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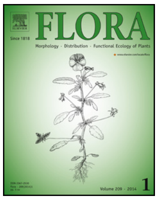

Reproductive biology of *Ziziphus mistol* Griseb. (Rhamnaceae), a wild fruit tree of saline environments

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ABSTRACT

Ziziphus comprises important fruit trees that inhabit arid environments of tropical and subtropical regions of the world and are able to adapt to drought and salt stress. Species of this genus are characterized by synchronous protandrous dichogamy and self-incompatibility; however, reduced seed set after hand self-pollination indicates that self-fertilization is possible and that it causes a strong inbreeding depression. We characterize the reproductive biology of *Ziziphus mistol*, a species typical of saline environments of Chaco American forests. For this purpose, we studied floral biology, observed foraging behavior and pollen load of captured floral visitors, and tested the breeding system through the assessment of fruit and seed set and pollen tube growth following self-, cross-, and open pollinations treatments. In addition, the dependence of a pollinator and autonomous self-pollination were evaluated by excluding floral visitors and airborne pollen. Cross-compatibility between individuals was tested through controlled hand pollinations. Results indicate that *Z. mistol* is predominantly outcrossing and depends on its pollinators to produce fruits and seeds. We observed high synchrony of flowering between individuals, as well as in floral anthesis between/within inflorescences within individuals. The flowers exhibit complete intrafloral dichogamy that fully prevents autonomous self-pollination, while sexual phases overlap within inflorescence; thus geitonogamy can easily occur. Manipulative experiments show that *Z. mistol* can set fruits and seeds after self- as well as cross-pollinations. However, reduction in fruit set and seed set after selfing and/or cessation of pollen tube growth in the style suggest a self-incompatible system. From reciprocal crosses tested ($N = 95$), we have identified only 32.6% compatible crosses via fruit and seed trials. We suggest that in *Z. mistol*, as well as in other species of the genus, both synchrony in floral development and partial self-incompatibility would be evolved to reduce the costs of inbreeding.

Introduction

Ziziphus Mill. comprises approximately 170 pantropical species, 25 of which are native to America and the Caribbean comprising the New World clade according to Islam and Simmons (2006). This genus contains trees and shrubs that inhabit arid environments on every continent due to their versatility in being able to adapt to drought stress (Arndt et al., 2001). *Ziziphus* species are important fruit trees with food and medicinal value and are commercially cultivated in hot and arid regions (Mizrahi et al., 2002). In China and India, species such as *Ziziphus mauritiana* Lam. and *Ziziphus jujuba* Mill. have a long tradition of selection and cultivation; as a result, species occurring in these countries are better known and more researched than those in other regions (Arndt et al., 2001). *Ziziphus mistol* Griseb., widely distributed in Brazil, Paraguay, and Argentina (Tortosa, 1995), is an important genetic resource of saline environments of Chaco forests (Ragonese, 1967). It fruits, prepared in different ways, have traditionally been used by humans as food and in popular medicine was mentioned the use of its bark and leaves (Scarpa, 2004). Currently, these are obtained in local markets extracted from natural populations, as there are no commercial orchards of the species.

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Studies on reproductive biology have shown an appreciable diversity in the breeding system of *Ziziphus*. Most species of this genus that were subjected to research are xenogamous and self-incompatible (SI) (Asatryan and Tel-Zur, 2013; Weekley and Race, 2001; Zietsman and Botha, 1992). Indeed, based on the presence of binucleate pollen grains and the cessation of pollen tube growth in the style, it has been suggested that SI system is gametophytically controlled (Asatryan and Tel-Zur, 2013). However, the strength of SI in this genus appears to be a variable trait because self-fertilization and parthenocarpy have been reported in cultivars of *Z. jujuba*, *Z. mauritiana* and *Ziziphus spina-christi* (L.) Desf. (Asatryan and Tel-Zur, 2013), and wild individuals of *Ziziphus celata* Judd. and D. W. Hall (Weekley and Race, 2001). Furthermore, the existence of self-compatible (SC) genotypes and varieties for *Z. jujube* (Ackerman, 1961) and *Z. mauritiana* (Mehrotra and Gupta, 1985; Rama Devi et al., 1989; Teaotia and Chauhan, 1964) were also mentioned. Finally, the failure in the style elongation in numerous flowers has led some authors to suggest the presence of andromonoecy (Galil and Zeroni, 1967; Tel-Zur and Schneider, 2009).

Reports on phenology of *Ziziphus* stated that hermaphrodite flowers display synchronized protandry; that is, all open blossoms on a ramet are in the same phase and anther dehiscence precedes stigmatic receptivity (Lloyd and Webb, 1986). In fact, the presence of genetical morphs that differ in the timing of anthesis during the day (Asatryan and Tel-Zur, 2013; Galil and Zeroni, 1967; Lyrene, 1983; Zietsman and Botha, 1992) was reported. If there are two morphs at a 1:1 ratio, where each morph is SI and reproduction occurs between contrasting morphs, the phenomenon is called heterodichogamy (Lloyd and Webb, 1986; Renner, 2001) or temporal dioecism (Cruden, 1988). Pollination studies of cultivated species of *Ziziphus* mention honeybees (*Apis* sp.) as the most common visitors (Pham, 2012), while data for wild genotypes in *Z. mauritiana* (Mishra et al., 2004; Rama Devi et al., 1989) and *Z. joazeiro* (Nadia et al., 2007) suggest species of wasps, flies, and bees as their major pollinators.

Hermaphroditism provides advantages such as economy of resources because allocation to floral attraction and reward benefits both maternal and paternal function, and reproductive assurance through self-fertilization within mates and/or pollinators is rare or absent (Charlesworth and Charlesworth, 1987). However, it has negative consequences for individuals (Barrett, 2002). Costs of hermaphroditism, in SI and SC taxa (Culley et al., 1999; Dudash, 1990; Vogler et al., 1999), include sexual interference within as well as between flowers of the same plants (Lloyd and Yates, 1982), and consequently stigma clogging (Lloyd and Webb, 1986), pollen discounting (Harder and Wilson, 1998), and ovule discounting (Barrett et al., 1996); and inbreeding depression (Charlesworth and Charlesworth, 1987). Thus, mechanisms of temporal and physiological separation of sexual functions within the same individual are considered to have evolved to reduce the disadvantages associated with hermaphroditism.

Dichogamy, the temporal separation of male and female phases in hermaphrodite flowers, has been historically interpreted as a mechanism for avoiding inbreeding (Lloyd and Webb, 1986). Recently, correlation analyses (Bertin, 1993; Routley et al., 2004) and experimental works (Dai and Galloway, 2011; Harder et al., 2000; Jersáková and Johnson, 2007; Routley and Husband, 2003) have supported that protandry, the most common form of dichogamy, evolved through male fitness by interference avoidance between male and female functions, rather than female fitness. Moreover, other studies (Harder and Aizen, 2004; Narbona et al., 2011) have demonstrated that protandry may complement physiological SI by reducing selfing when the latter is incomplete (Becerra and Lloyd, 1992; Poehlman and Sleper, 1995). Therefore, in SC species or with incomplete SI, both mechanisms are involved in overcoming hermaphroditism cost of inbreeding depression (Charlesworth and Charlesworth, 1987).

Here, we present the reproductive behavior of *Ziziphus mistol*, and we document floral phenology, potential pollinators, and breeding system as a function of the following predictions: (1) *Z. mistol* is pollinated by a small–medium insect functional group, (2) *Z. mistol* exhibits intrafloral dichogamy, and (3) *Z. mistol* is an SI species but can set fruits and seeds through self-fertility.

Materials and methods

Study area

Fieldwork was conducted in the springs (September–December) from 2010 to 2013 in a natural population of *Z. mistol* located at "Colonia La Brava" (30°51′08.63″ S, 60°15′56.37″ W), Santa Fe Province, Argentina. Observations and experiments were repeated in four seasons. The study site is within ecotonal woods between Chaco and Espinal regions dominated by *Prosopis* L. spp., together with *Geoffroea decorticans* (Gillies ex Hook. et Arn.) Burkart, *Phytolacca dioica* L., *Vachellia caven* (Molina) Seigler and Ebinger, and *Erythrina crista-galli* L. (Hilgert et al., 2003). The chaquenean component is represented by Z. *mistol* and *Schinopsis balansae* Engl. populations. The average annual rainfall amounts to 1200 mm, and the average temperatures range from 16 to 24 °C in the autumn–winter and spring–summer seasons, respectively (INTA, 2013).

Studied species

Individuals of *Z. mistol* are large shrubs or trees, deciduous, 4–8 (15) m tall. Branches have spines in pairs and leafy short shoots at nodes. Leaves are 2–6 cm long, alternate, broadly elliptic or oblong, broadest at the middle, basally three-nerved: margin serrated, crenate, or entire. Inflorescences are axillary umbelliform cymes consisting of yellowish green flowers. Flowers are bisexual, 4–6 mm across, with five triangular sepals 1.5–2 mm long, five oblong-spatulate petals 1 mm long, and five petal-opposed stamens attached at the base to a yellowish disk. The ovary has 2–3 chambers, each with a single ovule, sunk in the disk and one 2- or 3-lobed style. The ovary develops into a drupaceous fruit with one to three seeds (Medan and Schirarend, 2004; Tortosa, 1995).

Floral phenology: population, inflorescence, and flower level

Flowering phenology at population level was described in three inflorescences from ten individuals, based on the number of scars of fallen flowers, buds, open flowers, and fruits present in selected trees. Sampling dates were evenly spread over each reproductive season in 2010 and 2011.

Flowering synchrony between individuals was estimated using Augspurger's index (Augspurger, 1983). This measure focuses on the between-individual synchronization through the number of days in which an individual overlaps flowering with the rest of the individuals of the population. The synchronization level (S_i) of individuals is calculated with the following formula: $S_\mathrm{i} = (1/{-1})(1/f_\mathrm{i})\sum e_{j \neq \mathrm{I}}$, where *i* is a function of the number of individuals in the population *n*, the number of days the individuals is flowering f_i, and the number of days individuals *i* and *j* ($i \neq j$) are flowering simultaneously (e_i). S_i may vary between 0 and 1; when $S_\mathrm{i} = 0$, no synchrony occurs, and when $S_\mathrm{i} = 1$, perfect synchrony occurs. The index of population synchrony (Z) is the average of the S_i of all plants within the population.

To evaluate floral development within and between inflorescences, we used a modified method from Narbona et al. (2011).