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The genetics of resistance to lettuce drop (*Sclerotinia* spp.) in lettuce in a recombinant inbred line population from Reine des Glaces x Eruption

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Abstract Lettuce drop, caused by *Sclerotinia minor* and *S. sclerotiorum*, is an economically important disease of lettuce. The association of resistance to lettuce drop with the commercially undesirable trait of fast-bolting has hindered the integration of host resistance in control of this disease. Eruption is a slow-bolting cultivar that exhibits a high level of resistance to lettuce drop. Eruption also is completely resistant to Verticillium wilt caused by race 1 of *Verticillium dahliae*. A recombinant inbred line population from the cross Reine des Glaces × Eruption was genotyped by sequencing and evaluated for lettuce drop and bolting in separate fields infested with either *S. minor* or *V. dahliae*. Two quantitative trait loci (QTLs) for lettuce drop resistance were consistently detected in at least two experiments and two other QTLs were identified in another experiment; the alleles for resistance at all four QTLs originated from Eruption. A QTL for lettuce drop resistance on linkage group (LG) 5, *qLDR5.1*, was consistently detected in all experiments and explained 11 to 25% of phenotypic variation. On LG1, *qLDR1.1* was detected in two experiments explaining 9 to 12% of the phenotypic variation. Three out of four resistance QTLs are distinct from QTLs for bolting; *qLDR5.1* is pleiotropic or closely linked with a QTL for early bolting; however, the rate of bolting shows only a small effect on the variance in resistance observed at this locus. The SNP markers linked with these QTLs will be useful in breeding for resistance through marker-assisted selection.

Key words Lettuce drop, *Sclerotinia*, Genotyping by sequencing, QTL mapping, Breeding for resistance

Key message Two QTLs for resistance to lettuce drop, *qLDR1.1* and *qLDR5.1*, were identified. Associated SNPs will be useful in breeding for lettuce drop and provide the foundation for future molecular analysis.

Author contribution statement RJH and KVS conceived the lettuce drop study and obtained funding; RJH generated the population; BEM carried out experiments (phenotyping, mapping, data analyses) and drafted the paper; MJT carried out genotyping and marker identification; KDP conducted genotyping of the *Vr1* locus; RWM contributed to data interpretation; RJH, KVS, and IS contributed to phenotyping and data analyses. All authors contributed to writing the paper and approved the final manuscript.

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Introduction

Lettuce drop, caused by two species of the fungal pathogen *Sclerotinia* [*S. sclerotiorum* (Lib.) DeBary) and *S. minor* (Jagger)], is one of the most widespread and destructive diseases of lettuce (*Lactuca sativa* L.) in coastal California and other major lettuce producing regions of the world (Purdy 1979; Subbarao 1998). The disease is predominantly caused by *S. sclerotiorum*, except in Canada, New Zealand, and in the Salinas Valley of California and surrounding areas, where *S. minor* is the predominant species (Subbarao 1998). Lettuce drop was first reported in the United States in 1890 (Stevens and Hall 1911; Subbarao 1998) and has since established itself wherever there is intensive commercial production of lettuce. *S. sclerotiorum* causes new infections mostly from airborne ascospores but also from myceliogenic germination of sclerotia (Purdy 1979), whereas infections of *S. minor* mainly originate from the latter because it is not known to produce aerial spores. Both *S. sclerotiorum* and *S. minor* are capable of infecting all types and cultivars (cvs.) of lettuce. Successful infection results in complete decay of the crown tissue, wilting of leaves, and ultimately total collapse of the entire plant before harvest (Subbarao 1998; Isnaini and Keane 2007). Large numbers of propagules of both species are formed on infected plants that survive in soil as resting sclerotia, which may remain viable for up to 10 years (Sherf and MacNab 1986).

The generalist, necrotrophic mode of pathogenesis, dispersal, and survival of *S. sclerotiorum* and *S. minor* have made lettuce drop a difficult disease to control. The pathogen uses oxalic acid as a key pathogenicity factor for which resistance is generally limited in economically important crops (Cessna et al. 2000). Fungicides and cultural methods have traditionally been used (Subbarao 1998; Hao et al. 2003; Saharan and Mehta 2008) and the effectiveness of biological control for lettuce drop has also been investigated (Chitrampalam et al. 2008; Chen et al. 2016). However,

these methods require continuous monitoring, multiple applications, and do not reduce lettuce drop to desired levels (Matheron 1989; Subbarao 1998; Matheron 2004). Their application incurs extra input costs for growers. In addition to their adverse effects on the environment and human health, repeated applications of fungicides are also associated with the risk of fungicide-resistance development by the pathogen (Zhou et al. 2014; Lehner et al. 2015; Lehner et al. 2017; Fisher et al. 2018). Therefore, the most practical mechanism of lettuce drop control should involve the use of an integrated disease management strategy (Subbarao 1998; Hayes et al. 2010). Host resistance would be the most convenient, sustainable, and environmentally-friendly component to incorporate into an integrated strategy for controlling lettuce drop. Lettuce cultivars resistant to lettuce drop would also be the preferred option for organic farming.

The non-specialized nature of the pathogen, the association of resistance with plant development traits (Hayes et al. 2010), the lack of useful protocols for screening large populations for resistance that yield reproducible results (Grube and Ryder 2004), and the influence of the environment on disease development (Subbarao 1998) have made it difficult to identify and breed for resistance to lettuce drop. As in other host species, resistance against *Sclerotinia* spp. in lettuce germplasm is rare and no complete resistance to lettuce drop has been identified. Partial resistance to lettuce drop was observed in some cultivars, primitive forms, and wild relatives with reduced disease incidence at market maturity (Chupp and Sherf 1960; Elia and Piglionica 1964; Newton and Sequeira 1972; Abawi et al. 1980; Madjid et al. 1983; Sherf and MacNab 1986; Subbarao 1998; Whipps et al. 2002; Grube and Ryder 2004; Hayes et al. 2010). Resistance in these cases was often associated with traits such as rapid bolting, low leaf area, and upright growth habit (Newton and Sequeira 1972; Grube 2004; Hayes et al. 2010). Resistance in some of these

accessions is perhaps due to a mechanism related to plant architecture and growth rather than a physiological mechanism that operates throughout plant development.

The goal of multiple programs has been to identify lettuce drop resistance that is independent of plant architecture or development so that it could be used to breed cultivars of multiple market types. This type of resistance is believed to exist in lettuce because variation for disease incidence occurs among cultivars with similar plant architectures and market types, although this variation has not typically included accessions with economically meaningful levels of resistance (Grube and Aburomia 2004; Hayes et al. 2010). A high level of resistance to *S. minor* and *S. sclerotiorum* was identified in the slow-bolting, small-statured, and dark red colored Latin type *L. sativa* cv. Eruption (Hayes et al. 2010, 2011a). Hayes et al. (2010) determined the independence of plant morphology and resistance to *Sclerotinia* spp. in Eruption using residuals from regression analysis of rate of bolting on lettuce drop incidence. The relationship between plant height and *S. minor* resistance was studied further using segregating populations developed after crossing two romaine lettuce cultivars with cv. Eruption. Results of field trials showed no difference in disease incidence between families of different height classes, indicating the feasibility of developing commercial romaine cultivars with resistance to *Sclerotinia* spp. introgressed from Eruption (Hayes et al. 2011a; Hayes 2017). It was therefore important to determine the genetic basis of lettuce drop resistance in cv. Eruption and investigate the possible genetic association between resistance and plant development. Understanding the genetic basis of resistance to lettuce drop in cv. Eruption is essential for the successful deployment of this resistance using marker-assisted selection.

Eruption also is resistant to Verticillium wilt (Hayes et al. 2007; Sandoya et al. 2017), caused by race 1 of the soil borne fungus *Verticillium dahliae* Kleb., another highly destructive disease of many crop species including lettuce. The disease first appeared on lettuce in California in 1995

and has increasingly become an economically important problem. The *Verticillium resistance 1* (*Vr1*) locus first described in cv. La Brillante on linkage group (LG) 9 of lettuce provides complete resistance to race 1 of *V. dahliae* (Hayes et al. 2011b). Allelism tests between cv. La Brillante and several other resistant cultivars, including cv. Eruption, indicated that all tested cultivars carried the same resistance locus (Sandoya et al. 2017).

Cultivated lettuce exhibits considerable variation in leaf color and other morphological characteristics. The dark red leaf coloration of cv. Eruption is due to high anthocyanin content (Simko et al. 2016). Anthocyanin is a secondary metabolite important in plant defense against pathogens and abiotic stressors (Winkel-Shirley 2002; Lorenc-Kukuła et al. 2005; Zhang et al. 2013). A major locus represented by candidate genes leading to the biosynthesis of anthocyanins was identified in soybean (*Glycine max* L. *Merr*.) that may also be involved in resistance to *S. sclerotiorum* (Zhao et al. 2015). The involvement of anthocyanin in resistance to *Sclerotinia* spp. in lettuce is unknown. However, Newton and Sequeira (1972) reported that red pigmentation appeared to be correlated with lettuce drop resistance. Anthocyanins also have health benefits to consumers (Morais et al. 2016; Qin et al. 2018). The coloration of leaves due to anthocyanins was one of the early Mendelian traits studied in lettuce and other plants. In lettuce, Durst (1915) first reported the inheritance of anthocyanin formation as a single dominant gene. Subsequent studies demonstrated the involvement of additional genes controlling anthocyanin formation (reviewed in Robinson et al. 1983). Leaf color in lettuce is now known to be controlled by several genes encoding biosynthetic enzymes and transcription factors (Zhang et al. 2017; Tao et al. submitted). Identification of genes and molecular markers linked to beneficial horticultural quality traits and disease resistance would enable the development of lettuce cultivars with increased value.

The primary objectives of this study were to: (i) determine the genetic basis of resistance to *Sclerotinia* spp. in cv. Eruption, (ii) assess the number and chromosomal locations of quantitative resistance loci (QTLs) and identify linked molecular markers, and (iii) characterize the relationship between lettuce drop resistance with bolting and anthocyanin pigmentation. The paper also describes confirmation of the *Vr1* allele for resistance in cv. Eruption and identification of QTLs for other morphological traits segregating in this population.

Materials and methods

Plant materials

Lettuce is a diploid (2n = 2x = 18) autogamous species and cultivars are highly homozygous and phenotypically homogenous. One hundred sixty-two $F_{6:8}$ recombinant inbred lines (RILs) derived from a cross between cvs. Eruption and Reine des Glaces (RG) were used for the study. RG is a French cultivar developed by Vilmorin in 1883 (Wehner 2002). The cultivar is plant introduction (PI) 634668 in the United States Department of Agriculture, National Plant Germplasm System (USDA-NPGS) collection, where it is listed under the name Batavia Reine des Glaces. RG is a slow-bolting *L. sativa* cultivar susceptible to both species of *Sclerotinia* and to race 1 of *V. dahliae*. It is a light green heirloom Batavia type of lettuce with incised and undulated leaf margins; it is black-seeded. Eruption was developed by Enza Zaden (Wehner 2002) and is found in the USDA-NPGS as PI 613577. It is a slow-bolting dark red Latin type cultivar with generally entire and undulating leaf margins; it is white-seeded.

Population development

175 F_1 seed from RG × Eruption was produced using the method of Ryder and Johnson (1974) and all
176 F_2 and later generations were produced through self-pollination. Seed from each plant was kept
177 separate, unless otherwise noted. A RIL population was developed by inbreeding the population
178 up to the F_6 generation using single seed descent (Fehr 1991). $F_{6:8}$ seed lots of each RIL were
179 produced from pooling seed from approximately 20 field grown $F_{6:7}$ plants. The $F_{6:8}$ seed lots were
180 used in all field experiments.

181 **Phenotyping for resistance to lettuce drop and rate of bolting**

182 The mapping population and parents were evaluated for resistance to lettuce drop and rate of
183 bolting in spring 2016, summer 2016, and spring 2017 (hereafter Spr16, Sum16, and Spr17,
184 respectively). The common commercial cultivars of romaine lettuce (Green Forest, Hearts Delight,
185 and Brave Heart) were included as controls in all three experiments. Experiments were conducted
186 in an infested field dedicated to evaluation of lettuce drop at the USDA-ARS station in Salinas,
187 California. The experiments were arranged in an alpha design with three replications per line. Two
188 seed lines from each RIL and parental line were planted in ~9 m long rows that were 1 m wide.
189 Per plot, 25 to 40 plants were evaluated for lettuce drop. The field was artificially infested with a
190 mixture of sclerotia of four isolates of *S. minor* (BM001, BM004, BM005, and BM010)
191 immediately prior to planting of Spr16 and Spr17 experiments. The sclerotia were produced and
192 used for infesting the field using the method described by Hayes et al. (2010, 2011a). The Sum16
193 experiment relied on the resident sclerotia in the soil following the Spr16 season. Lettuce drop
194 incidence (number of plants diseased out of the total) was evaluated weekly, six times in each
195 experiment, starting from the first appearance of symptomatic plants. The rate of bolting of each
196 RIL was evaluated when the RILs exhibited maximum variation for the trait, which was towards
197 the end of the experiments. The rate of bolting was evaluated on a scale of 1 to 6 (1 = rosette stage,

198 2 = expanded leaves, 3 = a bud beginning to emerge, 4 = a bud and internodes emerged, 5 =
199 multiple extended buds emerged, and 6 = first flower emerged).

200 The following variables derived from the combined weekly mortality data were used for
201 genetic analyses:

202 • disease incidence (proportion mortality);
203 • disease rating (DR) generated by arcsine transformation of the proportion mortality to
204 achieve normality of data distribution;
205 • standardized area under the disease progress stairs (sAUDPS) score calculated from the
206 weekly proportion mortality evaluations (Simko and Piepho 2012); and
207 • sAUDPS residual: residual resistance score calculated from the sAUDPS regressed on
208 bolting score (see next section).

209 To reduce repetitive details, only DR and sAUDPS are presented in this paper because the
210 results of these variables were representative of results with the other variables.

211 **Relationship between lettuce drop and rate of bolting**

212 Mapping QTLs for disease resistance and correlated development traits can determine if resistance
213 QTLs are unlinked, closely linked, or possibly have pleiotropic effects on plant architecture or
214 development. The level of resistance not accounted for by a correlated trait can be estimated by
215 regression analysis followed by collection of the residuals. Thus, regression analysis was
216 conducted to determine the relationship between lettuce drop and the rate of bolting using sAUDPS
217 as the dependent variable and bolting score as the explanatory variable using the R software
218 (www.r-project.org/; R Core Team 2017). Residual resistance scores (residual sAUDPS) were
219 calculated as the difference between the actual and the predicted sAUDPS for each RIL. This
220 variable was generated with the assumption that regression of the sAUDPS values on bolting score

removes the possible contribution of rapid bolting towards lettuce drop resistance. Resistance QTLs detected using residual sAUDPS in this population would not be because of the lettuce drop resistance enhancing effect of "rapid bolting-associated factors" observed in other lettuce accessions because, in principle, regression would have removed the portion of resistance contributed by rate of bolting. This was a modified approach (Hayes et al. 2010) of the methods described by Visker et al. (2003) and Bradshaw et al. (2004), which analyzed residuals from regression of vine maturity on severity scores of late blight of potato and found that resistance to the disease was not due to the inherent effect of plant maturity. In a similar approach, Wisser et al. (2011) incorporated plant maturity in a multivariate mixed model to characterize the levels of quantitative resistance of maize to three fungal diseases independent of maturity effects and identified pleiotropy. The residual sAUDPS thus can be interpreted as a measurement of true resistance/susceptibility (Bradshaw et al. 2004) that is independent of the rate of bolting.

Evaluation of anthocyanin content

Parents and RILs were evaluated for relative anthocyanin concentration [leaf anthocyanin content index (ACI)] using an Anthocyanin Content Meter (ACM-200plus; ADC BioScientific Ltd., Hoddesdon, UK) on plants in the Spr17 lettuce drop experiment. The instrument provides an estimate of anthocyanin content that correlates well with chemical testing (for details: https://www.optisci.com/acm-200.html). The ACI measurement was conducted on leaves of three different plants in each plot in two replications of the experiment. Leaves towards the middle of the leaf canopy that were neither the youngest nor the most mature on the plant were used for the measurement. The mean of the ACI for each plot was used in further analyses.

Evaluation for reaction to *Verticillium dahliae* race 1

243 The RILs and parents were evaluated in a field infested with a race 1 isolate (VdLs16) of *V. dahliae*
244 at the USDA-ARS station in Salinas, California. The parental lines and the commercial romaine
245 cultivars used as controls in the lettuce drop experiments also were included in this experiment.
246 The seeds were planted on June 20, 2017 in ~5.5 m long plots with 1 m wide beds with two seed
247 lines. The experiment was arranged in an alpha design with three replications. Disease incidence
248 was assessed on September 12 to 14, 2017 by evaluating ten plants per plot that were past market
249 maturity for vascular discoloration symptoms (of xylem tissue) typical of infection by *V. dahliae*
250 (Inderbitzin and Subbarao 2017). The rate of bolting was also evaluated in this experiment using
251 the scale described above.

252 **Evaluation of other morphological traits**

253 The mapping population was evaluated for the following morphological traits 41 days after
254 planting on a single $F_{5:6}$ plant of each RIL grown in a six-inch pot in the greenhouse in the spring
255 of 2015: anthocyanin (red color), tinged coloration, plant stature (Hayes et al. 2011a), leaf margin
256 undulation (0 = none to 3 = extreme), margin serration (0 = none to 3 = extreme), and glossiness
257 of leaf. Anthocyanin, tinged coloration, short plant stature, and glossiness of leaf were recorded as
258 binary presence (1) or absence (0) traits. Seed weight and seed coat color were recorded after seed
259 harvest. For anthocyanin pigmentation, intensity of red color was coded as no red (0) or red (1).
260 For tinged coloration, genotypes with red pigmentation were coded as red (0) or tinged (1). RILs
261 that lacked red color marks (i.e., dark green, green, and light green) were considered missing data
262 points. For QTL mapping of chlorophyll content, which has a strong relationship with intensity of
263 green color (Simko et al. 2016), the tinged red color was disregarded. Thus, leaf color was coded
264 as light green (0), green (1), or dark green (2). Genotypes with intense red color, for which the
265 intensity of green color could not be determined, were considered missing data points.

Statistical analysis of phenotypic traits

Pearson correlation coefficients among traits were calculated using the R statistical software. For traits with a non-normal data distribution, results of the Pearson's correlation test were confirmed using Spearman's rank-order correlation (*rs*). However, because correlations calculated using the two approaches were similar, only results of the Pearson's test are presented. Broad-sense heritability (h^2_B) was used to estimate the genetic reproducibility of the phenotypic traits across environments (seasons) based on RIL means. To estimate h^2_B, variance components of each trait were generated by the "proc varcomp" procedure in SAS 9.4 (SAS Institute, Cary, NC) using the mixed model: response = general mean + genotype + environment (season) + replication + error. Heritability was calculated using the formula $h^2_B = \sigma_G^2 / (\sigma_G^2 + \sigma_e^2/r)$ for a single environment, or $h^2_B = \sigma_G^2 / [(\sigma_G^2) + (\sigma_E^2/e) + (\sigma_e^2/re)]$ for multiple environments (seasons) combined, where σ_G^2 is genetic variance, σ_E^2 is the variance of the environments (seasons), σ_e^2 is the error variance, r is the number of replications in each environment, and e is the number of experiment environments (seasons). Means of phenotype data calculated from the replicates were used for QTL mapping.

Genotyping by sequencing (GBS), SNP identification, and map construction

DNA was extracted from ~50 $F_{5:6}$ seeds of each RIL and parental line using GenElute Plant Genomic DNA Miniprep kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions and digested with *AvaII* to reduce genomic complexity. Unique adaptors were then ligated to each RIL and parental line. Individual libraries were constructed according to protocols for GBS (Elshire et al. 2011); the libraries were then pooled and sequenced using an Illumina HiSeq 4000 platform (Illumina, San Diego, CA). The TASSEL package (Bradbury et al. 2007) was used for read mapping and SNP calling. Custom scripts were applied that assessed multiple contiguous SNPs to obtain single haplotypes per scaffold (Truco et al. in preparation). Scaffold

haplotypes were used to construct a genetic map using MSTmap (Wu et al. 2008). Markers were clustered based on the reference map (Truco et al. 2013) and the position (bp) identified for each marker.

A framework set of evenly distributed markers was developed for QTL analysis by imputing haplotypes for 1 centiMorgan (cM) windows along each LG and used to construct a second linkage map using MSTmap (Wu et al. 2008). The resultant map was checked for collinearity with the reference map (Truco et al. 2013) and was oriented to be consistent with the latter. The linkage map (in cM) is presented along with the coordinates of the scaffolds (in bp) on the genome assembly (Reyes-Chin-Wo et al. 2017) in Supplemental material 1. MapChart was employed to draw the LGs (Voorrips 2002). A chi-square test was used to determine whether the segregation ratio of each marker fit the expected ratio of 1:1 ($\alpha < 0.05$). Genetic positions on each LG where one or more adjacent markers deviated from a 1:1 ratio were considered regions with segregation distortions (RSDs).

QTL analyses

QTL analyses were conducted using the qtl library of the R/qtl software v. 1.41-6 (http://www.rqtl.org/; Broman et al. 2003). To calculate the QTL genotype probabilities, analyses were conducted along the LGs at 1 cM intervals assuming a genotyping error rate of $1.0e^{-4}$ and using the Kosambi map function (Kosambi 1944). For QTL detection, composite interval mapping (Zeng 1994) was performed using the Haley-Knott regression (Haley and Knott 1992) selecting three markers as cofactors by forward selection to control genomic background effects. The genome-wide significance thresholds for the logarithm of odds (LOD) scores ($\alpha < 0.05$) were determined for each trait by permutation tests (1,000 times) (Churchill and Doerge 1994). The confidence intervals for each QTL were estimated using the "*lodint*" function that calculates the

1.5 LOD support intervals. The percentage of the phenotypic variance explained (PVE) and effects of QTLs in combination, individually, or in interactions were obtained by fitting a mixed linear model using the "*fitqtl*" function.

Effect of bolting QTL alleles on the rate of bolting

The RG parent carries alleles for early bolting on LG1 and LG4, while Eruption has alleles for early bolting on LG5 (shown in results). Therefore, this RIL population exhibited eight bolting QTL genotypes with some genotypes that bolted earlier or later than the parents. The rate of bolting for the eight QTL genotypes were compared to commercial romaine cultivars and the parents (cvs. RG and Eruption). A two-way analysis of variance (ANOVA) was conducted using the software JMP v. 11.1.1 (SAS Institute, Cary, NC). Multiple comparisons were made based on the Tukey-Kramer honest significant difference (HSD) test to determine significant differences.

Genetic analysis of the *Verticillium resistance 1* (*Vr1*) locus

A polymerase chain reaction (PCR)-based assay was used to determine the genotype at the *Vr1* locus as described in Inderbitzin et al. (2018, submitted). The parents (Eruption and RG), the resistant and susceptible cvs. (La Brillante and Salinas, respectively), and ten each of homozygous resistant and susceptible RILs were screened using the assay based on the amplification of the resistant allele of the candidate *LsVer1* gene.

Data availability

The marker sequences used in this study can be obtained using information in the lettuce genome assembly (Reyes-Chin-Wo et al. 2017). The authors declare that all other data supporting the findings of this study are included in the main manuscript file, Supplemental material, or are available from the corresponding author upon request.

335 **Results**

336 **Phenotypic variation for lettuce drop resistance and rate of bolting**

337 All lettuce cultivars included as controls reacted as expected to lettuce drop during all three
338 experiments. The two parents exhibited distinctly different levels of lettuce drop incidence (high
339 in RG, low in Eruption), while the RIL population showed continuous phenotypic variation in
340 response to infection by *S. minor* (Fig. 1A and B). The infection level in Spr16 was slightly higher
341 and more variable than in the other two seasons. Eruption and RG had mean DR scores ranging
342 from 0.31 ± 0.04 (Spr17) to 0.69 ± 0.06 (Spr16) and from 1.08 ± 0.16 (Sum16) to 1.14 ± 0.18
343 (Spr16), respectively (Fig. 1A). The mean sAUDPS for Eruption ranged from 0.03 ± 0.01 (Spr17)
344 to 0.19 ± 0.03 (Spr16) and from 0.41 ± 0.12 (Spr17) to 0.55 ± 0.01 (Spr16) for RG. The RIL
345 population had the highest mean and level of variation both for DR scores (1.07 ± 0.17) and
346 sAUDPS (0.35 ± 0.09) in Spr16. The lowest mean DR scores (0.86 ± 0.17) and sAUDPS (0.28 ±
347 0.09) for the RILs were recorded in Sum16. The genetic reproducibility of the lettuce drop
348 incidence and derived parameters was high (h^2_B from 0.66 to 0.82) as estimated by broad-sense
349 heritability (Table 1).

350 In the lettuce drop experiments, the two parents showed similar rates of bolting across the three
351 environments (seasons) (Fig. 1C). In Spr16, the two parents did not exhibit observable differences
352 in bolting at the time of evaluation; also, there was limited variation among the progeny. Eruption
353 had a mean rate of bolting ranging from 1.00 ± 0.00 (Spr16) to 1.50 ± 0.00 (Spr17), while RG had
354 a rating of 1.00 ± 0.00 in all three environments. The RILs showed a low to moderate variation in
355 rate of bolting. Mean rate of bolting of the RILs ranged from 1.26 ± 0.33 in Sum16 to 1.78 ± 0.77
356 in Spr17. There was transgressive segregation among the RILs (Fig. 1C), indicating the
357 complementary action of alleles for early bolting inherited from both parents. This was confirmed

by QTL analysis and ANOVA. The heritability estimate for rate of bolting was high ($h^2_B = 0.78$, Table 1). Significant positive correlations were detected within ($r = 0.80$ to 1.00, $p < 0.001$) and between environments ($r = 0.43$ to 0.72, $p < 0.001$) for lettuce drop phenotypes (Table 2). A linear relationship was detected between sAUDPS-based disease resistance and rate of bolting in all three experiments (Supplemental material 2). The effect of bolting on resistance was statistically significant ($p < 0.001$) and explained 8.25% (Spr16), 6.39% (Sum16), and 6.04% (Spr17) of the variability in sAUDPS. However, the results of the QTL analysis using sAUDPS residual indicate that the effect of bolting on resistance was minimal.

Significant negative correlations were detected between bolting and lettuce drop within ($r = -0.19$ to -0.30, $p < 0.05$) and between environments ($r = -0.12$ to -0.33, $p < 0.05$) (Table 2), which is in agreement with previous reports (Hayes et al. 2010). RIL progenies with relatively higher bolting scores had lower disease levels and vice versa. To test whether the high correlation coefficients detected between lettuce drop and bolting are due to a few individuals with outlying rates of bolting, additional statistical analyses were performed using DR and bolting data. The results of Pearson's correlation in all three experiments were confirmed using Spearman's coefficient of rank correlation. When progenies with rates of bolting ≥ 2 (16, 8, and 57 RILs in Spr16, Sum16, and Spr17, respectively) were removed, the Pearson's correlations were reduced from -0.28 to -0.14 ($p = 0.10$; Spr16), from -0.24 to -0.23 ($p = 0.0048$; Sum16), and from -0.28 to -0.17 ($p = 0.076$; Spr17). These results indicate that the observed relationship between the rate of bolting and resistance to lettuce drop is not exclusively caused only by a few early bolting RILs, though they influenced the size of the correlation coefficients. The correlation between bolting measured in the lettuce drop and Verticillium experiments were 0.54 (Spr16), 0.45 (Sum16), and 0.64 (Spr17). These correlation coefficients are highly significant at $p < 0.001$, similar to the

correlations of bolting between seasons in the lettuce drop experiments. In addition, identical bolting QTLs were detected in both the lettuce drop and Verticillium wilt experiments. Thus, the measurements of bolting in the lettuce drop experiments were not biased due to disease incidence.

Phenotypic variation for anthocyanin content

The parental lines and RILs showed moderate-to-high levels of variation in anthocyanin content (Fig. 1E). Eruption and RG had mean ACI values of 38.65 ± 1.25 and 4.49 ± 0.35, respectively. The mean ACI value for the RILs was 14.45 ± 15.33, with h^2_B of 0.93 (Table 1). Moderate to high (negative or positive) correlations were detected between ACI and other traits, except in a few cases (Table 2). ACI had strong negative correlations (r = -0.26 to -0.36, $p < 0.001$) with lettuce drop incidence (and rating) and strong positive correlations (r = 0.20 to 0.31, $p < 0.001$-0.010) with rate of bolting.

Phenotypic variation for resistance to Verticillium wilt and rate of bolting

The parents and the RIL population exhibited extensive genetic variation in reaction to Verticillium wilt. Eruption and RG had low (0.00) and high (0.75 ± 0.05) DR, respectively (Fig. 1D). Verticillium wilt incidence had h^2_B of 0.65 (Table 1). In the Verticillium wilt experiment, Eruption and RG had mean bolting scores of 1.83 ± 0.24 and 3.00 ± 0.00, respectively (Fig. 1E). The RIL population had a mean bolting score of 2.50 ± 1.00. The relatively greater differences in rate of bolting observed in this experiment were likely because the test materials were more mature at evaluation. In this experiment, bolting heritability was relatively lower than in the lettuce drop experiments (h^2_B of 0.66 vs. 0.78, Table 1). The incidence of Verticillium wilt was not significantly correlated with any of the other traits (Table 2).

Genetic linkage map

After quality control analysis of the GBS data, 840 SNP markers were selected for construction of the genetic map and QTL analysis. All SNPs resulted in reliable genotype data with > 91% call rate for all the RILs and 67.26% (565) of the SNPs had > 95% call frequency. The genetic map for the RIL population covered 1,574.4 cM, which is very close to the 1,579 cM reported for the reference map (Truco et al. 2013). This genetic linkage map provided good coverage of all nine LGs of lettuce for QTL analysis (Table 3; Fig. 2; Supplemental material 1). Six hundred and ten (72.62%) of the SNP markers mapped to unique positions. The distance between unique adjacent markers ranged from 0.32 to 38 cM with a mean of 2.58 ± 3.77 cM and > 86% (529/610) of the SNPs had < 5 cM distance between them. Segregation distortion ($p < 0.05$) was observed in all LGs similar to Truco et al. (2013) involving ~10% (86/840) of SNP markers (Table 3). A total of six regions with segregation distortions (RSDs) longer than 3 cM were detected in the genome, with the highest number of RSDs on LG2 (three RSDs), and others located on LGs 1, 5, and 8.

QTL analysis of lettuce drop resistance traits

Four QTLs associated with lettuce drop resistance traits were detected using 12 variables (Table 4; Fig. 3). These QTLs were located on LGs 1, 4, 5, and 7. The QTLs on LGs 1 (42–54 cM) and 5 (132–147 cM) were consistently detected using multiple parameters and were repeatedly detected in two or all three field experiments, respectively; these QTLs were named *qLDR1.1* (*first QTL for Lettuce Drop Resistance on LG1*) and *qLDR5.1*, respectively. In combination, *qLDR1.1* and *qLDR5.1* had a major effect (QTL effect size classification according to Burke et al. 2002) explaining 30 to 41% of the phenotypic variation in resistance depending on the experiment. Individually, these QTLs were of minor to intermediate effect; *qLDR1.1* and *qLDR5.1* explained 9 to 12% and 11 to 25% of the phenotypic variation in resistance, respectively. The QTLs on LGs 4 (*qLDR4.1*; 36–44 cM) and 7 (*qLDR7.1*; 65–78 cM) were both detected in association with at

least two of the resistance variables in one environment and each explained ~9 and ~11% of the phenotypic variation. Two other putative QTLs, one on LG1 (56–68 cM) and one on LG5 (*qLDR5.2*; 89–102 cM) were also detected, with each explaining ~11% of the variation. These hypothetical QTLs were associated with at least one resistance measurement in the Spr17 experiment. The putative QTL at the 56–68 cM interval on LG1 was very close to that of *qLDR1.1*. Also, the residual sAUDPS in the Spr17 experiment, when the 56–68 cM interval was detected, was associated with *qLDR1.1*. Thus, this interval is likely a shifted position of the *qLDR1.1*. The *qLDR5.2* interval is 30 cM away from the *qLDR5.1* and is possibly a different QTL that is perhaps environment-dependent. Decreased disease levels were associated with the alleles from Eruption at all the QTLs detected for lettuce drop resistance.

QTL analysis of rate of bolting, anthocyanin content, and resistance to Verticillium wilt

Three QTLs were detected in the rate of bolting analysis in the RIL population, one each on LGs 1, 4, and 5 (Table 4 and Fig. 3). These QTLs were named *qBLT1.1*, *qBLT4.1*, and *qBLT5.1*, respectively, referring to *first QTL for Bolting on LGs 1*, *4*, and *5*, respectively. All three QTLs were detected in at least two environments. The QTLs on LGs 1 (*qBLT1.1*; 22–34 cM) and 5 (*qBLT5.1*; 137–148 cM) were both detected in three environments and explained 8 to 12% and 8 to 14% of the variation in rate of bolting, respectively. The QTL on LG4 (*qBLT4.1*; 193–202 cM) was identified in two environments and explained 11 to 16% of the variation in bolting. At the *qBLT1.1* and *qBLT4.1* loci, the alleles for earlier bolting came from the RG parent. At the *qBLT5.1* locus, the alleles from Eruption had additive effects that increased the rate of bolting. In Spr16, no significant QTLs were detected for bolting because the RILs did not exhibit sufficient variation in rate of bolting at the time of measurement.

448 A single QTL for leaf anthocyanin content measured quantitatively was detected on LG5 in
449 the 56–59 cM interval; this was designated *qACI5.1* and explained 18% of the variation. The red-
450 colored parent, Eruption, contributed the allele for increased ACI values. The interval of *qACI5.1*
451 overlapped with the QTL for the qualitatively assessed tinged red color; therefore, both were
452 considered manifestations of the same trait. For Verticillium wilt resistance, one large effect QTL
453 was identified on LG9 both for disease incidence and DR traits (Table 4 and Fig. 3). This QTL
454 mapped to the 34–44 cM interval of LG9 and explained 47 to 51% of the phenotypic variation.
455 The allele inherited from Eruption was associated with decreased levels of disease. This
456 Verticillium wilt resistance locus coincided with the previously described *Vr1* locus (Hayes et al.
457 2011b). Both the *Vr1* locus and the anthocyanin content QTL were independent of the QTL for
458 resistance to lettuce drop.

459 **QTL analysis of additional morphological traits**

460 A 1:1 segregating ratio was observed for seed coat color ($\chi^2 = 0.73$, $p = 0.39$; Thompson 1943),
461 anthocyanin ($\chi^2 = 0.08$, $p = 0.78$; Durst 1915), leaf tinged coloration ($\chi^2 = 0.03$, $p = 0.87$), and leaf
462 glossiness ($\chi^2 = 1.26$, $p = 0.26$), suggesting that each of these traits segregated as single genes in
463 this population. Twelve QTLs co-locating in eight genomic regions were detected associated with
464 the additional morphological traits (Supplemental materials 3 and 4). Most of the QTLs mapped
465 in genomic regions where QTLs were reported previously (Table 5).

466 **Co-location of QTLs for lettuce drop resistance and bolting**

467 A comparison of the QTL location for lettuce drop resistance and bolting indicated QTLs
468 associated with the two traits at close genomic positions on LGs 1 and 5 (Table 4 and Fig. 3).
469 *qLDR5.1* and the *qBLT5.1* on LG5 were mapped at the genetic intervals from 132–148 cM, flanked
470 by the SNP markers Lsat_1_v5_g_5_1002 and Lsat_1_v5_g_5_892. The overlapping region of

the two QTLs spanned 9.3 cM (137.2–146.5 cM), indicating that the same QTL with a pleotropic effect, or two tightly-linked QTLs control(s) these traits. The two QTLs contributed a considerable proportion (ranging from 8 to 25%) of the phenotypic variation for the respective traits. The analysis of the residuals from the regression of sAUDPS on rate of bolting identified significant QTLs at the *qLDR5.1/qBLT5.1* location (Table 4 and Fig. 3) that explained 16 to 22% of the trait variation. These results confirm that QTLs in the *qLDR5.1/qBLT5.1* region affect both the rate of bolting and lettuce drop resistance, but they also indicate that the component of resistance associated with the rate of bolting was small, accounting approximately for only 6–8% of the total variance of sAUDPS.

The *qLDR1.1* and the *qBLT1.1* were identified on adjacent regions of LG1 with their peaks ~30 cM apart and their supporting intervals separated by 8 cM, which is a distance large enough to consider them separate loci. The beneficial alleles are in *cis* at the *qLDR1.1* and *qBLT1.1*, as alleles for both low disease levels and slow bolting were inherited from Eruption. QTL alleles for higher resistance and slow rate of bolting are in *trans* at the *qLDR5.1/qBLT5.1* region as the allele from Eruption enhances both the resistance and the rate of bolting. *qLDR4.1* and *qLDR7.1* were mapped in genomic regions of LGs 4 and 7 where no bolting QTL was identified.

Effect of bolting QTL alleles on rate of bolting

The RIL genotype with all three early bolting alleles at the *qBLT1.1*, *qBLT4.1*, and *qBLT5.1* (called 'BBE' genotype due to the combination of alleles from RG and Eruption) had a significantly higher rate of bolting than all romaine cultivars and the parents (Table 6). The 'BBE' and 'EEB' genotypes are the only ones exhibiting transgressive segregation, bolting significantly earlier ('BBE') or later ('EEB') than both parents, thus confirming the results of the QTL analyses. The

493 *qBLT5.1*/*qLDR5.1* locus significantly enhances the rate of bolting in the RIL population (as
494 compared to commercial cultivars) only in combination with the other two QTLs for bolting
495 located on LG1 and LG4 ('BBE' genotype). The other three genotypes with the Eruption allele at
496 the *qBLT5.1*/*qLDR5.1* locus ('EEE,' 'EBE,' and 'BEE'), which includes the genotypes expected
497 to have the highest level of resistance, had a similar rate of bolting as the romaine cultivars (Table
498 6). Therefore, seven out of eight bolting QTL genotypes from this population provide bolting
499 phenotypes that are similar to commercial romaine cultivars.

500

501 **Presence of the Verticillium race 1 resistance *Vr1* locus**

502 The locus for resistance to race 1 of *V. dahliae* mapped to the identical location with the *Vr1* locus
503 on LG9 (Hayes et al. 2011b). Using a PCR-based assay for *Vr1*, all ten homozygous resistant RILs
504 showed a PCR product corresponding to the functional allele of the candidate *LsVer1* gene
505 (Inderbitzin et al. 2018, submitted). The same product was detected in Eruption and cv. La
506 Brillante, consistent with the RILs being homozygous for the functional allele of *LsVer1*. No PCR
507 product was detected for the ten susceptible RILs, RG, or cv. Salinas. This co-segregation between
508 resistance to race 1 of *V. dahliae* in Eruption and *Vr1*-mediated Verticillium wilt resistance is
509 consistent with the presence of the same *Vr1* locus in La Brillante being responsible for race 1
510 resistance in Eruption.

511

512 **Discussion**

513 Resistance to lettuce drop has previously been associated with premature bolting, which is an
514 undesirable trait in modern lettuce cultivars. The rarity of useful resistance genes to *Sclerotinia*
515 spp. has been a major constraint to the use of genetic resistance for control of lettuce drop. To our

knowledge, this is the first report on the inheritance of lettuce drop resistance in *Lactuca* spp. We detected two consistent QTLs on LGs 1 and 5, *qLDR1.1* and *qLDR5.1*, which together explained up to 41% of phenotypic variation in lettuce drop caused by *S. minor*. Two additional QTLs were detected in a single field experiment. The polygenic inheritance identified in this study is consistent with the genetic basis of resistance to diseases caused by *Sclerotinia* spp. reported in most other host species; for example, resistance to *Sclerotinia* stem rot is polygenic in *Brassica* spp., sunflower (*Helianthus annus* L.), dry beans (*Phaseolus vulgaris* L.), and soybean [*Glycine max* (L.) Merr.] (Fuller et al. 1984; Kim and Diers 2000; Castaño et al. 2001; Li et al. 2015). Dominant monogenic resistance conferred to *S. sclerotiorum* has been reported in only a few species; for instance, in faba beans (*Vicia faba* L.; Lithourgidis et al. 2005) and common bean (*P. vulgaris* L.; Schwartz et al. 2006).

The RIL population exhibited a sufficient level of variation in response to *S. minor* infection to map segregating QTLs for resistance. The variability observed in the incidence of lettuce drop between field experiments was expected because of variation in environmental conditions and associated factors that greatly influence the growth of *Sclerotinia* spp. (Imolehin et al. 1980; Subbarao 1998; Hao and Subbarao 2005). Despite this variability, significant correlations were found between experiments, consistent with QTLs for lettuce drop resistance segregating among the RILs. The broad-sense heritability of resistance was 0.66 to 0.82 (Table 1), indicating that a large portion of the observed phenotypic variance is explained by the genetic variance. In other host species, similar levels of heritability to our results were reported; in *B. napus* for instance, broad-sense heritability ranging from 0.57 to 0.84 was observed for *Sclerotinia* stem rot resistance (Wu et al. 2016).

Bolting is a developmental trait that affects the reaction of lettuce germplasm to *Sclerotinia* spp. (Grube 2004; Grube and Ryder 2004; Hayes et al. 2010). It may contribute to resistance through lignification of lettuce stems or modification of the microenvironment making it unsuitable for pathogen development or infection initiation (Grube 2004; Grube and Ryder 2004). In our study with parents that did not differ greatly for bolting, analyses of QTL intervals, linkage phase of QTL alleles, and statistical inference using residual resistance indicated that resistance conferred by *qLDR1.1*, *qLDR4.1*, and *qLDR7.1* is not associated with early bolting. At all of these QTLs, the Eruption allele confers higher resistance to lettuce drop. Combining alleles at these loci from Eruption would be beneficial to cultivar development. Beneficial alleles for resistance to *S. minor* and bolting are in *trans* at the *qLDR5.1*/*qBLT5.1* locus. However, the analysis of the residuals from the regression of sAUDPS on the rate of bolting identified a significant QTL at the *qLDR5.1/qBLT5.1* location with a similar PVE effect (16–22%) to that determined using sAUDPS data (11–25%). In addition, analysis of the effect of the *qBLT5.1*/*qLDR5.1* locus on bolting confirmed that the Eruption allele for bolting at this locus alone does not significantly increase the rate of bolting compared to commercial romaine cultivars in the RG × Eruption genetic background. These results provide evidence that the rate of bolting plays only a minor role in the variance of resistance observed at the *qLDR5.1* locus. Thus, resistance to *Sclerotinia* spp. can be introgressed into commercial cultivars from Eruption without any or with only a minimal effect on earliness of bolting.

Some of the QTLs we detected are located in the same chromosomal regions with QTLs reported in other studies. The *qLDR1.1* locus mapped to a region containing the lettuce downy mildew resistance genes *Dm17* and *Dm43* (McHale et al. 2009). This locus also co-locates with the position of a candidate gene, flavonol quercetin-3-malonylglucoside (Q-3MG), underlying

561 total bioflavonoid content on LG1 (Damerum et al. 2015). Flavonoids are one of the hydroxylated
562 polyphenolic compounds important for multifaceted functions in plants and combating
563 environmental stressors, including microbial infection (Kumar and Pandey 2013). Phenolics may
564 play a role in forming a hardened wall that cannot be easily degraded by pathogen enzymes,
565 thereby directly inhibiting their growth (Underwood 2012). The *qLDR1.1* locus also maps in a
566 similar region with previously reported QTLs for chlorophyll *a* and chlorophyll *b* content (Hayashi
567 et al. 2012). However, in our study, a QTL for chlorophyll (light green color; *qLG4.2*) was not
568 linked to *qLDR1.1* as it mapped to a different LG. The QTL for short plant stature (*qsl1*)
569 overlapped with *qLDR1.1* in the current study but does not affect lettuce drop resistance
570 introgressed from cv. Eruption (Hayes et al. 2011a). *qLDR4.1* mapped to the same region as the
571 downy mildew RGC4 (McHale et al. 2009; Simko et al. 2015) and the light green color QTL,
572 *qLG4* (Simko et al. 2016). The *qLG4.2* for light green leaves in our study was mapped on the same
573 LG but at a position distant from the *qLG4* locus. The *qBLT1.1* maps in a location where a QTL
574 was identified for luteolin (a flavone) derivative compound luteolin-7-O-glucoside (L-7G), a
575 nutritional trait (Damerum et al. 2015). Flavones are a subclass of flavonoids that are known to be
576 involved in plant physiological and developmental processes, including individual organ and
577 whole-plant development (for review, see Brunetti et al. 2013). *qLDR7.1* is within the large QTL
578 interval for resistance to downy mildew *qDM7.1* (Simko et al. 2015), which also encompasses the
579 QTL for resistance to powdery mildew *pm-7.1* (Simko et al. 2014) . Resistance to downy mildew
580 *RBQ1* (Jeuken and Lindhout 2002; McHale et al. 2009) also maps to this region but its precise
581 position is unclear because it was mapped with earlier technologies.

582 The genetics of anthocyanin pigmentation has been the subject of multiple classical studies
583 and more recently molecular characterization (reviewed in Robinson et al. 1983; Zhang et al. 2017;

584 Tao et al. submitted). Five genes (*Red Lettuce Leaves 1* to *5*; *RLL1* to *RLL5*) contributing to
585 variation in red leaf color in lettuce were characterized recently (Tao et al. submitted). The QTL
586 for anthocyanin content identified in our study mapped to the same region of LG5 as the *RLL2*
587 gene and was designated *qACI5.1* but should be renamed as *RLL2* if future studies show the two
588 loci to be allelic. The candidate *RLL2* gene was identified as encoding a R2R3-MYB transcription
589 factor (MYB113, LG5_426271) (Zhang et al. 2017; Tao et al. submitted). Current data do not
590 allow *RLL2*/*qACI5.1* to be designated as any of the genes previously described in classical genetic
591 studies (Robinson et al. 1983). In the present study, anthocyanin content index was positively
592 correlated with lettuce drop resistance. However, *qACI5.1* did not co-locate with the resistance
593 QTL (*qLDR5.1*), indicating that genes controlling these two traits are not the same in Eruption.
594 Thus, it should be possible to develop both red and green leaf lettuce cultivars with improved
595 resistance to lettuce drop from Eruption.

596 In summary, lettuce cv. Eruption is resistant to the soil borne diseases lettuce drop and
597 Verticillium wilt. Partial resistance of Eruption to lettuce drop is controlled by at least two genes
598 of small to medium effect located on LGs 1 and 5 that together explain up to 41% of the trait
599 variation. The resistance allele at *qLDR1.1* is not associated with early bolting (an undesirable
600 trait), while the resistance allele at *qLRD5.1* appears to be pleiotropic, but only minimally affecting
601 the rate of bolting. We cannot, however, exclude the possibility of two closely linked genes, one
602 for resistance and the other for rate of bolting. Both of the resistance QTLs, potentially together
603 with *qLDR4.1* and *qLDR7.1*, are vital in lettuce breeding programs. The SNP markers closely
604 linked to the QTLs could facilitate genotyping assays to assist in transferring lettuce drop
605 resistance alleles from Eruption to modern genotypes of lettuce. The Verticillium wilt resistance
606 in Eruption was confirmed as being determined by the single dominant *Vr1* gene previously

described in cv. La Brillante (Hayes et al. 2011b; Sandoya et al. 2017). Thus, Eruption could serve as a source of resistance against both *Sclerotinia* spp. and *V. dahliae.*

Conflict of interest The authors state that there is no conflict of interest.

Ethical standards The experiments comply with the laws of the USA, the country in which the study was performed, and the ethical standards of the respective university and employers of the authors.

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Figure legends

Fig. 1 Boxplots of lettuce drop disease rating, standardized area under the disease progress stairs (sAUDPS), rate of bolting, Verticillium wilt disease rating and anthocyanin content index in the Reine des Glaces × Eruption recombinant inbred lines (RILs) and parents evaluated in Salinas Valley, California, in 2016 and 2017. Cultivars Reine des Glaces (RG) and Eruption are the susceptible and resistant parents, respectively. **a** lettuce drop disease rating data generated by arcsine transformation of the proportion mortality of RILs and parents in spring 2016 (Spr16), summer 2016 (Sum16), and spring 2017 (Spr17); **b** sAUDPS data of RILs and parents in Spr16, Sum16, and Spr17; **c** rate of bolting data on scale of 1 (rosette stage) to 6 (emergence of first flower) of RIL and parents in Spr16, Sum16, and Spr17; **d** Verticillium wilt disease rating data generated by arcsine transformation of the proportion mortality of RILs and parents in 2017; **e** rate of bolting data of RIL population and parents in Verticillium wilt trial in 2017; and **f** anthocyanin content index (ACI) of RIL population and parents in Spr17 lettuce drop trial. Five statistics (bars) are represented in each boxplot from bottom to top: the smallest observation, lower quartile,

median, upper quartile, and largest observation. Data points positioned outside this range and depicted as circles are outliers.

Fig. 2 Linkage groups (LGs) and map locations for 840 SNP makers in the recombinant inbred line population of the Reine des Glaces × Eruption. The numbers on the left side of each LG are genetic distances in centiMorgans (cM).

Fig. 3 Distribution of putative QTLs for lettuce drop resistance, rate of bolting, Verticillium wilt, and anthocyanin content index identified in the Reine des Glaces × Eruption recombinant inbred line population.

Table legends

Table 1 Components of variance and broad-sense heritability of lettuce drop disease incidence, disease rating, standardized area under the disease progress stairs (sAUDPS), sAUDPS residual, Verticillium wilt (disease incidence and rating), anthocyanin content index, and rate of bolting in the Reine des Glaces × Eruption recombinant inbred line population.

Table 2 Pearson correlation coefficients for lettuce drop disease incidence (LDDI), disease rating (LDDR), standardized area under the disease progress stairs (sAUDPS), sAUDPS residual, rate of bolting in lettuce drop trials (RBLD), Verticillium wilt (disease incidence and rating; VWDI and VWDR), rate of bolting in a Verticillium wilt trial (RBVW), and anthocyanin content index (ACI) in the Reine des Glaces × Eruption recombinant inbred line population evaluated in Salinas Valley, California, in 2016 and 2017.

Table 3 Description of molecular linkage groups calculated from the Reine des Glaces × Eruption recombinant inbred line population.

Table 4 Summary of QTLs for lettuce drop resistance, rate of bolting, Verticillium wilt, and anthocyanin content index (ACI) identified in the Reine des Glaces × Eruption recombinant inbred line population.

Table 5 Overview of QTLs for morphological traits identified in the Reine des Glaces × Eruption recombinant inbred line population.

Table 6 Mean values of bolting rates of commercial lettuce cultivars, parental lines Reine des Glaces (RG) and Eruption, and progenies of the Reine des Glaces × Eruption recombinant inbred lines (RILs) with alleles of cultivars Eruption or Reine des Glaces (RG) at the three bolting QTLs in field experiments in Salinas Valley, California, in 2016 and 2017.

Supplemental materials

Supplemental material 1 Genetic linkage map of the Reine des Glaces × Eruption recombinant inbred line population with scaffold coordinates on lettuce genome assembly (Reyes-Chin-Wo et al. 2017). Strand column indicates if the sequence of the scaffold corresponds to the reference (+) or to the reversed transcribed strand (-).

Supplemental material 2 Linear regression of standardized area under the disease progress stairs (sAUDPS) score calculated from weekly evaluations of mortality due to lettuce drop in *Sclerotinia minor*-infested field experiments on rate of bolting of the Reine des Glaces × Eruption recombinant inbred line population evaluated in Salinas Valley, California. **a** spring 2016; **b** summer 2016; and **c** spring 2017 experiments. Shown on figures are linear regression equations and correlation coefficients (*r*). The sAUDPS score was calculated from the weekly mortality data (Simko and Piepho 2012). Rate of bolting was scored towards the end of each experiment on a scale of 1 (rosette stage) to 6 (first flower emerged).

Supplemental material 3 Summary of QTLs/genes for morphological traits [seed weight, seed coat color, anthocyanin (red color), chlorophyll (green color), tinged coloration, short plant from *sl*, margin undulation, margin serration, and glossiness of leaf] identified in the Reine des Glaces × Eruption recombinant inbred line population.

Supplemental material 4 Distribution of putative QTLs on linkage groups 1, 2, 4, 5, 7, and 9 for seed weight, seed coat color, and other morphological traits identified in the Reine des Glaces × Eruption recombinant inbred line population. The blue horizontal dashed line corresponds to the LOD score thresholds ($\alpha = 0.05$).