Chapter 5

Seed Quality Testing

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The success of seed producer depends on its product quality which is the first and unchanging principle. Quality is a relative term which means the degree of excellence expressed as a rating when compared with an acceptable standard. It may be better, equal or worse in comparison, depending on the criteria and wording used. Seed quality describes the potential performance of a seed lot. Trueness to variety; the presence of inert matter, seed of other crops, or weed seed; germination percentage; vigor; appearance; and freedom from disease are important aspects of seed quality. Thus the high-quality seed lots should meet minimum standards for each of these characteristics.

Seed quality is essential to assess the physical and biological aspects of seed. These tests are commonly done immediately after seed processing, before sowing and then periodically on seed lots kept for long storage. Several techniques have been designed to evaluate the seed quality. These quality tests are essential at several stages during the progress of seed from the parent tree to the seed bed. Efficiency and success for establishment in plantations also depends on the quality of the seeds used.

The essence of good seed testing is the application of reliable standard methods of examination to ensure that uniform and reproducible results are obtained (Justice, 1972; Turnbull, 1975). Analyses of many samples can be facilitated by removing the lightweight material as mentioned in the previous chapter. The quality tests performed in the laboratory exhibit the main aim of provision of accurate and reproducible results which predict how the seed will perform in the field. It also says about the storage

potential of seed, like germination test and thousand seed rate test (Number of seed per weight) together give the actual seed sowing rate. In addition to it, when several seed lot of equal germination potential are together present then TTZ (Tetra zolium) test along with thousand seed rate test helps in deciding the seed sowing rate.

The tests which may be required are purity, authenticity, seed weight, germination, testing of viability, moisture content, and seed health and damage. A prerequisite for all testing is good seed sampling. According to Harrington (1972) maximum seed quality is achieved at the end of seed filling period, which is termed as physiological maturity (Shaw and Loomis, 1950), thereafter seeds begin to age, losing viability and vigor. There are many characteristics which can be considered as measurements of seed quality which are also referred as attributes of seed quality. Some of these characters like seed density and germination percentage are also indicative of reproductive potential of the species.

Materials and Method

The major characteristics considered for measurements of seed quality were:

- Physical purity
- Maturity index
- Germination
- Seed lot screening
- Seed density
- Viability
- Vigor

Standard methods were followed for all the above tests, except for seed lot screening, for which special X-ray techniques were used.

Physical purity

Purity analysis was done for the sample seed lot which was already subjected to the seed processing. Thus, the previously cleaned seeds were further subjected to the purity analysis and the applied procedure was as follow:

- Previously cleaned seeds were weighed (W_1).
- Weighed seed sample was subjected to manual separation.
- During manual separation the contaminant associated with the pure seeds were removed.
- The separated cleaned seeds were reweighed (W_2).
- Seed purity % was calculated.

$$\text{Purity \%} = \frac{\text{Weight of pure seeds } (W_2) \times 100}{\text{Total weight of original sample } (W_1)}$$

Seed density

It was calculated by taking 10 random samples of 100 seeds (i.e. caryopses for grasses and seed from legume pod) from a pure lot. If the difference between any two replicates exceeded 10% of the mean weight, additional replicates were drawn.

Seed weight ratio was calculated with manual counting 1 gm pure seeds were taken.

Maturity index

Maturity indices are totally based on the moisture content that seeds have. It is necessary to check the moisture content of the seed at regular interval till the constant moisture content was obtained. For our requirement, maturity index was calculated on the constant weight basis using following procedure.

- Visually mature, 1 gm of seeds was taken.
- Seeds were dried in oven at 58°C for 3 hours for grasses and for 5 hours for legumes.
- The oven dried sample was reweighed.
- Moisture content (MC **%**) was calculated.
- The procedure was repeated for further collected seeds.
- When the MC % remains constant, the seeds were considered as mature.
- The maturity indices were calculated.

A maturity index was calculated as per following formula:

Moisture content % = $(W_1 - W_2) \times 100$

Where, $W1$ = initial weight, W_2 = weight after oven drying

Viability

Prior to the viability test, seed conditioning was done. In this, seeds were soften in water before staining to facilitate enzyme activation which permits the activation of germination enzymes and makes the seed tissues less fragile. For grasses the caryopses were directly cut while legume seeds were soaked in water overnight as they have hard seed coat. The seed coat was removed manually.

The tetrazolium (TZ) staining test was conducted, it indicates the presence of live tissue and the % of viability was checked through following procedure:

- 25 seeds / caryopses were cut opened length-wise without damaging the embryo
- 0.1% solution of TTZ (2,3,5 – triphenyl tetrazolium chloride) was applied to fully imbibed seeds

- The grass seeds were left for 2 hours in the dark at ~30° C; The legume seeds were left for 3 hours in the dark at ~30° C
- The live embryo, cotyledons and other tissue stain pink to red indicating that the seeds are viable

The % of viability was calculated

Viability %= (A+B) x 4

Where; A = No. of fully red colored stained seeds

B = No. of pink colored stained seeds

Here, red and pink both are considered as viable

Germination

Germination experiments were conducted in laboratory for grass as well as legume seeds with two purposes, one to know the germination index and other to find out the viable storage period.

Protocol followed was as below:

- 100 seeds from each seed lot were placed under moist conditions (100 ml of water) on filter papers in plastic trays (35 x 23 x 6 cms.) for 15 days.
- Subsequent watering was done to maintain the humidity.
- Any kind of pre-treatment to the seeds was not given for grass seed.
- For improving the germination of legumes seeds having hard seed coat, they were soaked in water for 24 hours.
- Mechanical scarification was also applied to the same seeds by putting a small cut by a sharp blade.
- Care was taken to avoid any damage to the embryo.
- The study was conducted at room temperature.

The first and foremost step is to draw a true representative sample from the seed lot, for this seeds were randomly selected from the collected lot.

As we required both purity and germination tests:

1. Seeds for germination tests were taken from the pure seed fraction, after conducting the physical purity analysis which was done manually.
2. The counting of the seed was done without discrimination as to the size and appearance.
3. Germination experiment was continued for two years at the interval of 2 months.

Germination results are expressed as percentage.

$$\text{Germination (\%)} = \frac{\text{Number seeds germinated}}{\text{Number seeds on tray}} \times 100$$

Vigor

The test is done along with the regular germination test. The number of normal seedlings, germinated on the first count day, as specified in the germination test for each species, is counted. The number of normal seedlings gives an idea of the level of seed vigor in the sample. Higher the number of normal seedlings greater is the seed vigor. The applied method is as follow.

- 10 seeds were kept for germination
- The observation of seedlings were recorded at the interval of 5 days
- The seedlings were categorized into different categories i.e. normal and abnormal seedlings.
- The % of vigor was calculated

$$\text{Vigor \%} = \frac{\text{Number of normal seedlings x 100}}{\text{Number of total seedlings}}$$

Seed lot screening- X-ray radiography

The x-ray studies were carried out at the Dept. of Radiology and Imaging, The Gujarat Cancer Research Institute, Ahmedabad, Gujarat. The protocol followed was:

- Seeds / fruits of forestry species of grasses and legumes collected from the study sites were investigated by x-ray radiography.
- The seeds were not pretreated in any way for taking the radiographs.
- A representative portion of each sample was utilized i.e. 100 seeds.
- Seeds were linearly arranged on the white paper and were sealed with cellophane tape. The sequence of the seeds was numbered.
- Since the radiographs were to be enlarged and copied on photographic paper a very slow industrial x-ray film with ultra-fine grain was used.
- The exposure conditions applied were as follow:
  kV (kilovolt) = 22, ffd (focal film distance) = 58 cms for all seeds. While mAs (mili amperes) were different for different species i.e. depending upon their seed size
- The films were processed with x-ray developer.
- After the radiographs were taken, the seeds were compared with the image developed.
- Ratio of filled, empty or damaged seeds was calculated

With this comparison, the germinative capacity, viability and vigor of each seed was examined. The germination test was then carried out to detect possible relationships between seed anatomy and resulting seedling/seed ratio, as revealed by radiographs.

Results and Discussion

Purity test

The primary emphasis of the seed quality analysis is given on their physical purity. Thus any kind of pasture establishment will depend on seeds that are planted. The purity analysis involved steps like separation of pure seeds, inert matter, other crop seeds, etc. from the same seed lot. Forage grass seed samples contain impurities such as weed seeds, seeds of other species, detached seed structures, leaf particles and other material. The aim of purity analysis is to determine the composition by weight of the sample. For this, the sample is separated into its different component parts. When purity analysis is done, it is the first step to be carried out because subsequent tests are made only on the pure seed component.

According to ISTA (1976), pure seed refers to seed lot of a species having mature and undamaged seed along with the removal of undersized, shriveled, immature and germinated seeds. Thus the seeds for working sample containing all the impurities are weighed and then the pure seed is removed and weighed separately. Thus, the main objective of this test is to determine the composition by weight of the variety and contaminants in the sample**.**

In present study, when harvesting was going on, care was taken to avoid mixing of different species samples. Thus, when we applied the harvested material for the seed processing, there were not much impurities of other species except the inert matter of the same species. As soon as the cleaning process was completed, purity % was checked for the cleaned sample lot. The purity analysis was done only for the grasses. Obtained results are shown in the table 5.1. For all species mentioned in the table total weight of original sample (W_1) was 1 gm, W_2 represents amount of pure seed per gm. Purity

analysis was done only for the 25 species which were subjected to mechanical seed cleaning process.

Table 5.1 Purity Analysis Data for Selected Grasses

Sr. No.	Species Name	W_2 gms.	Purity %
1	*Apluda mutica*	0.9 ± 0.02	90
2	*Arthraxon lanceolatus*	0.6 ± 0.01	60
3	*Cenchrus ciliaris*	0.7 ± 0.02	70
4	*Chloris barbata*	0.9 ± 0.01	90
5	*Chloris virgata*	0.9 ± 0.01	90
6	*Chrysopogon fulvus*	0.4 ± 0.01	40
7	*Dactyaloctanium aegyptium*	0.8 ± 0.01	80
8	*Desmostachya bipinnata*	0.2 ± 0.01	20
9	*Digitaria adscendens*	0.5 ± 0.01	50
10	*Echinochloa colonum*	0.6 ± 0.01	60
11	*Echinochloa crus-galli*	0.5 ± 0.01	50
12	*Eleusine indica*	0.7 ± 0.01	70
13	*Eragrostis tenella*	0.8 ± 0.01	80
14	*Eragrostis unioloides*	0.7 ± 0.01	70
15	*Ischaemum pilosum*	0.2 ± 0.01	20
16	*Ischaemum rugosum*	0.4 ± 0.01	40
17	*Panicum trypheron*	0.5 ± 0.02	50
18	*Paspalidium flavidum*	0.3 ± 0.01	30
19	*Schoenefeldia gracilis*	0.7 ± 0.01	70
20	*Sehima ischaemoides*	0.4 ± 0.01	40
21	*Sehima nervosum*	0.3 ± 0.01	30
22	*Sehima sulcatum*	0.2 ± 0.01	20
23	*Sorghum helepanse*	0.5 ± 0.01	50
24	*Thelepogon elegans*	0.4 ± 0.02	40
25	*Themeda cymbaria*	0.3 ± 0.01	30

The results showed that in almost all species purity level was high. The high purity level is mainly due to the use of proper cleaning technique used earlier. The species which shows less purity level had more amount of inert matter; this inert matter included mainly the outer appendages i.e. non-seed material of the grass floret.

Purity determination is based on what proportion of the seed sample by weight has pure seed and what proportion is other material. There are four main different components of a seed lot viz: pure seeds, other seeds, damaged seeds and inert matter or other non-seed materials. Here, the separation was done manually by placing seeds on a working table. In this separation; immature, shriveled, cracked, damaged seeds, etc. were removed. Purity analysis must be done before the other quality tests are started. By this test we can surely increase the germination % of the species in the field.

Seed purity denotes the composition of a particular seed lot. It is based on physical determination of the components present and includes percentage by weight of pure seeds, other crop seeds, weed seed and inert matter (Copeland, 2001). The purity test is perhaps the most complex and exacting of all tests for seed quality. A seed analyst must have a comprehensive knowledge of seed structure and function and must be able to identify a wide array of differing species.

Seed Density

Seed density is generally assumed to be an ecologically important life history trait in plants because it influences both dispersal ability (Harper et al., 1970; Baker, 1972; Platt, 1975; Werner and Platt, 1976) and seedling establishment (Gross and Werner, 1982; Winn, 1985). It significantly affects species richness and composition in experimental communities (Munzberg, O. 2012). Relatively few studies, have examined whether seed weight effects on seedling growth and survival are comparable in different environments or not (Gross, 1984; Winn, 1985; Wulff, 1986), but the results were promising and positive.

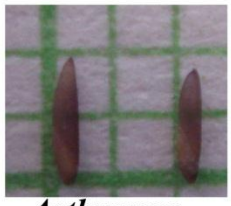
Measurement of seed weight is made on the pure seed component resulted from the purity test, and is normally expressed as the weight of 1000 pure seeds. This figure can be readily converted to number of pure seeds per g or per kg as

Andropogon pumilus

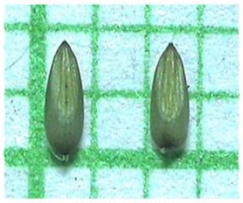
Apluda mutica

Aristida adscensionis

Aristida funiculata



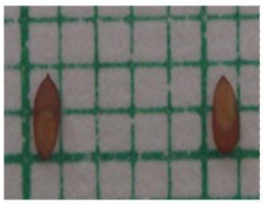
Arthraxon lanceolatus



Bothriochloa pertusa

Brachiaria eruciformis

Brachiaria reptans

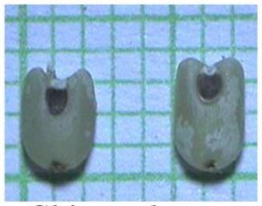


Capillipedium huegelii

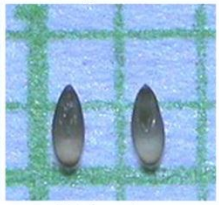
Cenchrus biflorus

Cenchrus ciliaris

Cenchrus setigerus



Chionachne koenigii



Chloris barbata

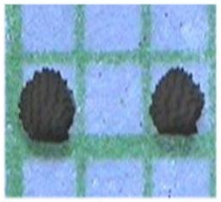
Chloris virgata

Chrysopogon fulvus

Coix lachryma-jobi

Cymbopogon martinii

Cynodon dactylon

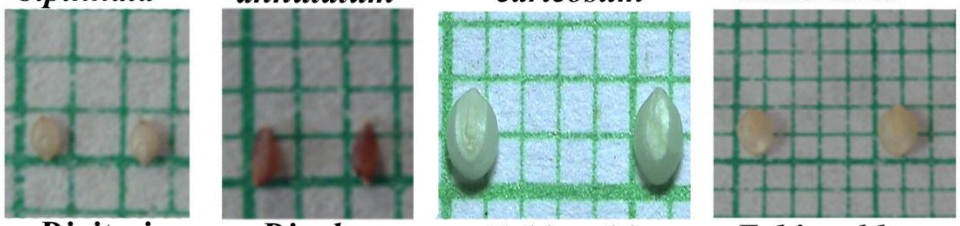


Dactyaloctanium aegyptium

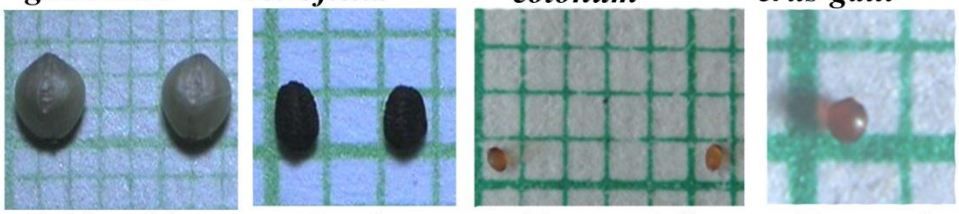
Plate 5.1 Caryopses dimension

(1 square = 1 milimeter square)

Desmostachya bipinnata | *Dichanthium annulatum* | *Dichanthium caricosum* | *Digitaria adscendens*

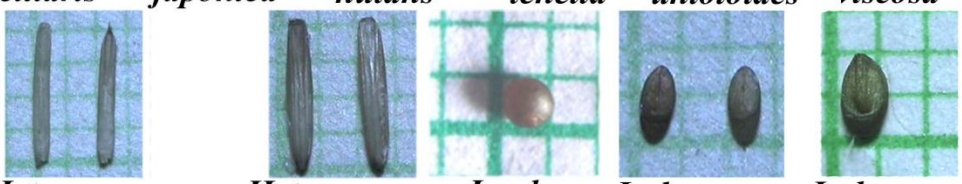


Digitaria granularis | *Dinebra retroflexa* | *Echinochloa colonum* | *Echinochloa crus-galli*



Echinochloa stagnina | *Eleusine indica* | *Eragrostiella bifaria* | *Eragrostis cilianensis*

Eragrostis ciliaris | *Eragrostis japonica* | *Eragrostis nutans* | *Eragrostis tenella* | *Eragrostis unioloides* | *Eragrostis viscosa*



Heteropogon contortus var. contortus sub var. typicus | *Heteropogon contortus var. contortus sub var. genuinus* | *Isachne globosa* | *Ischaemum indicum* | *Ischaemum molle*

Plate 5.2 Caryopses dimension

(1 square = 1milimeter square)

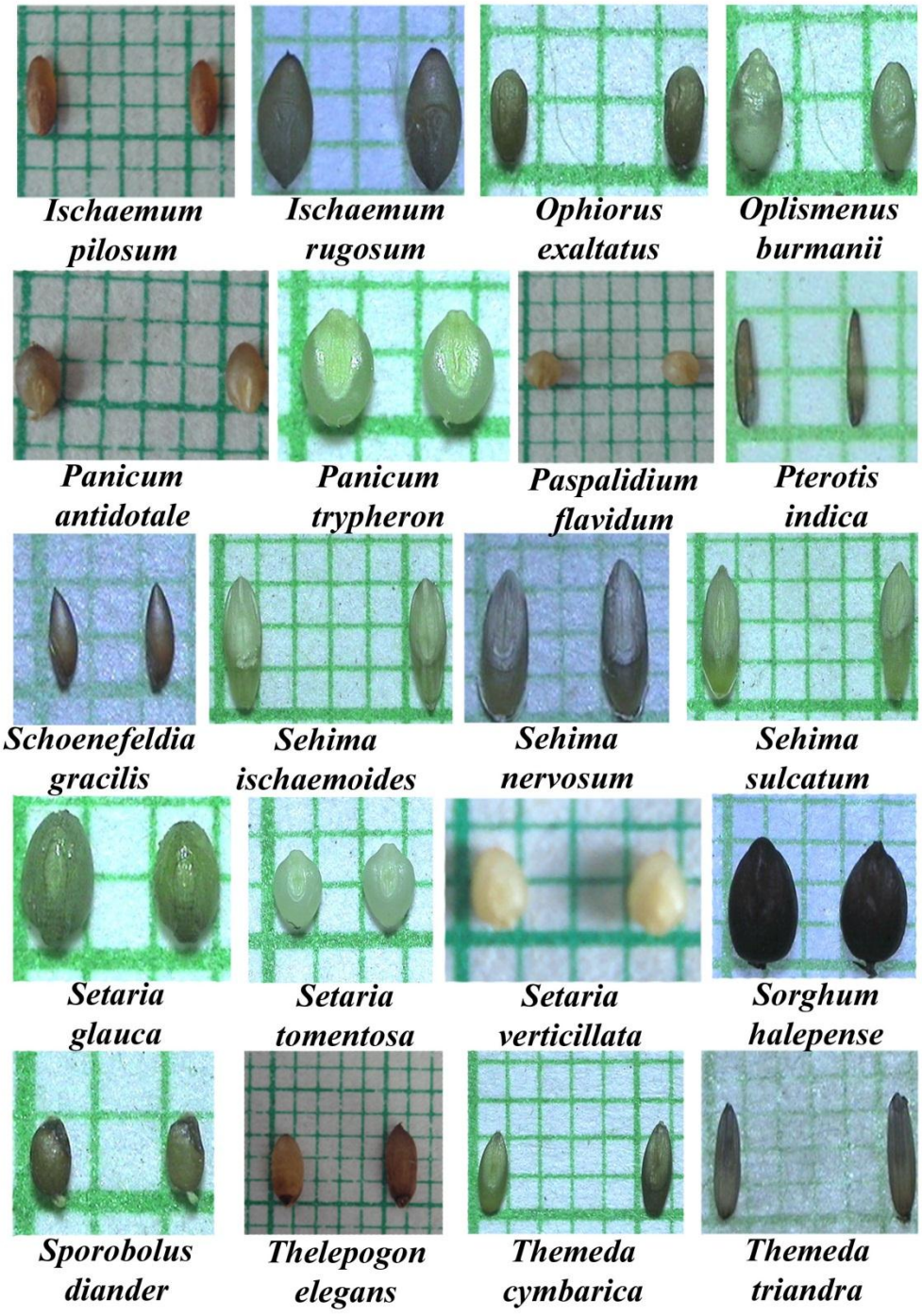


Plate 5.3 Caryopses Dimension

(1 square = 1 milimeter square)

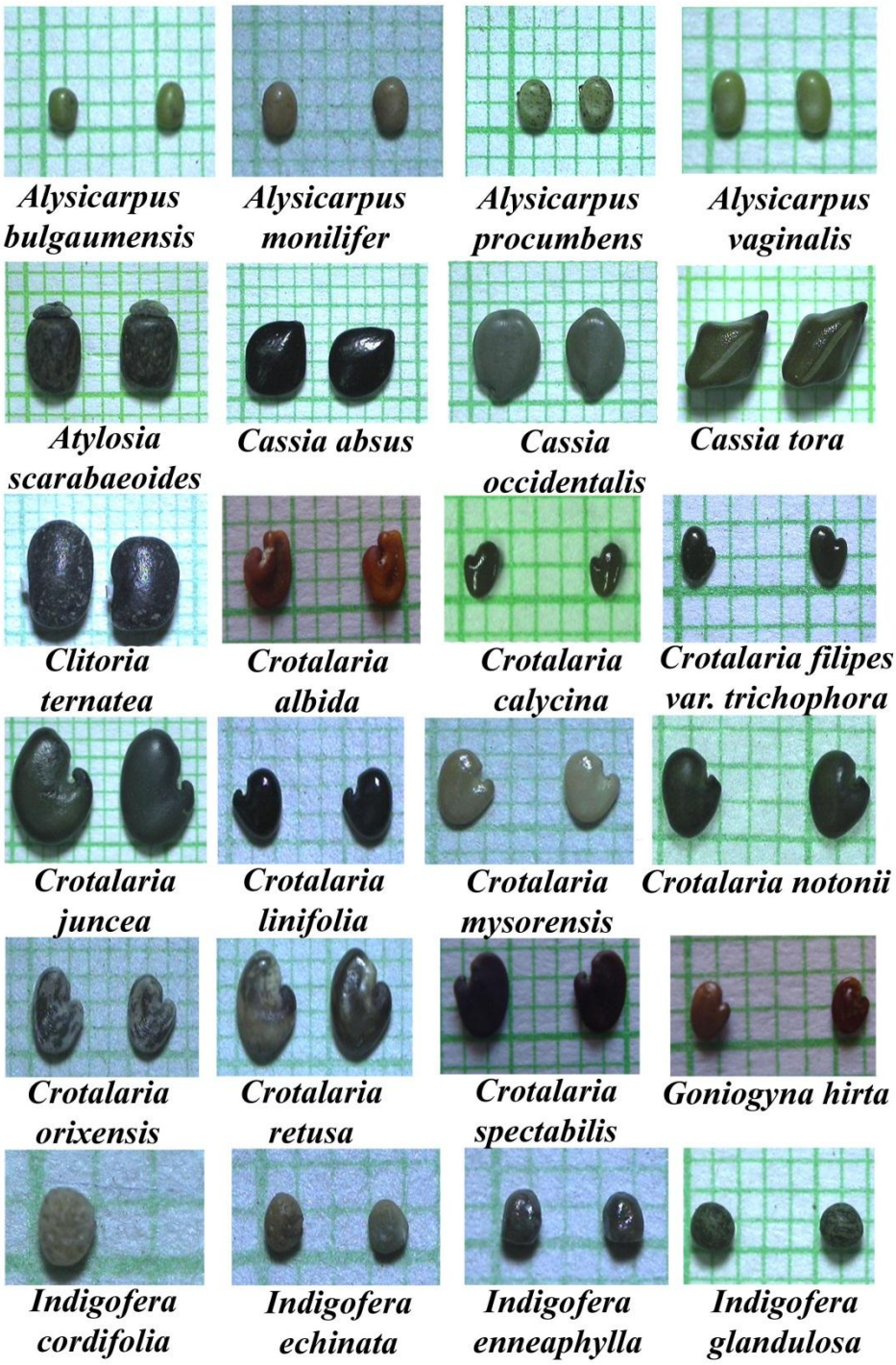


Plate 5.4 Legume Seed Dimension

(1 square = 1 milimeter square)

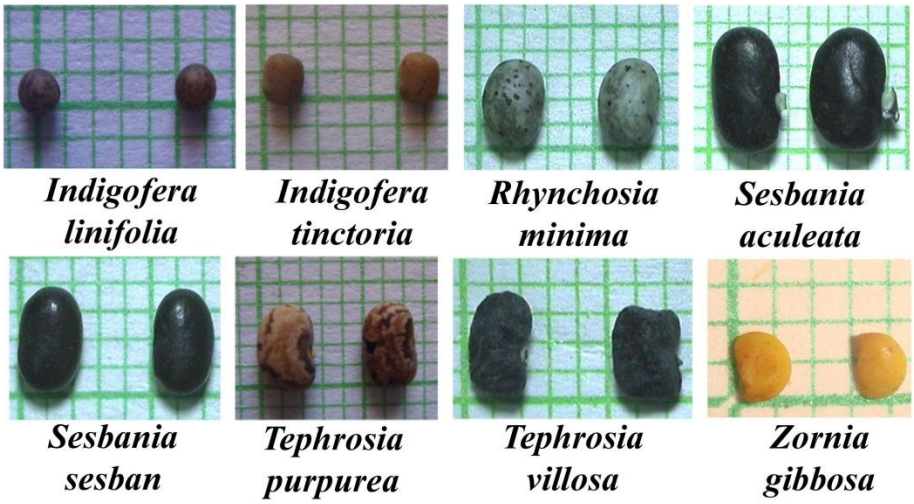


Plate 5.5 Legume Seed Dmension
(1 square = 1 milimeter square)

required. Weight may be determined simply by counting out 1000 seeds and weighing them (Bonner, 1974; Paul, 1972), but the use of several smaller samples enables the analyst to estimate variation within the sample. It is normally expressed for 1000 pure and full seeds.

The counting of seeds for weight was done manually. The main purpose of this study was to calculate the number of seeds per gram, which then can be converted into required unit of weight. Along with seed weight, seed size and seed vigor were also measured (Table 5.2). And seed size for selected species given in Plate 5.1 – 5.5.

Table: 5.2 Density, weight, size and vigor of grass and legume seeds

Sr. No.	Species Name	Seed density (No. of caryopsis / gm)	1000 seed weight (gm)	Seed dimension (mm)			Seed Vigor (%)
				L	T	W	
Grasses							
1	*Andropogon pumilus*	19350	0.05	2.7	0.4	0.2	100
2	*Apluda mutica*	1480	0.68	2.1	0.6	0.9	75
3	*Arthraxon lanceolatus*	23500	0.04	1.8	0.3	0.3	100
4	*Bothriochloa pertusa*	1694	0.59	1.8	0.7	0.4	-
5	*Brachiaria eruciformis*	6973	0.14	1.1	0.6	0.4	-
6	*Brachiaria reptans*	4674	0.21	1.9	1.4	0.2	-
7	*Capillipedium hugellii*	1986	0.50	1.4	0.6	0.3	100
8	*Cenchrus biflorus*	1082	0.92	1.5	1.0	0.7	100
9	*Cenchrus ciliaris*	1136	0.88	1.9	0.8	0.5	100
10	*Cenchrus setigerus*	948	1.05	1.8	1.0	0.7	100
11	*Chionachne koenigii*	158	6.33	3.4	2.1	1.3	100
12	*Chloris barbata*	4968	0.20	1.4	0.5	0.4	71
13	*Chloris virgata*	7000	0.14	1.5	0.4	0.5	40
14	*Chrysopogon fulvus*	1650	0.61	4.6	0.5	0.9	100

Sr. No.	Species Name	Seed density (No. of caryopsis / gm)	1000 seed weight (gm)	Seed dimension (mm)			Seed Vigor (%)
				L	T	W	
15	*Coix lachryma-jobi*	13	76.92	4.4	3.6	2.6	100
16	*Cymbopogon martinii*	926	1.08	2.5	0.8	0.6	100
17	*Cynadon dactylon*	6250	0.16	1.3	0.5	0.3	100
18	*Dactyloctanium aegyptium*	4082	0.24	0.9	0.9	0.5	-
19	*Desmostachya bipinnata*	8754	0.11	1.0	0.3	0.4	-
20	*Dicanthium annulatum*	1545	0.65	2.5	0.8	0.5	67
21	*Dicanthium caricosum*	1321	0.76	2.3	0.9	0.4	100
22	*Digitaria adscendens*	1334	0.75	2.2	0.8	0.4	85
23	*Digitaria granularis*	7496	0.13	0.9	0.6	0.3	-
24	*Dinebra retroflexa*	6280	0.16	1.2	0.3	0.6	100
25	*Echinochloa colonum*	1250	0.80	1.6	1.2	0.7	100
26	*Echinochloa crus-galli*	935	1.07	1.8	1.7	0.9	100
27	*Echonochloa stagnina*	147	6.80	2.7	2.3	1.7	100
28	*Eleusine indica*	4400	0.23	1.2	0.6	0.5	100
29	*Eragrostiella bifaria*	8963	0.11	0.6	0.5	0.3	-
30	*Eragrostis cilianensis*	8326	0.12	0.5	0.4	0.4	-
31	*Eragrostis ciliaris*	9240	0.11	0.4	0.2	0.2	100
32	*Eragorstis japonica*	9238	0.11	0.5	0.2	0.2	-
33	*Eragrostis nutans*	9239	0.11	0.5	0.2	0.2	-
34	*Eragrostis tenella*	9238	0.11	0.5	0.2	0.2	100
35	*Eragrostis unioloides*	8333	0.12	0.7	0.2	0.4	100
36	*Eragorstis viscosa*	9368	0.11	0.6	0.2	0.2	-
37	*Heteropogon contortus* var. *genuisus* subvar. *typicus*	1280	0.44	3.9	0.4	0.3	100
38	*Heteropogon contortus* var. *genuisus* subvar. *hispidissimus*	2248	0.78	4.2	0.6	0.5	100
40	*Ischaemum indicum*	4150	0.24	1.5	0.7	0.5	100

Sr. No.	Species Name	Seed density (No. of caryopsis / gm)	1000 seed weight (gm)	Seed dimension (mm)			Seed Vigor (%)
				L	T	W	
41	*Ischaemum molle*	1937	0.52	1.9	1.1	0.6	-
43	*Ischaemum pilosum*	1400	0.71	2.4	0.8	0.6	100
44	*Ischaemum rugosum*	1800	0.56	2.2	0.9	0.8	100
45	*Ophiorus exaltatus*	1692	0.59	1.4	0.7	0.4	-
46	*Oplismenus burmanii*	1397	0.72	1.6	0.8	0.7	-
47	*Panicum antidotale*	1263	0.79	1.6	0.9	0.7	100
50	*Panicum trypheron*	1394	0.72	1.6	1.2	0.4	100
51	*Paspalidium flavidum*	1560	0.64	1.2	1.2	0.4	100
52	*Pterotis indica*	9387	0.11	1.7	0.3	0.3	90
53	*Schoenefeldia gracilis*	6250	0.16	2.0	0.5	0.4	71
54	*Sehima ischaemoides*	468	2.14	1.8	0.4	0.5	100
56	*Sehima nervosum*	536	1.87	4.3	1.0	0.3	100
57	*Sehima sulcatum*	1250	0.80	2.6	0.9	0.6	100
58	*Setaria glauca*	1638	0.61	1.5	0.9	0.5	100
59	*Setaria tomentosa*	1985	0.50	1.6	1.2	0.3	100
60	*Setaria verticillata*	2083	0.48	1.5	1.0	0.6	-
61	*Sorghum halepance*	269	3.72	2.8	1.7	1.1	100
62	*Sporobolus diander*	9863	0.10	0.6	0.4	0.6	100
63	*Thelepogon elegans*	403	2.48	3.6	1.4	0.5	100
65	*Themeda cymbaria*	1967	0.51	2.3	0.9	0.3	100
66	*Themeda triandra*	269	0.48	3.2	0.9	0.6	90
	Legumes						
1	*Alysicarpus bulgaumensis*	468	2.14	1.3	1.0	0.7	-
2	*Alysicarpus monilifer*	408	2.45	2.0	1.0	0.7	100
3	*Alysicarpus procumbens*	365	2.74	1.9	1.5	0.3	-
4	*Alysicarpus vaginalis*	452	2.21	1.2	0.8	0.6	-
5	*Atylosia scarabaeoides*	62	16.13	4.6	2.9	1.1	80

Sr. No.	Species Name	Seed density (No. of caryopsis / gm)	1000 seed weight (gm)	Seed dimension (mm)			Seed Vigor (%)
				L	T	W	
6	*Cassia absus*	48	20.83	4.1	3.6	0.7	100
7	*Cassia occidentalis*	55	18.18	4.6	3.8	0.5	100
8	*Cassia tora*	21	47.62	6.0	2.0	2.0	90
9	*Clitoria ternatea*	16	62.50	5.5	4.0	2.6	100
10	*Crotalaria calycina*	23	43.48	1.6	1.4	0.4	80
11	*Crotalaria filipes*	38	26.32	1.8	1.3	0.4	100
12	*Crotalaria juncea*	23	43.48	6.8	5.0	1.2	90
13	*Crotalaria linifolia*	272	3.21	2.0	1.3	0.4	-
14	*Crotalaria mysorensis*	208	4.81	2.8	1.7	1.1	100
15	*Crotalaria notonii*	161	6.21	2.3	2.0	0.3	70
16	*Crotalaria orixensis*	115	8.70	3.0	2.5	0.8	90
17	*Crotalaria leptostachya*	73	13.70	1.5	3.0	0.9	80
18	*Crotalaria spectabilis*	272	3.68	2.9	2.3	0.3	80
19	*Indigofera cordifolia*	786	1.27	1.1	1.0	0.3	-
20	*Indigofera echinata*	774	1.29	1.3	1.0	0.4	80
21	*Indigofera enneaphylla*	800	1.25	1.4	1.0	0.9	90
22	*Indigofera glandulosa*	319	3.13	1.7	1.7	1.7	100
23	*Indigofera linifolia*	125	8.00	1.2	1.2	1.2	100
24	*Indigofera tinctoria*	371	2.70	1.6	1.3	0.9	90
25	*Rhynchosia minima*	51	19.61	3.6	2.8	1.9	100
26	*Sesbania aculeata*	36	27.78	5.7	3.3	2.0	100
27	*Sesbania sesban*	350	2.86	3.8	2.2	1.8	80
28	*Tephrosia purpurea*	90	11.11	4.0	2.5	1.5	100
29	*Tephrosia villosa*	163	6.13	3.6	2.2	0.7	90
30	*Zornia gibbosa*	513	1.95	1.8	1.3	0.4	-

The factors that affect seed weight are size, moisture content and proportion of fully filled seeds in the lot. Although seed weight is a relatively conservative character in plants it can vary depending on a variety of factors including age of the parent plant (Cavers and Steel, 1984), day length (Cook 1975), moisture and growing season (Schimpf, 1977) and temperature during development (Wardlaw and Dunstone, 1984). According to Fenner (1987), in some species there can be considerable size variation among seeds, formed under similar conditions.

The results show a direct relation between the seed size and seed weight i.e. seed weight is directly proportional to the seed size in most of the species. As the seed size is more the weight also become more. On the other hand some interesting observations were recorded in the present study specially in case of grasses when along with seed size and weight other two parameters viz: density (no. of seeds/gm.) and seed vigor were also assessed (Table 5.2).Some species showed lesser density, smaller size but higher seed weight (*Cenchrus setigerus*). Species like *Andropogon pumilus* and *Arthraxon lanceolatus* showed quite higher density of light weight, smaller seeds. Large and heavy weight seeds with very low density were observed in *Coix lachryma-jobi*. Inter varietal differences appeared in two varieties of *Heteropogon contortus.* Where, minor difference in the size of seeds showed makeable difference in the seed weight. Interestingly in all such cases the seed vigor was 100 %. Seed vigor variation was observed within species of genus *Dicanthium* and genus *Chloris.* Seeds of *D. annulatum* and *D. caricosum* were almost of same size with little difference in weight and density, but vigor of *D. caricosum* seeds was quite high. Between two species of *Chloris,* seeds of *C. barbata* exhibited higher vigor and lower density while higher density and lower vigor was observed in *C. virgata* though seeds of both the species were almost of same and weight. Variation in seed size or seed weight among different species is a natural fact as it is a species specific character, but this variation becomes significant when it is applied to

community regeneration or restoration. Guo (2011) in his biodiversity experiment observed that seed size, seed weight and plantation density all should be considered for such experiment. He further suggested that larger and heavier seeds will show greater germination rate and will bring successful restoration.

Large seeds of legumes weigh more per seed than small ones of the same specific gravity and, because they contain larger food reserves, they are likely to germinate better and produce initially more vigorous seedlings. Goor and Barney (1976) have reported that large seeds of *Eucalyptus citriodora* had a higher germination rate than medium-sized seeds which in turn had a higher rate than small seeds. Number of pure seeds per unit weight is therefore not in itself a good guide to potential for plant production and must be complemented by germination tests or indirect tests of viability.

The difference in seed weight of a same species seems to be adaptive and resulting in increased nutrition of the seedlings. These seedlings again resulting from larger food reserve of the heavier seed. While smaller seeds produced in larger number, their dispersabilty and reproductive output will increased. Literature and experiments show a general positive correlation between seed weights and rates of shoot and root growth, at least within species (Baker, 1972). Seed weight increases progressively in a series from annual grasses to tree.

The larger seeds produce larger seedlings than do smaller seeds of the same species (or variety) is a truism. The need to improve seedling establishment of perennial grass species sown for forage has motivated many studies (Kneebone and Cremer 1955) on the relationship between seed size and seedling size and vigor. Only the seedling stage

has generally been of concern in such studies of seed-size effects in grasses (Hutchinson, 1984).

Seed weight distribution patterns varied widely among plants within a species. The importance of these patterns in selecting for seedling vigor and in obtaining genetic equality in the contribution of plants to a composite variety is discussed (Carleton and Cooper, 1972**)**. Individual from a large seeds have an advantage early in the life cycle, particularly if seed weight affects seedling size and competitive ability (Howell, 1981; Gross, 1984) or resistance to abiotic stress (Weis, 1982). However, this size advantage may be relatively short lived if seedlings from small seeds have higher relative growth rates than those from large seeds (Zimmerman and Weis, 1983; Gross, 1984).

The evolutionary importance of seed size variation among and within populations will depend upon the degree to which observed seed weight differences among individuals are genetically or environmentally determined. Studies examining causes of seed weight variation in non-crop species have found that much of the observed variation is environmentally determined (Schaal, 1980) or due to developmental effects (Waller, 1982; Stanton, 1984; Winn, 1985).

Maturity index

Seed maturation is one of the main components of seed quality and a prerequisite for successful germination and emergence (Perry, 1982). Therefore, seed crops should be harvested when their maturity is maximal. However, the time of the achieving complete maturity during development and its association with seed and fruit features are greatly debated and also show variation among crops and growing locations as well. According to Shaw and Loomis (1950) the maximum seed dry mass is referred as its

physiological maturity which has also been described as a measure of maximum seed quality for a long period. According to Harrington (1972), maximum seed viability and vigor coincide with the attainment of maximum physiological maturity and decline thereafter. Other research also says that the seed longevity, along with other seed quality traits, continues to increase after the end of the seed filling phase i.e. after attaining its maximum physiological maturity (Pieto Filho and Ellis, 1991; Demir and Ellis, 1993). Thus seeds of any species show their important physiological changes for a significant period of time after attaining maximum dry weight which also termed as physiological maturity.

According to Reid (1992), maturity denotes the stage when the commodity has reached to the stage of development at which its quality will be at least the minimum acceptable to the ultimate consumer.

The estimation of maturity can also be done satisfactorily on the basis of fruit features. The index of maturity must meet two requirements: minimum acceptable eating quality and a long storage life. As fruit matures and ripens, color changes from green to red or yellow/brown but as an exception, in case of legumes, difficulties encountered is the lack of uniformity of seed-coat color.

Seed moisture content has been found to be the most reliable indicator of seed maturity and harvest timing in grass seed crops. Since pollination and seed maturation are not uniform processes in grass seed crops, a range of seed maturity can be found in a single field. Harvesting within the correct range of seed moisture contents will maximize harvestable seed yield and minimize losses of seed during harvest. Seed moisture content is also an important factor in the storability of harvested seed. High seed moisture content reduces longevity of seed in storage and reduces seed quality

(Silberstein, 2010). It is the most vital parameter, which influences the seed quality and storage life of the seed. Seed moisture content is closely associated with several aspects of seed quality like, seed maturity, optimum harvest time, mechanical damage, economics of artificial seed drying, seed longevity and insect and pathogen infestation, etc.

The aim of present study was to find out the time of the occurrence of most potential seed quality in wild grasses and herbaceous legumes during development and its association with seed dry mass. For our study, we collected the seed at different time and the moisture content of the collected seeds was checked. When the moisture content was observed to be constant, we considered the seeds as mature. The obtained results are given in table 5.3.

Table 5.3 Maturity index determination data

Sr. No.	Species Name	Moisture content % at different stages				
		15 Days	**30 days**	**45 Days**	**60 days**	**75 days**
Grasses						
1	*Andropogon pumilus*	30	20	10	10	10
2	*Apluda mutica*	40	30	20	10	10
3	*Brachiaria eruciformis*	30	20	10	10	10
4	*Brachiaria reptans*	20	20	10	10	10
5	*Cenchrus ciliaris*	20	20	20	10	10
6	*Cenchrus setigerus*	30	30	20	20	10
7	*Chloris barbata*	20	20	10	10	10
8	*Chloris virgata*	30	20	20	10	10
9	*Chrysopogon fulvus*	30	30	20	20	10
10	*Coix lachryma-jobi*	20	10	10	10	10

Sr. No.	Species Name	Moisture content % at different stages				
		15 Days	30 days	45 Days	60 days	75 days
11	*Cymbopogon martinii*	20	20	10	10	10
12	*Cynodon dactylon*	20	10	10	10	10
13	*Dactyaloctenium aegyptium*	10	10	10	10	10
14	*Desmostachya bipinnata*	30	20	20	20	10
15	*Dichanthium annulatum*	30	20	10	10	10
16	*Dichanthium caricosum*	30	20	20	20	10
17	*Digitaria adscendens*	20	20	10	10	10
18	*Digitaria granularis*	20	20	20	20	20
19	*Dinebra retroflexa*	10	10	10	10	10
20	*Echinochloa colonum*	30	20	10	10	10
21	*Echinochloa crus-galli*	30	30	20	10	10
22	*Eleusine indica*	20	10	10	10	10
23	*Eragrostis tenella*	20	10	10	10	10
24	*Heteropogon contortus* var. *contortus* subvar. *hispidissimus*	20	20	10	10	10
25	*Heteropogon contortus* var. *contortus* subvar. *typicus*	30	20	20	10	10
26	*Ischaemum pilosum*	30	30	20	20	10
27	*Ischaemum rugosum*	30	30	20	20	10
28	*Panicum antidotale*	40	20	20	10	10
29	*Panicum trypheron*	40	30	20	10	10
30	*Paspalidium flavidum*	30	20	10	10	10
31	*Schoenefeldia gracilis*	20	10	10	10	10
32	*Sehima nervosum*	40	30	20	10	10
33	*Setaria glauca*	20	20	20	10	10
34	*Setaria tomentosa*	30	20	20	10	10
35	*Sorghum halepense*	30	10	10	10	10

Sr. No.	Species Name	Moisture content % at different stages				
		15 Days	30 days	45 Days	60 days	75 days
36	*Thelepogon elegans*	20	10	10	10	10
37	*Themeda cymbaria*	30	20	20	10	10
38	*Themeda triandra*	30	20	20	10	10
Legumes						
1	*Alysicarpus monilifer*	20	10	10	10	10
2	*Alysicarpus procumbens*	20	10	10	10	10
3	*Alysicarpus vaginalis*	10	10	10	10	10
4	*Atylosia scarabaeoides*	30	10	10	10	10
5	*Cassia absus*	20	10	10	10	10
6	*Cassia occidentalis*	20	10	10	10	10
7	*Cassia tora*	20	10	10	10	10
8	*Clitoria ternatea*	10	10	10	10	10
9	*Crotalaria filipes* var. *trichophora*	30	20	10	10	10
10	*Crotalaria juncea*	20	10	10	10	10
11	*Crotalaria spectabilis*	20	10	10	10	10
12	*Crotalaria mysorensis*	20	10	10	10	10
13	*Crotalaria notonii*	10	10	10	10	10
14	*Crotalaria orixensis*	20	20	10	10	10
15	*Indigofera cordifolia*	20	10	10	10	10
16	*Indigofera echinata*	10	10	10	10	10
17	*Indigofera enneaphylla*	10	10	10	10	10
18	*Indigofera glandulosa*	20	20	10	10	10
19	*Indigofera linifolia*	20	20	20	20	10
20	*Indigofera tinctoria*	20	20	10	10	10
21	*Rhynchosia minima*	30	10	10	10	10
22	*Sesbania sesban*	20	20	10	10	10
23	*Tephrosia purpurea*	10	10	10	10	10

Sr. No.	Species Name	Moisture content % at different stages				
		15 Days	30 days	45 Days	60 days	75 days
24	*Tephrosia villosa*	20	10	10	10	10
25	*Zornia gibbosa*	30	20	10	10	10

In given result we considered the skin color of florets and pods as a visual maturity indicator to obtain accurate maturity index. 10-12% of moisture content is safe to store most of the seeds in open storage and in cloth bags or moisture-resistant containers. Thus we tried to collect the seeds having same moisture content. Immature seeds of many species will not germinate if they are placed on a moist substrate as they quickly become covered with fungi. It should be noted that these immature seeds will die if they are allowed to dry out (Harrington, 1972). As a fact, germination requirements and percentage of immature seeds may be different from those of mature seeds of same species. Mature seeds of some species do not germinate because seed coats are impermeable to water and Helgeson (1932) and Hyde (1954) have shown that this seed coat impermeability develops as seeds dry. Thus, the seeds of hard seeded species are collected before they have a chance to dry on mother plant**.**

The development of seed quality in wild species is poorly understood, and yet the wide temporal variation in flowering and seed development typical in wild plants means that seed collections intended for conservation in seed banks may contain considerable seed-to-seed variation in their physiological maturity and, therefore, seed quality.

Germination and Viability

One of the most fascinating stages of a plant life cycle is germination. A seeds ability to germinate is the most convincing and accepted index of its quality which is also a complex physiological process triggered by imbibition of water. Under favorable conditions rapid expansion growth of the embryo culminates in rupture of the covering layer and emergence of the radicle. Radicle emergence is considered as the completion of germination. Along with it, viability is the measure of the capability of seed to be germinating. These both parameters of seed quality testing are important and also depend on each other.

Germination is the main component of seed quality but it is not necessarily good indicator of seed vigor. Seed longevity in air-dry storage is claimed as a good, sensitive indicator of differences in seed quality among high viability seed lots and was shown experimentally in rice (Ellis et al., 1993) and soybean (Zanakis et al., 1994).

Three principal environmental factors control seed germination, viz: light, moisture and temperature and they vary with the type of ecosystems and habitat. Among them high moisture requirement of the seed for germination could be the major factor limiting germination, especially during the dry season (Tomado et al., 2002).

In the present study, a germination response of forage grass species and associated herbaceous legumes was assessed. Here, dominant species occur in varying proportions and are joined by other grasses and legume species, depending on macro and micro climatic conditions. The grasslands in the eastern part of India are essentially natural grasslands but the monsoon grasslands too, which show their best potential in the monsoon period only for about four months a year. After these four months owing to

moisture stress and advent of winter, the grass species enter to the dormancy period till the next monsoon.

Studies on germination response are conducted by many scientists. Jaiswal and Chaudhary (2005) tested germination behavior of trees and forage grasses by using different substrata; Beckstead et al., (1995) studied the after ripening effects on germination of *Bromus tectorum* and *Elymus elymoides* i.e. on viability basis; Andrews and Burrows (1972) checked the germination response of Dormoat (derivatives of crosses between *Avena sativa* L. and *A. fatua* L.) dormant seeds towards low temperature; Karlsson et al., (2006) examined the pattern of germination and dormancy simultaneously for *Galeopsis speciosa*. In the studied grassland; continual usage of such excellent grasses and total dependency of nearby tribal for their cattle; resulted in the dwindling of proportion of these grasses. This dwindling; finally results in the influence of annual grasses and sometimes inferior and less palatable grasses also. Thus, such important reservoirs of forage require careful attention and scientific management.

In the study area, availability of good quality seeds of such valuable grass species is the major constraint, which limits the increased production even by the Forest Department. To overcome such difficulties, an eco-physiological comparison of the mechanisms for deciding the season of emergence was performed with 62 grass and legume species co-occurring in a dry tropical grassland community of eastern India, for which the phenologies for seed germination, seedling emergence and the storage effect on seed lot along with the seasonal variation were recorded. Based on the data obtained, responses of individual species and their correlation to different physiological mechanisms for the seedling emergence were analyzed.

Species response patterns

A species response pattern in the present screening shows that immediately after seed collection, all the seeds in this seed population were dormant, with no germination occurring in the initial test. Thus along with germination tests, other physiological parameter i.e. viability was examined to know exact reasons behind germination response to different storage durations. Great variations in the response patterns were observed among the species in the present screening, based on this observation species were grouped into different categories. The different categories included species with:

1. Continuous Uniform Germination
2. Higher germination rate in early stages and then show decline
3. Low or zero germination in early stages and higher germination rate at later stages
4. Fluctuating germination rate
5. Very less germination
6. No germination

Germination is one of the most important stages of a plant's life cycle. Successful germination can be crucial for dispersal and then survival of a species in the natural environment. Therefore, it is of considerable interest to examine the mechanisms regulating seed germination in response to environmental parameters. The study was conducted with the same purpose; results of study are represented in table 5.4 and Fig. 5.1 – 5.8.

The higher percentage of germination recorded in the test performed after the 2nd and 4th months of storage, indicates that somewhat higher temperatures were needed for a complete removal of primary dormancy. Exposure to a higher temperature range probably induced a secondary dormancy, since considerably lower percentages of

germination were recorded during the DT regime in some of the species studied. As the storage durations increased, a low degree of relative dormancy was exhibited by all species. Great variations in the response patterns to the temperature were observed among the species in the present screening. Although all the species showed their higher germinability after 12-16 months of storage.

Table 5.4 Germination response of selected grass and legume species

IT = Increasing temperature (IT regime); DT = Decreasing temperature (DT regime); 'V'= viability %; 'G'= germination%

Sp. Index	Species Name		% germination and viability at different storage durations												
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Category 1. Continuous Uniform Germination															
Sp. 1	*Cenchrus ciliaris*	V	44	-	-	92	-	-	96	-	-	100	-	-	96
		G	0	40	90	84	85	85	80	90	95	90	80	95	70
Sp. 2	*Dichanthium annulatum*	V	76	-	-	92	-	-	96	-	-	92	-	-	60
		G	0	60	55	50	56	70	65	70	75	66	65	50	48
Sp. 3	*Echinochloa stagnina*	V	100	-	-	100	-	-	96	-	-	95	-	-	80
		G	2	95	100	100	92	100	90	95	100	98	92	95	90
Sp. 4	*Ischaemum indicum*	V	50	-	-	45	-	-	36	-	-	40	-	-	20
		G	5	45	50	40	38	35	35	40	42	32	30	20	15
Sp. 5	*Setaria glauca*	V	45	-	-	40	-	-	40	-	-	25	-	-	10
		G	0	34	30	35	35	30	25	30	25	25	20	15	10
Sp. 6	*Setaria tomrntosa*	V	40	-	-	44	-	-	28	-	-	20	-	-	10
		G	0	30	32	40	35	30	25	25	30	28	25	25	26
Sp. 7	*Cassia absus*	V	90	-	-	88	-	-	96	-	-	92	-	-	85
		G	0	10	20	25	30	24	30	40	40	35	42	43	32
Sp. 8	*Crotalaria filipes*	V	48	-	-	40	-	-	28	-	-	44	-	-	44
		G	0	0	0	0	10	12	10	15	10	15	18	12	15

Sp. Index	Species Name		% germination and viability at different storage durations												
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Sp. 9	*Crotalaria juncea*	V	100	-	-	100	-	-	98	-	-	100	-	-	98
		G	2	35	80	95	98	98	90	96	92	98	96	80	92
Sp. 10	*Rynchosia minima*	V	100	-	-	100	-	-	88	-	-	80	-	-	84
		G	0	0	10	45	40	38	40	45	42	35	30	32	30
Category 2. Higher germination rate in early stages and then show decline															
Sp. 11	*Apluda mutica*	V	40	-	-	75	-	-	90	-	-	100	-	-	60
		G	0	20	40	70	68	75	80	100	100	95	80	50	45
Sp. 12	*Aristida adscensionis*	V	65	-	-	70	-	-	35	-	-	0	-	-	0
		G	0	0	20	33	15	13	12	10	10	0	0	0	0
Sp. 13	*Aristida funiculata*	V	55	-	-	50	-	-	35	-	-	0	-	-	0
		G	0	0	10	35	26	30	30	20	10	0	0	0	0
Sp. 14	*Cenchrus biflorus*	V	95	-	-	28	-	-	0	-	-	0	-	-	0
		G	0	95	30	2	0	1	0	0	0	0	0	0	0
Sp. 15	*Chloris barbata*	V	92	-	-	80	-	-	65	-	-	0	-	-	0
		G	0	95	90	82	80	57	40	20	0	0	0	0	0
Sp. 16	*Chrysopogon fulvus*	V	8	-	-	50	-	-	70	-	-	88	-	-	12
		G	0	10	20	40	30	35	50	40	30	20	0	0	0
Sp. 17	*Cymbopogon martinii*	V	75	-	-	56	-	-	36	-	-	20	-	-	0
		G	0	10	50	16	15	21	20	10	5	0	0	0	0
Sp. 18	*Dinebra retroflexa*	V	10	-	-	20	-	-	0	-	-	0	-	-	0
		G	0	0	20	10	15	10	0	0	0	0	0	0	0
Sp. 19	*Echinochloa colonum*	V	90	-	-	88	-	-	76	-	-	60	-	-	40
		G	0	5	10	12	67	65	65	54	40	35	25	22	20
Sp. 20	*Echinochloam*	V	85	-	-	72	-	-	68	-	-	48	-	-	40
	crus-galli	G	0	10	25	90	80	75	70	75	55	40	42	30	10
Sp. 21	*Eleusine indica*	V	10	-	-	25	-	-	8	-	-	0	-	-	0
		G	0	0	10	20	40	30	15	5	0	0	0	0	0

Sp. Index	Species Name		% germination and viability at different storage durations												
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Sp. 22	*Eragrostis ciliaris*	V	30	-	-	25	-	-	20	-	-	10	-	-	0
		G	0	5	10	15	20	25	15	15	10	5	5	0	0
Sp. 23	*Eragrostis tenella*	V	35	-	-	33	-	-	35	-	-	12	-	-	4
		G	0	10	15	26	30	30	25	20	20	8	5	5	0
Sp. 24	*Eragrostis unioloides*	V	50	-	-	44	-	-	40	-	-	20	-	-	15
		G	0	5	12	20	22	25	30	25	20	15	5	0	0
Sp. 25	*Ischaemum pilosum*	V	60	-	-	52	-	-	44	-	-	24	-	-	20
		G	0	5	5	10	25	40	40	35	10	15	0	0	0
Sp. 26	*Panicum antidotale*	V	50	-	-	35	-	-	25	-	-	20	-	-	10
		G	10	25	48	45	30	30	28	25	20	20	18	15	10
Sp. 27	*Panicum trypheron*	V	65	-	-	60	-	-	55	-	-	55	-	-	50
		G	0	8	10	15	20	60	50	50	45	40	35	35	30
Sp. 28	*Paspalidium flavidum*	V	70	-	-	60	-	-	45	-	-	20	-	-	0
		G	0	55	45	52	40	42	38	22	20	15	5	0	0
Sp. 29	*Sehima ischaemoides*	V	55	-	-	50	-	-	48	-	-	40	-	-	20
		G	10	30	70	20	60	70	50	45	25	15	0	0	0
Sp. 30	*Sehima sulcatum*	V	65	-	-	35	-	-	25	-	-	10	-	-	0
		G	0	10	80	20	15	20	10	10	5	2	0	0	0
Sp. 31	*Thelepogon elegans*	V	95	-	-	96	-	-	88	-	-	50	-	-	30
		G	20	15	40	35	26	65	70	55	50	35	40	20	10
Sp. 32	*Themeda triandra*	V	60	-	-	84	-	-	100	-	-	80	-	-	40
		G	0	0	10	80	45	100	80	95	100	80	40	0	0
Sp. 33	*Crotalaria calycina*	V	28	-	-	24	-	-	28	-	-	20	-	-	28
		G	0	30	30	25	22	20	20	15	10	5	0	0	0
Category 3. Low germination in early stages and higher germination rate at later stages															
Sp. 34	*Andropogon pumilus*	V	65	-	-	48	-	-	40	-	-	36	-	-	30
		G	0	15	20	12	20	40	40	35	35	32	35	30	25

Sp. Index	Species Name		% germination and viability at different storage durations												
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Sp. 35	*Capilipedium hughellii*	V	80	-	-	70	-	-	65	-	-	40	-	-	30
		G	0	10	0	15	20	60	62	55	50	35	35	30	20
Sp. 36	*Cenchrus setigerus*	V	75	-	-	70	-	-	72	-	-	60	-	-	45
		G	0	10	0	20	57	60	50	45	40	20	20	10	0
Sp. 37	*Ciox lachryma-jobi*	V	70	-	-	56	-	-	60	-	-	52	-	-	40
		G	0	20	50	60	50	70	55	50	40	45	45	40	30
Sp. 38	*Dichanthium*	V	50	-	-	48	-	-	48	-	-	36	-	-	30
	caricosum	G	0	20	50	30	53	70	45	40	40	35	30	20	10
Sp. 39	*Digitaria adscendens*	V	100	-	-	96	-	-	92	-	-	80	-	-	70
		G	0	0	0	20	100	90	90	85	85	65	60	55	50
Sp. 38	*Heteropogon contortus* var. *genuisus*	V	72	-	-	96	-	-	92	-	-	96	-	-	40
	subvar. *genuinus*	G	0	0	60	52	85	80	65	90	45	80	30	10	20
Sp. 39	*Heteropogon contortus* var. *genuisus*	V	100	-	-	95	-	-	92	-	-	88	-	-	50
	subvar. *typicus*	G	0	0	60	52	85	80	75	80	85	75	65	45	40
Sp. 40	*Themeda cymbaria*	V	80	-	-	76	-	-	72	-	-	60	-	-	50
		G	0	25	30	65	40	80	55	65	68	55	54	50	45
Sp. 41	*Atylosia scarabaeoides*	V	85	-	-	76	-	-	72	-	-	84	-	-	80
		G	2	10	20	5	40	30	40	45	30	32	38	42	30
Sp. 42	*Cassia occidentalis*	V	96	-	-	96	-	-	92	-	-	80	-	-	90
		G	0	0	10	10	20	40	50	35	40	45	32	42	40
Sp. 43	*Cassia tora*	V	100	-	-	100	-	-	96	-	-	88	-	-	90
		G	0	3.3	10	20	30	10	35	40	32	34	20	30	38
Sp. 44	*Clitoria ternatea*	V	96	-	-	92	-	-	96	-	-	80	-	-	76
		G	0	5	10	0	20	10	45	42	48	42	35	40	25
Sp. 45	*Crotalaria spectabilis*	V	84	-	-	84	-	-	76	-	-	80	-	-	76
		G	0	0	0	0	2	10	30	25	32	15	40	35	25

Sp. Index	Species Name		% germination and viability at different storage durations												
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Sp. 46	*Crotalaria leptostachya*	V	92	-	-	88	-	-	92	-	-	80	-	-	78
		G	0	7	10	20	20	40	30	25	35	25	20	25	40
Sp. 47	*Indigofera linifolia*	V	55	-	-	40	-	-	44	-	-	32	-	-	40
		G	0	3	0	2	10	0	30	35	25	20	25	30	30
Sp. 48	*Indigofera tinctoria*	V	100	-	-	96	-	-	92	-	-	88	-	-	80
		G	0	0	0	10	10	12	20	25	30	35	30	30	45
Sp. 49	*Sesbaia aculeata*	V	96	-	-	96	-	-	92	-	-	88	-	-	90
		G	0	20	10	30	20	50	70	65	50	35	45	50	35
Sp. 50	*Tephrosia purpurea*	V	90	-	-	88	-	-	92	-	-	80	-	-	85
		G	0	0	5	30	20	40	30	35	40	25	30	20	25
Sp. 51	*Tephrosia villosa*	V	90	-	-	96	-	-	96	-	-	72	-	-	60
		G	0	0	2	10	25	30	40	45	65	50	45	55	50
Category 4. Fluctuating germination rate															
Sp. 52	*Arthraxon lanceolatus*	V	95	-	-	92	-	-	92	-	-	80	-	-	65
		G	0	30	15	0	73	55	60	55	50	50	45	45	40
Sp. 53	*Ischaemum rugosum*	V	75	-	-	64	-	-	55	-	-	48	-	-	25
		G	10	60	20	80	0	40	42	45	35	35	30	20	10
Sp. 54	*Pterotis indicum*	V	100	-	-	100	-	-	100	-	-	96	-	-	80
		G	10	100	20	85	40	75	95	82	55	70	65	45	62
Sp. 55	*Schenefeldia gracilis*	V	100	-	-	100	-	-	96	-	-	92	-	-	85
		G	0	40	0	20	36	45	85	45	60	35	45	40	40
Sp. 56	*Sorghum halepanse*	V	75	-	-	64	-	-	64	-	-	60	-	-	55
		G	0	70	10	32	60	42	54	55	58	55	45	45	40
Sp. 57	*Crotalaria mysorensis*	V	94	-	-	96	-	-	88	-	-	92	-	-	88
		G	0	5	30	0	20	0	10	0	2	5	0	0	0
Sp. 58	*Indigofera ennaephyalla*	V	50	-	-	44	-	-	28	-	-	36	-	-	44
		G	0	0	20	0	20	10	10	5	0	15	10	2	12

Sp. Index	Species Name		% germination and viability at different storage durations											
			DT 0	IT 2	IT 4	IT 6	DT 8	DT 10	DT 12	IT 14	IT 16	IT 18	DT 20	DT 22	DT 24
Category 5. Very less germination														
Sp. 59	*Chionachne koenigii*	V	55	-	-	50	-	-	40	-	-	20	-	-	0
		G	0	10	0	25	30	25	20	20	20	15	10	0	0
Sp. 60	*Dactyloctenium*	V	15	-	-	12	-	-	8	-	-	4	-	-	0
	aegyptium	G	0	2	2	4	0	10	5	2	0	0	0	0	0
Sp. 61	*Crotalaria orixensis*	V	76	-	-	80	-	-	84	-	-	92	-	-	70
		G	0	0	0	0	0	10	10	0	0	5	2	0	0
Sp. 62	*Indigofera glandulosa*	V	50	-	-	52	-	-	52	-	-	32	-	-	40
		G	0	0	0	0	10	0	10	5	2	15	10	12	10

According to the above categories, hardly few species among grasses e.g. *Cenchrus ciliaris, Dichanthium annualtum, Echinochloa stagnina, Ischaemum indicum, Setaria tomentosa, Setaria glauca,* etc. and species among legume namely *Cassia absus, Crotalaria juncea, Crotalaria filipes and Rhynchosia minima* showed uniform germination. The main reason behind this type of performance is the adaptive ability of species to fluctuating environmental conditions. All these grasses had good potential to withstand shortage of water. As we conducted our germination tests in laboratory at ambient condition, the results can be correlated the with the field conditions to a great extent. The germination test reflected immense ability of these species to survive under uncertain environmental conditions prevailing in rangeland conditions.

Species like *Apluda mutica, Aristida adscensionis, Aristida funiculata, Cenchrus biflorus, Chloris barbata, Chrysopogon fulvus, Cymbopogon martinii, Dinebra retroflexa, Echinochloa colonum, Echinochloa crus-galli, Eleusine indica, Eragrostis ciliaris, Eragrostis tenella, Eragrostis unioloides, Ischaemum pilosum, Panicum antidotale, Panicum trypheron, Sehima*

ischaemoides, Sehima sulcatum, Thelepogon elegans, Themeda triandra, Crotalaria calycina, etc. showed high degree of germination in early stages and then it declined. This declination of germination shows the dependency of the plant on the environmental conditions. The temperature responses of germination of a number of species are briefly surveyed with particular reference to interactions between temperature and other factors of the environment. Comparisons are drawn for the germination strategies of species and we observed that the environmental fluctuations had a significant effect on the germination.

Species like *Andropogon pumilus, Capillipedium hughellii, Cenchrus setigerus, Coix lachryma-jobi, Dichanthium caricosum, Digitaria adscendens, Heteropogon contortus* var. *genuisus* subvar. *hispidissimus, Heteropogon contortus* var. *genuisus* subvar. *typicus, Themeda cymbaria, Atylosia scarabaeoides, Cassia occidentalis, Cassia tora, Clitoria ternatea, Crotalaria spectabilis, Crotalaria leptostachya, Indigofera linifolia, Indigofera tinctoria, Sesbania aculeata, Tephrosia purpurea, Tephrosia villosa,* etc. showed less or no germination in early stages then progressively improvement in germination was observed. These types of results indicate that the species may have some dormancy period after the collection or it may be influence of the environment. As these species were collected in post monsoon period, the coming drier season might have not been favorable for the germination.

Species like *Arthraxon lanceolatus, Ischaemum rugosum, Pterotis indicum, Schoenefeldia gracilis, Sorghum halepanse, Crotalaria mysorensis, Indigofera ennaephylla,* etc. showed fluctuating germination. These fluctuations prove that the minor change in the optimal environmental conditions for the proper germination performance fluctuate the plant life which in future may affect its density in its habitat also.

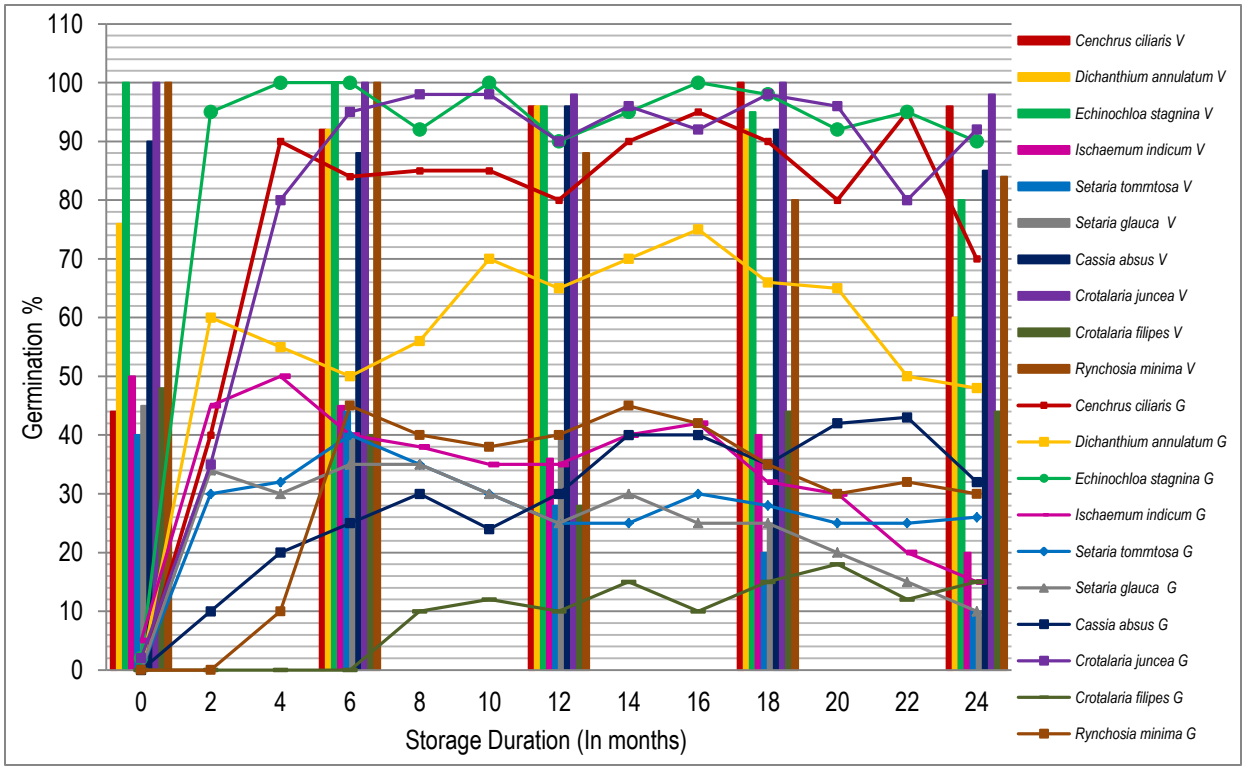


Fig. 5.1 Continuous Uniform Germination

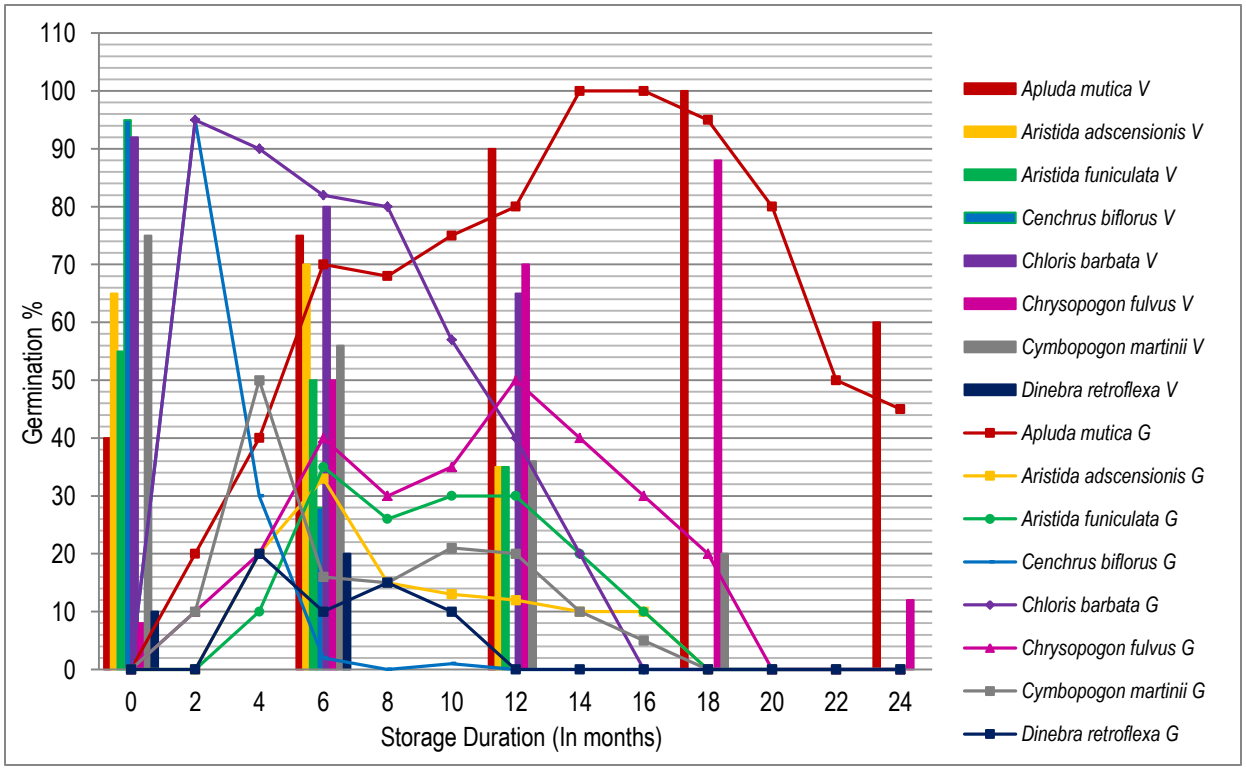


Fig. 5.2 Higher germination in early stages and then show decline - 1

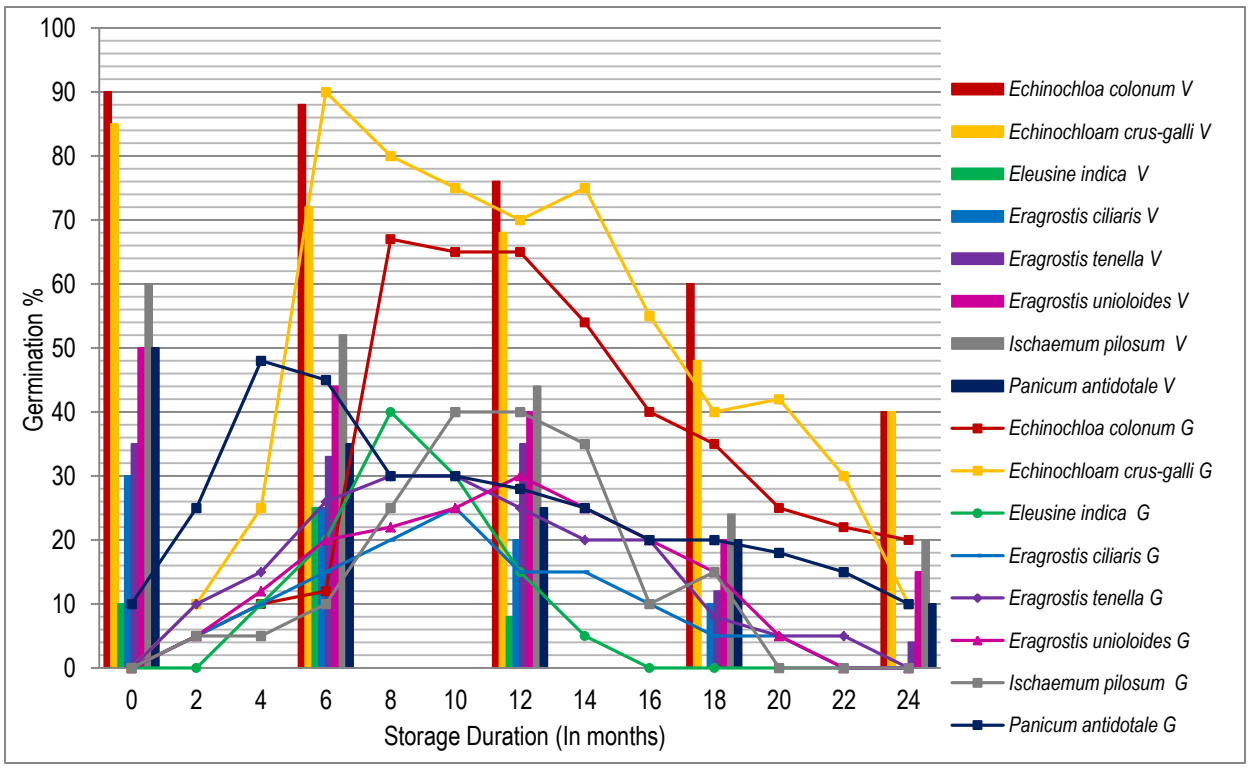


Fig. 5.3 Higher germination in early stages and then show decline - 2

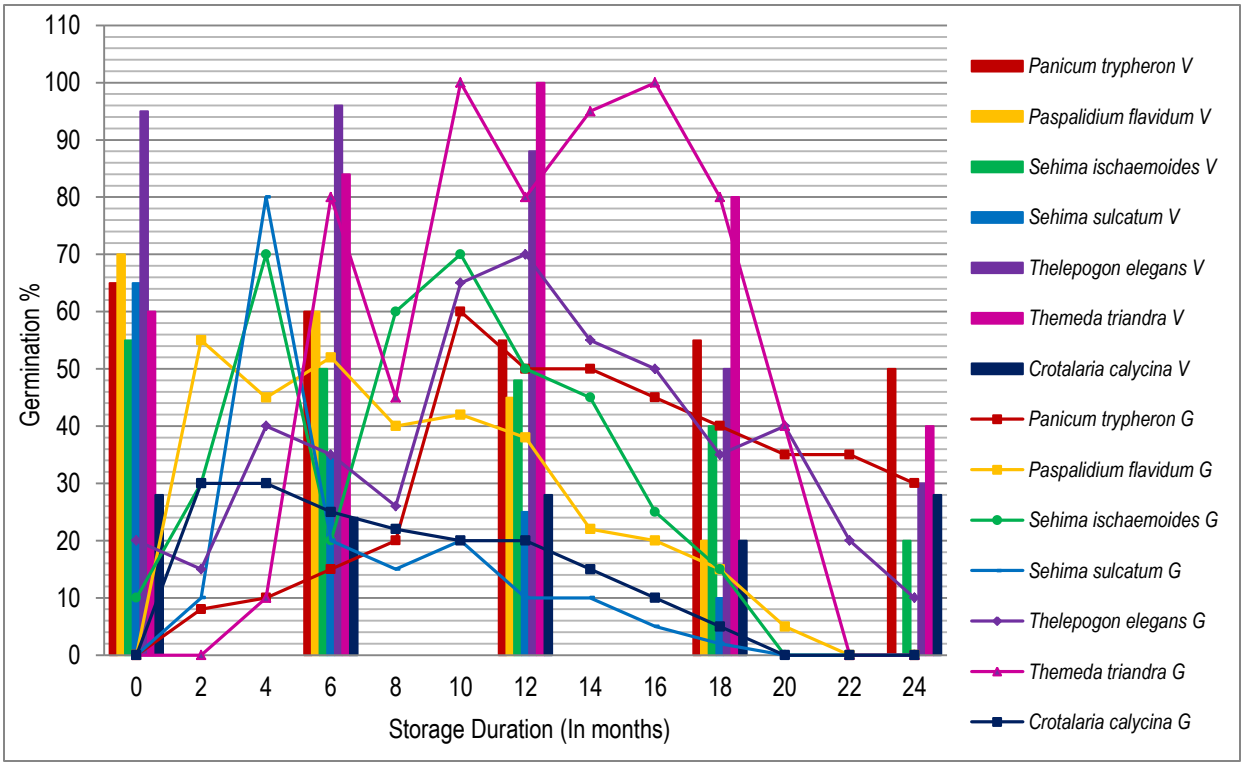


Fig. 5.4 Higher germination in early stages and then show decline - 3

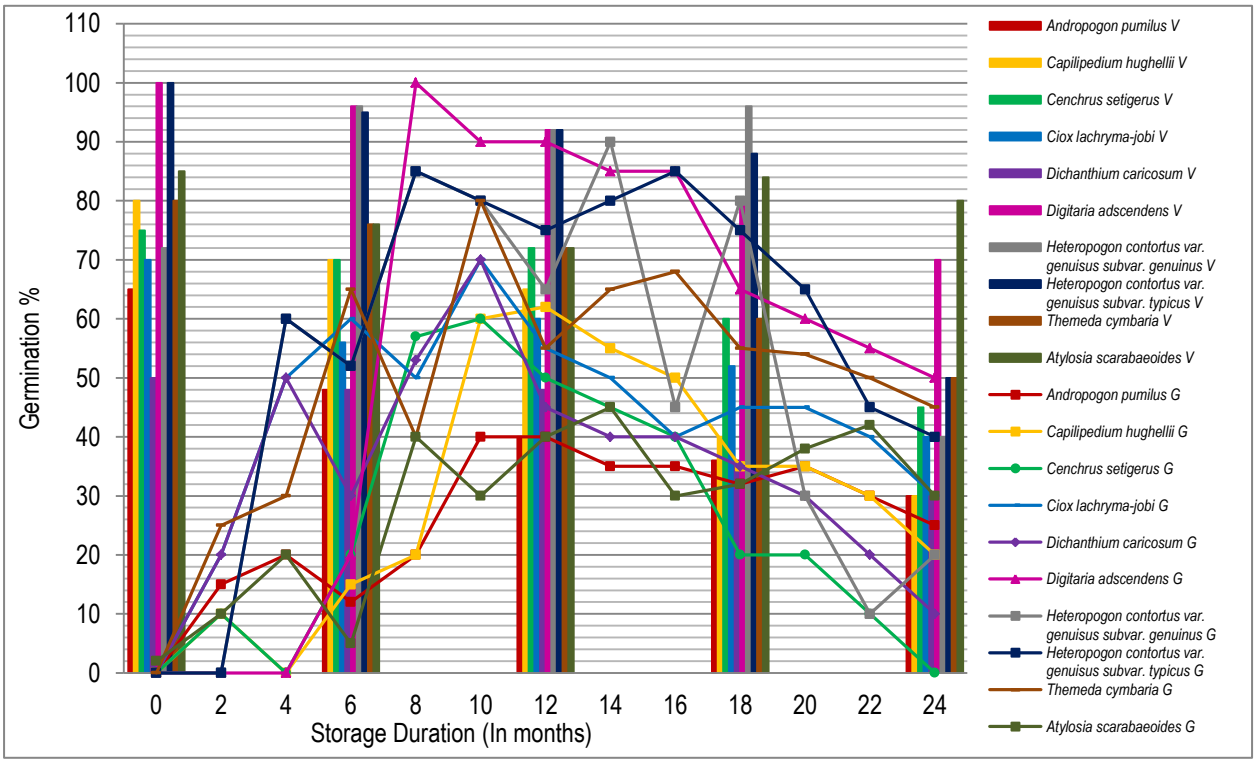


Fig. 5.5 Low germination in early stages and higher germination rate at later stages - 1

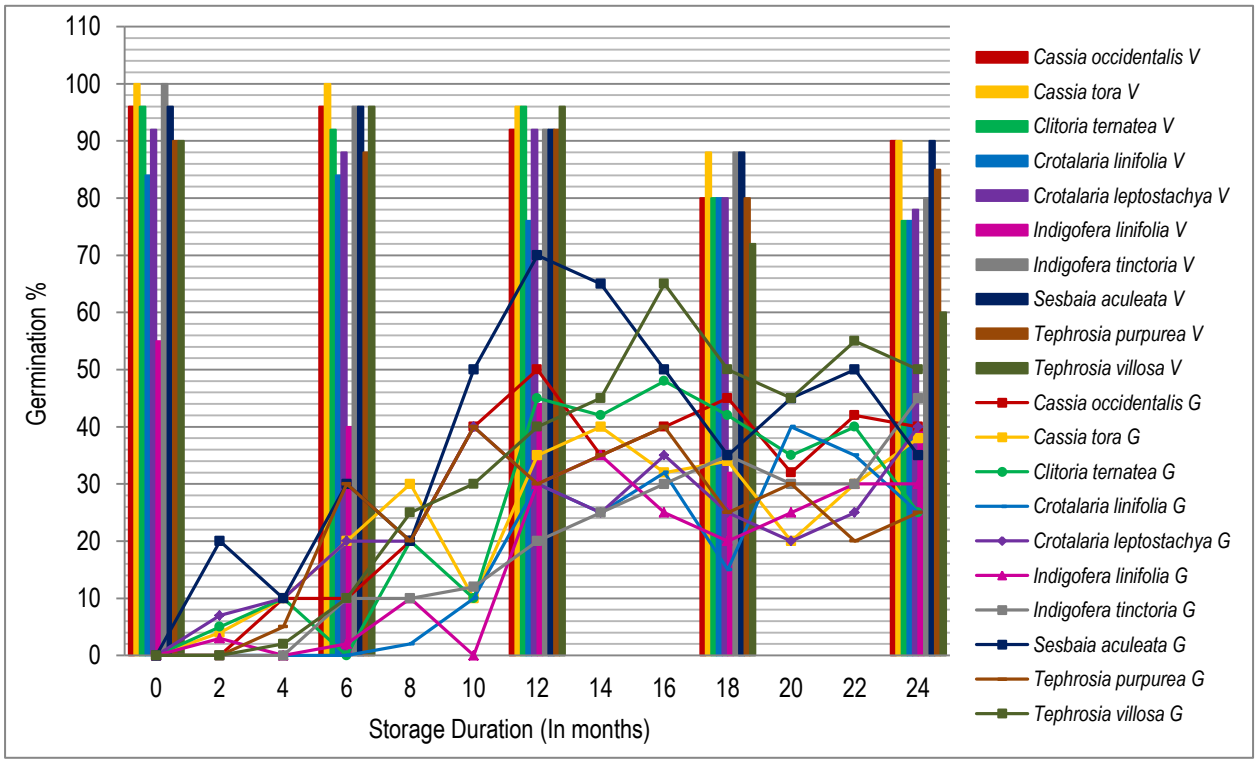


Fig. 5.6 Low germination in early stages and higher germination rate at later stages - 2

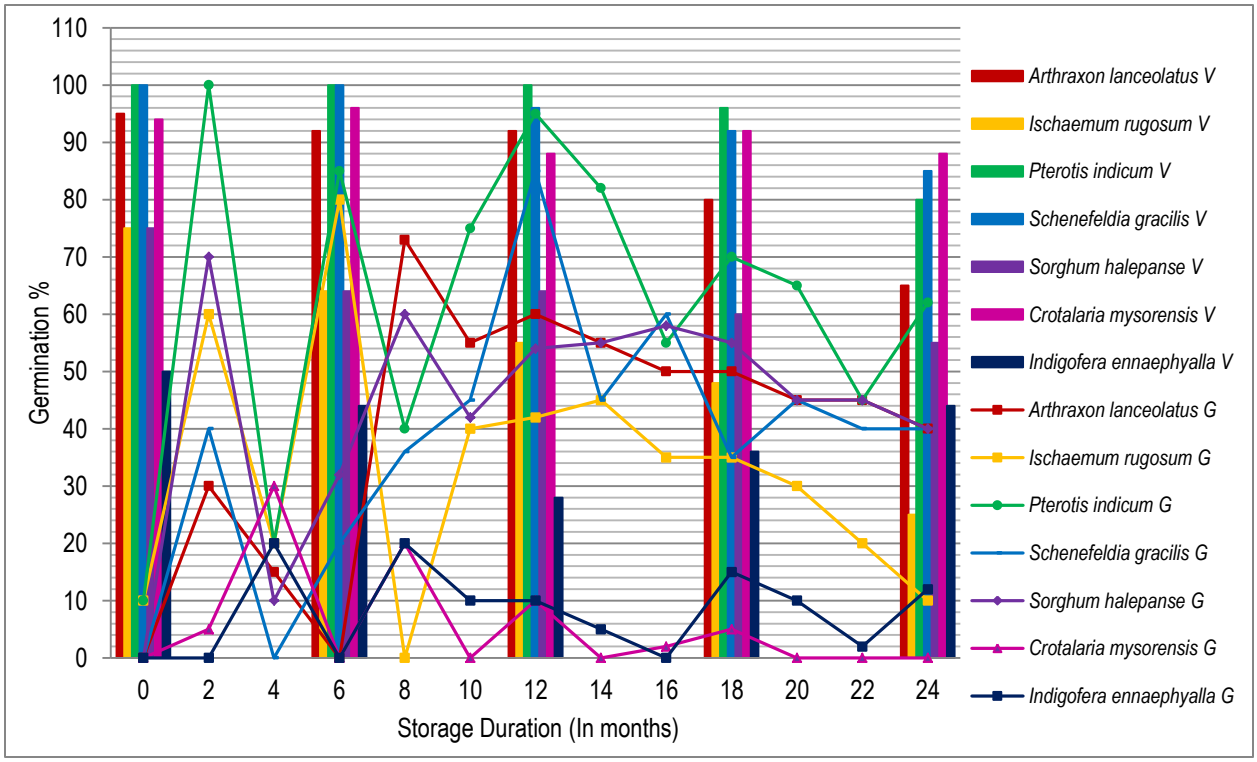


Fig. 5.7 Fluctuating germination rate

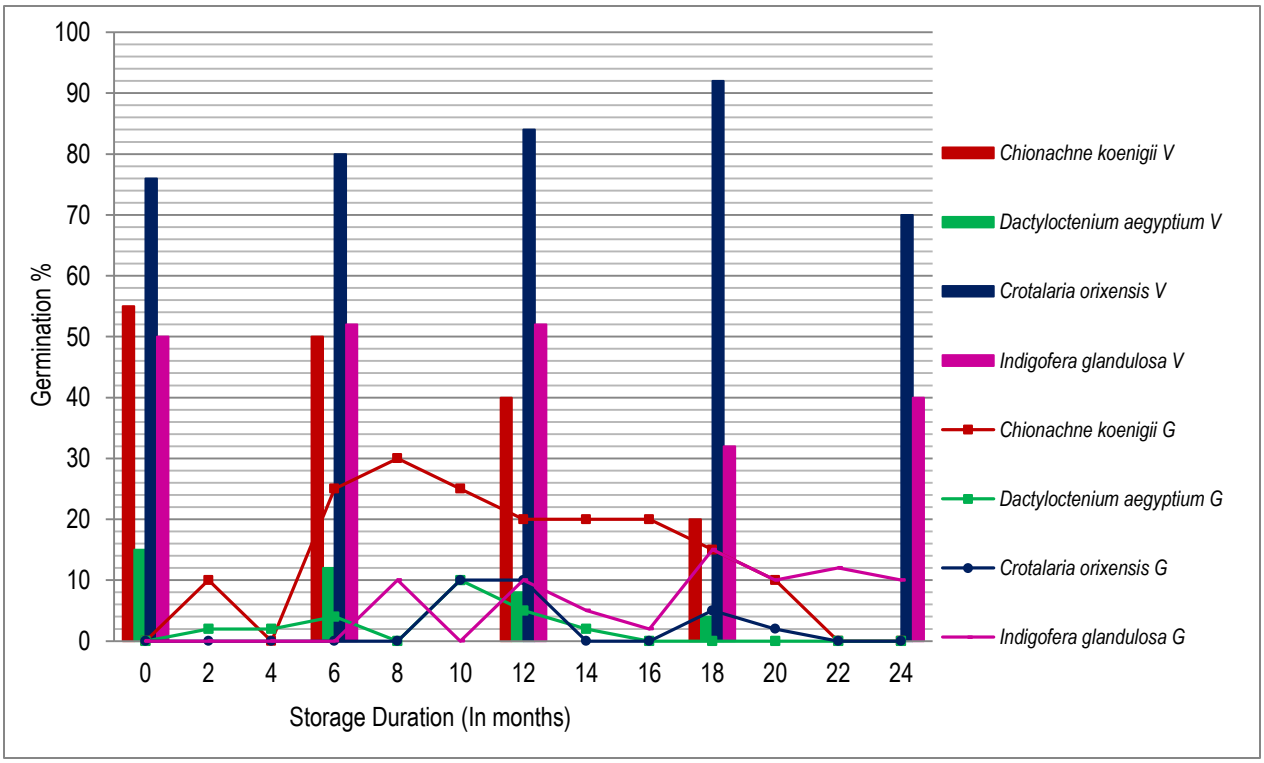


Fig. 5.8 Very less germination

Species like *Chionachne koenigii, Dactyloctenium aegyptium, Crotalaria orixensis, Indigofera glandulosa,* etc. exhibited very less percent germination that hardly reached up to 20–30%. Though these species have mature seeds but environment might not support their physiology of germinating capacity.

Species like *Bothriochloa pertusa, Desmostachya bipinnta, Digitaria granularis, Eragrostis biferia, Sehima nervosum, Setaria verticilata, Sporobolus diander, Alysicarpus bulgamensis, Alysicarpus procumbens, Alysicarpus vaginalis, Indigofera cordifolia, Zornia gibbosa,* etc. showed no germination or almost near to it of which all having immature seeds. Species like *Melanocenchris jacquemontii, Imperata cylindrica* and *Pennisetum setosum* etc. having no seed setting at all; while *Hackelochloa granularis* was seen to be smut infected.

Dominant grass species of the study area are *Apluda mutica, Cenchrus ciliaris, Chrysopogon fulvus*, *Dichanthium annulatum, Heteropogon contortus* var. *contortus* subvar. *genuinus, Heteropogon contortus* var. *contortus* subvar. *typicus*, *Themeda triandra, Crotalaria juncea,* etc. They are naturally growing as well as they are planted also. All the species are widely growing, and are more in demand for cattle feed and are stored for scarcity period. The germination performance of such species was almost uniform throughout the study period i.e. for 24 months. It indicates that seeds of these demandable species can be stored for longer period and can subsequently be utilized for propagation for the livestock.

When we compared the germination performance of legumes with grasses, they showed very poor germination. The main reason behind the poor germination is hard seed coat. Along with this, the immaturity of seeds also might have affected the average germination rate. Results of the legume germination showed that initially almost all legumes showed very less or zero germination as at that time any type of pretreatment

was not given. It was confirmed that the seeds were viable and thus it was thought that the nil germination is only because of dormancy and to break it mechanical scarification was given by putting a cut without damaging the embryo which finally resulted into the increase in the germination rate.

Seed dormancy could be considered simply as a block to the completion of germination of an intact viable seed under favorable conditions. Dormancy should not just be associated with the absence of germination; rather, it is a characteristic of the seed that determines the conditions required for germination (Vleeshouwers et al., 1995; Fenner and Thompson, 2005).

Dormancy characters are often reduced or eliminated by selection in cultivated forage grasses but are still operative in most species in natural ecosystems. Generally, cool-season grasses have low amounts of seed dormancy and differ in seed longevity. Such inhibition of dormancy, we observed in the germination performance of some species.

Capacity for immediate germination

Although, for about all of the species examined, the germination of a fraction of the seed population was recorded in the initial test performed immediately after the seed collection and the results showed that seeds of most of the species seemed to be 100% dormant as they did not germinate. Their dormancy was exhibited up to 2-4 months of storage, though the seeds were viable. Only few species like *Echinochloa stagnina, Ischaemm indicum, Ischaemum rugosum, Sehima ischaemoides, Thelepogon elegans, Pterotis indicum, Atylosia scarabaeoides, Crotalaria juncea,* etc. showed immediate germination after the seed collection. While species like *Digitaria adscendens, Crotalaria filipes, Crotalaria spectabilis, Crotalaria orixensis, Indigofera glandulosa* and *Indigofera tinctoria* showed seed dormancy up to six months.

Response to dry storage

The dry storage showed various effects on germination patterns in the present test system like generally increasing the germinability, improvement of germinability, or dormancy breaking. In all species, storage was associated with a progressive increase in germination percentage and rate. Thus, as mentioned earlier, from the results total six groups could be distinguished (Table 5.4). Beneficial effects of dry storage upon germinability were observed for many forage species. Seeds of selected species are shed during the post monsoon. The after ripening, functions as the mechanism for prevention of pre-mature germination in dry habitats. The effect of premature germination may result in the production of plants with low vigor. The species, which displayed a distinct improvement in germinability during dry storage, are small seeded. Thus, the consideration of possibility was occurred that in certain related species a major effect of delayed ripening and germination is to facilitate seed burial**.**

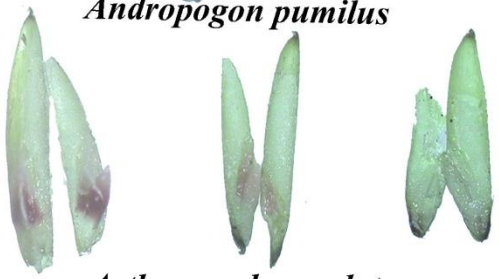
Response to temperature fluctuation

The requirement of temperature fluctuation for dormancy breaking is known to be common in wetland species and arable weeds (Thompson and Grime, 1983). If a species subjected to the present screening had a low or moderate response to temperature fluctuation, it was fulfilled by the gradual change of temperature in the test system. In species with a greater response to temperature fluctuation, a considerable increase in germination occurred under the alternating temperature regimes immediately following the IT regime. Thus, the rates of germination, recorded in the present study, may vary being achieved in the field. Such difference in germination rates with variable temperature was observed by Yuan and Shi (2009) in germination rate of *Spartina alterniflora***.**

From the results we can see that among all 62 species most of them showed considerable increase in germination rate as the temperature increased. But during the testing after 8-10 months of storage, some kind of fall in germination in each species was seen and that is because the environmental conditions reached at the peak of IT regime, where temperature almost reached above 40°C. As the temperature again fall < 40°C seeds started germinating, which actually shows the minor sensitivity to the fluctuating temperature. Among grasses a high proportion of species was capable of germination over a wide range of temperature i.e. >20°C. This feature is particularly evident in species of dry habitats which suggests that relative insensitivity to temperature is characteristic of seed in which water supply acts as the primary determinant of the timing of germination in the laboratory condition as well as field (Panchal et al., 2011).

In the present study, other eco-physiological parameter studied was viability. Though the germination test is the main test for viability, it does not always provide an accurate assessment of the plant producing capacity of the seed lot. In many cases, seeds may be alive but they fail to germinate because of the intense dormancy at the time of germination. This dormancy however, may be short lived, and those seeds which did not germinate may produce seedlings at the time of planting. Considering this as the main need of viability testing, we checked viability at the regular interval of 6 months, and obtained results are given in the Table 5.4.

Andropogon pumilus



Arthraxon lanceolatus

Cenchrus biflorus

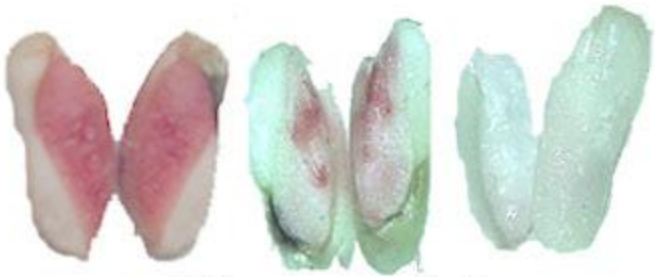
Apluda mutica

Brachiaria reptans

Cenchrus ciliaris

Plate 5.6 Viability test

Cenchrus setigerus



Chionacnne koenigii

Chloris barbata

Chrysopogon fulvus

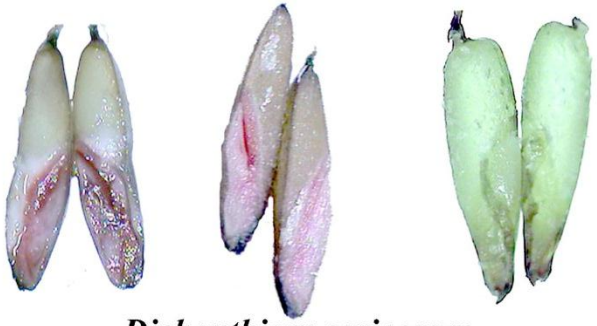
Coix lachryma-jobi

Cymbopogon martinii

Plate 5.7 Viability test

Dactyaloctanium aegyptium

Dichanthium annulatum



Dichanthium caricosum

Digitaria adscendens

Echinochloa colonum

Echinochloa crus-galli

Plate 5.8Viability Test

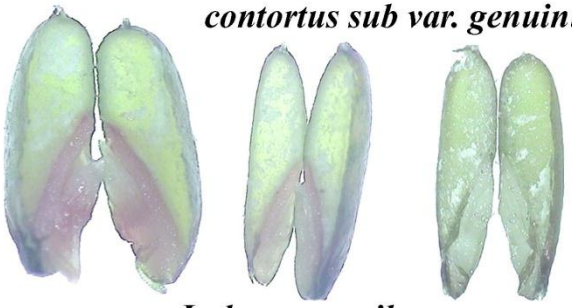
Echinochloa stagnina

Eleusine indica

Heteropogon contortus
var. contortus sub var. typicus

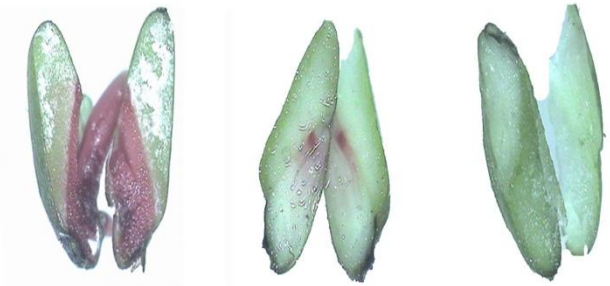
Heteropogon contortus
contortus sub var. genuinus

Ischaemum indicum



Ischaemum pilosum

Plate 5.9 Viability Test

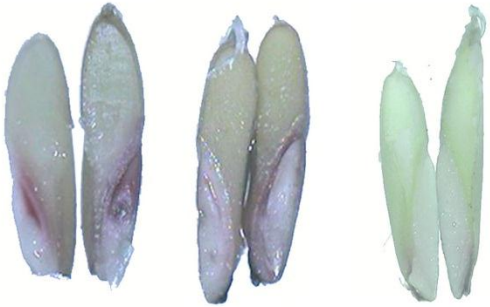


Ischaemum rugosum

Panicum antidotale

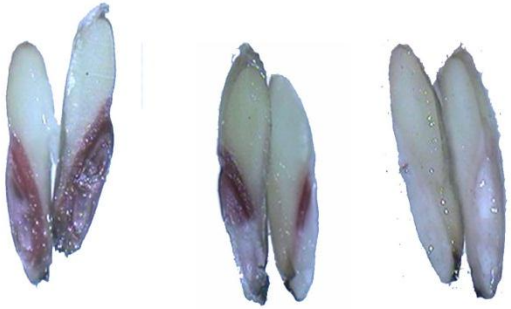
Panicum trypheron

Paspalidium flavidum

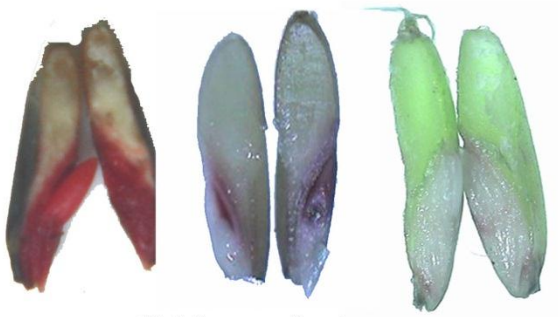


Sehima ischaemoides

Plate 5.10 Viability Test



Sehima nervosum



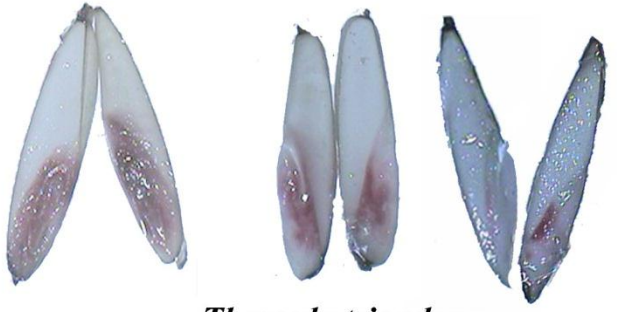
Sehima sulcatum

Setaria tomentosa

Sorghum halepense

Thelepogon elegans

Themeda cymbarica



Themeda triandra

Plate 5.11 Viability Test

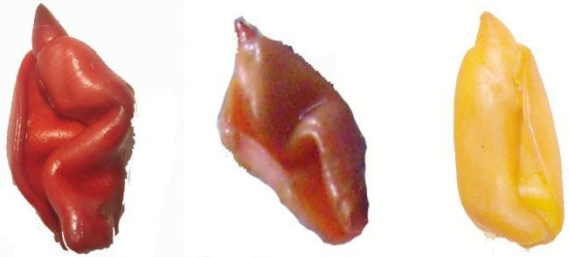
Alysicarpus monilifer

Alysicarpus procumbens

Atylosia scarabaeoides

Cassia absus

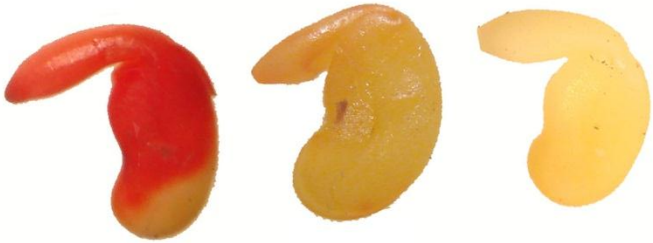
Cassia occidentalis



Cassia tora

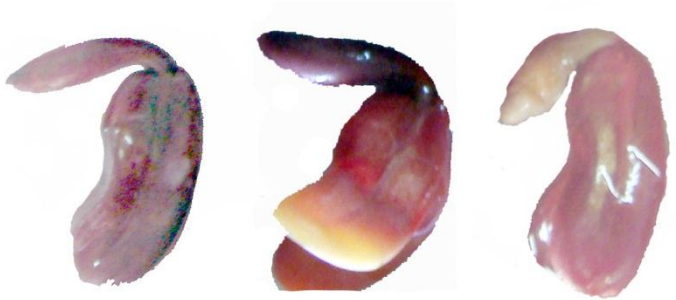
Plate 5.12 Viability Test

Clitoria ternatea



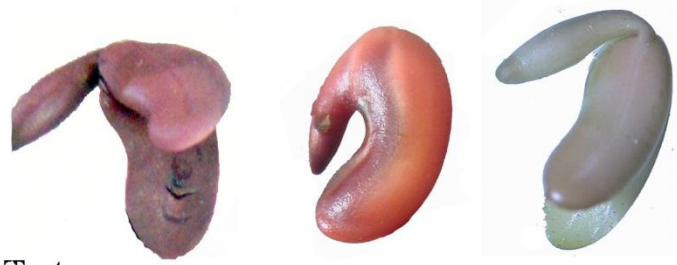
Crotalaria linifolia

Crotalaria notonii



Crotalaria juncea

Crotalaria mysorensis



Crotalaria orixensis

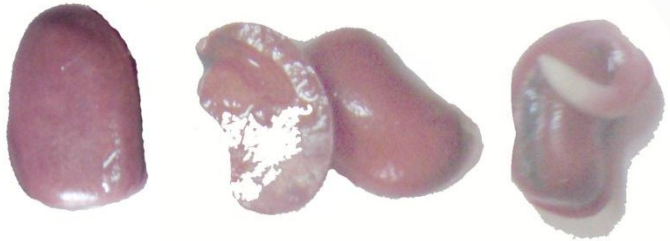
Plate 5.13 Viability Test

Crotalaria retusa

Indigofera cordifolia

Indigofera enneaphylla

Indigofera tinctoria

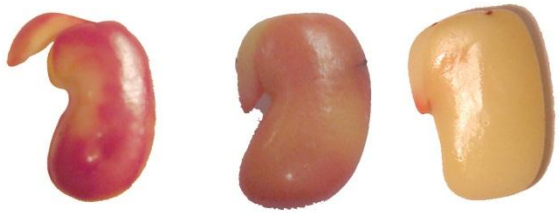


Rhynchosia minima

Sesbania aculeata

Plate 5.14 Viability Test

Sesbania sesban



Tephrosia purpurea

Tephrosia villosa

Plate 5.15 Viability test

Seed viability is affected by many factors like; time, temperature and moisture, nature unexplained, Storage conditions, pollination, pre-treatments given before seed testing, erratic environmental conditions, water availability, immature harvesting etc. Seeds that have high initial viability maintain their quality for longer period than seeds with low viability. This high initial viability factor has been one of the key factors that contribute to a successful supply of high quality seeds year after year. Since most grass seeds are being tested for germination or TZ right after harvest, it is easy to know what seed lot has high viability (good candidate for storing) and which have low viability (higher risk in storage).

Seed viability can be defined as "the capacity of a seed to germinate under favorable conditions in the absence of dormancy" (Copeland and McDonald, 1985). And the difference between viability and germination tests represents the percentage of dormant seeds. In present study we used the TTZ method to check the viability % of stored forage seeds. We also tried to correlate the viability % on the basis of color intensity developed by TTZ solution. The correlation between viability and germination shown by the selected grass species is graphically represented in the Fig. 5.9 and the images of the seeds (TTZ tested) with all three categories (intensity of color developed through TTZ staining) are represented in **Plate 5.6 to 5.15**.

The results show (Fig. 5.9) that among all categories, species of 'Continuous uniform germination' and 'Low germination in early stages and higher germination rate at later stages' showed positive correlation. *Cassia absus* and *Crotalaria filipes* from the 1st category and *Atylosia scarabaeoides, Cassia occidentalis, Cassia tora, Clitoria ternatea, Crotalaria spectabilis, Crotalaria leptostachya, Indigofera linifolia, Indigofera tinctoria* and *Tephrosia villosa* from the 3rd category showed positive correlation. Other than this *Indigofera glandulosa* from the 5th category i.e. 'Very less germination which hardly

reached up to 20-30%' also showed the positive correlation. The germination rates of mentioned species are linearly dependent on viability that the seed have. They showed less influence of the relative fluctuating environmental factors. Thus, such species can be stored for longer period and frequently can be used for community regeneration in pasture development. In contrast to that other species showed positive correlation but the degree of correlation was very weak. Some kind of environmental factors put influence on their germinability that they couldn't show dependable germinability.

The experimental conditions used throughout this study were extremely simple and are comparable to the natural conditions. Thus the results obtained can be applied to the natural regeneration for same seed population. The findings of the present study suggest that most of the seed lots show considerable viability up to 18 months of storage. These seeds remained dormant for initial storage of 2-4 months then after they show considerable germination.

By comparing the germination curves for IT and DT regimes and also by examining the effects of other eco-physiological parameter (viability) on the germination patterns in the test system, we could extract information on the germination characteristics of individual seed populations: the presence or absence of induction or breakage of dormancy by certain thermal (temperature) regimes, the permissible or optimal temperature range for the germination of non-dormant seeds and, the range of thermal time required for germination in different storage durations. As suggested by Geneve (2005), the secondary dormancy could be induced due to an unfavorable environmental condition which was exhibited in results as sudden fall in the germination curve. This secondary dormancy might be induced due to effect of dry storage also which has ability to alter the pattern of dormancy and germination (Dasti et al., 2001). Similar

kinds of results have been reported by Bennington et al., (1991) by using the seeds of *Luzula parviflora*.

The results of the present study show numerous instances where the different species showing similar kind of seed characteristics reoccurs in association with species of same ecology. And such information can be used through the extrapolation in regeneration activities. It also can be a satisfactory analysis in which laboratory results are complemented by studies of production and chance of seeds under natural conditions. Such results are shown by Tobe et al., (2005) in which they studied the dependence of seed germinability on the temperature, dormancy along with seed viability and precipitation available in the field condition.

Seed dormancy is an adaptation that prevents the germination of newly dispersed seed and, based on the length and type of dormancy, may help to preserve a supply of seed in the soil seed-bank. Seed growers need to understand the potential for seed-bank persistence due to seed dormancy attributes which control the timing of germination to maximize the probability of seedling survival (Tarasoff et al., 2007).

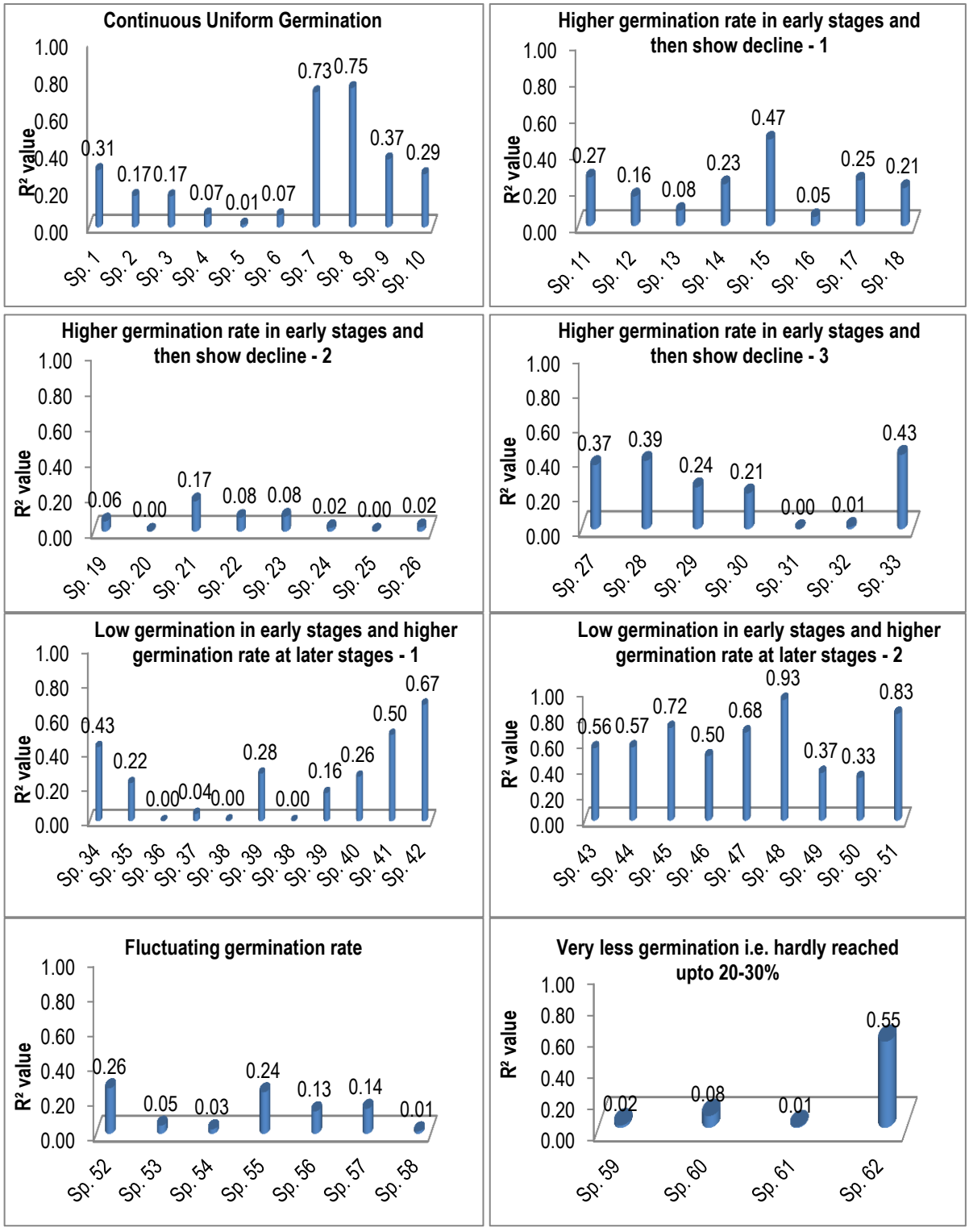


Fig. 5.9 Comparison of correlation Co-efficient

The time-course of the germination response to temperature in each species has been presented in the Table 5.4. All species shows almost bell shaped curve for germination. At this type of curve, the upper limit of temperature range became constant after the initiation of germination and also shows progressive extension with time of the lower limit. The establishment of a contact between seed and substrate is a critical factor determining imbibition and germination possibility of the seed. However, during higher temperatures rapid evaporation of moisture from the substrate or the soil surface becomes a severe hazard to the seedlings which are established in response to temporary available water/moisture to the substrate (Panchal et al. 2011). A selective advantage can be gained from the mechanism which restricts germination until the constant desirable source of moisture or low evaporating losses, or both show potential for the seedling survival. Despite of waiting for such favorable sources of environmental factors for germination, human induced supply of moisture, could also help in gaining maximum output in the field conditions.

The present study indicated that, along with temperature and dormancy, seed longevity i.e. seed viability play a crucial role in determining germinability due to which seedling establishment can be regulated in the field condition. Thus, the seed distribution of seeds in field condition is expected to determine the proportion of germination and also act to maintain seed banks over a time. Germination preference varied between genus, species even taxa. This might be resulting from differing optimum temperature required by different seed populations, intervals for germination, and dormancy strength which is also taxon specific and highly variable. These dormancy dependent germination preferences, basically explained how the taxa can perform in colder or warmer climates. In the field, an entire seed cohort will not germinate during a single season.

From the eco-physiological studies of seed germination we can suggest that temperature, its changes, and its fluctuation can be the most reliable environmental signals to indicate the appropriate timing for germination. Thus, for the successful community development, dry storage and timely utilization of the stored seeds, can help in community restoration mainly when there is a continual dwindling in the palatable forage resource.

Seed vigor

Seed vigor refers to the ability of seed to germinate, emerge, and produce a good crop under a wide range of environmental conditions. In 1876, Fredrich Nobbe first distinguished the concept of seed vigor from that of germination. He introduced the term 'triebkraft' which means driving force or shooting strength to convey the idea that, in addition to germinate, speed and uniformity of emergence was important parameters of seed quality. In 1957, Isely defined seed vigor as "the sum total of all seed attributes which favor stand establishment under favorable conditions." Deloache and Caldwell (1960) stated that, "seed vigor is the sum of all seed attributes which favor rapid and uniform stand establishment." It comprises of those properties, which determines the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions. It is rather a loose term used to describe observable germination differences in seed lots of similar or different genetic makeup.

The point at which the seed achieves its maximum dry weight is called physiological maturity. At this point, it has its greatest potential for maximum germination and vigor. However, since seeds generally achieve physiological maturity at high moisture levels unsafe for storage, seed is typically not harvested until it attains harvest maturity, which is low enough for storage, but high enough to minimize mechanical injury.

Between physiological maturity and harvest maturity, the seed is essentially stored on the plant where it may be exposed to severe environmental conditions that adversely affect seed quality. The factors influencing the seed vigor are genetic constituent, environment during seed development and seed storage environment.

In a germination test, some seedlings may sprout and appear to germinate, but they may be missing critical parts, have serious lesions or such arrested development they are not classified as normal seedlings based on the AOSA Rules for Testing Seeds. On this basis we classified the seedlings and calculate the vigor %. The obtained results are given in the table 5.2.

Normally the abnormal seedlings show features like twisted coleoptile, split and twisted coleoptile, lacking of primary root growth, absence of shoot development, etc. In our study we did not get high ratio of such abnormal seedlings, the reason for this was that we used cleaned and good quality seeds. The abnormal seedlings were rarely recorded, and if recorded the percent of the same was very low; hardly went up to 20 % and the vigor % of the seeds ranged between 70-100%.

Growth tests are based on the principle that vigorous seeds grow at a faster rate than poor vigor seeds even under favorable environments. Vigorous seeds rapidly germinate, metabolize and establish in the field. Therefore, any method used to determine the rapidity of growth of the seedling will give an indication of seed vigor level.

Seed vigor is an important component of seed quality and satisfactory levels are necessary in addition to traditional quality criteria of moisture, purity, germination and seed health to obtain optimum plant stand and high production of crops. As

agricultural and horticultural techniques become progressively more sophisticated, the need for high vigor seeds will increase and testing standards, similar to those recognized for germination will be required. The technology of seed vigor testing has not been perfected so far, so much so that there is not a single universally accepted seed vigor test method. Research is needed to further refine the current seed vigor test methods and to develop new methods which are more related to field/storage conditions.

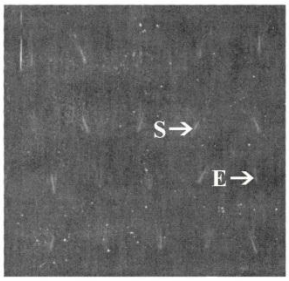
When we compared the purity % with the vigor %, we are able to know that as the purity % are high; the vigor % is also high. Thus to gain maximum vigor, the purity analysis should be the 1st step of seed quality testing, and the seed lot which we are using should be 100% pure.

Seed lot screening through x-ray radiography

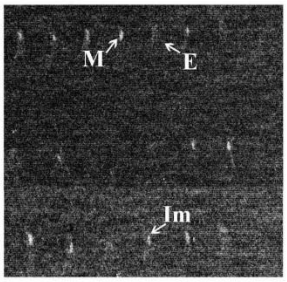
The use of x-ray radiography has been widely applied in seed testing of forest trees as well as agricultural species (Bino et al., 1993; Tigabu, 2003; Skrzyszewska and Chłanda, 2009) In many species it implies a rapid and a definite method of determining seed development and potential rate of germination. The observations of radiographic films show full or empty seeds, good or bad seed morphology, damaged or destroyed seeds etc. Many applications can be developed in the future and several are already operational for: controlling the quality of seed, a sample or a seed lot; guiding the processing or storage condition of a lot immediately after harvesting; homogenizing a work lot; controlling a pelleted seeds; observing the seed evolution during storage or germination. The x-ray technique cannot solve all the seed problems, but, it still gives some new, useful or essential information for scientific work and the quality control of the seed lot in the horticulture (Chavagnat, 1987).

In the present study, non-destructive imaging of the seeds was demonstrated using the synchrotron-based x-ray imaging technique. The seed images obtained had good contrast and definition, and physiological events were also observed. X-rays have found extensive use in the forage seed quality assessment. The observations of radiographic films show full or empty seeds, good or bad seed morphology, damaged or destroyed seeds. The ratio or percentage of different classes along with the possible germination percent is given in the table 4.5 and 4.6. While physiological features like filled and empty seeds, insect damaged seeds, healthy and unhealthy seeds (on the basis of seed size), etc. are evaluated with the x-ray films (**Plates 5.16 to 5.22**) and graphical representation is given **in** Fig. 5.10 and 5.11**.**

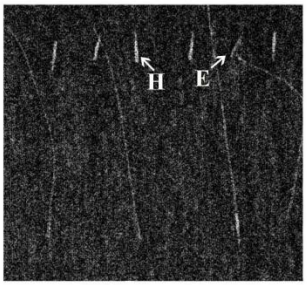
The results of the present study show how useful x-rays can be as a seed quality test even for small sized seeds. Another potential use of x-rays and the objective of this study was to classify seeds according to the size of embryo in the embryo cavity. In the images we can easily distinguish these classes. Here, the results obtained from the germination test correspond to the expected germination based on the classes obtained with the radiography of the seeds (Table 5.5 and



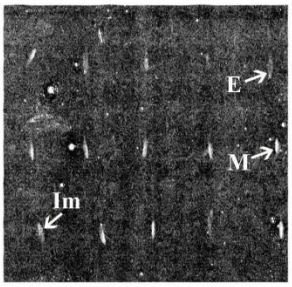
Andropogon pumilus



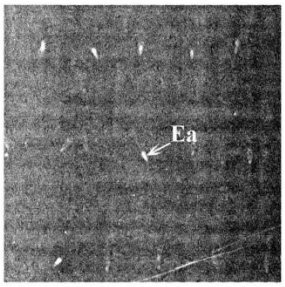
Apluda mutica



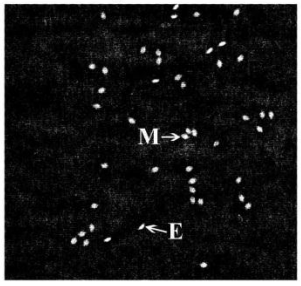
Aristida funiculata



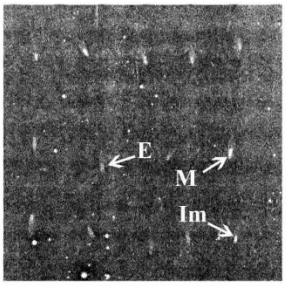
Arthraxon lanceolatus



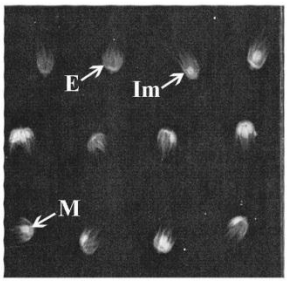
Bothriochloa pertusa



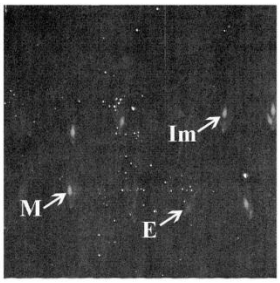
Brachiaria reptans



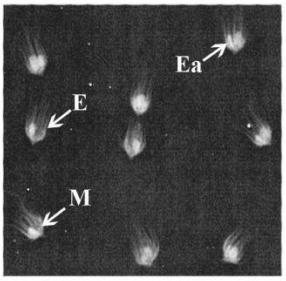
Capillipedium huegelii



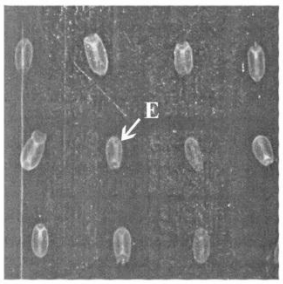
Cenchrus biflorus



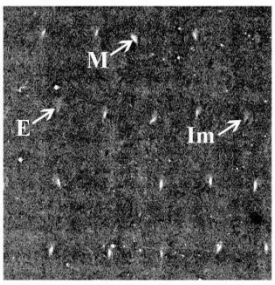
Cenchrus ciliaris



Cenchrus setigerus

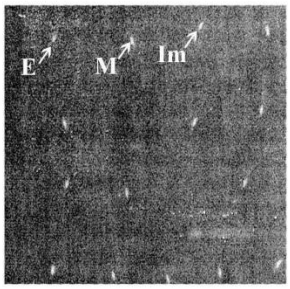


Chionachne koenigii

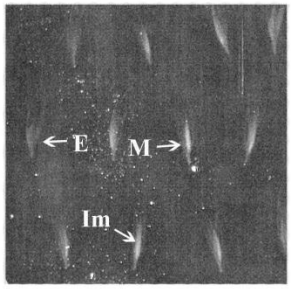


Chloris barbata

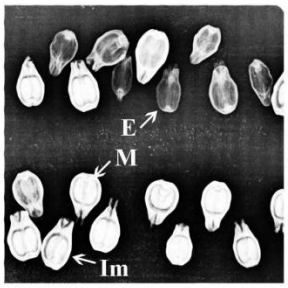
Plate 5.16 X-Ray analysis
(E-empty, M-mature, Im-immature)



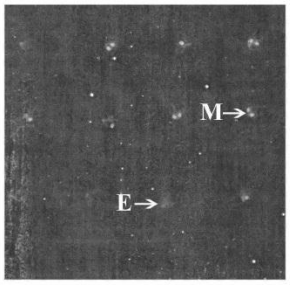
Chloris virgata



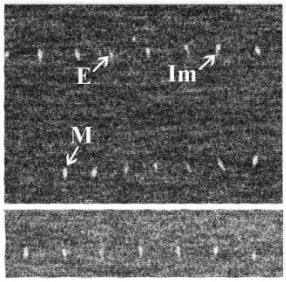
Chrysopogon fulvus



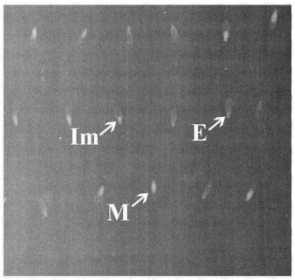
Coix lachryma-jobi



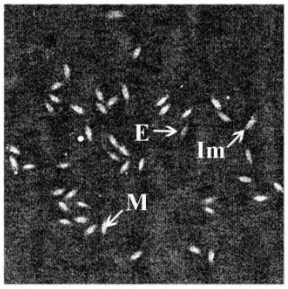
Dactyaloctanium aegyptium



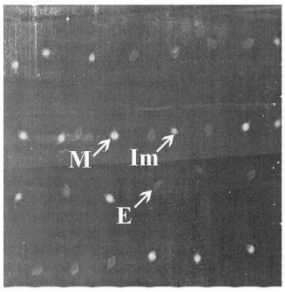
Dichanthium annulatum



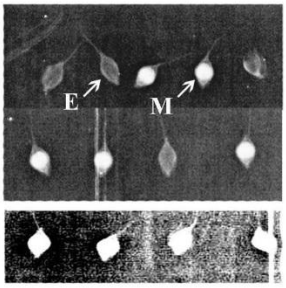
Dichanthium caricosum



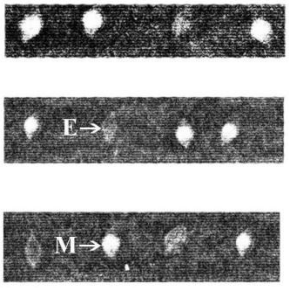
Digitaria adscendens



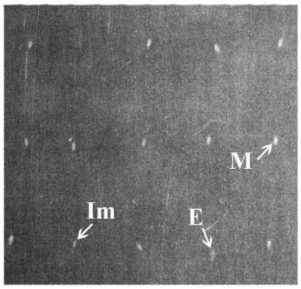
Echinochloa colonum



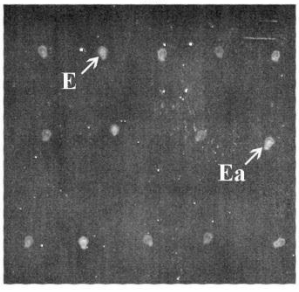
Echinochloa crus-galli



Echinochloa stagnina

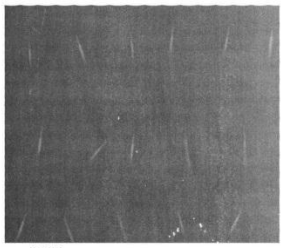


Eleusine indica

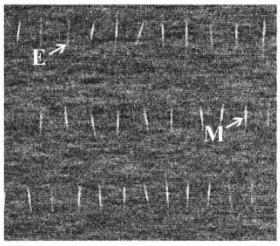


Hackelochloa granularis

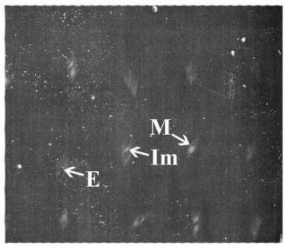
Plate 5.17 X-Ray analysis
(E-empty, M-mature, Im-immature)



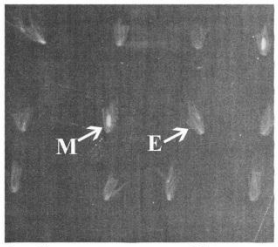
Heteropogon contortus var. contortus sub var. typicus



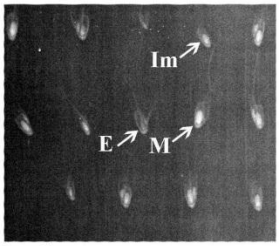
Heteropogon contortus var. contortus sub var. genuinus



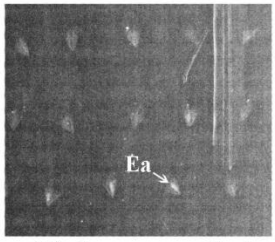
Ischaemum indicum



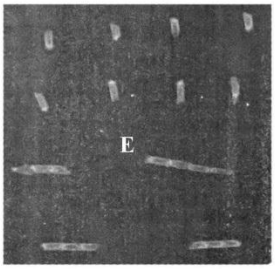
Ischaemum pilosum



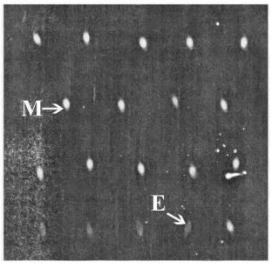
Ischaemum rugosum



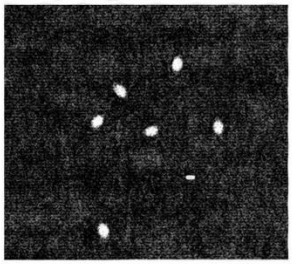
Melanocenchris jacquemontii



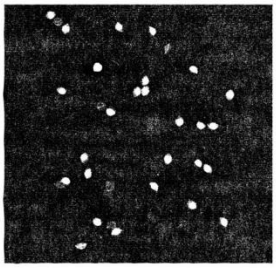
Ophiorus exaltatus



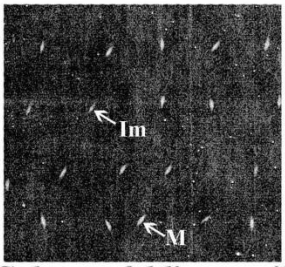
Panicum antidotale



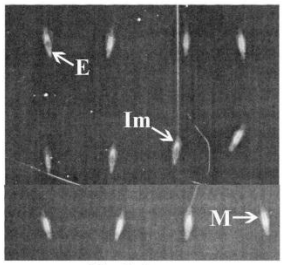
Panicum trypheron



Paspalidium flavidum

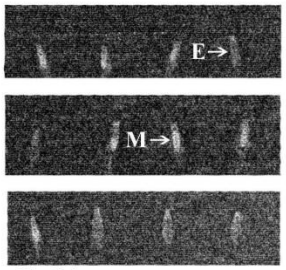


Schoenefeldia gracilis

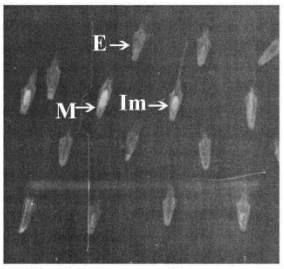


Sehima ischaemoides

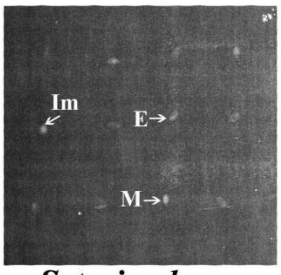
Plate 5.18 X-Ray analysis
(E-empty, M-mature, Im-immature)



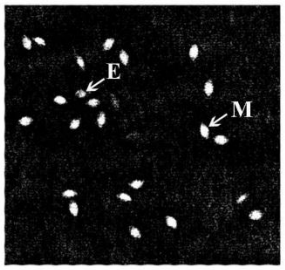
Sehima nervosum



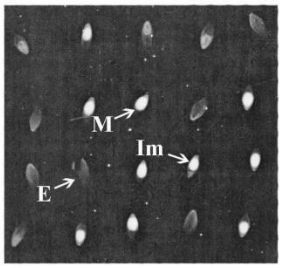
Sehima sulcatum



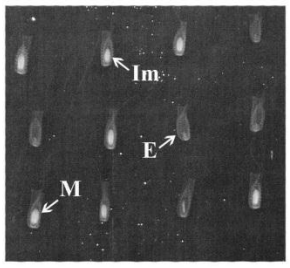
Setaria glauca



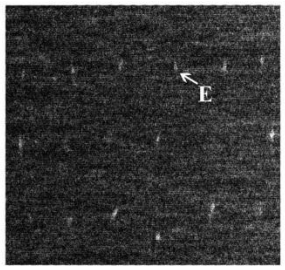
Setaria tomentosa



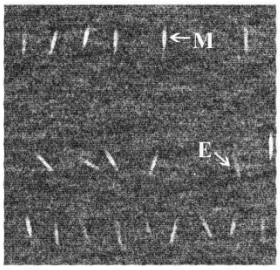
Sorghum halepense



Thelepogon elegans

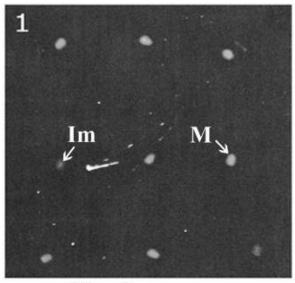


Themeda cymbarica

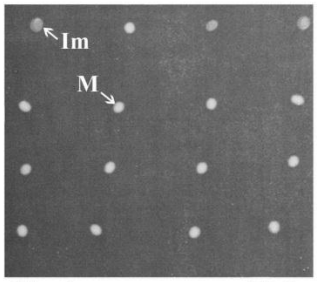


Themeda triandra

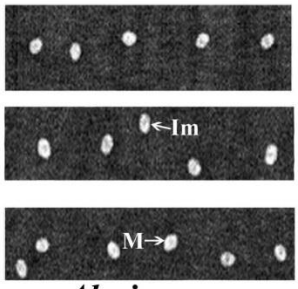
Plate 5.19 X-Ray analysis
(E-empty, M-mature, Im-immature)



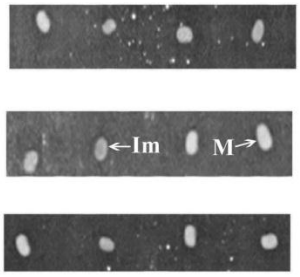
Alysicarpus bulgaumensis



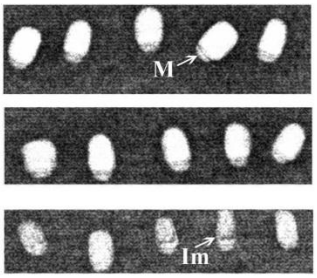
Alysicarpus monilifer



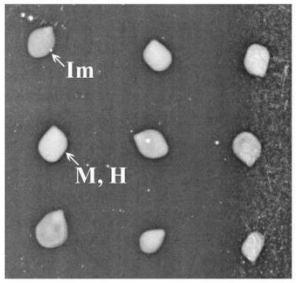
Alysicarpus procumbens



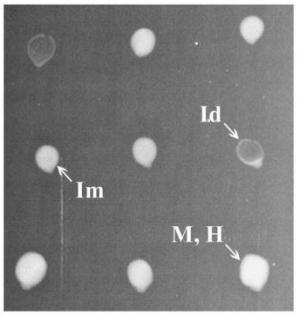
Alysicarpus vaginalis



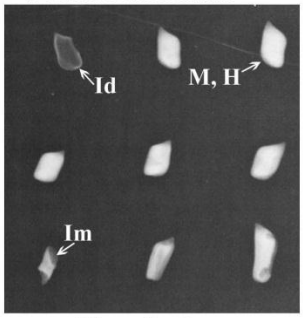
Atylosia scarabaeoides



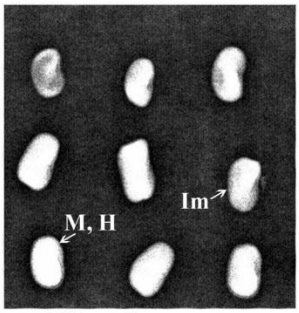
Cassia absus



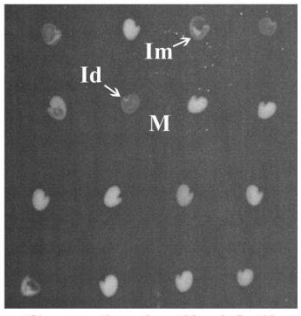
Cassia occidentalis



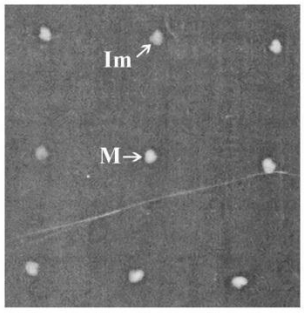
Cassia tora



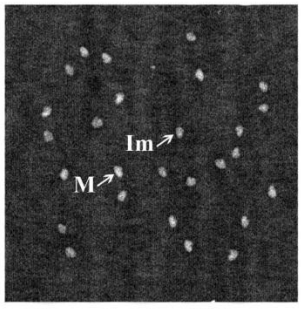
Clitoria ternatea



Crotalaria linifolia



Crotolaria calycina



Crotolaria filipes var. trichophora

Plate 5.20 X-Ray analysis

(E-empty, M-mature, Im-immature, M,H-mature and healthy, Id-insect damaged)

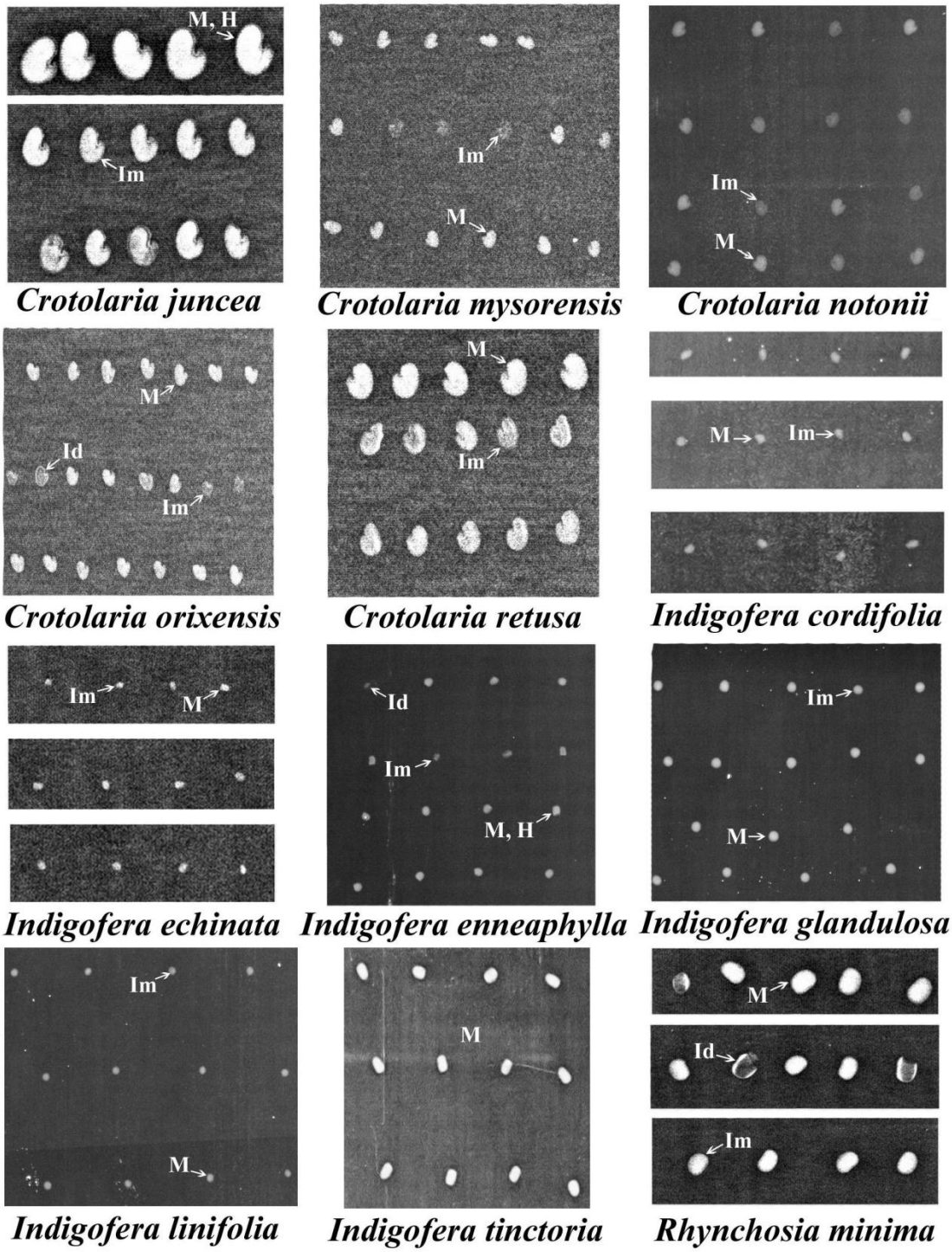
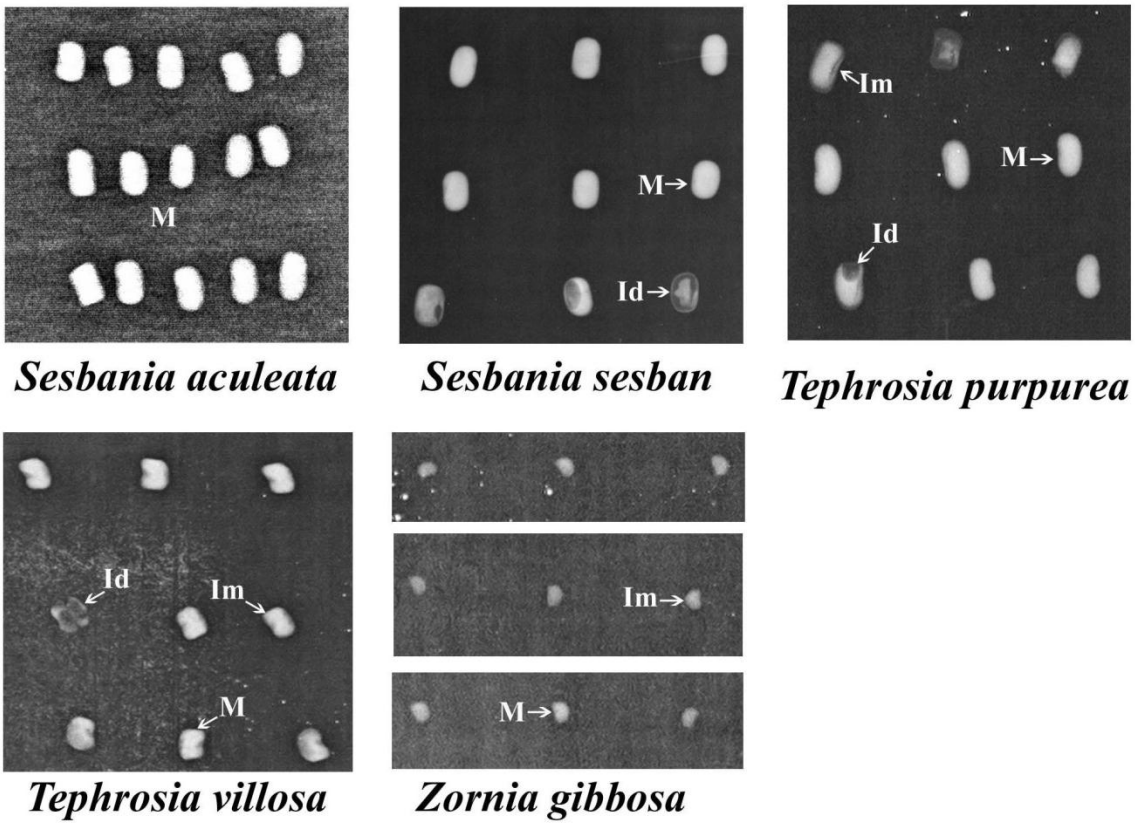


Plate 5.21 X-Ray analysis

(E-empty, M-mature, Im-immature, M,H-mature and healthy, Id-insect damaged)



Sesbania aculeata *Sesbania sesban* *Tephrosia purpurea*

Tephrosia villosa *Zornia gibbosa*

Plate 5.22 X-Ray analysis

(E-empty, M-mature, Im-immature, Id-insect damaged)

5.6). The germination test revealed that seeds which are healthy enough and are exposed to the minimum dosage of soft x-rays are able to germinate and their germinability correlate with that of untreated seeds of the same species and same seed lot. The physiological features like filled and empty seeds, insect damaged seeds, healthy and unhealthy seeds (on the basis of seed size), etc. were also evaluated with the x-ray films and were denoted as E = Empty, M = Mature, Im = Immature, Id = Insect damaged, M and H = Mature and healthy seeds. (**Plates 5.16 to 5.22**).

Table 5.5 X-ray analysis and germination performance of selected forage grasses.

Sr. No.	Botanical name	Applied mAs	Seed Size (mm) (LxTxW)	Mature Caryopses %	Immature Caryopses %	Injured/ damaged seeds	Germination % Treated seeds	Germination % Untreated Seeds
Sp. 1	*Andropogon pumilus*	10	2.7x0.4x0.2	34	44	0	38	40
Sp. 2	*Apluda mutica*	2	2.1x0.6x0.9	36	34	0	85	90
Sp. 3	*Aristida funiculata*	2	1.8x0.8x0.5	36	50	0	35	35
Sp. 4	*Arthraxon lanceolatus*	2.2	1.8x0.3x0.3	32	48	0	75	90
Sp. 5	*Bothriochloa pertusa*	2.2	1.8x0.7x0.4	0	1	0	0	0
Sp. 6	*Brachiaria reptans*	5	1.9x1.4x0.2	28	56	0	38	40
Sp. 7	*Capillipedium huegellii*	2.2	1.4x0.6x0.3	24	49	0	30	40
Sp. 8	*Cenchrus biflorus*	36	1.5x1.0x0.7	11	31	0	0	20
Sp. 9	*Cenchrus ciliaris*	22	1.9x0.8x0.5	41	5	0	75	95
Sp. 10	*Cenchrus setigerus*	36	1.8x1.0x0.7	79	9	0	0	70
Sp. 11	*Chionachne koenigii*	45	3.4x2.1x1.3	0	2	0	0	0
Sp. 12	*Chloris barbata*	2.8	1.4x0.5x0.4	57	25	0	75	75
Sp. 13	*Chloris virgata*	2.8	1.5x0.4x0.5	41	36	0	24	30
Sp. 14	*Chrysopogon fulvus*	22	4.6x0.5x0.9	9	33	0	42	50
Sp. 15	*Coix lachryma-jobi*	2	4.4x3.6x2.6	41	24	0	55	60
Sp. 16	*Dactyaloctenium aegyptium*	36	0.9x0.9x0.5	35	19	0	8	10
Sp. 17	*Dichanthium annulatum*	2.2	2.5x0.8x0.5	47	12	0	88	90

Sr. No.	Botanical name	Applied mAs	Seed Size (mm) (LxTxW)	Mature Caryopses %	Immature Caryopses %	Injured/ damaged seeds	Germination % Treated seeds	Germination % Untreated Seeds
Sp. 18	*Dichanthium caricosum*	36	2.3x0.9x0.4	27	35	0	0	45
Sp. 19	*Digitaria adscendens*	5	2.2x0.8x0.4	46	27	0	78	85
Sp. 20	*Echinochloa colonum*	36	1.6x1.2x0.7	18	20	0	0	75
Sp. 21	*Echinochloa crus-galli*	36	1.8x1.7x0.9	52	9	0	0	65
Sp. 22	*Echinochloa stagnina*	2.8	2.7x2.3x1.7	95	5	0	100	100
Sp. 23	*Eleusine indica*	11	1.2x0.6x0.5	57	28	0	4	5
Sp. 24	*Hackelochloa granularis*	11	-	0	0	0	0	0
Sp. 25	*Heteropogon contortus*	2.2	3.9x0.4x0.3	46	15	0	80	80
Sp. 26	*Imperata cylindrica*	22	-	0	0	0	0	0
Sp. 27	*Ischaemum indicum*	36	1.5x0.7x0.5	17	30	0	0	40
Sp. 28	*Ischaemum pilosum*	36	2.4x0.8x0.6	5	2	0	0	50
Sp. 29	*Ischaemum rugosum*	22	2.2x0.9x0.8	39	24	0	75	75
Sp. 30	*Melanocenchris jacquemontii*	16	-	0	0	0	0	0
Sp. 31	*Ophiorus exaltatus*	36	1.4x0.7x0.4	0	0	0	0	0
Sp. 32	*Panicum antidotale*	36	1.6x0.9x0.7	42	27	0	12	15
Sp. 33	*Panicum trypheron*	5	1.6x1.2x0.4	23	35	0	20	20
Sp. 34	*Paspalidium flavidum*	5	1.2x1.2x0.4	61	32	0	24	25
Sp. 35	*Pennisetum setosum*	22	-	0	0	0	0	0
Sp. 36	*Schoenefeldia gracilis*	2.8	2.0x0.5x0.4	59	33	0	94	95
Sp. 37	*Sehima ischaemoides*	15	1.8x0.4x0.5	35	44	0	45	45
Sp. 38	*Sehima nervosum*	2	4.3x1.0x0.3	55	20	0	8	10
Sp. 39	*Sehima sulcatum*	36	2.6x0.9x0.6	11	8	0	0	5
Sp. 40	*Setaria glauca*	11	1.5x0.9x0.5	23	31	0	15	25
Sp. 41	*Setaria tomentosa*	5	1.6x1.2x0.3	35	39	0	10	45
Sp. 42	*Sorghum halepense*	36	2.8x1.7x1.1	47	16	0	0	60
Sp. 43	*Thelepogon elegans*	36	3.6x1.4x0.5	28	28	0	0	85
Sp. 44	*Themeda cymbaria*	10	2.3x0.9x0.3	89	7	0	55	60

Sr. No.	Botanical name	Applied mAs	Seed Size (mm) (LxTxW)	Mature Caryopses %	Immature Caryopses %	Injured/ damaged seeds	Germination % Treated seeds	Germination % Untreated Seeds
Sp. 45	*Themeda triandra*	2.2	3.2x0.9x0.3	60	21	0	87	95

Table 5.6 X-ray analysis and germination performance of few wild legumes.

Sr. No.	Botanical name	Applied mAs	Seed Size (mm) (LxTxW)	Mature seeds	Immature seeds	Injured/ damaged seeds	Germination % Treated seeds	Germination % Untreated seeds
Sp. 1	*Alysicarpus bulgaumensis*	11	1.3x1.0x0.7	0	100	0	0	0
Sp. 2	*Alysicarpus monilifer*	40	2.0x1.0x0.7	83	16	1	0	75
Sp. 3	*Alysicarpus procumbens*	5	1.9x1.5x0.3	61	39	0	57	58
Sp. 4	*Alysicarpus vaginalis*	36	1.2x0.8x0.6	0	100	0	0	0
Sp. 5	*Atylosia scarabaeoides*	5	4.6x2.9x1.1	72	28	0	55	80
Sp. 6	*Cassia absus*	45	4.1x3.6x0.7	13	87	0	0	90
Sp. 7	*Cassia occidentalis*	63	4.6x3.8x0.5	66	11	23	0	90
Sp. 8	*Cassia tora*	63	6.0x2.0x2.0	61	33	6	0	98
Sp. 9	*Clitoria ternatea*	5	5.5x4.0x2.6	78	22	0	71	90
Sp. 10	*Crotalaria spectabilis*	2.2	2.9x2.3x0.3	64	36	0	59	80
Sp. 11	*Crotalaria calycina*	25	1.6x1.4x0.4	75	24	1	74	75
Sp. 12	*Crotalaria filipes*	5	1.8x1.3x0.4	51	49	0	51	50
Sp. 13	*Crotalaria juncea*	5	6.8x5.0x1.2	88	12	0	88	98
Sp. 14	*Crotalaria mysorensis*	2.2	2.8x1.7x1.1	71	27	2	65	92
Sp. 15	*Crotalaria medicaginea*	45	2.3x2.0x0.3	55	45	0	0	90
Sp. 16	*Crotalaria orixensis*	2.2	3.0x2.5x0.8	62	38	0	57	85
Sp. 17	*Crotalaria leptostachya*	5	1.5x3.0x0.9	48	52	0	46	90
Sp. 18	*Indigofera cordifolia*	36	1.1x1.0x0.3	72	28	0	0	25
Sp. 19	*Indigofera echinata*	5	1.3x1.0x0.4	60	40	0	30	25

Sr. No.	Botanical name	Seed Applied mAs	Size (mm) (LxTxW)	Mature seeds	Immature seeds	Injured/ damaged seeds	Germination % Treated seeds	Untreated seeds
Sp. 20	*Indigofera enneaphylla*	40	1.4x1.0x0.9	70	30	0	0	40
Sp. 21	*Indigofera glandulosa*	40	1.7x1.7x1.7	92	8	0	0	50
Sp. 22	*Indigofera linifolia*	45	1.2x1.2x1.2	73	7	0	0	40
Sp. 23	*Indigofera tinctoria*	32	1.6x1.3x0.9	93	7	0	0	90
Sp. 24	*Rhynchosia minima*	5	3.6x2.8x1.9	43	36	21	40	98
Sp. 25	*Sesbania aculeata*	5	5.7x3.3x2.0	100	0	0	98	95
Sp. 26	*Sesbania sesban*	63	3.8x2.2x1.8	50	41	9	0	90
Sp. 27	*Tephrosia purpurea*	40	4.0x2.5x1.5	44	52	14	0	90
Sp. 28	*Tephrosia villosa*	45	3.6x2.2x0.7	57	37	6	0	95
Sp. 29	*Zornia gibbosa*	32	1.8x1.3x0.4	67	33	0	0	35

The higher energy of 100 kV as well as high exposure time has been found to be unsuitable for radiography of food products. Mammography is a specific type of imaging that uses a low-energy (usually around 30 kV) x-ray system to examine biological material. It is a specific type of imaging that uses a low dose x-ray system and high contrast, high-resolution film for examination of biological material.

Along with kV and mAs, application of specific contrast chemical i.e. $BaCl_2$ to the seed before x-ray enhances the possibility of evaluating viability of tissue, because this chemical stains, live and dead tissue differently. The x-ray contrast (XC) method gives a different image of live and dead tissue of seed similar to the TTZ test (Saelim et al., 1996). However as x-ray radiographs are black and white, interpretation of the results requires even more experience than TTZ test.

The seed containing fully developed embryos or 100 % filled with storage food material germinated to a large extent (table 5.6 and 5.7). The empty or fungus attacked seeds did

not germinate at all. Nevertheless the germination rate of the best material amounted to around 90 % which is quite significant.

Laedem et al., (1995) found good correlation between x-ray radiography and germination test in *Dalbergia cochinchinensis* and *Pinus kesiya,* both of which had a high seed quality, while there was low correlation for *Pinus merkusii* in which the germination percentage was low. From these observations, it was generalized that the method is less applicable to seed lots for low physiological quality. X-ray with energy ranging from 15 to 80 kV at various current levels has been reportedly used. The X-ray exposure time as high as 90 seconds has been reported. Thus, the optional technique is using.

The present study focused on using mammography technique to observe internal seed characteristics and its relativity with the germination. Features that were observed using this technique included the mature, immature, healthy seeds; filled and empty seeds, cavities due to insect infestation in the seeds, etc. Although this study has focused on seeds, the images presented here and the demonstrations revealed that how this technique can be used in pasture development, revegetation programs, etc. as a base tool for seed quality assessment.

The range of light and dark shades observed in radiographic images of seeds (**Plate 5.16 to 5.22**) is defined as a function of the level of absorption of x-rays in distinct regions of the seed, which is determined by the thickness, density and composition of the tissues. Seed radiography can be of help to evaluate seed viability also. Therefore, it is necessary to establish a relationship between the internal structures of the seeds and the corresponding seedlings that are produced, which was done by the comparison of germinating both the x-ray treated and non-treated seeds (Table 5.5 and 5.6).

Conventional x-ray imaging has been used to reveal insect infestation (Schatzki and Fine, 1988; Karunakaran et al., 2003; Haff and Slaughter, 2004) and seed damage (Milner et al., 1952, de Carvalho et al., 1999; Letang et al., 2002), and to indicate seed quality (Simak and Sahlen, 1981; Fouct et al., 1993; Downie et al., 1999); the mammography technique is similar to that but one step advanced in detecting such minute seeds along with more clearer images. The x-ray image analysis technique is a precise method which enables examination of regions that are damaged and their location. It is a non-destructive method allowing the x-ray treated seed to be submitted to quality physiological tests.

From the available literature survey, we tried to x-raying with same kV and different mAs and the results showed that the seeds x-rayed with lowest mAs were not affected with the radiations and shows normal germination when compared to the untreated seeds. For few species having very small size of seeds, it was not possible to take radiographs, as these seeds require still lower kV and mAs as well.

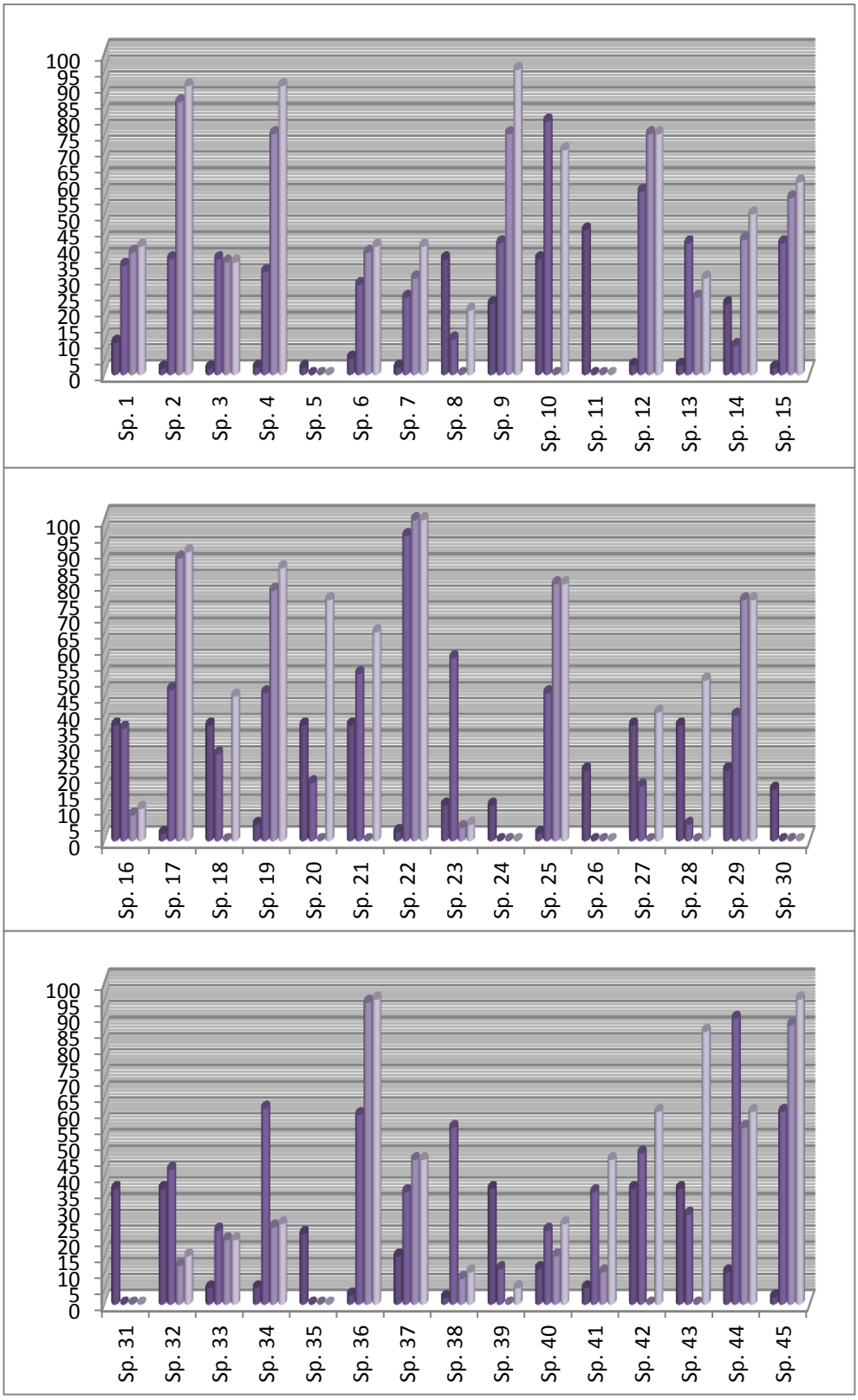


Fig. 5.9 graphical presentation for details obtained through Grass X-Rays.

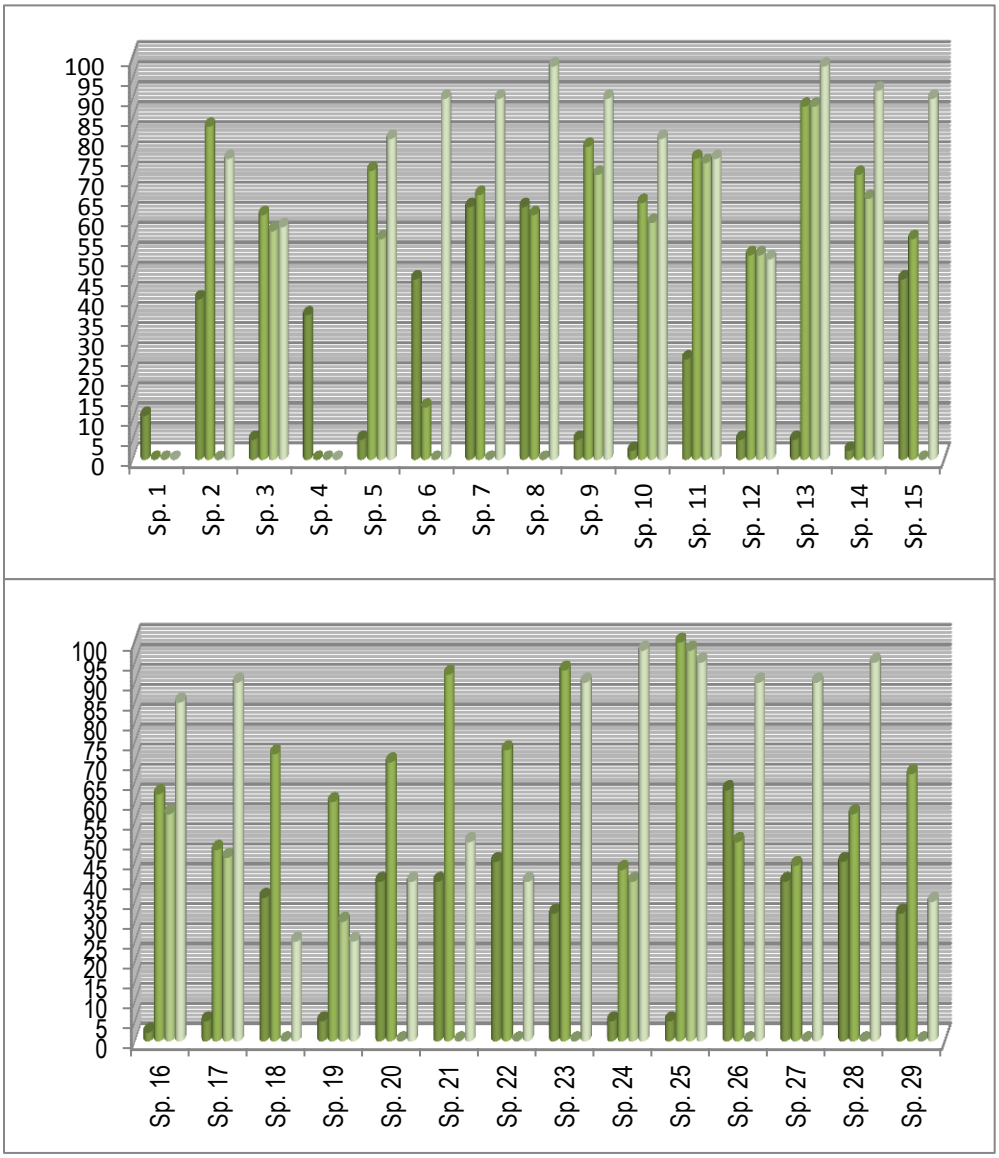


Fig. 5.10 graphical presentation for details obtained through Legume X-Rays.

Correlation of x-ray imaging and germination:

Since x-rays are non-destructive method, seeds examined by the low energy x-rays may also be used in direct germination tests. Chaichanasuwat et al., (1990) found good conformity between x-ray radiography and germination tests for *Peltoforum pterocarpum.* In comparative studies of different viability tests, Bhodthipuks et al (1996) found that x-ray radiography overestimated viability, as compared to the germination tests. In present study, the seed containing fully developed embryos or 100 % filled with storage food material germinated to a large extent (table 5.5 and 5.6). The empty or fungus attacked seeds did not germinate at all. Nevertheless the germination rate of the best material amounted to around 80-90 %. Part of this, inferior germination may be due to embryo damage caused during seed processing. Other reasons for inferior germination even though lower mAs were applied may be: 1) Insect infested seeds where no entry hole is viable, e.g. legume seeds infested by bruchids. 2) Seeds with shrunken or underdeveloped embryos, e.g. immature seeds.

In the present study, x-ray imaging of such smaller sized grass caryopses was done for seed lot screening and for such a small sized seeds study with normal characterization was not possible. The findings showed that the method is reliable and can be used for detecting empty, filled and mechanically damaged seeds or fruits. The applications of x-ray radiography in pasture development for good quality seed collection, seed processing, nursery practice, seed trade and plant quarantines, and for research etc. is now possible.

The main benefit of this study is that, on the basis of % of mature seeds, we can assume that how much seeds may be viable. The ration of the mature seeds can predict the quality of seed lot of the particular species.

In the present study, x-ray imaging of seeds was done. Almost all grass seeds and many of legume seeds studied were very small and study with normal characterization was not possible. For these efforts were done with modifying the specifications, successful results could be obtained and results stands as one of the major achievement and can be successfully used in the field of pasture development.

Poor germination and development of important palatable forage grasses in the study area is the major problem for pasture development. These grasses are very important for the cattle of surrounding areas during summer and scarcity periods. Good seed quality is the indispensible premise to guarantee high performance. Therefore the quality tests carry great importance; nevertheless most of them only offer information on the germination percentage or the degree of purity of the sample. Seed quality is an important factor in stand establishment and varies greatly among seed lots, especially during seed harvesting and processing. The main objective of the present study was to analyze the relationship between x-ray image pattern and germination percentage. X-ray analysis can be used to determine the quality of seeds, showing the cause of bad germination. X-ray images provide information on the internal structure and morphology of seeds, mechanical damage, percentage of empty and filled seeds, micro fractures, possible embryo deformations and insect infestation, and thus, the relationship between internal structures and seed viability. In present study we describe different suitable working conditions for successful x-ray imaging of 45 forage grasses and 32 wild forage legume species. In present study, explanations are also given for the main damages to seeds and their consequences for germination. Our results confirm the seed lot screening of collected raw sample lot of forage grass seeds and demonstrate the potential of x-ray image analysis to provide a rapid and nondestructive means to successful predict it. The possibility of using x-ray radiography technique for assessing

the quality of seeds is very promising; it is a precision method that enables one to examine in detail the damaged or altered region, its location and extent.

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