# Exploring Crossability Among *Rudbeckia* L. Species

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**Significance to Industry:** Rudbeckia are valuable nursery crops that offer broad adaptability and exceptional ornamental merit. Interspecific hybridization between durable, perennial species and showy, annual species could lead to the development of valuable new cultivars.

**Nature of Work:** The genus *Rudbeckia* L. is widespread throughout North America and contains approximately 30 species of annuals, biennials, and perennials known for their colorful ray corollas and prominent disk-shaped receptacles. The annual species *R. hirta* L. includes cultivars with a diverse range of flower colors and forms; however, this species is short lived and susceptible to diseases including rhizoctonia rot (*Rhizoctonia* sp.) and cercospora leaf spot (*Cercospora* sp.) (3,4). Other rudbeckia species, including *R. fulgida* Ait., *R. missouriensis* Engelm. ex C.L. Boynton & Beadle, and *R. subtomentosa* Pursh., are reliable perennials with superior disease resistance (1).

There has been little published on the genetics and breeding of rudbeckia. Urbatsch (6) reported that the *Rudbeckia* subgenus *Rudbeckia* (including *R. hirta, R. fulgida*, *R. missouriensis,* and *R. subtomentosa*) has a base chromosome number of x=19. Polyploids have been reported to exist in *R. hirta* and *R. fulgida* var. *speciosa* (5). However, information on interspecific crossability and ploidy levels of specific cultivars is lacking. The objectives of this study were to determine the ploidy levels and DNA contents of selected species and cultivars, and to evaluate self-compatibility and crossability among species to facilitate the future development of new hybrids.

Holoploid, 2C DNA contents (i.e., DNA content of the entire non-replicated, chromosome complement regardless of ploidy level) were determined via flow cytometry for all the parental taxa and progeny. Approximately 0.25 $in^2$ (1.6 $cm^2$) of leaf or petal tissue was chopped with a razor blade in a petri dish containing 400 µL of extraction buffer (CyStain UV Precise P, Partec, Münster, Germany). The suspension was filtered through 50-µm nylon mesh and nuclei were stained

using 1.6 mL staining buffer containing 4', 6-diamidino-2-phenylindole (DAPI) (CyStain UV Precise P, Partec). The suspension was analyzed using a flow cytometer with fluorescence excitation provided by a mercury arc lamp (PA-I Ploidy Analyzer, Partec). The mean fluorescence of each sample was compared with an internal standard of known genome size [*Pisum sativum* L. 'Ctirad', 2C = 9.09 ρg; (2)]. Chromosome counts were performed on *R. hirta* 'Toto Gold', *R. hirta* 'Indian Summer', *R. missouriensis*, *R. subtomentosa*, and *R. fulgida* var. *sullivantii* 'Goldsturm' to calibrate DNA content with ploidy level for each species. Root tips were collected and placed in 2 mM 8-hydroxyquioline for 3-5 h at 54F (12C). Roots were then rinsed with cold (39F, 4C) distilled water and placed in 6:3:1 solution of 95% ethanol: chloroform: glacial acetic acid fixative for 24 h at room temperature. Samples were rinsed with a 70% ethanol solution, placed in 70% ethanol solution, and stored at 39F (4C). The following week, samples were removed from storage and transferred to 30% aqueous ethanol solution for 12 min, followed by two 15 min rinses in distilled water. Roots were then hydrolyzed for 1 h at room temperature in 1 N HCl, followed by a quick rinse in distilled water, were placed in Feulgen stain for 2 h at room temperature. Root tips were excised and placed on a glass microscope slide with a drop of 1% aceto-carmine stain, squashed with a coverslip, and viewed at 1,500 ×. Base 1Cx monoploid DNA content (i.e., DNA content of the non-replicated base set of chromosomes with x = 19) was calculated for each species as 2C DNA content / ploidy level. DNA content data were subjected to analysis of variance and means separation using the Tukey HSD procedure.

Taxa selected for breeding included ten *R. hirta* cultivars, three *R. fulgida* varieties, *R. missouriensis*, and *R. subtomentosa* (Table 1). Forty-three interspecific crosses were completed in a greenhouse with at least eight pollinated inflorescences per cross. Pollinations included reciprocal crosses between *R. hirta* cultivars and the five remaining taxa. Inflorescences were pollinated daily until all ray flowers passed anthesis. Self-pollinations were also performed on separate inflorescences of the same taxa. Achenes were collected after flower senescence and sown.

**Results and Discussion:** Chromosome counts documented that *R. hirta* 'Toto Gold' was a diploid (2*n* = 2x = 38) and *R. hirta* 'Indian Summer' was a tetraploid (2*n* = 4x = 76) with an average 1Cx value of 3.91 ρg (Table 1). Our seedlings of *R. missouriensis* and *R. subtomentosa* were diploids with 1Cx values of 4.6 ρg and 5.7 ρg, respectively. *Rudbeckia fulgida* var. *sullivantii* 'Goldsturm' was a tetraploid with a 1Cx value of 9.28 ρg. Based on these standards, ploidy levels of the remaining cultivars were then estimated for each species (Table 1). Mean 1Cx DNA content (genome size) was similar among cultivars of *R. hirta*, regardless of ploidy level (i.e., there was no apparent genome downsizing at higher ploidy levels). However, 1Cx DNA content varied close to 300% among species, emphasizing the need to calibrate DNA content with ploidy level separately for each species within this subgenus.

The self-pollinated inflorescences produced no viable seed indicating a high level of self-incompatibility (data not shown). Crosses between species yielded 0 to 38 seedlings per inflorescence with a total of 844 seedlings. However, 2C DNA contents of all but one of these seedlings were similar to the maternal parent, suggesting that these plants arose through pseudogamy: a process whereby pollination stimulates apomixis. Apomixis has been recorded previously in *R. triloba* L. (3x) and *R. fulgida* (4x) (5). Overall, interspecific crossability among these species was found to be extremely low, with the production of only one successful hybrid: *R. subtomentosa* (2C = 22.8 ρg) × *R. hirta* 'Toto Gold', (2C = 7.4 ρg) which yielded a seedling with an intermediate 2C DNA content of 15.2 ρg, confirming hybridity. Cytology also documented two sets of 19 chromosomes of disparate size, consistent with both parents (Fig. 1).

Results from the cytology and cytometry component of this project documented ploidy levels and DNA contents of selected species and cultivars. Breeding and crossability components determined a high level of self-incompatibility among these taxa, but found that pseudogamy appears to be prevalent following interspecific pollination. Although successful interspecific hybridization was rare and difficult to achieve, it is possible and future efforts may lead to the development of improved cultivars.

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**Table 1.** DNA content, ploidy level, and chromosome number of selected *Rudbeckia* taxa.

| Taxa | 2C DNA Content (ρg) | 1Cx DNA Content (ρg) | Ploidy Level | 2*n* Chromosome Number |
|---|---|---|---|---|
| *R. hirta* 'Goldilocks' | 7.1[Z]± 0.10 a | 3.5[Z]± 0.05 a | 2x | 38 |
| *R. hirta* 'Marmalade' | 7.3 ± 0.03 a | 3.6 ± 0.02 ab | 2x | 38 |
| *R. hirta* 'Toto Gold' | 7.4 ± 0.07 a | 3.7 ± 0.04 ab | 2x[Y] | 38[Y] |
| *R. hirta* 'Toto Rustic' | 7.4 ± 0.15 a | 3.7 ± 0.07 ab | 2x | 38 |
| *R. hirta* 'Sonera' | 14.2 ± 0.34 b | 3.5 ± 0.08 a | 4x | 76 |
| *R. hirta* 'Cherokee Sunset' | 14.6 ± 0.17 bc | 3.6 ± 0.04 ab | 4x | 76 |
| *R. hirta* 'Autumn Colors' | 15.4 ± 0.26 bc | 3.8 ± 0.07 ab | 4x | 76 |
| *R. hirta* 'Prairie Sun' | 16.0 ± 0.23 bcd | 4.0 ± 0.06 abc | 4x | 76 |
| *R. hirta* 'Indian Summer' | 16.4 ± 0.32 bcd | 4.1 ± 0.08 abc | 4x[Y] | 76[Y] |
| *R. hirta* 'Tetraploid' | 16.7 ± 0.44 cd | 4.2 ± 0.11 bc | 4x | 76 |
| *R. missouriensis* | 18.3 ± 0.02 d | 4.6 ± 0.01 cd | 2x[Y] | 38[Y] |
| *R. subtomentosa* | 22.8 ± 0.18 e | 5.7 ± 0.04 e | 2x[Y] | 38[Y] |
| *R. fulgida* var. *fulgida* | 35.2 ± 1.10 f | 8.8 ± 0.27 f | 4x | 76 |
| *R. fulgida* var. *speciosa* | 36.8 ± 0.15 f | 9.2 ± 0.39 f | 4x | 76 |
| *R. fulgida* var. *sullivantii* 'Goldsturm' | 37.1 ± 0.56 f | 9.3 ± 0.14 f | 4x[Y] | 76[Y] |

[Z]Values are means, n = 2 to 5 ± SEM. Values followed by a common letter, within a column, are not significantly different, P ≤ 0.05.
[Y]Confirmed through cytology.