Chapter 5: Discussion

5.1 GROWTH HABIT AND PLANT MORPHOLOGY

The present study and previous works have shown that *A. sessilis* 'Green' could be found in both terrestrial and aquatic habitats (Backer, 1949; Henderson, 1959; Maheshwari, 1963; Merrill, 1912; Pancho & Soerjani, 1978; Ridley, 1924; Trimen, 1980; Womersley, 1978). The origin of *A. sessilis* 'Red' remains unknown. It could be an introduced species owing to its popular medicinal value.

Studies showed that *A. paronychioides* could be found in dry sandy areas, damp places or areas subjected to periodical inundation (Chen, 2008; Mishra, 1994; Trimen, 1980; Veldkamp, 1971). Likewise, almost all of the specimens for the present study are collected from dry sandy areas except for one single collection from the mangrove area. Previous study reported *A. brasiliana* as a herb (Backer, 1949) but this is distinctly different from the present study where *A. brasiliana* is a shrub.

The present study proposes to divide *Alternanthera* species into two groups based on the inflorescence structure. Group A is made up of *A. brasiliana* which has pedunculate inflorescence while group B is made up of *A. sessilis*, *A. paronychioides*, *A. ficoidea* and *A. bettzickiana* that have sessile inflorescence (Table 13). Similar studies have been reported recently and it is further supported by molecular phylogenetic study (Sánchez-del Pino *et al*., 2012).

Besides having pedunculate inflorescence, *A. brasiliana* also differ from the other species studied in several other morphological aspects. These are: appressed straight to curved hairs scattered on the stem internodes and leaves; obtusangular and articulated stem; dentate crest on the bracteoles; sterile stamens; cylindrical tube formed by filaments and obovoid ovary. Most of the characters are similar to the previous

studies except for two characters (Backer, 1949; Sánchez-del Pino *et al.*, 2012; Wagner *et al.*, 1990). For instance, fertile stamens have been reported in specimens collected in Hawaii (Wagner *et al.*, 1990) whereas all the present specimens from different localities are found to be sterile. Moreover, hairy rachis has so far not been observed although it was reported in specimens collected in Malesia (Backer, 1949).

Two subgroups could be formed in group B based on the morphology of ovary, bracteole and petal. *Alternanthera sessilis* and *A. paronychioides* are placed in subgroup 2A as having obcordate ovary, absence of bracteoles, petal with acute apex. *Alternanthera ficoidea* and *A. bettzickiana*, placed in subgroup 2B are characterized in having subconical ovary, presence of bracteoles, inner petal with a mucronate apex.

Within subgroup 2A, *A. sessilis* and *A. paronychioides* could be further delimited based on six morphological characters. These are: hair structure and distribution on the stem internodes, leaves and petals; leaf margin; rachis; bract shape and apex; petal shape and number of nerves; number of stamens and pseudostaminodes apex.

Most of the characters reported in *A. sessilis* in the present study are in line with previous studies especially those from Malesia (Backer, 1949) and the Malay Peninsula (Henderson, 1959; Ridley, 1924). Nevertheless, *A. sessilis* from the present study is slightly different from earlier reports in the number of stamens. Three stamens are noted in the present study while five stamens were reported. Among the five stamens, two or three are anantherous (Backer, 1949; Henderson, 1959; Trimen, 1980; Womersley, 1978). In addition, bracts with mucro and presence of bracteoles were previously reported but not noted in the present study (Trimen, 1980).

In *A. sessilis* ‘Green’, the leaf from the aquatic area is longer and narrower as compared with the leaf from the terrestrial area. Leaf and stem variation correlates to

habitat has been reported in the previous study (Backer, 1949; Trimen, 1980). Similarly, *A. philoxeroides* also shows different phenotypes in different habitats and this is mainly due to phenotypic plasticity rather than low genetic variation (Geng *et al*., 2006; Li & Ye, 2006; Wang *et al*., 2005; Xu *et al*., 2003). The causes of the broad ecological breadth and morphological variation of the green remain unclear until further study is carried out.

Aside from the colour of stem, leaf, flower and ovary, *A. sessilis* 'Red' was identical to 'Green' in both qualitative and quantitative aspects (Table 13). These two leaf forms do not show significant difference in the length and width of leaf and petal as well as the stamen length. The colour difference could be due to genetic variation as three quantitative trait loci are responsible for different pigments on different parts of *Amaranthus* species (Kulakow *et al.*, 1985).

The morphology of *A. paronychioides* in the present study is also in accordance with that reported in earlier studies (Mishra, 1994; Sánchez-del Pino *et al.*, 2012; Trimen, 1980), especially those from Indo-Malesia (Veldkamp, 1971). A minor difference is noted when bracteole was reported (Veldkamp, 1971) but its presence is uncertain in the present study. This is because the differences between the bracteole and bract are indistinctive in the present study. Another difference is stiff hairs on the upper half of the outer petal (Veldkamp, 1971) which are not noted in the present study.

Within group 2B, *A. ficoidea* and *A. bettzickiana* could be further delimited based on five morphological characters. These are: hair structure and its distribution on the stem internodes, bract, bracteoles, and petal; leaf margin and style length. These characters have also been documented in species collected from Malesia (Backer, 1949) and Papua New Guinea (Womersley, 1978). However, the leaf colour of *A. ficoidea* was described as variegated with colour (Womersley, 1978) but this is not observed in

the present study. Hairy rachis is not observed in the present study although it has been reported in specimens found in Malesia (Backer, 1949).

Among all the morphological characters, hair morphology and distribution pattern at different part of the flower plays an important role in species delimitation in genus *Alternanthera*. This character has been suggested as a useful tool in species delimitation especially for the *Alternanthera* species in Malaysia (Backer, 1949).

Table 13: Key to species of *Alternanthera* collected in the present study.

1A. Pedunculate inflorescence. Stem internodes with appressed straight to curved hairs scattered all over. Stems obtusangular and articulate above the node. Lower and upper leaf surface with minute dentate hairs all over. Stamens empty (without pollen grains). Filaments fused for most of their length, forming a cylindrical tube. Ovary obovoid……..……..…….…….*A. brasiliana*

1B. Sessile inflorescence. Stem internodes with short erect stiff hairs concentrated along the interpetiolar median, nodes and leaf axils, or with long hairs all over. Stems terete. Lower and upper leaf surface with either flexuous long hair or appressed long hair. Stamens with pollen grains. Filaments fused at their base, forming a short cup. Ovary obcordate or subconical. ……..……..…….…….…….....(2)

2A. Ovary obcordate. Bracteoles absent. Petal equal or subequal, apex acute……....……..……...…….……...(3)

2B. Ovary subconical. Bracteoles present. Petal subequal, apex mucronate……. …….…….……..……...(4)

3A. Stem internodes with short erect stiff hairs concentrated along the interpetiolar median, nodes and leaf axils, otherwise glabrescent. Lower leaf surface with a few long hairs especially on the midrib and no or minute barb-like hairs scattered over lamina. Leaf margin sparsely serrate and occasionally fimbriate. Rachis hairy. Bract, deltoid with acute apex. Bracteoles absent. Petals equal, 1-nerved, abaxially glabrous, adaxially a few minute hairs or glabrous. Stamens 3. Pseudostaminodes, white, as long as stamens, apex narrowly to broadly triangular and entire-subentire along the margin. ……..……*A. sessilis*

3B. Stem internodes are densely covered with long flexuous hairs all over. Lower leaf surface densely scattered with appressed long hair all over. Leaf margin entire. Rachis not hairy. Bract absent. Bracteoles 3, convex-lanceolate with mucronate apex. Petals unequal. Outer petal 3-nerved, abaxially long-hairy at the lower half, adaxially glabrous. Inner petal abaxially hairy, adaxially glabrous. Stamens 5. Pseudostaminodes, green, half the length of stamens, apex broadly short-dentate…….*A. paronychioides*

4A. Stem internodes are densely covered with flexuous long hair all over. Leaf margin sparsely serrate. Bract abaxially long-hairy at the upper part. Bracteoles abaxially hairy along the midrib. Outer petal abaxially hairy along the margin. Inner petal abaxially hairy. Stamens 5, all fertile. Style shorter than 0.2 mm……*A. ficoidea*

4B. Stem internodes are densely covered with compressed long hairs all over. Leaf margin undulate. Bract abaxially short-hairy at the lower part. Bracteoles abaxially hairy at the lower part. Outer petal abaxially hairy to at the lower half. Inner petal glabrous. Stamens 5, 3–4 sterile. Style longer than 0.2 mm……*A. bettzickiana*

5.2 CHRONOLOGY OF FLOWER AND FRUIT DEVELOPMENT

Alternanthera sessilis and *A. bettzickiana* bloom throughout the year while *A. brasiliana* only blooms at the end of the year. *A. sessilis* is a fast growing species. The flowering period of the offsprings of *A. brasiliana* is two months longer than the parent plants. The reasons of the prolonged flowering period remain unknown and further study is needed. A single flower of the red and green leaf forms of *A. sessilis* takes 19–40 and 21–50 days to develop from bud to seedling respectively. The parent plants of *A. brasiliana* take a longer period as compared with *A. sessilis*, which is 31–99 days.

The flowering phenology of both *A. sessilis* 'Red' and 'Green' is similar. Among the species studied, only the flowers of *A. brasiliana* reach anthesis at midnight. Flower anthesis of *A. sessilis* and *A. bettzickiana* is similar to other *Amaranthus* species in the family, which occurs between 0700–0800 hours (Peters & Jain, 1987). *Alternanthera sessilis* and *A. bettzickiana* resemble *Alternanthera littoralis* var. *maritima* in having small flowers with diurnal anthesis, white tepals and yellow stamens (Antonucci *et al.*, 2011).

All the anthers fully dehisce in *A. sessilis* whereas only 2–3 anthers partially dehisce in *A. bettzickiana*. Although *A. bettzickiana* has produced pollen grains, they are sterile as evidenced in the embryological study. As compared to *Amaranthus hypochondriacus* L., anther dehiscence commences much earlier in *A. sessilis* and *A. bettzickiana* (Peters & Jain, 1987) (*A. sessilis*: 0800 hour; *A. bettzickiana*: 0700 hour; *A. hypochondriacus*: 1000 hour). Anther dehiscence has never observed to occur in *A. brasiliana* probably due to lack of fibrous thickening in the endothecium as evidenced in the embryological study.

Abnormal anther phenotype (brown and membranous) in *A. brasiliana* and *A. bettzickiana* (brown and shrunken) has indicated that these two species are male sterile

species (Kaul, 1988). Another male sterile indication could be the diameter of the open flowers during flower anthesis. The flowers of *A. brasiliana* only open slightly during flower anthesis and similar descriptions have been reported in other male sterile species such as *Amaranthus hypochondriacus* (Peters & Jain, 1987); *Corunastylis apostasioides* Fitzg. (Sorensen *et al.*, 2009); *Allium cepa* L., *Beta vulgaris* L., *Glycine max* (L.) Merr., *Pennisetum typhoides* (Burm.*f.*) Stapf & C.E.Hubb. (Kaul, 1988) and *Helianthus annuus* L. (Meric *et al.*, 2003). The causes of high flower abortion in *A. brasiliana* and *A. bettzickiana* remain unknown and further study is needed.

Alternanthera sessilis and *A. brasiliana* take less than 15 days to develop a mature fruit. These two species produce fruit in a shorter period as compared with *Amaranthus* species. For instance, *A. albus*, *A. blitoides* and *A. blitum* take 20–30 days (Costea & Tardif, 2003) while *A. retroflexus, A. powellii* and *A. hybridus* take 30 days (Costea *et al.*, 2004). The fruit morphology of *A. sessilis* and *A. brasiliana* in this study was similar to that reported in previous work, especially those reported in Malesia (Backer, 1949).

The present study and previous work have confirmed that fruits are not produced in *A. bettzickiana* (Backer, 1949). This is mainly due to male sterility and egg cell abortion which would be discussed in the embryological studies. Apart from *A. bettzickiana, A. philoxeroides* is another species in the genus that rarely set fruits or viable seeds (Julien, 1995; Xin *et al.*, 2008) due to sterile pollen grains (Liu *et al.*, 2008).

5.3 EMBRYOLOGICAL STUDY

5.3.1 Embryology of *Alternanthera sessilis*

Generally, the embryology of the species studied conforms well to the embryology of Amaranthaceae (Davis, 1966; Johri *et al.*, 1992) and *Alternanthera* (Kajale, 1935). Nevertheless, several differences are noted and will be discussed in the following paragraphs. The embryological studies have further confirmed that the *A. sessilis* 'Red' and 'Green' resemble each other. This present study confirms for the first time that *A. brasiliana* is an apomict while *A. bettzickiana* is a sterile plant.

In the past, it has been generally reported that the anther development of Amaranthaceae conforms to the Monocotyledonous type (Davis, 1966; Johri *et al.*, 1992). The present study, on the other hand, shows that the Dicotyledonous type of anther development is occasionally observed in all the species studied except in *A. brasiliana* and *A. sessilis* 'Red'. Fibrous thickening in the endothecium of *A. sessilis* (present study, at the stage of one-celled microspore) is developed slightly earlier as compared to previous study (at the stage of two-celled microspore) (Kajale, 1935).

In the *Alternanthera* species studied, the ovule is campylotropous, bitegmic, crassinucellar and the micropyle is formed by the inner integument only. Similar descriptions of these species have been reported (Kajale, 1940; Pal, 1968; Pal *et al.*, 1990; Sachar & Murgai, 1959). The presence of floral nectary in the species studied represents another new finding. The location of the nectary glands is similar to previous work in Amaranthaceae, which is at the inner base of the filament (Zandonella as cited in Bernardello, 2007). However, floral nectary is absent in *A. brasiliana*.

In the present study, one-celled archesporium, linear tetrad megaspores and pear-shaped egg cell are commonly found in *A. sessilis* (both leaf forms) whereas in previous studies, multicellular archesporium, three megaspores and broadly flask-

shaped egg cell have been reported (Davis, 1966; Johri *et al*., 1992; Kajale, 1935). In Amaranthaceae, one-celled archesporium is not always observed except in some *Amaranthus* (Narahara & Pullaiah, 1986; Pullaiah & Narahara, 1987; Sebastian & Deshpande, 1974) and present study. Filiform apparatus is not commonly found in the synergids of Amaranthaceae except in *Achyranthes aspera* (Kajale, 1935 & 1940; Johri, *et al*., 1992) and *Alternanthera* species (present study).

In addition, more than one pollen grain has been observed germinating on the stigma before fertilization. Thus, more than one accessory tube has also been observed penetrating the embryo sac via the micropyle (porogamous fertilization). In fact, this phenomenon is common in Amaranthaceae (Bakshi, 1952; Johri *et al*., 1992; Joshi & Kajale, 1937; Kajale, 1940; Narahara & Pullaiah, 1986; Padhye, 1962; Pullaiah & Narahara, 1987). In *Gomphrena celosioides* (Padhye, 1962) and *Psilostachys sericea* (Bakshi, 1952), two synergids are destroyed during penetration of accessory tube. However, only one synergid is destroyed in *A. sessilis* (present study).

In Amaranthaceae, the time of embryo sac elongation is not clearly stated (Bakshi, 1954; Kajale, 1940; Padhye, 1962). Nevertheless, the embryo sac of *Gomphrena celosioides* elongated after fertilization (Padhye, 1962) and this pattern of development is also observed in the present study. Similar to the previous studies, the antipodals are pushed laterally due to embryo sac elongation (Joshi, 1936; Kajale, 1935; 1937b).

The embryogeny *A. sessilis* 'Red' and 'Green' follows a transition form between the Chenopodiad-type and Solanad-type (Johansen, 1950). However, this is in contrast to the previous work on *A. sessilis* (Joshi & Kajale, 1937) and other members in Amaranthaceae. Earlier studies show that the embryogeny of Amaranthaceae follow that of the Chenopodiad-type (Davis, 1966; Johri *et al.*, 1992; Joshi & Kajale, 1937).

Members of the Amaranthaceae investigated so far have not shown polyembryonic seeds (Davis, 1966; Johri *et al.*, 1992). Thus, the incident of three zygotes in *A. sessilis* (present study) is a rare case. Moreover, the possibility of all these three zygotes developing into embryos is low since polyembryos have not been observed in the embryological study and seed germination experiments (results obtained from Chapter 4.7).

The endosperm development of the species studied follow *ab initio* Nuclear-type agreeing with the previous reports in Amaranthaceae (Davis, 1966; Johri *et al.*, 1992) and *Alternanthera* (Joshi & Kajale, 1937a). Wall formations in the nuclear endosperm do not proceed to the chalazal end and this is a unique feature of *Alternanthera* (Davis, 1966; Johri *et al*., 1992; Kajale, 1940). It has been suggested that the one or two layers of endosperm surrounding the hypocotyl-radicle region may act as an additional protection for the embryo (Prego *et al.*, 1998). This has also been observed in the species studied.

The seed coat of most Amaranthaceae species is two or three layers which are derived from both the outer and inner integuments (Bakshi, 1952; Padhye, 1962; Pal, 1968; Pal *et al.*, 1990; Sachar & Murgai, 1959) except in the species studied, and *Amaranthus hypochondriacus* (Pal *et al.*, 1990) in which the seed coat is single-layered.

The outer layer of the seed coat elongates developing a conspicuous thickened wall which is filled with tanniferous materials. This was observed throughout the family Amaranthaceae (Bakshi, 1952; Padhye, 1962; Pal, 1968; Pal *et al.*, 1990; Sachar & Murgai, 1959) including *Alternanthera* in the present study. An interesting feature is a layer of cuticle in between the inner integument and nucellus, as observed in *A. paronychioides* and *Psilostachys sericea* only (Bakshi, 1952).

5.3.2 Embryology of *Alternanthera brasiliana, Alternanthera bettzickiana* and *Alternanthera ficoidea.*

From the embryological studies, it is confirmed that *A. brasiliana* has adventive nucellar embryos which develop into mature embryos in the absence of pollen grains and egg cell. Three common features of adventive embryos have also been observed in the present and previous work; i.e. the absence of the suspensor in the embryo, the occurrence of polyembryony (Naumova, 1993) and early egg cell abortion (Gupta *et al.*, 1996; Kaur *et al.*, 1986; Lim, 1984; Naumova, 1993). In the family, *Aerva tomentosa* and *A. javanica* are the other examples of obligate apomict, these two species produce mature embryos via diplosporous parthenogenesis and this is different from the present study (Khan *et al.*, 1970; Sachar & Murgai, 1959).

Alternanthera brasiliana is a cytoplasmic male sterile species that does not produce any viable pollen grains. This is due to microspore abortion caused possibly by the early degeneration of the tapetum, either before meiosis or when the tetrads are released. Since the main function of the tapetum is to provide nutrients to developing pollen grains (Maheshwari, 1950), both microsporocytes and microspores of *A. brasiliana* degenerate at about the same time as the degeneration of the tapetum. This is supported by various studies that show either early (Chauhan & Singh, 1966; Horner Jr, 1977; Jones & Peterson, 1976; Sun & Ganders, 1987) or delayed degeneration (Pritchard & Hutton, 1972; Sandal & Alam, 1967) of the tapetum would result in microspore abortion.

Another possible factor that contributes to microspore abortion in *A. brasiliana* could be the absence of ubisch granules on the inner wall of the tapetum and endothecium. Ubisch granules consist of structural protein (RAFTIN) which is essential for pollen grain development (Wang *et al.*, 2003) and are involved in the development

of exine (Huysmans *et al.*, 1998). Therefore, in the absence of ubisch granules, the exine of the microspores is unable to develop. Most of the members of the Amaranthaceae have ubisch granules (Johri *et al*., 1992; Kajale, 1940; Padhye, 1962; Pullaiah & Narahara, 1987) except in *A. brasiliana* and *Amaranthus leucocarpus* (Sebastian & Deshpande, 1974). The lack of ubisch granules in *A. leucocarpus* is probably due to the short-lived endothecium (Sebastian & Deshpande, 1974).

Meanwhile, embryological observation also shows that the cause of indehiscent anther in *A. brasiliana* is probably due to the lack of fibrous thickening in the endothecium. This is a common characteristics in the male sterile flower (Dawson *et al.*, 1999; Meric *et al.*, 2003; Pritchard & Hutton, 1972; Villarreal *et al.*, 2009). Within the Amaranthaceae, most of the members are reported to have fibrous endothecium except in *Achyranthes aspera* (Johri *et al.*, 1992) and *Amaranthus leucocarpus* (Sebastian & Deshpande, 1974).

Nucellar embryo initials could be stimulated in the absence of fertilization as seen in *A. brasiliana* and other reported species (Desai, 1962; Gupta *et al.*, 1996; Kaur *et al.*, 1986). Nucellar embryo initial cells have been observed at different parts in the embryo sac, ranging from the micropylar to the chalazal region as well as along the length of the embryo sac (Desai, 1962; Kaur *et al.*, 1986; Koltunow, 1993). The presence of adventive nucellar embryony confirms the presence of apomixis as indicated by the presence of fruit set in the bagged flowers (results obtained from Chapter 4.6).

The lack of suspensor in the adventive embryo has become a controversial point when some authors claimed that the suspensor is seen after the globular stage (Maheshwari & Swamy, 1958; Wakana & Uemoto, 1987). In *A. brasiliana*, the suspensor is not seen throughout the embryogeny even after the globular stage. This is

very different from the zygotic embryos found in the other species studied in which the suspensor in present (*A. sessilis* and *A. paronychioides*).

Another typical character of the adventive embryo is polyembryony (Desai, 1962; Naumova, 1993). In the polyembryonic seed, the nucellar embryos compete among themselves to absorb nutrients from the endosperm during development. In this connection, not all the initiated nucellar embryos are able to develop into mature embryos successfully. For instance, two initiated nucellar embryos are noted but only one nucellar embryo managed to develop in *Shorea ovalis* (Ha *et al.*, 1988). From two to eleven nucellar embryos initiated, only two to six of them reach globular or older stage in *Commiphora wightii* (Gupta *et al.*, 1996). In *A. brasiliana*, on the other hand, two nucellar embryos are noted and both of them are able to develop into mature embryos. This is further evidenced in the seed germination experiment that shows two seedlings growing out from a single seed.

Nevertheless, zygotic embryo and adventive embryo could also occur concurrently within the same seed (Bacchi, 1943; Naumova, 1993). Undoubtedly, *A. brasiliana* does not produce zygotic embryo as fertilization could not occur. The actual type of embryogeny in *A. brasiliana* could not be determined due to the irregular sequence of cell divisions or uncertain position of the nucellar initial buds. This is commonly reported in adventive embryony (Lim, 1984; Naumova, 1993).

In the development of the endosperm, autonomous endosperm formation in *A. brasiliana* is less common as compared to amphimictic among the species that exhibit adventive embryony (Naumova, 1993). Similar observation is also noted in other apomict plants (Desai, 1962; Gorham, 1953; Gupta *et al.*, 1996).

Present embryological study shows that *A. bettzickiana* is a sterile plant as the embryo does not develop due to cytoplasmic male sterility and egg cell abortion. Unlike

A. brasiliana, *A. bettzickiana* produces coenocytic microspores resulting from incomplete cytokinesis (Albertsen & Palmer, 1979). Although studies have shown that these coenocytic microspores could develop into mature pollen grains (Albertsen & Palmer, 1979; Masamichi, 1986), this did not happen in the present study. In fact, these coenocytic microspores degenerate before the formation of exine. Although some tetrads produce microspores after the callose dissolution, these microspores are probably sterile since they are shrunken and devoid of cytoplasm (Kaul, 1988). The cause of microspore degeneration remains unknown as the tapetum and ubisch granules behaved normally in the present study.

Although the egg cell and zygote degenerate at an early stage, *A. ficoidea* is able to produce mature embryos. However, the origin of the mature embryo remains unknown as neither adventive nucellar initial cells nor integumentary buds have been observed. Unlike *A. brasiliana*, the endosperm development of *A. ficoidea* might be formed by amphimixis in which pollination and fertilization are needed (Naumova, 1993). This is postulated based on the presence of a conspicuous primary endosperm nucleus beside the degenerated zygote and the presence of pollen grains on the stigma. Similar observations have been reported in Rutaceae and Anacardiaceae such as *Aegle marmelos* (Johri & Ahuja, 1956); *Citrus* species and *Mangifera* species (Maheshwari & Swamy, 1958).

5.4 PALYNOLOGICAL STUDY

5.4.1 Comparative pollen morphology of *Alternanthera* species

On the whole, the pollen grains of the three species studied conform well to the *Gomphrena*-type of Erdtman (1966) which corresponds to the *Pfaffia*-type of Borsch (1998). The pollen is small and the pore structure is similar to Type I of Borsch (1998). Similar observations have been reported in the pollen grains from the New World (Müller & Borsch, 2005b; Borsch, 1998; Eliasson, 1988; Nowicke & Skvarla, 1979) and China (Li *et al*., 1993; Liang *et al*., 1978).

Variation in the quantitative characters in Amaranthaceae is fairly common and often the quantitative characters are not significant enough to define a pollen type (Borsch, 1998). Therefore, qualitative characters such as sexine ornamentation should be included. In fact, the present study is in line with this by using *A. sessilis* as an example. The pollen morphology of *A. sessilis* 'Red' and 'Green' is remarkably similar as shown by the result of the one-way ANOVA and t-test in which the polar length, equatorial diameter, the number of ektexinous bodies per pore and the height of the microspines has no significant difference. This is further strengthened by the similarity in the sexine ornamentation of these two leaf forms. Their sexine ornamentation is metareticulate, tectum perforate with a row of microspines attached distally.

Interestingly, the pollen grains of *A. ficoidea* seem to be identical with those of *A. sessilis* (both leaf forms) in having dodecahedric pollen grains with twelve pores. However, the aperture number alone should not be used to define a particular pollen type in this genus. This is because *Alternanthera* is known to be stenopalynous in terms of the aperture number (Borsch, 1998; Srivastava *et al*., 1977). This is in agreement with the existing report as most of the species examined have twelve to fourteen pores (Müller & Borsch, 2005b; Borsch, 1998; Li *et al*., 1993; Eliasson, 1988; Nowicke & Skvarla, 1979; Liang *et al*., 1978; Mittre, 1963).

The pollen grains of *A. ficoidea* could be distinguished from *A. sessilis* by the combined quantitative and qualitative characters. The result of the t-test suggests that the pores of *A. ficoidea* are larger (*A. ficoidea*: 4.83 µm; green and red leaf forms of *A. sessilis*: 4.17 µm and 4.48 µm respectively) and are covered by slightly more ektexinous bodies (*A. ficoidea*: 33; green and red leaf forms of *A. sessilis*: 29 and 28 respectively). The other characters that distinguish these two species are the type and height of microspines as well as the distribution of perforations. The microspines are longer and sharper in the pollen of *A. ficoidea* as compared to the pollen of *A. sessilis* (*A. ficoidea*: 0.29 µm; green and red leaf forms of *A. sessilis*: 0.24 µm and 0.22 µm respectively). The perforations are distributed at the top of the mesoporia only in *A. ficoidea* but they are distributed all over the mesoporia in *A. sessilis*.

The pollen grains of *A. paronychioides* are remarkably different from those of *A. sessilis* and *A. ficoidea* in both the quantitative and qualitative characters. The most obvious difference noted between *A. paronychioides* and the other two species is in the shape of the pollen grains. *A. paronychioides* is the only species which does not have the dodecahedric pollen grains. Although the pollen of *A. paronychioides* and *A. ficoidea* are characterized by similar sexine ornamentation, they could be distinguished by the pore structure. All the pores of *A. ficoidea* are round and are situated in a pentagonal face but a few are observed as oval and are situated in a hexagonal face as in *A. paronychioides*.

The pollen of *A. sessilis*, *A. ficoidea* and *A. paronychioides* can be distinguished as follows:

Table 14: Summary of comparative pollen morphology of the three species studied.

CHARACTERS	*A. sessilis* (both leaf forms)	*A. ficoidea*	*A. paronychioides*
Shape	dodecahedron	dodecahedron	slightly spheroidal
Polar length (μm)	< 20.0	< 20.0	> 20.0
Equatorial diameter (μm)	< 20.0	< 20.0	> 20.0
Pores			
• shape	round	round	round and oval
• number	12	12	14–18
• diameter (μm)	4.17 ± 0.35 (red) 4.48 ± 0.28 (green)	4.83 ± 0.20	6.06 ± 0.83
Sexine ornamentation			
• Shape of mesoporia	pentagonal	pentagonal	pentagonal and hexagonal
• End of microspines	blunt	sharp	blunt
• Perforation distribution	at the top of mesoporia and the area nearer to the pores	at the top of the mesoporia only	at the top of the mesoporia only

An attempt is made to observe the pollen morphology of *A. sessilis* (both leaf forms) from different localities and habitats. Unfortunately, the polar length results could not be used as the homogeneity assumption of ANOVA has been violated. For the equatorial diameter, the difference is noted only in *A. sessilis* 'Red' from three different habitats. However, the pollen grains of these three different habitats appear similar in the LM study. They are dodecahedric; pantoporate with twelve pores and each of the pores is situated in a pentagonal face.

Pollen sterility is not a common trend in *Alternanthera* and it is reported in the present study (*A. brasiliana* and *A. bettzickiana*) and *A. philoxeroides* (Liu *et al.*, 2008). Pollen grains are totally absent in *A. brasiliana* whereas pollen grains in *A. bettzickiana* are sterile and usually without exine (results from the embryological studies). The sterile pollen grains of *A. philoxeroides* are probably destroyed during the preparation

procedures for observations under scanning electron microscopy and light microscopy (Liu *et al.*, 2008). Under scanning electron microscopy, the development of the aperture wall is incomplete and ruptured.

5.4.2 Comparative pollen morphology of *Alternanthera* species from different localities

When comparing the present data and studies in the other countries, the pollen grains of *A. sessilis* in the present study are distinctly different from those reported in Pakistan and India in terms of aperture and sexine ornamentation. The number of pores is reported as six in the grains of *A. sessilis* from the Upper Gangetic plain (Rao & Shukla, 1975) and 3–3.2 from Pakistan (Perveen & Qaiser, 2002) whereas the number of pores is 12 in the present study. The sexine ornamentation of *A. sessilis* from India is reported as granulated (Rao & Shukla, 1975) or simplibaculate without spinules (Mittre, 1963) while that of the present study is metareticulum. However, this kind of apparent contradiction, especially the study from India, is difficult to resolve without further confirmatory work because the methodology and voucher specimens of *A. sessilis* were not mentioned by these authors and therefore taxonomic verification could not be carried out.

Furthermore, the pollen grains of *A. ficoidea* in the present study are not similar to those reported in China (Li *et al*., 1993) in having bigger pollen and pores (present study, polar length = 15.79 µm, pore: 4.83 µm) while those from China are smaller (polar length = 10.90 µm, pore = 3.60 µm). Moreover, only a single row of spinules is observed in the present study whereas one to two rows of spinules are observed in the pollen from China.

Data obtained in the previous study of *A. paronychioides* are different from the present findings as well. For instance, the pollen grains from Pakistan (Perveen & Qaiser, 2002) are smaller (15.34 µm) with six to nine pores while those from this study are bigger (21.85 µm) with around 14–18 pores. Furthermore, the size of the pores in the present study is about twice that of those from Pakistan, which are 6.06 µm and 3.44 µm respectively.

5.4.3 Comparative pollen morphology of *Alternanthera* and other genera in Amaranthaceae

Recently, findings in phylogenetic studies within the subfamily Gomphrenoidea (Sánchez del-Pino *et al*., 2009; Müller & Borsch, 2005a) have reaffirmed the role of palynological data of this genus. For instance, the parsimony and Bayesian analysis show a close relationship among the three monophyletic genera in *Alternantheroid* clade *(Alternanthera*, *Pedersenia* and *Tidestromia)* as previously established by pollen morphology studies. The pollen grains in these three genera are characterized as either the *Pfaffia*- or *Tidestromia*-type. The pollen in the present study is generally in agreement with the *Pfaffia*-type and thus may conform to *Alternantheroid* clade.

Although *Pfaffia* is reported to have the same pollen type as *Alternanthera*, it may be possible to distinguish between these two genera by taking into account the number and shape of aperture on the pollen grains. Instead of having spheroidal grains with more than 20 pores as reported in the *Pfaffia* species, most of the *Alternanthera* species (including the present specimens) have dodecahedric pollen with less than 20 pores (Borsch, 1998). The exceptions are *P. aurata* (Mart.) Borsch, *P. completa* (Uline & W.L. Bray) Borsch, *P. costaricensis* (Standl.) Borsch and *P. densipellita* Borsch (Borsch 1998; 1995) as these species are also reported to have dodecahedric pollen with twelve to fourteen pores. The arrangement of the microspines on the sexine could be

used to resolve this problem. The microspines are occasionally arranged in an undulating row or side by side as seen in *P. aurata* and *P. costaricensis* (Borsch, 1995) but are distally and regularly arranged in most of the *Alternanthera* species. Furthermore, the perforations tend to be distributed at the base of the mesoporia in the pollen grains of *Pfaffia* (Eliasson, 1988) but the perforations are either distributed all around the mesoporia or along the microspines in *Alternanthera* species (present study).

In addition, the pollen of *Kyphocarpa angustifolia* (Moq.) Lopr. is quite similar in shape and sexine ornamentation to the pollen of *Alternanthera*. Both of these taxa are dodecahedric, tectum punctuate with a row of microspines arranged distally but are set apart by the structure of pore and microspines. The pollen of *Kyphocarpa angustifolia* has cylindrically elongated microspines and pores of Type VIII (Borsch, 1998). The pores of Type VIII have less than ten ektexinous bodies. The ektexinous body is plate shaped and regular with one tooth-shaped microspine attached on to it. While the pollen of *Alternanthera* has conical elongated microspines and pores of Type I (Borsch, 1998). The pores of Type I are characterized as being covered by 20–60 ektexinous bodies. The shape of the ektexinous body is rectangular, sinuous or elongated in outline with one to four distinct microspines attached onto it. The size of the ektexinous body is 1.5–4 times as long as broad (Borsch, 1998).

5.5 POLLEN GERMINATION AND VIABILITY

In the present study, the optimum sucrose concentration for pollen germination in *A. sessilis* is lower than *Celosia argentea* var. *cristata* (L.) Kuntze, a member of Amaranthaceae (Bodhipadmaa *et al.*, 2010) (*A. sessilis*: 'Red' –16%; 'Green'–14%; *Celosia argentea* var. *cristata*–20%). However, *A. sessilis* shows a higher optimum sucrose concentration when comparing with the other tri-nucleate pollen grains. For examples, the optimum sucrose of *Arabidopsis thaliana* (L.) Heynh. (Boavida & McCormick, 2007) and *Annona cherimola* Mill. (Rosell *et al.*, 1999) is 10% and 5–10% respectively.

The germination percentage of *A. sessilis* is also lower than *Celosia argentea* var. *cristata* (Bodhipadmaa *et al.*, 2010) and *Annona cherimola* (Rosell *et al.*, 1999) (*A. sessilis*: 'Red'–19.70 ± 6.24%; 'Green'–24.31 ± 11.56%; *Celosia argentea* var. *cristata*–26.90 ± 8.50%; *Annona cherimola*–38% in 5% of sucrose concentration and 22% in 10% of sucrose concentration). Low germination percentage in the present study could be attributed by two factors. Firstly, the Brewbaker and Kwack's medium (Brewbaker & Majumder, 1961) might not suitable for tri-nucleate pollen grains of *A. sessilis*. Tri-nucleate pollen grains of *Capsella bursa-pastoris* (L.) Medik. only show 16% of germination rate in Brewbaker and Kwack's medium (Leduc *et al.*, 1990). Secondly, pollen density greatly affects the germination percentage. Low density results in low germinations percentage and short pollen tubes and this is has been reported in *Petunia inflata* R.E.Fr. (Brewbaker & Majumder, 1961); *Betula pendula* Roth (Pasonen & Kapyla, 2008) and *Arabidopsis thaliana* (Boavida & McCormick, 2007). In addition, several studies have reported that in vitro pollen germination using tri-nucleate pollen grains always show a low germination percentage (Brewbaker & Majumder, 1961; Preuss *et al.*, 1993; Taylor & Hepler, 1997).

In the present study, lowest germination percentage is recorded in the sucrose concentration of 10% and 12%. This is probably due to lack of sucrose as a nutrient source as similar result has been reported in *Persea americana* Mill. (Alcaraz *et al.*, 2011). Sucrose also play an osmoregulatory role during germination (Taylor & Hepler, 1997). In the higher sucrose concentration, the pollen tubes of *Annona cherimola* are short or burst (Rosell *et al.*, 1999). Similarly, the pollen tubes of *A. sessilis* in the present study are the shortest in the sucrose concentration of 20% and 22%.

5.6 POLLINATION

Two types of breeding system are noted in the present study; facultative xenogamy in *A. sessilis* and obligate apomixis in *A. brasiliana*. Hence, the present study is the first to record facultative xenogamy and obligate apomict breeding system in *Alternanthera*.

5.6.1 *Alternanthera sessilis*

According to Cruden & Lyon (1989), facultative xenogamous plants could adapt to cross pollination and have delayed self pollination in the absence of pollinators. Flowers of *A. sessilis* are morphologically adapted to cross pollination by producing nectar and emitting scent to attract small insects such as *Formicidae* (ants), *Apis florea* (honeybee) and *Syrphidae* (hoverfly) (Proctor *et al.*, 1996). The presence of nectar in *Alternanthera* is especially interesting when most of the taxa do not possess nectaries in the family (Zandonella as cited in Bernardello, 2007).

In Indo-Malaya, bees are an important pollinator in the lowland forests, orchards and cultivate field crops. *Apis cerana* and *A. florea* are often recorded as the pollinators for crops, ornamental plants and weeds in the non-forest habitats (Crane, 1990; Kiew & Conner, 1993; Tilde & Cervancia, 2003).

Although *A. sessilis* does not produce perceivable odour, it is also visited by *Apis florea*. Honeybees are polylectic insects that seldom show strong preferences and collect food from a wide range of flowers (Branquart & Hemptinne, 2000). They are attracted to flowers by their scent, colour and shape. Scent plays the most important role in attracting bees although most of the flowers visited do not seem to be strongly scented (Free, 1982; Michael *et al.*, 1996). For instance, *Amaranthus lividus* and *A.*

spinosus (common weed in Malaysia) attract *A. cerana* even tough the flowers are not strongly scented (Kiew & Conner, 1993).

In addition, the white cup shaped flower of *A. sessilis* 'Green' could have been responsible in attracting honeybees from far. In the lowland forest of Lambir, *Apis* is attracted by the rotate or cup shaped, yellow or white flowers, i.e. *Dryobalanops aromatica* C.F.Gaertn. (Momose *et al.*, 1998). Another study in Malaysia shows that *A. cerana* prefers the white or creamy flower more than the other colours such as yellow-orange, pink, red, purple, green and blue (Kiew & Conner, 1993).

Similar to honeybees, most of the hoverflies are also polylectic. The type of flower visited correlates strongly with the length of proboscis. Hoverflies with short proboscis show preference for simple, actinomorphic and shallow flowers due mainly to the easily accessible nectar or pollen grains (Gilbert, 1981; Goulson & Wright, 1998; Proctor *et al.*, 1996; Sajjad & Saeed, 2010). Similar flower morphology has also been observed in *A. sessilis* and therefore, hoverflies are also recorded as one of the flower visitors.

The small size of ants could be the pollinators for small flowers as they come into contact with the anthers and stigma while foraging for nectar and therefore help in pollination (Garcia *et al.*, 1995; Gómez & Zamora, 1992; Hickman, 1974; Peakall & Beattie, 1989; Petersen, 1977; Svensson, 1985). Current field observation of *A. sessilis* concurs with the above as the mouth part of ants is observed to touch the dehisced anthers and receptive stigma during food collection.

Conventionally, ants are considered as nectar thieves and not a true pollinator as they secrete antibiotic substances (from metapleural glands) which could cause a decrease in pollen viability (Beattie *et al.*, 1984; Gómez & Zamora, 1992; Ramsey, 1995). As research progresses, ants have been suggested as pollinators depending on

their interactions with the plants. Positive reaction to frequent visitation of viable pollen grains during mass flowering could improve reproduction especially when other winged pollinators were scarce (Gómez & Zamora, 1992; Ramsey, 1995).

The character of matting and mass flowering of *A. sessilis* could be another insect attraction. For example, hoverflies usually visit the plants that grow in a mat or patches with mass flowering as they offer sufficient nectar or pollen grains (Colley & Luna, 2000; Momose *et al.*, 1998; Sajjad & Saeed, 2010; Sutherland *et al.*, 1999). The ants could help to facilitate cross pollination and self pollination (geitonogamy) at reclining stems where the flying insects hardly visit (Gómez & Zamora, 1992; Peakall & Beattie, 1989). Therefore, the effectiveness of insect pollination is increased when the plant is matted.

Pollinator effectiveness in cross pollination could not be determined in the present study owing to the small flowers of *A. sessilis*. The flowers are emasculated and exposed for open pollination (Cruden & Lyon, 1989). However, it is not advisable to perform this on *A. sessilis* because emasculation could destroy the flowers. This was experienced in the emasculated and bagged flowers experiment as well as cross pollination experiment.

According to Lloyd & Schoen (1992), delayed self pollination takes place at the end of flower anthesis when the dehisced anthers and receptive stigmas come into contact and thus, pollinate the flowers which have not been cross pollinated. Similar observations have been noted in *A. sessilis* and some reported species, in which the anthers bend downwards and touch the stigma at the end of flower anthesis 5.3.2(Dole & Ritland, 1993; Faegri & Pijl, 1979; Klips & Snow, 1997; Lyon, 1992; Rathcke & Real, 1993).

Additionally, the flowers must be homogamous and both the anthers and stigma located at the same height in order to facilitate self pollination (Dafni & Firmage, 2000; Proctor *et al.*, 1996). This has been observed in the present study when the stigma of *A. sessilis* is receptive (positive result from 3% hydrogen peroxide test) and the pollen grains are still viable (showing high germination rate and long pollen tube) when the anthers touch the stigma in the evening.

One of the disadvantages of self pollination is inbreeding depression (Husband & Schemske, 1996; Keller & Waller, 2002; Richards, 1997). Inbreeding depression is defined as the reduction in the fitness of progeny derived from inbreeding relative to those derived from out crossing. Inbreeding depression is expressed at four stages in the life cycle of a plant which are parental seed fecundity, seed viability, progeny survival and progeny growth rate (Husband & Schemske, 1996; Stevens & Bougourd, 1988). The greatest inbreeding depression is expressed during parental seed fecundity and progeny reproduction rather than seed viability and progeny survival. This could be due to the shorter development period that requires fewer genes in seed viability and progeny survival (Husband & Schemske, 1996).

However, the facultative xenogamous species could outweigh the effects caused by inbreeding depression since a low outcrossing rate is sufficient to maintain heterozygosity (Kalisz *et al.*, 1999; Lyon, 1992; Richards, 1997). Theoretically, inbreeding depression would decrease with increased inbreeding since the deleterious recessive alleles would be purged from the genetic load due to selection (Charlesworth & Charlesworth, 1990; Husband & Schemske, 1996). Outcrossing pollination, on the other hand, promotes gene flow and maintains heterozygosity (Proctor *et al*., 1996).

In both leaf forms, the fruit set obtained from the open pollination is significantly higher than the self pollination experiment, geitonogamy; cross pollination

and emasculated and bagged flower experiment (apomixis). This may be attributed to four factors. Firstly, lack of pollinators in the bagging experiment. Secondly, wounding resulted from handling technique as the flowers are very small. Thirdly, pollination bags might cause side-effects such as build-up of humidity or heat and infection by fungi or bacteria. Lastly, inbreeding depression could have resulted from self pollination.

In addition, the fruit set obtained from the untreated and bagged inflorescences experiment is higher than the untreated and bagged individual flowers experiment. This could be due to wounding while removing the unwanted flowers from the inflorescence in the untreated and bagged individual flowers experiment. Although fruit set is obtained from the emasculated and bagged flower experiment (apomixis), the presence of adventive embryo has not been observed in the embryological study.

A. sessilis 'Red' and 'Green' are of the same species because they could interbreed and produce fruit as shown in the cross pollination experiment. According to Mayr (1963), species are groups of interbreeding natural populations that are reproductively isolated from other such groups (biological species concept).

Although the status of invasion of *A. sessilis* 'Green' in Malaysia has not been seriously studied, it could be a potential invasive weed due to the higher fruit set in the open and self pollination experiment as compared with *A. sessilis* 'Red'. The delayed self pollination provides reproductive assurance even when pollinators are scare and cross pollination increases the ability of the plant to adapt to a new environment (Cruden & Lyon, 1989; Etcheverry *et al*., 2003; Kalisz *et al*., 1999). Furthermore, *A. sessilis* also reproduces vegetatively by stem cutting and this will probably enhance the onset of invasion of the plant (Pancho & Soerjani, 1978).

5.6.2 *Alternanthera brasiliana* and *Alternanthera bettzickiana*

Fruit set obtained from the open and self pollination experiment in the absence of pollen grains has indicated *A. brasiliana* is an obligate apomict. This was strengthened by the presence of adventive nucellar embryony from the embryological study. As such, pollinators have not been observed since the nectar and scent are not produced. Within the genus, *Alternanthera littoralis* var. *maritima* reproduces via apomixis if pollination does not happen (Antonucci *et al.*, 2011).

Obligate apomixis would benefit the commercial propagation of *A. brasiliana* in two ways. First, the potential good genotype could be identified and thus, help in commercial propagation (Koltunow *et al*., 1995). Second, disease transmission via vegetative cultivation could be prevented (Asker & Jerling, 1992).

In *A. bettzickiana*, fruits did not develop in both the open pollination and bagging experiment. The failure in fruit production could have resulted from several factors. Firstly, the partially dehisced anthers do not expose the pollen grains and the flower could not be pollinated. Secondly, failure in fruit production could be due to wounding in the bagging experiment as the flowers are small. Result obtained from the embryological study reveal that failure in fruit production is due to cytoplasmic male sterility and egg cell abortion.

5.7 SEED GERMINATION

In general, all the seeds of *A. sessilis* and *A. brasiliana* show epigeal germination. Contrary to the present study, it was reported that both sexual and asexual derived embryo (apomict) show vivipary in *Alternanthera littoralis* var. *maritima* (Antonucci *et al.*, 2011). Polyembryonic seeds are only noted in *A. brasiliana* and have never been observed in *A. sessilis*. The germination percentage of the seeds obtained from the open pollination experiment was more than 70% in these two species studied except in the parent plants of *A. brasiliana*. Higher germination percentage (80–90%), on the other hand, has been reported in *Amaranthus* species (Costea *et al.*, 2004, 2005).

5.7.1 *Alternanthera sessilis*

Seed germination percentage, day of seedling emergence (incubation period) and seedling height are used as indicators of seedling vigour (Acevedo *et al.*, 1991; McKenzie *et al.*, 1980; Regan *et al.*, 1992).

In *A. sessilis* 'Red', higher seed germination percentage and shorter incubation period indicate higher seedling vigour in the seeds collected from the open pollination experiment as compared with the self pollination experiment (Ghassemi-Golezani *et al.*, 2010). Deterioration of the seeds obtained from the self pollination experiment could be attributed to two reasons. Firstly, it could be due to ageing, bacterial or fungal growth since they are not collected from the bag immediately when they are mature (Ghassemi-Golezani & Hosseinzadeh-Mahootchy, 2009). Secondly, it could be due to inbreeding depression (Cisse & Ejeta, 2003; Keller & Waller, 2002; Roach & Wulff, 1987; Steiner, 1990).

Seedling vigour in *A. sessilis* 'Green' in the seeds obtained from the self pollination experiment is higher as compared with the seeds obtained from the open pollination. Although the germination percentage of the self pollination is not significantly higher than the seeds obtained from the open pollination, the seedlings are significantly taller.

When comparing the two leaf forms of *A. sessilis*, the red leaf form has a higher seedling vigour than the green leaf form in the seeds obtained from the open pollination experiment. The red leaf form shows a higher germination percentage and shorter incubation period. The green leaf form show a better seedling vigour in the seeds obtained from the self pollination. The green leaf form shows a higher germination percentage, taller seedling and shorter incubation period. High seedling vigour allows *A. sessilis* 'Green' to colonize an area rapidly and could become an invasive weed (Ghassemi-Golezani *et al.*, 2008a; Ghassemi-Golezani *et al.*, 2008b).

Although the cross pollinated seedlings show the lowest germination percentage and long incubation period, success in seed germination has further confirmed that the red and green leaf forms of *A. sessilis* are of the same species.

The temperature at the sowing site is in accordance with the constant temperature that shows the highest germination percentage in *Amaranthus* species (Cristaudo *et al.*, 2006; Ghorbani *et al.*, 1999; Steckel *et al.*, 2004). In fact, the optimum temperature for non-dormant seeds of *Amaranthus* species ranges from 25–40°C (Ghorbani *et al.*, 1999; Matsuo & Kubota, 1993). In *A. sessilis*, the sowing temperature is in line with the optimum temperature and this may be one of the factors that contribute to high germination.

The highest germination percentage is recorded on the soil surface in *A. tenella* (Canossa *et al*., 2007) and some *Amaranthu*s species (Chauhan & Johnson, 2009).

Seeds of *A. sessilis* in the present study are also sown on the soil surface. Thus, suitable soil depth is also an important factor contributing to high germination percentage. This is further evidenced by field observations where numerous germinated seedlings are seen on the soil surface near to the parent plants.

Previous study on *A. sessilis* has shown that an alternation of light and temperature is needed for dormancy break (Datta & Biswas, 1968). It has been shown that light is more successful in stimulating germination (Datta & Biswas, 1968). Since the alternation of temperature is not needed to stimulate germination in the present study, the seeds of *A. sessilis* do not experience dormancy.

Another study states that naked seeds of *A. sessilis* produce a higher germination percentage (Kaul, 1967). However, some of the germination percentage of *Amaranthus* seeds is unaffected even when the pericarp is left intact during seed germination (Costea & Tardif, 2003). Likewise, the present study finds that the pericarp of the seeds in *A. sessilis* does not affect the germination percentage.

5.7.2 *Alternanthera brasiliana*

When comparing the seedlings from seeds of the parent plants and their offsprings, the seedling vigour seems to be similar. Both of them show no significant difference in the germination percentage, seedling height and incubation period. In addition, the seedling vigour in the seeds obtained from the open and self pollination experiment is also similar. Again, there are no significant differences in the germination percentage, seedling height and incubation period in the seeds from both pollination experiments. These similarities could possibly be due to the lack of gene flow as

obligate apomict individuals have a genome derived entirely from the female parent (Hwa & Yang, 2008; Koltunow *et al.*, 1995).

Chapter 6: Conclusion and Future Study

Alternanthera sessilis ‘Green’ and *A. ficoidea* are common weeds found in terrestrial and aquatic area. *Alternanthera sessilis* ‘Red’ is a medicinal herb while *A. brasiliana* and *A. bettzickiana* are ornamental plants. All the three species are cultivated in shady or open areas.

Alternanthera species in the present study are perennial herbs or shrubs showing decumbent, creeping, prostrate or ascending growth. The stem is usually hairy when young and glabrescent when mature. The leaf is simple, opposite, hairy and varying in shape and size. The inflorescence is either pedunculate or sessile. The bisexual sessile flower is subtended by scarious bracts or bracteoles, either glabrous or hairy. Five free petals are either equal or subequal in size, glabrous or hairy. The androecium is made up of three to five stamens that are either fertile or sterile. The filaments fuse into a tube or a short cup and alternate with pseudostaminodes. The length, apex and colour of these pseudostaminodes also vary according to species. The gynoecium is made up of a unicarpellate pistil which consists of a capitate stigma, short style and a superior ovary. The shape of the ovary ranges from obcordate, obovoid to subconical.

The species studied are divided into two groups based on the inflorescence morphology. Group A includes *A. brasiliana* which is characterized by having pedunculate inflorescence while group B is characterized by having sessile inflorescence and this includes *A. sessilis* (both leaf forms); *A. paronychioides*, *A. ficoidea* and *A. bettzickiana*.

Further, *A. sessilis* and *A. paronychioides* are placed in subgroup 2A as they have obcordate ovary, absence of bracteoles and petals with acute apex. The morphology of *A. sessilis* ‘Red’ and ‘Green’ is remarkably similar except in the colour

of leaf, stem, flower and ovary. *Alternanthera ficoidea* and *A. bettzickiana* in subgroup 2B, are characterized by having subconical ovary, presence of bracteoles and petals with mucronate apex.

The time taken for a single flower to develop from a bud to young seedling for the red and green leaf forms of *A. sessilis* is 19–40 and 21–50 days respectively while it is 31–99 days in *A. brasiliana*. *Alternanthera brasiliana* and *A. bettzickiana* show a high flower abortion percentage which is 89.02 ± 10.18% and 74.36 ± 14.61% respectively.

The flower anthesis in *A. sessilis* ranges from 0730–1000 hours and in *A. bettzickiana* 0700–1200 hours. *Alternanthera brasiliana* takes two to three days to complete flower anthesis. Anther dehiscence accompanies flower anthesis in *A. sessilis* and the flower is homogamous throughout the flower anthesis. The brown and membranous anthers of *A. brasiliana* do not dehisce and are devoid of pollen grains. The brown and shrunken anthers of *A. bettzickiana* partially dehisce but do not expose the pollen grains. The life span of a flower of *A. sessilis* and *A. bettzickiana* is one day and two to three days in *A. brasiliana*. The fertilized flowers develop into mature fruits in about 15 days for both *A. sessilis* and *A. brasiliana*.

The anther is bisporangiate and the wall development usually conforms to the Monocotyledonous type and occasionally to the Dicotyledonous type (Davis, 1966). The mature anther wall consists of a single layer of epidermis, fibrous endothecium with ubisch granules, an ephemeral middle layer and a multinucleate tapetum with ubisch granules. Cytokinesis during microsporogenesis is simultaneous forming mostly tetrahedral and rarely isobilateral or decussate tetrads. The mature pollen grains are shed at the three-celled stage.

The embryology of *Alternanthera* conforms well to the embryology of reported Amaranthaceae. The embryological data obtained for both the red and green leaf forms of *A. sessilis* are identical.

The ovule is campylotropous, bitegmic, crassinucellate and the micropyle is formed by the inner integuments only. The nectar glands are located at the inner base of the filament in all the species studied except in *A. brasiliana*. The development of the embryo sac conforms to the monosporic *Polygonum* type (Maheshwari, 1950). The synergids are hooked and consist of filiform apparatus. Two polar nuclei fuse before fertilization. As the embryo sac elongates, the antipodals degenerate and are left in a lateral position. The fertilization is porogamous.

The endosperm development is of the *ab initio* Nuclear type. Wall formation commences at the micropylar region but does not proceed to the chalazal region. The embryo development in *A. sessilis* follows the transitional form between the Chenopodiad-type and Solanad-type (Johansen, 1950). Nevertheless, the precise mode of embryogeny of the genus *Alternanthera* remain to be determined as it is not fully established in *A. paronychioides* and *A. ficoidea*.

Alternanthera brasiliana is an obligate apomict due to cytoplasmic male sterility and early egg cell abortion. The fibrous thickening does not develop in the endothecium and this probably caused the indehiscence of anther. Absence of ubisch granules on the endothecium and early degeneration of the tapetum caused microspore abortion. The embryo is an adventive nucellar embryo and often the embryos are without suspensor. Polyembryony is occasionally noted and the endosperm development is of the autonomous type.

Alternanthera bettzickiana is a sterile plant due to cytoplasmic male sterility and egg cell abortion. Neither coenocytic microspores nor the normal microspore tetrads

develop into mature pollen grains despite the presence of the tapetum and ubisch granules. In *A. ficoidea*, the mature embryo sac frequently aborted and the origin of the adventive embryo remains to be determined.

Palynological characters of the three *Alternanthera*, especially *A. sessilis*, conform well to those from the New world and China but are different from the species from India and Pakistan. The pollen morphology of the red and green leaf forms of *A. sessilis* is remarkably similar except in the pore diameter. *Alternanthera sessilis* 'Green' has the bigger pore than the *A. sessilis* 'Red'.

The pollen grains resemble the *Gomphrena*-type of Erdtman (1966) which corresponds to the *Pfaffia*-type of Borsch (1998). These pollen grains are dodecahedric, isopolar and small (12.56–23.57μm). The pores of the pollen grains of all the species studied are covered with rectangular, sinuous, or elongated ektexinous bodies. The sexine is metareticulate.

Pollen grains of *A. sessilis*, *A. ficoidea* and *A. paronychioides* can be differentiated mainly by the number and size of apertures, number of ektexinous bodies and distribution of perforations at the mesoporia. The apertures of *A. sessilis* and *A. ficoidea* are pantoporate with 12 round pores whereas the pollen grains of *A. paronychioides* have 18 oval pores. *Alternanthera paronychioides* has the largest pore and the highest number of ektexinous bodies, followed by *A. ficoidea* and *A. sessilis*. The perforations are distributed unevenly at the top and base of the mesoporia, except in the pollen grains of *A. ficoidea* and *A. paronychioides*, where the perforations are distributed along the microspines at the top of the mesoporia.

The optimum sucrose concentration for pollen germination in the red and green leaf forms of *A. sessilis* is 16% and 14–18% respectively. In *A. sessilis*, the breeding system is facultative xenogamy. The flowers are adaptive to cross pollination.

They are white; cup-shaped, matted and provide nectar as well as pollen grains to attract pollinators. In addition, the anthers and stigma are homogamous and locate at the same height making the delayed self pollination possible.

Both leaf forms of *A. sessilis* show a significant higher fruit set in the open pollination experiment as compared to the other pollination experiments. *Alternanthera sessilis* 'Green' significantly produced more fruit than *A. sessilis* 'Red' in both experiments. Fruit set obtained from the cross pollination experiment between the red and green leaf forms of *A. sessilis* further show that the two leaf forms are of the same species as they could interbreed.

In *A. brasiliana*, the breeding system is obligate apomixis. Fruit set is recorded from the open and self pollination experiment despite the absence of nectar, scent and pollen grains. *Alternanthera bettzickiana* is a sterile plant as fruits are not produced from both the open and self pollination experiment.

Both the sexually derived embryo and adventive embryo are characterized as dicotyledonous, curved and annular. The seed coat is made up of the outer layer and a small part of the inner layer of the outer integument.

The seeds of *A. sessilis* and *A. brasiliana* show epigeal germination. The germination percentage of the seeds obtained from the open and self pollination experiment is more than 70% in the two species studied. Polyembryonic seeds are only noted in *A. brasiliana* and have never been observed in *A. sessilis*.

Seed germination percentage, incubation period and seedling height are used as indicators of seedling vigour. In *A. sessilis* 'Red', the seedling vigour is more in the seeds obtained from the open pollination experiment as compared to those from the self pollination experiment. In *A. sessilis* 'Green', seedling vigour is higher in the seeds

obtained from the self pollination experiment. When comparing the two leaf forms of *A. sessilis*, the red leaf form showed better seedling vigour than the green leaf form in the seeds obtained from the open pollination experiment. The red leaf form showed a higher germination percentage and shorter incubation period. For the seeds obtained from the self pollination experiment, *A. sessilis* 'Green' has better seedling vigour that showed a higher germination percentage, taller seedling and shorter incubation period. The cross pollinated seedlings showed the lowest germination percentage and longest incubation period.

To sum up, the study of morphology, embryology and palynology show that *A. sessilis* 'Red' and 'Green' are identical. Furthermore, these two leaf forms are able to interbreed and produce fruit and viable seed. The combined evidence from the flowering phenology, fruit development, breeding system and seed germination studies has shown that *A. brasiliana* is an obligate apomict. The offspring is actually a clone of the parent plant. Last but not least, the present study also establishes that cytoplasmic male sterility and egg cell abortion are the causes of sterility in *A. bettzickiana*.

In order to design a better eradication or control program for *A. sessilis* 'Green', a detailed future study on the reproductive biology of the plant growing in aquatic habitats is recommended. Furthermore, the genetic variation of *A. sessilis* (both leaf forms) should be investigated in order to determine whether its broad ecological breadth and morphological variations are due to genetic variation or phenotypic plasticity.

In *A. brasiliana*, it would be beneficial if detailed study on the causes if high flower abortion and prolonged flowering period (in the offspring) is carried out so as to improve yielding. A survey on the reproductive biology from the other habitat could also be carried out to determine whether obligate apomict is also present. Environmental parameter such as rainfall and mean temperature should be included in the study.

To improve our understanding on the origin of the adventive embryos of *A. ficoidea*, more study on the embryology is needed. In addition, the flowering phenology, pollen viability, pollination experiments and seeds germination should also be carried out to elucidate the reproductive biology of *A. ficoidea*.

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