A Simple Chromosome Doubling Technique Is Effective for Three Species of Cupressaceae

Ryan N. Contreras1,2
Department of Horticulture, Oregon State University, 4017 Agricultural and Life Sciences Building, Corvallis, OR 97331-7304

Abstract. Platycladus orientalis (L.) Franco (syn. Thuja orientalis L.), Thuja occidentalis L., and T. plicata D. Don. are conifers often used in the landscape. Most of the available cultivars of these species share the character of having foliage that turns an off-color during winter as a result of photoinhibition. Tetruploids of the related Japanese-cedar [Cryptomeria japonica (L. f.) D. Don.] have exhibited greener color retention than diploids during winter and a recent report described a simple technique to double its chromosomes. The technique used to double the chromosome number of C. japonica was applied to the three species mentioned to determine if it would be effective for inducing polyploidy and, if so, optimal duration of treatment. Seedlings were treated at the cotyledon stage for 0 (control), 10, 20, or 30 days with an aqueous solution containing 150 μM oryzalin + 0.1% Tween 20 using a standard household spray bottle that created a fine mist. No tetraploids were observed for any species in control treatments, indicating all recovered tetraploids resulted from applying oryzalin. Tetruploids were observed for all other treatments except T. plicata at 30 days. Efficacy ranged from 0% to 27.1% of transplanted seedlings being tetraploid. There was a quadratic relationship between duration of treatment and percent tetraploids in T. occidentalis and T. plicata and a linear relationship for P. orientalis. Based on regression analysis, the optimal duration of treatment was 20.5 days for T. occidentalis and 13.9 days for T. plicata. The highest percent tetraploids recovered for P. orientalis was at 30 days and it is unclear if increasing duration beyond this would continue increasing percent tetraploids recovered. Morphology was not useful in early identification of tetraploids for any species.

Materials and Methods

Plant material. Seeds labeled as Platycladus orientalis ‘Compactus’, Thuja occidentalis, and T. plicata were received from Lawyer Nurseries, Inc. (Plains, MT). The seeds of oriental arborvitae labeled as ‘Compactus’ likely were collected from the cultivar Sieboldii, which has the synonym ‘Compactus’ (Krüssman, 1985). There is no evidence for a cultivar named ‘Compactus’. In this report I refer to the cultivar as Compacta as a result of its prevalence in the trade and its similarity to the labeled cultivar name. With the exception of controls, ~1000 seeds of each species were sown in germination trays containing 6 Douglas fir bark [Pseudotsuga menziesii (Mirbel) Franco]–3 peat:1 pumice (v/v) and germinated under laboratory conditions in humidity chambers (100% relative humidity) with constant light (32 μmol·m–2·s–1) supplied by cool-white fluorescent lamps at 20 °C. Controls of the three species were grown under the same conditions; however, only 100 seeds were sown.

Inducing polyploidy. Beginning at germination (cotyledon stage), seedlings were sprayed to runoff daily for 0 (control), 10, 20, or 30 d with an aqueous solution containing 150 μM oryzalin (supplied as Surflan® AS; United Phosphorus, Trenton, NJ) + 0.1% Tween 20 (Aeros Organics, Geel, Belgium) using a standard household spray bottle that created a fine mist. Each species was replicated once per treatment duration for a total of 12 trays. After each treatment the seedlings were moved to a glasshouse with day/night set temperatures of 27/20 °C. When seedlings were 4 or 5 cm, they were transplanted into 32-cell trays (T.O. Plastics, Clearwater, MN) containing 1 bark mix abg:1 SB40 patio mix (Sun Gro Horticulture, Bellevue, WA) and fertilized weekly with 100 ppm nitrogen with Jack’s Professional® 20-8.7-16.6 (J.R. Peters, Inc., Allentown, PA).

Ploidy analysis. Flow cytometry was used to screen all seedlings that survived treatments. Approximately 0.5 cm of leaf tissue was finely chopped in an extraction buffer (CyStain® Ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany) with a double-sided razor blade to extract nuclei. The nuclei suspension was passed through a 30-μm filter (Partec), nuclei were stained with 4,6-diamidino-2-phenylindole (CyStain® ultraviolet Precise P Staining Kit; Partec) and nuclei were analyzed using a CyFlow® Ploidy Analyzer (Partec). All samples were analyzed with an internal standard (Pisum sativum L. ‘Cirrad’; 2C = 8.76 pg) (Greilhuber et al., 2007) to correct for peak shifting and ensure correct interpretation of peak location.

Results and Discussion

The number of tetraploids induced in Platycladus orientalis, Thuja occidentalis, and T. plicata after treatment with oryzalin...
for 10, 20, or 30 d ranged from 0 to 107 (Table 1). No tetraploids were recovered in the control (0 d) treatment for any species, indicating that tetraploids observed in the study were the result of treatments and not reduced gametes or spontaneous chromosome doubling in embryonal initials (Khoshoo, 1959). Only diploids and tetraploids were recovered in the current study. In contrast, Contreras et al. (2010) observed 9.3% mixploids after 30 d treatment of Japanese-cedar. It is unclear why no mixploids were recovered in the current study.

Tetraploids were observed for all three species in each of the treatment durations except the 30-d treatment of western red cedar. There was a quadratic relationship between percent tetraploids recovered and treatment duration for Thuja plicata (y = -0.060x^2 + 2.47x - 0.86; R^2 = 0.96) and T. plicata (-0.032x^2 + 0.89x + 1.08; R^2 = 0.65) and a linear relationship for P. orientalis ‘Compacta’ (y = 0.22x - 0.3) (Fig. 1). Observed values of percent tetraploids for American arborvitae increased from 15.3% at 10 d, to 27.1% at 20 d, and declined to 16.3% at 30 d (Table 1). By solving for the derivative of the quadratic formula, the optimal treatment duration for American arborvitae was determined to be 20.5 d. Observed values for percent tetraploids in Oriental arborvitae increased from 1.5% at 10 d, to 3.8% at 20 d, and reached 6.4% at 30 d (Table 1). It remains to be seen if increasing treatment duration beyond 30 d will continue to increase the percent tetraploids recovered. Observed values for percent tetraploids of western red cedar were 10% at 10 d, 2.8% at 20 d, and 0% at 30 d (Table 1). By solving for the derivative of the quadratic formula, the optimal treatment duration for inducing tetraploidy in western red cedar was 13.9 d.

It was not possible to select tetraploids based on phenotype as can be done in pines, larch, Japanese-cedar, and Japanese cypress. A great deal of morphological variation among all seedlings including controls was observed, which likely contributed to the inability to select tetraploids based on phenotype as can be done in pines, larch, Japanese-cedar, and Japanese cypress. However, Kanezawa (1951) reported tetraploids of Japanese-cypress [Chamaecyparis obtusa (Sieb. & Zucc.) Endl.], a species with scale-like leaves, to have coarser branches that were more erect, broader leaves that were squatter than diploids, and leaves roughly twice as thick as diploids. An important distinction is that Kanezawa (1951) was reporting on trees at least 3 years old, whereas the current study focused on examining seedling morphology for altered phenotypes to reduce the number of seedlings to be screened using flow cytometry.

Contreras et al. (2010) previously reported on effectively using oryzalin to double the...
chromosomes of Japanese-cedar using the method described here. Applying oryzalin as a mist to seedlings of three conifer species for 10, 20, or 30 d resulted in the recovery a total of 308 tetraploids, demonstrating the efficacy of oryzalin using this treatment to induce tetraploidy among three species in two genera of Cupressaceae.

Literature Cited


