Chromosome Karyotypes of Echinacea angustifolia var. angustifolia and E. purpurea

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Additional index words. medicinal plants, chromosome variation

Abstract. Chromosome karyotypes of the most commonly cultivated and medicinally used Echinacea taxa, E. angustifolia DC. var. angustifolia and E. purpurea (L.) Moench., were analyzed. The chromosomes of both taxa are medium in length, ranging from 4.12 to 5.83 µm in E. angustifolia var. angustifolia and 3.99 to 6.08 µm in E. purpurea. No abrupt length changes in the chromosomes were noted. The karyotypes of the two species are generally similar, but a distinguishable feature exists in each pair of chromosomes. The centromere of chromosome pair 10 is subterminally located in E. purpurea, but terminally located in E. angustifolia var. angustifolia, which can be readily recognized in mitotic metaphase cell plates. This finding may provide useful information for Echinacea evolutionary, genetic, and breeding studies.

Chromosome size and morphology may help indicate evolutionary relationships among plant species (Clark and Wall, 1996). In plant taxonomy, breeding, and genetic studies, information about chromosome karyotypes can be useful in species identification and analysis of hybrid populations. Native to North America, Echinacea species have an important place in herbal medicine. Echinacea has long been used by Native Americans to treat many conditions, including venous bites, rashes, cold, headache, and stomach cramps ( Foster, 1991; Kindscher, 1989; Li, 1998). Its non-selective, immune-enhancing properties have promoted the use of and demand for Echinacea products in recent years and, consequently, its cultivation has gained international attention and has been conducted in many countries, including the United States, Canada, Germany, Norway, Romania, Finland, Australia, Poland, Russia, New Zealand, Egypt, and China (Do et al., 2001; El-Gengaith et al., 1998; Finger et al., 1998; Li, 1998). Cultivation of Echinacea has gained international attention and has been conducted in many countries, including the United States, Canada, Germany, Norway, Romania, Finland, Australia, Poland, Russia, New Zealand, Egypt, and China (Do et al., 2001; El-Gengaith et al., 1998; Finger et al., 1998; Li, 1998).

Echinacea angustifolia var. angustifolia and E. purpurea are diploids with a somatic chromosome number of 2n = 22. Echinacea pallida is a tetraploid (2n = 44) and was suspected to be derived from chromosome doubling of a hybrid between E. simulata McGregor and E. sanguinea Nutt. ( McGregor, 1968). Chromosome karyotypes of Echinacea species have been reported to be quite similar ( McGregor, 1968); however, precise descriptions and visual presentation of karyotypes of these species have not been published. This research was to analyze the karyotypes of E. angustifolia var. angustifolia and E. purpurea.

Materials and Methods

Root tips from germinating seeds and young leaves from field-grown plants were used for chromosome preparation. Echinacea angustifolia var. angustifolia and E. purpurea seeds were provided by the North Central Regional Plant Introduction Station (NCRPIS), U.S. Dept. of Agriculture (USDA) ( Ames, Iowa), and purchased from Johnny’s Selected Seeds (Albion, Maine), Wind River Seed (Manson, Wy.,) and Richters (Goodwood, Ont., Canada) (Table 1). Seed germination was conducted in a growth chamber held at 25 °C. Seeds were soaked with deionized (DI) water in petri dishes on two layers of Whatman no. 1 paper for 30 min. Chromosome slides were prepared by macerating the tips on slides in carbol fuchsin stain ( Li and Zhang, 1991) and squashing under cover glass. Chromosome examination and photography were conducted using a microscope (Zeiss IM35) under 1000× magnification.

For each seed source, chromosome evaluation was conducted from at least five root tips (five seeds) and young leaves from two plants. Chromosome measurements were made on the enlarged prints and converted to micrometers by relating measurements made in the microscope with a micrometer. Karyotype analysis was based on at least seven high-quality metaphase cell plates.

Table 1. Seed sources and origins of E. angustifolia var. angustifolia and E. purpurea used in chromosome evaluation.

| Species        | Accession no. | Seed origin       | Provider          |
|----------------|---------------|-------------------|-------------------|
| E. angustifolia | Ames23930     | Iowa              | NCRPIS, USDA      |
| E. angustifolia | Ames24060     | Kansas            | NCRPIS, USDA      |
| E. angustifolia | 13370         | Wyoming           | Wind River Seed   |
| E. angustifolia | 16420         | Minnesota         | Johnny’s Selected Seeds |
| E. purpurea    | Ames23967     | North Carolina    | NCRPIS, USDA      |
| E. purpurea    | Ames25104     | Louisiana         | NCRPIS, USDA      |
| E. purpurea    | 52280         | Oregon            | Richters          |
| E. purpurea    | 12448         | Colorado          | Wind River Seed   |

North Central Regional Plant Introduction Station.
Results and Discussion

Typical mitotic metaphase cell plates are shown in Figure 1. As previously reported (McGregor, 1968), chromosome numbers of *E. angustifolia* var. *angustifolia* and *E. purpurea* are 2n = 22. Results of the karyotype analysis are presented in Figure 2 and Table 2.

In general, the chromosomes of both species are medium in length, ranging from 4.12 to 5.83 μm in *E. angustifolia* var. *angustifolia* and from 3.99 to 6.08 μm in *E. purpurea*. No abrupt length changes in the chromosomes were noted. Both species have four pairs of chromosomes with median-region centromeres and two pairs with submedian-region centromeres. The remaining five pairs have subterminal-region centromeres in *E. purpurea*. In *E. angustifolia* var. *angustifolia*, four of the five remaining pairs have subterminal-region centromeres, but chromosome pair 10 has its centromeres in the terminal region. Pair 10 can be readily recognized in *E. angustifolia* when compared with those of *E. purpurea*. The other pairs of chromosomes of the two species cannot be readily distinguished without detailed measurements. Within-species karyotypic variation was not observed in this study.

The readily distinguishable chromosome pair 10 in *E. angustifolia* might be used in hybrid identification in crosses of these two species. Although some quantitative differences in chromosome length and arm ratios exist in other pairs of the variety and the species, the overall similarity of the chromosomes and their subtle differences may make them unsuitable in species identification. Further research using chromosome banding (Tuna et al., 2001) or fluorescent dye staining (Schwarzacher et al., 1992) may help discriminate these pairs in more detail.

The plant materials used in this research represent four different growth locations (four states in the United States) for both taxa (Table 1), and no within-species karyotypic variation was observed. This may indicate that karyotypic differentiation, which is particularly prominent due to the presence of the pair of terminal-region centromere chromosomes in *E. angustifolia* var. *angustifolia*, is broadly true in the two taxa. It may be interesting to examine whether chromosome karyotype differences exist among other species in *Echinacea*.

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**Fig. 1.** *Echinacea* metaphase chromosomes prepared from root tips of germinating seeds: (a) early metaphase stage and (b) metaphase stage of *E. angustifolia* var. *angustifolia*; and (c) early metaphase stage and (d) metaphase stage of *E. purpurea*. Arrows indicate the chromosomes with a terminal centromere region. Bar = 10 μm.

**Fig. 2.** Chromosome karyogram of: (a) *E. angustifolia* var. *angustifolia* prepared from Fig. 1b; and (b) *E. purpurea* prepared from Fig. 1c. Bar = 10 μm.
Table 2. The chromosome (Chr.) karyotypes of *E. angustifolia* var. *angustifolia* (E.a) and *E. purpurea* (E.p).[^1]

| Chr. | Total length | Long arm | Short arm | Arm ratio[^y] | Chr. type[^x] |
|------|---------------|----------|-----------|---------------|--------------|
|      | E.a           | E.p      | E.a       | E.p           |              |
| I    | 5.83 ± 1.03   | 6.08 ± 1.02 | 3.36 ± 0.61 | 3.53 ± 0.59 | 2.47 ± 0.43 | 2.55 ± 0.42 | 1.36 ± 0.06 | 1.38 ± 0.07 | m m             |
| II   | 5.61 ± 0.98   | 5.61 ± 1.08 | 3.14 ± 0.56 | 3.27 ± 0.64 | 2.47 ± 0.42 | 2.33 ± 0.43 | 1.27 ± 0.05 | 1.40 ± 0.07 | m m             |
| III  | 4.93 ± 1.01   | 5.16 ± 0.96 | 3.81 ± 0.79 | 3.59 ± 0.69 | 1.12 ± 0.21 | 1.57 ± 0.29 | 3.40 ± 0.13 | 2.29 ± 0.09 | st sm          |
| IV   | 4.93 ± 0.89   | 5.15 ± 0.84 | 2.73 ± 0.69 | 2.80 ± 0.48 | 2.20 ± 0.21 | 2.35 ± 0.39 | 1.24 ± 0.08 | 1.19 ± 0.05 | m m             |
| V    | 4.89 ± 0.76   | 4.75 ± 0.91 | 3.62 ± 0.55 | 3.27 ± 0.61 | 1.27 ± 0.18 | 1.48 ± 0.28 | 2.85 ± 0.07 | 2.21 ± 0.06 | sm sm          |
| VI   | 4.84 ± 0.73   | 4.57 ± 0.82 | 3.45 ± 0.53 | 3.79 ± 0.69 | 1.39 ± 0.21 | 0.78 ± 0.14 | 2.48 ± 0.10 | 4.86 ± 0.08 | st m           |
| VII  | 4.82 ± 0.85   | 4.50 ± 0.89 | 2.53 ± 0.44 | 2.67 ± 0.54 | 2.29 ± 0.42 | 1.83 ± 0.34 | 1.10 ± 0.05 | 1.46 ± 0.10 | m m             |
| VIII | 4.53 ± 0.92   | 4.48 ± 0.76 | 3.81 ± 0.77 | 3.81 ± 0.63 | 0.72 ± 0.14 | 0.67 ± 0.12 | 5.31 ± 0.07 | 5.69 ± 0.06 | st st          |
| IX   | 4.31 ± 0.83   | 4.22 ± 1.02 | 3.32 ± 0.65 | 3.21 ± 0.74 | 0.99 ± 0.18 | 1.01 ± 0.27 | 3.36 ± 0.04 | 3.18 ± 0.05 | st st          |
| X    | 4.30 ± 0.79   | 4.12 ± 0.73 | 3.99 ± 0.74 | 3.25 ± 0.59 | 0.31 ± 0.06 | 0.87 ± 0.14 | 12.87 ± 0.12 | 3.73 ± 0.13 | t st           |
| XI   | 4.12 ± 0.81   | 3.99 ± 0.99 | 3.36 ± 0.67 | 3.00 ± 0.73 | 0.76 ± 0.16 | 0.99 ± 0.26 | 4.42 ± 0.08 | 3.03 ± 0.09 | st st          |

[^1]: Chromosome type nomenclature is based on Levan et al. (1964), and chromosome length is in mm.
[^y]: Arm ratio = length of the long arm : length of the short arm.
[^x]: m = median region centromere; sm = submedian region centromere; st = subterminal region centromere; t = terminal region centromere.

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