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RESEARCH ARTICLE

FTIR SPECTROSCOPIC ANALYSIS OF BIOCHEMICALS SYNTHESIZED DURING EMBRYOGENY AND EMBRYO DESICCATION IN *SYZYGIUM CUMINII* (L.) Skeels

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ABSTRACT

Seed recalcitrance is a major challenge in the conservation of many tropical species. *Syzygium cuminii* (L.) Skeels is a threatened species due to problems associated with natural regeneration and seed recalcitrance. The study was carried out to understand the physiology and biochemistry of jamun embryos during embryogeny and embryo desiccation. Maintenance of higher moisture content in the embryos was observed throughout embryogeny and lacked an embryo desiccation phase. Seeds germinated immediately after shedding without any dormant phase and desiccated very quickly and no other innate mechanisms in the seeds to prevent water loss. The critical threshold water level in the embryos was found to be 45% and a drop in water content significantly decreased the percentage of germination. The seeds remained viable for ten days and polyembryony was a frequent occurrence. FTIR analysis showed variations in biochemical composition during embryogeny and embryo desiccation. Embryo desiccation resulted in the lack of production of many biomolecules but induced the production of certain other compounds.

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INTRODUCTION

Syzygium cuminii (L.) Skeels is an evergreen tree belonging to the family Myrtaceae, commonly known as jamun, Malabar plum or Indian black berry. It is native to India, Bangladesh, Nepal, Pakistan, Sri Lanka, Philippines and Indonesia. It flowers during April-May and sets seeds in June-July. The species is classified as a threatened one due to problems associated with its natural regeneration and seed recalcitrance. Seeds are highly sensitive to desiccation and lose viability very quickly soon after their shedding. Therefore, the seeds cannot be stored for long period (Engelman, 2011) and it is a major challenge for germplasm conservation. Seed recalcitrance is a major concern in many tropical tree species and it is characterized by the sensitivity of seeds towards desiccation (Berjak *et al*., 2007). The exact molecular mechanism involved in seed recalcitrance has not been studied completely and there are reports highlighting the change in the physiology and biochemistry of recalcitrant seeds during desiccation (Xia *et al*., 2012; Delahaie *et al*., 2013; Kermode *et al*., 2002; Pammenter *et al*., 1999).

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Fourier transform infrared spectrometry (FTIR) is widely used to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated molecular composition or content of the chemical group (Surewicz *et al*., 1993; McCann *et al*., 1992; Eberhardt *et al*., 2007; Egwaikidi *et al*., 2009). The objective of the present investigation was to understand the change in molecular composition during embryogeny and embryo desiccation in the recalcitrant seeds of *Syzygium cuminii*. The work also focused on the change in moisture content of the embryo during maturation and desiccation. The percent of germination at different moisture regimes of the embryo was also studied and the threshold moisture content of the embryo for optimal germination was also recorded.

MATERIALS AND METHODS

Seed collection

Young, mature and just fallen seeds were collected from healthy *Syzygium cuminii* during the month of June. Ten healthy young seeds were randomly taken from the seedlot and were used for the determination of moisture content.

Another ten healthy seeds were dissected to remove embryos for FTIR analysis. From the mature seed lot, twenty seeds were randomly taken for viability test using tetrazolium salt. Ten seeds were randomly taken for the determination of moisture content. Fifty mature seeds were arranged in five rows in a plastic tray containing sterile coir pith and the tray was kept in a growth chamber for germination studies (Fig. 1). Another ten healthy seeds were dissected to remove embryos for FTIR analysis. The same methodology was adopted for just fallen seeds also. The remaining mature seeds were allowed to desiccate under natural conditionsfora period of twelve days. Every three days, ten desiccated seeds were randomly taken for the determination of moisture content. Fifty desiccated seeds were taken for germination studies and ten seeds were dissected to remove embryos for FTIR analysis.

FTIR Spectroscopic analysis

For FTIR analysis, the seeds were thoroughly washed in distilled water and the seed coat was removed. The embryo was chopped and ground in methanol using mortar and pestle. The homogenized sample was centrifuged and the supernatant was taken. The methanol was evaporated in a rotary evaporator and the sample was dispersed in dry potassium bromide. The mixture was mixed in a mortar and pressed at pressure of six bars to form a KBr thin disc. The disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 Infrared spectrophotometer. The sample was scanned from 400 to 4000cm^{-1} for sixteen times to increase the signal to noise ratio.

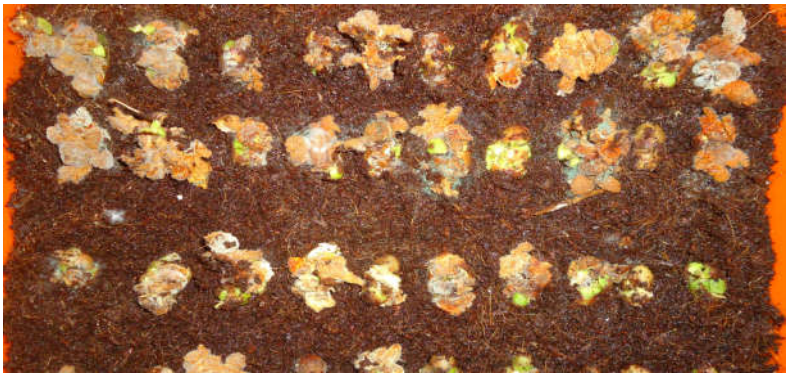

Fig. 1. Seeds of *Syzygium cuminii*arranged in rows in the tray containing sterile coir pith medium

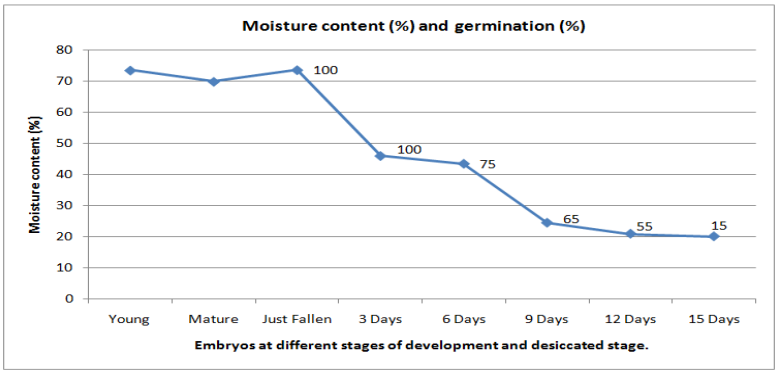

Fig. 2. The moisture content ofthe embryos of *Syzygium cuminii*during embryogeny and desiccation. Percentage of germination at different moisture content has been shown

Fig. 3. Polyembryony is a frequent occurrence in *Syzygium cuminii*. Five seedlings from a single seed

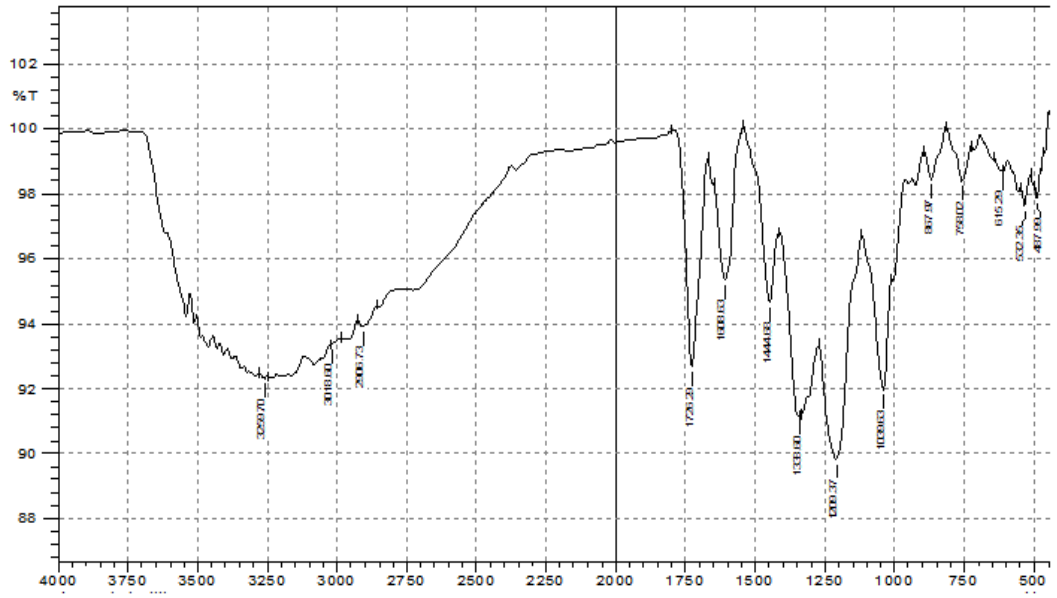

Fig. 4. FTIR Spectrum of young embryonic tissue of *Syzygium cuminii*(methanolic extract)

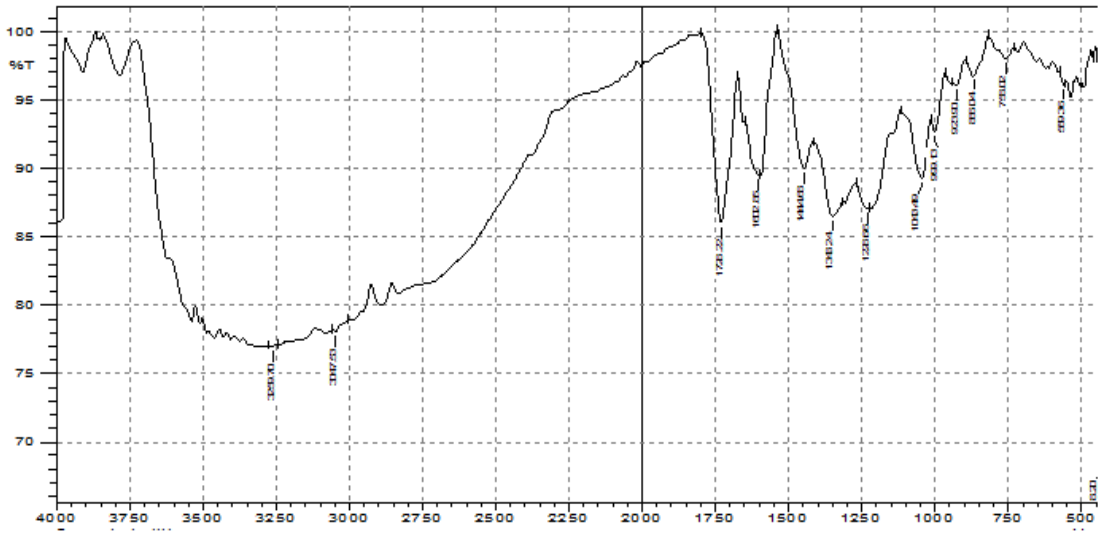

Fig 5. FTIR Spectrum of mature embryonic tissue of *Syzygium cuminii*(methanolic extract)

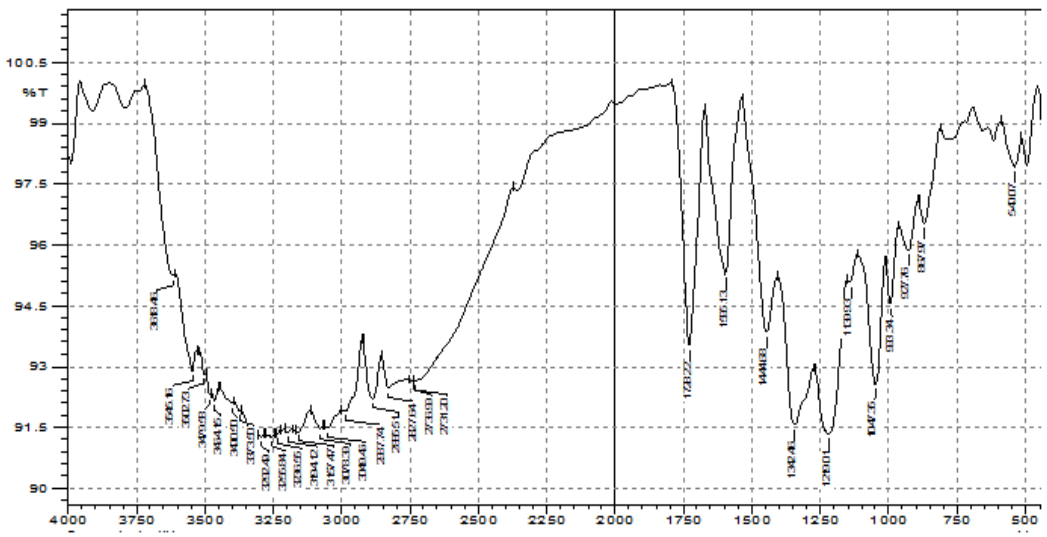

Fig 6. FTIR Spectrum of mature embryonic tissue in the just fallen seeds of *Syzygium cuminii* (methanolic extract)

Samples were run in triplicate and data were collected within one day (Manigandan *et al*., 2015; Marimuthu and Gurumoorthi, 2013)

Statistical analysis

All statistical analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Change in moisture content in the developing as well as desiccated embryos of *Syzygium cuminii*: Significant changes have been observed in the moisture content of embryos during embryogeny and subsequent embryo desiccation in *Syzygium cuminii* (Fig.2).

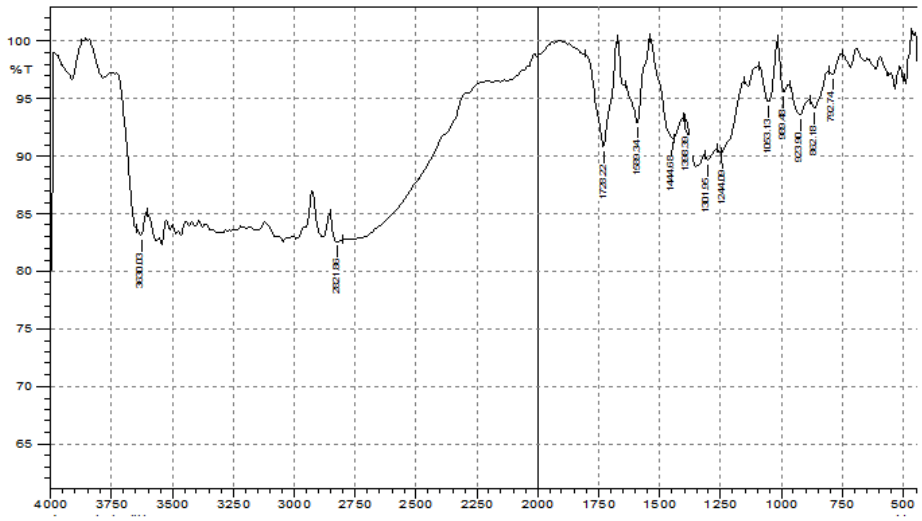

Fig 7. FTIR spectrum of three days desiccated embryonic tissues ofSyzygium cuminii (methanolic extract)

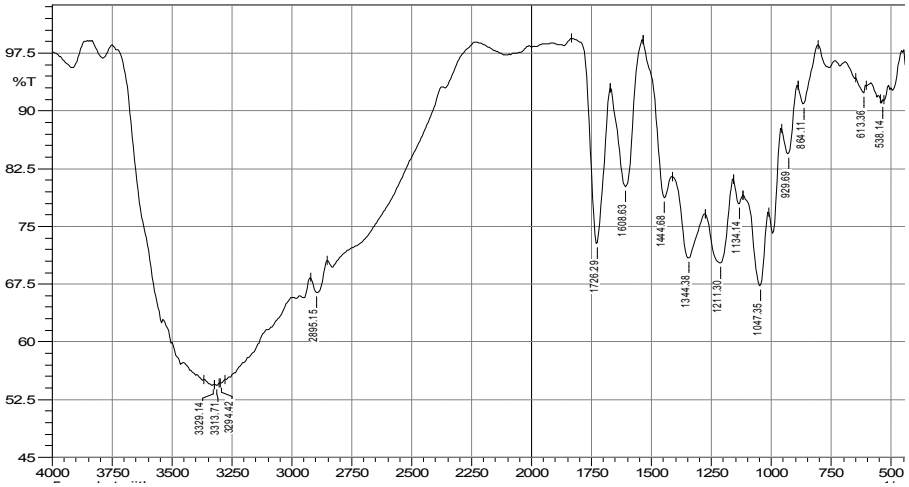

Fig 8. FTIR spectrum of six days desiccated embryonic tissues of*Syzygium cuminii* (methanolic extract)

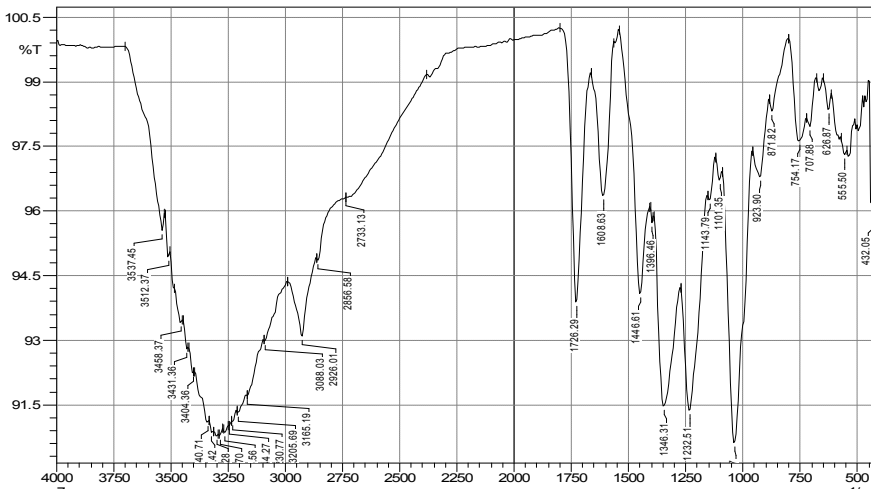

Fig 9. FTIR spectrum of nine days desiccated embryonic tissues of*Syzygium cuminii* (methanolic extract)

Table 1. FTIR Spectral analysis of the embryos of *Syzygium cuminii*

Functional Groups	Stretch/Bend	Stages of seed maturation			Embryo desiccation (days)			
		Young	Mature	Just Fallen	3	6	9	12
Alkanes	C-H stretch			2885.51	2821.86	2895.15	2926.01	
							2856.58	
	CH2 Bend			1047.35	1053.13	1047.35	1446.61	
				993.34	989.48	929.69	1035.77	
				927.76				
	CH3 Bend			1444.68	1444.68	1444.68	1446.61	
Alkenes	C-H Strech							
	C=C Strech							
	C-H Bend							
Aromatic rings	C-H Bend			1219.01				
				1139.93				
				1047.35				
				993.34				
				867.97				
	C=C strech			1595.13				
	C-H Strech			3078.39				
Aldehydes	C=O Strech			1728.22			1726.29	
	C-H Strech			2736.99			2733.13	
				2731.2				
Ketones	C=O Strech							
Quinones	O-H strech			3259.7				
	C-O Strech			1602.85				
Esters and Lactones	C=O Strech	1726.29	1728.2	1728.22	1728.22	1726.29	1726.29	
	C-O Strech			1139.93	1053.13	1134.14	1143.79	
Carboxylic acids and alts	O-H strech	3259.7	3259.7	3292.49	3630.03	3329.14		
	C=O Strech	1338.6	1348.2	1595.13	1589.34	1608.63	1608.63	1552.7
		1608.63	1602.9	1342.46	1398.39	1344.38	1396.46	1392.61
					1301.95		1346.31	
	C-O Strech			1139.93	1053.13	1134.14	1143.79	1114.86
								1087.85
Ammonium salts	N-H4 Strech	3259.7	3259.7	3292.49		3294.42	1396.46	1392.61
				3194.12				
	C=O Strech							
Esters	C=O Strech	1726.29	1728.2	1728.22	1728.22	1726.29	1726.29	
	C-O Strech			1139.93	1053.13	1134.14	1143.79	
Amides	C=O Strech							1653
	N-H Strech							
	N-H Bend							
Alcohols	O-H strech	3259.7	3259.7	3545.16	3630.03	3329.14		
				3618.46		3313.71		
				3502.73		3294.42		
Pri/sec/tertiary alcohol	O-H Strech			3618.46	3630.03			
				3545.16				
	C-O Strech			1047.35	1053.13			
				1139.93				
Phenols	O-H Strech			3618.46				
				3545.16				
	C-O Strech			1219.01				
	O-H Bend			1342.46				
Carbohydrates	O-H Strech	3259.7	3259.7	3373.5		3329.14		
				3292.49		3313.71		
						3294.42		
	C-O Strech			1139.93		1134.14		
Ethers	C-O Strech			1139.93	1053.13	1134.14	1232.51	1114.86
					1244.09		1143.79	1087.85
							1101.35	
							871.82	
Pri/secondary amines	N-H strech			3502.73				
				3479.58				
				3464.15				
				3400.5				
	C-N Strech			1342.46				
				1139.93				
				1047.35				
	N-H Bend			1595.13				
Nitriles	C=N Strech	1608.63	1602.9	1595.13		1608.63	1608.63	1653

Continue ……..

Sulphides	C-S Strech						707.88	644.22
							626.87	609.51
Disulphides	S-S Strech	615.29				613.36	432.05	609.51
Polysulphides	S-S Strech	487.99						
Sulphur-oxy compounds	SO2 Strech			1342.46	1398.39	1134.14	1346.31	1114.86
					1301.95	1344.38	1143.79	1392.61
Peroxides	C-O-O- Strech	867.97	866.04	867.97	862.18	864.11	871.82	
Organic Phosphates	P=O Strech	1338.6	1348.2	1342.46	1301.95	1344.38	1346.31	
Aromatic Phosphates	P-O-C Strech	867.97	923.9	993.34	862.18	1211.3	1232.51	
		1209.37	866.04	927.76	923.9	929.69	923.9	
			1228.7	1219.01	989.48	864.11	871.82	
				867.97				
Aliphatic Phosphates	P-O-C Strech	1039.63	999.13	1047.35		1047.35	1035.77	1014.56
			1043.5	993.34				
Halides	C-F	1039.63	1043.5	1139.93	1053.13	1134.14	1101.35	1114.86
				1047.35		1047.35	1143.79	1087.85
							1035.77	1014.56
	C-CI	758.02	758.02		792.74		754.17	798.53
							707.88	
	C-Br	615.29				613.36	626.87	644.22
	C-I	532.35	559.36	540.07		538.14	555.5	514.99
Silicon -oxy compounds	Si-O-C						1101.35	1087.85
	Si-O-Si	1039.63	1043.5	1047.35	1053.13	1047.35	1035.77	
Aromatic nitro compounds	N=O	1338.6	1348.2	1342.46		1344.38	1346.31	1552.7
Aliphatic nitro compounds	N=O							1552.7

Table 2. Phytochemicals detected during embryogeny and embryo desiccation

Functional Groups	Stages of seed maturation			Embryo desiccation (days)			
	Young	Mature	Just Fallen	3	6	9	12
Alkanes							
Alkenes							
Aromatic rings							
Aldehydes							
Ketones							
Quinones							
Esters and Lactones							
Carboxylic acids and their salts							
Ammonium salts							
Esters							
Amides							
Alcohols							
Pri/sec/tertiary alcohol							
Phenols							
Carbohydrates							
Ethers							
Primary, Secondary amines							
Nitriles							
Sulphides							
Disulphides							
Polysulphides							
Sulphur-oxy compounds							
Peroxides							
Organic Phosphates							
Aromatic Phosphates							
Aliphatic Phosphates							
C-F							
C-CI							
C-Br							
C-I							
Silicon -oxy compounds							
Aromatic nitro compounds							
Aliphatic nitro compounds							

The moisture content was as high as 73.47% in the young embryos but it dropped to 69.85% at maturity and later it increased to 73.59% at the time of seed shed. This indicates the recalcitrant nature of seeds as the embryos continue to accumulate water bypassing maturation drying as orthodox seeds do.

Maintenance of higher moisture content during embryogeny is considered to be a unique feature of recalcitrant seeds (Elizabeth Farnsworth., 2000). The just fallen seeds germinated within a period of three days and showed cent percent germination indicating the total viability of seeds in hydrated state.

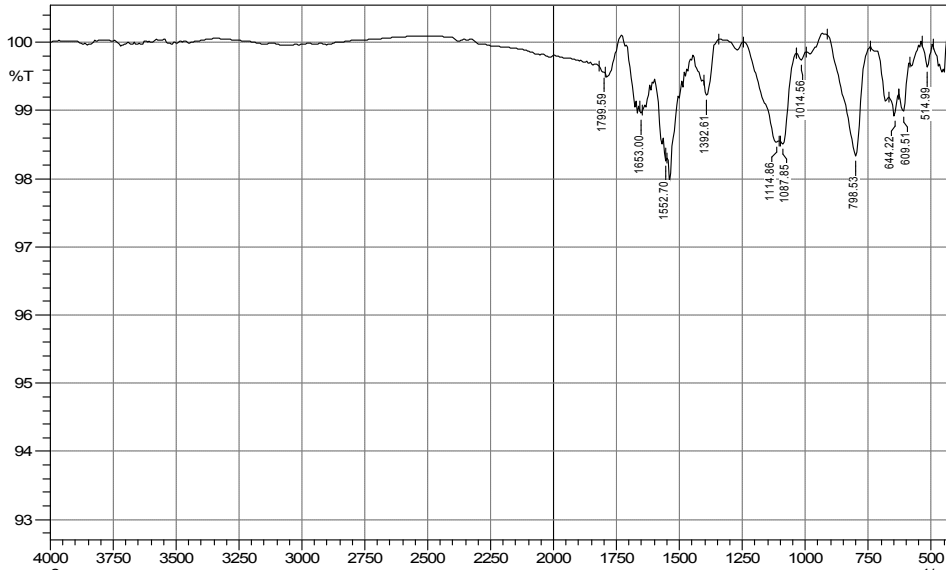

Fig 10. FTIR spectrum of twelve days desiccated embryonic tissues of *Syzygium cuminii* (methanolic extract)

The seeds did not show any maturation drying and the subsequent metabolic quiescence and proceeded directly to the germination phase. This particular physiological behaviour is shown by many recalcitrant seeds (Elizabeth Farnsworth., 2000). The seeds desiccated for three days showed a drastic decrease in the moisture content from 73.59% to 45.97% indicating the lack of innate mechanism to prevent rapid loss of moisture from the embryonic tissues even under natural environmental conditions. However, the drop in moisture content was not accompanied by any change in the percentage of germination indicating the ability of embryonic tissues to maintain growth and development even under mild water stress. Further desiccation did not change the moisture content significantly (43.49%) but decreased the germination to 75%.

This indicates that in the *Syzygium cuminii*, the recalcitrant seeds started losing their viability when the moisture content dropped from a critical threshold level of 45%. It is reported that the seed germination, growth, DNA integrity, protein synthesis, membrane structure, organellar formation, and normal embryo development are disrupted when internal hydration levels drop below critical thresholds (Artlip TS *et al*., 1995; Kermode AR., 1990; MacIntyre GI., 1987; Osborne *et al*., 1994).

The loss of viability in the recalcitrant seeds is usually associated with the change in pressure potentials and the subsequent perturbation in the cell membrane. Hence, it is assumed that the decrease in embryonic pressure potentials could be the reason for the drop in germination percentage.Nine days of natural desiccation decreased the moisture content to 24.51% with a germination percentage of 65. Twelve days of desiccation made a further decrease of moisture content to 20.97% with 55% germination. Further desiccation could not significantly decrease the moisture content (20.14%) but lethal to the viability of seeds with a poor germination percentage of 15. Poor germination during severe drying could be attributed to the fusion of vesicles, vesiculation of the endoplasmic reticulum or free-radical peroxidation of lipid and protein components of cell membranes (Elizabeth Farnsworth., 2000).Polyembryony was frequent in this species in which two, three, four or five seedlings could be observed per seed (fig 3).

FTIR spectral analysis of phytochemicals synthesized during embryogeny and embryo desiccation in *Syzygium cuminii*: Methanolic extracts of embryos of *Syzygium cuminii* during embryo development and desiccation were analyzed with FTIR Spectroscopy and are presented (Fig. 4,5,6,7,8,9,10). The phytochemicals, identified based on the characteristic stretch and bend of their functional groups, have been shown in the tables (1 and 2). The embryos of just fallen seeds showed the presence of alkanes, aromatic ring compounds, aldehydes, esters and lactones, carboxylic acids and their salts, ammonium salts, primary/secondary/tertiary alcohols, phenols, carbohydrates, ethers, primary/secondary amines, nitriles, sulphur-oxy compounds, peroxides, organic phosphates, aromatic phosphates, aliphatic phosphates, C-F and C-I compounds, silicon-oxy compounds and aromatic nitrocompounds. These compounds are assumed to be vital for faster germination and also for preventing microbial infection due to their fully hydrated state. Alkanes, aromatic ring compounds, aldehydes, primary/secondary/tertiary alcohols, phenols, ethers, primary/secondary amines and sulphur-oxy compounds were detected only in the mature embryos of just fallen seeds and these could not be detected in other stages of embryogeny. Natural desiccation resulted in the decreased production or complete inhibition in the synthesis of phenols, primary/secondary/tertiary alcohols, carbohydrates, aromatic ring compounds and primary/secondary amines. Peroxides, organic phosphates, aromatic phosphates, esters and lactones, primary/secondary/tertiary alcohols, carbohydrates, phenols, alkanes were undetected in the embryos desiccated for 12 days. The poor germination of seeds after 12 days of desiccation and the loss of viability could be attributed to the inability of the desiccated embryos to synthesize these compounds.

However, mild desiccation resulted in the production of certain class of compounds in the embryos such as sulphides, disulphides, amides and aliphatic nitro compounds. Amides and aliphatic nitro compounds were synthesized in response to severe desiccation.

Conclusion

The following conclusions are drawn from the present study

- In *Sygium cuminii*, like many recalcitrant seeds, there is a continuous accumulation of water in the embryos during embryogeny. Higher moisture content is maintained throughout embryo development. There is no embryo drying phase during embryogeny in this species.
- Just fallen seeds show immediate germination without any embryo dormancy. The fresh seeds show cent percentage viability and germination but it decreases upon desiccation.
- The critical threshold water content in the embryonic tissue of this species for cent percent germination is 45%.
- The seeds do not have any innate mechanisms to prevent water loss form the embryonic tissues. They desiccate very quickly compared to other recalcitrant species.
- The seeds remain viable for a period of 10 days under natural conditions after that viability is greatly decreased.
- Polyembryony is frequent in *Sygium cuminii*. Two, three, four or five seedlings may develop from a single seed.
- FTIR analysis of embryonic tissues shows variation in biochemical compositionduring embryogeny and embryo desiccation.
- Lack of synthesis of peroxides, organic phosphates, aromatic phosphates, esters and lactones, primary/secondary/tertiary alcohols, phenols, carbohydrates and alkanes in severely desiccated embryos in association with the loss of seed viability.
- Desiccation induces the production amides, aliphatic nitrocompounds, sulphides and disulphides.

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