DISCUSSION

Wide variations are observed in the fruit/seed morphology, seed viability, storability, mode of germination, time taken for germination and in the biochemical processes associated with reserve mobilization during germination and seedling development in the seeds of the five species of palms selected for the present investigation. Fruits of all the five species are drupaceous (Fig. 1). The drupe of *Borassus flabellifer* is large sized and three seeded, while that of *Caryota urens* is two seeded. Only one seed is found to develop in the fruits of *Corypha umbraculifera*, *Licuala peltata* and *Livistona rotundifolia*. Variation in the number of seeds per fruit is a common character of palms. Koebernik (1971) suggested that, some palm species produce single seeded fruits while others produce multi-seeded fruits with variable number of seeds as in *Scheelea leandroana*. According to Davis (1978) and Padmanabhan and Raghupathy (1981), in single seeded fruits of palms, either only one carpel is fertile or all the three are fertile with two of them getting aborted during development.

The mesocarp varies from fleshy as in *C. umbraculifera* and *C. urens* to very fibrous as in *B. flabellifer*. The endocarp is differentiated into a hard stony structure in all palms in the present study, except *C. urens*. Similar endocarp is seen in *Cocos nucifera* and *Jубaeopsis caffra* (Moore and Uhl, 1982) while in others it is papery and undifferentiated as in *Caryota mitis* (Murray, 1973). Such an undifferentiated papery endocarp is seen in *Caryota urens*.

In the seeds of all palm species included in the present study, the embryo is very small. Such small sized embryos have already been reported
in Corypha umbraculifera (Fisher et al., 1987), Elaeis guineensis (Davis et al.; 1978) and in Phoenix dactylifera (Chandra Sekhar and DeMason, 1988a).

Based on the mode of germination, Gatin (1912) following Martius (1823) divided the palm seeds into two categories such as “germination admotive”, in which the elongation of the cotyledon is slight and “germination remotive”, in which the elongation of the cotyledon is marked (adjacent and remote respectively, according to Uhl and Dransfield, 1987). In remotive germination, proximal end of the cotyledon elongates, grows downwards into the soil after emerging out of the seed, and widens like a sheath, some distance off the fruit/seed. The embryo proper, thus, moves away from the seed to the bottom of the cotyledonary sheath. Remotive type of germination has been reported in palms like Borassus flabellifer (Tomlinson 1961), Phoenix sylvestris (DeMason, 1988) and in Euterpe edulis (Moegenburg, 2003). Some common palms with admotive type of germination include Areca catechu, Cocos nucifera and Archontophoenix alexandreae (Meerow, 1991).

All the palm species selected for the present study are of remotive germinating type and the young seedlings are pushed down to various lengths by the growing cotyledonary sheath, also called cotyledonary petiole or cataphyll (Tillich, 1995) depending upon the species as well as the size of the seed. In B. flabellifer, the cotyledonary sheath is elongated up to 60-90 cm. In C. umbraculifera, the sheath shows an elongation up to 11 cm, in C. urens and L. rotundifolia up to 6-8 cm each and in L. peltata to about 2-3 cm. Tomlinson (1961) observed in Lodoicea that the cotyledonary sheath elongates up to about 3.6 m. According to Tomlinson (1961), the remotive type of germination might be an adaptation to the relatively dry habitat. However, the wide variations among the length of the cotyledonary sheath of five palm species in the present study cannot be directly correlated to the dry
habitat since all are growing under more or less the same climatic conditions. So, the variation in the length and morphology of the cotyledonary sheath appears to be related to the size of the seed/fruit and may not necessarily be related to the habitat.

Germination of fresh seeds of all palms investigated is slow and highly erratic. Seeds of most palms are found to take months for the commencement of germination. The seeds of *B. flabellifer* started to germinate after 43±2 days while those of *C. umbraculifera* took 76±3 days, *C. urens* 127±4 days, *L. peltata* 66±3 days and *L. rotundifolia* 60±3 days (Table 4). The results of the present study on seed germination in palms are in agreement with the view of several authors who observed wide variation in the time taken for the initiation of germination. Most palm seeds are found to be highly erratic in this regard (Hussey, 1958; Koebernik, 1971; Basu and Mukherjee, 1972). Several years have been reported to be needed for the germination of all the seeds of *Acrocomia sclerocarpa*, *Arenga engleri* and *Astrocaryum aculeatum* (Koebernik, 1971).

Removal of the husk/pulp is found to shorten the period required for germination in all the palm seeds studied. Depulped/dehusked seeds germinated more rapidly than those with pulp/husk (Table 6A-E, Fig. 9A-E). According to Broschat and Donselman (1987), an inhibitor may be present in the mature fruit tissues of palms that may prevent easy germination. Removal of fruit tissues is reported to promote palm seed germination (Broschat and Donselman, 1986, 1987; Ehara et al., 2001). Studies on *Chrysalidocarpus lutescens*, *Phoenix roebelenii* and *Elaeis guineensis* have revealed that removal of fleshy pericarp is essential for storage as well as germination (Broschat and Donselman, 1988). Inhibitory effect of pericarp and sarcotesta of *Metroxylon sagu* on seed germination is reported to be due to their impermeable nature or endogenous inhibitors leaching from the seed coat.
tissues (Ehara et al., 2001). The fresh, depulped seeds of *Livistona rotundifolia* germinated within 14 days of sowing while those, which were not depulped, took 60 days to germinate. This is in agreement with the view of Barbier (1985), who noted that seed germination in *Livistona carinensis* occurs within 2 or 3 months of sowing.

As mentioned earlier, fresh seeds of all the palms investigated in the present study are not readily germinable and show varying degrees of dormancy. They took several days for the initiation of germination (Table 4). Kozlowski and Gunn (1972) stated that, as a rule, true seed dormancy is absent among palms and in many instances development of the embryo continues even after fruit ripening. So, the underdeveloped embryo may be the apparent cause of dormancy in palm seeds. Odetola (1987) also suggested that true seed dormancy is generally absent among the members of Palmae. Yet, some species show varying periods of delay in the initiation of germination. Dormancy may be related to the surrounding structures such as seed coat and endosperm or due to unfavourable environmental conditions (Bewley, 1997; Baskin and Baskin, 1998). It may also be physiological, since treatments with plant growth regulators like gibberellins have been proved to promote germination percentage or to increase the rate of germination as reported in *Ptychosperma macarthurii* (Nagao et al., 1980), in the dormant seeds of several species of ornamental palms (Odetola, 1987) and in *Elaeis guineensis* (Chin, 1988).

Morphophysiological dormancy has been reported in palm seeds such as *Caryota mitis* in which germination starts after 40 or more weeks of incubation (Raich and Khoon, 1990), *Livistona kingiana* and *Corypha umbraculifera* (Manokaran, 1979). According to Baskin and Baskin (2001), morphophysiological dormancy is described on the basis of phenology of embryo growth and germination, especially temperature in the habitat during
the period between seed maturation and the time of germination. However, the relationship between phenology of seed maturation and temperature in the habitat, which is believed to affect morphophysiological dormancy, is not applicable in the manifestation of variable duration of dormancy observed in the seeds of palms in the present study, since all the seeds except those of *C. urens* mature during almost the same period of rainy season that follows fruit ripening.

Even though dormancy has been reported in many palm seeds (Odetola, 1987; Kozlowski and Gunn, 1972), only fragmentary information is available on this aspect and most of the views are ambiguous. Instead of the word “dormancy”, several authors have expressed or described the phenomenon as “delay in the initiation of germination” (Nagao *et al*., 1980; Doughty *et al*., 1986; Carvalho *et al*., 1988; Dickie *et al*., 1993).

In addition to depulping/dehusking, various treatments like soaking in water, hot water scarification, application of growth regulators and mechanical scarification have been reported to promote seed germination in *Chrysalidocarpus lutescens* and *Syagrus romansoffiana* (Broschat and Donselman 1986, 1987), *Dypsis lutescens* (Meerow, 1991) and in *Metroxylon sagu* (Ehara *et al*., 2001). According to Doughty *et al*., (1986) and Odetola (1987), mechanical scarification promotes germination of a variety of palms, especially, in which the seed coat is hard. Results of various treatments by these authors are in agreement with the findings of the present investigation, which show that time taken for germination is shortened when dehusked/depulped seeds are used for germination studies.

Baskin and Baskin (2001) proposed that, generally, palm seeds are characterized by morphophysiological dormancy in which germination is initiated after 4 or more weeks. As the name indicates, morphophysiological dormancy is a combination of morphological and physiological dormancy.
According to Côme and Thevenot (1982) and Baskin and Baskin (1991), seeds with morphophysiological dormancy may constitute linear or rudimentary embryos, which have physiological dormancy, and the primary reason for the morphological dormancy is the impermeability of the seed/fruit coat to water and oxygen. Corner (1976) opined that in many single seeded fruits, the embryo is protected by several layers of fruit wall consisting of epicarp, mesocarp and endocarp which are mostly impermeable to water. Fruit wall morphology of palm seeds investigated in the present study is in agreement with the view of Corner (1976) and Baskin and Baskin (2001) because pericarp of all the species consisting of epicarp, mesocarp and hard endocarp is found to be a hindrance for germination.

In addition to the fruit/seed coat, the endosperm of many seeds also is expected to offer considerable constraint to germination. Some seeds contain relatively reduced endosperm causing dormancy in seeds like lettuce (Halmer and Bewley, 1979) and white spruce (Downie et al., 1997). In some seeds it may be a constraint to germination that must be overcome before the radicle can emerge (Bewley, 1997). According to Bewley and Black (1994), the embryos with physiological dormancy cannot generate enough force to penetrate the tissue layers and this mechanical restraint altogether imposes morphophysiological dormancy.

Dehusking and depulping of palms seeds are found to induce earlier germination in all the seeds in the present study. But dormancy is not fully broken by the depulping process due to the constraint of hard endosperm. In the present study, the endosperm of all the seeds is mannan rich and hence very hard and stony due to the non-absorbant nature of the mannan walls. So, the role of endosperm in imposing morphological dormancy in palm seeds cannot be ruled out.
As mentioned earlier, palm seeds in general are characterized by delayed germination, which can be interpreted in terms of physiological dormancy. Even though most of the palm seeds are with high moisture content, the physiological dormancy is seemed to be correlated to water relations. Stored seeds, irrespective of the storage conditions, show significant reduction in MC, resulting in earlier germination (Table A-E). Wood and Pritchard (2003) reported earlier and enhanced germination in stored seeds of bottle palm \((Hyophorbe lagenicaulis)\) in comparison with fresh seeds. This observation confirms the physiological role of water relations in the manifestation of morphophysiological dormancy in palm seeds.

Occurrence of physiological dormancy in palm seeds is indirectly evident in the studies on \(Ptychosperma macarthurii\) treated with 1000 ppm GA resulting in accelerated germination (Nagao et al., 1980; Doughty et al., 1986). Induction of germination by treatment with 1000 ppm GA was reported in \(Chrysalidocarpus lutescens, Elaeis guineensis\) and \(Phoenix roebelenii\) (Broschat and Donselman, 1988).

Germination of palm seeds is reported to be erratic by several authors (De Leon, 1958; Koebernik, 1971; Meerow, 1991). The germination of all the palm seeds investigated in the present study show inconsistency in the initiation of germination as well as the germination behaviour. The dormancy in palm seeds is attributed to the delay in the initiation of germination. The seed/fruit morphology leading to dormancy is seemed to be imposed by the pericarp of the fruit consisting of fleshy/fibrous mesocarp and hard endocarp layers. In addition to this, the hard and stony endosperm also is involved in imposing seed dormancy. In addition to morphological dormancy, physiological dormancy associated with water relation is also very evident in the palm seeds included in this study.
Seeds of many important plantation crops inclusive of palms are grouped under recalcitrant/intermediate category due to their short lifespan and desiccation sensitivity. The inability to store palm seeds is a serious problem since vegetative propagation is not possible for most of the palms.

Palm seeds included in the present study are short lived and storage conditions are found to have significant effects on the storability of *Borassus flabellifer*, *Corypha umbraculifera*, *Caryota urens*, *Licuala peltata* and *Livistona rotundifolia*. According to De Leon (1958), palm seeds are generally short lived and often lose viability after storage for 2 weeks to 3 months. The results obtained show that storability and viability of palm seeds are affected by factors like nature of the seeds sown, whether entire or dehusked/depulped, and storage period/conditions. Depulping also shows significant effect on storability in the seeds of all the species kept under different conditions. For instance, the depulped seeds could be successfully stored for more than a year in sealed polythene bags kept at room temperature as observed in *C. umbraculifera*.

Of the three different storage conditions employed, seeds maintained in polythene bags at room temperature yielded maximum storability since these seeds are less vulnerable to desiccation stress than the fresh seeds kept in the open at room temperature. According to Broschat (1994), seeds of many palm species lose viability within 3-6 weeks of harvest due to the deleterious effects of desiccation, revealing their recalcitrant nature. The seeds of all palms kept in polythene bags at 4°C did not germinate at all, except that of *C. urens* and *L. rotundifolia*. These results are in agreement with the view of Broschat and Donselman (1988) that palm seeds stored in polythene bags under chilled conditions do not germinate. According to Andrade (2001), *Euterpe edulis* seeds are recalcitrant and at low temperature between 12-15°C, the seeds suffer from chilling injury and lose viability.
Based on longevity, De Leon (1961) classified the palm seeds into short-lived, intermediate, and long lived. According to Donselmann (1982) and Meerow (1991), palm seeds should be planted fresh as viability is lost within relatively short period. Broschat (1994) stated that seeds of many palm species lose viability with in a few weeks after harvest due to deleterious effect of desiccation. Almost all the authors are of the opinion that palm seeds exhibit many characteristic features of recalcitrant seeds as described above.

Roberts (1973) categorized the seeds into orthodox and recalcitrant, based on their storage behaviour. Orthodox seeds are long lived, desiccation tolerant and can be stored for prolonged period while recalcitrant seeds are those which cannot be dried to MC below 30% without any injury and cannot be stored successfully because high MC results in rapid deterioration of seeds. There is a third category of seeds that are tolerant to dehydration to a certain extent but sensitive to low temperature. Such seeds are classified as intermediate (Ellis et al., 1990).

Most palm seeds have high moisture content at the time of shedding. In *Borassus flabellifer* the MC of fresh entire seeds is about 66% and the seeds are large sized (Table 5A, Fig 8A). When stored in open at RT, cent percent germination is found to occur in seeds stored up to 6 weeks with negligible reduction in MC and viability is retained to 70% up to the 14\textsuperscript{th} week, even with considerable reduction of MC. Further desiccation below this MC results in a reduction of viability to less than 70%. This moisture content at which seed viability is significantly reduced is similar to other tropical recalcitrant seeds such as *Euterpe catinga* in which drastic reduction in viability was found to occur below an MC of 22% (Andrade 1994).

Generally recalcitrant seeds are heavier due to their large size and high moisture content, which may range from 30% to 70% of the fresh weight
(King and Roberts, 1979; Chin et al.; 1984; von Teichman and van Wyk, 1994). The high moisture content at which viability is lost and large sized seeds of *B. falbellifer* agree with the recalcitrant nature. However, seed behaviour such as storability up to 14 weeks at open RT, loss of viability at low temperature are not strictly complying with the characteristics of recalcitrant seeds. All fruits of *B. flabellifer* kept in polythene bags at RT completed germination within one week. Rapid germination under moist storage is an important characteristic of recalcitrant seeds (Chin et al., 1981; Bewley and Black, 1994; Copeland and McDonald, 1995; Baskin and Baskin, 2001). The seeds stored in polythene bags at 4°C showed signs of decay and became nonviable, revealing another feature of recalcitrant seeds as opined by many investigators (Chin et al., 1984; Anilkumar et al., 1997, 2002; Danthu et al., 2000). Based on the storage behaviour in general and desiccation tolerance in particular, *B. flabellifer* seeds can be included under intermediate category of seeds because these seeds are tolerant to desiccation up to 14 weeks of storage with a corresponding MC of 47%. Nevertheless, these seeds are intolerant to storage at 4°C, which is an important feature of intermediate seeds (Chin et al., 1981). More or less similar behaviour is noticed in the case of dehusked seeds under the same set of storage conditions. These seeds are characterized by an initial MC of 50% and a slightly higher percentage of germination compared to that of fresh entire seeds (Table 6A).

Cent percent germination is not shown by fresh seeds of *Corypha umbraculifera*. These seeds, with 60% MC, show only 80% germination when kept in the open at RT, probably due to pulp-induced inhibition of germination (Table 5B, Fig. 8B). The viability is lost within one week with a concomitant but significant reduction in MC (Table 6B, Fig.9B). The fruits kept in polythene bags at RT showed only 60% germination after one week of storage and viability is lost thereafter, even though there is only negligible reduction in MC. The seeds stored in polythene bags at 4°C did not germinate.
at all. The short life span of *C. umbraculifera* seeds under all the different storage conditions reveals the recalcitrant nature.

Dehusked seeds of *Corypha umbraculifera* show enhanced viability when stored in the open at RT with cent percent germination, probably due to the removal of pulp-induced inhibition of germination as reported in palm seeds (Broschat and Donselman, 1987). In seeds stored for one week, the germinability is found to be 100%, which is slowly reduced to 60% after 3 weeks of storage. Even when the MC is reduced to 20%, the germination is above 60% revealing slight desiccation tolerance of dehusked *C. umbraculifera* seeds. However, the dehusked seeds stored in polythene bags at room temperature maintained 100% germination up to 4 weeks with the MC remaining unchanged. Even after this period, a high percentage of germination is retained up to 14 weeks of storage and the corresponding reduction of MC is found to occur only gradually. But, the viability is not lost even when the MC is reduced to 31%. This observation confirms the comparatively greater longevity due to considerable desiccation tolerance of the seeds under wet storage. The seeds stored in polythene bags at 4°C failed to germinate totally, probably due to chilling injury which is a well documented characteristic feature of recalcitrant seeds (Chin *et al*., 1984; Corbineau and Côme, 1988; Danthu *et al*., 2000; Le Tam *et al*., 2004). In spite of considerable desiccation tolerance exhibited by depulped seeds under storage in polythene bags at RT, pulpy seeds are highly vulnerable to desiccation. Based on all these characters, *C. umbraculifera* seeds can be considered as recalcitrant. Nevertheless, long-term storability is possible in the case of dehusked seeds when kept in sealed polythene bags at RT. So this storage protocol can be recommended as a method of *ex situ* preservation of *C. umbraculifera* seeds.
Fresh seeds of *Caryota urens*, have an initial MC of about 60% (Table 5C, Fig. 8C) and show 100% viability for 3 weeks when stored in the open at RT with a concomitant reduction of MC to less than 50% of the initial value. Fruits kept in polythene bags at room temperature are found to lose germinability only after storage for 4 weeks with negligible reduction of MC. A rapid reduction in viability was seen after 6\(^{th}\) week of storage in polythene bags at 4\(^{\circ}\)C (Table 6C, Fig. 9C). The loss of moisture content reaches the lowest rate in this condition, and the seeds retain viability up to 11 weeks of desiccation. Thus *C. urens* seeds can be included under intermediate type due to moderate tolerance to desiccation.

The depulped seeds of *Caryota urens* with an initial MC of 38\% showed more or less the same pattern of moisture loss as that of the pulpy seeds. The seeds are ascertained to belong to the intermediate category of storage behaviour as they are found to maintain high percentage of viability even after desiccation for 10 weeks in the open at RT, and high germination percentage up to 14 weeks in polythene bags at room temperature. Moreover, seeds stored in polythene bags at 4\(^{\circ}\)C exhibited a reduction in viability to less than 30\% after 4 weeks. These characteristics such as considerable storability of the pulpy seeds and prolonged viability of depulped seeds under all the different storage conditions confirm the intermediate behaviour of *C. urens* seeds.

Fresh pulpy seeds of *Licuala peltata* with an initial MC of 60\% (Table 5D, Fig. 8D) exhibited only 80% germination. The loss of MC is found to occur at a very high rate when kept open at RT, eventually becoming reduced to about one-half of the initial value after one week with a concomitant decline in germination percentage to 30. This behaviour is typical of highly recalcitrant category of seeds. Contradictory to this character, seeds stored in polythene bags at RT retained viability up to three weeks with only slight
reduction in MC. In dehusked seeds, viability is retained up to the 2\textsuperscript{nd} week. Hence in the former case, the inhibitory role of pulp/husk cannot be ruled out in the manifestation of abrupt loss of seed viability. Those seeds stored in polythene bags at 4\textdegree C, retain viability up to 5 weeks of storage with a negligible reduction of MC. The seeds stored in polythene bags at 4\textdegree C do not show the inhibitory effect of the fruit pulp. This feature is unique to \textit{L. peltata}.

Depulped seeds of \textit{L. peltata} have an initial MC of 38\% and exhibited 75\% germination. After one week of storage, the MC is reduced to 12\% with a concomitant reduction of germination to 60\% (Table 6D, Fig.9D). Germinability is declined to 65\% after desiccation for 1 week in seeds stored in polythene bags at room temperature even though the change in MC is negligible. Viability is completely lost within one week when stored in polythene bags at 4\textdegree C while MC remained unchanged. The lack of desiccation tolerance exhibited by fruits when stored under open RT, loss of viability when stored in polythene bags at RT and in polythene bags at 4\textdegree C show that the seeds of \textit{L. peltata} belong to recalcitrant category.

In \textit{Livistona rotundifolia}, the fresh pulpy seeds with a moisture content of 58\% (Table 5E, Fig. 8E) exhibit only about 78\% germination (Table 6E, Fig. 9E). Those stored in polythene bags at RT are viable up to 4 weeks even though MC remained unchanged. In the fruits kept in polythene bags at 4\textdegree C, rapid reduction in germinability is found to occur and the germination percentage is reduced to 30\% in one week, though at elevated MC levels.

The depulped seeds of \textit{L. rotundifolia} exhibit a reduction of 50\% in MC compared to the initial values during storage for 2 weeks in the open at RT, without any decline in germinability and retain a higher percentage of germination for 10 weeks. In polythene bags at RT, the seeds show relatively higher percentage of germination for 14 weeks with only gradual reduction in
MC. In the seeds kept in polythene bags at 4°C the germination percentage is reduced gradually and the seeds become nonviable after 4 weeks of storage. Considerable tolerance towards desiccation during storage under open RT as well as low temperature indicates the intermediate nature of *L. rotundifolia* seeds. More or less similar storage behaviour has been reported in oil palm seeds by Ellis *et al.* (1991), who proposed that oil palm seeds could be included under intermediate category.

Classification of palm seeds into orthodox, recalcitrant or intermediate is still ambiguous because most of the views are not fully substantiated or obeyed by the specific characteristic of the various seed categories. Seeds of all the palm species investigated exhibit many typical characters of recalcitrant seeds such as high moisture content, sensitivity towards desiccation and chilling, short storability, big seed size, phenology of flowering, seed maturity etc. Hong *et al.* (1997) studied the seed behaviour in 21 palm species and reported 16 species to be orthodox and the others as recalcitrant.

Desiccation sensitivity is the most studied and well-interpreted aspect of recalcitrant seeds (Lin and Chen 1995; Bonner, 1996; Danthu *et al.*, 2000; Chaitanya *et al.*, 2000; Greggain *et al.*, 2001; Malik *et al.*, 2005). On the basis of desiccation sensitivity, pulpy seeds of *Corypha umbraculifera* are highly sensitive, since their viability is lost within one week with the MC being reduced to almost 75% of the fresh seeds. However, in the case of dehusked seeds of *C. umbraculifera*, the desiccation tolerance is exhibited in spite of significant reduction in MC. *L. peltata* seeds also are highly sensitive to desiccation, since viability is lost within one week and hence recalcitrant. But storability is possible up to three weeks in the case of depulped seeds.

Quantitative distribution of storage reserve galactomannan and mannan of the five seeds reveals their role in storage behavior and classification.
According to Meier and Reid (1982), the role of galactomannan/mannan of seeds is directly related to water relations in fenugreek and these authors suggested that galactomannan reserve might represent an adaptation towards desiccation. The seeds of *C. umbraculifera* and *L. peltata* contain low amount of galactomannan and may lead to their recalcitrant nature in comparison with other three species which contain more galactose and obey many characters of intermediate nature with considerable desiccation tolerance.

The seeds of *B. flabellifer*, *C. urens*, and *L. rotundifolia* are tolerant to desiccation to considerable extent because their viability is retained up to 15, 7 and 4 weeks respectively when pulpy seeds are subjected to desiccation whereas desiccation tolerance is found to be enhanced in depulped seeds. Hence these seeds can be categorized under intermediate types as suggested by Ellis *et al.* (1991) and Bewley and Black (1994). According to these authors, intermediate seeds survive desiccation to considerable extent, but become damaged during dry storage in open air for prolonged period. Even though seeds categorized as intermediate storage behaviour are relatively desiccation tolerant, they will not withstand removal of water to levels as that of orthodox seeds (Ellis *et al.*, 1990; Hong and Ellis, 1996).

Das and Ray (1985) suggested that the main factors influencing palm seed longevity are seed MC and storage temperature. In *Areca catechu* seeds, the higher the initial seed moisture content, the more rapid is the decrease in germination capacity, confirming the recalcitrant nature of *A. catechu* seeds. Contradictory to the views of Das and Ray (1985), the longevity and initial MC of the palm seeds are not directly correlated. *C. umbraculifera* and *L. peltata*, which are found to be highly recalcitrant, contain 60% initial MC where as intermediate seeds of *B. flabellifer*, *C. urens*, and *L. peltata* showed 66%, 60% and 58% MC respectively (Table 5A-E). Hence, discernible
differences do not exist between the classifications of the five species as far as the moisture content distribution is concerned (Das and Ray, 1985; Muralikrishna et al., 2001; Dickie et al., 1993; Andrade, 2001).

An important variance with recalcitrant nature observed in palm seeds is the occurrence of seed dormancy (Odetola, 1987; Raich and Khoon, 1991) because recalcitrant seeds never go into dormancy but continue development and progress towards germination (Berjak et al., 1990; Berjak and Pammenter, 1999). However, not even a single seed of the five palm species included in the present study is reported so far as showing continuum between maturation and germination.

In addition to the important diagnostic features such as intolerance to desiccation and chilling, Ellis et al. (1990) and Pammenter et al. (1994) stated that recalcitrant seeds are metabolically active when shed and the embryonic axes show ultrastructural changes that are similar to those occurring during germination. However, in palm seeds, despite the manifestation of several typical recalcitrant behaviour, germination associated changes are not obvious because of inordinate delay in the initiation of germination observed in all seeds. Such a delay in germination has been reported in *Acrocomia sclerocarpa* (Koebernik, 1971), *Chrysalidocarpus lutescens* (Broschat and Donselman, 1986).

According to Hong and Ellis (1996), the main feature of intermediate seed storage is often associated with desiccation damage at low moisture content of 7-12%, which is species specific. However, desiccation damage does occur in all the palm seeds in the present investigation at very high MC level (Table 5 A-E & 6A-E). Hong and Ellis (1996) opined that the critical levels of moisture content of intermediate seeds at which more rapid loss in viability occurs during hermetic storage is found to vary with species, degree of maturity, and method of seed extraction/handling. In the present study, this
species specific nature of critical MC, characteristic of intermediate seeds is shown by *Borassus flabellifer*, *Caryota urens* and *Livistona rotundifolia* with critical seed MC of 48%, 26% and 28% respectively. But in the depulped seeds of this intermediate category, the critical MC is 28% in *B. flabellifer*, 24% in *C. urens* and 14% in *L. rotundifolia*. Hence, the critical MC of intermediate seeds not only varies with species but also differs with seed extraction methods as opined by Hong and Ellis (1996). The seeds of the palms studied such as *B. flabellifer*, *C. urens* and *L. rotundifolia* show slow and inconsistent germination, majority showing intermediate seed storage behaviour.

Generally palm seeds are short lived and on the basis of storage behavior, several authors have put forth contradictory views. Storability of *Corypha umbraculifera* and *Licuala peltata* seeds are very short and are classified as recalcitrant and *Borassus flabellifer*, *Caryota urens* and *Livistona rotundifolia* are grouped under intermediate category due to their moderate tolerance to desiccation and hence storability is not possible for prolonged periods. Enhanced rate of germination is found to be a character of all the stored seeds, presumably due to changes in moisture content which controls the physiology of germination.

Observations of the present study confirmed many variations in the storage behaviour of palm seeds and some of their characters are inconsistent with either orthodox or recalcitrant seeds. It appears that palm seeds fail to satisfy recalcitrant seed behaviour because they show considerable desiccation tolerance than the typical recalcitrant seeds as shown by *B. flabellifer*, *C. urens* and *L. rotundifolia*. Many of the results of the present study are in disagreement with the definition of recalcitrant and orthodox seeds and hence indicative of intermediate storage behaviour of these species.
Controversial views exist regarding the storage behaviour and classification of palm seeds. One and the same seed has been classified under different categories by different authors. For instance, King and Roberts (1979) classified *Elaeis guineensis* as recalcitrant. From desiccation experiments, Grout *et al.* (1983) suggested the orthodox nature of oil palm seeds. Oil palm seeds have been proved to be intermediate between orthodox and recalcitrant on the basis of storage behaviour under different temperatures and MC (Ellis *et al.*, 1993). Similarly according to Ellis *et al.* (1985) *Phoenix dactylifera* seeds are orthodox due to their longevity up to 1 year at -20°C with 5% MC. According to some authors, the *P. dactylifera* seeds cannot withstand any degree of drying and hence are recalcitrant (Al-Madeni and Tisserat, 1986).

The present investigation has confirmed the various inconsistencies in the behaviour of palm seeds manifested as storage effect, erratic germination pattern and delay in the initiation of germination of palm seeds collated previously (Kobernik, 1971; Das and Ray, 1985; Carvalho *et al.*, 1988; Dickie *et al.*, 1993; Ellis *et al.*, 1995; Muralikrishna *et al.*, 2001; von Fintel *et al.*, 2004).

The reserve materials of the palm seeds are stored in the endosperm, which is hard and ivory like on maturation, almost filling the seed cavity. The endosperm of all the five species is found to contain very little amount of storage starch (Table 10A-E). The seeds of *B. flabellifer* have the highest starch content of about 1.5%. Galactomannans occur in abundance in the endosperm as the reserve material of all the seeds (Table 9A, Fig. 12A) and the quantity of this polysaccharide is estimated and expressed in terms of its hydrolytic products such as mannose and galactose. Mannans as the principal reserve material in the endosperm of palm seeds have been reported in *Phytelephas macrocarpa* and *Phoenix dactylifera* (Meier, 1958), in
Phytelephas macrocarpa (Aspinall et al., 1958) and in Cocos nucifera (Mukherjee and Rao, 1962). Pure mannans constitute a backbone of linear mannose chain (1-4) –β linked while in glucomannan, some residues are replaced by glucose and in galactomannan, the backbone carries (1-6)-α linked galactosyl substitutes (Meier and Reid, 1982). Endosperm of all palm seeds included in the present study constitutes abundant quantity of mannose and comparatively reduced amount of galactose. The ratios of mannose:galactose are 8:1, 5.5:1, 10:1, and 34:1 in B. flabellifer, C. umbraculifera, C. urens and L. rotundifolia respectively while L. peltata is devoid of galactose.

Replacement of starch as the major carbohydrate reserve by thick deposits of polysaccharides on the endosperm cell wall is a widespread phenomenon in seeds. Many of the deposits have been shown to be rich in mannans in the seed reserves of several monocots and some dicots (Halmer and Bewley, 1979). Some of the polysaccharides are pure mannans as seen in the endosperm of Phoenix (Meier and Reid, 1982).

Members of the palm family are among the many known species of flowering plants, which store polysaccharides in the form of thickened cell walls in their endosperm rather than in the form of starch (Meier and Reid, 1982; DeMason et al., 1989). Even though many palm seeds are reported to contain mannans as seed reserves, Meier and Reid (1982) opined that pure mannans contain less than 10% of nonmannose sugar residues. According to these authors, there is clear natural distinction between the pure mannans of seeds and the glucomannans and galactomannans both of which contain the proportion of >20% of nonmannose sugar residues. In the present study, seeds of all palms except L. peltata showed the presence of mannose and galactose, the amount of the latter being in minute quantities. B. flabellifer is found to have the highest amount. The galactose content of all the seeds are
less than 17% and galactose is totally absent in the endosperm of *L. peltata*. Meier (1958) suggested that the date endosperm walls contain 92% mannan (61% mannan A and 31% mannan B) and 8% cellulose. Accordingly, pure mannans are of general occurrence in the hard endosperms of palm seeds and contain less than 10% of non-mannose sugar residues. Mukherjee *et al.* (1961) stated that the endosperm of the immature seeds of *B. flabellifer* consisted of galactomannans where as mature seeds store pure mannans and galactomannan which is composed of galactose and mannose in the ratio 1:2.4. The kernel of *B. flabellifer* contains two polysaccharides galactomannan and mannan (Anonymous, 1988). Keusch (1968) noticed the depolymerization of mannan into mannose and their mobilization into the haustorium during germination of date seeds. Ashford and Gubler (1984) stated that mannan rich cell wall deposits are found as reserves in the endosperm of *Phoenix dactylifera, Carum carvi* and in the perisperm of *Coffea arabica*. Recently, Srivastava and Kapoor (2005) stated that galactomannans are found in many palm species such as *Phoenix dactylifera, Elaeis guineensis, Phytelephas macrocarpa* etc.

In addition to the abundant occurrence of galactomannans and reduced quantities of starch, the endosperm of *Caryota urens* and *Licuala peltata* contains high amount of proteins, 167 and 175mg g\(^{-1}\) dw respectively. Only lesser amount of protein (<4%) is found to occur in *Corypha umbraculifera* and *Livistonia rotundifolia*. Of the five species of palms studied, the lowest protein content (only 1%) is found in the endosperm of *Borassus flabellifer*.

Lipids are found to occur in relatively low amounts (<6%) in the endosperm of all palm seeds investigated in the present study (Table 19, Fig 24). However, many palm seeds such as coconut is oil rich and 68-70% of the endosperm is lipid (Balasubramaniam *et al.*, 1973) and the endosperm in oil palm also is rich in oil content (Oo and Stumpf, 1983). A gradual and
progressive reduction in the lipid content is found to occur in the endosperm of all seeds studied throughout the process of germination and seedling development.

Given the occurrence of very low amount of lipids in the palm seeds, which are found to be mannan/galactomannan rich, considerable rate of lipids utilization is observed during germination. In lipid rich seeds, it provides an important source of energy by lipase activity and the resultant degradation (Bewley and Black, 1994). According to Harwood (1980), lipase activity is not usually limited to the degradation process alone but also in the formation of membranes. Hence glycolipid and phospholipid synthesis also are taking place during germination of lipid rich seeds in general and lipid poor seeds in particular. The reduction of lipids in the endosperm of all palm seeds in the present study is in accordance with the view that energy rich lipids are utilized during early phase of germination in galactomannan rich palm seeds.

During seed germination in the palms studied, one half of the cotyledon is differentiated into a cotyledonary sheath that carries the embryonic axis during germination and the other half is modified as a haustorium. The haustorium is spongy and fibrous. In seedlings, it expands tremendously and eventually fills the seed cavity, as the major portion of the endosperm disappears (Fig. 7). The haustorium apparently absorbs degradation products from the endosperm, which may eventually be transported to the growing axis.

In the context of reserve mobilization, germination is defined as metabolic processes which occur in the seed during imbibition followed by radicle protrusion and reserve mobilization is therefore a post-germinative event associated with seedling development which continues up to several weeks/months in palm seeds (Meier and Reid, 1982).
As a result of mobilization of metabolites from endosperm to haustorium and to different parts such as cotyledonary sheath and plumular sheath during germination, significant changes are observed in the distribution of dry matter. The dry weight percentage of different tissues of the seeds during germination and seedling development varies considerably. The dry weight percentage of the endosperm shows no change up to the 3rd stage of germination (Table 8A, Fig. 11 A) and an insignificant but progressive decline occur during the course of germination in all the palm seeds. According to Meier and Reid (1982), seeds that contain deposits of cell wall polysaccharides as seed reserves, such as mannans of the endosperm are highly insoluble in water and occur at least in part as crystalline microfibrillar material in the cell wall. The absence of dry matter change of endosperm during earlier phases of germination in all the five palm species is evidently due to the lack of water uptake and this observation is in conformity with the view of Meier and Reid (1982), who emphatically stated that mannan rich endosperm cells of palm seeds scarcely imbibe water during germination and seedling development. Water is required in the endosperm only to maintain the very narrow dissolution zone, which surrounds the haustorium. The decrease in dry weight of the endosperm during later stages of germination is an indication that the mannans have been broken down by the enzymes and the products are mobilized to the growing parts.

The dry weight percentage of the haustorium in all the palms studied show a general pattern of an initial increase followed by a decrease in the later stages of germination and seedling development (Table 8B, Fig. 11B). Alang (1982) and Aspinall (1959) suggested that the initial rapid increase in the dry weight of haustorium was due to the synthesis of starch by utilizing mannose and galactose, the end products of galactomannan degradation. The increase in the dry weight of the haustorium is indicative of the progressive growth of tissues with differentiated vasculature and active translocation of the
hydrolytic products from the endosperm and the synthesis of transient storage metabolites, predominantly starch. The dry weight is found to decline in the later stages of germination mainly due to the translocation of materials from the haustorium to the developing tissues as seedling growth progresses. By this time, the haustorium becomes soft and appears as liquefied, since all the reserves are transported from the haustorium to the growing embryonic axis through the cotyledonary sheath. DeMason (1985) suggested in date seeds that the depletion of starch reserves occurs after 10 weeks of seedling growth. Nagarajan and Pandalai (1963) reported the involvement of numerous enzymes in the haustorium of *Cocos nucifera* at the time of germination and noted that the haustorium swells and continues to grow until it completely fills the cavity. The haustorium contains amylase, lipase, protease, invertase, peroxidase, catalase and dehydrogenases.

In the palm seeds included in the present study, the products of hydrolysis of mannan or galactomannan such as mannose and galactose are found to be mobilized into the haustorium. According to Reid (1971), in *Trigonella foenum-graecum*, galactomannan degradation in the endosperm and starch formation in the cotyledons occurs concurrently. In palms, starch accumulates in the haustorium with a concomitant increase in dry weight. Apart from the contribution of starch, tissue differentiation consisting of well-developed vasculature also is responsible for the increase in the dry weight of the haustorium. In the final stages of seedling growth, starch degradation and translocation into the cotyledonary sheath followed by cellular degradation occur resulting in a decrease in dry weight of the haustorium.

The dry weight percentage of the cotyledonary sheath showed the same pattern of changes as that of the haustorium (Table 8C, Fig. 11C). Therefore, the cotyledonary sheath also functions as a transient storage organ, which
receives metabolites from the haustorium and transfers them to the other seedling parts.

The plumular sheath (cataphyll) encloses a very small leafy shoot (eophyll) within. There is an initial increase in dry weight percentage followed by a decrease in the plumular sheath of *B. flabellifer* and *C. urens*. But in *C. umbraculifera and L. peltata*, the values show a gradual increase (Table 8D, Fig. 11D). This discrepancy in the distribution of dry matter content of plumular sheath between different species is probably due to the difference in the differentiation/growth phases of the seedlings. Sampling of seedlings at different stages was done on the basis of morphological differentiation into haustorium, cotyledonary sheath and plumular sheath. Apart from this, the growth of the plumular sheath is dependent on the differentiation of the plumule. Even though the sampling is done at approximately comparable intervals, the stage of growth of different species may be different from one another and hence significant variations may occur between the species. The increase in the dry weight of the plumular sheath in *C. urens* during germination is indicative of the lack of complete development of plumule and hence dry matter is not much mobilized. The decrease in dry weight, seen in species such as *C. umbraculifera and L. peltata* might be due to the fact that the plumule is fully differentiated and plumular sheath dries off when the leafy shoot develops from within.

The mobilization of mannan/galactomannan, the principal reserve material in the endosperm is presumed to be the most conspicuous biochemical change occurring in the tissue during germination of palm seeds. In the present investigation, the mobilization of mannan/galactomannan is assayed only in the endosperm of *Corypha umbraculifera*, since this species is unique in its monocarpic nature and sufficient seeds were available during the
The analysis of the endosperm of *C. umbraculifera* at various stages of germination showed a gradual but significant decrease in the hydrolytic products of galactomannan - mannose and galactose - during successive stages of germination indicating its degradation and mobilization (Table 9B, Fig. 12B). Biochemical aspects of mannan/galactomannan degradation and the enzymes involved are not widely investigated in palm seeds and so a comparison of the present data with relevant references is not possible. Even though *C. umbraculifera* seeds are mannan rich, maximum quantity of galactose also is present when compared with other palm species and this monosaccharide is found to be reduced significantly during seedling growth. This is an adaptation seen in *C. umbraculifera* seeds because plant tissues containing galactose as an important constituent are equipped with a salvage pathway for reutilization of the galactose during metabolic turn over (Feingold and Avigard, 1980).

Studies on hydrolysis and mobilization of reserve carbohydrate galactomannan and/or mannan have been carried out elaborately in the endospermic legume, *Trigonella foenum-graecum* (Reid, 1971). Seed reserves as cell wall hemicelluloses consisting of mannan and galactomannan in the endosperm of palm seeds and their hydrolysis and disappearance following germination have been reported (Meier and Reid, 1982). Yet, a study on the physiology of mannan degradation has been done only in the seeds of *Phoenix dactylifera* (Keusch, 1968). In cereal grains and endospermic legumes, after the emergence of the radicle, the galactomannan of the endosperm are hydrolysed by α-galactosidase, β-mannanase and β-mannosidase, releasing galactose and mannose which are absorbed by the cotyledon (Malek and Bewley, 1991; Bewley and Black, 1994).
According to Keusch (1968), microscopic and biochemical investigations of mannan mobilization in the date endosperm following germination exclusively revealed that mannan chains are depolymerized in the dissolution zone of the endosperm surrounding the haustorium and decomposition of cell wall polysaccharides is brought about by hydrolytic enzymes. The end product of mannan hydrolysis is mannose and this sugar is rapidly converted to sucrose. Bewley and Black (1994) stated that hydrolysis in the endosperm of date palm occurs when haustorial projection grows into the endosperm. The hydrolytic enzymes released from the protein bodies in the endosperm come in contact with the cell wall, which is rich in galactomannan. The galactomannan is converted into the constituent monomers - galactose and mannose - that are absorbed by the haustorium and transported to the growing seedling. The distribution pattern of galactose and mannose in *Phoenix dactylifera* seeds following germination is in agreement with the views of Keusch (1968), Reid (1971), Meier and Reid (1982) and Bewley and Black (1994).

Hydrolytic products such as galactose and mannose are depleted in the endosperm due to translocation into the haustorium, which is reported to occur by passive diffusion (Uebelmann, 1978). In *Corypha umbraculifera*, the translocation of monomers from the endosperm is found to be passive because the endosperm cells are nonliving. However, the rate of diffusion is extremely rapid, resulting in an exorbitant accumulation of sugars in the haustorium (Table 14B, Fig. 18B) while the concentration of sugars in the endosperm shows only negligible changes. Similar mobilization has been reported in *Trigonella foenum-graecum*, where translocation of galactose and mannose from the endosperm to the cotyledon occurs (Reid, 1971; Reid and Meier, 1972).
The mobilization of mannan/galactomannan in fenugreek and cereal endosperms occurs by enzymatic hydrolysis and in both cases, the living aleurone layer is responsible for the synthesis and secretion of polysaccharide-degrading enzymes (Meier and Reid, 1982). But in palm seeds, the enzymes are reported to be produced from the proteins of the endosperm, which in turn are liberated from the protein bodies as suggested by Bewley and Black (1994). However, the protein content of the endosperm tissues is progressively depleted during germination, probably due to degradation of protein bodies in accordance with the view of DeMason (1988). Hence, mobilization of proteins from the endosperm cannot be ruled out (Table 18A, Fig. 22A).

Mobilization of mannose and galactose from the endosperm of *Corypha umbraculifera* is found to occur gradually from stage to stage, retaining 40% and 20% respectively in the last stage. However, the dry weight reduction was not related to the depletion of galactomannans. But starch mobilization also is found to be contributing considerably. Even with mannose occurring in five-fold quantity of the lipids in the endosperm of *C. umbraculifera*, significant amount of lipid is found to be mobilized following germination. Similarly, proteins and total sugars also exhibit significant mobilization from the endosperm. The concomitant reduction of dry weight is only 15%, revealing the limited mobilization of storage reserves in general and galactomannan in particular during germination and seedling growth because dry weight of mature seeds of palms is accounted for by the two major storage reserves - carbohydrates consisting mainly of mannans and galactomannans and triacyl glycerols (neutral lipids).

In *C. umbraculifera*, following germination/seedling growth, the storage reserve galactomannan is degraded and 64% of the of the total reserve is seemed to be mobilized up to the 4th stage while the total sugar content of
the haustorium show only very low increase during the entire period of seedling growth. So also is the starch accumulation. But the amylase activity is very high which increased continuously throughout the seedling growth. Hence the turnover of starch metabolite is found to be very high. But the accumulation of starch is not directly related to it.

In storage organs of seeds, translocation of raffinose family of sugars and maltose formation during degradation of starch by amylases are well documented (Kandler and Hopf, 1980; Preiss and Levi, 1980). Maltose formed by amylase activity may be split by α-glucosidase (maltase) in germinating seeds, thus preventing the accumulation of maltose. The increase of reducing sugars in *C. umbraculifera* haustorium from the 3\(^{rd}\) to the 7\(^{th}\) stage of germination, which coincides with very high amylase activity and resultant starch depletion, are related to each other. However, the stability in the accumulation of total sugars, in spite of very high amylase activity and starch depletion is indicative of mobilization of soluble carbohydrates, mostly as sucrose, synthesized from the products of α-glucosidase activity on maltose. Under these circumstances, the synthesis/accumulation and degradation/mobilization of starch resulting in only a transient storage in the haustorial tissue is not clear. Even though, starch synthesis is well documented in plants in general (Preiss and Levi, 1980), and in the cotyledons of germinating lipid rich seeds in particular (Ashford and Gubler, 1984), seeds of palms which are galactomannan rich with well-developed haustoria during germination is not yet investigated to elucidate the carbohydrate metabolism.

Even though enzymatic changes of mannan or galactomannan during germination of palm seeds have not been assayed, the results of studies in *Trigonella foenum-graecum* (Reid and Meier 1972, 1977), *Asparagus officinalis* (Williams et al., 2001) and *Coffea arabica* (Giorgini and Campos,
1992) can be compared with that of palm seeds during germination, especially in the biochemistry of degradation of mannan/galactomannan. Following germination, hydrolytic degradation of stored polysaccharide results in the release of mannose and galactose in the endosperm. These are then mobilized to the haustorium, where they are utilized for the synthesis of starch. This is apparent from a significant hike of starch in the haustorium during the later stages of germination.

According to Meier and Reid (1982), in fenugreek, the galactomannans of the endosperm are degraded by β-mannosidase, β-mannanase and α-galactosidase and monomers of galactose and mannose are released. Then galactose is absorbed by passive diffusion to the cotyledons and mannose requires active uptake. These sugars are not accumulated in the cotyledon but may be phosphorylated and converted into sucrose and then to starch, which is, then mobilized to the axis when sugars are depleted in the axis.

Williams et al. (2001) opined that the seeds of Asparagus officinalis contain glucomannan as the major reserves in the endosperm along with proteins and lipids and complete mobilization these reserves occurs prior to that of galactomannans. Contradictory to this view, in Elaeis guineensis, the galactomannan located in the secondary walls of endosperm cells is the second largest component that is utilized more rapidly than lipid during early stages of germination (Alang et al., 1988). These authors suggested that gluconeogenetic formation of sugars from lipids accumulate in the haustorium. However, the constituents of endosperm reserves such as proteins, lipids, starch etc. of C. umbraculifera are very low (less than 1%) and their depletion during germination is insufficient for the metabolism. Hence, galactomannan degradation and mobilization are presumed to occur simultaneous with haustorial development. In Elaeis guineensis, although the
seeds are lipid rich, the galactomannans are degraded earlier than lipids due to the selective activity of enzymes (Alang et al., 1988).

The total starch content in the endosperm of fresh palm seeds studied is very low, which show a gradual but insignificant decline following germination (Table 11A, Fig. 14A). This indicates that even though meager in quantity, the starch is being hydrolyzed and the products may be utilized as source of energy during early phase of germination. Since the galactomannans/mannans are degraded well after radicle emergence, it is not considered to be important in germination sensu stricto (Ashford and Gubler, 1984). This view is true to the germination behaviour of palm seeds because germination is a very slow process in palm seeds. Perhaps, during initial stages of germination, starch breakdown is existing in the seeds even though mannans/galactomannans are the principal storage reserve material in the endosperm. On the contrary, starch synthesis was reported in the cotyledons of *Trigonella foenum-graecum*, which contain no starch in the endosperm, but is rich in galactomannan (Reid, 1971; Reid and Meier, 1972; 1973; Reid et al., 1977). Confirmatory evidence for the role of galactomannan degradation during germination of *Trigonella foenum-graecum* seeds in which galactose as the translocation form of sugar has been reported (Komor, 1982).

The soluble as well as reducing sugar content show a marked increase in the endosperm of *B. flabellifer, C. umbraculifera* and *C. urens* up to the 5th stage of germination and an insignificant decline in the last stage of germination studied (Table 14A, 16A). In *L. peltata* and *L. rotundifolia*, however, the total and reducing sugars show continuous decline from the fist stage of germination to the last stage studied. The increase in the sugar content in the endosperm shown by some palms indicates active degradation of starch, mannan/galactomannan and perhaps gluconeogenesis from the lipid component of the endosperm. The lack of increase in the sugar content in the
endosperm of *L. peltata* and *L. rotundifolia* is indicative of reduced rate of degradation of galactomannans, the quantity of which are comparatively less than that of the other three palms (Table 9A, Fig. 12A).

While the endosperm of *Borassus flabellifer* is found to contain the lowest amount of protein (11.55±0.38 mg g\(^{-1}\) dw), those of *Corypha umbraculifera* and *Livistona rotundifolia* are found to have relatively higher amount of proteins. *Caryota urens* and *Licuala peltata* show the maximum protein content in the endosperm with the values reaching 168 and 175 mg g\(^{-1}\) dw respectively. In all the species, the protein content is found to decline gradually during germination and seedling development (Table 18A, Similar to date seeds, in *Washingtonia filifera*, the endosperm cells are not metabolically active and no endoplasmic reticulum or membrane system for protein synthesis is present (DeMason, 1986). Continuous depletion of proteins in all the palm seeds in the present study is in conformity with the views of Dalling and Bhalla (1984), Demason *et al.* (1983) and Demason (1986).

Studies on electrophoretic analysis of proteins in the seeds of date palm (Chandra Sekhar and DeMason, 1988b) revealed that storage proteins specific to endosperm and embryo are present in these seeds. The proteins specific to the endosperm of date seeds are source of many hydrolytic enzymes that are involved in autocatalytic reactions during germination. DeMason and Stillman (1986) opined that the activity of hydrolytic enzymes such as acid phosphatases are active in the endosperm cells where the activity is associated with protein bodies in *Phoenix dactylifera* whereas in *Washingtonia filifera* the enzymes are associated with plasma membrane. Those authors concluded that in palm seeds the enzymes are stored in the protein bodies during endosperm development and are activated during germination. So it is evident that no protein synthesis occurs in the endosperm of palm seeds as
observed in the present study in which the quantitative depletion of proteins reveals the cessation of enzyme activity in the endosperm following germination.

SDS PAGE profile of soluble proteins in the endosperm of *B. flabellifer* showed new bands indicating the presence of new proteins in the tissues of the endosperm during germination (Fig. 23A 1-4). But in *C. umbraculifera* certain bands disappeared while others made their appearance during successive stages (Fig. 23B 1-5). This observation corroborates with the view of Chandra Sekhar and DeMason, (1988a; 1988b) and DeMason et al. (1989b) who reported proteins unique to the endosperm as well as the embryo exist in *Phoenix dactylifera* and *Washingtonia filifera*.

The protein content in the haustorium is found to increase in the initial stages of germination and was found to decline later on (Table 18B, Fig. 22B). In *B. flabellifer* and *L. rotundifolia* the increase is found to occur up to the 3rd stage of germination and in *C. umbraculifera, C. urens* and *L. peltata* up to the 4th stage. In the samples of earlier stages of haustorium, a gradual increase in protein content is observed and a subsequent decline. This suggests that, synthesis of the protein/enzymes in the haustorium is an essential prerequisite for the tissue differentiation and active metabolism of the haustorium and during later stages of germination, the metabolites are mobilized from the haustorium to the cotyledonary sheath and the haustorium becomes shrunken and dried. Enhanced activity of four acid phosphatases has been identified in the haustorium of *Phoenix dactylifera* and *Washingtonia filifera* (DeMason and Stillman, 1986).

A gradual and progressive reduction in the lipid content is found to occur during the process of germination and seedling development. Balasubramaniam (1983) suggested that during the early stages of germination, the coconut seeds depended mainly on hemicellulose of the
kernel and did not utilize lipid, its major reserve. Contradictory to this observation, it is found in the present study that degradation of lipid occurs to some extend in Corypha umbraculifera during the early stages of germination along with the degradation of galactomannan. Lipid utilization in the early stages is also evident in other palm seeds such as B. flabellifer, C. urens and L. peltata. Lipid has been found to exhaust almost completely during early stages, particulary in B. flabellifer. Almost similar observation has been noticed in the case of L. pelata (Table 19).

Mobilization of lipid reserves provides chemical energy and carbon skeleton for embryonic growth during germination in oil seeds (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1994). Baleroni et al. (1997) reported a rapid decline in lipid content during germination of Brassica napus seeds evidencing the use of these reserves in the cotyledons. According to Li and Ross (1990), mobilization of storage lipids is initiated prior to germination, although a greater part of the hydrolysis takes place during the post-germinative growth.

In palm seeds, the distal end of the single cotyledon remains inside the seed and is presumed to function in absorbing the degradation products from the endosperm resulting in the formation of the haustorium during germination (DeMason, 1984; 1985). A rapid and significant increase in starch content is noticed in the haustorium as seedling growth advanced with a drastic decline in the later stages. Among the five species studied, the haustorium of B. flabellifer is found to have the highest quantity of starch, which exhibits a continuous increase up to the 5th stage of seedling growth followed by a decline (Table 10A, Fig. 13A). The starch content in the haustorium showed an increase followed by a decline in all the other species as seedling growth advanced. The increase in the starch content in the haustorium indicates active synthesis of starch as a transient reserve material,
using the hydrolytic products of galactomannan translocated from the endosperm. When thickened mannan-rich cell walls of endosperm of *Phoenix dactylifera* and *Washingtonia filifera* are degraded, starch granules are built up in the haustorium (DeMason 1985). The monomers required for the synthesis of starch might have produced mainly by the degradation of galactomannan as suggested by Meier and Reid (1982) and DeMason (1985) and to a much lesser extent by gluconeogenesis in oil palm (Oo, and Stumpf, 1983). The present study is in agreement with the view of all these authors. Balasubramaniam et al. (1973) also observed in *Cocos nucifera* that, during early stages of germination, the total starch content in the haustorium increased linearly. In date seeds, depletion of starch reserves occurs after ten weeks of seedling growth and eventually the haustorium dries (DeMason, 1985).

In *Corypha umbraculifera* seeds, the depletion of galactose and mannose in the endosperm and a concomitant increase in reducing and total sugars as well as an accumulation of starch in the haustorial tissues show that the hydrolytic products from the endosperm are translocated to the haustorium and starch is synthesized as a transient storage material. This process is a sequestering of sugars as a strategy for the removal of potentially osmotically damaging monomers in the cells of the haustorium, which contains very high moisture content content and hence metabolically active. DeMason (1988) opined that haustorium of *Washingtonia filifera* are active metabolically and the ultra structural features are similar to that of scutellum of grasses and after 10 weeks, the stored reserves disappear, and eventually the haustorium dries.

A more or less comparable observation has been reported in germinating coffee seeds (Georgini and Campos (1992). Starch content in the endosperm of resting seed is very low and contains relatively large amount of mannan and galactan. According to these authors, most of the soluble sugars,
which enter the cotyledons, and the starch consequently formed, could be derived from the degradation of non-amylaceous polysaccharides.

Preliminary studies undertaken on the distribution of starch have shown that during the germination of palm seeds, active starch metabolism is occurring in haustorium and also in the plumular sheath. Hence, the role of amylase was assayed in the tissues of the haustorium and plumular sheath of *Borassus flabellifer* and *Corypha umbraculifera*, which provided sufficient tissue samples for enzyme assays due to comparatively larger size of the seedlings. In the haustorium of *B. flabellifer*, amylase activity is found to increase and reach the maximum value by the 5\textsuperscript{th} stage of germination (Table 12A, Fig 16A-B). This coincides with the stage where the starch content starts to decline drastically. In the subsequent stages, the enzyme activity declines. So also is the starch content. In *C. umbraculifera*, the amylase activity show rapid increase in the haustorium and no decline in the activity is noticed till the last stage of study (Table 12 B, Fig. 16C-D). The amylase activity in the haustorium of *C. umbraculifera* is remarkably high compared to that of *B. flabellifer*. The relatively low amount of storage starch, which is coincided with, enhanced distribution of total and soluble sugars observed in the haustorium of *C. umbraculifera* can be attributed to the high amylase activity. In the haustorium, the soluble sugar content is found to increase steadily during the successive stages of germination in all the palm seeds. In *B. flabellifer* and *L. rotundifolia*, the values are found to increase through out the period of germination studied. In *C. umbraculifera*, *C. urens* and *L. peltata*, the amount of soluble sugars is decreased slightly in the last stage of germination. The reducing sugars follow the same pattern of change as the soluble sugar except that in *C. umbraculifera* in which no decline is observed in the last stage as in the case of soluble sugars. The increase in soluble as well as reducing sugars is presumed to be due to continuous translocation from the endosperm on one hand and degradation of starch within the
haustorium on the other. However, the decline in the amount of the sugars in the haustorium detected towards the last stage of germination in *C. umbraculifera, C. urens* and *L. peltata* may be due to the active transport to the plumular sheath through the cotyledonary sheath. The period of decrease in sugar content coincides with the plumular sheath development.

The present study is in agreement with the views of Balasubramaniam *et al.* (1973) who observed in *Cocos nucifera* that, during early stages of germination, the total starch content in the haustorium increased linearly where as reducing and soluble sugars rose rapidly and remained at a steady state thereafter. During germination, the embryo metabolizes the stored carbohydrates of the kernel. The excess carbohydrates are stored in the haustorium as starch. As the haustorium increases in size with the progress of germination, changes occur in the carbohydrate content. The amount of starch in the haustorium increased in a linear manner during the entire period and this corresponded closely with the decrease observed in the kernel. During germination, the amounts of total soluble and reducing sugars of the solid kernel decrease exponentially. Therefore, the authors assumed that the soluble sugars serve as the food for the growing embryo and the excess is stored temporarily as starch in the haustorium. Hence the mobilization of starch in the haustoria of the palm seeds in the present study which show remotive type of germination is similar to *Cocos nucifera* seeds.

Compared to the haustorium, cotyledonary sheath contains only negligible amount of starch, with only slight fluctuation during seedling growth (Table 11C, Fig. 14C). But the plumular sheath is rich in starch, the amount being greater than that of the haustorium. So, synthesis and accumulation of starch is found to occur again in the plumular sheath and the cotyledonary sheath is acting as a carrier of translocating form of soluble
sugars formed by the degradation of starch in the haustorium transporting them to plumular sheath.

Distribution of dry weight, starch and soluble sugar contents of the cotyledonary sheaths of all palm seeds show that only negligible fluctuations are observed during seedling growth except in *B. flabellifer* and *C. umbraculifera* in which significant increase in sugars is evident and a proportionality is maintained between the sugar content of haustorium and cotyledonary sheath in these two species. All these observations indicate the carrier or passage role of cotyledonary sheath, which shows only insignificant metabolic role, since it is the elongated proximal end of the cotyledon in which the growing axis is included. Nevertheless, protein content of the cotyledonary sheath of all seedlings is comparatively more than that of all other parts of the seedlings, which are metabolically active. The increase in protein may be related to the transport of metabolisable carbohydrates particularly sugars, because role of proteins are well documented for translocation as carriers, channels or porters or electrogeneric pumps.

The observation that the sugars formed in the haustorium are translocated to the plumular sheath is substantiated by an initial increase followed by a decrease in the sugar content of the cotyledonary sheath which functions as an intermediary between the haustorium and the plumular sheath following germination. All the palms investigated in the present study show more or less identical pattern of changes in the soluble as well as reducing sugars with the value reaching maximum by the 4th stage and declining subsequently (Tables 14B, 16B). The point at which the decline in the sugar content begins in the cotyledonary sheath coincides with the differentiation of the plumular sheath, indicating a strong source-sink relationship between the two tissues.
The stages of starch depletion in the haustorium of the palm seeds studied coincide with a rapid rise in the starch content in the plumular sheath. Thus it is inferred that starch synthesized in the haustorium is hydrolysed and is translocated as sugars through the cotyledonary sheath to the growing plumular sheath where starch is again synthesized. The low starch content observed in the cotyledonary sheath throughout the period of germination and seedling development corroborates the transport of sugars from the haustorium to the plumular sheath where it is metabolized as starch. The starch content in the plumular sheath of *B. flabellifer* and *C. umbraculifera* show a hike followed by a decline in the last stages of germination. Nevertheless, significant differences in the phases of differentiation following germination cannot be ruled out because the sampling was done at almost comparable intervals based on seedling morphology. The decline in starch content towards the later stages of germination can be attributed to the advancement of seedling growth and resultant mobilization of soluble sugars formed by the hydrolysis of starch by amylase activity.

In the plumular sheath of *B. flabellifer*, the amylase activity is increased initially and a decline is observed in the later stages of germination (Table 12A). This corresponds with the relatively very high starch content of the plumular sheath. Padmanabhan *et al.* (1978) reported the starch rich nature of tuberous seedling in *B. flabellifer*. In *C. umbraculifera*, the amylase activity showed rapid increase in the plumular sheath and no decline in the activity is noticed till the last stage of study (Table 12B). The ever-increasing enzyme activity in the plumular sheath is responsible for the rapid metabolism of starch, which is essential for seedling development until it attains autotrophy.

Total as well as reducing sugar content show a progressive increase in the plumular tissue of all the palm seeds under investigation (Table 14D,
16D). This is due to the mobilization of sugars from the endosperm and haustoria through the cotyledonary sheath and due to the breakdown of starch synthesized in the plumular sheath as transient storage molecules, which is mobilized for seedling development.

The total soluble sugars and reducing sugars in the different seeds tissues of the palms investigated show a parallel behaviour during different stages of seedling growth. According to Goldberg and Roland (1971), in Asparagus seeds soluble sugars are released as the endosperm cell walls are degraded. It has been suggested that hemicellulases are secreted by the haustorial cotyledon, which remains within the endosperm following germination, and that the hydrolytic products of the cell walls are then absorbed to support axial growth.

Starch synthesis occurring in the haustorial cells is very significant in palm seeds during germination, particularly in B. flabellifer and C. umbraculifera (Table 11B, Fig. 14B) and their immediate degradation is evident by high amylase activity (Table 12 A-B). Whenever the hydrolytic products of starch accumulate in the form of glucose, in the haustoria and cotyledonary sheath, they may be converted into sucrose, which is the important translocating form of sugars (Preiss and Levi, 1980).

According to Bewley and Black (1994) in legume seeds like Phaseolus vulgaris, starch hydrolysis by α and β amylases in the cotyledons, results in the synthesis of maltose. However, in B. flabellifer and C. umbraculifera, starch hydrolysis due to amylase activity is coincident with the increase in reducing sugars indirectly revealing the breakdown of maltose into glucose by α glucosidase activity.

The similarities between the metabolization of galactomannans in fenugreek and palms are unique in that the cotyledons do not contain starch
and it is synthesized as a temporary reserve following germination. The galactomannans content of cereals and endospermic legumes such as *Trigonella foenum-graecum* (Meier and Reid, 1982) is hydrolyzed by enzymes released from a living protoplastic layer of aleurone cells and the products are absorbed by the scutellum which is a reduced cotyledon in the cereals. In palm seeds, there is no such a distinct aleurone layer or scutellum. According to Buchanan et al. (2000), there are no secondary deposits of galactomannans in the cell walls in the aleurone layer, which remain alive, and hence they are the source of the enzymes that degrade galactomannan following germination. The functions performed by these organs are presumed to be carried out by the haustorium in palm seeds.

As mentioned earlier, the distal end of the cotyledon is differentiated into the haustorium while the proximal end is elongated as a cotyledonary sheath carrying the growing axis of the seedling. However, the source of the synthesis of enzymes for the degradation of galactomannan is not investigated in most palm seeds.

Keusch (1968) demonstrated that isolated haustorium of date seedlings were able to degrade mannans *in vitro* and concluded that the haustorium secretes the enzymes necessary for mannan breakdown. However, in the palm seeds of the present study, the presumption is not possible because the haustorium is developed from the distal end of the rudimentary cotyledon and contains abundant soluble carbohydrates and starch.

Seeds of all the five palm species contain galactomannan, the hydrolytic products of which consist mostly of mannose and some galactose (Table 9A). Reserve mobilization starts along with germination. As already described, the seedling consists of different morphological components, rather organs, and reserve mobilization pattern is very peculiar in palm seeds. Even though the present author chose the sampling of seed/seedling at different
intervals, utmost care was taken to draw a continuous spectrum of reserve mobilization.

Once the hydrolytic products start effluxing from the endosperm, the haustorium, which is formed as a transient storage organ, store carbohydrates as insoluble starch in order to maintain optimal osmoticum and their accumulation seems to be due to the slow rate of translocation to the elongating cotyledonary sheath carrying the growing embryo proper. However, as seedling growth proceeds, the starch content of the haustorium is hydrolysed as a result of exorbitant amylase activity. The soluble sugars so formed, enter the developing plumular sheath where again starch synthesis and accumulation occur with a concomitant depletion of starch in the haustorium that became shrunken and lose cellular integrity. The cotyledonary sheath acts only as a passage or intermediary organ for the efflux of soluble metabolites from the haustorium to the plumular sheath. Following further growth of seedling, actual shoot (eophyll) and roots develop resulting in the depletion of starch in the plumular sheath. Even though detailed study is conducted only in B. flabellifer and C. umbraculifera, almost similar pattern is shown by the other species also, with slight fluctuations, which are presumed to be species specific. Compared to the well-established gluconeogenetic origin of starch/sugars in lipid rich seeds, the palm seeds which are galactomannan rich, are characterized by intermittent accumulation of starch /sugars in the haustorium and plumular sheath, the cotyledonary sheath being an intermediary organ containing comparatively more proteins which are presumed to be involved in translocation of sugars.

For a comparative study of storage behaviour, dormancy and reserve mobilization of palm seeds, characterized by remotive type of germination; investigations are undertaken in five palm species – Borassus flabellifer,
Corypha umbraculifera, Caryota urens, Licuala peltata and Livistona rotundifolia. Although morphophysiological dormancy and short life span are common characters, the seeds show significant variations in aspects like thickness of pericarp, seed storage behaviour, desiccation and chilling sensitivity, time taken for initiation of germination, time taken for the development of different seed tissues etc. Moreover, moisture content, which is found to control dormancy, also varies among the five species. C. umbraculifera and L. peltata are characterized by the recalcitrant nature while the other three species show intermediate behaviour.

Mobilization of reserves following germination and during seedling growth accomplished by the estimation of metabolites in the components such as endosperm, haustoria, cotyledonary sheath and plumular sheath reveals almost similar pattern. Nevertheless, qualitative and quantitative differences in the distribution of dry matter, galactomannan/mannan, starch, total and reducing sugars, proteins and lipids do occur among the five palm seeds which vary in size and morphology and hence the differences may be species specific.