

Tipburn resilience in lettuce (*Lactuca* spp.) – the importance of germplasm resources and production system-specific assays

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Abstract

BACKGROUND: Tipburn is a physiological disorder of lettuce (*Lactuca* spp.). It causes discoloration and collapse of leaf margins, leading to unsaleable crops in both protected (glasshouse, hydroponic) and outdoor production systems. The occurrence of tipburn is hard to predict and is sensitive to environmental conditions. Phenotyping for tipburn resilience requires diverse germplasm resources and, to date, limited material has been investigated for this condition.

RESULTS: Using a *Lactuca* diversity fixed foundation set (DFFS) under glasshouse conditions, we identified a significant ($P < 0.001$) genotypic effect on tipburn resilience across both the entire population and across lines belonging to the cultivated species *L. sativa* alone. *Lactuca sativa* lines exhibited significantly ($P < 0.05$) higher average tipburn severity than those belonging to the wild species *L. saligna*, *L. serriola*, and *L. virosa* but we were able to identify both cultivated and wild tipburn-resilient lines. Leaf morphology factors, which included pigmentation, width, and serration, also significantly ($P < 0.05$) influenced tipburn resilience. Using a recombinant inbred line (RIL) mapping population derived from two DFFS lines, different small-effect quantitative trait loci (QTLs) accounting for 12.3% and 25.2% of total tipburn variation were identified in glasshouse and field conditions, respectively.

CONCLUSIONS: These results reflect the advantages of phenotyping under production-system-specific conditions for the examination of environmentally sensitive traits and highlight genetic markers and germplasm resources for the development of tipburn resilient lines for use in both protected and outdoor lettuce production.

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Keywords: lettuce; *Lactuca sativa*; *serriola*; *saligna*; *virosa*; tipburn; diversity set

INTRODUCTION

Lettuce (*Lactuca sativa*) is an important fresh produce crop with 27.7 m tonnes produced globally in 2020, of which 107 000 t were grown in the UK.¹ Lettuce is often produced in the field but is also commonly grown under glass using hydroponic culture systems, particularly in countries with less favorable or reliable weather conditions or where extension of the growing season is required.

Tipburn in lettuce is characterized by the browning of leaf tips, usually at the lettuce heart, either externally ('external tipburn'), on exposed leaf margins, or internally on the edges of leaves contained within the heart ('internal tipburn'), and is an important factor affecting marketability for both outdoor and indoor-grown crops. The incidence and severity of tipburn exhibits genetic (G), environmental (E) and interaction (G × E) influences.² Tipburn occurrence^{3,4} is a serious issue for the fresh produce industry and there are currently no completely effective control methods available. Increasing long- and short-term variation in weather

patterns associated with climate change,⁵ including day-to-day variability in growth conditions⁶ is likely to further confound the ability of growers to predict and prevent tipburn.

Tipburn appears to be associated with calcium deficiency in affected leaves and leaf margins, particularly in the inner tissue of the lettuce head, which is more prone to symptom development in most,⁷⁻¹³ but not all,¹⁴ cases. Insufficient calcium concentration in the leaf tissue may act to lower cell membrane integrity and weaken cell wall structure, leading to tissue collapse and

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discoloration due to the rupture of laticifer cells and resulting release of latex.^{15,16} This is then followed by a loss of turgor and necrosis, often associated with opportunistic infection by pathogens.

Inadequate calcium supply to leaves could result from insufficient uptake from the growing medium when calcium levels are low. However, calcium application often does not serve to reduce tipburn incidence^{14,17,18} and it has been suggested that tipburn could be unrelated to soil calcium availability.¹⁴

Environmental factors and their associated effects on calcium transport appear to influence tipburn occurrence and indeed may be more important in dictating symptom development than external calcium availability. Calcium transport occurs only in the xylem, so movement of the transpiration stream is required for its delivery to leaf tips, with calcium accumulating in those tissues transpiring most.¹⁹ Indeed, studies suggest a strong association between insufficient root water uptake and tipburn.²⁰ Reduced transpiration and so lowered calcium transport, for example arising from high external humidity through environmental conditions^{14,21} or the enclosure of leaves during hearting, appears to correlate with the most severe tipburn.^{2,17,22-24}

Tipburn may also be associated with increased growth rates.²⁵⁻²⁷ This may reflect insufficient calcium delivery for the rapid expansion of leaves, leading to increased tipburn incidence in larger leaves^{8,28} and whole plants;^{10,29,30} however, head size does not always correlate with calcium concentration.³¹ Furthermore, latex pressure in laticifer cells increases with plant development,³² perhaps increasing the tendency of cells to rupture as the plant grows.

Genotypic variability in tipburn susceptibility has been found in many studies.^{4,33-40} However, to date, a limited range of germplasm has been investigated for this condition.⁴¹ Increasing the chance of success of identifying beneficial traits of interest in plant collections through phenotyping approaches requires a diverse range of germplasm that maximizes variation between lines.⁴² This then raises the possibility of breeding from diverse lettuce sets to develop new tipburn-resilient cultivars. For example, tipburn-resilient varieties such as the leaf lettuce 'Hacheong' have been reported.⁴³ Quantitative trait loci (QTLs) for tipburn have been identified in a number of recombinant inbred line (RIL) mapping populations,^{44,45} yet very few genes with direct involvement in tipburn in lettuce have been identified to date.

Due to the influence of multiple environmental factors on the development of tipburn, it will likely prove necessary to develop phenotyping approaches that are tailored to reflect different commercial production systems and their associated growth conditions. Indoor hydroponic production of lettuce is an increasingly popular method of cultivation in both conventional glasshouse and controlled environment facilities; however field production is still widely used. Methods for rapid (approximately 28 days) phenotyping of tipburn have been reported but, to date, have focused on internal tipburn⁴⁶ or have used a limited range of germplasm.^{4,38,41,47,48} An assessment of resilience to external tipburn, which predominates in some growing environments, is therefore required. This study developed a hydroponic glasshouse tipburn assay to investigate external tipburn resilience in a wide range of germplasms. This assay was then used to phenotype a recombinant inbred line (RIL) mapping population and was compared to an in-field assay for this population. We hypothesized that the study populations would exhibit both genetic and environmental influences on tipburn resilience, which may lead to the identification of differing extreme lines between assays (indoor versus outdoor).

EXPERIMENTAL

Plant material

Seeds from the Vegetable Genetic Improvement Network (VeGIN) *Lactuca* diversity fixed foundation set (DFFS) and Saladin × Iceberg recombinant inbred line (RIL) mapping population (F8 generation) were obtained from the UK Vegetable Genebank at the University of Warwick Crop Centre (Wellesbourne, UK). The DFFS population was selected to maximize genetic, geographical origin, and phenotypic variability and contained 69 lines belonging to the domesticated *Lactuca sativa* species with an additional 17 lines belonging to the three wild relatives, *L. serriola*, *L. saligna*, and *L. virosa*. For the RIL mapping population, an F7 linkage map of 1040 cM covering all nine lettuce chromosomes was produced with 425 markers mapped. Lines of the DFFS were genetically fixed by rounds of inbreeding. Ninety-six lines of each of the two populations were used for experimentation. Together, these two populations represent important resources for this study due to their ability to maximize likelihood of trait discovery due to germplasm variability (DFFS) and ability to target loci contributing to tipburn resilience (RILs). The lettuce variety 'Little Gem' (Sutton's Seeds, Paignton, UK) was used as a control.

Glasshouse tipburn phenotyping

Lettuce lines were sown in '345' module trays containing Levington M2 compost (ICL, Ipswich, UK) and grown in temperature-controlled glasshouse facilities at Harper Adams University (HAU, Edgmond, UK) with a minimum day/night temperature of 15 °C/5 °C and supplemental high-pressure sodium lamp lighting set at a 16 h day where required. Seedlings were watered as required until the 2 true leaf stage, then transplanted into 65 × 65 × 80 mm plastic pots filled with coarse perlite (Westland Horticulture Ltd, Ellesmere Port, UK) over a thin base of gravel. The pots were submerged into 25 × 35 cm black plastic boxes (IKEA Ltd, Delft, Netherlands) with 65 × 65 mm holes in the lid of each box. Five pots were placed in each box. Each box was placed on a glasshouse bench in a frame constructed from Jablite Universal Insulation Board (B&Q UK Ltd, Eastleigh, UK) and filled with a 7 mL L⁻¹ solution of Ionic Hydrogrow (Growth Technology, Taunton, UK) HW hydroponic nutrient mixture (0.311 g kg⁻¹ nitrogen, 0.054 g kg⁻¹ phosphorus, 0.384 g kg⁻¹ potassium, 0.07 g kg⁻¹ calcium, 0.0002 g kg⁻¹ copper, 0.004 g kg⁻¹ iron, 0.001 g kg⁻¹ manganese, 0.0001 g kg⁻¹ molybdenum and 0.00025 g kg⁻¹ zinc) until the solution covered half of the depth of the pots. The nutrient solution was aerated using aquarium air bricks connected to a Resun Air 8000 aquarium pump using 6 mm outside diameter and 4 mm inside diameter tubing (Angels Aquatics, Sheffield, UK) and topped up with water as necessary. Tipburn was scored from 0 (absent), through mild (1) and moderate (3) to severe (5) (Fig. S1 in the supporting information). Plants were grown until the control variety (Little Gem, Sutton's seeds) showed a moderate tipburn score (3), by which time the rest of the population exhibited a spread of symptoms ranging from mild to severe (approximately 4 weeks from transplanting). The presence/absence of red pigmentation was also recorded. Plants were cut at the substrate surface level, the fresh weight was recorded, and then they were dried in an oven at 80 °C for 5 days, and the dry weight was recorded. One plant per line was used in each replicate of tipburn phenotyping and the entire experiment was repeated over time to obtain five replicates (four for the mapping population) to account for the influence of seasonal environmental variation on tipburn symptoms (Fig. S2A-

S2B in the supporting information). Samples of the nutrient solution were taken at the start and end of a trial replicate using the DFFS and they were analyzed for mineral content (L013 Standard Water with Alkalinity, NRM Laboratories, Bracknell, UK).

Field phenotyping

Lettuces were sown in commercial peat transplant blocks (G's Fresh Ltd, Barway, UK) and grown to the fourth true leaf stage in a polytunnel at HAU. Seedlings were then planted out into the field in groups of 12 plants per line in a 4 × 3 plant arrangement at 60 cm spacing, with 60 cm between adjacent lines in a completely randomized design. Three plantings were made on 16 June, 23 June, and 30 June 2015. For plantings 1–3, the mean daily dry bulb air temperature was 15.8, 15.8, and 15.4 °C, respectively and mean daily solar energy was 17.00, 15.59, and 14.72 MJ, respectively. Tipburn was scored as per glasshouse plants on 12 August, 26 August, and 4 September for each plant and calculated per line. The mean tipburn score across the population for each planting is shown in Fig. S2C in the supporting information. Scoring dates were chosen in order to obtain comparable symptom development.

Data analysis

All statistical analyses were performed using ANOVA and Pearson's correlation coefficient in GenStat 17th edition software (Genstat 17th Edition; VSN International Ltd, Hemel Hempstead, UK). Statistical analysis of each trial in the glasshouse and field settings took into consideration experimental design, replicate number, and hydroponic box (where relevant). Graphs indicate mean values ± 1 standard error of the mean (SEM) or one standard deviation (SD) (Fig. S1 in the supporting information). Means were compared using least significant difference (LSD) or Tukey's multiple comparison test at the 5% level.

Identification of QTLs

A genetic map of the Saladin × Iceberg mapping population was generated from Kompetitive Allele Specific PCR (KASP) marker data⁴⁹ using JoinMap 4.0 (Kyazma BV, Wageningen, Netherlands), based on an F8 RIL population with regression mapping using Kosambi's mapping function and grouping using an independence logarithm of odds (LOD). The parent lines were excluded from the map generation. A QTL analysis was performed using MapQTL6 (Kyazma BV) and mean tipburn score data. Interval mapping was first used to analyze the data for the presence of putative QTLs, followed by further analysis using cofactor markers in multiple QTL model (MQM) mapping of the data. The genome-wide statistical LOD score significance of 2.1, described by Van Ooijen (1999) for RIL populations of diploid species was used to highlight 'suggestive linkage' QTLs.

RESULTS AND DISCUSSION

Glasshouse-grown *Lactuca* DFFS

Assaying the *Lactuca* DFFS in the glasshouse hydroponic assay indicated a significant ($P < 0.001$) variation in tipburn resilience across the 96 lines of the DFFS and identified a number of lines exhibiting high levels of tipburn resilience (Fig. 1). When the DFFS lines were ranked by either mean or maximum tipburn observed, a number of lines were identified as being among the most resilient (Table 1). Such lines included several belonging to the cultivated species *Lactuca sativa*, plus a number of lines belonging to the wild species *L. serriola*, *L. saligna*, and *L. virosa*. These data

indicate the suitability of such populations for use in the identification of stress-resilient material that can be utilized in downstream breeding programs.

When the four *Lactuca* species were compared, *L. sativa* exhibited a significantly higher ($P < 0.05$) mean tipburn score than *L. saligna*, *L. serriola*, and *L. virosa* (Fig. 2, white bars). This result could partially reflect the significantly higher ($P < 0.05$) head density found for *L. sativa* in comparison with the other species (Fig. 2, black bars). Indeed, a significant correlation between tipburn score and head density was found across all 96 lines ($r = 0.56$, $P < 0.001$) and in *L. sativa* lines alone ($r = 0.51$, $P < 0.001$). A higher head density would be expected to result in increased humidity in the head as moisture is trapped between the leaves, reducing transpiration flow and calcium delivery to leaf margins, thereby increasing tipburn severity. Indeed, loose-leaf varieties of lettuce have been reported to contain higher levels of calcium than butterhead and romaine types.³¹ Furthermore, recently identified tipburn major QTLs in lettuce collocated with QTLs for head firmness.⁴⁵

The mean fresh weight of all three wild species and the dry weight of *L. saligna* and *L. virosa* (but not of *L. serriola*) was significantly lower ($P < 0.05$) than that of *L. sativa* (Fig. 3(A)), whereas the percentage dry weight content was significantly lower for *L. sativa* than for the three wild species (Fig. 3(B)). It is possible that these differences could also contribute to the difference in tipburn resilience observed between the species, the correlation between tipburn score and fresh weight, dry weight and % dry weight was $r = 0.40$ ($P < 0.001$), 0.26 ($P < 0.05$) and -0.25 ($P < 0.05$), respectively, providing significant but weaker correlations with tipburn score compared to head density. Fresh weight has previously been correlated with tipburn severity in other studies^{10,29,30} and may reflect the difficulty in delivering calcium to leaf margins further from the roots in larger plants.

However, analyzing the tipburn data for the *L. sativa* lines alone revealed significant variations in the tipburn symptom score ($P < 0.001$), head density ($P < 0.001$), fresh weight ($P < 0.05$) and dry weight ($P < 0.001$), but not percentage dry weight across this group of lines and the correlation between tipburn score and fresh weight or dry weight was $r = 0.35$ ($P < 0.01$) and 0.26 ($P < 0.05$), respectively. This indicates that not all variability in tipburn score across the DFFS is due to interspecies differences. Factors such as size variation between the lines may influence tipburn development but the coefficient of determination (r^2) between tipburn score and fresh weight, for example, in the *L. sativa* lines is 0.12, indicating that a large component of inter-line variation is due to other factors and does not merely reflect the influence of growth rate upon tipburn severity.

Comparing the leaf morphology of the lines across all four *Lactuca* species (data not shown) and for *L. sativa* DFFS lines alone (Fig. 4) revealed significant ($P < 0.05$) differences in tipburn resilience between morphology types for leaf width, margin, and color. Lines possessing narrower leaves, a serrated leaf margin, or lack of red (anthocyanin) pigment had significantly lower tipburn scores than lines with wider or smooth-edged leaves or leaves containing red pigmentation. In another study, a major tipburn QTL was found to collocate with a QTL for leaf crinkliness, amongst other traits.⁴⁵ Narrower leaves may contribute to lower tipburn by facilitating calcium delivery to leaf margins by reducing the length of transpiration stream from the roots. By comparison, serrated leaf margins and a lack of red pigmentation may affect the humidity of the local leaf external environment and biochemical pathways associated with leaf discoloration, such as the

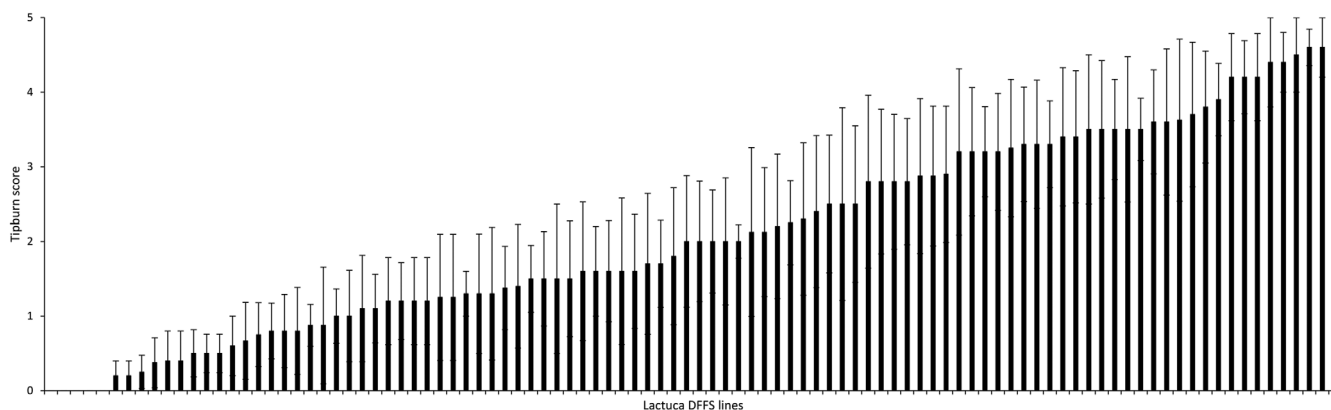


Figure 1. Mean tipburn score for the 96 diversity fixed foundation set (DFSS) lines showing the range of resilience present in this set (n = 5). Tipburn was scored from absent (0), through mild (1), moderate (3), to severe (5). Bars indicate means +/- 1 standard error of the mean (SEM).

Table 1. Lines from the *Lactuca* diversity fixed foundation set exhibiting the lowest mean and maximum tipburn scores. Lines shown are selected to have a mean tipburn score below 1 and a maximum tipburn score of 2 or lower (n = 5)

Accession no.	DFSS line no.	Species	Line name	Tipburn score (0–5)	
				Mean	Maximum
LJ10368	52	<i>L. sativa</i>	Kakichisha White	0	0
LJ14022	86	<i>L. serriola</i>	(no name)	0	0
LJ10404	88	<i>L. serriola</i>	(no name)	0	0
LJ14033	91	<i>L. serriola</i>	(no name)	0	0
LJ14041	96	<i>L. virosa</i>	(no name)	0	0
LJ10319	3	<i>L. serriola</i>	(no name)	0.2	1
LJ14038	94	<i>L. serriola</i>	(no name)	0.2	1
LJ10400	84	<i>L. serriola</i>	(no name)	0.25	1
LJ10411	95	<i>L. virosa</i>	(no name)	0.38	1.5
LJ14005	1	<i>L. sativa</i>	Saladin	0.4	2
LJ10381	65	<i>L. sativa</i>	White Lettuce	0.4	2
LJ10334	18	<i>L. sativa</i>	Waldermann's Dark Green	0.5	1.5
LJ10336	20	<i>L. saligna</i>	(no name)	0.5	1
LJ14006	22	<i>L. saligna</i>	(no name)	0.5	1
LJ14030	90	<i>L. serriola</i>	(no name)	0.6	2
LJ10409	93	<i>L. serriola</i>	(no name)	0.67	2
LJ10383	67	<i>L. sativa</i>	(no name)	0.75	2
LJ10363	47	<i>L. sativa</i>	(no name)	0.8	2
LJ10390	74	<i>L. sativa</i>	Simpson	0.8	2
LJ14007	26	<i>L. sativa</i>	Tardisix	0.88	1.5

phenylpropanoid pathway, for which anthocyanin biosynthesis forms an intermediate,⁵⁰ respectively.

Next, 96 recombinant inbred lines (RILs) of an F8 generation of a cross between the DFSS *L. sativa* Saladin (an iceberg type cultivar) and Iceberg (a Batavian type cultivar) lines, which had exhibited significantly ($P < 0.05$) different tipburn symptoms, were phenotyped for tipburn resilience (Fig. 5(A)). Due to a single Saladin plant exhibiting uncharacteristically severe tipburn in the RIL glasshouse assay, the difference in tipburn severity between the two parent lines (2.13) was not quite significant (LSD = 2.32, Fig. 5(A)); however, the results still revealed a significant ($P < 0.05$) variation in tipburn resilience across the 96 RILs tested, indicating the suitability of this population for the identification of QTLs contributing to resilience to tipburn in *L. sativa*. This

range of responses could be due to transgressive segregation in the mapping population. A QTL analysis of the glasshouse data revealed a suggestive small-effect linkage QTL on chromosome 1 (Table 2), accounting for 12.3% of the variation in tipburn resilience.

The role of calcium in tipburn development

Analysis of the nutrient solution indicated that only 22.1% of the calcium content of the solution had been depleted by the end of the experiment, with a final calcium concentration of 84.4 mg L⁻¹. This suggests that calcium availability was not the cause of external tipburn development in the assay presented here, agreeing with most,^{4,14} but not all,^{46,51} studies. Further investigation will aim to

determine the predominant genetic and molecular factors affecting tipburn resilience in this population.

These results underline the value of diversity sets for the identification of traits of interest through the provision of diverse germplasm. Assaying the population studied here identified a number of cultivated and wild relative lines with high levels of tipburn resilience, which may prove effective in the development of more durable varieties of lettuce for protected cultivation through crop breeding.

Field phenotyping of the F8 RIL mapping population

Finally, due to the known environmental sensitivity of tipburn development and different grower options for production, we assayed tipburn symptoms in the RIL mapping population in the field (Fig. 5(B)).

Tipburn severity of each of the parent lines differed in the field setting versus the glasshouse assay (Fig. 5(B)). In the field, the two parent lines were no longer significantly different from each other. However, again, the mapping population as a whole exhibited a significant ($P < 0.05$) range of tipburn scores across the 96 RILs examined. A QTL analysis of the field data revealed the presence of three suggestive small-effect linkage QTLs, this time on chromosomes 5, 6 and 8 (Table 2). Together, these three

small-effect QTLs account for 25.2% of the variation in tipburn resilience. The QTLs for tipburn in Canadian field-grown RILs from a cross between the iceberg type cultivars Emperor and El Dorado on chromosomes 2, 5 and 8, with a particularly strong effect QTL on chromosome 5, were previously identified.⁴³ In a more recent study of a number of RIL populations under a range of growth environments, two major tipburn QTLs were identified in linkage groups 1 and 5 of the cultivar Salinas, a variety similar to Saladina, responsible for up to 45% and 66% of the variation in phenotype, respectively.⁴⁵ The differences between these earlier studies and our own are likely to be due to the different parent lines and mapping populations used but also differences in the growing conditions and scoring system.

In earlier tipburn studies,^{4,7} only a limited subset of cultivars showed a consistent response between glasshouse and field. Another study was able to obtain comparable results between hydroponic and soil grown plants but in the same growing environment.⁴¹ The use of controlled environment growth chambers can increase the correlation with in-field data,⁵² but requires mature plants, limiting assay throughput and increasing cost. In this study, the QTLs identified in the glasshouse and field were located on different chromosomes. This suggests that different suites of genes contribute to tipburn resistance to different extents under particular growth settings. This may reflect the

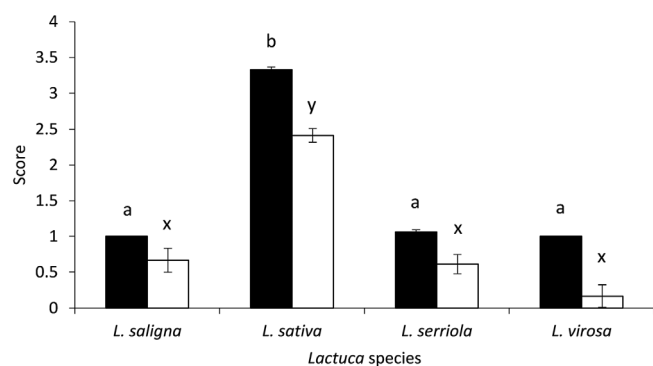


Figure 2. Mean tipburn (white bars) and head density (black bars) scores for the diversity fixed foundation set (DFFS) lines grouped by *Lactuca* species. Bars indicate means \pm 1 standard error of the mean (SEM). For each of the two variables, bars labeled with different letters are significantly different ($P < 0.05$).

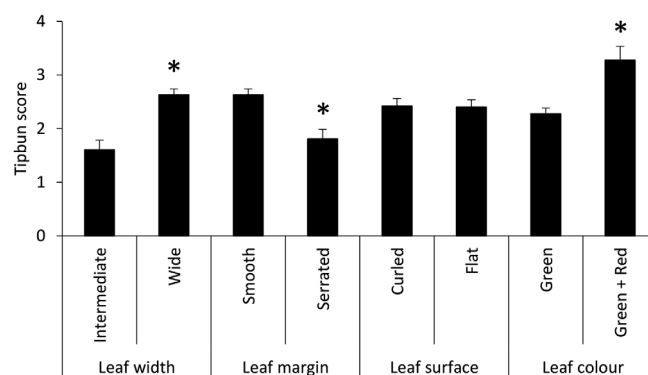


Figure 4. Mean tipburn score for the lettuce diversity fixed foundation set (DFFS) lines grouped by leaf morphology characteristics (data for *L. sativa* lines only). Bars indicate mean \pm 1 standard error of the mean (SEM). For each of the two variables, bars labeled with different letters or asterisks are significantly different ($P < 0.05$).

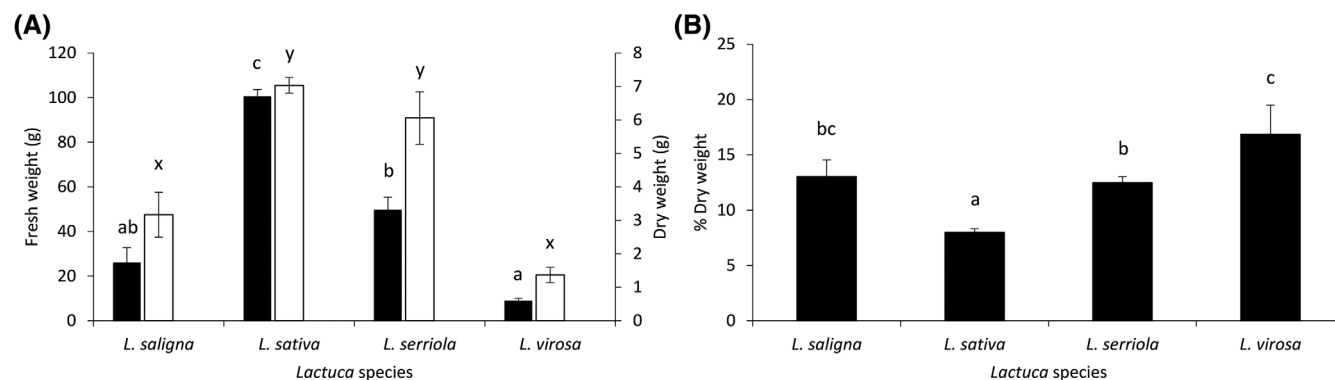


Figure 3. Fresh and dry weight analysis of the lettuce lines. (A). Mean fresh weight (black bars) and dry weight (white bars) for the lettuce diversity fixed foundation set (DFFS) lines grouped by *Lactuca* species. Bars indicate means \pm 1 standard error of the mean (SEM). For each of the two variables, bars labeled with different letters are significantly different ($P < 0.05$). (B). Mean percentage dry weight for the lettuce DFFS lines grouped by *Lactuca* species. Bars indicate mean \pm 1 SEM. For each of the two variables, bars labeled with different letters are significantly different ($P < 0.05$).

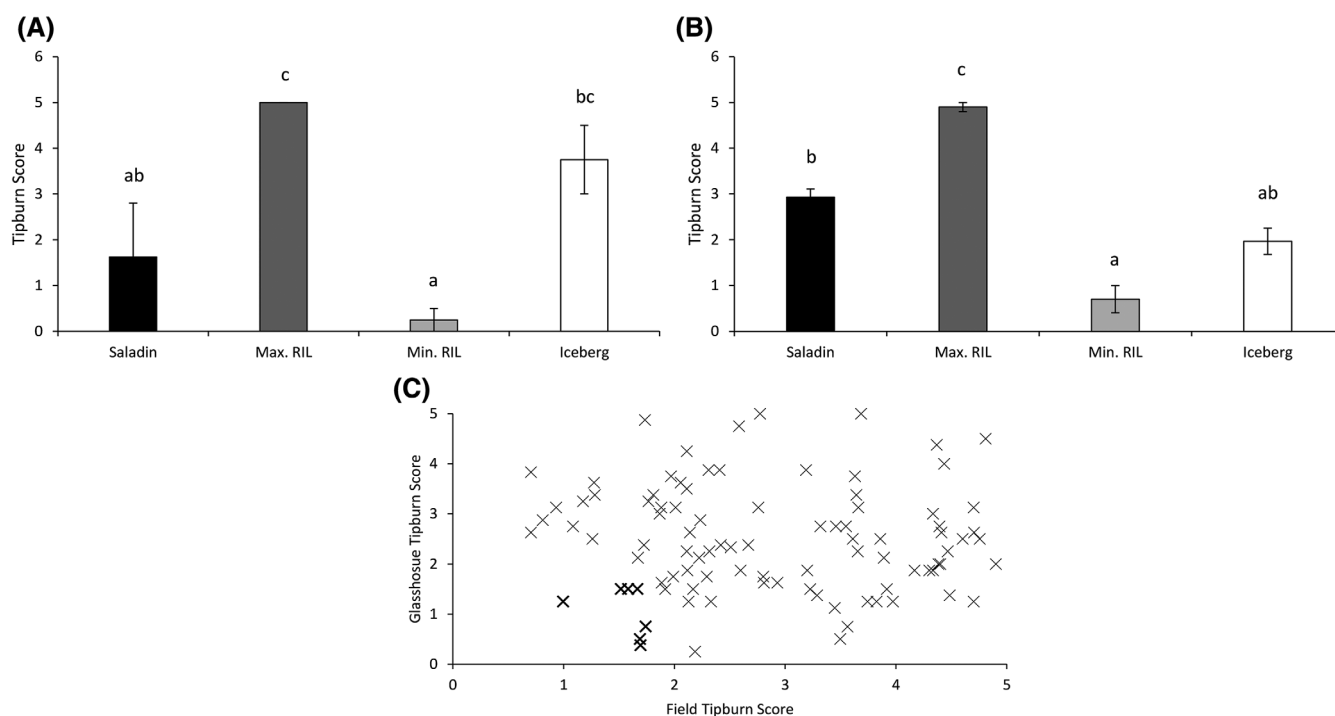


Figure 5. Tipburn scores for the parents and maximum and minimum recombinant inbred lines (RILs) of the Saladin × Iceberg mapping population, indicating the range of tipburn resilience present in this population ($n = 4$) in (A) glasshouse and (B) field conditions. Bars indicate mean \pm 1 standard error of the mean (SEM), bars labelled with different letters are significantly different ($P < 0.05$). (C) Correlation between mean field and glasshouse tipburn scores for RIL lines.

Table 2. Small-effect QTLs revealed in analysis of glasshouse-grown and field-grown lettuce belonging to the Saladin × Iceberg F8 RIL mapping population. U = unmapped

Environment	Chromosome	Linkage group	Position (cM)	Marker	LOD	1 LOD interval (cM)	Percentage variance explained	Contributing parent
Glasshouse	1	12	13.967	1_LS1_73;17	2.74	1.531	12.3	Iceberg
Field	8	6	22.377	8_LS1_381;46	3.62	0.32	10.2	Iceberg
	5	9	0	E35M47_191i	2.98	3.00	8.3	Saladin
	6	27	0	BLCL-OP3	2.45	1.36	6.7	Iceberg

relative importance of different factors contributing to tipburn development in indoor versus outdoor production, such as humidity and temperature. Furthermore, in the present study, four lines amongst those with the lowest tipburn scores in the field were amongst those with the highest scores in the glasshouse and three lines that had amongst the lowest scores in the glasshouse were amongst the highest scores in the field. The overall coefficient of determination of tipburn score between glasshouse and field-grown RILs was only 0.001, indicating no overall correlation between symptoms in the glasshouse and field across the population as a whole (Fig. 5(C)). However, it should also be noted that, of the 20 most resilient lines selected from each growing environment, seven lines were found to match between the glasshouse and field, suggesting the presence of environmentally independent genetic contributions to tipburn development (shown in bold in Fig. 5(C)).

These data confirm that, in agreement with earlier studies,² tipburn development is highly environmentally dependent and exhibits genotype (G), environment (E), and genotype × environment (G × E) effects. For this reason, we suggest that phenotyping

assays used to investigate such environmentally sensitive traits should be designed to reflect the commercial production system for which their data will be utilized.

CONCLUSIONS

This investigation has indicated the suitability and value of diverse *Lactuca* germplasm resources for the identification of significant variation in traits such as tipburn resilience. Several tipburn-resilient lines have been highlighted in the *Lactuca* DFFS, belonging to both the cultivated species and to wild relatives (Fig. 1). These represent promising resources for future breeding programs. The investigation has also revealed the influence of leaf morphological characteristics on tipburn development. Phenotyping of tipburn in a lettuce mapping population in glasshouse and field environments revealed putative tipburn QTLs for future investigation into the underlying mechanistic basis of tipburn occurrence. Finally, this study highlights the importance of tailoring assay design to chosen production systems when phenotyping environmentally sensitive traits.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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