RESULTS

1. Fruit Morphology

Fruits of all the five palm species studied are drupaceous. In *Borassus flabellifer*, the fruits are large with a length of about 7-9 cm and a breadth of 6-7 cm at maturity. They are three seeded and are dark purple in colour (Table 3, Fig. 1). The mature fruit of *Corypha umbraculifera* is dark green, 4-5 cm in diameter and contains a single seed while that of *Caryota urens* is purple coloured and double seeded with a fruit size of 5.0 – 5.5 cm length and 3.8- 4 cm breadth. The mature seeds of *Licuala peltata* are smaller, with a diameter of about 1.0 cm and are orange red in colour. Ripe fruits of *Livistona rotundifolia* are orange to dark red, with a diameter of about 2.0 cm. The endosperm is very hard and resembles ivory.

2. Germination Pattern

Fresh seeds of *B. flabellifer* are observed to germinate after about 43 ± 2 days of sowing while those of *C. umbraculifera* took 76 ± 3 days. Of the palms studied, the seeds of *C. urens* required the longest period to germinate, taking about 127 ± 4 days. A period of 66 ± 3 days was required for the germination of fresh seeds of *L. peltata* and 60 ± 3 days for those of *L. rotundifolia* (Table 4). All the palms selected for the present study showed remotive type of germination, with some variation in the size of the seedling and in the time taken for the completion of germination (Figs. 2-6).

As germination is initiated, several types of tissues are differentiated successively within the seeds of the palms studied, culminating eventually in the emergence of the seedling.
2.1. Endosperm

The endosperm in all the palms studied is hard and ivory like on maturation. The thickness of the endosperm varies in different species studied and is found to occupy the periphery of the seeds, leaving a narrow cavity in the center. During germination, the contents of the endosperm are depleted gradually, as the haustorium expands. This is most conspicuous in *Borassus flabellifer* and *Corypha umbraculifera* (Figs. 7). The endosperm is converted into a soft pulpy mass during the later stages of germination.

2.2. Embryo

The embryo of the mature seed is very small and is embedded in the endosperm near the apex of the kernel. As the seed germinates, the embryo is differentiated into a haustorium and a cotyledonary sheath that carries the embryonic axis deep into the soil (Figs. 2-6).

2.2.1. Haustorium

The distal part of the cotyledon is enlarged as the haustorium, a suctorial organ, which grows into the cavity of the endosperm and is seen eventually as embedded in the endosperm. It is spongy and fibrous in nature (Fig. 7). The haustorium is globular initially but later becomes bilobed. Towards the later stages of germination, the haustorium occupies greater part of the seed cavity and by consuming the degradation products of the endosperm renders it into a thin peripheral layer.

2.2.2. Cotyledonary sheath

The proximal part of the cotyledon is modified as a cotyledonary sheath or apocole, which elongates geotropically. It carries the embryonic axis and grows to some distance from the seed and gets buried deep in the soil. During later stages of germination, the apex of the cotyledonary sheath is enlarged slightly (Figs. 2-6). The sheath forms a bridge between the
haustorial organ embedded in the endosperm and the embryonic axis seen in the tip region. It forms a leathery and protective covering around the plumular sheath along with the primary seedling leaf. In the later stages of seedling development, the sheath becomes a thin and papery covering with the outer layer becoming brownish.

2.2.3. Plumular sheath

The plumule consists of a sheath enclosing the leafy shoot within (Figs. 2-6). It varies in size, depending upon the palm species. It is rather large in *Borassus flabellifer* and relatively small in others. The outer covering of plumular sheath is very thin, becomes brown coloured at maturity and encloses the primary palmate leaf.

3. Seed Moisture Content

The percentage of moisture content (MC) of fresh seeds of *Borassus flabellifer, Corypha umbraculifera, Caryota urens, Licuala peltata* and *Livistona rotundifolia* has been estimated. The percentage of MC of fresh seeds of all the five species at the time of harvest was very high but was found to decrease as storage and desiccation progressed. The fruits/seeds stored in open conditions at room temperature (RT) showed a gradual and significant decrease in MC throughout the period of storage and was reduced to minimum in the later stages of storage (Table 5A-E, Fig. 8A-E). But such a rapid change in moisture content was not observed in fruits stored in polythene bags, either at RT or at 4°C in a refrigerator. The moisture content of seeds kept in polythene bags at RT and at 4°C showed only negligible decrease throughout the period of storage. The fresh, dehusked/depulped seeds showed a lesser moisture content than the entire fruits. However, the pattern of decrease in moisture content in the de-husked/depulped seeds under the three storage conditions such as open, polythene bags at RT and polythene bags at 4°C was more or less the same as that of the fresh entire seeds.
Of the palms studied, the fresh fruits of *Borassus flabellifer* showed maximum moisture content and it was reduced to half the initial value in approximately 22 weeks when stored open at RT (Table 5A, Fig. 8A). Dehusked seeds showed lesser MC than entire fruits and the rate of reduction in seeds kept open at RT was lesser than that of the entire fruits (Table 5A, Fig. 8A). The seeds kept in polythene bags at RT started germinating within one week and those stored in polythene bags at 4°C showed signs of decay within two weeks. Therefore the MC of such seeds was not estimated.

The fresh fruits of *Corypha umbraculifera* were found to have an initial moisture content of about 60% (Table 5B, Fig. 8B). The moisture content of the fruits stored open at room temperature declined sharply and significantly and was reduced to less than one-third of the original value by 14 weeks. The entire fruits kept in polythene bags at RT and at 4°C exhibited negligible decline in moisture content. Even after 14 weeks of desiccation, the moisture content showed only a slight decline when compared to the initial value. The de-husked seeds, when desiccated under open conditions at room temperature, showed a rapid decline in the moisture content, a pattern similar to that of the seeds with the pulp. However, the depulped seeds stored in polythene bags at room temperature and at 4°C showed a considerably enhanced rate of desiccation than that of the intact fruits. The depulped seeds when stored in polythene bags at RT, showed a significant decline of MC after one week. Thereafter, the reduction became negligible and gradual. On the other hand, those stored in polythene bags at 4°C exhibited a gradual decrease in MC from the initial stage onwards.

In the unpulped seeds of *C. urens*, a rapid reduction in the moisture content was observed when kept open at RT (Table 5C, Fig. 8C). Within two weeks of storage itself, the MC was reduced to less than 50% of the initial value. Subsequent decline in MC was gradual and the value was reduced to one-third of the original value by the 14th week of storage. The MC of the
seeds kept in polythene bags at RT was lost gradually with only an insignificant reduction from stage to stage. The loss of moisture content was found to occur at the lowest rate when the unpulped seeds were kept in polythene bags at 4°C. The depulped seeds of Caryota urens showed more or less the same pattern of moisture loss as the unpulped seeds.

In Licuala peltata the fresh unpulped seeds showed an initial MC of 60% (Table 5D, Fig. 8D). The loss of MC was found to occur at a very high rate and eventually becoming reduced to about one-half of the initial value in when kept open at RT by the 1st week of desiccation itself. A sudden decline in MC was found to occur on the 2nd week but in the subsequent stages, the reduction was found to be more gradual. The value was reduced approximately to one-fourth of the initial value by the 12th week of storage. The reduction in MC content was less rapid in unpulped seeds maintained in polythene bags at RT and by the 12th week the value was reduced to about one-half of that of the fresh seeds. Only negligible loss in MC was noticed in fruits kept in polythene bags at 4°C during the period of study. The depulped seeds showed an initial MC of 37.8%. The pattern of reduction in moisture content under different storage conditions was almost similar to those of unpulped L. peltata seeds.

In Livistona rotundifolia, the fresh entire fruits and depulped seeds exhibited moisture contents of 58.0% and 37.8% respectively (Table. 5E, Fig. 8E). The entire fruits as well as depulped seeds showed a 50% reduction in MC compared to the initial values during a period of 2 weeks storage. Only negligible reduction was observed in the subsequent stages and the pattern of moisture loss in both types of seeds under different storage conditions was more or less similar to that of L. peltata seeds.
4. Percentage of Germination

Germination of the palm seeds under study was found to be highly erratic. It took several months for the initiation of germination in some species. Dehusked / depulped seeds germinated relatively quickly and showed a higher percentage of germination.

Hundred percent germination was found to occur in fresh seeds of *Borassus flabellifer* upon incubation (Table 6A, Fig. 9A). Unhusked seeds stored open at room temperature did not show any reduction in germination percentage up to 6 weeks of desiccation. A slight decline in the value was noted on the 8th week onwards. The germination percentage was found to decrease gradually in the subsequent stages of desiccation and by the 24th week it was reduced to zero. Those stored in polythene bags, either at room temperature or at 4°C, failed to germinate. De-husked seeds kept in the open at RT showed 100% germination for 6 weeks. Thereafter, a decline in the percentage of germination was observed. The seeds were found to germinate, albeit at a reduced rate, for about 24 weeks. All the dehusked seeds kept in polythene bags at room temperature started germinating within one week and those stored in polythene bags at 4°C showed signs of decay within two weeks. Hence, storage of *B. flabellifer* seeds was found to be unsuccessful under these two conditions.

The pattern of germination was very irregular in *C. umbraculifera* (Table 6B, Fig.9B). Fresh seeds with intact pericarp were found to exhibit only 80% germination. When such fruits were desiccated in the open at RT, viability was lost within one week. The fruits kept in polythene bags at RT showed 60% germination after one week but lost viability completely thereafter and those kept in polythene bags at 4°C did not germinate at all. In de-pulped seeds kept open at room temperature, 100% germination was observed only for one week. Subsequently, the percentage of germination
was reduced gradually up to 7 weeks. Dehusked seeds, stored in polythene bags at room temperature, maintained 100% germination up to 3 weeks and even after that a high percentage of germination was maintained up to 14 weeks of storage and beyond. However, the seeds stored in polythene bags at 4°C failed to germinate totally.

Fresh fruits and depulped seeds of *Caryota urens* showed 100% viability when stored in the open at RT for 2 weeks (Table 6C, Fig. 9C). A reduction in the percentage of germination was observed thereafter. The rate of germination was reduced to 20% when fresh fruits were desiccated for 12 weeks. Fruits kept in polythene bags at room temperature were found to lose germinability after storage for 4 weeks while those kept in polythene bags at 4°C retained viability up to 10 weeks of desiccation. After this period, viability of the seeds was found lost. Depulped seeds of *C urens* maintained high percentage of viability after desiccation for 12 weeks in the open at RT. Seeds maintained in polythene bags at room temperature retained high germination percentage up to 24 weeks. The percentage of germination declined below 50% only after the 24th week of storage. Seeds stored in polythene bags at 4°C showed a reduction in viability to less than 30% after 6 weeks.

Unpulped seeds of *Licuala peltata* exhibited only 80% germination initially. The value was reduced to 30% when desiccated for one week in open at RT (Table 6D, Fig. 9D). Germinability was found to decline after desiccation for 3 weeks in seeds stored in polythene bags at room temperature and after 2 weeks in seeds stored in polythene bags at 4°C. Depulped fresh seeds of *L. peltata* showed 75% germination, which was reduced to 30% when desiccated for 3 weeks in the open at RT. Those kept in polythene bags at RT showed gradual reduction of viability up to 3 weeks. Depulped seeds of *L. peltata* kept in polythene bags at 4°C lost the germinability in the first week of germination itself.
Pulpy fresh seeds of *Livistona rotundifolia* exhibited about 78% germination (Table 6E, Fig. 9E). However, in the subsequent stages of storage, the percentage of germination was found to decline and reached 40% by the 4th week. The pulpy seeds stored in polythene bags at RT reached the same percentage of germination (40%) only by the 6th week of storage. In seeds kept in polythene bags at 4°C, rapid reduction in germinability was found to occur and the germination percentage reached a value of 10% in 4 weeks. The depulped seeds retained a higher percentage of germination for 10 weeks and became nonviable afterwards when kept open at RT. In polythene bags at RT, the seeds retained relatively higher percentage of germination for 24 weeks. In the seeds kept in polythene bags at 4°C; the germination percentage was found to be reduced gradually and became nonviable after 6 weeks of storage.

5. Biochemical Studies

5.1. Dry weight

Change in the dry weight percentage of various tissues of the seeds of the palms under study through successive stages of germination was estimated. In the fresh seeds, the endosperm was found to have a higher dry weight percentage than that of the other tissues such as haustorium, cotyledonary sheath and plumular sheath.

In *Borassus flabellifer* maximum dry weight was shown by the endosperm tissue, which exhibited a gradual decline during seedling development (Table 7 A, Fig. 10A). The dry weight of the haustorium was comparatively lesser than that of the endosperm. The values showed an increase followed by a decrease in the final stage studied. The cotyledonary sheath registered minimum dry weight percentage with marginal increase during final stages. Plumular sheath had a higher value of dry weight in
comparison with haustorium and cotyledonary sheath which showed an increase (p<0.01) followed by a significant decrease.

Though the endosperm of *Corypha umbraculifera* exhibited greater dry weight than that of *B. flabellifer*, the pattern of decline during the successive stages of seedling development was more or less similar. (Table 7B, Fig. 10B). The dry weight of the haustorium was lesser than that of the endosperm. The values showed a decline after the 3rd stage of seedling growth. The dry weight percentage of cotyledonary sheath was only half that of the haustorium in the first stage and showed an increase up to the 3rd stage, beyond which the values showed a decline. The plumular sheath showed an insignificant increase in dry weight percentage in the 5th stage but showed a rapid and significant increase (p<0.01) in the next stage of germination.

In *Caryota urens* also, endosperm tissue was found to have maximum dry weight which showed a gradual decline during the successive stages of seedling development. The dry weight of the haustorium in fresh seeds was approximately one-half that of the endosperm. The values showed an insignificant increase up to the 4th stage of germination, followed by a gradual decrease (Table 7C, Fig. 10C). The cotyledonary sheath as well as the plumular sheath of fresh seeds showed lesser dry weight percentage than that of the endosperm. In both the tissues, the dry weight percentage showed an increase in the initial stages of germination and a subsequent decline.

Among the different palm species studied, the endosperm of *Licuala peltata* exhibited the maximum dry weight percentage, which was found to decline gradually, but significantly during successive stages of germination (Table 7D, Fig. 10D). The dry weight percentage of the haustorium was only half that of the endosperm in the initial stage. The value then showed a gradual increase up to the 4th stage of germination and a subsequent decline. The dry weight percentage of the cotyledonary sheath remained more or less
constant throughout the period of development. The dry weight of the plumular sheath showed a slight increase in the 6th stage and remained more or less the same in the next stage also.

*Livistona rotundifolia* also showed a more or less similar pattern of changes in dry weight percentage in different tissues, with the endosperm having the maximum value and plumular sheath exhibiting the minimum. The endosperm showed a gradual but negligible reduction in the dry weight percentage throughout the period of germination where as in the haustorium and cotyledonary sheath, the values showed negligible increase up to the 4th stage and declined significantly afterwards. The dry weight percentage of the plumular sheath remained more or less unchanged (Table 7E, Fig.10E).

When a comparison is made between the dry weight percentage of individual seedling parts of different palms of the present study, significant variation was observed.

### 5.1.1. Endosperm

The dry weight percentage of the endosperm of all the palms such as *Borassus flabellifer*, *Corypha umbraculifera*, *Caryota urens*, *Licuala peltata* and *Livistona rotundifolia* under study was found to decrease steadily and significantly from the initial stages to the final stages of germination (Table 8A, Fig. 11A.).

### 5.1.2. Haustorium

The dry weight percentage of haustorium in all the palms studied showed a general pattern of an initial increase followed by a decrease (Table 8B, Fig. 11B). In *Borassus flabellifer*, the dry weight percentage reached the maximum value in the 5th stage of germination and showed an insignificant decline thereafter. In *Corypha umbraculifera* and *Livistona rotundifolia*, a decline in dry weight percentage was observed after the 3rd stage whereas in
Caryota urens and Licuala peltata a marked decline was noticed after the 4th stage.

5.1.3. Cotyledonary sheath

The dry weight percentage of the cotyledonary sheath showed the same pattern of change as that of the haustorium (Table 8C, Fig. 11C). An increase in the dry weight percentage was observed in the cotyledonary sheath of all the palm seeds studied in the initial stages of germination, with the maximum value being reached in the 5th stage in Borassus flabellifer, 4th stage in Caryota urens, Licuala peltata and Livistona rotundifolia and 3rd stage in Corypha umbraculifera. In the subsequent stage, the values showed a decline, which was significant in C. umbraculifera and L. rotundifolia and insignificant in others.

5.1.4. Plumular sheath

Since plumule development was observed only after the 3rd stage of germination, sampling for analysis started only afterwards (Table 8D, Fig. 11D). In B. flabellifer, the dry weight percentage of the plumular sheath showed a significant increase (P<0.01) in the 5th stage and a rapid decline in the subsequent stages of seedling growth. The same pattern of change in dry weight percentage was observed in the plumular sheath of C. urens. In L. rotundifolia, an initial increase and a subsequent decrease, both negligible, were observed, in the different stages of plumular sheath development. In C. umbraculifera, the dry weight percentage showed significant increase from the 4th stage to the 6th stage. In L. peltata on the other hand, a significant increase (P<0.01) was noticed in the 5th stage of germination followed by a negligible increase in the next stage.

5.2. Galactomannan

Galactomannan was found to be the predominant storage component of the endosperm of the seeds of the palms under study. In L. peltata, pure
mannan was found to be present (Table 9A, Fig. 12A). Galactomannan was measured in terms of the HPLC estimation of its hydrolytic products – mannose and galactose. *B. flabellifer* was found to have the highest amount of galactomannan. In *C. umbraculifera*, endosperm of fresh seeds showed 198 mg g⁻¹ dw. mannose and 34 mg g⁻¹ dw. galactose. During germination, the mannose sugar was depleted continuously throughout the period of seedling growth (P<0.01) and was reduced to 78 mg g⁻¹ dw in the final stages of study. But galactose was only 34 mg g⁻¹ dw and was reduced gradually to 7 mg g⁻¹ dw during final stages (Table 9B, Fig. 12B).

### 5.3. Starch content

Among the different tissues whose starch content was determined, the endosperm and the cotyledonary sheath contained only minimal quantities with the haustorium and plumular sheath having higher amounts.

In *Borassus flabellifer*, the endosperm showed very low amount of starch and was found to decrease insignificantly throughout the process of germination and seedling development (Table 10A, Fig. 13A). The haustorium was found to have very high starch content from the initial stage onwards which increased rapidly up to the 5th stage of germination and declined sharply afterwards. Only lesser amount of starch was observed in the cotyledonary sheath of *B. flabellifer* and the value showed a significant increase up to the 3rd stage of germination and declined afterwards. In the case of plumular sheath, there was a very rapid increase in the starch content in the initial stages, reaching the maximum value at stage 6 and declining in the subsequent stages.

The endosperm of *C. umbraculifera* (Table 10B, Fig. 13B) is found to have very low starch content, which declined gradually during the process of germination. The starch content of the haustorium was greater than that of the endosperm, the values showing an initial increase up to the 3rd stage of
germination and decreasing significantly thereafter. The cotyledonary sheath contained starch in very low amounts and exhibited only negligible variation during seedling development. Among the various tissues, plumular sheath contained the maximum amount of starch, the values peaking at stage 5, and declining significantly afterwards.

The starch content in the endosperm of Caryota urens was also very low and it exhibited a decline at negligible rate throughout the process of germination (Table 10C, Fig. 13C). The haustorium contained a higher amount of starch; the pattern of change during consecutive stages of germination being more or less similar to that of the other two genera mentioned earlier. In the cotyledonary sheath of Caryota urens, a slow and gradual increase in the starch content was noticed up to the 5\textsuperscript{th} stage and a decline in stage 6. The plumular sheath contained remarkably high starch content in the 4\textsuperscript{th} stage at which its differentiation was initiated. The starch content was found to increase very rapidly in the next two stages with the value increasing almost six-fold of the initial value by the 6\textsuperscript{th} stage.

Compared to the other palms studied, Licuala peltata showed lesser amounts of starch in various tissues with somewhat higher values in the haustorium and plumular sheath (Table 10D, Fig. 13D). The starch content in the endosperm declined gradually and insignificantly from initial to final stage. In the haustorium, the starch content was found to increase significantly up to the 4\textsuperscript{th} stage of germination. The value declined rapidly (p<0.01) in the 5\textsuperscript{th} stage and showed a negligible decline in the next stage. In the cotyledonary sheath, maximum value was observed in the 3\textsuperscript{rd} stage with a subsequent reduction.

The pattern of change in the amount of starch in various tissues of L. rotundifolia during germination was almost similar to that of L. peltata (Table 10E, Fig. 13E). The starch content was minimum in the endosperm and
maximum in the haustorium. Endosperm showed a gradual but negligible decline in starch content throughout the period of germination while the haustorium showed an increase in the value up to the 5th stage and a decline thereafter. The starch content in the cotyledonary sheath showed a negligible increase in all the stages of germination and the plumular sheath showed a progressive increase from 4th stage to the 6th stage.

A comparative analysis of individual seedling parts with regard to the starch content in different palms studied showed more or less uniform pattern of changes with minor variations.

5.3.1. Endosperm

Only very low starch content (4-16 mg g⁻¹ dry weight) was observed in the endosperm of fresh seeds. The starch content was found to decline in the endosperm throughout the process of germination and seedling development of all palm seeds studied. The reduction in the starch content was gradual but negligible in all stages of germination (Table 11A, Fig. 14A).

5.3.2. Haustorium

A highly rapid and significant increase in starch content was noticed in the haustorium of the palms under study in the initial stages of germination followed by a significant decline in the later stages (Table 11B, Fig. 14B). Among the different palm seeds investigated, haustorium of B. flabellifer showed very high starch content (134-353 mg g⁻¹ dry weight). The increase in the starch content in the haustorium became highly noticeable up to the 6th stage of germination and the value was reduced sharply to less than half that of the previous stage. A very rapid and significant decrease in the starch content was observed in the subsequent stages of germination.

More or less similar pattern of mobilization was noticed in C. urens even though the starch content in the haustorium was lower than that of the other species. In C. umbraculifera, an increase in starch content was
observed up to the 4th stage and a significant decrease was observed later on (p<0.01). In *L. peltata* and *Livistona rotundifolia*, the starch content was observed to increase significantly up to stage 4 and to decline thereafter (p<0.01).

### 5.3.3. Cotyledonary Sheath

The cotyledonary sheath contained relatively low amount of starch in all the species studied (Table 11C, Fig. 14C). In *B. flabellifer*, the starch content was found to increase significantly up to the 4th stage of germination and then to decline gradually to the last stage. In *C. umbraculifera*, a negligible increase in the starch content was observed up to the 3rd stage of germination and an insignificant decline thereafter, whereas *L. peltata* showed a significant increase in starch content up to the 5th stage and a significant reduction later on. In the cotyledonary sheath of *C. urens*, the slow and gradual increase in the starch content continued up to the 6th stage of seedling development and then was found to decline. In *L. rotundifolia*, a negligible increase in the starch content was observed till the 5th stage of germination and a decline later on.

### 5.3.4. Plumular sheath

The plumular sheath was found to develop only in the 4th stage of germination in all palm seeds studied. In addition, from stage 6 onwards the tissue began to shrink when the leafy shoot developed. The plumular sheath is rich in starch in all the palm seeds investigated, but the amount is less than that of the haustorium. (Table 11D, Fig. 14D). In *B. Flabellifer, C. urens* and *L. rotundifolia*, the value was rapidly and significantly increasing from the 4th stage up to the 6th stage of germination. In the germinating seeds of *C. umbraculifera* and *L. peltata*, the initial spurt in the starch content was followed by a rapid decline in the 6th stage.
5.4. Amylase Activity

The assay for amylase activity during seed germination was carried out in starch rich tissues such as haustoria and plumular sheath of *B. flabellifer* and *Corypha umbraculifera*. Optimal conditions for enzyme assay were standardized.

5.4.1. pH Optimum

The optimum pH for amylase activity was determined by incubating the enzyme assay system in buffers of pH ranging from 4.4-7.8 at interval of pH 0.4 and the maximum activity was obtained at pH 5.0 (Fig. 15A).

5.4.2. Temperature Optimum

When assay was conducted at the optimum pH at different temperatures ranging from 21°C-41°C, highest activity was obtained at 37°C. Hence the optimum temperature for the amylase assay was confirmed as 37°C (Fig. 15B).

5.4.3. Enzyme Proportionality

The assay was conducted at optimum pH (5.0) and at optimum temperature (37°C) using different volumes of the enzyme extract (10% w/v) ranging from 50μl-400 μl. The assay system showed maximum activity at 200 μl enzyme extract. Hence the optimum enzyme concentration for the amylase assay was confirmed as 200 μl of 10% (w/v) enzyme extract (Fig. 15C).

5.4.4. Substrate Saturation

When the assay was conducted at optimum pH, optimum temperature and optimum enzyme concentration and at different quantities of substrate (4% soluble starch) ranging from 100-260 μg/ml, the assay system showed optimum activity at 200 μg ml⁻¹ substrate (Fig. 15D).
There was no significant difference in optimum pH, temperature and substrate and in enzyme proportionality range for the activity of amylase between the different tissues of haustoria and plumular sheath of the two palms studied such as *Borassus flabellifer* and *Corypha umbraculifera*.

**5.4.5. Unit Activity**

The unit activity of amylase in the haustoria showed a rapid and significant increase after the 2\(^{nd}\) stage of germination in *B. flabellifer* seeds and reached the maximum value on the 4\(^{th}\) stage and declined subsequently (Table 12A, Fig. 16A). The unit activity of amylase showed a significant increase in the initial stages of plumular sheath formation (p<0.01) and a decline was observed after stage 5 of germination process (Table 12A, Fig. 16A).

In the haustorial tissue of *C. umbraculifera*, on the other hand, the unit activity showed a continuous and significant increase from the second stage of germination onwards (Table 12B, Fig. 16C). In *C. umbraculifera* the unit activity in the plumular sheath showed a significant increase throughout the period of germination studied (Table 12B, Fig. 16C).

**5.4.6. Specific Activity**

In *B. flabellifer*, the specific activity of amylase in the haustoria was found to increase after stage 2 reaching the highest value in the 5\(^{th}\) stage of germination and declining subsequently (Table 12A, Fig. 16B). In the plumular sheath, the specific activity of amylase showed an increase in the 5\(^{th}\) stage and was found to decrease in the later stages of germination (Table 12A, Fig. 16B). In *C. umbraculifera*, the change in the specific activity of amylase in the haustorium showed a continuous increase throughout the process of seedling growth (Table 12B, Fig. 16D). The increase in the specific activity was negligible in the initial stages, but became more rapid and significant later on (p<0.01). In the plumular sheath of *C. umbraculifera*, the specific
activity showed an insignificant increase in the 5th stage of germination and a slight decline thereafter (Table 12B, Fig. 16D).

5.5. Total Soluble Sugar

A high degree of mobilization of sugars was observed in all seedling parts of the palms chosen for the present study.

In *Borassus flabellifer*, endosperm of fresh seeds was found to possess only minimum amount total soluble sugar and the value showed a significant increase up to the 5th stage of germination followed by a decline in the subsequent stages. In the haustorium, the values continued to increase steadily and significantly till the 6th stage of germination. The total sugar content in the cotyledonary sheath showed significant increase up to the 4th stage. A rapid and significant reduction in the values was observed in the later stages. The soluble sugar content in the plumular sheath showed a significant increase from the 4th stage of germination, at which the tissue began, to the 5th stage. The next stage of plumular sheath development showed nearly three-fold increase in the sugar content (Table 13A, Fig. 17A).

*Corypha umbraculifera* showed more or less similar amounts of total sugar in various seedling tissues as that of *B. flabellifer* in the initial stages. In the endosperm, the total soluble sugar content exhibited a gradual and insignificant decrease from the 1st stage of germination to the final stage. The soluble sugar content in the haustorium was found to increase in the initial stages of germination reaching the maximum value on the 5th stage of germination. The value showed a decline in the next stage. The cotyledonary sheath showed a rapid and significant increase in sugar content up to the 4th stage of germination followed by a significant reduction in the later stages. In plumular sheath, the total soluble sugar content increased rapidly from the 4th stage of germination to the 5th stage and the value remained more or less the same in the next stage (Table 13B, Fig. 17B).
The endosperm of *Caryota urens* exhibited relatively lesser amount of soluble sugars than that of the other palms studied. A significant increase in the total soluble sugar content was noticed in the endosperm up to the 4th stage of germination followed by a significant decline in the later stages. (Table 13C, Fig. 17C). The sugar content in the haustorium showed a steady and significant increase till the 5th stage of germination. A fall in the sugar content was observed in the remaining stage. In the cotyledonary sheath, the increase in the value remained insignificant up to the 3rd stage of germination and became pronounced in the very next stage (p<0.01). A rapid fall in the sugar content was noticed in the next stage with the value being reduced to approximately one-half as that of the previous stage. A progressive increase in the sugar content was noticed in the plumular sheath from the beginning of its differentiation in the 4th stage up to the last stage of seedling growth.

The endosperm of *Licuala peltata*, showed an insignificant decrease in total sugar content during initial two stages of germination (Table.13D, Fig. 17D). A sharp drop in the value was observed in the 2nd stage of germination. The decline in the sugar content was less pronounced in the subsequent stages. The amount of soluble sugar content was found to increase initially in the haustorium with the maximum value being reached during the 4th stage. The value was found to decline subsequently. The increase in the total sugar content observed in the cotyledonary sheath remained insignificant in the initial stages, but became significant in the third stage (P<0.01). A slight increase was found to occur in the 4th stage and the values declined insignificantly thereafter. The total soluble sugar content in the plumular sheath increased significantly from the 4th stage of germination up to the last stage.

In the endosperm of *Livistona rotundifolia*, the total soluble sugar content showed a continuous decrease throughout the period of germination (Table 13E, Fig. 17E). In haustorium, the values showed a steady and
progressive increase till the last stage of germination studied. The sugar content in the cotyledonary sheath showed an insignificant increase in the values in the initial stages and then a rapid increase (p<0.01) in the 4th stage. A sharp reduction was seen in the 5th stage (p<0.01) and remained more or less the same in the 6th stage also. The change in total soluble sugar content in the plumular sheath of L. rotundifolia was similar to that of L. peltata.

A comparative analysis of individual seedling parts with regard to change in the pattern of total soluble sugar content in different palms studied showed significant variations during the successive stages of germination.

5.5.1. Endosperm

The total sugar content showed a steady decrease in the endosperm of Corypha umbraculifera, Licuala peltata and Livistona rotundifolia during the entire period of germination, whereas in Borassus flabellifer and Caryota urens an increase in the sugar content was found to occur in the initial stages of germination followed by a decline (Table 14A, Fig. 18A). In B. flabellifer, there occurred a rapid increase of total sugar in the endosperm up to the 4th stage and a significant decline thereafter (p<0.01). In C. urens endosperm, a significant increase was noticed in the total soluble sugar content up to the 4th stage. A negligible decline in the value was found to occur in the soluble sugar content in the 5th stage and a significant decrease thereafter. In the endosperm of C. umbraculifera, L. peltata and L. rotundifolia the total soluble sugar content showed continuous decrease from the initial stage of germination to the final stage. In L. peltata, the total sugar content showed a significant decrease from the 2nd stage to the 4th stage of germination and the changes in the value in the other stages was negligible and in L. rotundifolia, the decline in the soluble sugar content of the endosperm was gradual and negligible throughout the period of seedling development.
5.5.2. Haustorium

The amount of total soluble sugars in the haustorium increased rapidly during the early stages of germination (Table 14B, Fig. 18B). In *B. flabellifer* and *Livistona rotundifolia* the values continued to increase steadily and significantly till it reached the maximum value in the 6th stage. In *Corypha umbraculifera, Caryota urens* and *Licuala peltata*, the concentrations of soluble sugar content were found to increase initially and to decrease in the later stages. In *C. umbraculifera* and *C. urens* the maximum value was reached in the 5th stage of germination whereas in *L. peltata*, it was attained in the 4th stage itself.

5.5.3. Cotyledonary Sheath

The total soluble sugar content in the cotyledonary sheath of all the palm species studied showed an increase up to the 4th stage of germination and a decline subsequently (Table 14C, Fig. 18C). In *B. flabellifer* and *C. umbraculifera* the total sugar content in the cotyledonary sheath showed nearly three-fold increase by this stage. A rapid and significant reduction in the values was observed in the later stages. In *C. urens*, the increase in the value remained insignificant up to the 3rd stage of germination and became pronounced in the 4th stage (p<0.01). The value declined drastically in the later stages (p<0.01). In *L. peltata* the increase in the total sugar content was insignificant up to the 2nd stage and then showed a sudden increase in the third stage (p<0.01). A slight increase was found to occur in the 4th stage and showed a negligible decrease thereafter. In *L. rotundifolia* the insignificant increase in the values continued up to the 3rd stage of germination and showed a rapid increase (p<0.01) in the 4th stage. The sugar content showed a significant decline in the 5th stage (p<0.01) and remained more or less the same in the 6th stage also.
5.5.4. Plumular sheath

The total soluble sugar content in the plumular sheath increased significantly from the 4th stage of germination up to the 6th stage in all the palm seeds studied (Table 14D, Fig. 18D).

5.6. Reducing Sugars

The total reducing sugar content in the various seed tissues of palm seeds investigated generally showed an increase during the initial stages of seedling growth and showed a decline in the later stages with minor variations.

The reducing sugar content in the endosperm of B. flabellifer was found to increase rapidly up to the 5th stage of germination and to decline later on (Table 15A, Fig. 19A). In the haustorium, the values continued to increase steadily and significantly throughout the process of germination. The cotyledonary sheath exhibited an initial increase in the reducing sugar content, which attained the highest value in the 4th stage and showed a rapid and significant decrease in the subsequent stages. The plumular sheath showed a significant increase in reducing sugar content from the 4th to the 5th stage of germination and subsequently to the 6th stage (p<0.01).

In the endosperm of Corypha umbraculifera, the reducing sugar content was found to increase gradually up to the 5th stage of germination (Table 15B, Fig. 19B). A negligible decline was observed in the last stage. In the haustorium, as in the other palms studied, the values of reducing sugars continued to increase steadily and significantly throughout the period of seedling growth. The cotyledonary sheath showed an increase in reducing sugar content up to stage 4 and a rapid decrease thereafter. In plumular sheath, the reducing sugar content showed a significant increase from the 4th to the 5th stage (p<0.01) and an insignificant increase in the next stage of germination.
As in other palm seeds, the endosperm of fresh seeds of *Caryota urens* contained only very low amount of reducing sugar and during germination it was found to increase rapidly up to the 5th stage (Table 15C, Fig. 19C). A slight decline was observed in the last stage of germination (p<0.01). The amount of reducing sugar in the haustorium of *C. urens* was found to increase initially and to decrease later on. The cotyledonary sheath exhibited an initial increase of reducing sugar up to stage 4 and a rapid decline thereafter and in the plumular sheath, an initial insignificant increase in the value in the 5th stage was found to be followed by a significant increase (p<0.01) in the very next stage.

The amount of reducing sugar in the endosperm of fresh seeds *Licuala peltata* was found to be relatively greater than that of the other palm seeds under study and showed a steady decrease during successive stages of germination (Table 15D, Fig. 19D). In the haustorium of *L. peltata*, the amount of reducing sugar was found to increase initially up to the 4th stage and to decrease in the subsequent stages. An increase in the reducing sugar content was observed up to the 4th stage of germination in the cotyledonary sheath. The values declined in the next two stages. The reducing sugar content of the plumular sheath showed a significant increase throughout the different stages of its existence.

The fresh seeds of *Livistona rotundifolia* had only very low amount of reducing sugar in the endosperm. The value was found to increase gradually during germination (Table 15E, Fig. 19E). A slight decline was observed in the last stage. The reducing sugar content in the haustorium continued to increase steadily and significantly during germination and the maximum value was observed in the 6th stage. In the cotyledonary sheath, the value was found to increase up to the 3rd stage before decreasing significantly till the 6th stage. The plumular sheath of *L. rotundifolia* showed a rapid and significant increase throughout.
A comparative analysis of individual seedling parts with regard to change in the pattern of reducing sugar content in different palm seeds studied exhibited more or less similar changes.

5.6.1. Endosperm

The reducing sugar content in the endosperm of *Borassus flabellifer* was found to increase rapidly up to the 5\(^{th}\) stage of germination (Table 16A, Fig. 20A). A significant decline was observed in the last stage (p<0.01). A similar pattern of change in reducing sugar content was observed in *Corypha umbraculifera*, *Caryota urens* and *Livistona rotundifolia*. In the endosperm of *Licuala peltata*, a steady decrease was observed from the first stage of germination to the last stage.

5.6.2. Haustorium

The amount of reducing sugars in the haustorium increased rapidly during the early stages of seedling development (Table 16B, Fig. 20B). In *B. flabellifer*, *C. umbraculifera*, and *L. rotundifolia*, the values of reducing sugars continued to increase steadily and significantly till it reached the maximum in the 6\(^{th}\) stage of germination. In *C. urens* and *L. peltata*, the amount of reducing sugar was found to increase initially, reaching the highest values in the 5\(^{th}\) and 4\(^{th}\) stages of germination respectively and to decrease in the later stages.

5.6.3. Cotyledonary Sheath

The cotyledonary sheath exhibited the same pattern of changes in reducing sugar content with an initial increase up to stage 4 in all the palms studied and a significant decrease thereafter (Table 16C, Fig. 20C).

5.6.4. Plumular sheath

In the plumular sheath of *Borassus flabellifer*, a significant increase in the reducing sugar content was observed from the 4\(^{th}\) to the 5\(^{th}\) stage of
germination and subsequently to the 6th stage (p<0.01each). The same pattern of change was observed in all the other palms such as *C. umbraculifera, Caryota urens, L. peltata* and *L. rotundifolia* (Table 16D, Fig. 20D).

**5.7. Mobilization of Proteins**

The total protein content in different tissues of the palm seeds studied was comparatively low and showed only minor changes during the successive stages of germination.

In *Borassus flabellifer*, the endosperm showed a continuous decrease in protein content during the process of germination and seedling development (Table 17A, Fig. 21A). The protein content of the haustorium showed an initial increase up to the 3rd stage and a sharp decline in the 4th stage. The decline in the value continued in the next two stages of germination also. In the cotyledonary sheath an initial increase in the protein content was noticed reaching the maximum value in the 2nd stage of germination. The values showed significant decline thereafter. In the plumular sheath, a gradual increase in the amount of protein was noticed in the 5th stage and a decrease afterwards.

In the endosperm of fresh seeds of *Corypha umbraculifera* the protein content showed a significant decrease from the first stage of germination to the final stage studied (Table 17B, Fig. 21B). In the haustorium, the protein content showed an initial increase up to the 5th stage and a decline during the subsequent stages. The values in the cotyledonary sheath were found to increase insignificantly till the 4th stage of germination. The values showed a sharp decrease from the 5th stage onwards (p<0.01). The plumular sheath of *C. umbraculifera* contained only low amount of protein, which showed a slight increase in the 5th stage and a rapid and significant increase in the next stage of seedling development.
The endosperm of *Caryota urens* contained a high amount of protein. The protein content was found to decrease rapidly in the endosperm during the successive stages of seedling development. In the haustorium, the protein content showed an initial increase up to the 4th stage and a decrease in the remaining stages. The protein content in the cotyledonary sheath increased initially up to the 3rd stage and decreased significantly thereafter. The amount of protein in the plumular sheath of *C. urens* showed a continuous decline from 4th to the 6th stage (Table 17C, Fig. 21C).

The endosperm of *L. peltata* contained the highest amount of protein among the different palms studied. The endosperm showed a decrease in the total protein content throughout the period of germination (Table 17D, Fig. 21D). An increase up to the 4th stage was observed in the protein content of the haustorium and a decline during the subsequent stages of germination. The protein content of the cotyledonary sheath was found to increase up to the 5th stage and to decline in the next stage. The plumular sheath exhibited a gradual increase in the amount of protein in the 5th stage followed by a significant decrease.

*Livistona rotundifolia* showed rather lesser amounts of protein in the endosperm. The amount was found to increase during the initial stages of germination and to decrease as germination proceeded (Table 17E, Fig. 21E). The haustorium showed an initial increase in protein content up to the 3rd stage and a decline during the subsequent stages of seedling growth. In the cotyledonary sheath, the values increased initially up to the 5th stage and decreased subsequently. A significant increase in the protein content of the plumular sheath was observed in the 5th stage of germination and in the 6th stage the value was reduced to less than one half of the previous stage.
The various seedling tissues of different palms such as endosperm, haustorium, cotyledonary sheath, and the plumular sheath showed much variation in the pattern of change in total protein content.

5.7.1. Endosperm

Considerable variation was found to occur in the amount of protein present in the endosperm of different palm species under study. *Caryota urens* and *L. peltata* were found to have very high amount of protein, 168 and 175mg g\(^{-1}\) dw respectively. *Borassus flabellifer* contained the lowest amount, about 12 mg g\(^{-1}\) dry weight. The endosperm in all the palm seeds showed a decrease in the total protein content during the process of germination and seedling development (Table 18A, Fig. 22A).

5.7.2. Haustorium

In the haustorium, the protein content showed an initial increase up to the 4\(^{th}\) stage in *Corypha umbraculifera, Caryota urens* and *L. peltata* (Table 18B, Fig. 22B) and a decline during the subsequent stages of germination. The changes were significant in all cases. In *B. flabellifer* and *L. rotundifolia* the protein content increased gradually up to the 3\(^{rd}\) stage beyond which the values declined. The decline in the value was very sharp and distinct in *B. flabellifer*. In *L. rotundifolia*, this decline was gradual.

5.7.3. Cotyledonary sheath

The cotyledonary sheath was found to have high protein content in all palm seeds studied. The values showed an initial increase and a subsequent decline during the period of existence of the sheath. The maximum value of protein in the cotyledonary sheath of *B. flabellifer* was seen in the 2\(^{nd}\) stage of germination. A rapid decrease was found to occur in the later stages. The protein content was found to increase up to the 4\(^{th}\) stage in *C. urens*, and *C. umbraculifera* and up to the 5\(^{th}\) stage in *L. peltata* and *L. rotundifolia*. 

73
Significant decline in the values was noticed in all the palms subsequent to the stages of maximum protein content (Table 18C, Fig. 22C).

5.7.4. Plumular sheath

The plumular sheath in all seeds was found to have a lesser amount of protein than the cotyledonary sheath on a dry weight basis. A gradual increase in the amount of protein present was noticed in the 5th stage of germination in most cases except *Caryota urens* in which the values showed a continuous decline (Table 18D, Fig. 22D). In the next stage the values were found to be decreasing except in *C. umbraculifera* where an increase in the value was noticed in stage 6.

5.8. Protein profile by Gel electrophoresis

SDS PAGE profile of proteins in the endosperm of fresh seeds *Borassus flabellifer* showed the presence of five bands in the gel, with molecular weight ranging from 21.9 KDa to 99.05 KDa (Fig. 23A 1). In the second and fourth stages of germination, no significant change was noticed in the number as well as molecular weight of the bands (Fig. 23A 2-3). In the 6th stage, two additional bands were identified (Fig. 23A 4).

In *Corypha umbraculifera*, no significant variation was noticed in the number and position of electrophoretic bands in the gel during successive stages of germination. In the fresh seeds 10 bands were seen with molecular weight ranging from 16.73- 59.37 KDa. Certain bands were found to disappear while other new bands became visible in the various stages such as 0, 1,3,5 and 6 (Fig. 23B 1-5).

5.9. Change in Lipid Content

The endosperms of the palm seeds selected for the present study were found to have rather low lipid content in fresh seeds (Table 19, Fig. 24). A gradual and progressive reduction in the lipid content was found to occur in
the endosperms of all seeds studied throughout the process of germination and seedling development. The rate of reduction was slow and insignificant. In *Caryota urens*, a rapid and significant decline was observed when germination just started. Thereafter the reduction was observed to be gradual.