Indian J. Plant Physiol., Vol. 9, No. 4, (N.S.) pp. 372-377 (Oct.-Dec., 2004)

DESICCATING SENSITIVITY OF *LITCHI CHINENSIS* SONN. SEEDS

J.S. PRASAD AND U.S. PRASAD*

Post Graduate Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur-812 007.

Recieved on 7 May, 2003, Revised on 31 Dec., 2004

SUMMARY

On air-drying, daily loss of moisture content was directly proportional to the degree of loss of seed viability in four cultivars, viz. Deshi, Kasba, Purbi, and Early Bedana of *Litchi chinensis* Sonn. Their inability to withstand desiccation was the most pronounced in Early Bedana and the least in Deshi which may be due to cultivar differences. Moisture loss led to rising trend in starch and total sugar contents in these litchi cultivars. Both α-amylase and invertase enzyme activity exhibited parallel trends in each cultivar. However, in cultivars Deshi, Kasba and Purbi, these enzymes exhibited minimum activity whereas in cv. Early Bedana they increased considerably on the 4th and 5th day of air drying. Concurrently, soluble protein content underwent a rapid linear decline in Early Bedana whereas in Purbi and Kasba decline occurred at a relatively slow rate. On the other hand, in Deshi which retained greater viability, the protein content increased appreciably and was not adversely affected by moisture stress even after 72 hours of air-drying when IAA-oxidase activity declined to its minimum. This was in sharp contrast to Early Bedana exhibiting maximum loss of viability as indicated by no positive response with 2, 3, 5- triphenyltetrazolium chloride even after 12 hours of air-drying.

***Key words*: Desiccation, *Litchi chinensis*, protein, seed viability, starch, sugars.**

INTRODUCTION

Seeds of *Litchi chinensis* Sonn.were widely reported to be reacalcitrant and rapidly lost their viability under desiccation (Menzel 1983, Fu *et al.* 1990, Kumari-Singh and Prasad 1991, Xia *et al.*1992 a and 1992 b). Cull and Paxton (1982) observed viability of litchi seeds for one or two weeks and reported when these were best stored while inside fruits or in moist peat moss in a freezer.

According to Menzel (1985), litchi seeds kept well for four weeks in fruit after harvest but lost viability within a day after separation from the fruit. Prasad *et al.* (1996) achieved optimum litchi seed germination immediately after removal of the seed from the fruit. Notwithstanding several reports on recalcitrant behaviour in the recent past (Chin *et al.* 1984, Fu *et al.* 1990, Xia *et al.* 1992b). Chin *et al.* (1984) reported recalcitrant behaviour of seeds of litchi and several other plant species. Fu *et al.* (1990) examined desiccation pattern, viability prolongation and preservation methods of litchi seeds. Xia *et al.* (1992a and 1992b) studied the effect of desiccation. temperature and other factors on their germination and suggested their most storage, consistent with the observation of Fu *et al.* (1990). Physiological biochemical causes concerning loss of viability of litchi and other seeds still remain to be thoroughly investigated which form the basis for the present study.

MATERIALS AND METHODS

Fruits of four cultivars Deshi, Kasba, Purbi and Early Bedana of *Litchi chinensis* Sonn. were procured from the College of Agriculture, Sabour, Bhagalpur (India) for

* Corresponding author

the present work. They were collected in the morning hours between 7-10 A.M. from the trees which were 15 years old or more (Deshi 16 years, Kasba 20 years, Purbi 15 years and Early Bedana 21 years). Thier physical attributes are presented in Table 1.

Eight hundred seeds of each cultivar, soon after separation were divided into two lots in equal numbers, one lot being placed in a humidity chamber (70% RH at 35°C) and another lot at room temparature (35+2°C) and 70% RH. Seed viability was tested at 0, 2, 4, 6, 8, 12, 24, 48 and 72 hours after removal (HAR) from the fruit by placing seeds without seed coat in 0.1% 2,3,5-triphenylterazolium chloride (TTC) for 24 hours in the dark. A red colord embryonic axis (positive TTC response) was considered as the criterion for seed viability and the percentage of seed with colored axes was noted. Degree of dehydration of seeds at room temperature was assessed by weighing them separately and percent moisture content was deatermined each day upto 5 days after removal (DAR) from the fruit.

On each day, embyonic axis tissue of dehydrating seed was homogenized in a pre-chilled all-glass mortar and pestle and subjected to biochemical analysis.

Precipitated protien by trichloroacetic acid was dissolved in sodium hydroxide and bluse colour developed with Folin-Ciocalteu reagent was read at 600 nm (Lowry *et al.* 1951). For estimation of total sugar, the homogenate was deproteinized by zinc sulphate and sodium hydroxide. After centrifugation at 3000 rpm for 15 minutes, the clear supernatant was used for determination of total sugar by phenol-sulphuric acid method when orange colour developed (Dubios *et al.* 1956) was read at 490 nm. Starch content from the residue was digested in 72% perchloric acid and then blue colour developed by anthrone reagent was read against reagent blank at 625 nm (McCready *et al.* 1950).

α-amylase was assayed at 37°C according to the method of Bernfeld (1955). The reaction mixture consisted of 1% starch solution in phosphate buffer (0.02M, pH 6.9; 0.7mM anhydrous $CaCl_2$). After addition of I-KI solution (Iodine 254 mg, KI 4.0 g dissolved in 100 ml distilled water), optical density was read at 620 nm. The difference between the optical densities of control and experimental tubes gave the enzyme activity which was expressed as mg starch hydrolysed mg^{-1} protein h^{-1}. For the assay of invertase, the reaction mixture consisting of 0.4M sucrose in acetate buffer (pH 4.8) and the enzyme source was incubated for 30 min. at 37°C and the reducing sugar content was determined by the method of Somogyi (1945). The difference between experimental and cotrol tubes gave the enzyme activity which was expressed as mg glucose mg^{-1} protein h^{-1}. For the assay of indole-acetic acid (IAA) oxidase, the reaction mixture consisting of 0.15 mM IAA and 0.1 mM magnesium chloride in acetate buffer was incubated at 37°C for 1h. The amount of IAA oxidized mg^{-1} protein h^{-1} was estimated at 530 nm with Salkowski reagent (Gordon and Weber 1951).

Table 1. Physical characteristics of litchi seeds

Culti-vars	Fruit shape	Fruit wt. (g)	Rind colour	Pulp thickness, (cm) at base	at apex	Seed coat colour	Seed surface	Seed length, (cm)	Seed wt. (g)
Deshi	Oblong	14.56	Red	0.9	0.3	Dark brown	Plain	2.8	3.0
Kasba	Conical with blunt apex	18.61	Blood red	0.9	0.4	Dark brown	Plain	4.1	3.2
Purbi	Conical with pointed apex	16.32	Red	0.7	0.3	Dark brown	Plain	2.2	2.4
Early Bedana	Oval	14.20	Reddish green	1.3	1.1	Blackish brown	Granulated, chicken tongue	1.4	0.9

All the chemicals used were of analytical grade. Data presented at each stage were mean of six replicates (± standard error, S.E.). Data were subjected to randomized block design and analysis of variance (ANOVA) with the help of Indostate satatistical package according to Snedecor and Cochran (1961).

RESULTS AND DISCUSSION

The loss of viability in the seeds of litchi cvs. Kasba, Purbi and Early Bedana was initiated even at 2 h after removal from the fruits on the basis of TTC-test (Table 2). It continued further at seed storage for longer period but loss of viability was slow in the seeds stored in a humidity chamber (70 % RH). Whether stored in a humidity chamber or at room temperature, Deshi seeds retained viability for longer duration. After seed storage for 8 h, TTC viability in cv. Early Bedana declined to 2 % at room temperature (35 ± 2°C). In other cultivars, TTC test showed a slower decline in seed viability (Table 2). Loss of viability was directly proportional to loss in seed moisture content (Table 3). In litchi cv. Early Bedana seeds were nonviable at 12 HAR both at room temperature as well as in humidity chamber. In cv. Kasba, viability was lost at 48 HAR at room temperature but was retained in Purbi (2 %) and Deshi (57 %) even at 72 HAR at room temperature (Table 2). Thus, litchi seed in general and Early Bedana in particular was sensitive to moisture loss. In the present study loss of moisture in the seed varied from 35.3 % in Deshi to 48.6 % in Early Bedana on 5 DAR with a total loss of TTC-response (Table 2). Thus, litchi cultivars were unable to withstand desiccation below 50 % moisture content and the seeds of Early Bedana were the most sensitive to moisture loss which consequently had a profound effect on biochemical changes (Table 3). On 1 DAR, seeds of all cultivars showed rise in starch content and reached its maximum on 5 DAR in the seeds except Early Bedana. The rising pattern of starch content did not present a true increase as it occurred due to loss in internal moisture content of seed tissue.

Similarly, the apparent increase in total sugar content in dehydrating seeds of the four litchi cultivars was associated with rapid moisture loss. On all days (from 0 DAR to 5 DAR) Purbi had the highest sugar content followed by Deshi, Early Bedana and Kasba in decreasing order. Koster and Leopold (1988) studied the relationship betweep soluble sugar content and desiccation tolerance of the axis of germinating seeds in soybean, pea and maize. According to them the accumulation of reducing sugar in a drying seed could lead to Maillard reaction causing protein and nucleic acid damage which in turn terminated the viabilities of seeds.

The fresh seeds of Early Bedana (0 DAR) were different from the remaining three cultivars in having a high protein content which was high on 1 DAR but progressively declined from 2 to 5 DAR. In sharp contrast, Deshi seeds showed a low protein content on 1 DAR which increased and reached its maximum value on 5

Table 2. Percentage of TTC-positive seeds of litchi cultivars under two different environmental conditions

Hours after removal from the fruit (HAR)	In humidity chamber (70 % RH, 35°C)				At room temperature (35 ± 2°C)			
	Deshi	Kasba	Purbi	Early Bedana	Deshi	Kasba	Purbi	Early Bedana
0	100	100	100	75	100	100	100	75
2	100	90	100	75	100	90	95	65
4	100	65	100	63	100	62	95	55
6	100	65	100	36	100	62	90	25
8	100	60	92	04	100	55	70	02
12	100	55	85	00	97	50	55	00
24	90	42	58	00	85	40	55	00
48	80	12	38	00	65	00	45	00
72	65	00	12	00	57	00	02	00

Table 3. Changes in contents of starch, protein, total sugars and percentage moisture contents and α-amylase, invertase and IAA-oxidase in dehydrating seeds of four litchi cultivars.

DAR*	Cultivar	Starch (mg 100^{-1} mg tissue)	Total Sugar (mg 100^{-1} mg tissue)	Moisture content (%)	α-Amylase (mg starch hydrolysed mg^{-1} protein h^{-1})	Invertase (mg glucose mg^{-1} protein h^{-1})	IAA-oxidase (mg IAA-oxidised mg^{-1} protein h^{-1})
0	Deshi	28.0 ± 2.0	2.6 ± 0.2	60.2 ± 6.2	2.5 ± 0.0	2.3 ± 0.1	31.5 ± 0.7
	Kasba	28.9 ± 2.5	2.5 ± 0.2	59.1 ± 4.6	0.8 ± 0.0	7.2 ± 0.1	112.9 ± 0.4
	Purbi	27.7 ± 2.5	3.6 ± 0.2	52.2 ± 3.4	1.5 ± 0.1	7.5 ± 0.1	55.7 ± 1.5
	Early Bedana	29.2 ± 2.1	2.4 ± 0.2	52.4 ± 4.6	0.8 ± 0.0	1.9 ± 0.0	18.2 ± 0.4
1	Deshi	32.8 ± 3.1	4.0 ± 0.3	52.6 ± 5.5	1.8 ± 0.0	3.9 ± 0.1	55.9 ± 0.9
	Kasba	32.7 ± 3.5	3.0 ± 2.8	51.5 ± 3.9	0.7 ± 0.0	3.1 ± 0.0	92.0 ± 1.6
	Purbi	28.9 ± 2.8	4.6 ± 0.3	57.3 ± 3.4	2.4 ± 0.0	3.7 ± 0.1	76.4 ± 1.1
	Early Bedana	33.1 ± 3.8	4.1 ± 0.4	46.9 ± 4.6	1.7 ± 0.3	2.7 ± 0.1	10.6 ± 0.6
2	Deshi	35.1 ± 3.6	6.3 ± 0.5	49.5 ± 3.4	0.4 ± 0.0	3.0 ± 0.1	53.9 ± 0.0
	Kasba	33.8 ± 3.1	2.9 ± 0.1	48.1 ± 3.5	0.6 ± 0.0	2.1 ± 0.1	18.9 ± 0.5
	Purbi	30.5 ± 3.2	6.8 ± 0.5	47.1 ± 4.0	1.1 ± 0.0	1.3 ± 0.1	116.6 ± 3.0
	Early Bedana	30.3 ± 3.5	6.2 ± 0.5	46.0 ± 4.0	1.7 ± 0.0	4.6 ± 0.1	24.0 ± 0.5
3	Deshi	35.9 ± 4.1	6.4 ± 0.5	44.7 ± 3.4	1.0 ± 0.0	5.3 ± 0.1	16.1 ± 0.8
	Kasba	35.5 ± 3.8	4.9 ± 0.3	43.9 ± 3.9	0.6 ± 0.0	2.0 ± 0.1	33.1 ± 0.7
	Purbi	34.4 ± 3.6	7.0 ± 0.4	43.8 ± 3.0	1.3 ± 0.2	1.6 ± 0.1	189.8 ± 3.1
	Early Bedana	32.8 ± 2.9	6.3 ± 0.5	41.2 ± 3.3	1.0 ± 0.0	7.1 ± 0.0	64.9 ± 1.8
4	Deshi	36.1 ± 4.0	7.4 ± 0.4	42.5 ± 2.9	0.4 ± 0.0	2.8 ± 0.1	1.2 ± 0.1
	Kasba	35.9 ± 3.9	4.5 ± 0.3	42.1 ± 2.9	0.2 ± 0.0	1.4 ± 0.1	92.1 ± 0.6
	Purbi	35.2 ± 3.2	9.9 ± 0.7	37.9 ± 2.9	1.1 ± 0.0	1.6 ± 0.1	102.1 ± 1.6
	Early Bedana	36.8 ± 4.1	6.8 ± 0.6	36.2 ± 3.1	1.7 ± 0.1	7.8 ± 0.2	161.3 ± 1.2
5	Deshi	37.1 ± 2.4	7.4 ± 0.5	39.5 ± 3.3	0.4 ± 0.0	0.3 ± 0.0	1.5 ± 0.4
	Kasba	46.7 ± 3.3	7.1 ± 0.6	39.7 ± 4.1	0.1 ± 0.0	1.3 ± 0.1	55.7 ± 1.1
	Purbi	36.5 ± 3.4	9.4 ± 0.7	34.7 ± 2.9	0.1 ± 0.0	1.1 ± 0.1	269.8 ± 3.6
	Early Bedana	36.2 ± 4.7	7.2 ± 0.6	34.5 ± 2.7	4.9 ± 0.0	17.6 ± 0.2	284.7 ± 1.5

*Days after removal of seed from fruit

DAR exhibiting high protein content on all days and underwent a slow decline on desiccation. It is, thus, evident that in spite of cultivar differences, increasing dehydration resulted in increased proteolysis in litchi seeds except Deshi. The rising trend in protein content of Deshi suggested the retention of the protein synthesizing system in spite of moisture-stress. Deshi seeds therefore were better adapted to withstand desiccation, as was evidenced by greater viability on the basis of TTC response.

α-amylase exhibited its highest activity either on 0 DAR (i.e. freash seeds as in Deshi and Kasba) or on 1 DAR in Purbi and Earli Bedana (Table 3). Enzyme activity in Kasba, in comparison to other cultivars was low on all days and whereas in Early Bedana, it was high on 1 DAR, declined on 2 DAR and 3 DAR when no TTC response was observed but increased again on the last two days of dehydration (i.e. 4 DAR and 5 DAR). Advancing desiccation of seeds might have caused breakdown of

membrane system which in turn led to an increase in contact between the substrate and the enzyme exhibiting greater amylosis on 4 and 5 DAR in Early Bedana. Consistent with this view, Krishnamurthy *et al.* (2000) reported increase in membrane damage under water stress in black pepper.

Among the litchi cultivars tested, Early Bedan exhibited an increasing trend of invertase activity which reached its highest on 5 DAR when the seed did not show a positive TTC response. This observation of Early Bedana coincided with high α-amylase activity as well as greater loss in moisture content, being higher on 4 and 5 DAR, as compared to other cultivars. Consistent with observation on litchi cv. Early Bedana, Saxena *et al.* (1985) also reported increased invertase activity on accelerated ageing of sesamum seeds. High enzyme activity in litchi seeds suggested production of reducing sugars which might lead to a Maillard reaction in drying seeds, eventually terminating seed viability, as emphasized in other seeds (Koster and Leopold 1988).

The fresh litchi seeds (0 DAR) of cv. Kasba exhibited highest IAA-oxodase activity which declined to its minimum on 2 DAR (Table 3). On the other hand, in Early Bedana this enzyme, after its low activity on 0 and 1 DAR, underwent a rapid increase on 4 and 5 DAR. However, the enzyme activity underwent a considerable increase upto 2 DAR in Deshi and upto 3 DAR in Purbi before declining in an identical manner on 4 DAR in these cultivars except Kasba. On 5 DAR, maximum IAA-oxidase activity was observed in E. Bedana followed by Purbi but activity in Deshi was very low.

Deshi seeds with low IAA-oxidase activity on 5 DAR could, therefore, withstand desiccation to a greater extent than the other cultivars in which high IAA-oxidase activity might be related to the rapid loss of viability as in Early Bedana seeds as a result of progressive desiccation. In black pepper, activities of certain enzymes like peroxidase and polyphenol oxidase have been observed to increase under water stress (Krishnamurty *et al.* 2000). Several other reports on IAA oxidase activity on seed storage and germination are also available. Rodriguez and Tames (1982) studied the activities of peroxidase and IAA oxidase enzymes in relation to optimum pH, stability against temperature and time of storage in seeds of *Cicer arietinum*. In a study of salt (sodium chloride) stress on seedling growth in relation to salt tolerance of three cultivars of *Ipomea batatas*, Ke and Pan (2002) observed increase in peroxidase and IAA oxidase activities which were more in salt sensitive cultivars than tolerant ones. Qiang *et al.* (2004) investigated the combined effect of cadmium and UV-B radiation and reported inhibition of root growth along with significant decline in peroxidase and IAA oxidase activities which were reported to be crucial for soybean growth. All these reports are more or less consistent with the present study on litchi seeds.

It may be concluded that greater sensitivity to desiccation of litchi seeds in general and cv. Early Bedana in particular brought about apparent increase in starch content and true increase in sugar as a result of amylosis and invertase action before commencement of final loss of seed viability. Results suggested a role for IAA-oxidase which appeared to be a regulatory factor in litchi seed viability and it seems possible to utilise some of the parameters as a measure of water stress. However, it needs to be further elaborated in a precise manner.

ACKNOWLEDGEMENT

The authors are thankful to the Principal and the Head of the Dept. of Botany, T.N.B. College, Bhagalpur University, Bhagalpur-812 007 (India) for providing necessary laboratory facilities.

REFERENCES

Bernfeld, P. (1955). Amylases α and β. In: S.P. Colowick and N.P. Kaplan (eds.), Methods in Enzymology, Vol. 1, 149-158. Academic Press, New York.

Chin, H.F., Hor, Y.L. and Lassim, M.B.M. (1984). Identification of reclacitrant seeds. *Seed Sci. & Technol.* **12**: 429-436.

Cull, B.W. and Paxton, B.F. (1982). Litchi (*Litchi chinensis*). *Yearb West Aust. Nut Tree Crop Asoc.*, 7: 13-21.

Dubois, M., Gilles, K.S., Hamilton, J.K., Robers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350-356.

Fu, J.R., Zhang, B.N., Wang, X.F., Qiao, Y.Z. and Huang, X.L. (1990). Physiological studies on desiccation, wet storage and cry preservation of reclacitrant seeds of three fruit species and

their excised embyonic axes. *Seed Sci. & Technol.* **18**: 743-754.

Gordon, S.A. and Weber, R.P. (1951). Modern Methods of Plant Analysis VII. Springer-verlag, Berlin.

Ke, Y. and Pan, T. (2002). Effects of NaCl stress on seedling growth and IAA metabolism of sweet potato and its relation to salt tolerance. *Ying Yong Sheng Tai Xue Bao*. **13**: 1303-1306.

Koster, K. and Leopold, C. (1988). Sugars and desiccation tolerance in seeds. *Plant Physiol.* **88**: 829-832.

Krishnamurthy, K.S., Ankegowda, S.J. and Saji, K.V. (2000). Water stress effects on membrane damage and activities of catalase, peroxides and superoxide dismutase enzymes in black pepper (*Piper nigrum* L.). *J. Plant Biol.* **27**: 39-42.

Kumari-Singh, A. and Prasad U.S. (1991). Dehydration pattern and viability loss in seeds of two cultivars of litchi (*Litchi chinensis* Sonn.). Seed Res. **19**: 41-43.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin-phenol reagent. *J. Biol. Chem.* **193**: 265-271.

McCready, R.M., Guggoiz, J., Siluira, V. and Owens, H.S. (1950). Determination of starch and amylase in vegetable application of peas. *Anal. Chem.* **22**: 1150-1158.

Menzel, C.M. (1983). The control of floral initiation in lychee: a review. *Scientia Hort.* **21**: 201-215.

Menzel, C.M. (1985). Propagation of lychee: a review. *Scientia Hort.* **25**: 31-48.

Prasad, J.S. (1991). Physiological and Biochemical Studies on Litchi Seed Germination. Ph.D. thesis, Bhagalpur University, Bhagalpur, India.

Prasad, J.S., Raj Kumar, Mishra, M., Kumar, R., Singh, A.K. and Prasad, U.S. (1996). Characteristics of litchi seed germination. *Hort. Sci.* **31**: 1187-1189.

Qiang, W., Yang, H., Chen, T., An, L. and Wang, X. (2004). Effect of the combination of cadmium and UV-B radiation on sybean growth. *Ying Yong Sheng Tai Xue Bao*. **15**: 697-700.

Rodriguez, R. and Tames, R.S. (1982). Peroxidase and IAA Oxidase in germinating seeds of *Cicer arietinum L. Rev. Esp. Fisiol.*, **38**: 183-188.

Saxena, O.P., Pakeeraish, T. and Lakshmi. P. (1985). Studies on accelerated ageing in sesamum. *Indian J. Plant Physiol.* **28**: 35-42.

Snedecor, G.W. and Cochran, W.G. (1961). Statistical Methods, Allied Pacific Private Limited, Bombay.

Somogyi, M. (1945). A new reagent for estimation of sugars. *J. Biol. Chem.* **160**: 61-68.

Xia, Q.H., Chen, R.Z. and Fu, J.R. (1992a). Effects of desiccation, temperature and other factors on the germination of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan* Steud.) seeds. *Seed Sci. & Technol.* **20**: 119-127.

Xia, Q.H., Chen, R.Z. and Fu. J.R. (1992b). Moist storage of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan* Steud.) seeds. *Seed Sci. & Technol.* **20**: 269-279.