— Short Communication —

TEMPORARY INCIDENCE OF POLYGAMOMONOECY IN THE BIODIESEL PLANT *JATROPHA CURCAS* L. IS ASSOCIATED WITH STYLE POLYMORPHISM PROMOTING AUTOMATIC SELF-POLLINATION

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Abstract—Polygamomonoecy – the occurrence of perfect flowers alongside staminate and pistillate ones – in the monoecious biodiesel plant *Jatropha curcas* L. was reported as rare and often dismissed as having no specific functionality. Here we report that the incidence of perfect flowers is almost exclusively limited to a short period at the beginning of the blooming season and is directly associated with stigmas bending over fertile anthers, as early as in the mature bud stage. All perfect flowers have bent stigmas, while this trait is completely absent from female flowers produced later in the blooming season. In light of this species' self-compatibility, we suggest that the occurrence of perfect flowers with bent stigmas promotes automatic self-pollination, thus enhances reproductive success when conditions are unfavourable for pollination.

Keywords: Automatic self-pollination, hermaphrodite flower, Jatropha curcas, perfect flower, polygamomonoecy, sexual expression

INTRODUCTION

Jatropha curcas L., a native to tropical America, is a perennial shrub in the Euphorbiaceae. It was once used as a live fence to contain or exclude farm animals and as an oil source for soap production and lamp fuel (Openshaw 2000). Now it is cultivated as an oil crop for biodiesel production (de Jongh 2006; Divakara et al. 2010).

Jatropha curcas blooms in two major periods: the first in late spring to mid summer and the second in autumn (Wu et al. 2011; Wang & Ding 2012; Vaknin, personal information). It is monoecious with unisexual flowers, at various staminate (male) to pistillate (female) ratios, ranging from 4:1 to 29:1 (Raju & Ezradanam 2002; Bhattacharya et al. 2005; Chang-wei et al. 2007; Tewari 2007). The ratio decreases as the age of the plant increases (Prakash et al. 2007). Staminate flowers are green, plate shaped with ten diadelphous stamens, arranged in two tiers of five each (Fig. 1a). Pistillate flowers are also green, but are relatively larger with three bifid stigmas and a three-carpel ovary, which matures into a three-seeded capsule (Raju & Ezradanam; 2002; Fig. 1b). Both staminate and pistillate flowers have nectar glands in their base and are insect pollinated (Raju & Ezradanam 2002; Bhattacharya et al. 2005). The plant is reported to be self-compatible, possibly with a small rate (12%) of apomixis (Chang-wei et al. 2007). Previously, we

described the pollination biology and breeding system of *J. curcas* (Samocha et al. 2014; Samra et al. 2014). Perfect (hermaphrodite) flowers (Fig. 1e) were reported by Dehgan & Webster (1979) as a rare occurrence, and since then no detailed analysis of these flowers has been provided other than by Abdelgadir et al. (2012), reporting on pollen from perfect flowers having lower viability, lower germination rate and shorter pollen tubes, compared to staminate flowers. However, our preliminary observations, during 2007 to 2009, revealed that perfect flowers were almost exclusively limited to a short period of 2-4 weeks, in late spring, at the beginning of the first blooming season. In 2010 we undertook more thorough observations of phenology and sexuality.

Our aim was to elucidate the significance of temporal polygamomonoecy in *J. curcas* by characterizing the occurrence of perfect flowers in association with seasonal changes in stigmatic traits.

MATERIALS AND METHODS

The studied plants were a mix of 4-year-old plants of wild germplasm originating from Brazil, Suriname, Niger, South Africa, India and China, none of which were ever bred or selected for a desirable trait. The plants were grown at the ARO research institute in Bet-Dagan, Israel (31°59' N, 34°49'E) at a density of 2 × 2 m. Local climate conditions are classified as Mediterranean, with hot dry summers and mild wet winters with an average of 524 mm rainfall per year (Israel Meteorological Service). Irrigation and fertilization
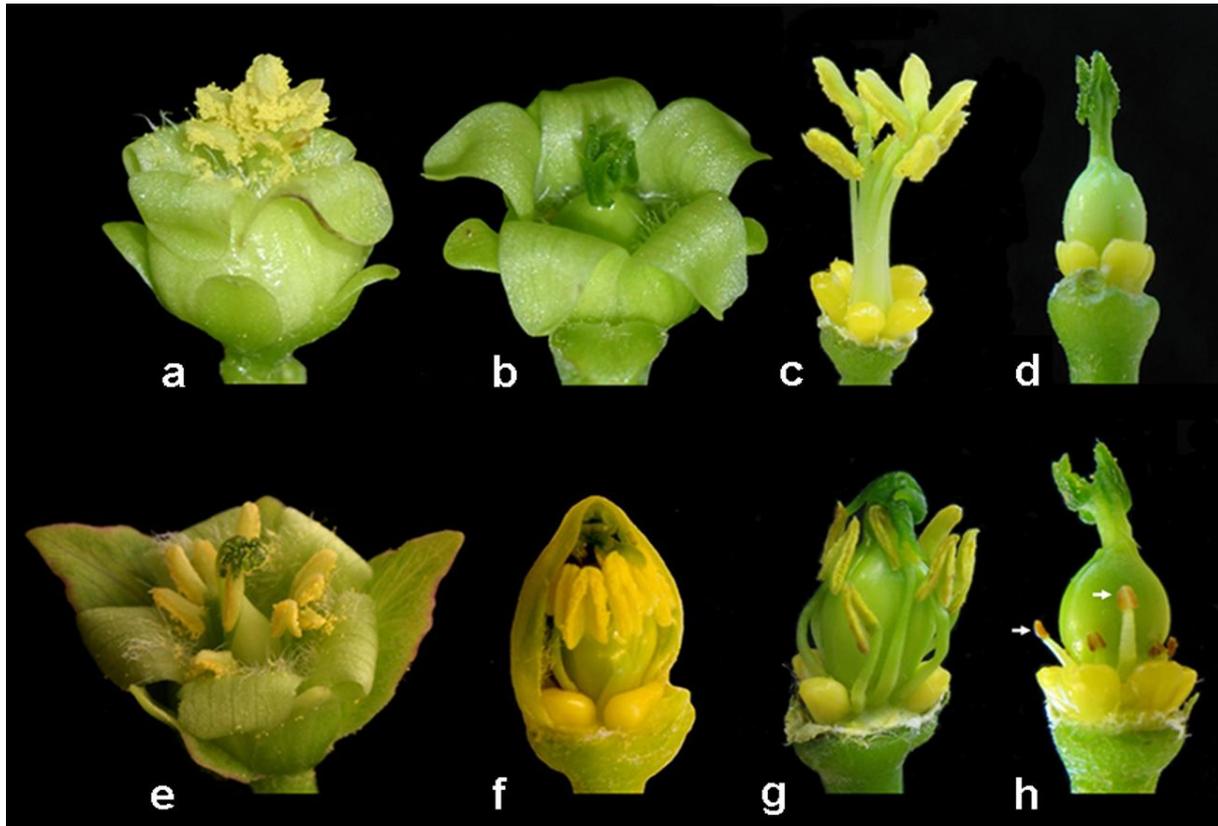
FIGURE 1.   *Jatropha curcas* flower types: Regular staminate (a) and pistillate (b) flowers and the same flowers with the sepals and petals removed (c and d, respectively). Early-blooming perfect flower, when fully open (e), at the mature bud stage (f) and with the sepals and petals removed (g). Early blooming pistillate flower with the sepals and petals removed (h). Arrows point at vestigial anthers.

were applied via drip irrigation throughout the summer and early autumn (30 cubic meters per day per hectare, with NPK fertilizer (20-10-20 0.1 kg per 10 litres).

Bloom phenology and floral traits

In early April 2010, the plants in the study site were surveyed daily. Plants with early developing inflorescences ($N = 50$) were marked and counted and one inflorescence was removed from each plant, for further analyses. Summer blooming, staminate and pistillate flowers, were similarly sampled. All inflorescences were analyzed for number of open staminate, pistillate and perfect flowers, number of flower buds, number of normal and/or aborted stamens for each flower type ($N = 10$), and number of bent stigmas for pistillate and perfect flowers (Fig. 2) ($N = 20$). Corolla width of all flower types was measured using digital callipers having an accuracy of $\pm$ 0.1 mm ($N = 10$). Pollen viability in all early-blooming-flower types as well as summer-blooming staminate flowers was estimated using the DAB (3.3' Diaminobenzidine) staining technique (Dafni et al. 2005) ($N = 10$). Stigmatic pollen germination was estimated using Aniline Blue Epifluorescence (Martin 1959) for early blooming pistillate and perfect flowers and for summer-blooming pistillate ones ($N = 10$). Crosses were made by hand, and total number of germinating pollen grains was scored. Stigmatic pollen germination was recorded with an Olympus IX 81 fully automated laser scanning confocal microscope (Olympus, Tokyo, Japan).

Fruit and seed traits

In early April 2010, a sample of 20-30 early-blooming pistillate and perfect flowers was marked. In late June 2010, all mature fruits, developed from these flowers, were harvested. Percentage fruit set and seed set were calculated. Fruit set is the proportion of carpels with at least one seed. Seed set is reported based on those fruits that set 1-3 seeds, i.e., given that the fruit as a whole set. Twenty seeds from each flower type were weighed and following the removal of seed coats, the kernels were weighted. Pistillate flowers ($N = 20$) were also marked during the summer bloom (June) and the developing fruits and seeds were similarly analyzed.

Statistical analyses

Statistical analyses were preformed with StatView® 5.0 (SAS Institute Inc. Cary, NC, USA) according to Sokal and Rohlf (1995). One-way analysis of variance (ANOVA) was used in order to compare how flower types differed in flower size, number of normal and aborted stamens, percentage of viable pollen, pollen germination on the stigma, seeds per fruit, and seed and kernel weight. Tukey-Kramer HSD was performed in order to compare the means two samples at a time. In order to normalize the data, arcsine square-root and square-root transformations were applied on percentages and counts, respectively. Differences are reported as significant when $P < 0.05$.

FIGURE 2. The stigma of a perfect flower bending over a fertile anther. Scale bar is 1 mm.

TABLE 1. Comparisons of various flower types during the early-bloom period (perfect, pistillate, and staminate flowers) and during the summer bloom period (pistillate and staminate flowers).

Bloom period Flower type	Early-bloom Perfect	Pistillate	Staminate	Summer Pistillate	Staminate
Corolla width (mm)	[a] 10.3 ± 0.5 a	8.9 ± 0.4 b	8.2 ± 0.4 bc	8.6 ± 0.1 bc	7.5 ± 0.2 c
Normal stamens (no.)	6.4 ± 1.1 b	0.0 c	9.3 ± 0.3 a	0.0 c	9.8 ± 0.1 a
Aborted stamens (no.)	2.4 ± 1.1 b	9.0 ± 0.3 a	0.1 ± 0.1 c	0.0 c	0.0 c
Bent stigmas (%)	100.0	36.0	-	0.0	-
% of seed-bearing flowers	38.7	61.3	0.0	100.0	-
% of open flowers	16.7	14.9	68.4	25.5	74.5
Staminate/pistillate ratio	5988.0	3685.0	-	26.0	-
Pollen viability (%)	94.4 ± 2.2 a	-	81.8 ± 4.4 b	-	95.9 ± 1.8 a
Stigmatic pollen germination (no.)	8.4 ± 3.6 a	4.2 ± 1.8 a	-	4.6 ± 1.0 a	-
Fruit set (%)	46.4	64.3	-	90.0	-
Seed set (%) in set fruits	33.3	57.1	-	90.2	-
Seeds per fruit in set fruits	2.20 ± 0.30 a	2.70 ± 0.10 a	-	2.70 ± 0.10 a	-
Seed weight (gr)	0.66 ± 0.01 a	0.71 ± 0.02 a	-	0.59 ± 0.02 b	-
Kernel weight (gr)	0.41 ± 0.01 a	0.43 ± 0.01 a	-	0.36 ± 0.02 b	-

[a] Data are shown as means ± SE. Post hoc comparisons between flower types were carried out for the entire year, early bloom and summer bloom. Means marked with the same letter were not significantly different.

RESULTS AND DISCUSSION

Bloom phenology and floral traits

During winter and early spring, *J. curcas* did not bloom. A relatively small portion of the plants (~10%) started blooming as early as April. From then through May was denoted as the early bloom period. During this period, the open flowers ($N = 516$) were of three types: perfect, pistillate and staminate (Fig. 1e, h and a, respectively) accounting for 17, 15 and 68% of the open flowers, respectively (Table 1). An additional 6023 flower buds were counted and sorted as 98.2% staminate, 1.5% pistillate, and 0.3% perfect flower buds. All open flower types developed stamens: normal fertile stamens, aborted non-fertile vestigial stamens or a combination of both (Table 1). The vestigial anthers were smaller than fertile ones and did not produce viable pollen. As the summer bloom period got well underway in June, with data gathered until July, the majority of the new inflorescences were composed only of pistillate and staminate flowers (Fig. 1b and a, respectively; $N = 330$ open flowers), and no vestigial stamens were observed. An additional 3382 flower buds were counted and sorted as 96% staminate and 4% pistillate flower buds.
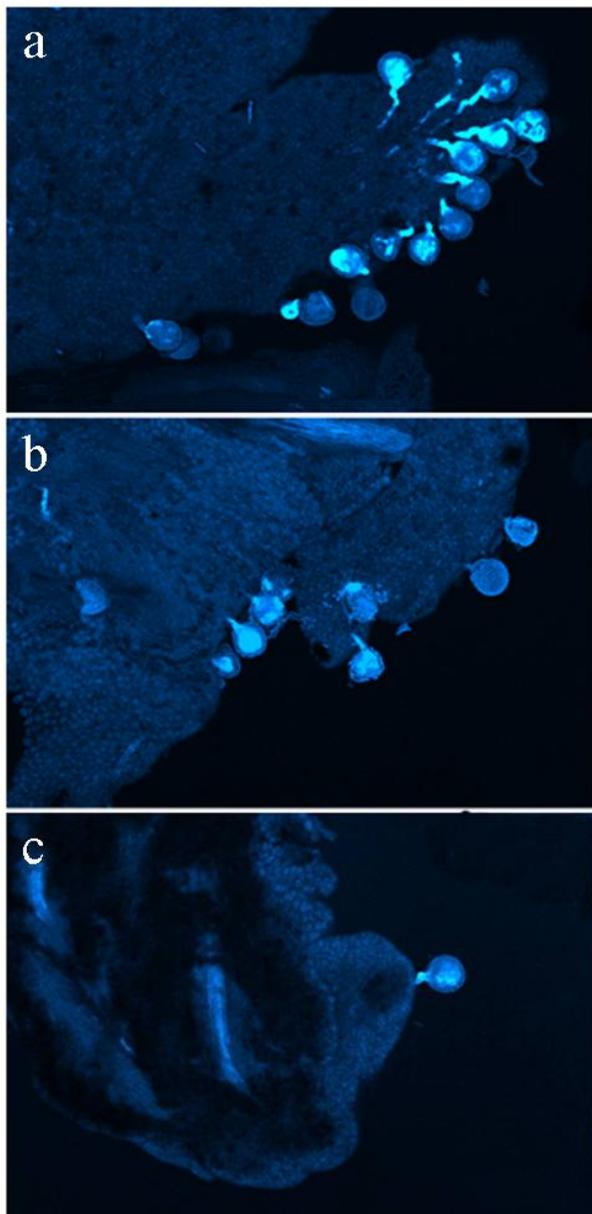
FIGURE 3.　Pollen germination on the stigma of early blooming perfect flower (a), pistillate flower (b), and perfect flower at the bud stage (c). Scale bar is 100 μm.

Recent studies on *J. curcas* flowers revealed that pistillate flowers originate from bisexual primordia, with pistils and stamens growing simultaneously and the stamens stopping their development, either very early, leaving no trace in the mature flowers, or much later, thus forming antherodes with aborted pollen (Wu et al. 2011; Wang & Ding 2012).

Perfect flowers were significantly larger than other flower types (Table 1). According to Horovitz & Galil (1972), very often flowers of hermaphroditic plants have much larger petals than those of male-sterile plants. Early-blooming and summer-blooming pistillate flowers, were relatively similar in size, and were significantly larger than summer-blooming staminate flowers (Table 1). Similarly, Raju & Ezradanam (2002) described pistillate flowers as relatively larger than staminate ones.

Pollen viability of all pollen-producing flowers was relatively high (> 80%) with slightly lower values recorded for early-blooming staminate flowers (Table 1). Stigmatic pollen germination for perfect flowers (Fig. 3a) was higher than for early-blooming (Fig. 3b) and summer-blooming staminate flowers. However, the differences were not significant. Stigmatic pollen germination was also recorded on perfect flower buds (Fig. 3c); in other words, the flowers were cleistogamous. Our results were in contrast to those of Abdelgadir et al. (2012) who reported that pollen from perfect flowers has lower viability, lower germination rate and shorter pollen tubes, compared to staminate flowers. Possibly, the rare perfect flowers reported by Abdelgadir et al. (2012) were distinctly different from the ones we have encountered due to differences in environmental conditions.

Perfect flowers in *J. curcas* have been considered rare and abnormal with no specific function (Luo et al. 2007). Here we report on a unique phenomenon of early-blooming perfect flowers with stigmas bent over fertile stamens (Fig. 2) and pollen grains were deposited on the receptive surface (Fig 1e, f, and g) as early as in the mature bud stage (Fig 1f). Practically 100% of all early-blooming perfect flowers and about a third of the early-blooming pistillate flowers presented bent stigmas, whereas it was completely absent from summer-blooming pistillate flowers (Fig 1b and d). Automatic self-pollination is a known strategy adapted by plants to provide reproductive assurances when pollinators are scarce as in some orchids (Catling 1990) and the cleistogamous flowers of *Lamium amplexicaule* L. (Galil & Eisikowitch 1964; Lord, 1979).

An exogenous application of the plant growth regulator 6-benzyladenine (BA), a synthetic compound with cytokinin activity, on *J. curcas* (Pan & Zu 2011) and male grapevines (Negi & Olmo 1966) resulted in the induction of perfect flowers. Cytokinins are important factors in the regulation of flower bud initiation and development, and floral sex expression in certain fruit trees (Pan & Zu 2011). Therefore, the induction of perfect flowers in *J. curcas* could be associated with elevated cytokinins as the plant "breaks" its cold-induced dormancy in late spring.

Fruit and seed traits

Fruit set and seed set for summer-blooming pistillate flowers were ~50% higher than for early-blooming pistillate flowers and ~100% higher than for perfect flowers (Table 1). Pistillate flowers often produce more seeds than perfect ones, most likely due to the reallocation of energy away from pollen production to seed production (Shykoff et al. 2003). The reduction in fruit set and seed set in perfect flowers could not be explained by reduced pollen viability or stigmatic pollen germination (Table 1), but rather by factors that remain to be elucidated such as reduced embryo fertilization or increased level of aborted embryos or other forms of inbreeding depression.

Perfect flowers produced significantly lighter seeds than early blooming pistillate flowers. These results are in accordance with other plant species characterized by lighter seeds originating from perfect flowers (Shykoff et al. 2003). In *J. curcas*, smaller seeds will eventually develop into lower

quality seedlings (Zaidman et al. 2010), a form of inbreeding depression (Charlesworth & Charlesworth 1979). This is not surprising as we have already shown that selfing in *J. curcas* will eventually lead to the production of smaller seeds (Samoch et al. 2014). Early-blooming perfect and pistillate flowers produced significantly heavier seeds and seed kernels in comparison with summer-blooming pistillate flowers.

As in *J. curcas*, most gynodioecious species are self-compatible. The perfect flowers more often self-fertilize (Meagher 2007; Ehlers & Schierup 2008), while the female flowers usually produce higher quality seeds resulting from being forced to outcross thereby limiting inbreeding depression in their offspring (e.g. Thompson & Tarayre 2000; Chang 2007). Our results, however, did not support that assumption, presumably since geitonogamous selfing levels were relatively high during the summer bloom period.

Summary

Perfect flowers with varying levels of male sterility and bent stigmas over fertile stamens occurred during a relatively short period in late spring when pollination services by the local fauna was scarce and opportunities for cross-fertilization were rare. The placement of a stigma and anthers in the same flower greatly enhances the chances of successful pollination and fertilization in one single floral visit, and the bending of the stigma over the anthers seems to act as a fail-safe mechanism to ensure reproductive success in the absence of pollinators. We suggest that the ability to change sex expression may be used in future breeding programs to enhance yield in this important biodiesel feedstock.

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