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ISSN 2320-5407 International Journal of Advanced Research (2013), Volume 1, Issue 10, 304-309

ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

SEED RECALCITRANCE: A MAJOR CONSTRAINT IN THE REGENERATION OF A CRITICALLY ENDANGERED MANGROVE SPECIES *CYNOMETRA IRIPA* KOSTEL

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Manuscript Info

Manuscript History:

Received: 12 November 2013
Final Accepted: 18 November 2013
Published Online: December 2013

Key words:
Mangrove, *Cynometra iripa* Kostel., Seed recalcitrance.

Abstract

Cynometra iripa Kostel is one of the narrowly distributed mangrove species in India. It belongs to family Caesalpiniaceae without typical vivipary or cryptovivipary. Natural regeneration of the species is very poor. The seeds are recalcitrant, lacking seed dormancy. The present paper reports eco-physiological aspect of seed recalcitrance in the species.

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Introduction

Seed dormancy is an adaptive trait that improves survival of the next generation by optimizing the distribution of germination over time. There are critical checkpoints at the transactions from dormancy to germination and from germination to growth. Most of the species exhibit a critical capacity to control the timing of their reproduction and the establishment of a new generation of off-springs. For exhibiting the dormancy the seed tissues show outstanding feature of desiccation.

Non dormant or desiccation intolerant seeds are of two types - viviparous and recalcitrant. Plant species in which the embryo grows sufficiently to enlarge visibly from within the seed tissues before dispersal are termed as viviparous (Tomlinson, 1986). In another set of species the embryo sustains metabolic activity throughout ontogeny but bursts the seed tissues shortly after dispersal. In natural populations, these seeds may germinate readily within the fruit or soon after dehiscence, and they do not persist in the soil bank. These type of embryos rapidly loose viability if they are dried or chilled; hence they are termed as recalcitrant to storage (Roberts, 1973). The term recalcitrant is generally applied to seeds that have been systematically tested to determine their ability to tolerate desiccation (Ellis et al., 1985). The inability to store seeds of these species creates challenges for germplasm conservationists; foresters concerned with tropical and temperate forest regeneration and restoration ecologists. Therefore, in devising seed storage schemes, substantial efforts are essential to systematically diagnosing types and causes of recalcitrant behaviors (Pritchard et al., 1996).

Material and Methods

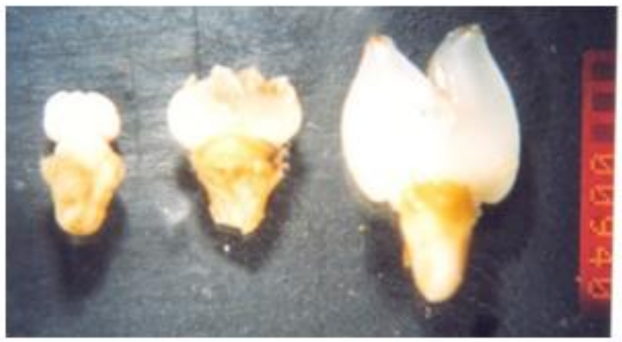
The fruits of *Cynometra iripa* Kostel were collected from the population at Achara estuary (Sindhudurga District of State Maharashtra, India). Fruits are non-dehiscent single seeded pods; fruit wall and seed coat are fused. Therefore, entire fruit is the propagatory material. Different developmental stages of fruits were identified as follows (Figure I).

Stage	Description
I	About a month after fertilization, fruit 0.9 to 1.2 cm in length with greenish, photosynthetic fruit wall, embryo axis and cotyledons are clearly visible and separable.
II	About three months after fertilization, fruits 1.5 to 2.1 cm in length with brown

colored tough fruit wall lacking chlorophyll.

III At the time of fruit shading (about 5.5 to 6 months after fertilization), Fruits mature, 2.5 to 3.5 cm in length with tough, leathery, thick fruit wall without chlorophyll.

Figure 1: Three stages of embryo development considered for present study



Fruits of respective stage were brought to the laboratory, embryo axis and cotyledons were separated and analyzed for proline (Sadasivam and Manickam, 1991), proteins (Lowry et al., 1951), free amino acids (Sadasivam and Manickam, 1991), carbohydrates (Nelson, 1944), α-amylase (Sadasivam and Manickam, 1991), Lipid peroxidation (Cacmak and Horst, 1991) and Abscisic acid (ABA) contents (Nayyar and Walia , 2004; Wang et al, 2002). Moisture contents were estimated by oven drying the embryo and cotyledons separately in oven.

The mature fruits were kept in the lots of 30 each, in polythene bags under laboratory conditions. Three lots differently were analyzed for moisture content and germination percentage immediately and after each week of storage. Germination was studied on soil. Root: Shoot ratio was studied at the interval of 5 days.

Results and Discussion

Regeneration of mangrove species proceeds along the four different lines viz. vivipary, crypto-vivipary, seed germination on soil and vegetative mode of propagation. Physiology and ecology of vivipary and crypto-vivipary in mangroves is well discussed topic. But regeneration of species in which seeds germinate on soil, is crucial. The present paper reports ecophysiology of seed recalcitrance in *Cynometra iripa*. It is one of the back mangal narrowly distributed in India. It is critically endangered at some places (Bhosale, 2002). It requires standardization of protocols for large scale regeneration in the estuarine ecosystem.

Phenology of *C. iripa* is discussed by Chavan and Gokhale (2013). At the time of fruit shading, seeds exhibit fully mature embryos (Figure II). If the mature fruits kept in moist conditions the seeds germinate and radicle emerges out (Figure III). Observations on Root: Shoot ratio in the seedlings, up to 65 days are recorded in Table 1. Here root length increases but strikingly shoot length (Plumule) remain constant almost up to a month. Later on it starts to increase and grow very slowly.

Immediately after fruit fall, the seeds exhibit about 50% moisture content. But the seeds get dry very rapidly and after storage of a month the moisture levels decrease up to 21%. As per decrease in the seed moisture content, germination percentage also decreases. It is to be noted here that after the period of about 3 weeks (21 days) seeds cannot germinate. Therefore 37% seed moisture level is the critical checkpoint in retarding germination in *C. iripa* (Table 2).

Stage wise physiological analysis of seeds was carried out separately for embryo axis and cotyledons.

Concentration of proline, proteins and free amino acids is depicted in Table 3. Embryo axis is rich in proline than cotyledons. Proline accumulation is observed in embryo axis at the time of fruit detachment. In cotyledons, proline content decrease from young stage to mature stage of fruit.

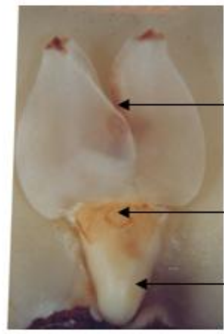


Figure 2: Mature embryo axis at stage III

Figure 3: Growth of radical

Table 1: Observations on Root: Shoot ratio in seedlings up to 65 days of growth.

Days of germination	*Root length	*Shoot length	Root: Shoot ratio	
1	0.3	0.5	0.6	Epicotylar dormancy period
5	2.3	0.5	4.6	
10	2.8	0.5	5.6	
15	3.1	0.5	6.2	
20	3.2	0.5	6.4	
25	3.5	0.5	7.0	
30	3.7	0.5	7.4	
35	4.1	0.6	6.8	Increment in shoot height
40	4.2	0.6	7.0	
45	4.5	0.9	5.0	
50	4.8	1.2	4.0	
55	5.2	1.5	3.4	
60	6.5	1.9	3.4	
65	7.1	2.3	3.0	
*Average of five seedlings				

Table 2: Effect of seed (as a whole, embryo axis + cotyledons) moisture content on germination percentage in *C. iripa*

Time period in days	Water content %	Germination %
1	49±3	70
7	45±5	65
14	37±2	30
21	30±5	00
28	28±2	00
35	21±3	00

Table 3: Concentration of proline, proteins and amino acids in embryo and cotyledons of *C. iripa*

	Proline mg/100 g		Proteins mg/100g		Free amino acids mg/100g	
Stage	Embryo axis	Cotyledons	Embryo axis	Cotyledons	Embryo axis	Cotyledons
I	180	123	2880	2130	790	738
II	137	67	3539	1893	815	530
III	360	58	630	970	2300	351

Table 4: Concentration of soluble sugars, starch and activity of enzyme α- amylase in embryo and cotyledons of *C. iripa*

	Soluble sugars mg/100 g		Starch g/100g		Amylase ΔOD/ hr/mg Protein	
Stage	Embryo axis	Cotyledons	Embryo axis	Cotyledons	Embryo axis	Cotyledons
I	2193	1321	1.030	3.781	0.21	0.01
II	2835	2207	0.958	10.938	1.08	0.35
III	2600	3765	0.840	7.125	0.73	1.70

Table 5: Moisture contents, concentration of Abscissic acid (ABA) and lipid peroxidation in embryo and cotyledons of *C. iripa*

	Water content %		ABA ng/1g fresh wt		Lipid peroxidation M mole MDA/g fresh wt	
Stage	Embryo axis	Cotyledons	Embryo axis	Cotyledons	Embryo axis	Cotyledons
I	43	51	61.24	228.02	50	60

II	60	57	76.00	80.30	80	90
III	47	63	108.20	15.39	190	130

Embryo axis is also rich in proteins and free amino acids than cotyledons. Both, proteins and free amino acids are decreasing as per development in cotyledons.

Soluble sugars, starch contents and α-amylase activity is recorded in Table -4. Highest concentration of soluble sugars is recorded in cotyledons at maturity. Cotyledons are rich in starch also.

Activity of an enzyme α-amylase is increased in cotyledons but in embryo axis primarily it is low, then increases and again decreases.

Table 5 depicts the moisture content, concentration of ABA and lipid peroxidation. In cotyledons moisture content increase as per the development of fruit, but in embryo axis it decreases at maturity. ABA accumulates in embryo axis at maturity while it slowly decreases in cotyledons. Lipid peroxidation increases in both as per the progress in development. Embryo axis shows maximum lipid peroxidation at maturity.

The results suggest that, physiological behavior of embryo axis and cotyledons is different. At maturity, cotyledons start starch hydrolysis at very faster rate when due to accumulation of ABA embryo axis becomes physiologically inactive. Nutritionally rich cotyledons readily attacked by insects and fungi followed by drying and seeds fail to germinate.

Root: Shoot ratio in the seedlings and accumulation of ABA in embryo axis provide sufficient ground to classify it as physiological epicotylar dormancy. It is prevalent in other species *Cynometra cauliflora* L. (Jayasuria et al., 2012).

Effect of seed moisture content on germination percentage suggests the desiccation intolerance of seeds. This phenomenon is also observed in *Cynometra bauhinifollia* Benthan by Nazario et al. (2008).

One unique phenomenon was observed in *Cynomera iripa* during field visits. The seeds in the fruits matured during heavy rain, germinate when the fruits are attached to the mother plant similar to crypto-vivipary or vivipary. If these fruits are kept in moist conditions remain viable up to 25 to 30 days. During storage, radical grows but plumule remains as it is.

As a backmangal, *C. iripa* has narrow ecological amplitude in the costal ecosystems. Moreover, desiccation intolerance and epicotylar dormancy may be the prevalent reasons behind rarity of species. Further research is needed to break physiological epicotyle dormancy to ensure regeneration of the species.

Acknowledgements

M. V. Gokhale is thankful to University Grants Commission, New Delhi, for providing generous grant.

References

Bhosale, L.J., Banik, S., Gokhale M.V. and Jayapp M.A. (2002). Occurrence of *Xylocarpus granatum* Koen and *Cynometra iripa* Kostel along the coast of Maharashtra. J. Econ. Taxon. Bot. 26, 82-87.

Chavan, N.S. and Gokhale, M.V. (2013). Phenological events of critically endangered mangrove species along the coast of Maharashtra. In : Bhatt J.R., Ramakrishna, Sanjapp M., Remadevi O.K., Nilaratha B.P. and Venkataraman K. (eds) Mangroves of India : their biology and uses, Zoological Survey of India, XVI, 640 pp.

Cakmak, I. and Horst, J.H. (1991). Effect of alluminium or lipid peroxidation, superoxide dismutage, catalase and paroxidation activities in root tip of soybean (*Glycine max*). Physiologia Plantarum, 83 : 463-468.

Ellis, R.H., Hong T.D. and Roberts E.H. (1985). Handbook of Seed Technology for Genebanks Volume I. Principles and Methodology. Rome, Italy. IBPGR.

Jayasuria Gchan K.M.G., Wijetunga A.S.T.B., Baskin J.M. and Baskin C.C. (2012). Physiological epicotyl dormancy and recalcitrant storage behaviour in seeds of two tropical Fabaceae (Subfamily Caesalpinioideae) species. AoB PLANTS : Pls 044, doi : 10.1093/aobpla.

Lowry, O.H., Rosenbrough, N.T., Farr, A.I. and Randall, R.J. (1951). Protein Measurement with Folin Phenol Reagent. J. Biol. Chem. 1993 : 262-275.

Nayyar, H. and B.P. Walia (2004). Genotypic variation in wheat in response to water stress and abscisic acid induced accumulation of osmolytes in developing grains. Agronomy Crop Science. (190) : 39 to 45.

Nazario, P., Ferreira A.D.N., Reboucas, E.R. (2008). Germination of *Cynometra bauhiniifolia* Benthan (Jutairana) seeds in terms of desiccation and maintenance under humid conditions. Acta Amazonica Vol. 38 (3) 439-444.

Nelson, N. (1944). A Photometric adaptation of the Somogy method for the determination of glucose. J. Biol. Chem., 153 : 375-380.

Pritchard, H.W., Tompse H.P.B. and Manger K.R. (1996). Development of a thermal time model for the quantification of dormancy loss. Seed Science Research. 6 : 127-135.

Roberts, E. H. (1973). Predicting the storage life of seeds. Seed Sci. Technol. 1 : 499-514.

Sadasivam, S. and Manickam, A. (1991). Biochemical Methods for agricultural Sciences, Willey Eastern Limited.

Tomlinson, P.B. (1986). The Botany of Mangroves Cambridge University Press, 419 pp.

Wang, Z. S., Mambelli and E.L. Setter (2002). Abscisic acid metabolism in maize kernels in respect to water deficient at early endosperm development. Ann. Botany. 19 : 623-630.