Inheritance and Phenocopy of a Red Beet Gibberellic Acid-deficient Dwarf Mutant

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ABSTRACT. A severe dwarf mutant affecting vegetative and reproductive growth arose spontaneously in our red beet (*Beta vulgaris* L. subsp. *vulgaris*) breeding nursery and was used in crosses with inbred lines to characterize its inheritance. Segregation data in backcross and F₂ generations were collected. Chi-square goodness-of-fit tests did not deviate significantly from the expected ratios for a monogenic character for each genetic background-generation combination. We propose the symbol *dw* to describe the genetic control of this dwarf phenotype. Greenhouse experiments were conducted to determine whether the mutant was sensitive to exogenous application of gibberellic acid (GA). GA₃ and GA_{4/7} in concentrations of 0 to 1000 ppm were applied to apical meristems during flower stem development in vernalized dwarf plants. Data on flower stem length and leaf length were collected over a 6-week period during reproductive growth. Recovery of wild-type flower stem length was obtained with application of both types of GA. A 30-fold increase in flower stem length over untreated plants was accomplished by GA application. Results of these phenocopy experiments suggest the mutant gene is involved in GA synthesis.

Red beet (*Beta vulgaris* subsp. *vulgaris*) is an important vegetable crop in eastern and central Europe and parts of Asia. The red beet was originally selected for its use as a leaf vegetable in the Mediterranean region and then later for use as a fresh or stored root crop (Campbell, 1976). In the 18th century, the use of beet root was expanded to include animal feed. The fodder beet became an important component of European agriculture by the 19th century and was the progenitor of the sugar beet (Pink, 1993).

Although substantial breeding efforts have been conducted with sugar beet to improve sucrose content and agronomic performance, few such efforts have been conducted with red beet. Several genes from sugar beet have been transferred to red beet in an effort to enhance breeding efficiency (Goldman, 1996); however, very few genes have been identified in red beet. The only two classes of genes studied to date are those conditioning the kinds and amounts of betalain pigment in the beet plant (Keller, 1936; Wolyn and Gabelman, 1989) and those responsible for male sterility (Bliss and Gabelman, 1965). Only two loci conditioning pigment shade and distribution and two loci governing male sterility have been described. Although these two classes of genes have been of great importance in beet breeding, additional markers are needed for genetic study and to gain a better understanding of the red beet genome.

Dwarfing genes have made important contributions to agriculture via the development of shortened-stature varieties of many cereal grains (Borlaug, 1983). The extensive use of these genes in plant breeding programs has led to improved cultivars and a better understanding of plant growth and development. In this paper we report our efforts to characterize the inheritance of a severe dwarf phenotype in red beet. Experiments were also conducted to assess the effect of exogenous gibberellic acid (GA) application on flower stem and leaf length during reproductive growth in mutant plants.

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Materials and Methods

Genetic material

The dwarf mutant arose spontaneously in a segregating population derived from a cross of W365B x W399B. The mutant was detected in the red beet breeding nursery at Madison, Wis. Dwarf plants were harvested, vernalized, and used as pollinators, with the sterile inbred line W336A as a female. Matings were also performed using the dwarf as a female parent with the F₁ plant (W429A x W364B) and as a male and female parent with the F₁ plants (W429A x W357B). The sterile 'A' lines used in the above crosses were of the genetic constitution *Sxxzz*. The 'B' lines were maintainer lines of the genetic constitution *Nxxzz*. The *X* and *Z* alleles are nuclear and condition fertility restoration in sterile cytoplasm. All F₁ plants were sterile. The dwarf was detected in self-compatible breeding material in *N* cytoplasm and was therefore self-fertile. Plants from these five matings were grown in the breeding nursery, harvested, vernalized, and used as female parents in backcrosses with the dwarf mutant or self-pollinated. BC₁ or F₂ plants were grown in seedling trays in the greenhouse and were evaluated phenotypically 3 weeks after planting. Segregation data were subjected to chi-square analysis.

Phenocopy experiments

EXPERIMENT 1. Beet plants expressing the dwarf phenotype were vernalized for 8 weeks and planted in clay pots in the greenhouse. Pots were arranged in a randomized complete-block design with two replications. Three plants comprised each replicate. Daylength was maintained at 16 h and temperature was maintained at 26 ± 3 °C. Ten milliliters of GA_{4/7} at concentrations of 0, 1, 5, 25, 50, 100, 250, and 1000 ppm was applied to the apical meristem of flower stems of dwarf plants during reproductive development. Two applications of GA_{4/7} were made 7 d apart. Height of the flower stem from the stem-hypocotyl junction to the apical meristem and length of the longest leaf were recorded at 6 weekly intervals 1 week after the second application. Length of the longest leaf was assessed on the same leaf from each plant throughout a 6-week period.

EXPERIMENT 2. Plants expressing the dwarf phenotype were

Table 1. Goodness of fit of the observed BC₁ and F₂ segregation data from three crosses to a monogenic model for the inheritance of the dwarfing trait in red beet.

Cross	Generation	Tall	Dwarf	Expected ratio	Expected progeny count	χ^2	P ^z
W336A x Dwarf	BC ₁	1436	1447	1:1	1442:1442	0.042	>0.90
(W429A x W357B) x Dwarf	F ₂	66	21	3:1	65:22	0.06	>0.75
Dwarf x (W429A x W357B)	F ₂	174	55	3:1	172:57	0.09	>0.75
Dwarf x (W429A x W364B)	F ₂	242	87	3:1	247:82	0.41	>0.50

^zProbability of a larger χ^2 .

vernalized and planted in clay pots in the greenhouse. Pots were arranged on the greenhouse bench in a completely randomized design with four replications under the same light and temperature regimes described above. To evaluate the comparative effect of other forms and quantities of GA, application of 1 mL GA₃ at concentrations of 0, 25, 50, 100, and 1000 ppm to the apical meristem of the flower stem were made on alternate days for a 6-week period. Length of the flower stem after 6 weeks of treatment was recorded as described above.

Results and Discussion

Chi-square goodness-of-fit tests revealed the data closely fit expected ratios for a monogenic character for each genetic background-generation combination (Table 1). No reciprocal effects were detected for the dwarf phenotype. Data from BC₁ and F₂ generations did not deviate significantly from the expectation that the dwarf phenotype is conditioned by a single recessive gene. We propose the symbol *dw* to describe the genetic control of this dwarf phenotype.

Many dwarf mutants described in other plant species are deficient in GA. Gibberellic acid is a plant hormone primarily responsible for cell elongation. Deficiencies of this hormone often result in a collapsed phenotype with shorter leaf internodes. The severe dwarf phenotype studied in this investigation is characterized by a rosette appearance with very short internodes and a dramatically shortened flower stem. Control *dw dw* plants (not treated with gibberellic acid) exhibited mature flower stem heights of only 2.25 cm, whereas *DWDW* plants routinely exhibited flower stem heights of 100 cm under identical conditions. The rosette of leaves in the dwarf plant are thick and waxy and display slower vegetative and reproductive growth relative to wild-type plants. In addition, the swollen hypocotyl and root characteristic of the red beet plant is greatly reduced, typically not exceeding 2 cm in diameter for field-grown plants. The *dw* gene clearly reduces leaf,

Fig. 1. Effect of GA₄₇ treatment at various concentrations on flower stem length in greenhouse-grown dwarf beet plants measured at weekly intervals. Two GA₄₇ treatments were applied 14 and 7 d before the first measurement (week 3). Bars indicate SE.

Table 2. Mean flower stem height in greenhouse-grown dwarf red beet plants at 6 weeks posttreatment following exogenous 1-mL GA₃ application at various concentrations.

GA ₃ concn (ppm)	Flower stem ht (cm)	SD
0	2.3	0.7
25	4.3	2.2
50	7.8	2.7
200	10.6	3.1
1000	11.6	4.1

hypocotyl, and root elongation, in addition to affecting leaf morphology. Pleiotropic effects of the *dw* gene on leaf characteristics suggest a role for this gene in leaf development.

This is the first report of a dwarfing gene in red beet. A plethora of dwarf mutants in rice (*Oryza sativa* L.) has been identified, characterized, and grouped into various classes by Mitsunaga et al. (1994). Waycott and Taiz (1991) evaluated gibberellic acid sensitivity of four dwarf mutants of lettuce (*Lactuca sativa* L.) and determined variable elongation response among these genes at low concentrations of GA₃. Although most dwarf mutants lack gibberellins, some workers have identified dwarf mutants that do not respond to exogenous application of gibberellic acid. Gibberellin-nonresponsive maize (*Zea mays* L.) dwarf mutants such as Dwarf-8 and Dwarf-9 are conditioned by dominant alleles (Winkler and Freeling, 1994), whereas most gibberellic-sensitive dwarf mutants are recessive in nature. Zanewich et al. (1991) identified and classified various *Brassica napus* and *Brassica rapa* mutants by

gibberellin sensitivity. A GA₃-sensitive Brassica mutant was identified by Rood et al. (1989). This mutant phenotype is conditioned by the *ros* allele, which reduces endogenous GA production yielding a rosette phenotype with delayed germination, flowering, and shoot growth; characteristics similar to the dwarf phenotype described here.

Linear growth responses to exogenous GA_{4/7} application were observed in all experiments (Fig. 1). Plants treated with 1000 ppm gibberellic acid were >11-fold longer than plants in control treatments (no GA_{4/7}) by 4 weeks posttreatment and a clear dose-response curve was indicated for intermediate concentrations. Longest leaves in gibberellic acid-treated plants were more than 3-fold longer than plants in control treatments by 4 weeks posttreatment (Fig. 2), indicating GA_{4/7} affects flower stem and leaf length in a similar manner. Application of GA₃ increased flower stem length 6-fold compared to control treatments (Table 2). Flower stem lengths in 1000 ppm GA treated plants were similar to wild-type plants at the same developmental stage, which suggests that restoration of the wild type phenotype can be accomplished with gibberellic acid application. Flower stems of wild-type plants typically reach 100 cm in length during the 3-month flowering period in the greenhouse. These lengths can be reached in *dw dw* plants following continuous gibberellic acid application throughout the growing season (data not shown). Indeed, two 10-mL applications of 1000 ppm GA_{4/7} increased flower stem length to

Fig. 2. Effect of GA_{4/7} treatment at various concentrations on length of longest leaf in greenhouse-grown dwarf beet plants measured at weekly intervals. Two GA_{4/7} treatments were applied 14 and 7 d before the first measurement (week 3). Bars indicate SE.

nearly 70 cm (Fig. 1). The 1-mL applications of 1000 ppm GA₃ were successful at inducing a similar 6-fold increase over non-treated plants; however, flower stem lengths were substantially less than in GA_{4/7} treated plants.

These data demonstrate the effects of the *dw* gene can be mediated by regular application of GA to meristematic tissue. Red beet plants are typically induced to flower in the greenhouse by planting vernalized roots in pots. Pollination is accomplished by placing pollen-shedding flower stems together in paper bags. Because flower stems of similar lengths are necessary for complete pollination, modification of flower stem length in *dwdw* plants can be easily accomplished with gibberellic acid application, and the *dw* gene can be introgressed into other backgrounds or used in crosses with other genetic stocks.

Gibberellins, which can in some circumstances substitute for the vernalization requirement of plants, often promote flowering when applied exogenously in experiments such as those described here. In Expts. 1 and 2, vernalized plants were studied and, thus, reproductive growth was expected. Parallel studies were conducted to determine the effect of exogenous gibberellic acid application on plant height and leaf morphology in *dwdw* seedlings (i.e., in a nonreproductive state). Regular application of GA₃ increased plant height and leaf length in a predictable manner (data not shown), suggesting that vegetative and reproductive phases are similarly affected by exogenous gibberellic acid. In contrast, Rabinowitch and Peters (1991) identified a dwarf scape (flowering stem) mutant in onion (*Allium cepa*) that was sensitive to application of GA₃. No effect of the dwarf scape mutant was detected during vegetative growth, thereby indicating distinct control of these two developmental phases. Application of GA₃ to *dwdw* plants in their vegetative phase also readily promoted flowering, suggesting a potential mechanism for circumventing the vernalization requirement of red beet. Confirmation of this speculation would require additional investigation in a range of genetic backgrounds and environmental conditions.

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