Mian and Coffey (1960) in various cereals seed.

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