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Morphological and Cytological Characterization of Six Porterweed (*Stachytarpheta* spp.) Selections

S. Brooks Parrish[1a], Renjuan Qian[2], Sandra B. Wilson[3], and Zhanao Deng[1]

[1]University of Florida, Department of Environmental Horticulture, Gulf Coast Research and Education Center, 14625 County Road 672, Wimauma, FL 33598; [2]Zhejiang Institute of Subtropical Crops, 334 Xueshan Road, Wenzhou, Zhejiang 325005, China; [3]University of Florida, Department of Environmental Horticulture, P.O. Box 110675, Gainesville, FL 32611

brooks.parrish@ufl.edu

[a]First Place- Charlie Parkerson Graduate Student Research Paper Competition

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Summary

Porterweed (*Stachytarpheta* spp.), a member of the verbena family, is a common ornamental plant in warmer parts of the U.S. that is frequently used in pollinator gardens to attract many species of butterflies and hummingbirds. Much floral diversity exists within the genus and hybrid forms. This study was conducted to assess the growth habit, flowering, DNA content, and chromosome number of six porterweed selections to explore the relationship among species. Results identified three distinct porterweed growth habits (upright, semi-upright, and prostrate) and showed that nuclear DNA content ranged from 2.95 to 3.79 pg/2C. Chromosome counting revealed that all porterweed accessions tested were polyploid (tetraploid, pentaploid and hexaploid), with the exception of dwarf blue porterweed (*Stachytarpheta* spp) that was darkly stained chromosomes as they become organized in the metaphase stage of cell division. Subsequent cytological and morphological comparisons can be used to not only readily distinguish invasive and non-invasive forms of porterweed, but aid in future breeding programs.

INTRODUCTION

Porterweed (*Stachytarpheta* spp.) is an ornamental that is desired for its abundant brightly colored flowers that attract a diverse array of pollinators throughout much of the year. It is a drought tolerant, low maintenance plant commonly used in the southern United States as a perennial in warmer climates (USDA cold hardiness 9a), an annual in more temperate climates, or in container gardens. The *Stachytarpheta* genus is vast with 133 species identified in Australia (Munir, 1992) and 79 species classified in Brazil (Atkins, 2005). Seed is readily available to the public and can be found from many nurseries and online sellers.

In Florida, the most commonly sold porterweed species are jamaican porterweed (*Stachytarpheta jamaicensis),* nettleleaf porterweed (*Stachytarpheta cayennensis),* coral porterweed *(Stachytarpheta mutabilis),* purple porterweed (*Stachytarpheta frantzii*) and dwarf red porterweed (*Stachytarpheta sanguinea*). Jamaican porterweed is native to dunes, shell middens, pine rocklands, and disturbed sites of the central and southern Florida peninsula (Wunderlin and Hansen, 2020); whereas, nettleleaf porterweed was introduced to the United States from Central and South America and has escaped cultivation. While it has not yet altered native plant communities in Florida, nettleleaf porterweed is listed as a Category II invasive plant by the Florida Exotic Plant Pest Council (FLEPPC) due to its increased abundance or frequency (FLEPPC, 2019). The University of Florida Institute of Food and Agricultural Sciences Status Assessment of Non-native Plants recommends its "use with caution" (UF/IFAS Assessment, 2020). Hybridization potential between the native and invasive congeners is of concern.

In prior work, Wilson et al. (2009) evaluated seed production and viability of eight porterweed selections in Florida and found 'Violacea' (*S. mutabilis*), 'Naples Lilac' (*S. cayennensis* × *S. mutabilis* 'Violacea') and 'Mario Pollsa' (*Stachytarpheta* spp.) porterweed to be highly female sterile. Also in their study, through controlled manual crosses, the potential for *S. cayennensis* to hybridize with × *S. jamaicensis* was realized.

Chromosome number and ploidy level are important plant characteristics, and the latter is an important factor determining hybridization potential. Fedorov (1974) and Sanders (2001) reported that the porterweed genus has varying numbers of chromosomes from $2n = 18$ to $2n = 160$ and varying levels of ploidy. However, these reports did not publish chromosome images likely due to low quality resolution. The use of acids for cell wall degradation and stains such as crystal violet or acetocarmine for staining have been popular choices for chromosome counting, but sometimes lack clarity with certain samples (Dalgaard, 1986). When plant cells contain large numbers of chromosomes, it is especially important and critical to have effective chromosome squashing techniques that can make chromosomes well spread and produce clear images of chromosomes.

The purpose of this study was to characterize growth habits and cytological features of six porterweed selections. The main objective was to confirm the ploidy level of common porterweed cultivars by chromosome counting and understanding the relationship between chromosome number and

nuclear DNA content determined by flow cytometry. This information is critical for future porterweed plant breeding programs, and also for the identification of hybrids. Ornamental plant breeders could benefit greatly from a reliable chromosome squashing protocol that will produce high quality metaphase images.

MATERIALS AND METHODS

Plant materials. Six porterweed selections were evaluated in this study. Jamaican, coral, nettleleaf, and 'Naples Lilac' porterweed plants were obtained from a previous study conducted by Wilson et al. (2009). U*J 3-2 resulted from manual crossing of *S. cayennensis* and *S. jamaicensis* in a greenhouse located at the Gulf Coast Research and Education Center (Wimauma, FL). Dwarf blue porterweed plants were obtained from Grandiflora Nursery, Inc. (Gainesville, FL). Vegetatively propagated porterweed plants were grown in gallon plastic containers filled with Fafard 2P mix (Florida Potting Soil, Orlando, FL).

Growth Habit and Flowering. Fully mature flowering plants were used to assign categories of growth habit and flowering. Growth habit was identified as upright, semi-upright, or prostrate. Flower production was quantified as low and high, where high flowering plants had more than 60 florets on a single spike. Five replicates were accessed for each porterweed selection.

Determining nuclear DNA content. An Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) was used to determine nuclear DNA content. The flow cytometry protocol recommended by Doležel et al. (2007) was followed using rye [*Secale cereal* 'Daňkovské' (16.19 pg·$2C^{-1}$)] as the internal standard. Three flow cytometrical analyses were run for each porterweed selection, and a minimum of 3000 nuclei were counted per run. Nuclear DNA content (pg/2C) was calculated according to Doležel et al. (2007).

Squashing and counting chromosomes. The chromosome squash protocol was adapted from Chen et al. (1982). Before 10:00 AM, vigorously growing root tips (1cm) were excised from porterweed plants and treated in 0.002 M 8-hydroxyquinoline for 4 h in the dark. Root tips were immersed in a fixative solution (3 methanol: 1 acetic acid, v/v) for at least 2 h. The fixed roots were rinsed three times in deionized water before a much smaller section of the root tips (approximately 1 mm) was excised and macerated in an enzyme solution containing 2.5% cellulase and 2.5% pectinase for 3 h 15 min inside an incubator at 27 °C. Macerated root tips were washed in deionized water for 10 min and then fixed in a fixative for 0.5 h. Root tips were squashed in a drop of the fixative solution on a pre-chilled microscopic glass slide. The prepared slide was heated over an alcohol burner for a few seconds and stained with a 2.5% Giemsa solution (Sigma, St. Louis, MO) for 10 min. Stained glass slides were rinsed in distilled water, air-dried, and then observed at 1000× magnification under a BX41 microscope with an Olympus Q-color 5 camera (Olympus America Inc., Melville, NY).

RESULTS

Three growth habit categories were identified from the six porterweed selections. Coral and 'Naples Lilac' grew upright; nettleleaf and dwarf blue grew semi-upright, and jamaican and U*J3-2 porterweed grew prostrate (Fig. 1). Nettleleaf, U*J3-2, and jamaican porterweed recorded 65-70 flowers at the time of evaluation. Coral, 'Naples Lilac', and dwarf blue porterweed had much fewer flowers with only 10-30 flowers each. Based on plant growth rather than flower number, these results emphasize how the native jamaican porterweed can be phenotypically distinguished from the invasive nettleleaf porterweed.

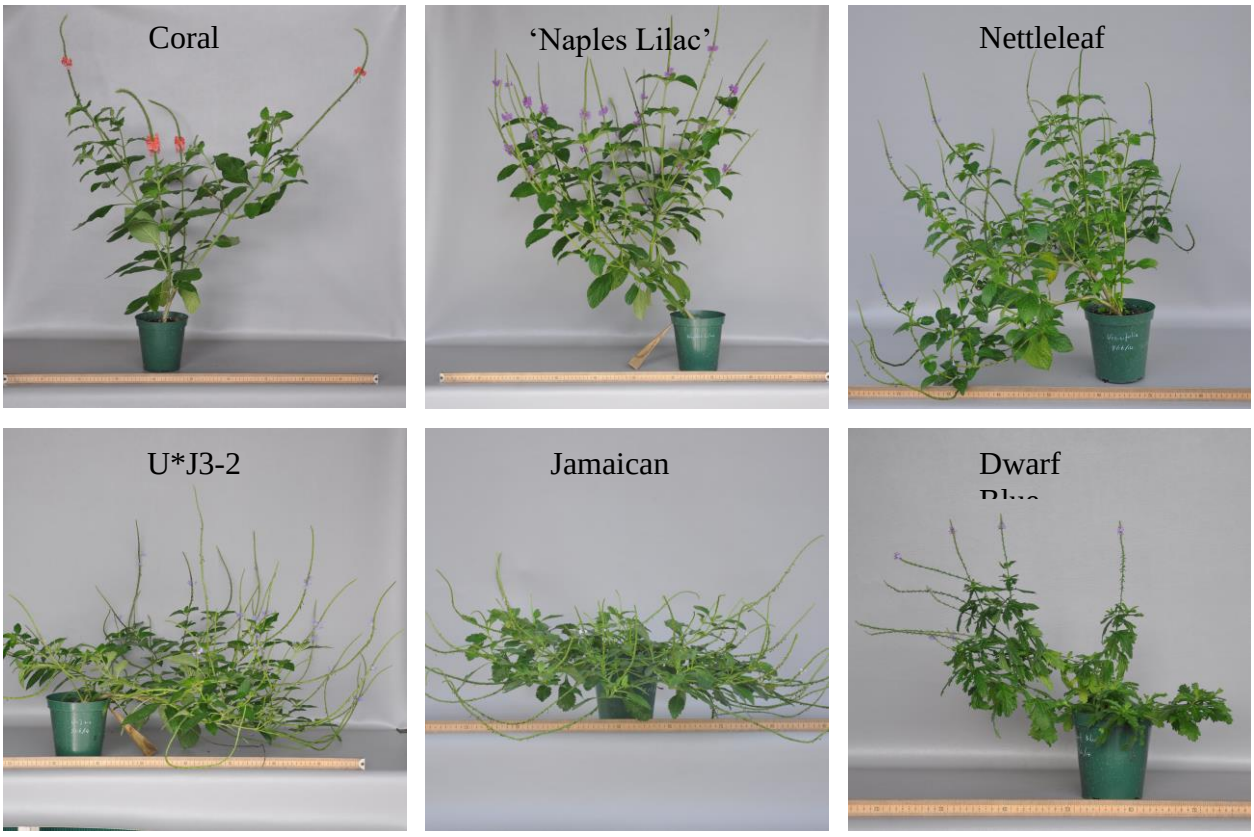

Figure 1. Images of each porterweed accession at the time of data collection. Plants were propagated at the same time and grown under the same conditions.

Nuclear DNA content of the selections had a range of 0.98 pg. At least 31 metaphases were observed for each accession which revealed four chromosome numbers (*2n* = 42, 112, 140, and 168) (Table 1). Chromosome analysis categorized accessions from diploids to hexaploids with two different base chromosome numbers (Table 1).

Table 1. Nuclear DNA content and chromosome number of six porterweed selections.

Taxa	Species	Nuclear DNA content ± SD (pg/2C)	Metaphases observed	Chromosome number (2n)	Base chromosome number	Ploidy level
Coral	*S. mutabilis*	3.66 ± 0.05	38	168	28	6x
Naples Lilac	*S. cayennensis* × *S. mutabilis* 'Violacea'	3.79 ± 0.04	41	168	28	6x
Nettleleaf	*S. cayennensis*	2.81 ± 0.03	36	112	28	4x
U*J 3-2	*S. cayennensis* × *S. jamaicensis*	3.28 ± 0.05	36	140	28	5x
Jamaican	*S. jamaicensis*	3.73 ± 0.09	31	168	28	6x
Dwarf Blue	*S.* spp.	2.95 ± 0.03	35	42	21	2x

The ploidy level of the native Jamaican porterweed was 6x, whereas the ploidy level of the invasive nettleleaf porterweed was 4x. Images of chromosomes of each accession can be clearly seen in Figure 2. Results from this study not only serve as a first report of chromosome numbers of specific porterweed cultivars; but present a successful new technique to study chromosomes in other ornamental species.

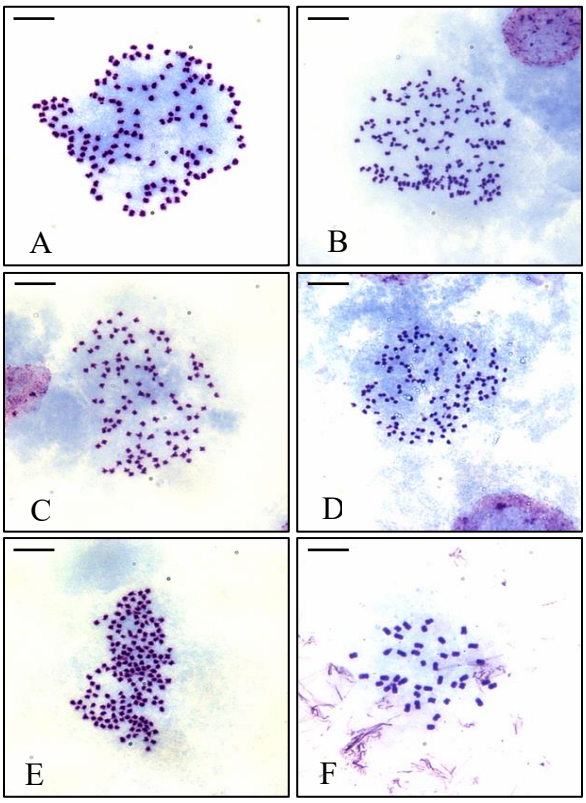

Figure 2. Micrographs (×1000) of somatic chromosomes observed in root tip cells stained in giemsa. A: coral porterweed (*2n* = 168), B: 'Naples Lilac' porterweed (*2n* = 168), C: nettleleaf porterweed (*2n* = 112), D: U*J3-2 porterweed(*2n* = 140), E: jamaican porterweed (*2n* = 168), and F: dwarf blue porterweed(*2n* = 42); scale bar = 10 µm.

DISCUSSION

All porterweed accessions evaluated in this study had purple to violet flower colors, with the exception of coral porterweed which had dark pinkish colored flowers (Fig. 3). With only small differences in flower color, the growth habit of each porterweed selection proved to be a feature that could aid in their identification. It is of interest to note that U*J3-2 took on the prostrate growth habit of its male parent (jamaican porterweed) but was considerably wider than both parents. A similar segregation of traits was observed in a hybridization of *S. angustifolia* × *S. cayennensis* produced by Solanke et al. (2019).

Flow cytometry yielded sharp peaks for both the internal standard and each of our samples. Standard deviation (SD) values for the mean nuclear DNA content were ≤ 0.09 pg (Table 1). Nuclear DNA content for the accessions ranged from 2.81 pg/2C in nettleleaf porterweed to 3.79 pg/2C in 'Naples Lilac'. This is of interest as 'Naples Lilac' is reported to be a cross between the invasive nettleleaf porterweed and the non-invasive 'Violacea' porterweed (*Stachytarpheta mutabilis*) (Kastenholz, personal communication).

A total of 217 cells in the metaphase stage of cell division were observed, photographed, and counted to determine the somatic chromosome number for all six selections. The stained cells produced had exceptional clarity that allowed the precise counting of the many small chromosomes. Surprisingly, dwarf blue porterweed recorded a higher average nuclear DNA content than nettleleaf porterweed, but had less than half the number of chromosomes, differing by 70 chromosomes. The identification of polyploids will be essential for breeders to use in generating non-invasive sterile cultivars. Sterile cultivars of *Lantana camara* have successfully been developed by the crossing of tetraploid and triploid cultivars (Czarnecki et al., 2014). Wilson et al. (2009) identified 'J.P's Pink' (*S. speciosa*) and 'Red Compact' (*S. speciosa*) porterweed as diploid species, making them potential candidates for crosses with now identified tetraploid nettleleaf porterweed. Furthermore, confirming polyploids through chromosome staining has laid the groundwork for determining the ploidy of other porterweed species with flow cytometry.

DNA content and chromosome counts presented for the six porterweed selections will undoubtably pave the way for the production of sterile porterweed cultivars as safe alternatives to the invasive form. The chromosome squashing protocol identified within has high potential for adaptation to other plant species. The technique requires few resources and can be completed in just two days yielding chromosome spreads with high clarity. Stained cells produced could further be used in chromosome measurements and forming karyotypes for plant species. Ornamental plant breeding programs often lack access to expensive genomic analysis. The use of this high throughput chromosome visualization protocol could be a major leap in the development of new ornamental hybrids.

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