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Experimental protocol for data collection of Datura spp.

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ABSTRACT

Experimental design employed to obtain biological materials for RNA seq analysis. We collected leaf tissue to examine gene family evolution of four *Datura* species.

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MATERIALS TEXT

Seeds from four species of the plant genus *Datura* (*D. stramonium, D. pruinosa, D. inoxia and D. wrightii*) collected in natural populations of central and southern Mexico, and southern United States, and stored for no longer than five years, were employed to produce experimental plants.

Germination

1 Plants were started from seed between March-April, growing under 16:8, L:D cycle with 25:20°C (L:D) in the glasshouse of the Institute of Ecology, National Autonomous University of Mexico. Experimental plants were obtained by sowing seeds of maternal families (natural progenies). Once the true leaves appeared and seedlings reached 3-5 cm long, they were planted in single 150 mL pots (Figure 1), in sterilized soil, and watered *ad libitum.*

Figure 1. A) Germination and B) seedling transplant of *Datura* sp.

Leaves collection

2 Nine plants (of different maternal families) were collected at two developmental stages. To capture developmental variation 10 fully expanded leaves from each plant were harvested either at the vegetative or reproductive stage as follows:
When plants reached at least 15 cm and/or started dichotomous branching (~40 days after germination), three juvenile plants were defoliated, whereas the six other plants remained undamaged until flowering.

Since leaf age of vegetative plants at the time of first harvesting differed from mature plants at the final harvesting, we controlled for leaf age differences at the two collection episodes by marking the new leaves produced after the first harvesting, in both treatment-allocated plants so that they could be compared.

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When the first flower emerged, at the reproductive stage (~ 60 days after germination), leaves of the remaining six plants were harvested. All leaves were flash-frozen after collection and stored at -80°C until RNA extraction.

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