A Monogenic Recessive Gene, *fw*, Conditions Flower Doubling in *Nicotiana glauca*

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Abstract. A double-flower form of *Nicotiana glauca* Link & Otto was characterized genetically as a monogenic recessive trait expressed when homozygous. Reciprocal crosses demonstrated no maternal effect on expression of double flowers. A single dominant gene expressed in the homozygous or heterozygous state caused the single-flower phenotype. The symbol *fw* is proposed to describe the gene controlling double-flower phenotype.

Nicotiana glauca Link & Otto (Solanaceae), known as 'Jasmine tobacco', is a flowering ornamental tobacco native to tropical north-eastern Argentina and southern Brazil (Griffiths, 1994). Plants average 1.5 m in height with sticky, hairy stalks, ovate to lanceolate leaves, and 5- to 8-cm long trumpet-shaped flowers with pale purple or white interiors, and yellowish exteriors (Graft, 1980; Kohlein and Menzel, 1994). Crosses of *N. glauca* × *N. forgetiana* Sander ex Hemsl. produce hybrids with white, greenish, pink, and red flowers (Kohlein and Menzel, 1994) commonly used as bedding plants.

A white, double-flowered form of *N. glauca* was found in an F₂ population of single, white flowering plants (Fig. 1). Anatomical analysis of flowers revealed petal-like outgrowths arising from the anther, connective and filament tissues (Zainol et al., 1998). Petal-like outgrowths can create unique flower types to enhance desirable floral qualities for ornamental use.

Occurrence of flower-doubling has been reported in several solanaceous species, including *N. langsdorffii* J.A. Weinm. hybrids (White, 1914), *N. tabacum* L. (Hitier, 1950; Komari, 1990) and *Petunia ×hybrida* Hort. (Saunders, 1910; Scott, 1937). In *N. tabacum* and *P. ×hybrida*, flower doubling has been reported to be conditioned by single genes, either recessive (Hitier, 1950; Saunders, 1910) or dominant (Hitier, 1950; Ntarella and Sink 1971; Scott, 1937; Sink, 1973). Herein, we report on the inheritance of the double-flowering phenotype in *N. glauca*.

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were categorized as normal and double flowers as possessing petal-like outgrowths on anthers and/or filaments (Table 1). Evaluation was conducted in Sept. 1994 (fall) and repeated with a different subsample of seed from the original cross populations in Jan. 1995 (winter). Data were subjected to chi-square analysis using Yates correction term for small populations (Strickberger, 1976).

Results

Self-pollinated NA produced all double-flowered progeny and self-pollinated single-flowered DS and ML produced all single-flowered progeny (Table 1). Reciprocal matings of DS or ML × NA produced single-flowered progeny. The F₂ generations, derived from DS or ML × NA, fit a 3 single : 1 double segregation ratio when grown in the fall (Table 1) and were close to a 3:1 ratio when grown in winter. Progeny from backcrosses of the F₁ generations to NA (BC_{P1}) segregated 1:1 in the fall, but one backcross failed to fit the expected 1:1 segregation in the winter. F₁ plants backcrossed to ML (BC_{P2}) produced single-flowers in both seasons. Intercrosses of F₁ plants (double cross) fit 3:1 segregations except when ML was present in both parents of the intercross grown in winter. Self-pollinations of single- and double-flowered selections derived from multiple cross matings fit 1:1 or 0:1 segregations (Table 2). Backcrosses of single-flowered selections to NA fit 1:1 segregations (Table 2).

Generally, plants exhibited reduced expression of flower-doubling in the winter. Also, expressivity varied between lines but was similar within a line (data not collected). Additionally, a degree of doubling stronger than the parent was observed in some lines among the first generation plants of self-pollinated NA. Flowers on these plants were

Materials and Methods

A double-flowered mutant of *N. glauca* 'White' (NA) was discovered in an F₂ population growing in a research test plot at the Univ. of Wisconsin, Madison (Fig. 1). The double-flowered NA was self-pollinated and outcrossed with single-flowered *N. glauca* 'Domino Salmon' (DS) and 'Metro Lime' (ML) using standard hybridization methods for *Nicotiana*. F₁ plants and both F₂ progeny and reciprocal backcrossed (BC) progeny were produced. Additional crosses were made to facilitate further determinations of inheritance (Tables 1 and 2).

Seeds were germinated and plants grown according to established procedures (Ball, 1998). Plants were arranged in a completely random design, and scored phenotypically for single or double flowers. Single flowers

Fig. 1. Double-flowering phenotype of *Nicotiana glauca* 'White'.

Table 1. Segregation for double-flower form in matings of *N. alata* Link & Otto.

Parents/Generation	Test ratio ^z	Fall 1994 Flower form ^y				Winter 1995 Flower form			
		—	+	χ^2	P	—	+	χ^2	P
<i>Parents self-pollinated</i>									
NA	0:1	0	27	All double	0	30	All single		
DS	1:0	13	0	All single	13	0	All single		
ML	1:0	27	0	All single	31	0	All single		
<i>F₁, F₂, and BC</i>									
NA (P1) x DS (P2)									
F ₁	1:0	29	0	All single	30	0	All single		
F ₂	3:1	19	6	0.07 0.82	25	6	0.39 0.55		
BCP1	1:1	12	15	0.46 0.50	8	22	6.57 0.01		
BCP2	1:0	No seed	---	---	No seed	---	---		
<i>NA (P1) x ML (P2)</i>									
F ₁	1:0	28	0	All single	34	0	All single		
F ₂	3:1	21	7	0.05 0.85	20	10	1.12 0.29		
BCP1	1:1	13	13	0.04 0.87	19	16	0.31 0.59		
BCP2	1:0	28	0	All single	30	0	All single		
<i>Reciprocal</i>									
DS x NA	1:0	No seed	---	---	No seed	---	---		
ML x NA	1:0	28	0	All single	31	0	All single		
<i>Double cross</i>									
(NA x DS) x (NA x DS)	3:1	20	7	0.14 0.71	22	5	0.90 0.36		
(NA x ML) x (NA x ML)	3:1	21	7	0.05 0.85	29	3	5.57 0.03		
(NA x DS) x (NA x ML)	3:1	22	8	0.03 0.88	21	7	0.05 0.85		

^zNA, DS, and ML = *N. alata* 'White' double-flowered, 'Domino Salmon' single-flowered, and 'Metro Lime' single-flowered, respectively.

^ySingle (–) or double (+) flower form.

Table 2. Segregation for double-flower form in *N. alata* Link & Otto after various matings of single- and double-flowered selections in Spring 1996.

Parent ^z	Flower form ^y		Test ratio		
	–	+	–:+	χ^2	P
<i>S₁ of single selections</i>					
NA x [NA x (NA x DS)]	25	26	1:1	0.02	0.89
NA x [NA x (NA x ML)]	21	25	1:1	0.35	0.76
<i>S₁ of double selections</i>					
NA "least" double	0	32	0:1	All double	
NA "most" double	No seed				
NA x DS	0	14	0:1	All double	
NA x ML	No seed				
NA x (NA x DS)	0	46	0:1	All double	
NA x (NA x ML)	0	50	0:1	All double	
(NA x DS) x (NA x DS)	0	50	0:1	All double	
(NA x ML) x (NA x ML)	0	11	0:1	All double	
[(NA x DS) x (NA x DS)] x [(NA x ML) x (NA x ML)]	0	51	0:1	All double	
<i>Single selections BC₁ to double selections</i>					
NA x [NA x (NA x DS)]	25	26	1:1	0.02	0.89
NA x [NA x (NA x ML)]	21	25	1:1	0.35	0.56

^zNA, DS, and ML = *N. alata* 'White' double-flowered, 'Domino Salmon' single-flowered, and 'Metro Lime' single-flowered, respectively.

^ySingle (–) or double (+) flower form.

deformed slightly, nearly cup-shaped, and yielded no seed because of poor pollen production.

Flowering plants with white, red, salmon, green, or bicolor double corollas were identified among the populations. All color forms expressed strong degrees of doubling.

Discussion

A nuclear recessive gene conditions the double-flowering phenotype of *N. alata*. The dominant allele conditioned single flowers in either the homozygous or heterozygous state. Segregation data obtained from F₁, F₂, back-cross families, and intercrosses between F₁

populations largely support the genetic model proposed (Table 1). The failure of two crosses [BC₁ DS and (NA x ML) x (NA x ML)] to fit expected segregation ratios was probably the result of sampling errors or small population size. We do not believe environmental variables caused significant deviation from expected ratios, since plants evaluated from other crosses fit expected segregation ratios under similar environmental conditions. We propose the symbol *fw* for the gene controlling the double-flower phenotype in *N. alata*.

In Solanaceae, flower-doubling has been reported in *N. langsdorffii* (White, 1914), *N. tabacum* (Hitier, 1950; Komari, 1990) and *P. ×hybrida* (Saunders, 1910; Scott, 1937).

Petal-like outgrowths developed from the filament in progeny of self-pollinated *N. langsdorffii grandiflora* Cav. and in the F₂ generation of *N. langsdorffii* x *N. forgetiana* (White, 1914). Flower doubling in the F₂ generation of *N. langsdorffii* x *N. alata* segregated 35 normal : 15 abnormal (White, 1914). Crosses in our studies of double-flowered by single-flowered plants produced F₁ progeny of either intermediate doubling or normal flower characteristics. In *N. tabacum* 'Corolle Double X', flower doubling was controlled by a recessive gene (Hitier, 1950). The origin of this plant remains unknown, but it is believed to have resulted from a cross with *N. tabacum* 'Java Iser're'. A double-flower form resulting from transformation of *N. tabacum* 'Xanthi' NC was characterized as being controlled by a dominant gene *Df*, homozygous *Df/Df* or heterozygous *Df/+*.

Flower doubling in *Petunia ×hybrida grandiflora* was originally determined to be controlled by recessive genes (Saunders, 1910). Later, *P. ×hybrida* flower doubling was attributed to a single dominant gene *D* (Natarella and Sink 1971; Scott, 1937; Sink, 1973).

In contrast, double flowering response of *N. alata* in the present study was controlled by a monogenic recessive character *fw*. Petal-like outgrowths originated from the androecium, leaving the gynoecium unaffected (Zainol et al., 1998). Also, self-pollinated NA produced progeny with varying degrees of flower doubling originating from the androecium (data not presented), suggesting that minor gene action may be involved in double-flower expression. However, male sterility prevented further investigation into plants with high expression of doubling.

Reynolds and Tampion (1983), reported that plants that produced double flowers were both male- and female-sterile. They stated that when petaloid development from stamens was strong, anthers failed to develop and function normally. Also, the carpel may be affected if petaloidy occurs in the center of the flower as in sterile double-flowering *P. ×hybrida* (Natarella and Sink, 1971). In *N. alata* (Zainol, et al., 1998), it was noted that, as degree of flower doubling increased, the anthers became more deformed and led to increased sterility. In addition, pollen from these plants was largely nonfunctional.

Observed seasonal differences in expressivity of flower doubling suggest that environmental variables influence gene expression, as reported by others (Garrod and Harris, 1974; Kohl, 1961). Transferring the *fw* gene to lines with other flower colors could increase the ornamental value of *N. alata*. However, challenges to commercial application may exist in the form of monogenic recessive inheritance and sterility factors.

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