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ORIGINAL ARTICLE

Influence of Desiccation and Associated Metabolic Changes During Seed Germination in *Corypha umbraculifera* Linn.

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Corypha umbraculifera L. is a monocarpic palm, which usually flowers after 30-60 years of growth. In *C. umbraculifera* seeds are the only propagating unit, but the germination rate is very low and seeds are highly recalcitrant. In this context, it was imperative to investigate the desiccation sensitivity, physiological and biochemical changes accompanying the desiccation and germination in *C. umbraculifera* seeds. Therefore, to make a detailed study, freshly collected *C. umbraculifera* seeds were desiccated at room temperature for a period of 35 d and physiological and biochemical changes during desiccation and germination were monitored at an interval of 7 d. It was observed that there was a sharp decline in the moisture content of the seed as desiccation proceeded. As the desiccation period progressed, the germination percentage decreased which was below 50% after 35 d. The dry weight percentage of the embryo and endosperm increased with the desiccation period and the increase in dry weight of embryo was significant in comparison with the endosperm. Total protein content of embryo was more compared to that of the endosperm. Peroxidase activity in the embryo was increased up to 28 d of desiccation and decreased further. The endosperm registered a gradual reduction of peroxidase activity during desiccation. In contrast, SOD activity in the embryo was comparatively higher in the fresh seeds and further declined during desiccation, while that of the endosperm remained almost unaltered. The results give a strong indication that desiccation in *C. umbraculifera* is accompanied by abundant activity of peroxidase in embryo, thereby viability is retained up to 35 days. Whereas, feeble activity of SOD is not seen to be linked with seed viability of *C. umbraculifera.*

Key words: Corypha umbraculifera, desiccation, Peroxidase, SOD

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In the entire flora, palms ranks second in economic importance. *Corypha umbraculifera* L., commonly called the “Talipot Palm” belongs to the palm family (Arecaceae), is a native of SriLanka and South India. In Kerala it is represented in Malappuram, Kozhikode, Palakkad and Kottayam district (Renuka, 1999).

This plant is at the verge of fast depletion in Kerala and has been included under endangered species.

Corypha is monocarpic and usually flowers after 30-60 years of growth. Mature plant occupies large area and therefore under canopy region of this plant becomes uncultivable for any other crops. The scarcity of inhabitable land is another major factor preventing the propagation of this species. In *C. umbraculifera* the seeds are highly recalcitrant and germination rate is very low. Monocarpic nature of *C. umbraculifera* palm, recalcitrant behaviour of seeds, desiccation sensitivity, sparse germination and peculiar seedling morphology are found to be the limitations or obstacles to a detailed study on seed biology of *C. umbraculifera*. In this context a detailed study on desiccation sensitivity and resultant metabolic changes during germination is required to recommend some conservation strategies to preserve the plant from extinction. Hence the objective of the present investigation include the analysis of correlation between desiccation sensitivity, moisture content and germination. Also elucidation of the metabolic changes of both embryo and endosperm in general and stress enzymes in particular during desiccation and following germination is examined.

MATERIALS AND METHODS

The fruits of *C. umbraculifera* were collected from Azhinjilam, Kozhikode district in July 2010. Ripe fruits were collected and was dehusked by removing the mesocarp and cleaned in running tap water and these fresh seeds were used for further investigation.

Desiccation Study: The seeds were desiccated in open tray at room temperature (30±3ºC).

Sampling: The desiccated seeds were sampled at an interval of seven days up to 35 days, beyond which the seed viability was decreased and the germination percentage was found to be lower than 50%. Fresh seeds served as the control.

Germination: To test the seed viability, fresh and desiccated seeds were collected and kept for germination in earthen pots filled with sand, soil and dry cow dung in 1:1:1: ratio. The soil in the pot was watered regularly.

Determination of dry weight of tissue: The seed coat of fresh and desiccated seeds were scraped off and further the embryo and endosperm was separated. The embryo and endosperm from 100 seeds were pooled together and sampled at specific interval. Dry weight of embryo and endosperm were carried out by oven dry method.

Analysis of Protein: Total Protein and soluble protein content of embryo and endosperm tissues of all the samples were analysed following the method of Lowry et al., (1951).

Enzyme Assay: Assay of guaiacol peroxidase activity was done according to Abeles and Biles (1991). Superoxide dismutase was assayed following the method of Giannopolitis and Ries (1977). All experiments were conducted at least five times and mean values ± standard error was tabulated.

RESULTS

Percentage of germination: Fresh seeds germinated relatively faster and showed higher percentage of germination. In general, the germination percentage of seeds was between 53-73% during the period 0-35 d of desiccation. Further, the germination percentage decreased below 50% (Table 1).

Moisture content: The fresh seeds contained 33% moisture content and during desiccation moisture content was decreased gradually and after 35 days only one half was retained. (Table 1).

Dry weight of tissues: The embryo of fresh seeds showed 40% of dry weight and it gradually increased in successive stages of desiccation and become nearly 80% after 35 days of desiccation. The dry weight of endosperm of fresh seed was 74% and it was gradually increased to 83%. The increase in dry weight of endosperm is negligible compared to that of embryo (Fig. 1).

Protein: Total Protein in embryo was more compared to endosperm. Quantity of protein was high

in the embryos of fresh seeds and it gradually decreased afterwards. The protein content of the endosperm remained unaltered up to 21st day of desiccation and later increased significantly.

In the embryo a sharp reduction was observed in the quantity of soluble protein after 7 days of desiccation and afterwards remained unaltered up to 28th day followed by a significant reduction. The endosperm showed very low quantity of soluble protein and the changes were insignificant up to 21st day. But considerable increase was occurred during 28th and 35th days of desiccation (Fig.2).

SOD activity: Superoxide dismutase activity was very high and maximum in the embryos of fresh seeds. But during desiccation up to 7 days, significant reduction was occurred and change in the activity was negligible from 7th day up to 35 days of desiccation. The endosperm showed low activity compared to the embryo and only negligible reduction was observed during desiccation (Fig. 3).

Peroxidase activity: Activity of Guaiacol peroxidise was very feeble in the embryo of fresh seeds. But there occurred a sharp increase on 7th day and only negligible change was noticed up to 14th day. But a significant ($p<0.01$) increase was observed on 21st day followed by significant reduction (Fig. 4).

Peroxidase activity of the endosperm was very feeble compared to the embryo and gradual reduction was occurred during desiccation. Specific activity also showed more or less similar trend in the embryo and endosperm.

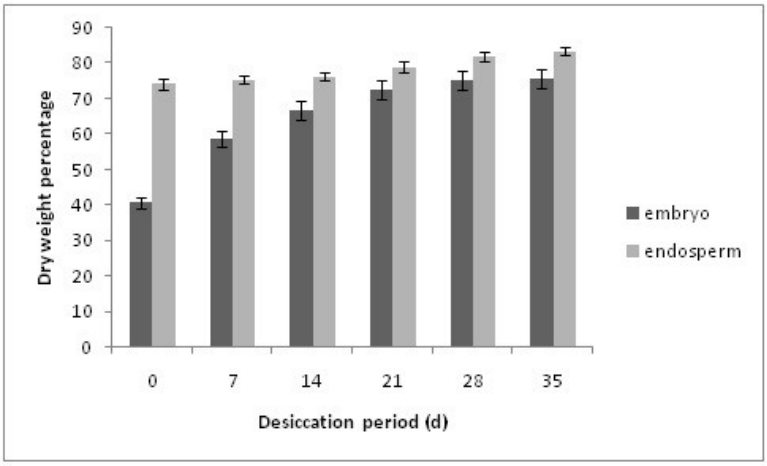

Figure 1: DW percentage of embryo and endosperm of *C. umbraculifera* during different periods of desiccation. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates (i.e. *n*=9).

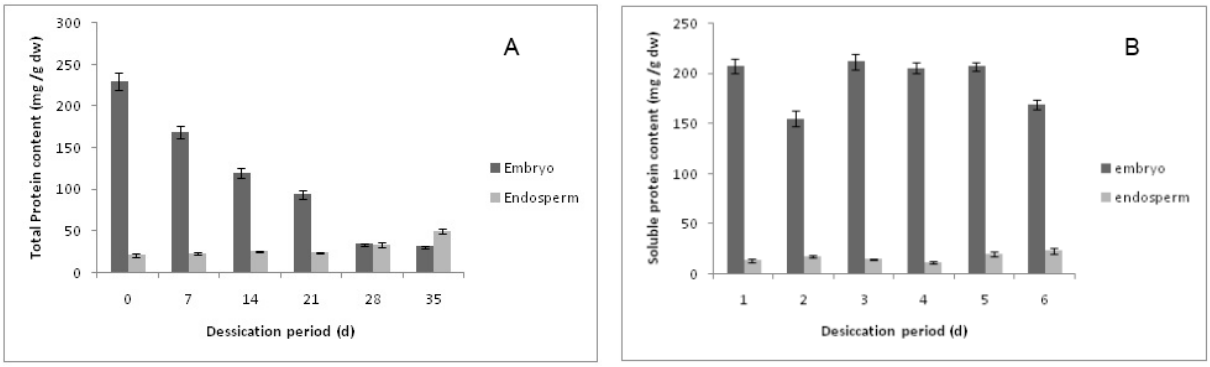

Figure 2: Total protein content (A) and soluble protein content (B) (mg/g d.w) of embryo and endosperm of *C. umbraculifera* during different periods of desiccation. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates (i.e. *n*=9).

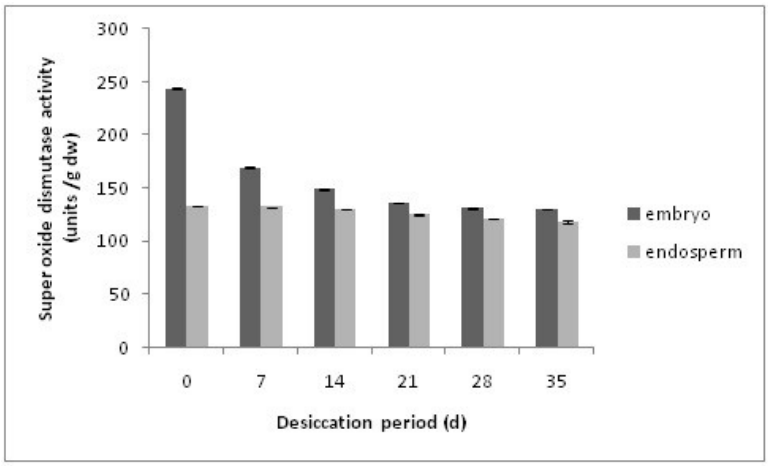

Figure 3: SOD activity (units/g d.w.) in the embryo and endosperm of *C. umbraculifera* during different periods of desiccation. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates (i.e. *n*=9).

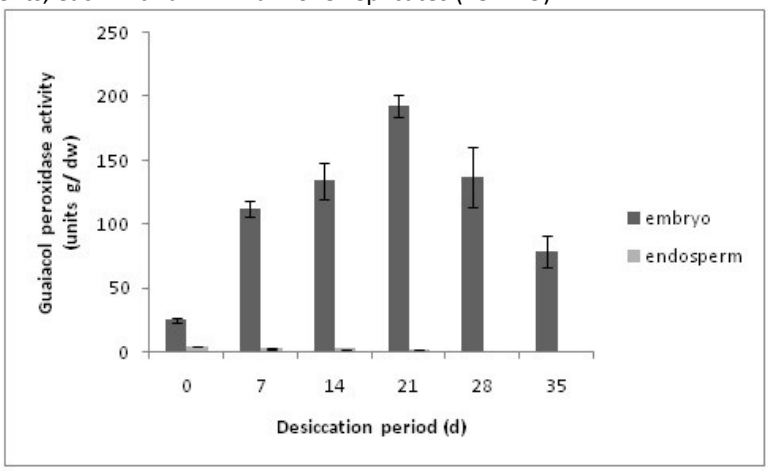

Figure 4: Guaiacol peroxidase activity (units/g d.w.) in the embryo and endosperm of *C. umbraculifera* during different periods of desiccation. The vertical lines represent SE of the mean value of recordings from 3 independent experiments, each with a minimum of 3 replicates (i.e. *n*=9).

Table I- Germination% and Moisture Content of Seeds during desiccation of *C. umbraculifera* seeds. The data is an average of recordings from three independent experiments each with three replicates (i.e. N=9). The data represent mean±standard error.

Desiccation Period(Days)	Germination%	MC%
0	72.77±1.47	32.82±1.45
7	61.66±1.66	31.60±1.56
14	58.33±1.66	28.97±1.24
21	63.88±2.03	24.85±1.36
28	72.22±4.08	19.38±0.91
35	52.77±1.46	16.62±0.68

DISCUSSION

As similar to other palm seeds, germination percentage is very low in *C. umbraculifera* and germination is found to be highly erratic. Despite the recalcitrant behaviour, germination percentage, spread over of germination varies considerably in a population of seed sample (Fig. 1). Wide variation in germination time among palm seeds have been earlier reported by Wagner, 1982 and Endt, 1996 including those from the same environment. Seeds of *Metraxylon warburgii* and *M. vitiense* germinated simultaneously after falling to the ground (Doren, 1997) while in *Ceroxylon ceriferum, Chamaedorea*

elegans, Elaeis guineensis, Gronophyllum ramsayi and *Pelagodoxa henryana,* germination is erratic and several years may be required for all seeds to germinate (Wagner, 1982; Philips 1996). Delayed and erratic germination of palm seeds may be due to dormancy imposed by immature embryo (Kozlowsky and Gong, 1972). According to Broschat and Donselman (1987), an inhibitor may be present in the mature fruit tissue of palm seeds that may prevent normal germination. Depulping shortens the period of germination as suggested by Hnatiuk, (1977). Sometimes dormancy may be related to the surrounding structure such as pulpy or fibrous fruit wall, hard seed coat and endosperm of seeds (Bewley, 1997; Baskin, 1998).

Moisture content of fresh seeds and rate of reduction in moisture content as well as germination rate are not comparable with the characteristics of recalcitrant seeds. But seeds of *C. umbraculifera* are desiccation sensitive because germination percentage is reduced during open air storage (Table I). More over during desiccation the moisture content of embryo is reduced drastically leading to the loss of viability where as moisture content of the endosperm exhibits only negligible reduction revealing the hard galactomannan rich cell, which are dead consisting of long microfibrillar polysaccharides that prevents imbibition (Meier and Reid, 1982).

Most of the palm fruits and seeds have high moisture content at the time of shedding. It was 55% and 33% in the fruit and seed respectively in fresh *Corypha* since fruit wall is fleshy and retain more MC. As the moisture content decreases the viability also decreases.

The total protein content in the embryo decreased during desiccation but the soluble protein content is increased. This may be due to the synthesis of more protein which constitute stress protein and stress enzymes.

The active metabolism in the embryo of control seeds due to the natural desiccation as a part of seed maturation may result in the generation of free radicals. During open air storage and desiccation the resultant activity of free radical scavenging enzyme-peroxidase activity increases proportional to the stress during desiccation whereas superoxide dismutase is very active in fresh seeds and drastically reduced during desiccation.

Generation of free radicals and reactive oxygen species has an established impact of stresses in plants (Halliwell and Gutteridge, 1993). Peroxidases are important stress enzymes involved in the elimination of reactive oxygen (Radotic *et al.*, 2000). In *C.umbraculifera* seeds very high activity of Guaicol peroxidase during desiccation protect the viability of seeds up to 28 days and loss of viability and reduced activity coincides afterwards. SOD also is another stress enzyme which detoxify reactive oxygen species. Nevertheless, in *C. umbraculifera* seeds SOD is significantly reduced during desiccation and apparently do not confer any desiccation tolerance. Similarly, low SOD activity permits the accumulation of superoxides of oxygen which are destined to destroy plant cells (Bowler *et al.*, 1992) and hence the role of superoxides of oxygen cannot be ruled out in the loss of viability of *C.umbraculifera* seeds. However, accumulation of superoxides is not envisaged in the embryo of *C.umbraculifera* seeds because generally superoxides are formed due to misdirected electrons donated to oxygen during metabolic reactions of photosynthesis and respiration (Bowler *et al.*, 1992) and these seeds never perform photosynthesis and respiratory reactions are restricted due to desiccation stress. On the other hand desiccation stress is apparent in the embryo of desiccating seeds, and in the detoxification of the reactive oxygen species, peroxidases are involved. In plants, Guaicol peroxidase is considered as most important in scavenging hydrogen peroxide formed due to stressful condition (Noctor and Foyer, 1998; Zhang *et al.*, 2007).

So the oxidative stress due to reactive oxygen species in the seeds of *C.umbraculifera* during desiccation is found to be imposed mainly due to the abundance of hydrogen peroxide compared to super oxides evidenced by reduced activity of SOD. Activity of SOD and peroxidase enzymes can be compared rather correlated in *C.umbraculifera* seeds in such a way that occurrence of very high activity of SOD in the control seeds revealing the active metabolism due to the recalcitrant behaviour in which development and germination are in continuum as opined by Farrant *et al*.,(1988) and Pammenter *et al*., (1994) and resultant production of H_2O_2 since one of the products of the former functions as the substrate for the latter.

Even though the endosperm consists of non viable cells in *C.umbraculifera,* feeble activity of enzymes like SOD and Peroxidase is observed, plausibly showing the presence of remnant constitutive enzymes which have had been very active during natural desiccation of the seed on the plant resulting in hardening of the endosperm from liquid syncytium texture.

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