The six species of present investigation were *Cassia fistula* Linn., *Cassia siamea* Lam., *Cassia alata* Linn., *Cassia occidentalis* Linn., *Cassia tora* Linn. and *Cassia hirsuta* Linn. under genus *Cassia* Linn. belong to the sub-family Caesalpinioideae of family Fabaceae. They were distributed all over Assam, India both in plain as well as in hilly areas up to the elevation of 900 meters above the sea level. Out of these six species *Cassia fistula* Linn. and *Cassia siamea* Lam. were perennial and tree. In between these two, *Cassia fistula* Linn. was deciduous, while *Cassia siamea* Lam. was evergreen in nature. Among the other four species *Cassia tora* Linn. was herb, but *Cassia alata* Linn. was a large shrub, while *Cassia occidentalis* Linn. and *Cassia hirsuta* Linn. were shrub. In all the six species the type of leaf was found to be unipinnate and peripinnately compound with pulvinous leaf bases. But the number of leaflets varies from species to species and their shape and size also different. Flowers were found in either raceme or panicle type of inflorescence in all the investigated species except *Cassia tora* Linn. where 1-3 flowers were found at the axils of the leaf without any definite type of inflorescence. In all the taxa flowers were yellow in colour and flowering time was at summer. Stamens were found to be heterodynamous and in *Cassia fistula* Linn. all the 10 stamens were fertile, while in other five species out of the 10 stamens each, 7 were fertile and rest 3 were sterile. Except shape and size, gynoecium characters were almost same in all the studied taxa. Though the type of fruit in all the species were represented by pod (legume), still some of their features were different. Pods of
*Cassia fistula* Linn. were indehiscent and in others dehiscent. Again in *Cassia fistula* Linn., *Cassia tora* Linn. and *Cassia hirsuta* Linn. shape of the fruit was cylindrical; in *Cassia siamea* Lam. and *Cassia occidentales* Linn. flat pod; while in *Cassia alata* Linn., it was found as winged pod.

Present day knowledge on classical taxonomy is greatly enhanced by the detail studies on micromorphology of leaves, chromosomes, pollen grains, fruit and seed morphology. Thus the microtaxonomic studies immensely helpful in solving many intricate taxonomic problems involving proper identification and speciation of many plants. (Stebbin, 1958; Carlquist, 1961; Stace, 1965; Ramayya, 1969; Dilcher, 1974; Bush et al., 1977; Sehithe, 1979; Bessis and Guyot, 1979; Wilkinson, 1979; Clark et al. 1980; Metacalfe and Chalk, 1980; Solomon, 1983; Cutler, 1984; Gbile, 1986; Knop et al. 1996; Bordolo, 2004; Fawzi, 2011; Sevik, 2012).

Microtaxonomic studies couples with information derived from micromorphological, foliar epidermal, cytological, palynological, fruit and seed morphological studies made in the present investigation revealed some interesting observations. Significant differences could be noticed among the investigated taxa in the features like presence and absence of stomata, stomatal frequency, nature of the epidermal cells, chromosome number, position of the centromere on the chromosomes, pollen surface, characters of fruit and seeds.

**Epidermal morphology**

Micromorphological features, particularly those of foliar epidermis, have been proved to be useful in delimiting many taxa (Radford, 1986). Many workers have emphasized the use of epidermal features like type and nature of stomata, epidermal cells, epidermal hairs and papillae in angiosperm systematic (Stace, 1965;
Paliwal and Anand, 1978; Sharma and Shiam, 1984; Radford, 1986; Sigaimani, 2005; Ogundipe et al., 2009; Saheed and Illoh, 2010; Awarinde et al. 2012). In the present investigation, the different epidermal characters particularly the presence and absence of stomata, nature and type of stomata, epidermal hairs and epidermal cells, venation pattern have been employed judiciously in differentiating the different species of *Cassia* Linn. In two tree species, *Cassia fistula* Linn. and *Cassia siamea* Lam., stomata were completely absent from the upper epidermis of the leaf, these are present only on lower surface; while in other four species, stomata were present on both the surfaces of the leaf. As far as the guard cell of the stomata is concerned, in *Cassia tora* Linn. and *Cassia hirsuta* Linn., both the guard cells of each stomata were found to be equal in size. But in other four species along with equal guard cells, sometimes unequal also found. Epidermal cells were always polygonal in *Cassia occidentalis* Linn., pentagonal in *Cassia fistula* Linn. and in other four species a single specific type was not found. They may be the combination of pentagonal, rectangular, and oval. Epidermal cell walls were straight in *Cassia siamea* Lam. and in *Cassia tora* Linn., sinuous in *Cassia occidentalis* Linn., while in *Cassia fistula* Linn., *Cassia alata* Linn. and *Cassia hirsuta* Linn. these were found to be both straight and sinuous. Cuticular examination revealed the presence of stomata on the surfaces of the leaflets in most of the species with higher frequency on the lower epidermis. The paracytic type of stomata was common to all the studied species is an agreement with the work of Okpon (1969 a & b). Actinocytic condition (stomata surrounded by many subsidiary cells) is considered as probably derived from the paracytic form and this have so far been found in a few rare cases in *Cassia* Linn. Okpon (1969 b) reported actinocytic type of stomata in
Cassia alata Linn. distributed in Tropical Australia, South East Asia and some islands of the Pacific.

Though the stomatal frequency (SF) varies considerably with the age of the leaf, stomatal index (SI) is highly constant for any given species (Olatunji, 1983; Adedeji and Jewoola, 2008). In the present study, SI was found to be helpful in species delimitation. Cassia occidentalis Linn. had the lowest SI on the upper epidermis (23.38%) while Cassia siamea Lam had the lowest on the lower epidermis (25.31%). It was highest in Cassia alata Linn. on both the epidermis (Upper – 35.29%, Lower – 42.15%) (Table-3).

Though ecological variations may affect the degree of hairiness, the type of hair is usually constant in many species (Okpon, 1969 a) and many workers have found the presence or absence and type of trichomes on epidermal surfaces as classificatory tools ( Lavania, 1990; Adedji et al., 2007). Metacalfe and Chalk, 1979 has long suggested that the types of epidermal trichomes can frequently delimit species, genera or families in plant. Differences in trichome types were employed by Isawumi (1989) to delimit species in genus Vernonia. In this study, the presence or absence of trichomes as well as their types can be useful in characterizing the genera and species. In Cassia occidentalis Linn. trichomes were absent from both the surfaces, while in Cassia tora Linn. these were present only on the lower surface and in other species on both the surfaces (Table 2). The presence of glandular/non-glandular trichomes in Cassia hirsuta Linn. separates it from Cassia fistula Linn., Cassia siamea Lam, Cassia occidentalis Linn., Cassia alata Linn. and Cassia tora Linn. that possess only non-glandular trichomes. Although quantitative, the variations in trichome length observed in this study can be reasonably employed in delimiting the species. On the basis of the leaf surface features Cassia hirsuta Linn.
was totally separate from other five species as it has the hirsute surface, while others it was mainly glabrous except *Cassia alata* Linn., in which both glabrous and pubescent surfaces were present.

Leaf venation pattern is a genetically controlled character (Roth-Nebelsick *et al.*, 2001) and tends to be species specific (Zetter, 1984; Kohler, 1993). In the present study, each *Cassia* species exhibited their own specific leaf venation pattern. The venation characters have been advantageously used for establishing the distinctiveness of all the studied *Cassia* members. Therefore it is quite logical to consider the leaf venation pattern as a key in the systematics of all the six species of *Cassia* Linn. in the present study. Roth- Nebelsick *et al.* (2001) also advocated the use of leaf venation pattern as a taxonomic tool.

**Karyomorphology**

Cytological characteristics are of immense importance in interpreting a classification and establishing relationships. The information collected from chromosomal features has been found to be valuable at all levels of taxonomic hierarchy (Jackson, 1971; Barma, 2007) and the usefulness of cytological data varies from group to group. Each plant species or variety is known to be featured by its karyotypes. Chromosome morphology also helpful in the process of identification of species level, even in variety level and to establish relationship among the allied species (Patil and Jadhav, 1985; Na and Kondo, 1994). Karyotypes of six species of genus *Cassia* Linn. of the family Fabaceae were studied in the present investigation to interpret their karyomorphological interrelation and affinity.

A few literatures on the karyomorphological details of the studied species of *Cassia* Linn. are available. Most of the works were restricted to chromosomes count
only in the previous works. A systematic attempt has been made in the present study to observe the morphological details of the chromosomes. It has been noticed that in earlier investigations by different workers the somatic chromosome numbers were found to be different for the same species. Nanda (1962), Datta and Jena (1974) reported \( n=12 \) chromosomes for *Cassia fistula* and Bir and Sidhu (1966) has reported \( n=13 \) for the same species. Again Sareen and Pratap (1975) found \( n=14; 2n=24 \) was observed by Irwin and Turner (1960), Mehra (1972), Das and Chatterjee (1994) and \( 2n=28 \) was reported by Pantulu (1946), Bir (1967), Mehra and Hans (1971), Mehra and Sareen (1973) and Sarkar and Chetterjee (1973) for the same species *Cassia fistula* Linn. For *Cassia siamea* Lam. same findings were reported as \( n=14 \) chromosomes by Sareen and Pratap (1975); \( 2n=28 \) chromosomes by Jacob (1940), Irwin and Turner (1960), Chatterjee, (1969), Datta and Datta (1973) and Das and Chatterjee (1994). In case of *Cassia alata* Linn. the basic chromosome number has been reported as \( n=14 \) by Pantulu (1960), Datta and Datta (1973); \( 2n=12 \) by Senn (1938), while \( 2n=24 \) by Irwin and Turner (1960). On the other hand \( n=13 \) chromosomes have been reported for *Cassia occidentalis* Linn. by several workers including Muto (1929), Frahm – Leliveld (1960), Bir and Sidhu (1966), Sinha and Acharia. (1972), Gill and Husaini (1981). But according to Singhal *et al.* (1990) it was \( n=12 \). On the other hand \( n=14 \) was observed by Irwin and turner (1960) and Sareen and Pratap (1975); \( 2n=24 \) by Ramnathan (1955) and \( 2n=28 \) by Pantulu (1942), Tandon and Bhat (1971), Bir and Kumari (1979). The works of Bir and Sidhu (1966), Colman and De Meneges (1980) revealed that the basic chromosome number in *Cassia tora* Linn. was \( n=13 \), but Sarkar *et al.* (1973) found it as \( 2n=24 \); Chatterjee (1969), Tandon and Bhat (1971) as \( 2n=26 \) and Jacob (1940), Sareen *et al.* (1974), Bir and Sidhu (1980) as \( 2n=28 \). Katayama (1953) and Miege (1960)
reported an abnormally higher number of chromosomes in *Cassia tora* Linn. which was 2n=52. In *Cassia hirsuta* Linn. also different workers have reported variable number of chromosomes after their study. Irwin and Turner (1960) has reported that n=14, while Sugiura (1931) and Diers (1961) found it as 2n=24. Frahm – Leliveld (1953) has found two different numbers of the chromosomes in the same study on *Cassia hirsuta* Linn. as 2n=28 and 56. According to Raman & Kesavan (1963), Mehra and Bawa (1969), Rye (1979) and Stace *et al.* (1997) the inconsistency in the chromosome number may be attributed to the role of aneuploidy and polyploidy. Darlington and Wylie (1945) also not reported a constant basic chromosome number for a particular species of genus *Clerodendrum* Linn. of family Verbenaceae. Variation in the basic chromosome number for the same genus also reported by Vijayavalli and Mathew (1989) for *Smilax* Linn. and Greizerstein and Poggia (1994) for the genus *Amaranthus* Linn.

The findings of present study on the somatic chromosome number in *Cassia fistula* Linn. 2n=28 was found to be the same as those of previous record of Pantalu (1946), Bir (1967), Mehra and Sareen (1973) and Sarkar *et al.* (1973). But it is sharp contrast to the earlier findings of Nanda (1962) and Bir and Sidhu (1966) where *Cassia fistula* Linn. was reported to possess n=12 and n=13 respectively. The somatic chromosome number of *Cassia siamea* Linn. was found as 2n=28, which was also in conformity with few of the earlier works including Irwin and Turner (1960), Datta and Datta (1973), Das and Chatterjee (1994).

In the present study the chromosome number of *Cassia alata* Linn. was found as 2n=26 which was a sharp contrast to the earlier report where n=14 was recorded by Pantalu (1960), Datta and Datta (1973); 2n=12 by Senn (1938) and 2n=24 by Irwin and Turner (1960). No report was found to be similar to that of
present study as far as the chromosome number is concerned. The somatic chromosome number in *Cassia occidentalis* Linn., *Cassia tora* Linn. and *Cassia hirsuta* Linn. were found as 2n=24 which was similar with some of the previous works like Ramnathan (1955), Singhal *et al.* (1990) for *Cassia occidentalis* Linn.; Sarkar *et al.* (1974) for *Cassia tora* Linn. and Sugiura (1931), Diers (1961) for *Cassia hirsuta* Linn. But some of the findings reported by workers like Bir & Sidhu (1966), Gill and Husaini (1981) as n=13 chromosomes; Irwin and Turner (1960), Sareen and Pratap (1975) as n=14 and Tandon and Bhat (1970), Bir and Kumari (1979) as 2n=28 for *Cassia occidentalis* Linn. Some of the previous works done on *Cassia tora* Linn. showed that n=13 (Bir and Sidhu, 1966), 2n=26 (Tandon and Bhat, 1971), 2n=28 (Sareen *et al*., 1974; Bir and Sidhu, 1980) and 2n=52 (Miege, 1960) which were not similar with the present work. On the other hand sharp contrast reports were forwarded by several workers in which chromosome number was shown as n=14 (Irwin and Turner, 1960) and 2n=28, 56 (Frahm – Leliveld, 1953). As the variable number of chromosomes was found in all the six investigated taxa, a conclusion can be drawn only after karyomorphological study.

Chromosomes were found to be the combination of both long and extremely short in size in all the six investigated species. The longest chromosome was found in *Cassia fistula* Linn. with 5.90µm in length and shortest one was observed in *Cassia tora* Linn. with a length of 0.90 µm. the shorter size of the chromosomes had placed limitation on further detailed study on the chromosome morphology. No secondary constriction could be detected in any one of the chromosomes of the six taxa under study. The non detection of secondary constriction may be due to lack of proper staining technique as well as the shorter size of the chromosome. Therefore the detection of secondary constriction in the chromosomes may not be ruled out. In
spite of these limitations, attempts were made for detail karyomorphology of all the six species under study as far as possible.

The Karyomorphological studies of the six species of genus Cassia Linn. of Family Fabaceae (Sub-family Caesalpinioideae) revealed a number of notable features. Three distinct groups were noticed with reference to their chromosome number. In first group with woody habit showed chromosome number as 2n=28. Cassia fistula Linn. and Cassia siamea Lam. belong to this group. In second group the chromosome number was found to be 2n=26 and large shrub Cassia alata Linn. was the lone member of this group. In third group three species with shrub and herb viz. Cassia occidentalis Linn., Cassia tora Linn. and Cassia hirsuta Linn. were included, in which the number of chromosomes were counted as 2n=24.

Among the six taxa, the variations in the chromosome size were found to be distinct. In Cassia fistula Linn. and Cassia siamea Lam. type A (long) chromosomes were found, but in other four species, no chromosome of this type was observed. On the other hand in Cassia tora Linn. type F (shortest) chromosomes were visualized, but in rest five species this type was completely absent. As far as the type of the centromeric position was concerned, in most of the cases chromosomes were found either metacentric or sub metacentric type. But in Cassia alata Linn. and Cassia occidentalis Linn. along with metacentric and sub-metacentric type, sub-telocentric type also found. Thus the karyotype in each species may be described as symmetrical, since the karyotype is consisting of chromosomes all essentially similar to each other in size and with median, sub-median and sub-terminal position of the centromere may be considered as symmetrical (Stebbins, 1950, 1971).
Pollen grains

Palynology played a significant role in angiosperm systematic (Nair, 1980; Cronquist, 1981). Pollen features have been used as an additional tool for elucidating taxonomic status of Caryophyllidae (Cronquist, 1981); *Cyperus* (Sharma, 1987) and others. Although the pollen morphology provides an important aid in distinguishing different species (Erdtman, 1952; Nair and Sharma, 1962; Nair, 1966), yet the present investigation did not reveal much distinct pollen morphological differences among the six species of genus *Cassia* Linn. Pollen grains in all the taxa were found to be trizonocolpate and colpi never meet each other at either pole. The shape of the grains were observed as prolate to subprolate in *Cassia fistula* Linn., *Cassia siamea* Lam. and *Cassia alata* Linn.; while in *Cassia hirsuta*, it was subprolate to spheroidal. On the other hand in *Cassia occidentalis* Linn. the shape was found to be only subprolate and in *Cassia tora* Linn. only prolate type. Pollen grains showed some differences in their size. Largest pollen grains were observed in *Cassia tora* Linn. with a length of 52.38 µm (±0.063) and smallest grains in *Cassia alata* Linn. with a length of 19.44 µm (±0.063). The surface of the pollen grains appeared to be smooth without any ornamentation under compound microscope. But under electron microscope distinct exine ornamentation can be observed. In *Cassia fistula* Linn., and *Cassia tora* Linn.; the exine ornamentation was observed as mesocolpial. But in *Cassia alata* Linn. and *Cassia hirsuta* Linn., it was found to be quite distinct and not identical to others. In *Cassia alata* Linn., ornamentation was identified as scabrate while in *Cassia hirsuta* Linn. it was perforate. Both the ends of the pollen grains were observed as blunt in all the species except *Cassia hirsuta* Linn. in which one end was blunt but the other end was slightly pointed. Wodehouse (1936) considered that thinner the exine, highly
evolved is the grain. In the present study it was found that all the pollen grains bear thin exine. Out of all, *Cassia occidentalis* Linn. had the most thin exine with 1.68 µm (±0.004). Hence, *Cassia occidentalis* Linn. may be considered as highly evolved among all the six species of *Cassia* Linn. under study as far as the exine character is concerned. Considering all the factores, the degree of differences among the six taxa reveal that – *Cassia fistula* Linn. and *Cassia siamea* Lam. was widely apart from other four species if we consider the shape, size and exine sculpture. Based on aggregate differences of pollen grains the six species of genus *Cassia* Linn. under study can be arranged as *Cassia fistula* Linn., *Cassia siamea* Lam., *Cassia tora* Linn., *Cassia occidentalis* Linn., *Cassia alata* Linn. and *Cassia hirsuta* Linn.

**Fruit and seed morphology**

Study of fruit and seed morphology also included to access the possible consideration of the characteristics to be used in the interrelationship and phylogeny of the six species of genus *Cassia* Linn. under study of family Fabaceae of sub family Caesalpinioideae. Though the type of the fruit in all the studied taxa was found to be pod (legume), all were not dehiscent. *Cassia fistula* Linn. had the non dehiscent pod while in others they were dehiscent. The pods of *Cassia hirsuta* Linn. were found to be cylindrical while in *Cassia siamea* Lam. and *Cassia occidentalis* Linn., these were flat pod and in *Cassia alata* Linn., it was four ribbed pod. Except *Cassia siamea* Lam. seeds were compactly arranged within pod, here depressions were found in between seeds. Highest number of seeds per pod was found in *Cassia fistula* Linn. with a total of 94 (±0.140) seeds and least number was observed in *Cassia siamea* Lam. with 15 (±0.120) seeds only. The difference in function of the dehiscence of pods as well as the number of seeds was used by Bisby (1981) to
segregate the genera *Genistai* and *Retama*. The size of the pods varies widely in the studied taxa (Table-14). Longest pod was observed in *Cassia fistula* Linn. with a length of 62.50cm (±0.150) while shortest pod with a length of 12.20cm (±0.080) was measured in *Cassia occidentalis* Linn. There exists a positive correlation between pod size and corolla size in the taxa under study, so that the species with larger flowers produce large fruit. This seems an obvious relationship since there also in general seems to be a positive correlation of flower size (Lopez et al., 1999).

Morphologically fruits of *Cassia fistula* Linn. and *Cassia alata* Linn. found to be straight and slightly curve in other species. In *Cassia hirsuta* fruits were covered by thick coat of hairs while in others the surface was found as pubescent or glabrous.

In *Cassia siamea* Lam. seeds were not compactly arranged, depressions were found in between seeds. In all other studied species seeds were compactly arranged. But the shape, size, weight, colour, length – breadth, germination characteristics of the seeds were differing in all the species under study. Colour and shape of the seeds were seems to be species specific and these two characters may be fruitfully used in the delimitation of the species. The surface of the seeds in hirsute shrub *Cassia hirsuta* Linn. was at par with two tree members *Cassia fistula* Linn. and *Cassia siamea* Lam. and large shruby member *Cassia alata* Linn. seems to be rugulate while in *Cassia occidentalis* Linn. and *Cassia tora* Linn. it was found to be tuberculate which indicates its closeness. Length and breadth of the seeds were found to be varied and species specific. Largest seed with 8.00mm (±0.024) x 6.00 mm (±0.020) was found in *Cassia fistula* Linn. and smallest with 3.00 mm (±0.008) x 3.00 mm (±0.008) was observed in *Cassia hirsuta* Linn. One interesting observation about the seed coat of two tree species *Cassia fistula* Linn. and *Cassia siamea* Linn.) and the lone large shruby species (*Cassia alata* Linn.) was the
presence of thick seed coat. For the germination of the seed in *Cassia fistula* Linn. and *Cassia siamea* Lam. concentrated acid treatment was required followed by water soaking, while in *Cassia alata* Linn. hot water treatment should be followed by normal water soaking for the purpose. But in two shrubby species, *Cassia occidentalis* Linn. and *Cassia hirsuta* Linn. along with lone herb species *Cassia tora* Linn., simply water soaking of seed was enough for the germination. Germination percentage was quite high throughout the taxa under study. It was as high as 98% in *Cassia hirsuta* Linn. and as low as 63% in *Cassia siamea* Lam.

From the aggregate similarity and differences it has been observed that *Cassia occidentalis* Linn. and *Cassia tora* Linn. were related, while *Cassia hirsuta* Linn. distantly related. *Cassia fistula* Linn., *Cassia siamea* Lam. and *Cassia alata* Linn. have less relation with the other three species.

**********