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Effect of Temperature on Viability and Biochemical Changes During Storage of Recalcitrant Seeds of *Vatica chinensis* L.

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Abstract: *Vatica chinensis* L. (Sannadhupa) is one of the important and critically endangered species of Dipterocarps present in North-East of the Udupi district Karnataka. The large seeds exhibit recalcitrant nature. The germination, viability and the biochemical changes during storage of the seeds of *Vatica chinensis* was carried out at different temperatures. The stored seeds were allowed to germinate using sand bed method, biochemical changes were determined following standard procedures. The seed did not germinate at the moisture lower than 61%. The germination percentage decrease with laps of time and stopped completely at a 5 days in open environment, 17 days at 12±2°C and 19 days at 20±2°C and 28±2°C. The decrease in the vigour index was also noticed with laps of time. There was also increase in free amino acid and reducing sugar both in endosperm and seed coat. The electrolyte leakage was also increase during lap of time. The moisture content during storage was found to be a critical factor influencing the viability. The synergistic effect of reduction in moisture, increase in electrolyte leakage, protein and sugar modification may be responsible in reducing the viability of the seeds of *Vatica chinensis*.

Key words: Biochemical changes, germination, storage, temperature, *Vatica chinensis*, viability

INTRODUCTION

The Dipterocarpaceae an important family of tropical rain forests with 680 species belonging to 16 genera is distributed in Malaysia, South East Asia, India, Sri Lanka and Africa (Meher Homiji, 1979). In India 31 species of Dipterocarps have been reported of which 14 species belonging to 5 genera are present in the Western Ghats, which is one of the global biodiversity hotspots for conservation (RAPA Monograph, 1985). Dipterocarps is one of the most important commercial timber sources in these forests (Yap, 1981; Ashton, 1982). One-quarter of the production of hard timber in the world is provided by this family (FAO, 1985) the diversity of the Western ghats forests with special reference to Dipterocarps species has been carried out by Bhandary and Chandrashekar (2003), Shivaprasad *et al.* (1999, 2002), Vasanthraj *et al.* (2001, 2005, 2006).

Dipterocarps seeds are short-lived. Most of them are very hydrous, sensitive to desiccation and low temperature and they do not exhibit any dormancy with germination occurring immediately after ripening. In logged-over hilly zones, though the germination rates are high, survival and growth of seedlings pose problems. During preservation, seeds loose viability rapidly if moisture content decreases. Storage is impossible by classic techniques such as low water content and low temperatures. The maximum period of conservation for dipterocarps seeds is, in most cases, from 2 weeks to 2 months (Yap, 1981; Maury-Lechon *et al.*, 1991; Mori, 1980; Sasaki, 1980).

There are a few reports on the requirement of optimum water content in seed and temperature for their conservation (Dayal and Kavirappa, 2000). Efforts also have been made to understand the biochemical mechanism responsible for the rapid loss of viability. Biochemical changes during the germination and storage of the recalcitrant seeds of endemic Dipterocarps of the Western Ghats such as *Hopea ponga* have been reported by Sukesh and Chandrashekar (2011). The storage of these seed at lower temperature reported to reduce the viability of the seeds as reported *Shorea* species (Purohit *et al.*, 1982; Yap, 1986), *Theobroma cocoa* (King and Roberts, 1979), *Hopea* species (Song *et al.*, 1984). The tropical recalcitrient seeds with high moisture contents were reported to sensitive low temperature (Chin and Roberts, 1980; Yap, 1986).

Vatica chinensis L. (Sannadhupa) is one of the important species of Dipterocarps present in North-East of the Udupi Taluk (Bhat, 2003; RAPA Monograph, 1985).

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The extensive forest survey conducted during 1991 and 1996, for the national conservation review did not record *Vatica chinensis* in the forest of Sri-Lanka and therefore it is consider to be extinct, because of its scattered distribution Western Ghats, now it is classified as critically endangered species (Ashton, 1982, 1998). The present study was undertaken to investigate the effect of temperature on viability and biochemical properties during seed storage and to find out any possible reasons for the poor viability of *Vatica chinensis* seeds.

MATERIALS AND METHODS

The mature seeds of *Vatica chinensis* were collected from Petri, near Hebri, Karkala taluk on plastic sheets by shaking the branches, which were immediately brought to the laboratory and used for the experiment. One set of 1000 seeds were incubated in open environment at 28±2°C. Fourteen sealed polythene bags containing 80 seeds each were incubated separately at different temperatures like 12±2, 20±2 and 28±2°C, respectively.

Every alternate day, one bag each from different temperature was used for the determination of moisture, germination and biochemical parameters. Moisture content was determined at 105±5°C in hot air oven. Germination percentage was determined by using sand bed method (ISTA, 1985). The germination percentage, root length and shoot length were measured on 30th day after sowing. The changes in the level of reducing sugar were in endosperm and embryo and seed coat were estimated separately (Sadasivam and Manickam, 2009).

The percentage electrolyte leakage was measured immediately after removing from the storage by using conductivity meter. For this, two embryo of the matured seeds excused and placed in 5 mL Double Distilled (DD) water for 1 h with occasional agitation and further 5 mL of DD water was added and the conductivity was measured. The conductivity of the seeds was measured once again after homogenizing the axis. The percentage leakage was calculated (Stewart and Bewley, 1980) as:

$$C = \frac{Cl}{Ch} \times 100$$

where, C is Percentage leakage, Cl is Conductivity of leachate, Ch is Conductivity of homogenate.

The correlation analysis was carried out with SPSS software package version 12.0. The relationship between the parameters observed was determined by Pearson's correlation analysis (Zar, 1999).

RESULTS

The seeds of *Vatica chinensesis* without any wing or appendages weighed between 8-15 g. The initial moisture of seed was 65.82% on the first day which reduced rapidly in the seeds stored under open condition (Table 1). The moisture content reduced gradually in the seeds stored in all other temperatures. The seeds retained the moisture more than 59% on 21th day.

The highest germination percentage on the first day was 83.3% (Table 2). The germination percentage decreased with increase in the number of days of storage. The germination percentage gradually decreased in open condition up to 15 days. The seeds stored in open condition readily germinated under in stored condition only after 13th day. Approximately 70% of the seeds germinated in stored condition. The germination percentage of the seeds stored in different temperature in polythene bags varied. The germination percentage reduced almost to 50% on 5th day in the seeds stored at 28±2°C and 20±2°C, thereafter decreased rapidly later and lost their complete viability by 19th day and 21st day in the seeds stored at 28±2 and 20±2°C, respectively. The germination percentage of seeds stored at 12±2°C decreased to 18.3% by 5th day and lost the complete viability by 15th day.

The Vigour index of the seeds kept for germination on the first day was 1389.03 and increased till 3rd day in all the storage conditions except the seeds stored at 28±2°C in open condition where vigour index was highest on 5th day (Table 3). The vigour index decreased from 5th days onward in all the stored conditions.

The average number of leaves per plant produced in growing seedlings of *Vatica chinensis* is given in Table 4. The highest average number of leaves (5.41±2.14) was observed in the seeds germinated immediately after harvest more or less similar number of average leaves (5.05± 0.25-5.75±0.25) were observed in seeds stored for seven days under all stored condition. The number of leaves produced in the plants decreased with increased in the number of days of storage. The average number of leaves varied in seed stored at 28±2°C. The leaf production reduced to almost 50% by the end of 15th day of storage in open condition and 9th day in the seeds stored at 20±2°C in the polythene bags. However, the lower temperature did not favour the leaf production after 9 days.

There was a slight initial increase in the total free amino acids of endosperm and embryo which decreased with aging of seeds (Table 5). The initial total free amino acids of endosperm and embryo was 6.47 and increased rapidly in seeds stored under open condition and it

Table 1: Moisture content of the seeds of *Vatica chinensis* in percentage

	Moisture content			
Days	28±2°C open Open environment	28±2°C Sealed polythene bag	20±2°C Sealed polythene bag	12±2°C Sealed polythene bag
1	65.82±1.82	65.82±1.82	65.82±1.82	65.82±1.82
3	63.72±0.66	65.76±2.06	67.78±0.28	65.82±0.37
5	62.31±1.03	65.46±2.10	66.67±0.58	65.50±1.48
7	61.69±4.42	65.33±0.58	66.22±3.50	64.55±0.79
9	59.19±0.55	64.53±0.69	65.02±2.50	64.00±1.10
11	57.75±1.65	63.78±1.71	63.61±2.67	63.87±3.90
13	55.34±2.15	63.44±1.13	63.55±2.64	63.57±2.10
15	52.24±1.85	62.52±1.41	63.03±0.21	62.30±1.98
17		61.77±1.71	62.85±1.07	62.07±0.56
19		61.68±2.13	62.28±3.44	61.69±1.34
21		59.23±0.54	62.22±0.62	61.27±2.10
23		57.23±1.12	62.55±0.34	58.72±2.50

Table 2: Germination percentage of seeds of *Vatica chinensis*

	Germination percentage			
Days	28±2°C open Open environment	28±2°C Sealed polythene bag	20±2°C Sealed polythene bag	12±2°C sealed polythene bag
1	83.3±6.7	83.3±6.7	83.3±6.7	83.3±6.7
3	81.65±3.33	75±3.33	80.3±1.67	83.3±10
5	91.65±5.00	50±13.33	44.95±15	18.30±6.67
7	79.95±6.67	15±11.67	36.6±3.33	16.6±5.00
9	81.6±1.67	1.67±1.67	31.65±6.67	8.3±3.33
11	75±3.33	1.67±1.67	23.3±6.67	3.3±1.67
13	73.3±8.33	1.67±1.67	28.3±11.67	3.3±0.00
15	69.95±6.67	3.3±0.00	31.65±16.67	1.67±1.67
17		1.67±0.00	29.95±10	0±
19		0±0.00	14.95±3.33	0
21		0	0	0

Table 3: Vigour index of seeds of *Vatica chinensis*

	Vigour index			
Days	28±2°C open Open environment	28±2°C Sealed polythene bag	20±2°C Sealed polythene bag	12±2°C Sealed polythene bag
1	1389.03±30.41	1389.03±30.41	1389.03±30.41	1389.03±30.41
3	1529.6±129.06	1876.36±11.65	1896.29±336.91	1678.85±11.65
5	2281.37±128.55	424.145±94.98	1019.44±67.28	943.00±93.5
7	2011.73±124.65	326.19±10.79	803.92±19.20	346.7±70.8
9	2007.73±136.27	121.16±81.64	271.6±22.22	33.3±33.3
11	1935.05±107.35	57.75±5.8	287.6±88.19	0
13	1657.85±21.58	47.88±9.7	248.14±31.77	0
15	1262.00±149.56	10.19±2.6	211±85	0
17	0	2.856±1.2	173.8±11.23	0
19	0	0	222.1±11.2	0
21	0	0	0	0
23	0	0	0	0

Table 4: Average number of leaves of *Vatica chinensis* seedlings after 30 days

	Avg. No. of leaves			
Days	28±2°C open Open environment	28±2°C Sealed polythene bag	20±2°C Sealed polythene bag	12±2°C Sealed polythene bag
1	5.14±0.14	5.14±0.14	5.14±0.14	5.14±0.14
3	3.68±0.25	2.85±0.12	3.63±0.43	3.88±0.25
5	5.14±0.14	4.93±0.43	5.10±0.6	2.27±2.0
7	5.29±0.25	5.75±0.25	5.05±0.25	5.60±0.4
9	4.04±0.04	2.50±2.5	4.43±0.08	3.00±0.45
11	5.13±0.09	2.00±0.14	2.30±1.2	0
13	5.30±0.3	3.00±1.1	5.21±0.045	0
15	2.30±2.4	3.00±0.14	4.80±0.4	0
17	0	2.00±0.34	0	0
19	0	0	0	0
21	0	0	0	0
23	0	0	0	0

Table 5: Total free amino acids in endosperm and embryo and seed coat of *Vatica chinensis* seeds (μg/100mg)

	Total free amino acids							
	28±2°C open Open environment		28±2°C Sealed polythene bag		20±2°C Sealed polythene bag		12±2°C Sealed polythene bag	
Days	endosperm and embryo	seed coat	endosperm and embryo	seed coat	endosperm and embryo	seed coat	endosperm and embryo	seed coat
1	6.47±2.32	2.59±0.62	6.47±2.32	2.59±0.62	6.47±2.32	2.59±0.62	6.47±2.32	2.59±0.62
3	7.61±3.42	5.14±1.85	6.63±2.13	5.46±1.49	7.75±1.52	6.07±1.94	7.56±2.52	3.67±0.28
5	8.29±2.53	6.18±1.20	6.18±2.14	5.74±1.14	7.06±2.14	8.69±2.64	7.3±1.56	4.05±0.42
7	8.16±3.12	7.16±0.37	4.85±0.95	6.39±0.79	6.7±1.23	9.68±0.73	7.16±0.6	4.81±0.18
9	5.37±1.9	7.82±0.77	4.3±1.94	10.22±4.44	5.92±0.56	9.83±2.64	6.87±1.88	5.2±0.25
11	5.06±2.12	7.92±0.45	4.20±1.2	10.70±2.10	5.77±1.2	9.95±0.24	6.01±0.5	5.16±0.54
13	5.32±0.52	7.93±0.27	4.10±1.82	11.35±4.01	5.65±1.36	11.06±0.21	5.87±0.85	5.28±0.62
15	4.58±0.95	9.67±0.06	6.89±4.32	11.65±1.80	5.05±1.15	11.7±0.34	5.85±1.78	6.07±0.79
17			9.88±3.12	12.36±3.92	4.8±0.4	14.14±1.13	5.01±0.57	6.61±0.47
19					4.46±1.23	14.05±1.08	4.81±1.36	6.84±0.22
21					4.42±1.15	14.2±3.55	5.59±0.46	7.2±0.34

Table 6: Reducing sugar in endosperm and embryo and seed coat of *Vatica chinensis* seeds (μg/100mg)

	Reducing sugar							
	28±2°C open Open environment		28±2°C Sealed polythene bag		20±2°C Sealed polythene bag		12±2°C Sealed polythene bag	
Days	Endosperm and embryo	Seed coat	Endosperm and embryo	Seed coat	Endosperm and embryo	Seed coat	Endosperm and embryo	Seed coat
1	6.62±1.95	12.85±3.23	6.62±1.95	12.85±3.23	6.62±1.95	12.85±3.23	6.62±1.95	12.85±3.23
3	7.46±0.31	12.62±2.58	5.48±1.02	12.62±2.05	6.76±0.04	12.87±1.4	6.15±0.52	16.3±2.48
5	8.18±0.89	11.47±2.49	6.15±1.16	10.90±3.05	5.33±0.81	11.17±0.59	6.28±1.06	14.55±4.03
7	8.18±3.91	7.93±1.03	6.26±1.95	9.66±2.91	6.7±0.16	11.26±3.27	6.44±0.31	14.17±2.10
9	9.13±0.38	6.94±1.27	6.61±1.65	9.41±1.72	7.23±0.53	11.57±0.3	6.52±0.13	13.72±1.78
11	10.29±0.6	6.91±2.1	6.51±0.58	9.11±3.21	7.23±0.24	7.89±1.6	6.6±0.18	12.41±0.93
13	10.52±0.59	6.94±1.48	6.86±1.33	7.63±1.5	7.6±0.32	7.54±1.36	6.95±0.18	10.45±1.7
15	10.82±0.6	6.81±0.25	6.98±0.3	7.95±0.9	7.62±0.27	6.33±1.32	7.42±0.51	12.66±1.36
17			8.31±1.86	5.15±0.69	8.01±0.6	5.97±1.16	7.61±0.5	8.377±1.30
19			9.22±0.28	6.22±2.12	8.04±0.44	5.88±0.23	7.64±0.39	8.077±0.74
21			7.44±0.29	6.19±0.46	8.85±0.2	9.36±0.36	8.9±0.20	8.037±2.65

Table 7: Percentage of electrolyte leakage of *Vatica chinensis*

	Percentage of electrolyte leakage			
Days	28±2°C open Open environment	28±2°C Sealed polythene bag	20±2°C Sealed polythene bag	12±2°C Sealed polythene bag
1	0.56	0.56	0.56	0.56
3	0.79	1.27	0.49	1.67
5	6.46	3.4	0.85	3.98
7	6.84	7.07	5.0	4.85
9	9.71	8.0	5.59	7.34
11	13.25	11.54	9.426	11.35
13	23.34	11.85	10.24	12.73
15	27.65	14.09	13.69	12.78
17		17.07	15.5	13.9
19		23.81	15.54	22.4
21		32.21	15.74	26.7

reached 8.29 on 5th day and then decreased. The total free amino acids of seed coat increased with aging of seeds. The initial total free amino acids was 2.59 and increased in seeds kept under sealed condition at 28±2°C and it reached 13.48 on 15th day. In open condition, it was 9.671 on 15th day and at 12±2°C it was 7.2 on 21th day. The total free amino acids reached up to 14.2 in seed coat of *Vatica chinensis* stored at 20±2°C.

The reducing sugar contents of endosperm and embryo increased from first day onwards (6.62±1.95 μg/100mg) in all the storage conditions (Table 6). The maximum reducing sugar was observed on 15th day (10.82±0.6 μg/100mg) in the case of seeds stored at 28±2°C. The initial reducing sugar of seed coat of *Vatica chinensis* was 12.85 on the first day which reduced in all the storage conditions.

The percentage electrolyte leakage of the seeds of *Vatica chinensis* is given in Table 7. The percentage electrolyte leakage was 0.56 in first day, which increased in the seeds stored at different storage condition. A rapid

Table 8: Pearson correlation table

Correlations	RSE	MC	COND	GR	NL	VI	AAE	AAS
RSC	-0.6301**	0.5890**	-0.7143**	0.2178	0.2480	0.2521	0.4395**	-0.7612**
RSE	1	-0.8749**	0.6692**	0.1660	-0.1352	0.1768	-0.3063	0.3445*
MC		1	-0.7503**	-0.1014	0.1696	-0.1211	0.3629*	-0.2711
COND			1	-0.4196**	-0.5703	-0.4057*	-0.6005**	0.5365*
GR				1	0.5970**	0.9492**	0.4171	-0.3907
NL					1	0.5747**	0.4765**	-0.3140
VI						1	0.4177	-0.4315**
AAE							1	-0.5534**
AAS								1

**Correlation is significant at 0.01 levels (2-tailed). *Correlation is significant at 0.05 levels (2-tailed). RSE: Reducing sugar of endosperm and embryo, RSC: Reducing sugar of seed coat, MC: Moisture content, TP: Total Phenolics, NL: Average number of leaves, COND: % of electrolyte of leakage, GR: Germination (%), VI: Vigour index, AAE: Total Free Amino acids of endosperm and embryo, AAS: Total Free Amino acids of seed coat

increase in the electrolyte leakage was observed in the seeds stored in open condition, which was gradual in all other storage conditions. The highest electrolyte leakage of 32.21% was observed on 21st day in the seed stored polythene bag at 28±2°C followed by 26.75% in the seed stored at 12±2°C. However, the lowest electrolyte leakage 15.74 was observed in the sealed polythene bag 20±2°C.

There was a significant positive correlation between the germination and vigour index/number leaves; reducing sugar of seed coat and moisture content/total Free Amino acids of endosperm and embryo; Reducing sugar of endosperm and embryo and electrolyte of leakage/Total Free Amino acids of seed coat; Moisture content and Total Free Amino acids of endosperm and embryo; electrolyte of leakage and Total Free Amino acids of seed coat; Average number of leaves and Vigour index/Total Free Amino acids of endosperm and embryo (Table 8). There was a significant negative correlation between the Reducing sugar of seed coat and Reducing sugar of endosperm and embryo/ electrolyte of leakage/ Total Free Amino acids of seed coat; Moisture content and Reducing sugar of endosperm and embryo/electrolyte of leakage; electrolyte leakage and germination/Vigour index/Total Free Amino acids of endosperm and embryo; Total Free Amino acids of seed coat and Vigour index/Total Free Amino acids of endosperm and embryo.

DISCUSSION

Recalcitrant seeds are those which will germinate immediately soon after they are separated from parent plants. It is widely believed that the seeds of Dipterocarpaceae are generally short lived and sensitive to desiccation (Ashton, 1982; Gunn, 1988). Tompsett, 1987) has described two types of seeds in dipterocarps; -one type of seed which could not be dried below 45% moisture content without causing damage, while the 2nd type could be safely dried to 10-15% moisture content. The term “critical water content” has been defined the limit of seed moisture content which seed tissues can be dried without apparent damage. Thus, the critical water content marks the level of dehydration stress that the tissue can tolerate. Dayal and Kavirappa (2000) reported that the critical moisture contents for *Hopea parviflora* and *Hopea ponga* as 26 and 27%, respectively below which they fail to germinate. The recalcitrant seeds are reported to contain high mean moisture content of 67% in *Symphonia globulifera*, 39% *Simarouba amara* 33% in *Hopea odorata* 54% and *Hopea ponga* 54%. The high mean moisture is reported to induce the germination in storage at favourable conditions (Sukesh and Chandrashekar, 2011; Corbineau and Come, 1989), Similar observation were made in the present study with *Vatica chinensis* where the seed did not show germination below the moisture content of 62% in stored condition.

The reducing sugar content increased with lapse of time both in endosperm and embryo and seed coat. The germination percentage of *Vatica chinensis* decline in the seeds incubated at low temperature. This may be due to the chilling effect on the seeds. Many Recalcitrant seeds of dipterocarps such as *Drybalanops aromatica* (Jensen, 1971) *Shorea curtesii, S. platycladus* (Tang, 1971) *Shorea ovalis* (Sasaki, 1976) and *Hopea odorata* (Tang and Tamari, 1973) are reported to suffer from chilling injury. According to Roberts (1972), freezing damage in moist seed is presumably associated with the formation of ice crystals. The reason for the deleterious effect of sub ambient temperature could be due to protein denaturation (Simon *et al*., 1976) or change in membrane thickness and permeability (Wolfe, 1978). It is indicated that lipid peroxidation products in seed increased with of ageing. It may be stated that ageing had a damaging effect on seed membrane and resultantly lipid peroxidation products and electrical conductivity was increased. On the basis of these findings, it is postulated that there is a strong relationship between lipid peroxidation and electrical conductivity of seeds and these factors responded significantly to stress environment. Cell membranes contain high proportions of lipids which are susceptible to oxidative damage

(Sung, 1996). Free radicals are produced in cell membranes during the accelerated ageing treatments which are very harmful to living cells of membranes and may cause membrane damage. This membrane damage results in the ion leakage of membranes of seeds (Parrish and Leopold, 1978). Damaged membranes are also considered to be responsible for the delayed germination percentage and slower relative growth rate (Khan *et al.*, 2003). Our results showed that accelerated ageing treatments leads to the increased lipid peroxidation value. Peroxidative changes in the fatty acid composition of membrane lipids lead to enhanced bilayer permeability, mitochondrial swell and lysis occur in severe cases (Priestley, 1986). Oxygen can add to the fatty acid chain to form peroxides and hydroperoxides. Peroxides undergo cleavage to produce bad smelling aldehydes, ketones and acids. The peroxide value is a measure of the amount of these products (Basra *et al.*, 2003). The increased seed leachates are attributed to cell membrane disruption associated with the loss of membrane phospholipids. The loss of phospholipids in deteriorated seeds is due to lipid peroxidation. There was an increment in total free amino acids and reducing sugar in embryo and endosperm during the course of storage. The increased amino acid content indicated the breaking of proteins. The leaching of soluble sugars and amino acids during the course of incubation was observed by Gupta and Aneja (2004) in soybean verities. They also observed less leakage of sugars and amino acids in cold condition. Similar results were obtained by Copeland and McDonald (1995).

CONCLUSION

There was decrease in germination percentage with decrease in the storage temperature. The moisture contents decreases with storage period. The seed of *Vatica cheninsis* may be stored for only 10 to 15 days under normal condition. There was increase in the reducing sugar content in endosperm and embryo and a decreased in seed coat whereas, total free amino acids decreased in endosperm and embryo and increased in seed coat. Therefore decrease in moisture, changes in carbohydrates and modification of protein may together responsible for the reduction in the viability of the seeds of *Vatica chinensis*.

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