

Interspecific Hybridization Studies of Three *Stachytarpheta* Species from Nigeria

Damilola G. Solanke., Matthew Oziegbe* and Sekinat O. Azeez

Department of Botany, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.

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Abstract

Interspecific hybridization studies involving reciprocal crosses were carried out on three *Stachytarpheta* spp. to investigate the genetic relationship among the three spp. The interspecific cross of *S. angustifolia* x *S. cayennensis* produced an F₁ hybrid that was vigorous, floriferous, and partly fertile, but the seed setting percentage was low (29.83 %) despite the fairly high pollen fertility (63.14 %). The F₂ seeds recovered from this cross failed to germinate. Interspecific crosses of *S. cayennensis* x *S. angustifolia* and *S. cayennensis* x *S. indica* produced F₁ seeds which did not germinate. The interspecific crosses of *S. angustifolia* x *S. indica* and *S. indica* x *S. angustifolia* failed, and no seed set was observed, but *S. indica* x *S. cayennensis* produced F₁ seeds which failed to germinate. The results from the hybridization studies showed that postzygotic-isolating mechanism has set a strong reproductive barrier among the three studied *Stachytarpheta* spp. manifested as embryo abortion in *S. angustifolia* x *S. indica* and *S. indica* x *S. angustifolia*, F₁ seed inviability in *S. indica* x *S. cayennensis*, *S. cayennensis* x *S. angustifolia* and *S. cayennensis* x *S. indica*, F₂ seed breakdown in *S. angustifolia* x *S. cayennensis*. The current study concluded that *S. angustifolia* is more closely related to *S. cayennensis* than *S. indica* and the three species are reproductively isolated.

Keywords: Genetic relationships, Interspecific hybridization, Nigeria, Reproductive barriers, *Stachytarpheta*

1. Introduction

The genus *Stachytarpheta* belongs to the *Verbenaceae* (Cantino *et al.*, 1992) which is widely distributed in tropical and subtropical America, and few species (mostly naturalised) can be found in tropical and subtropical Australia, Asia, Africa and Oceania (Munir, 1992). *Stachytarpheta* is a genus of about 133 species almost exclusively from the New World (only *S. indica* (L.) Vahl occurs in the Old World) (Atkins, 2005); the genus is represented in West Africa and in Nigeria by three species: *Stachytarpheta indica* (L.) Vahl, *Stachytarpheta angustifolia* (Mill.) Vahl, and *Stachytarpheta cayennensis* (Rich.) Vahl (Hutchinson and Dalziel, 1963). *Stachytarpheta* has several species named on the basis of collections from outside the New World, but all species except *S. indica* have been shown to be synonyms to New World species that had colonized Old World locations following European contact (Atkins, 2005.). They also reported that *S. indica* is very similar to *S. angustifolia* which also occurs in Africa as a weed, and that *S. indica* is likely either a divergent phenotype of *S. angustifolia* that was established early after contact or a hybrid with another weedy species, *S. jamaicensis* (L.) Vahl, which is also established in Africa. Thus, *Stachytarpheta* is probably restricted in its pre-Columbian distribution to the New World. The genus *Stachytarpheta* are herbs or low shrubs, glabrous or variously pubescent to tomentose with simple

hairs. The stem and branches almost terete or tetragonal. The leaves are simple, mostly decussate-opposite, petiolate or sometimes sessile, and the lamina is dentate to serrate, somewhat rugose (Munir, 1992). The pollen of *Stachytarpheta* is 3-colpate, with an exine sculpture made up of verrucae, and this distinguishes it from all other genera in the family *Verbenaceae* (Atkins, 2004). Adedeji (2010) observed pollen grains of the genus *Stachytarpheta* to possess a multicolpate and multiporate attribute which indicates that the genus is not primitive in evolutionary history and these spp. probably, evolved around the same time. She reported the pollen forms to be acolpate (i.e. without apertures), monocolpate, bicolpate, tricolpate (these were observed to be occurring at the highest frequency in all the species studied), and tetracolpate.

According to Sanders (2001), the chromosome number of the genus *Stachytarpheta* was reported to be within the range $2n = 18, 48, 56, 112, 160$ which may be based on $x = 8$ or its hexaploid-derived base $x = 24$. It was also reported that the chromosome numbers in this genus are incompletely known, and are based mostly on observations on *S. cayennensis* and *S. dichotoma*. Within this species complex, there is a range of polyploids, $2n = 18, 48, 56, 112, \text{ and } 160$, suggesting an aneuploidy series from the base numbers 8 and 9. Munir (1992) also reported $2n = 160$ in *S. indica* and the same chromosome count was reported by Fedorov (1974). For *S. angustifolia*, $2n = 56$ was reported by Mangenot and Mangenot (1962) cited in Siedo (2006). Moldenke (1958) carried out hybridization

* Corresponding author e-mail: oziegbem@oauife.edu.ng.

studies on some species of the genus *Stachytarpheta* and proposed that $\times S.$ is *abortive* for a hybrid which was produced from *S. cayennensis* (L.C. Rich) Vahl. (Which is actually *S. australis*) and *S. mutabilis* (Jacq.) Vahl. The cross was not successful when the pollen of *S. australis* was placed on the stigma of *S. mutabilis*, but 200 seeds were recovered from the reciprocal cross. Out of the 200 seeds recovered, only 98 plants were produced in which ninety-four were typical *S. australis* and only four were hybrids. Urban and Ekman (1929) collected $\times S.$ *adulterina* from the wild that was obtained from a cross between *S. jamaicensis* and *S. mutabilis*. This same hybrid was also described by Danser (1929), which was reported to be produced from *S. indica* and *S. mutabilis*, and was named $\times S.$ *trimeni* (but the one named *indica* was *jamaicensis*). $\times S.$ *adulterine*, that was produced in the laboratory by Danser (1929) from the reciprocal cross between *S. jamaicensis* and *S. mutabilis* because crosses carried out by pollinating the pistil of *S. mutabilis* by the pollen of *S. jamaicensis* did not produce any seed. $\times S.$ *gracilis*, was found growing as a natural hybrid between *S. australis* and *S. urticaefolia* (Salisb). He reported that it was commonly found where the parents are growing together in large quantities. A hybrid $\times S.$ *debilis* is also a natural hybrid between *S. australis* and *S. jamaicensis* (Linn.) Vahl. $\times S.$ *trimeni* was also found in the wild as a result of crosses between *S. mutabilis* and *S. urticaefolia*. The hybrid $\times S.$ *hybrida* is a name that was proposed by Moldenke (1940) for the natural hybrid between *S. jamaicensis* (Linn.) Vahl and *S. strigose* Vahl. $\times S.$ *intercedens* is a name proposed by Danser (1929) to the natural hybrid between *S. jamaicensis* and *S. urticaefolia* (Moldenke, 1958; Munir, 1992). Atkins (1991) came across a natural hybrid between *S. sericea* and *S. chamissonis* that were growing together while curating and naming the family Verbenaceae. The hybrid was observed to have the same morphological characteristics with *S. sericea*, while the inflorescence, calyx, and flower size resemble those of *S. chamissonis*. A pollen study to confirm the hybridity was carried out and the hybrid *S. sericea* \times *S. chamissonis* showed a very reduced and often absent protoplasm suggesting infertility which is a good indicator of hybridity (Atkins, 1991).

All the *Stachytarpheta* Spp. have been reported to be used ethnomedically as anti-malaria (Okokon *et al.*, 2007; Adebayo and Krettli, 2011), for the treatment and cure of diabetes. (Soladoye *et al.*, 2012), as anti-ulcer (Sandhaya *et al.*, 2013) antihelminthic and as an antimicrobial agent which can be used for the treatment of dysentery, eye troubles (e.g cataract), gonorrhoea, syphilis and other venereal infectious diseases (Mohammed *et al.*, 2012). The leaves of *S. jamaicensis* are used as tea in Brazil, and are referred to as Brazilian tea. They are used to treat sores in children's ears, heart trouble, and are also anti-asthmatic, sedative and anti-hypertensive (Duke and Vazquez, 1994; Adedeji, 2012). Experiments have been carried out to confirm its wound-healing capability (Pandian *et al.*, 2013). Although some species of *Stachytarpheta* are considered ornamental or medicinal, they occur usually as weeds in crops and pasture (Atkin *et al.*, 1996; Barbola *et al.*, 2006). They are cultivated as ornamental or hedge plants (Munir, 1992), and also as a result of their desirability as butterfly attractants they are planted in the garden (Sanders 2001). The genetic relationship among the three species of *Stachytarpheta* in Nigeria has not been

investigated. This study has investigated the genetic relationship among the three *Stachytarpheta* spp. from Nigeria.

2. Materials and Methods

Seedlings of the three *Stachytarpheta* species (*S. indica*, *S. angustifolia* and *S. cayennensis*) were collected in March, 2016 from various locations: Obafemi Awolowo university campus, Iwo, and Erin-Ijesha waterfall area, Osun State, Nigeria. The collected seedlings were transplanted into a seven-litre plastic bucket, and were raised to maturity from March –July, 2016 at the Department of Botany Obafemi Awolowo University, Ile-Ife. The matured plants were authenticated taxonomically at the IFE herbarium and voucher specimens of *S. indica* (U.H.I 7762), *S. angustifolia* (U.H.I 13413) and *S. cayennensis* (U.H.I 17324) were deposited at the herbarium. Seeds were collected from the mature plants, and were germinated and sub-cultured in the screen house for obtaining seedlings which were transplanted ten weeks after germination into seven-litre plastic buckets filled with top soil at the rate of one plant per bucket with five replicates for each species. After that, the quantitative and morphological characteristics were recorded. Some biological criteria prior to crossing were investigated including time of anthesis, the time anther dehiscence, availability of pollen, and the time the stigma is receptive. The pollen fertility test was also carried out by staining the pollen grains from just dehisced anthers in the parents and the F_1 hybrids with Cotton Blue in Lacto-phenol for forty-eight hours. Full pollen grains with the cytoplasm contents becoming stained uniformly blue were counted as viable pollens, while those that could not stain or were partially stained with a collapsed outline were counted as non-viable. (Jackson, 1962; Olorode and Baquar, 1976). The percentage of pollen fertility was determined by dividing the total number of fertile pollen by the total number of pollen counted and multiplying by 100 for each species and hybrids. Interspecific hybridization studies involving reciprocal crosses were carried out among the three species. Five inflorescences (Spikes) were selected randomly from each species and tagged for identification. As flower buds matured sequentially along the spike of the ovulate parent, they were physically emasculated before anther dehiscence at anthesis. Pollen grains from the specific pollen parents (staminate parents) were transferred to the ovulate parents between 10:00 am-12:00 pm when the stigma were observed to be receptive. Half of the emasculated flowers were not pollinated, and were kept for seed production in order to test for apomixis. Each crossed flower spike was bagged to prevent contamination by external pollens. The pollinated flowers were monitored for seed setting. Spikes of *S. cayennensis* and *S. indica* were harvested at maturity when they were brown and brittle and could be detached easily from the plant, but the seeds of *S. angustifolia* were collected sequentially as the seeds matured and dried up to prevent losing them. Mature seeds were harvested separately and enveloped for further studies. The F_1 seeds produced from successful crosses were planted along with their parents, and were monitored and evaluated for all identifiable characters. All quantitative vegetative and floral characters were compared among the three species and also among the

parents and their F₁ hybrids using one-way Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also applied at 0.05 level to compare the means.

3. Results

A high percentage of seed sets was observed in all of the species studied for self-pollination. (98.23 % was observed in *S. angustifolia*, 93.93 % in *S. cayennensis* and 98.61 % in *S. indica*). All emasculated non pollinated flowers dropped indicating that there was no apomixis.

3.1. Crosses Involving *S. angustifolia* as Ovulate Parent

All interspecific crosses of *S. angustifolia* x *S. indica* failed, but a swelling was observed in the ovary showing that fertilization had occurred, though seeds were not formed from the swollen ovary. The interspecific crosses of *S. angustifolia* x *S. cayennensis* was successful, and seeds were formed, but the percentage of crossability was low (6.82 %). Qualitative and quantitative characters of this F₁ hybrid and their parents are shown in (Tables 1 and 2). The F₁ hybrid of *S. angustifolia* x *S. cayennensis* exhibited perennials habit as observed in the staminate parent (*S. cayennensis*). The morphological characters in the F₁ hybrid varied. The height of the hybrid at the first flowering and the leaf length were not significantly different from those of the staminate parent (*S. cayennensis*). The F₁ hybrid plants were vigorous, floriferous, and erect like the staminate parent (Figure 1 a, b and c). The number of primary branches and the seed length were not significantly different from the staminate parent (Table 2). Some characters including leaf breadth and the seed breadth were not significantly different from the ovulate parent (*S. angustifolia*). The pigmented part (petiole and calyx) that was observed in the staminate parent was also observed to be pigmented in the hybrid plants (Figure 1 d, e, f, g, h and i). The floral characters (i.e. Petal length and breadth and the calyx length and breadth) of the hybrid flower were observed physically to

Table 1. Qualitative Characteristics of *S. indica*, *S. angustifolia*, *S. cayennensis* and the F₁ Hybrid.

Characters	<i>S. indica</i>	<i>S. angustifolia</i>	<i>S. cayennensis</i>	<i>S. angustifolia</i> x <i>S. cayennensis</i>
Petal colour	Deep purple with a white center	Light purple with a white center	Purple with a white center	Faint light purple with a white center
Calyx	Green with a purple tip	Green	Green with a purple tip	Green with a purple tip
Filament Colour	White	White	White	White
Anther Colour	Yellow	Yellow	Yellow	Brownish White
Stigma Colour	Green	Green	Yellow	Green
Style Colour	White with a purple tip	White	White	White
Ovary Colour	Green with a yellow base	Green with a yellow base	Yellow	Green with a yellow base
Petiole Colour	Purple	Green	Purple	Purple
Anther sacs	Dehiscent	Dehiscent	Dehiscent	Barely dehiscent
Habit	Erect Shrub, Perennial	Low-lying Herb, Annual	Erect Shrub, Perennial	Erect Shrub, Perennial

resemble those of the ovulate parent with regard to the pigmentation at the tip of its calyx (Table 2 and Figure 1); however, the floral characters of the hybrid plant were not significantly different statistically from the ovulate parent. The length of inflorescence was significantly different in the two parents and their hybrid which was observed to be an intermediate between the two parents. Pollen shapes in the F₁ hybrid (Acolpate) differed from those of the two parents (Tricopate). The anthers of the hybrid plants were brownish white in colour, they are mostly shrunken and failed to dehisce in many flowers. The F₁ hybrid pollen is whitish in colour while that of the parents was yellow. The percentage of pollen stainability in the F₁ hybrid was fairly high (63.14 %) but despite that, the percentage of seed setting was observed to be lower in the hybrid (29.83 %). This is significantly lower than 96.56 % observed in *S. angustifolia* and 87.76 % in *S. cayennensis*. The F₂ seeds of the F₁ hybrid (*S. angustifolia* x *S. cayennensis*) failed to germinate after several trials. The F₁ plant was propagated successfully through stem cuttings.

3.2. Crosses Involving *S. cayennensis* as Ovulate Parent

The crosses carried out between *S. cayennensis* and *S. angustifolia* (*S. cayennensis* x *S. angustifolia*) set seeds but the percentage of crossability was low (11.59 %). The F₁ hybrid seeds produced from these crosses failed to germinate. The interspecific crosses carried out between *S. cayennensis* and *S. indica* (*S. cayennensis* x *S. indica*) were successful, and F₁ hybrid seeds were produced, but failed to germinate. The percentage of crossability was low (12.33 %).

3.3. Crosses Involving *S. indica* as Ovulate Parent

The crosses carried out between *S. indica* and *S. angustifolia* (*S. indica* x *S. angustifolia*) was not successful, and no seed was formed. The crosses that were carried out between *S. indica* and *S. cayennensis* (*S. indica* x *S. cayennensis*) set seeds with a low percentage of crossability (2.07 %). The F₁ hybrid seeds produced from these crosses failed to germinate.

Table 2. Quantitative Characteristics of *S. indica*, *S. angustifolia*, *S. cayennensis* and the F₁ Hybrid.

Characters	<i>S. indica</i>	<i>S. angustifolia</i>	<i>S. cayennensis</i>	<i>S. angustifolia</i> x <i>S. cayennensis</i>
Height at first flowering(cm)	21.76±0.42 ^b	8.72±0.14 ^c	30.38±1.69 ^a	28.88±2.64 ^a
Height at maturity (cm)	43.84±1.65 ^a	8.72±0.14 ^b	40.02±1.42 ^a	40.44±4.32 ^a
Leaf length (cm)	17.14±0.30 ^a	10.30±0.10 ^b	10.80±0.09 ^b	8.00±0.49 ^b
Leaf breadth (cm)	13.85±2.93 ^a	3.20±0.03 ^b	5.03±0.05 ^b	3.57±0.25 ^b
Days to First Flowering	101	87	86	131
Number of primary Branches	14.20±0.34 ^a	10.20±0.27 ^b	15.00±0.78 ^a	13.20±0.71 ^b
Inflorescence length (cm)	56.26±0.43 ^a	47.20±1.28 ^a	20.10±0.62 ^c	31.01±0.85 ^b
% Seed sets	98.61	98.23	92.93	29.83
% Pollen fertility	96.29	96.56	87.76	63.14
Seed Length (mm)	4.59±0.02 ^a	4.08±0.02 ^a	5.10±0.78 ^a	3.21±0.06 ^a
Seed Breadth(mm)	1.56±0.03 ^a	1.70±0.02 ^a	1.11±0.01 ^b	1.02±0.01 ^b
Petal length(mm)	15.36±0.08 ^a	10.07±0.06 ^b	7.54±0.02 ^c	9.60±0.11 ^b
Petal breadth(mm)	11.52±0.09 ^a	8.72±0.05 ^b	5.50±0.06 ^c	8.00±0.12 ^b
Calyx length(mm)	6.44±0.05 ^a	5.08±0.04 ^a	4.36±0.02 ^b	5.36±0.04 ^a
Calyx breadth(mm)	2.00±0.00 ^a	2.00±0.00 ^a	1.80±0.01 ^b	1.95±0.01 ^a

^a Means followed by the same letter in each row are not significantly different at 0.05 level of probability.

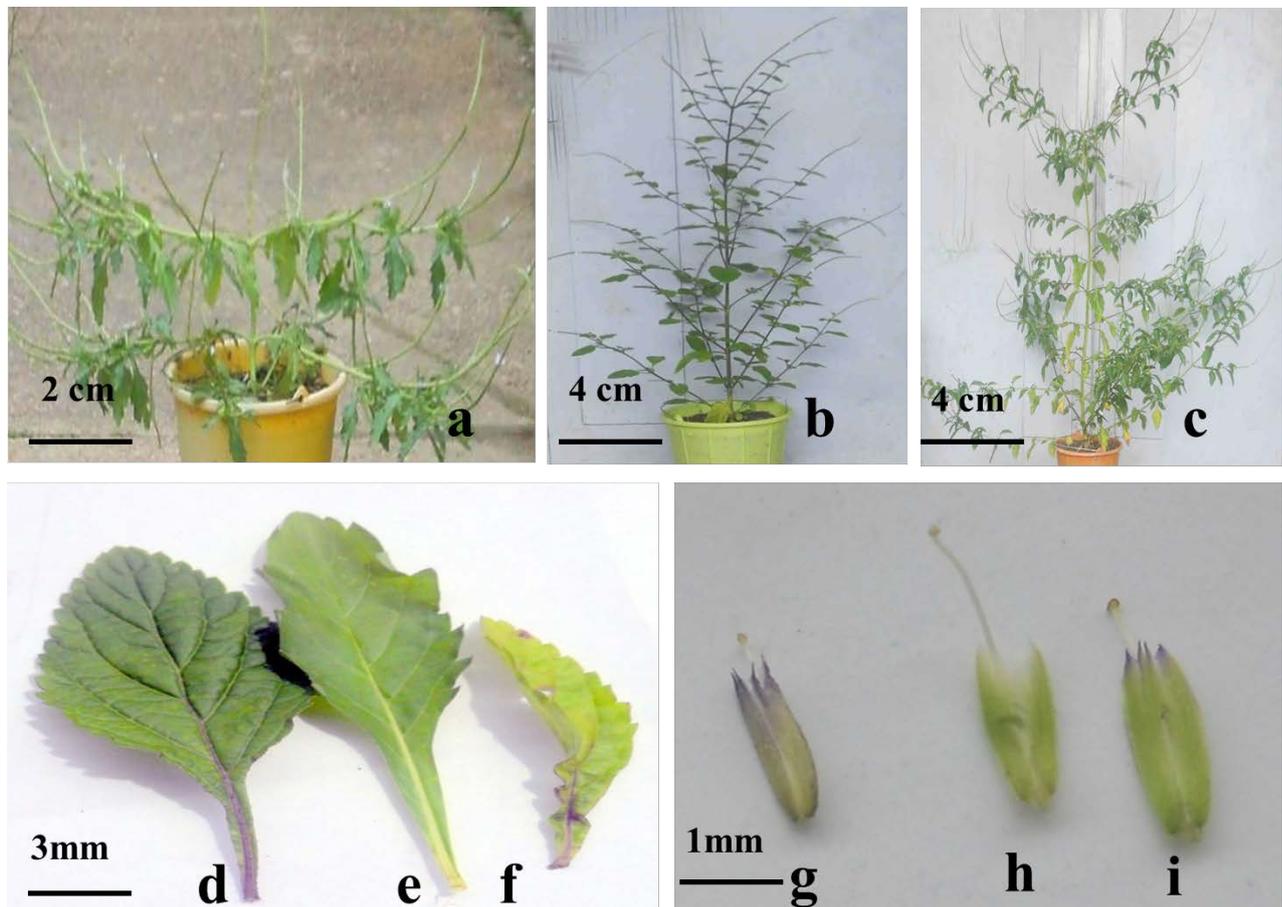


Figure 1. Morphological features of *S. angustifolia*, *S. cayennensis* and their F₁ hybrid (*S. angustifolia* x *S. cayennensis*). **a.** Habit of *S. angustifolia*, **b.** Habit of *S. cayennensis* **c.** Habit of *S. angustifolia* x *S. cayennensis* **d.** Pigmented petiole in *S. cayennensis* **e.** Acyanic petiole in *S. angustifolia* **f.** Pigmented petiole in *S. angustifolia* x *S. cayennensis* **g.** Calyx pigmentation in *S. cayennensis* **h.** Acyanic calyx in *S. angustifolia* **i.** Calyx pigmentation in *S. angustifolia* x *S. cayennensis*.

4. Discussion

Interspecific crosses between *S. angustifolia* (2n = 56) and *S. indica* (2n = 160) were not successful. Seeds were not produced; however, a considerably swollen ovary was

observed after fertilization which degenerated later. This failure might have occurred at the growing embryo stage. The failure observed may be as a result of disharmony between F₁ and the maternal tissue or genic disharmony where the parental genes in the cells of the hybrids are

unable to work together properly. The chromosome numbers reported for the two species also differ widely from one another which may affect the pairing pattern. This might be a result of the differences between the two parental genomes in the embryo (i.e. the F_1 genome). The two species can be said to have been isolated genetically based on their habitat preference (because though they are allopatric, they were brought together artificially, but no hybrid was produced). Carolin (1957) reported the same isolation barriers in species of the genus *Dianthus* of Caryophyllaceae. The inability of F_1 seeds produced from crosses between *S. cayennensis* and *S. indica* to germinate may be attributed to the disparity in their chromosome numbers which may cause pairing difficulties, or it may be attributed to the inviable hybrid seeds. Stebbins (1971) reported that hybrid inviability may be a result of a breakdown in the early stage of embryo development which is often due to the disharmony between the embryo and the endosperm which provides food material for its growth and the maternal tissue. Interspecific crosses between *S. angustifolia* ($2n = 56$) and *S. cayennensis* ($2n = 56$) and their reciprocal were successful and seeds were produced. The F_1 plants grown from the seeds recovered from these crosses were floriferous, and the purple pigmentation that was observed to be absent in the ovulate parent was found at the tip of the calyx and the petiole of the staminate parent in the hybrid. This character can be described as dominant, a character inherited from the pollen donor (*S. cayennensis*). The light purple colour of the corolla in the F_1 hybrid is similar to that of the ovulate parent (*S. angustifolia*), but is slightly lighter. This is in line with the observation of Danser (1929) who also observed that the hybrid $xS. gracilis$ produced between *S. australis* and *S. urticaefolia* have a corolla similar to that of *S. urticaefolia* but a slightly lighter or pale in colour. The anther sacs barely dehisce and the pollen productivity was very low in the F_1 hybrid plant studied; a similar state in $xS. intercedens$ was observed, while the pollen were absent in the hybrid produced between *S. jaimaicensis* and *S. urticaefolia*. The pollens in the genus *Stachytarpheta* are usually tricolpate and rarely acolpate, monocolpate, bicolpate, and tetracolpate (Adedeji, 2010), but in this investigation the hybrid produced displayed a totally different pollen type (acolpate) from the two parents. The F_1 hybrid (*S. angustifolia* x *S. cayennensis*) is partly fertile (63.14% pollen fertility) with a low seed set (29.83 %). The seeds produced from the reciprocal crosses (*S. cayennensis* x *S. angustifolia*) did not germinate, which may be attributed to the inviable hybrid seeds. The maternal environment (i.e. maternal tissue) may not be conducive for the growth of the embryo. This might be attributed to the fact that the pollens do not contain enough food reserves to support the pollen tube growth through their style because the reciprocal cross results in seed setting. Isolation factors between the species seem to be spatial and reproductive. The habitat preferences of the two species are different, one occurring in areas close to water (*S. angustifolia*) and the other in drier regions. The ability of *S. angustifolia* and *S. cayennensis* to produce vigorous and floriferous hybrids suggests a closer genetic relationship between the two species. Moreover, since their chromosome numbers have been reported to be the same, the sterility factors in these crosses are probably genic rather than chromosomal resulting from small structural

differences which are not sufficient to prevent pairing, but gives inviable chromosome combinations with decreased fertility of gametes and low pollen fertility in hybrids. The sterility observed may also be due to segregation problems resulting from the incompatibility of the parental chromosomes in the hybrid genomes. Stebbins (1971) stated that if the parental species are strongly differentiated, their chromosomes cannot pair at all, leading to an abnormal segregation at meiosis of either whole chromosomes or blocks of genes contained in the chromosomal segments resulting in hybrid sterility. He stated that when the parents of the hybrids are more closely related to each other, their chromosomes may pair at meiosis, but because the arrangement and structures of genes of the chromosomes are different, their pairing is imperfect and results in the segregation of the gametes of abnormal, disharmonious combinations of genes leading to the hybrid breakdown that is delayed until after fertilization. This also confirms that the species is reproductively isolated. A strong barrier to gene exchange exists between *S. angustifolia* and *S. cayennensis* in the form of F_2 hybrid breakdown leading to F_2 seed inviability in *S. angustifolia* x *S. cayennensis* and F_1 hybrid seed inviability in the reciprocal cross. Balafama (1982) reported a similar observation between the North African and European *Leucanthemum* species of the Asteraceae family. He observed that the species have equal chromosome numbers, but the hybrids recovered were with a reduced fertility. He suggests that the cytogenetic, distributional and morphological data of the North Africa group has evolved by hybridization at the diploid level followed by spatial isolation which is a pattern very different from that observed in European *Leucanthemum*. He confirmed that the species has been isolated spatially and reproductively.

Based on the recent hybridization study, *S. angustifolia* and *S. cayennensis* are genetically close to each other compared to *S. indica*. This agrees with Adedeji (2010) who carried out palynology studies on the three *Stachytarpheta* species in Nigeria and concluded that *S. cayennensis* and *S. angustifolia* are the closest, and *S. indica* is closer to *S. angustifolia*, but is farther from *S. cayennensis*. The hybrid plants were propagated vegetatively and established, but with a little pruning, they can form a small garden plant which blooms profusely all year and makes a splendid yield in tropical gardens.

5. Conclusion

The hybridization studies showed that the isolating mechanism (post-zygotic) has set a strong reproductive barrier among the three *Stachytarpheta* (such as embryo abortion in *S. angustifolia* x *S. indica* and *S. indica* x *S. angustifolia*; inviable F_1 seed in *S. indica* x *S. cayennensis*, *S. cayennensis* x *S. angustifolia* and *S. cayennensis* x *S. indica*; F_2 seed breakdown in *S. angustifolia* x *S. cayennensis*) to prevent exchange of genes in order to maintain their individualities.

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