CHAPTER - VI

STUDY ON SEED GERMINATION BEHAVIOUR AND SEEDLING GROWTH OF TEPHROSIA CANDIDA DC. WITH SPECIAL REFERENCE TO NON-VIABILITY OF SEED DURING STORAGE

INTRODUCTION

The seeds of Tephrosia candida DC. do not germinate even when favourable conditions are prevailed. The seeds are supposed to be in a state of dormancy. Besides this, seeds of this plant were observed to lose their viability during their storage for a certain period of time in laboratory condition. Dormancy of seed is a common phenomenon in various leguminous plants (Mayer & Poljakoff-Mayber, 1978) and various means for overcoming dormancy are well established. It is therefore very essential to study the effect of various treatments and conditions including growth regulators on the germination of seeds from biochemical point of view so that the determination of the optimum conditions to show maximum percentage of germination is possible. Besides it will help in understanding of overcoming of dormancy of seed. This will ultimately be helpful to increase the production of seedling required for the aforestation programme in the region. As the plant yields good amount of naturally occurring insecticide it has the potentiality to be utilized in agroforestry. Thus, the present work deals with the study on the effects of various physical and chemical factors on seed germination behaviour of the plant with special emphasis on its non-viability during storage.

MATERIALS AND METHODS

Materials

All the seeds of T. candida DC. (2n=22) utilized for germination are collected from the experimental field, Centre

for Life Sciences, North Bengal University.

Methods

For studying the rate of germination, seeds were first treated with conc. H_2SO_4 for 25-30 minutes to eliminate seed coat dormancy. After thorough washing with water seeds were placed in petridishes containing cotton pad soaked with different test solutions. These were kept in temperature and light controlled BOD incubator at 30°C in white light supplied by incandescent lamp (200 lx). Each experiment included three replicates, each containing 100 seeds. The imergence of the radical was taken as criterion for seed germination.

Effect of storage on germination and viability of seed

Seeds were stored in glassstoppered bottle. They were taken out every month in a lot and after treatment with H_2SO_4, they were placed for germination at, 30 ± 10°C. This was continued till one year from the month of their collection. To study the loss of total viability, the stored seeds, were examined for their germination in each year upto the duration of total six years.

Measurement of leachates from the seeds

The seeds were soaked in water after H_2SO_4 treatment and the solution was measured in conductivity Meter Bridge after 72 hrs. and expressed in m mhos.

Effect of different quality of light on germination of seed

To study the effect of different quality of light intensity, the Petridishes were kept in different light conditions, viz., direct sunlight, diffused sunlight, red, yellow, green and blue light. The sources of red, yellow, green and blue light were produced by the application of standard cellophane papers (coloured). The data were recorded upto 6th day after showing germination.

Effect of pH on seed germination behaviour

Phosphate, carbonate and acetate buffer solutions of sodium and potassium were prepared following the method described by Plummer (1978). Solutions having different pH have been maintained after the treatment of sodium or potassium hydroxides (1%) with HCl (1%). Sodium and potassium phosphate (NaH_2PO_4, KH_2PO_4) solutions were prepared after dissolving 100 mg of each phosphate in 100 ml distilled water (10^3 ppm). Different concentrations (10^2 to 10^{-3} ppm) of phosphate solutions were then obtained by diluting the stock solution (10^3 ppm).

Effect of growth regulators and growth hormone

A stock solution of 10^3 ppm for each of the chemical was prepared. It was diluted with distilled water to make desired concentrations. The solutions of GA, IAA, ABA, Kn, Rutin, Morin, Quercetin, benzoic, caffeic, ferulic, gallic and cinnamic acids were utilised for the purpose.

Preparation of Rotenone Solution

50 mg of rotenone was taken and dissolved in minimum volume of ethanol. Distilled water was added to make the volume upto 50 ml making the concentration of 10^3 ppm. It was diluted to produce different grades of solution upto 10^{-3} ppm.

Effect of NPK on seed germination behaviour

Seeds were soaked in different combinations of N, P, K solutions and were allowed to germinate. To study the individual and combined effects of nitrate, phosphate and potassium deficiencies on vegetative performance of the species, 4 weeks seedling of T. candida were then transplanted into 10 cm diameter pots containing sand at a rate of 3 seedlings/pot but thinned to 1 plant/pot a week later. These were supplied with experimental solutions at an interval of two days. Growth performance were recorded for a period of 6 months at an interval of 30 days. Biomass of above and underground plant parts was estimated by the method of Vyas et al (1978).

Preparation of N,P,K solution

Different N,P,K solution were prepared following the method described by Pemadasa (1981).

Composition (Major Nutrients) in different culture solutions (gl^{-1}). the concentrations of N, P and K, if added, were 128, 64 and 79 mg^{-1} respectively

	Complete	-N	-P	-K	-NP	-NK	-PK	-NPK
KNO_3	0.20	-	0.20	-	-	-	-	-
$Ca(NO_3)_2$	0.48	-	0.48	0.48	-	-	0.48	-
$NaH_2PO_4,2H_2O$	0.32	0.32	-	0.32	-	0.32	-	-
$MgSO_4,7H_2O$	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
$CaCl_2,6H_2O$	-	0.44	-	-	0.44	0.44	-	0.44
KCl	-	0.15	-	-	0.15	-	-	-
NaCl	-	-	0.12	-	0.12	-	-	0.12
$NaNO_2$	-	-	-	0.17	-	-	0.17	-

Trace elements were supplied in the form and quantities given by Hewitt (1962).

Biochemical Estimations

Quantitative and qualitative estimations at different biochemical contents have been done following the methods described in Chapter IV and V.

Seeds soaked with distilled water were served as control.

Table - 26

Effect of H_2SO_4 (conc.) on the germination behaviour of seeds of T. candida DC.

Condition of seed	24			48			72			
Duration of H_2SO_4 treatment (minutes)	Imbibed to germination	Imbibed	Unimbibed	Imbibed to germination	Imbibed	Unimbibed	Imbibed to germination	Imbibed	Unimbibed	Remarks
5	10	20	70	23	47	30	53	20	27	Cotyledon healthy
10	27	43	30	53	28	19	67	11	22	-do-
15	30	35	35	53	33	14	80	10	10	-do-
20	36	55	9	55	40	5	80	20	-	-do-
25	66	30	4	87	11	2	89	11	-	-do-
30	76	24	-	86	14	-	90	10	-	-do-
35	40	60	-	88	12	-	92	8	-	-do-
40	42	58	-	72	28	-	80	20	-	Scar on 1 cotyledon
50	31	69	-	68	32	-	83	17	-	Scar on 1 cotyledon
60	30	70	-	80	20	-	82	18	-	Scar on 1 cotyledon

Table - 27

Effect of temperature on seed germination of *T. candida* DC.

Temperature (°C) \ Duration of germination (hrs)	24	48	72
0	0	0	0
5	0	5	12
10	1	9	29
15	4	20	48
18	19	50	72
20	18	40	73
22	20	40	73
24	25	45	74
26	40	52	78
28	62	82	90
30	74	86	98
32	76	83	95
35	46	50	77
40	42	57	74
50	40	53	71
60	22	30	35
S.E. (±)	26.93	26.49	28.99
C.D. at 5%	8.22	8.15	8.53

Table - 28

Effect of storage on the viability of seeds (% of germination) in T. candida collected on March 1985.

% of germination / Month	24	48	72	Leachates (m mhos)
March 1985	48	88	92	19
April 1985	92	94	95	21
May 1985	93	97	98	26
June 1985	40	81	81	29
July 1985	54	62	77	33
August 1985	20	50	75	36
September 1985	24	36	70	39
October 1985	22	40	70	43
November 1985	18	40	63	47
December 1985	12	44	58	49
January 1986	19	48	59	52
February 1986	20	46	51	53
March, 1986	16	33	49	56
S.E. (±)	27.84	23.3	16.12	
C.D. at 5%	3.04	2.77	2.31	

Table - 29

Effect of storage on the viability of seed (% germination) in Tephrosia candida DC.

Percentage of germination / Age of the seeds (yrs)	24h	48h	72h	Leachates (m mhos)
Fresh collection	48	88	92	19
1 yr.	16	33	49	56
2 yrs.	30	38	46	78
3 yrs.	24	30	35	89
4 yrs.	20	24	24	135
5 yrs.	12	12	12	182
6 yrs.	0	0	0	321
S.E. (±)	15.08	27.88	30.04	
C.D. at 5%	3.19	4.34	4.50	

Table - 30

Effect of storage and test with Tetrazolium Chloride solution in the seeds of T. candida DC.

	Total no. of seeds	Rating Category				Optical density
		I	II	III	IV	
1986	100	100	0	0	0	0.31
1985	100	67	32	1	0	0.25
1984	100	30	40	25	5	0.19
1983	100	20	10	56	14	0.16
1982	100	1	2	2	95	0.11
1981	100	0	0	1	99	0.09
S.E.(±)		39.67	17.62	22.64	47.92	0.08
C.D. at 5%		5.68	3.77	4.28	6.23	0.26

Rating Category :- I - Whole seed coloured

II - Embryo deeply coloured

III - Embryo & cotyledons lightly coloured

IV - No colouration.

Table - 31

Effect of different quality of light on seed germination of T. candida DC. in presence of different temperatures (data collected upto 6th day after sowing).

Percentage of germination (%) / Quality of light	Temperature (°C)	
	20	30
Dark	80	100
Blue	80	100
Green	79	100
Yellow	74	97
Red	57	92
Sunlight (control)	50	95
S.E. (±)	13.16	3.32
C.D. at 5%	3.26	1.64

Table - 32

Effect of different buffers on seed germination behaviour of T. candida DC.

Germination percentage (72h)	Hydroxide			Phosphate			Carbonate			Acetate		
	pH	Na-	K-	pH	Na-	K-	pH	Na-	K-	pH	Na-	K-
	3.0	76	87	5.8	22	76	3.6	0	0	3.6	0	0
	4.0	80	90	6.2	9	70	4.0	2	3	4.0	0	0
	5.0	63	80	6.6	6	30	4.4	9	15	4,4	0	0
	6.0	38	78	7.0	1	26	4.8	80	98	4.8	0	0
	7.0	38	78	7.4	0	18	5.2	78	92	5.2	0	0
	8.0	30	72	7.8	00	0	5.6	60	76	5.6	4	15
	9.0	30	68	8.2	0	0	6.0	42	58	6.0	2	9
S.E. (±)		21.71	7.72		8.12	30.72		36.44	42.29		1.57	6.11
C.D. at (5%)		3.82	2.28		2.34	4.55		4.96	5.34		1.03	2.03

Seed germination in control = 90%

Table - 33

Effect of growth regulators on seed germination of T. candida DC. (data collected after 72 hrs.

% germinations	LIGHT							DARK						
Growth regulators	10^{3}	10^{2}	10^{1}	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{3}	10^{2}	10^{1}	10^{0}	10^{-1}	10^{-2}	10^{-3}
GA	67	80	89	90	93	88	85	90	92	95	98	98	96	92
Kinetin	70	75	90	93	94	84	82	92	93	100	100	97	95	94
I A A	68	78	80	84	85	88	84	69	90	93	94	94	93	92
A B A	12	56	82	85	85	88	88	2	69	89	90	91	92	92
Ethylene	85	87	88	89	89	87	87	96	100	97	97	95	95	91
Control	82							88						
S.E. (±)	26.57	10.74	4.30	4.16	4.82	2.56	2.50	35.94	10.46	4.63	4.72	3.76	2.93	1.97
C.D. at 5%	4.64	2.95	1.86	1.84	1.97	1.44	1.42	2.90	8.47	1.93	1.95	3.05	2.37	1.26

Table - 34

Effect of phenolic acids and flavonoids on seed germination behaviour of Tephrosia candida DC. (data collected after 72 hrs of sowing)

	Germination percentage													
	L I G H T							D A R K						
	10^{3}	10^{2}	10^{1}	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{3}	10^{2}	10^{1}	10^{0}	10^{-1}	10^{-2}	10^{-3}
Gallic acid	57	69	81	82	85	85	84	63	82	90	92	92	92	90
Benzoic acid	64	67	82	85	85	86	83	70	82	91	92	94	94	91
Ferulic acid	67	73	84	85	85	85	82	75	82	90	92	94	95	88
Cinnamic acid	69	78	82	85	87	87	84	75	82	90	94	95	95	93
Caffeic acid	71	80	85	86	87	86	83	75	86	92	95	95	94	90
Rotenone	79	83	90	93	93	93	89	80	90	92	95	97	97	90
Rutin	90	91	96	96	94	90	86	93	95	97	98	100	94	92
Quercetin	81	82	85	96	97	88	85	87	92	95	100	100	100	93
Morin	80	82	85	88	96	82	84	88	92	94	95	97	94	90
Control	82							88						
S.E. (±)	10.01	7.21	4.58	5.33	5.36	3.40	2.09	9.46	4.99	2.72	3.38	3.61	3.09	1.78
C.D. at 5%	2.09	1.78	1.42	1.53	1.53	1.22	0.96	2.04	1.48	1.09	1.22	1.26	1.17	0.88

Table - 35

Germination percentage of T. candida seeds due to the effect of N P K solution.

Germination percentage / Treatments	Time (hours)		
	24	48	72
Complete soln. (Cn)	38	100	100
-N	27	97	100
-P	10	90	90
-K	20	93	100
-NP	33	87	90
-NK	57	93	95
-PK	4	77	80
-NPK	28	75	80
Control (W)	48	90	94
S. E. (±)	17.00	8.38	7.91
C.D. at 5%	2.91	2.05	1.99

Cn = Complete nutrient; -N = complete minus nitrate; -P = complete minus phosphate; -K = complete minus potassium; -NP = lacking nitrate and phosphate; -NK = lacking nitrate and potassium; -PK = lacking phosphate and potassium; -NPK = lacking nitrate, phosphate and potassium; W = receiving distilled water only.

Table - 36

Effect of different phosphates on seed germination, lengths and dry weights of root-shoot of T. candida DC.

Conc. (ppm)	Germination percentage (72h)		Length (cm) (168h)				Dry weight (mg) (168h)			
			Shoot		Root		Shoot		Root	
	NaH_2PO_4	KH_2PO_4	NaH_2PO_4	KH_2PO_4	NaH_2PO_4	KH_2PO_4	NaH_2PO_4	KH_2PO_4	NaH_2PO_4	KH_2PO_4
10^3	60	56	5.0	5.5	1.9	1.8	100	121	26	26
10^2	68	60	5.8	6.0	2.3	3.3	121	128	30	28
10^1	70	75	5.9	6.5	2.8	3.4	130	142	32	37
10^0	73	100	6.6	7.2	3.5	3.8	133	150	40	43
10^{-1}	85	82	6.8	6.8	4.0	3.6	140	140	42	40
10^{-2}	90	70	7.0	6.3	4.0	2.8	145	130	47	38
10^{-3}	70	60	6.5	6.3	3.3	2.7	130	127	30	28
Control	70		6.3		2.7		130		28	
S.E. (±)	11.54	16.36	0.65	0.51	0.77	0.64	13.63	9.56	7.59	6.67
C.D. at 5%	2.57	3.07	0.61	0.54	0.66	0.61	2.80	2.35	4.37	1.96

Table - 37

The effect of different nutrient regimes on biomass production: the values given are the mean weight of above and under ground plant parts/plant/pot/6 months.

Component	Treatment										
	Complete culture	-K	-N	-NP	-NK	-P	-PK	-NPK	Water only (control)	S.E. (±)	C.D. at 5%
Above ground biomass											
Stems(g/plant)	32	31	24	20	17	13	8	8	20	8.81	2.09
Leaf(g/plant)	25	24	17	10	10	5	7	5	15	7.64	1.95
Under ground biomass (g/plant)	9	8	5	4	5	3	4	2.6	6	2.16	1.04
T o t a l	66	63	46	34	32	21	19	15.6	41	18.31	3.02

Table - 38

Effect of storage on nitrogen, protein, amino acid, carbohydrate and phenol contents in T. candida seeds.

Seed	Total nitrogen %	protein %	aminoacids %	carbohydrates %		phenols %
				soluble	insoluble	
Freshly collected	4.50	39.75	2.97	2.93	6.92	2.20
1 year old	3.50	33.50	3.35	2.60	4.70	2.80
6 years old	2.00	25.00	4.62	1.69	2.83	3.50
S.E. (±)	1.25	7.40	0.86	0.64	2.05	0.65
C.D. at 5%	1.63	3.96	1.35	1.16	2.08	1.17

RESULTS AND DISCUSSION

Table-26 shows that with the increase of duration of acid treatment the percentage of germination has been noted to increase (92% at 72 hrs) during the treatment of 35 minutes of the acid and with the subsequent increase of the treatment, the percentage of germination decreases gradually. During the acid treatment of 40 to 50 minutes duration, several scares have been noted (Table-26) which is supposed to be the cause to show low percentage of germination as well as the decrease in growth rate of the seedling. Thus the optimum period of sulphuric acid treatment is supposed to be 30 to 35 minutes without any drastic effect of acid on seed coat. As enormous amount of heat is generated when conc. H_2SO_4 comes in contact with water, it is necessary that conc. H_2SO_4 should be decanted off completely and seeds should be poured immediately to a large volume of water so that heat, generated, may not damage the cotyledon.

In this experiment, lowering of the duration of acid treatment has not been able to improve the permeability of seed coat but longer duration of the treatment has been noted to effect not only the seed coat but the cotyledons and embryo also, as a result of which germination is found to be very low and prominent scars on cotyledons were observed. That sulphuric acid treatment is very necessary to overcome hard seed coat dormancy was noted in other

leguminous plants also (Waedyanatha et al, 1976; Farnandez, 1978; Bulmasova, 1976; Pradhan & Basu, 1980; 1981; 1982). It has been noted that 45 minutes of conc. H_2SO_4 treatment is necessary for showing good percentage of germination in Tephrosia purpurea pers (Basu, 1980) and in T. hamiltoni drumm (Basu, 1977).

The table-27 shows that the highest rate of germination is found at 30°C (98%), though the plant is capable to germinate well at the range of 15°C to 40°C. The seeds may tolerate even 60°C but the percentage of germination (Table-27) has been noted to become very low (35% at 72 hrs).

In nature the seedlings of Tephrosia candida DC. are found when they receive first shower of rain during the month of April-May and when the normal temperature has been noted to be at the range of 30-31°C (Appendix-I). Again from the table-27 it appears that 30°C is congenial for seed germination. It is expected that this favourable condition helps the plant to grow luxuriuntty in the ecological condition of North Bengal, India. Moreover, the seeds of this plant have the capacity to germinate well in cooler climates of Kurseong, Mirik, Soureni and Sukhiapokhri.

The highest rate of germination (95% - 98%) has been found just after one to two months of collection of seed (Table-28). 75% of germination is noted at the sixth month. After that the rate of germination is found to decrease gradually and the viability of the seeds has been reduced to 49% after twelve months. It has been observed that it will take 6 years to lose total viability of

Tephrosia candida seeds (Table-29). There are reports of various seeds having different periods of viability (Bequerel, 1934; Crocker, 1938). Storage of seed in dry condition is found essential for keeping the seeds in viable condition (Ching et al, 1959; Griffith, 1942; Barton, 1948). The seeds of Tephrosia candida DC. have been noted to lose total viability within 6 years of collection (Table-29). As North Bengal is a region having high humidity(Appendix-I) it is expected that some effect of humidity might be there in connection with the loosing of viability of seeds of T. candida. From the table-29 it appears that leaching is higher from the seed stored for a longer period in contrast with those of freshly collected seed. It has been noted earlier that permeability of seed is lost at the state of non-validity of seeds. Thus, the losing or viability in the seeds of T. candida DC. may be due to the effect of storage and that the lower percentage of germination of seeds stored for a longer period may be due to the change of normal condition of membrane affecting the permeability of normal functioning of cell. That the seeds stored for a longer period attains non-viability has been verified with the Tetrazolium test (Table-30). Result shows that the Fromosan colour complex in the seed increases during the storage of seeds (Table-30). Besides these, during the phytochemical screening of viable and non-viable seeds, it has been noted that a new chemical constituent (m.p. 212^{o}C) which is supposed to be dehydrorotenoid in nature has been isolated from non-viable seed only (Fig. 13-16). As this is not occuring in trace in viable seed, it is expected that the phenolic compound has been synthesized newly within the

seed during the storage or it is a product formed after breaking down of some other chemical component of the cell of the seeds of T. candida DC. when the seed attains non-viability. It has been reported that cell wall of plant materials includes some chemical constituents which are phenolic in nature (Harborne, 1975) and the phenolic part is associated with the cell wall of plant material for the normal functioning of cell (Harborne, 1982). It is possible that during the attainment of non-viability, this chemical is produced disturbing the normal function of the cell wall.

During the study of the photoperiodic effect on the germination, it has been found that the seeds show acceleration in germination percentage in dark in comparison to light. In this connection, highest percentage (95%) of germination has been observed in complete dark phase and that only 50% has been observed in continuous 24 hrs of light during cold (Table 31). That Tephrosia candida DC. has the ability to cope with prolonged dark period than a number of other legumes has been reported by Pradhan (1982). High temperature (30°C) also shows the same result (Table 31). In this connection, 100% seed germination has been noted in dark condition while sunlight shows 95%(of germination). In connection with the effect of different quality of light, blue and green light have been noted to accelerate the percentage over red light both at low and high temperatures (Table 31). In this connection, blue and green light also show 100% seed germination at high temperature (Table-31). That various seeds are found to germinate in dark at certain temperature and the photo-

periodic effect may be substituted by temperature has been reviewed by several authors (Kinzel, 1926; Black, 1969; Ishikawa & Ishikawa, 1960). The effect of light on seed germination is found specially due to the presence of plant pigments known as phytochrome and its transition from one form to another (Malcoste et al, 1970) and involvement of this phytochrome system needs investigation in connection with T. candida DC also. As the seeds have a wide range of tolerance from dark, sunlight and other quality of lights, the plant is expected to have the capacity to grow on the forest floor under various conditions of illumination and may tolerate wide range of temperature in different habitat conditions of North Bengal.

Table-32 shows that in presence of at least six different buffers of Na and K-hydroxide, phosphate and carbonate, the seeds of T. candida have been noted to prefer the lower range of pH and the highest percentage of germination has been noted to occur in 4.0, 5.8 and 4.8 pH respectively. Carbonate buffers show highest germination percentages (86 and 98% in Na^+ and K^+ respectively) followed by hydroxide (80 and 90%) and phosphate (22 and 76%). Acetate buffers show only 4 to 15% germination at 5.6 pH and the value is found to be significant statistically as compared to the results obtained from other buffers (Table-32). However, the value is found to be much higher in presence of K-ion in comparison to that found in Na ion (Table-32). As K-ion has been known to be effective for maintaining balance in optimal metabolic activities (Evans & Sorger, 1966) and in certain cases K is much preferable

than Na ion (El-sheikh et al. 1967; Peunarum et al. 1978), the higher rate of seed germination value is also expected to be due the effect of K ion in case of T. candida DC.

Table-33 shows the effects of different growth regulators on seed germination. It shows that the percentage of germination is less in control in comparison to that during GA treatment both in light and dark. GA has been noted to stimulate germination over control in light (at 10^{1} to 10^{-3} ppm) as well as in dark (at all concentrations from 10^{3} ppm to 10^{-3} ppm). GA is well established to enhance germination of seed in a number of plants (Lona, 1956; Chen & Park, 1973). It is found to reverse the inhibitory effect of light and dark (Evenari et al. 1958; Phinney & West, 1960). According to Phinney and West (1960) and Barendse et al (1968), Gibberellins are released from a bound form in the cotyledons of some leguminous species and move to the embryonic axis to stimulate germination. This experiment shows that the seeds of T. candida DC. required optimum concentration of GA to show the enhancement of germination in both the condition of light and dark. This requirement of critical level in GA is supported by other observations (Naguo and Mitsui, 1959).

Table-33 shows that there is a marked effect of Kinetin on the germination of seed of T. candida DC. Miller (1958) showed that Kinetin promoted germination of seed when the furfuryl group is replaced by other in a large number of derivatives of Kinetin (Mayer and Poljakoff-Mayber, 1978). Previously it was thought

that Kinetin substituted for light in germination, it was later shown that Kinetin sensitised the seeds so that smaller doses of light would induce their germination (Weiss, 1960). According to him, the seeds need not be kept continuously in a solution of Kinetin, as a few hours in the solution, at a suitable period is sufficient to cause sensitisation. That Kinetin does not substitute for light is also indicated by the fact that some light inhibited seed as well as light sensitive seeds are not affected by Kinetin at all (Mayer and Poljakoff-Mayber, 1978). According to Raynold and Thompson (1973), the requirement for the full expression of the stimulatory effect of Kinetin depends on the development of some factors in the seed immediately after illumination involving some direct or indirect effect of phytochroma reaction. In addition to their interaction with light, Kinetin also interact with other exogenously applied compounds such as ABA, GA and also with temperature and needs investigation in T. candida.

The effect of Indole acetic acid (IAA) on germination has long been in dispute (Mayer and Poljakoff-Mayber, 1978). Numerous workers have investigated the effect of IAA on germination of a variety of seeds and have obtained conflicting result; stimulation and inhibition being obtained depending on the concentration of IAA and the type of seed. In T. candida DC. stimulation as well as inhibition of germination of seed have been noticed at different concentration of IAA. 10^{0} ppm and lower concentrations show increased percentage of germination over control while 10^{3} to 10^{1}

ppm concentrations of IAA show an inhibition over control in presence of light. In dark, inhibitory effect has been found only in 10^{-3} ppm (69% germination). All other concentrations, starting from 10^{2} to 10^{-3} ppm, show an increase in germination percentage over control. But the stimulation of germination (both in light and dark) has been noted to be less in comparison to those of GA and Kinetin (Table-33).

Abscisic acid (ABA) is recognised as the most important germination inhibitor (Mayer and Poljakoff-Mayber, 1978). The presence of abscisic acid has been reported in many seeds and fruits (Addicot and Lyon, 1969; Milborrow, 1974). Exogenously applied ABA prevents the germination of many seeds (Mayer and Poljakoff-Mayber, 1978). In T. candida DC. strong inhibition has been found in 10^{3} ppm concentration in light and dark (12 and 2% germination respectively) in comparison to 82-88% in control (Table-33). However, a small amount of acceleration in germination has been noticed in lower concentrations of ABA both in light and dark (Table-33) and this may be due to some interaction of ABA and other metabolite in the imbibed seed.

The dynamic part taken by ethylene in the regulation of growth and of many diverse developmental phenomenon has forced the realisation that this tiny molecules must be accepted as a hormonal agent in plants. The existence of an ethylene-requiring mutant puts the final touch on the case (Abeles, 1972; 1973). In addition to the inhibitory effects of ethylene, as with the other plant hor-

mones, small but definite promotions of growth by this hormone have been reported for several species under certain conditions (Ku et al, 1969; Musgrave et al, 1972). Dormancy of buds and seeds is sometimes relieved by ethylene applications and has been reported by Rosa (1925), Vacha and Harvey (1927). In many of the earlier survey on chemicals which might break dormancy, ethylene ordinarily was reported as being ineffective. However, modern experiments have shown that not only ethylene can break dormancy under some conditions (Toole et al, 1964) but endogenous formation of ethylene may be responsible for breaking dormancy (Ketring and Morgan, 1969; Esashi and Leopold, 1969). In T. candida DC., stimulation of seed germination has been noticed at all concentrations of ethylene in presence of light as well as in dark (Table-33). 10^{0} and 10^{-1} ppm contrations of ethylene solution were found to be most effective in increasing the germination percentage in presence of light (Table-33). In dark condition 10^{2} and 10^{1} show maximum values (100 and 97% respectively).

Table-34 shows the effect of different phenolics and flavonoids on seed germination of T. candida DC. Out of the five phenolic acids, gallic acid shows the greatest degree of inhibition in germination percentage both in light and dark (Table-34). Gallic acid has been known to be a strong inhibitor to seed germination at higher concentration (Rice, 1982; 1984; Nandakumar & Rangaswami, 1985). In this respect, stimulation of germination percentage has been noted starting from only 10^{1} ppm in presence

of light and 10^{1} ppm in dark (Table-34).

Benzoic acid, has also been observed to show both stimulation as well as inhibition in connection with T. candida seed germination. In this regard, dark condition has been found to be much effective than light (Table-34). 10^{-1} and 10^{-2} ppm solution of benzoic acid shows a distinct increase over control in dark. Moreover, no inhibition (except at 10^{3} and 10^{2} ppm) in seed germination was observed in dark (Table-34). However, in presence of light, stimulation has been observed only at 10^{0} to 10^{-3} ppm and the value of seed germination ranges from 83 to 86% only (Table-34). Stimulation and inhibition of germination at different concentration of the phenolic acid are in conformity with the observation reported earlier (Evenari, 1949).

Ferulic acid has also shown inhibitory as well as stimulatory effect on seed germination behaviour of T. candida DC. (Table-34). In this respect, light shows much inhibition than dark at higher concentration (Table-34). The strong inhibitory action of ferulic acid has been reported by Evenari (1949), whitehead (1964); Muller (1970, 1974); Zenk and Muller (1963); Rice (1984); Chang (1983). However, Jutilla (1976) and Morris et al (1984) have observed little allelopathic effect of ferulic acid on the germination of sugar beet fruits.

The table also shows the effect of cinnamic acid on the seed germination behaviour of T. candida DC. In this connection, lower range of concentrations show acceleration in germination percentage

both in light and dark condition while, higher concentrations have been found to be inhibitory (Table-34). That cinnamic acid has phytotoxic activity to different seeds has been reported earlier (Chou and Patrick, 1976; Chang, 1983; Rice, 1984).

Caffeic acid has also shown stimulatory and inhibitory effect on seed germination of T. candida DC. at different concentrations (Table-34). Stimulation of seed germination has been noted at the range of 10^{1} to 10^{-3} ppm both in light and dark condition. In this connection, 10^{0} to 10^{-1} ppm show the maximum increase of seed germination (95%) in dark compared to light (87%). Akkerman and Veldsten (1947) claimed that caffeic acid was responsible for the inhibition of germination of seeds within the tomato fruit. Basu (1971) has shown that caffeic acid is responsible for inhibition of the germination of T. purpurea at higher concentration. Vendring and Beffel (1961) observed that commercial trans-caffeic acid, at the concentration of 1 gm/ml, enhanced the elongation of coleoptile section as much as did Indole 3-acetic acid. Thimann, et al (1962) suggested that apparent activity of caffeic acid at low concentration was due to synergism with Indole 3-acetic acid. Tomaszewski (1972) has shown that caffeic acid and other diphenolic substances produce marked synergism effects with Indole-3-acetic acid. Caffeic acid involved in allelopathic action was noted previously by several workers (Everari, 1949; Lodhi & Rice, 1971; Basu, 1977; Basu and Laha, 1985). Though stimulation of germination due to caffeic acid has been noticed in T. candida DC., it

needs investigation to see any synergistic action with IAA.

Table-34 shows the stimulatory action of rotenone on the seed germination behaviour of T. candida DC. 93 to 97% germination have been noted by the application of 10^{0} to 10^{-2} ppm solution of rotenone in comparison to control (82 and 88% respectively in light and dark). Thus, rotenone, in a dilute solution has been noted to stimulate germination of seed within 72 hrs (Table-34). That rotenone stimulates germination of seed has been reported by Pradhan (1982).

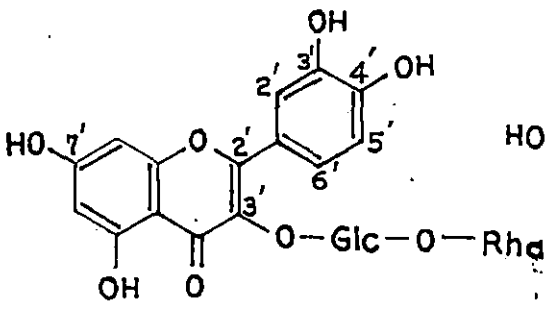
Rutin shows much stimulatory effect on seed germination. From Table-34, it appears that rutin (Quercetin-3 rutinoside) shows 100% seed germination as compound to control at 10^{-1} ppm in dark. 10^{0} ppm shows 96% and 98% germination in light and dark respectively. Only at lower concentration (10^{-3} ppm), the stimulatory activity is slightly lowered as compared to that of 10^{0} to 10^{-2} ppm (Table-34). On the other hand quercetin, the aglycone part of rutin shows distinct increase in germination percentage over control in light and dark at the concentration of 10^{0} to 10^{-2} ppm at 72 hrs (Table-34). It shows rapid decrease of activity as the concentration rises upto 10^{3} ppm. At 10^{-3} ppm concentration, 85 and 93% germination have been noted in light and dark conditions respectively (Table-34).

The stimulatory action of rutin and its aglycone, quercetin on germination of T. candida seed has not been mentioned earlier. Though it is clearly able to exert significant effect on seed germination within 24 hours when applied exogenously to the seed, it is

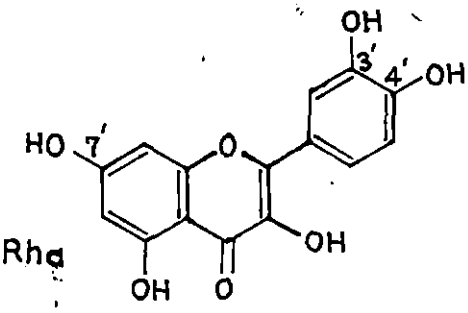
very difficult to say its endogenous role during germination of seed of T. candida DC.

Table-34 also shows the effect of morin on the seed germination behaviour of T. candida DC. Higher percentage of germination (96% and 97% in light and dark) has been observed at 10^{-1}ppm concentration (Table-34). In this connection, the higher concentrations (10^3 to 10^2 ppm) show minimum percentage of germination both in light and dark conditions (Table-34). The biosynthesis of rutin and quercetin has been studied by several workers (Underhill et al, 1957; Geissman and Swain, 1957; Shibata and Yamazaki, 1958). It is noted that caffeic acid is directly involved during the biosynthesis of those compounds and that light is essential for that purpose. The effect of rutin and quercetin on IAA oxidase activity has also been observed (Stenlid, 1963, 1968; Pradhan and Basu, 1980, 1982). Mestakov et al (1971) have determined the combined effects of flavonoids and abscisic acid on growth of several plants. Later, Pradhan and Basu (1981) have determined the effect on T. vogellii. Rutin and quercetin has been noted to relieve the inhibitory effect of abscisic acid (Mestakov et al, 1971). Stenlid and Saddik (1963) evaluated the effect of 20 flavonoids on IAA oxidase preparation from Pisum sativum roots. In every instance, a single 4'-hydroxy substituent increased enzyme activity while 3', 4' - dihydroxylated flavonoids are inhibitory. A free hydroxyl group at the 7-position is proved to be good co-factor nature of the flavonoids, 7-hydroxyl substituents is common in all these three growth regulators like

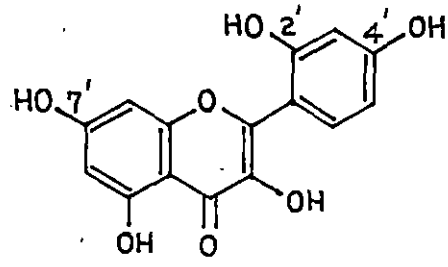
rutin, quercetin and morin.



RUTIN



QUERCETIN



MORIN

In spite of the presence of 3',4' - dihydroxyl substituents in quercetin, there is an overall stimulation of seed germination. That rutin and quercetin are produced in the seedling after germination and may react with other hormones to control the subsequent growth of the seedling of the plant has been reported by Pradhan & Basu (1980,1981,1982) in Tephrosia vogellii Hook.

Different N,P,K solutions show variation in germination of T. candida (Table-35). While complete nutrient solution (Cn) shows

100% germination within 48 hrs, control shows 90% in this respect (Table-35). Germination percentage has been noted to be decreased in solution where phosphorus is lacking. On the contrary complete solutions lacking nitrogen and potassium (-N and -K) have been recorded to show 100% germination indicating that phosphate may have some role on seed germination behaviour of the plant.

In this respect, addition of phosphates of sodium and potassium shows remarkable increase in seed germination percentage, root shoots lengths and dry weights over control and the values are found to be statistically significant (Table-36). Maximum lengths and dry weights of root shoots have been obtained from 10^{-2} and 10^{0} ppm concentrations of Na and K- phosphates when applied at the time of showing (Table-36).

Although, the deficiency of both phosphorus and nitrogen seem to affect growth, deficiency of potassium did not (Fig.-35). The seedlings given no extra potassium grew as vigorously as did the seedlings receiving complete nutrients (Fig.35). The total above and underground biomass of the specious at the end of six months growth (Table-37) was significantly higher (1% level) in the complete and -K cultures than in the -N, -NP and -NK cultures, which in turn produced more dry matter than did the remaining treatments (5% level). It is possible that the potassium requirement of the species examined is such that the available potassium though rather little in soil, is adequate in the plant itself for their growth. In contrast, the evidence from the experiment (Fig.35) is that the species demand

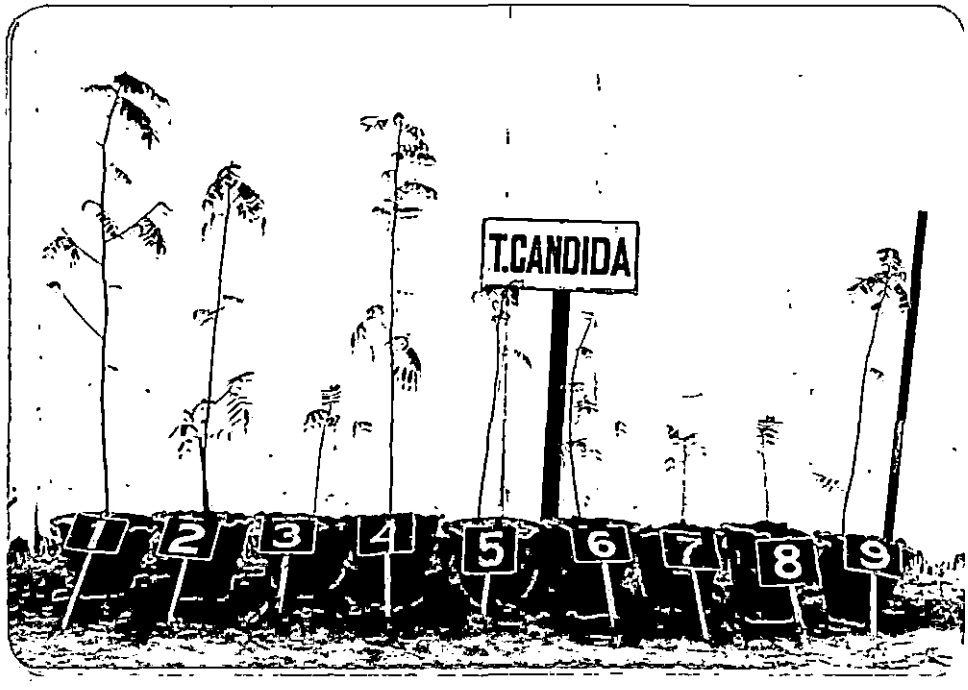


Figure 35 : The effects of N, P, K on T. candida DC.

1=Cn, 2= -N; 3= -P; 4= -K; 5= -NP;
6= -NK; 7= -PK; 8= -NPK; 9= Control.

much larger amounts of phosphorus and a little nitrogen for growth and development of the plant. Thus, the restricted vegetative performance of the species in this region can be related largely to the limiting effects and interaction of sub-tropical levels of these two nutrients. The phosphorus deficiency appears to be much more acute than nitrogen deficiency. That phosphorus is important for the growth of grain legume has been reported by many workers (Van Schreven, 1958; Garg et al, 1971; Rao and Singh, 1984). Leguminous crop applied with higher doses of P was stated to maintain maximum growth rate (Forada and Tomer, 1975). Several early workers have reported increased value of dry matter production along with protein content in grain legumes with increased P application (Sinha, 1971; Forada and Tomer, 1975; Rao and Singh, 1984). Application of N,P,K results better growth of different plants (Appadurai and Arsaratnam, 1969; Pemadasa, 1981; Agrawal et al, 1983; Omaliko, 1984; Prasad et al, 1984; Singh et al, 1984; Omaliko et al, 1987). The utility of the species for ecorestoration and forest management in North Bengal has been found noteworthy (Basu et al, 1986; Gupta and Basu, 1988; Basu and Gupta, 1988). The soil of the Balasan-Catchment area of North Bengal which is noted to be deficient in nutrients by leaching and erosion (De, 1977; Annual Report, 1986; Gupta and Basu, 1988), the supposedly slow rate of microbial activity due to acidic pH condition (Mandal et al, 1982) and rarety of legumes in acid pH of the soil (De, 1977; Basu et al, 1986) may thus be recovered by the large scale plantation of a species in the region. Moreover, the species which was once luxuriantly grown in

the region has become endangered now a days. As the species has been found to demand much larger amount of phosphorus, thus, it seems likely that prolonged nutrient-enrichment specially of phosphorus would help to re-establish the species in the region.

Leguminous seeds are regarded as store house of the plants (Bandemer, 1967) like other legumes, T. candida seeds are also enriched with huge amount of nitrogen, proteins, amino acids and carbohydrate contents (Table-38). That protein is the chief constituent of leguminous seeds has been reported by many authors (Bandamer, 1967; Kapoor et al, 1971). In this respect, 39.75% protein has been estimated from fresh seed (Table-38). High amount of free amino acids (2.9%) has also been estimated from T. candida fresh seed (Table-38). That leguminous seeds are rich in amino acid content has been reviewed by Bandemer (1967). In this connection, fresh seed has been found to contain a fair amount of total phenol content (Table-38).

During storage, T. candida seeds undergo a decrease in total nitrogen, protein, amino acid and carbohydrate contents within one year period (Table-38). In this connection, more significant decrease in all contents has been obtained from 6 years old non-viable seed (Table-38). It has been reported that the reduction in chemical constituents including protein contents in several legumes occurs during storage (Jackson and Bell, 1969; Maheshwari, 1986). That changes in protein contents during storage occurs in legumes due to the effect of fungi including Aspergillus has also been reported (Jackson and Bell,1969; Maheshwari, 1986). However, a significant

increase in total phenol content (3.5%) has been noted in T. candida seed during 6 years of storage (Table-38). Although the actual reason behind it is not known fully, it is assumed that free phenol in fresh seed may be converted into polyphenols during storage (Harborne, 1975; 1982). Moreover, the dehydrorotenoid structure which has been isolated from 6 years old non-viable seed of T. candida DC. but no trace of the chemical in fresh seed, may also be responsible for the increase in total phenol content in non-viable seed (Fig. 13-16). It is thus possible that during the attainment of non-viability, these chemicals are produced disturbing the normal function of the cell wall.

S U M M A R Y

During the study on the germination of seed, it was noted that the seeds of Tephrosia candida DC. at the time of their dispersal, were in a state of dormancy due to the presence of hard seed coat. The seed coat dormancy was noted to overcome by concentrated sulphuric acid treatment.

Seed was observed to show higher rate of germination in dark than in light.

30°C was noted to be the optimum showing highest percentage of seed germination (98%) in light at the end of 72 hours. 100% of seed germination was observed in dark.

Blue and green light have been noted to show stimulation of seed germination as compared to red and other light quality.

GA and kinetin were noted to accelerate the germination rate both in light and in dark as compared to IAA.

Rutin and quercetin were found to accelerate germination over control (100%).

Phenolic acids showed inhibition during seed germination at higher concentrations and in this respect, gallic acid was noted to show a marked inhibitory effect followed by benzoic and ferulic acids.

Seeds were found to prefer acidic pH for germination and seedling growth and was confirmed by using different buffer solu-

tions.

Phosphorus deficient solution showed much low percentage of seed germination compared to that of complete nutrient solution. However, 100% seed germination was observed in solution deficient in nitrogen and potassium.

Solution deficient in potassium showed no variation of seed germination as compared to complete nutrient solution.

Progressively greater germination percentage (90-100%) had been found when different phosphate salts were applied to the seeds.

Phosphorus deficiencies showed much less growth of seedling as compared to that of nitrogen deficiency.

92% of seed germination was observed in the month of harvest. Germination percentage increased to 98% after two month and became only 49% at the end of one year.

Duration of storage had an effect on seed germination behaviour. At the end of fifth year of storage the seed showed only 12% of germination as compared to 92% of seed germination observed immediately after harvest. The seed lost viability in the sixth year. The observation was supported by tetrazolium test.

Six year old seed was observed to show low contents of nitrogen, protein, carbohydrates as compared to freshly collected seeds. The content of total amino acid and phenols were observed to become high during storage.