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Glossy and Nonglossy Near-isogenic Lines USVL115-GL, USVL115-NG, USVL188-GL, and USVL188-NG of Broccoli

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Two pairs of near-isogenic lines of green-sprouting broccoli (*Brassica oleracea* L. var. *italica*), designated USVL115-GL and USVL115-NG, and USVL188-GL and USVL188-NG, were released by the Agricultural Research Service of the U.S. Department of Agriculture in 2008. Each of the released pairs (doubled haploids) includes two uniform and true-breeding broccoli lines that appear identical to one another except that one has normal, nonglossy leaves and the other has glossy leaves. Using the two pairs of broccoli lines that only differ for the glossy leaf trait, plant scientists can better study the effect of the trait on other plant attributes like resistance to lepidopterous caterpillar predation.

Origin

The broccoli breeding program at the U.S. Vegetable Laboratory (Charleston, SC) has developed a large number of doubled haploid (DH) lines of broccoli using anther culture. Typically, the lines that result from these anther cultures are highly uniform and can be presumed to be highly homozygous. In relatively rare instances in our program, lines arising from culture exhibit variants that are not observed when first regenerated (R_0 generation) but become evident in the first generation (R_1) from seed (previously unpublished). The most common variant observed in these DH lines is a plant with a glossy leaf type, which occurs at a frequency less than 0.5% when examining individual DH lines. By selecting and selfing glossy leaf mutants and nonglossy (normal or wild-type) plants from the same line, it has been possible to develop paired lines that appear identical except for the glossy leaf trait (Fig. 1). DNA marker analysis has verified that the paired lines can be considered near-isogenic for the glossy leaf trait. All cases observed in our program indicate the glossy leaf trait we observe is controlled by a single recessive gene. For example, all F_2 populations resulting from crosses of glossy by nonglossy lines always segregate ≈3:1 nonglossy to glossy. Tests have not been conducted to determine if the different glossy variants are the result of a change at the same locus or at different loci.

USVL115-GL and USVL115-NG are paired lines that originated as single glossy and nonglossy plants in the first seed generation of a DH line derived from anther cultures of the commercial hybrid 'Marathon' (Sakata Seed Inc., Morgan Hill, CA). The original pair of glossy/nonglossy selections was moved to pots in a greenhouse and each individual of the pair was self-pollinated to produce separate lots of seed (Fig. 2). The parental hybrid 'Marathon' is heterozygous and produces a segregating population when self-pollinated. Conversely, USVL115-GL and USVL115-NG lines are highly homozygous and phenotypically homogeneous as expected with DHs.

USVL188-GL and USVL188-NG are paired lines that originated as single glossy and nonglossy plants in the first seed generation of a DH line derived from anther cultures of an F_1 hybrid formed by crossing a 'Marathon'-derived DH line with an 'Everest' (Syngenta, Gilroy, CA)-derived DH line (Fig. 2). As described previously in this article, the original selections were moved to pots in a greenhouse and each individual was self-pollinated to produce separate lots of seed. Again, as expected for DHs, USVL188-GL and USVL188-NG exhibit a high level of homozygosity and homogeneity.

Description

USVL115-GL and USVL115-NG are partially self-compatible and will produce limited quantities of seed without manual pollinations; however, to maximize seed production of these lines, it is recommended to conduct manual self-pollinations (e.g., bud

Fig. 1. **(A)** USVL115-NG (left) and USVL115-GL (right) growing side by side in field plots at the U.S. Vegetable Laboratory, Charleston, SC. **(B)** USVL188-GL (left) and USVL188-NG (right) growing side by side in pots in a greenhouse.

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pollinations) that are standard for producing *Brassica* seed. USVL188-GL and USVL188-NG have a higher level of self-incompatibility than the other pair but will still produce a small amount of seed without being manually pollinated. Seed production from these lines can also be maximized by conducting manual bud self-pollinations.

When comparing phenotypic characteristics of the two lines that make up each pair, they are essentially identical except for the glossy leaf trait. The most distinguishing appearance of the glossy line of each pair is a shiny leaf that is deep green in color (Fig. 1). On the contrary, the nonglossy line has a more normal or wild-type broccoli leaf that is dull in appearance, rather than shiny, and has a blue–green color (Fig. 1). USVL115-GL and USVL115-NG are among the latest maturing lines grown in the U.S. Vegetable Laboratory broccoli program, producing market-sized (e.g., 10 to 12 cm diameter) broccoli heads anywhere from 80 to 100 d after transplanting in a fall (September through December) environment (Table 1). This maturity is very similar to the important commercial broccoli hybrids 'Marathon' and 'Legacy' (Seminis Vegetable Seeds, Saticoy, CA). USVL115-GL and USVL115-NG produce only marginal quality broccoli heads compared with commercial checks (Table 1). The mass of typical heads produced by these two lines is relatively small with heads cut to a 16-cm length usually weighing ≈160 to 170 g (Table 1). On average, this is ≈55% to 70% of the head weight for 'Marathon' or 'Legacy' grown in the same trials. Other head traits of USVL115-GL and USVL115-NG include a slight to moderate dome shape, intermediate bead size (1.50 to 1.80 mm), and a stem diameter of 23 to 31 mm. These two lines also have intermediate vigor and a high degree of uniformity in field trials.

USVL188-GL and USVL188-NG have much earlier maturity than the other pair producing market size heads in 65 to 80 d after transplanting in fall environments (Table 1). This maturity is very similar to the commercial hybrids 'Gypsy' (Sakata Seed Inc.) and 'Captain' (Seminis Vegetable Seeds). Under optimal conditions (e.g., cool maturation temperatures), USVL188-GL and USVL188-NG produce better than average heads, but these lines tend to be sensitive to high temperature and sometimes respond to this with bracting occurring in heads. However, head mass is comparable to many commercial hybrids, including 'Gypsy' and 'Marathon' (Table 1). Other head traits of this pair include

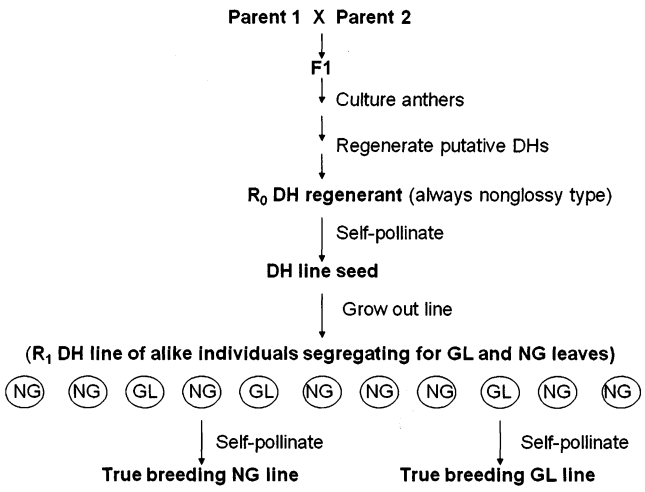

Fig. 2. Scheme followed in rare instances in which glossy (GL) and nonglossy (NG) individuals were identified in doubled haploid (DH) lines and self-pollinated to establish true breeding, homozygous individuals differing only for the leaf glossiness trait. In the case of USVL115-GL and USVL115-NG, which were derived from the commercial hybrid 'Marathon,' Parents 1 and 2 are a trade secret and unknown. With USVL188-GL and USVL188-NG, Parent 1 is a DH line derived from 'Marathon' and Parent 2 is a DH line derived from 'Everest'.

Table 1. Trait means and protected least significant differences ($P = 0.05$) computed from analysis of variance (SAS, Version 9.1; SAS Institute, Cary, NC) for days from transplant to harvest (DTH), degree of dome rating, bead size, head quality, head mass, and stem diameter for the glossy and nonglossy lines and four control commercial broccoli hybrids in fall environments for 3 years.

Variety	DTH (days)	Dome (rating[z])	Bead size (mm)	Head quality (rating[y])	Head mass (g)	Stem diam (cm)	Yr
USVL115GL	80	2.3	1.57	5.0	175	31.90	2006
USVL115NG	82	2.5	1.46	5.1	167	31.30	
USVL188GL	67	1.8	1.63	6.4	243	31.90	
USVL188NG	64	1.8	1.64	6.8	240	32.20	
CAPTAIN	64	2.0	1.47	3.9	194	34.20	
GYPSY	66	1.8	1.34	3.6	262	35.80	
MARATHON	78	1.9	1.24	3.6	288	36.20	
LEGACY	83	1.9	1.15	3.8	321	37.30	
$LSD_{0.05}$	5	0.3	0.23	0.8	79	3.00	
USVL115GL	93	2.7	1.72	5.6	170	28.50	2007
USVL115NG	90	2.3	1.82	6.3	160	27.90	
USVL188GL	76	2.4	1.60	5.0	208	30.30	
USVL188NG	72	2.3	1.40	4.7	218	30.40	
CAPTAIN	72	2.3	1.65	4.3	177	33.10	
GYPSY	75	1.9	1.70	3.4	240	31.20	
MARATHON	89	1.7	1.63	3.8	252	36.00	
LEGACY	89	1.9	1.51	3.5	222	35.60	
$LSD_{0.05}$	6	0.3	0.14	0.8	40	2.80	
USVL115GL	98	2.3	1.88	5.5	129	23.60	2008
USVL115NG	99	2.0	1.84	5.8	110	23.80	
USVL188GL	83	2.0	1.80	5.0	168	26.90	
USVL188NG	80	2.2	1.74	4.5	181	26.40	
Captain	72	2.2	1.59	4.0	141	29.40	
Gypsy	82	2.0	1.65	3.8	206	27.40	
Marathon	93	1.8	1.56	3.6	210	27.40	
Legacy	93	2.3	1.49	3.4	218	32.30	
$LSD_{0.05}$	6	NS	0.14	0.4	39	3.00	

[z]Dome rating on a 1–5 scale with 1 = very high dome, 3 = moderate dome, and 5 = flat.
[y]Quality rating on a 1–9 scale with 1 = excellent, 3 = very good, 5 = average, marketable 7 = poor, and 9 = nonmarketable.
LSD = least significant difference; NS = nonsignificant.

a moderate dome shape, intermediate bead size (1.60 to 1.80 mm), and stem diameter of 27 to 32 mm. These two lines are more vigorous than the USVL115 pair with a similar high degree of uniformity in field trials.

The quality characters of the two pairs of broccoli lines released here are not as important as the glossy leaf trait that distinguishes them. To date, comparisons between glossy and nonglossy phenotypes have been made using unrelated lines that differ for numerous characteristics. A number of these studies have shown that certain insects can exhibit modified behavior in response to glossy leaves compared with normal, nonglossy leaves with the net result of glossy types often having significantly less insect predation and appearing relatively resistant to attack (Dickson and Eckenrode, 1980). Such observations have been observed with several pests, including the imported cabbageworm (Stoner, 1990), diamondback moth (Eigenbrode et al., 1991), and silverleaf whitefly (Farnham and Elsey, 1995). Eigenbrode et al. (1995) also showed that some generalist insect predators that feed on problem species may be influenced by glossy leaves. Several *B. oleracea* sources of the glossy leaf trait have been cited; in some cases, resistance appears to be controlled by recessive gene action and in others by dominant gene action (Dickson and Eckenrode, 1980; Stoner, 1990). In the mentioned studies, it is impossible to rule out the possibility that other genes or traits that differ between unrelated lines could account for differences between the glossy and nonglossy types. In contrast, USVL115-GL and USVL115-NG, and also USVL188-GL and USVL188NG, provide researchers with advanced germplasm that will allow for the comparison of the glossy and nonglossy leaf traits in essentially the same genetic backgrounds.

Small quantities of USVL115-GL, USVL115-NG, USVL188-GL, and USVL188NG seed produced by manual self-pollinations may be obtained from Dr. Mark W. Farnham, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414-5334. It is requested that appropriate recognition be made if this inbred line contributes to the development of a new breeding line or hybrid.

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