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PLANT BREEDING

# Selection of biofortified mini lettuce progenies resistant to *Pectobacterium carotovorum* subsp. *carotovorum*

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**ABSTRACT.** Brazilian consumers are increasingly seeking unique vegetable products, such as mini lettuces. However, their production is hampered by a wide variety of pathogens, including *Pectobacterium carotovorum* subsp. *carotovorum*, which causes soft rot in lettuce. In this study, we aimed to select biofortified mini lettuce genotypes with good agronomic traits and resistance to *P. carotovorum*. A randomized block design consisting of 12 treatments, conducted in a field and greenhouse (Federal University of Uberlândia [UFU]), was used to select biofortified mini lettuce with good agronomic traits: SPAD index, stem and plant diameter, stem length, plant height, number of leaves, fresh weight, and bolt resistance. A completely randomized design consisting of 13 treatments was used for resistance or susceptibility testing. The bacterial suspension was inoculated and the following were assessed: disease severity, 16 days after inoculation; area under the disease progress curve, calculated separately at 4, 8, 12, and 16 days after inoculation as well as the sum of data calculated on all the assessment days; and the disease resistance class. The genotypes UFU 215#1 and UFU 215#2 had significantly high carotenoid concentrations. The genotypes UFU 66#4, UFU 215#1, and UFU 215#7 showed high bolt resistance. All genotypes were classified as resistant or moderately resistant to the *P. carotovorum* isolate, UFU A7.

**Keywords:** *Lactuca sativa*; soft rot; carotenoids; plant pathogenic bacteria.

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## Introduction

Lettuce (*Lactuca sativa* L.) is the most widely consumed and economically important leafy vegetable in Brazil (Gnoatto, Guerrra, Dantas Neto, Silva, & Ferruzzi, 2018; Gusatti, Zanuzo, Machado, Vieira, & Cavalli, 2019). The year 2019 was profitable for the sector, making lettuce a highly promising crop. Additionally, estimates for 2020 project a 12% increase in the cultivated area of lettuce-producing regions (Carvalho, Kist, & Beling, 2020).

The ease of acquisition of lettuce products, a pleasant taste, low production costs, and Brazilians' pursuit of a healthier lifestyle have led to its extensive production and widespread consumption. Lettuce can help prevent several diseases related to oxidative stress (Rocha & Reed, 2014) due to its high carotenoid content (Cassetari et al., 2015).

Carotenoids, which are precursors of vitamin A, can be found in high concentrations in the leaves of the dark green or purple lettuce varieties (Freitas et al., 2015).

As such, production of carotenoid-rich cultivars could contribute to human health through disease prevention by improvement of the nutraceutical properties of lettuce. Studies in Brazil aim to obtain cultivars that are able to grow in a range of environmental conditions and are resistant to disease-causing pathogens. These efforts contribute significantly to the sustainability of lettuce farming (Sala & Costa, 2012).

*Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of soft rot, is considered one of the most scientifically and economically important phytobacteria (Mansfield et al., 2012). The disease is difficult to control due to the wide variety of host plants and the ability to survive in crop residues (Félix, Oliveira, Mariano, & Souza, 2014). As such, the use of resistant cultivars is an effective alternative to pesticides, as they have a lower associated production cost and environmental impact. However, the lack of information on cultivars resistant to this pathogen has hampered this practice.

In this study, we aimed to identify biofortified mini lettuce progenies resistant to soft rot with desirable agronomic traits for commercialization.

## Material and methods

The experiments were conducted at the Experimental Vegetable Station of the Federal University of Uberlândia (UFU), Monte Carmelo Campus, Minas Gerais State, Brazil (18°43'36,03" S, 47°31'28,59" N, and 903 m a.s.l.). According to Köppen's classification system, the climate in the region is wet and temperate, with hot summers and dry winters.

The experiments consisted of two different stages: agronomic assessment of the mini lettuce genotypes and selection of genotypes resistant to *P. carotovorum* subsp. *carotovorum*.

Experiments for agronomic assessment were conducted in two growing seasons: fall/winter (GS1), from May 4 to July 30, 2019; and spring/summer (GS2), from November 14, 2019, to February 20, 2020.

A randomized block design was used, with 12 treatments and four repetitions. The treatments consisted of 11 genotypes from the $F_{5:6}$ line obtained by crossing the Uberlândia 10,000 (UDI 10,000) line and Pira 72 cultivar (Belíssima), namely, UFU 66#3, UFU 66#4, UFU 66#7, UFU 66#8, UFU 215#1, UFU 215#2, UFU 215#6, UFU 215#7, UFU 215#10, UFU 215#13, and UFU MC MINIBIOFORT2; and the Purpurita mini lettuce cultivar. The genotypes belonged to the UFU Vegetable Germplasm and were previously selected for their high leaf carotenoid content.

Each experimental plot contained four rows with a length of 1.05 m, spaced 0.15 m apart with 0.15 m distance between each plant. Ten plants from the two center rows of each plot were considered for assessment (study area).

Seeds were planted in 200-cell expanded polystyrene trays filled with Maxfertil® coconut husk fiber substrate. The seedlings were kept in a greenhouse (7 × 4 m) covered with clear UV-resistant plastic (150 micra) until transplantation.

When the seedlings displayed three to five true leaves, they were transplanted into the plots. Prior to transplanting, liming was performed in the experimental area of the spring/summer crop to raise the base saturation to 70%. For both the fall/winter and spring/summer experiments, base dressing consisted of 30 kg $ha^{-1}$ of N, 300 kg $ha^{-1}$ of $P_2O_5$, and 18 kg $ha^{-1}$ of $K_2O$, based on soil analysis results and the recommendations of Fontes (1999), with the top dressing consisting of 30 kg $ha^{-1}$ of N and 18 kg $ha^{-1}$ of $K_2O$ at 15 days after base dressing, and 45 kg $ha^{-1}$ of N and 27 kg $ha^{-1}$ of $K_2O$ at 30 and 45 days after transplanting (DAT) (Fontes, 1999).

Harvesting was performed when the plants reached maximum vegetative development, and the following agronomic traits were assessed: stem diameter (mm), plant diameter (cm), stem length (cm), plant height (cm), number of leaves per plant, and fresh weight (g). Part of the plots for all the treatments remained in the field to determine bolt resistance, namely the number of days from transplanting to tassel emergence. Bolting could not be assessed in genotype UFU 215#10 because the plants died from diseases before bolting occurred.

One day before harvesting, the chlorophyll content was measured at dawn in 10 plants from the study area of each plot, at four points in the middle third of each plant in recently mature leaves, using a SPAD chlorophyll meter (Minolta SPAD-502 CFL1030).

Experiments to test for resistance/susceptibility to *P. carotovorum* subsp. *carotovorum* were repeated twice, with the first carried out from May 4 to July 27, 2019 (fall/winter – GS1) and the second from November 14, 2019 to March 7, 2020 (spring/summer – GS2).

The *Pectobacterium carotovorum* subsp. *carotovorum* isolate used in this study, UFU A7, was collected on March 10, 2009, and was preserved in the UFU Plant Bacterial Laboratory (LABAC). The isolate was identified using biochemical methods and has not been used in any other literature study previously.

The isolate was grown in bacterial screening medium 523 (10 g sucrose, 8 g hydrated casein, 4 g yeast extract, 2 g biphasic potassium phosphate, 0.3 g magnesium sulfate, 15 g agar, and 1000 mL distilled water) for 48h at 28°C (Kado & Heskett, 1970).

The bacterial suspension was prepared in sterile filtered water and the suspension concentration was adjusted using a spectrophotometer at 570 nm to $A_{570} = 0.36$, corresponding to approximately $1 \times 10^9$ colony forming units (CFU) $mL^{-1}$.

A completely randomized design was used, with 13 treatments and three repetitions. Treatments consisted of the same 11 biofortified lettuce genotypes from the $F_{5:6}$ line used in agronomic trait assessment, and two commercial lettuce cultivars (Vitória de Santo Antão–considered moderately resistant according to Félix et al. (2014) and Purpurita — considered susceptible to Feltrin®).

Each experimental plot comprised three pots with three plants each and one pot containing the control (without bacterial suspension application).

Planting was performed as described for the agronomic assessment experiment, and at around 20 days after planting (DAP), the seedlings were transplanted into 5 L plastic pots containing substrate, soil, and sand at a ratio of 1:1:1 (v/v).

At 35 DAP, the plants were inoculated in the central region of the stem with 50 μL of bacterial suspension, using a Descarpack® insulin syringe, in line with the methodology of Mello, Silveira, Viana, Guerra, and Mariano (2011), with some adaptations. The inoculation region was marked with a toothpick to ensure that all the cuts made for lesion assessment were in the same direction.

After inoculation, the plants were placed in plastic bags in the humidity chamber of a greenhouse for 12h. Then, they were watered frequently to keep the soil moist, to favor disease development.

To assess lesion length, the plants were cut longitudinally, parallel to the direction of inoculation, and the following were assessed:

a) disease severity: assessed 16 days after inoculation, according to the rating scale proposed by Ren, Petzoldt, and Dickson (2001), with adaptations ranging from 1 to 8 (1 = no lesions at the inoculation site; 2 = lesions smaller than 5 mm; 3 = lesions between 5 – 10 mm; 4 = lesions larger than 10 mm, but not reaching the leaves; 5 = lesions larger than 10 mm, reaching the leaf blade near the inoculation site; 6 = lesions larger than 10 mm, reaching leaves near and far from the inoculation site; 7 = whole plant near death; and 8 = dead plant).

b) area under the disease progress curve (AUDPC): calculated separately at 4, 8, 12, and 16 days after inoculation, as well as the sum of data of all the assessment days, using the formula $AUDPC = \sum[((y1 + y2)/2) \times (t2 - t1)]$, where $y_1$ and $y_2$ are two consecutive assessments of the proportion of damaged tissue performed at times $t_1$ and $t_2$, respectively.

c) disease resistance class (DRC): from the sum of AUDPC scores obtained on all the assessment days; the genotypes were grouped into disease resistance classes of 1 to 9, where 1.0 – 2.0 = resistant (R), 2.01 – 4.00 = moderately resistant (MR), 4.01 – 7.0 = susceptible (S), and 7.01 – 9.00 = highly susceptible (HS).

The data from both experiments were tested to determine whether they met the ANOVA assumptions of residual normality, homogeneity of variances, and additivity at 1% probability.

Once the assumptions were met, a joint analysis was conducted for the agronomic assessment experiment and, when significant, the agronomic traits were submitted to decomposition and subsequently compared using the Scott-Knott test at 5% significance.

For resistance/susceptibility to *P. carotovorum* subsp. *carotovorum*, when the assumptions were met, treatment means for disease severity and the sum of AUDPC scores were compared using the Scott-Knott test at 5% significance. Statistical analyses were performed using the R Core Team (2019).

For each of the assessment days in both growing seasons, graphs were created to illustrate disease progression, as demonstrated by the area under the disease progression curve. Bidirectional graphs comparing all the genotypes with the Purpurita (considered susceptible by the Feltrin® company) and Vitória de Santo Antão cultivars (considered moderately resistant according to Félix et al., 2014). The figures were compiled using SigmaPlot® software version 10 (Systat Software Inc., 2006).

## Results and discussion

Significant interactions were observed between the genotypes and growing seasons for all agronomic variables assessed (Figures 1 and 2), except for shoot fresh weight (Figure 2D).

Larger stem diameters were observed in the fall/winter crop than in the spring/summer crop (Figure 1A). The stem diameter varied by 56% between the commercial Purpurita cultivar (9.42 mm) and genotype UFU 66#3 in the fall/winter crop (Figure 1A).

Similar behavior was observed for plant diameter: the fall/winter crop exhibited larger dimensions when compared to the spring/summer counterpart for all of the genotypes assessed (Figure 1B). The genotypes UFU 66#3, UFU 66#4, UFU 66#7, UFU 66#8, UFU 215#2, UFU 215#6, UFU 215#7, UFU 215#13, and UFU MC MINIBIOFORT2, displayed larger plant diameters than those observed in the Purpurita cultivar (Figure 1B), which is considered the market standard among mini lettuce cultivars. The plant diameters in the present study were similar to those reported by Takahashi and Cardoso (2014) with the Sartre cultivar (16.31 cm).

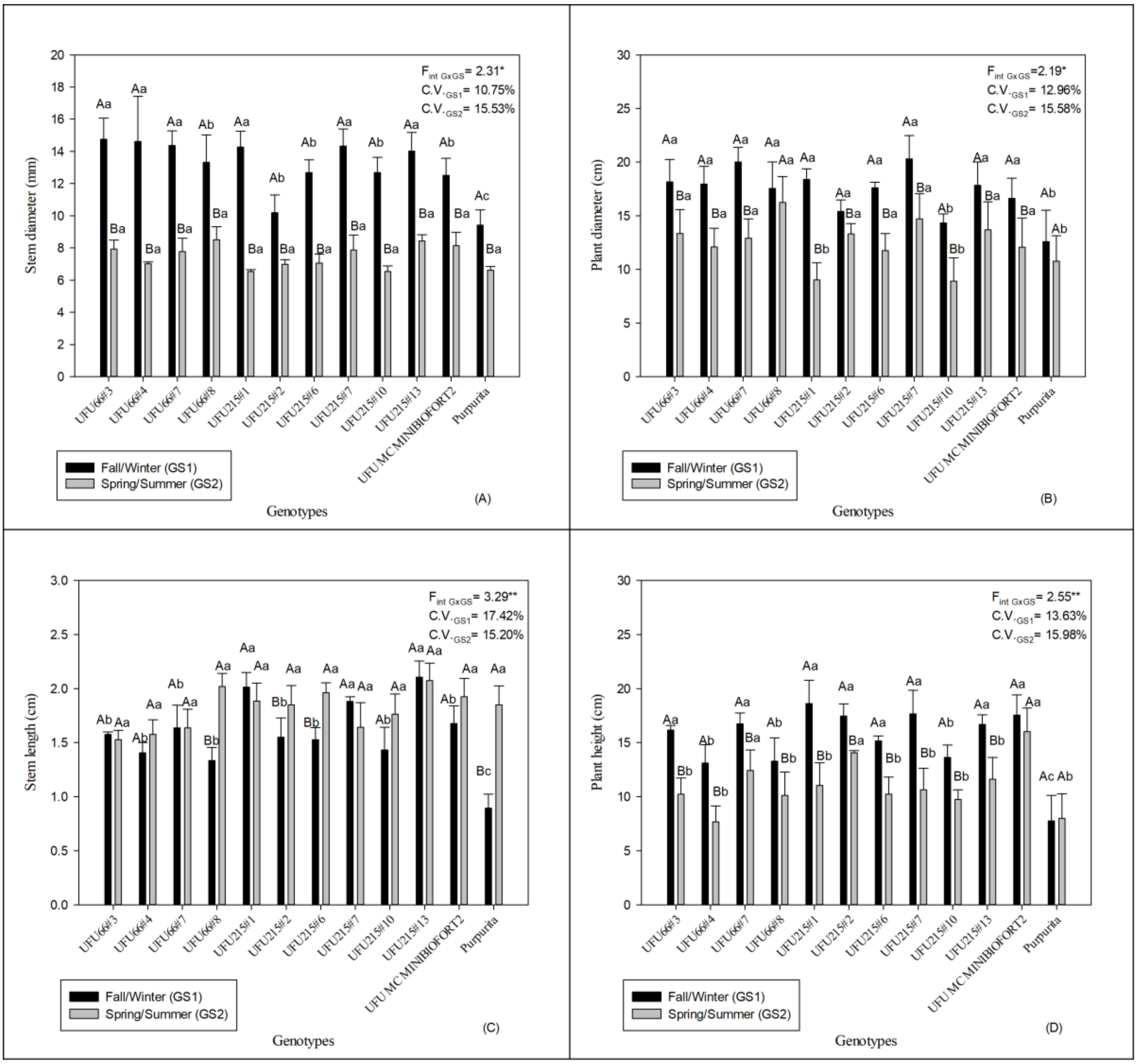


**Figure 1.** Average interaction decomposition values for stem diameter (mm) (A), plant diameter (cm) (B), stem length (cm) (C), and plant height (cm) (D) of 11 biofortified mini lettuce genotypes and the Purpurita mini lettuce cultivar in two growing seasons (GS1 = fall/winter, GS2 = spring/summer). Monte Carmelo, Minas Geris State, Brazil, UFU, 2019–2020. Uppercase letters on the bars compare the growing seasons of each genotype and lowercase letters the genotypes in each growing season.

The stem lengths of UFU 66#3, UFU 66#4, UFU 66#7, UFU 215#1, UFU 215#7, UFU 215#10, UFU 215#13, and UFU MC MINIBIOFORT2 were not influenced by the growing season (Figure 1C), indicating a possible high tolerance to early bolting. When bolting occurs, the stem length increases, resulting in an overall increase in plant height, within certain limitations. Stem length is related to bolting; as the plant begins to flower, the stem elongates until the tassel emerges (known as bolting). Thus, stem length is an important trait to assess when selecting tropical genotypes (Resende, Costa, Yuri, & Mota, 2017). According to a previous study, the shorter the stem, the higher the quality of the vegetable for commercialization and the greater the resistance to bolting (Resende et al., 2017).

In the fall/winter season, plants were taller in all the genotypes assessed, except for the Purpurita cultivar and UFU MC MINIBIOFORT2 (Figure 1D). Genotypes UFU 66#7, UFU 215#2, and UFU MC MINIBIOFORT2 were taller than the other genotypes in both growing seasons (Figure 1D). Similarly, a larger number of leaves were observed in the fall/winter crop than in the spring/summer crops in all genotypes (Figure 2A). In both growing seasons, the genotypes developed at the Federal University of Uberlândia (UFU) produced more leaves than the Purpurita cultivar (Figure 2A), an important quality trait in mini lettuce.

Because of the larger stem diameter, number of leaves, and/or plant diameter in the fall/winter crop (Figures 1A, 1B, and 2A) for all the genotypes assessed, shoot fresh weight in the fall/winter crop was higher than that in the spring/summer crop (Figure 2D). Genotypes UFU 66#3, UFU 66#7, UFU 215#7, and UFU 215#13 exhibited the highest shoot fresh weight compared to the standard Purpurita cultivar, in both growing seasons (Figure 2D), indicating their high adaptability and phenotypic stability. The aforementioned genotypes are quite promising, since their biometric traits are similar to those of several commercial cultivars, which were assessed and reported by Castoldi, André, Braz, and Charlo (2012) and Takahashi and Cardoso (2014).

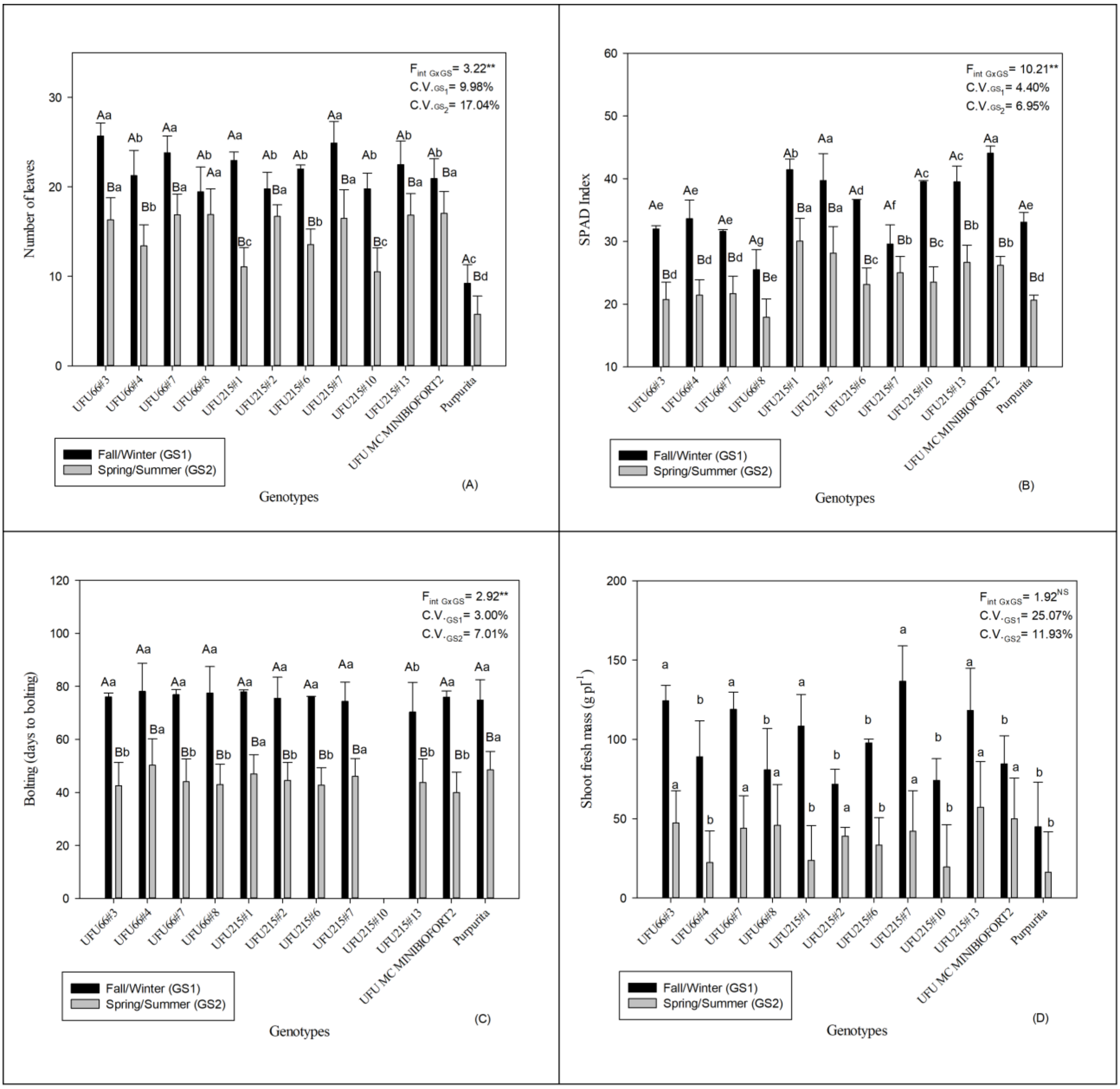


**Figure 2.** Average interaction decomposition values for number of leaves per plant (A), SPAD index (B), bolting (C), and fresh weight (D) of 11 biofortified mini lettuce genotypes and the Purpurita mini lettuce cultivar, in two growing seasons (GS1 = fall/winter, GS2 = spring/summer). Monte Carmelo, Minas Geris State, Brazil, UFU, 2019–2020. Uppercase letters on the bars compare the growing seasons of each genotype and lowercase letters the genotypes in each growing season.

It is important to emphasize that mini lettuce plants should have the same appearance as a standard lettuce plant, albeit with smaller sizes. Thus, stem and plant diameter, stem length, plant height, and number of leaves are important in establishing the architecture and appearance of mini lettuce plants, as well as being indicators of susceptibility to early bolting. Santana, Santi, Dallacort, Santos, and Menezes (2012) reported that the larger the stem diameter, the larger the plant architecture overall. However, plant architecture requires a more in-depth assessment, taking into account the number of leaves, plant diameter, and height, since together they provide specific size and compactness traits of mini lettuce. Thus, obtaining compact plants involves selecting genotypes with adequate stem and plant diameter, associated with a relatively reduced height and 15 or more leaves per plant, as they ensure plants with good commercial appearance. The abovementioned characteristics associated with shoot fresh weight and adaptation to tropical conditions are the main selection criteria for mini lettuce in Brazil. In the present study, genotypes UFU 66#3, UFU 66#7, UFU 215#7, and UFU 215#13 were the most promising commercial cultivars.

In addition to these biometric traits, one of the main goals of breeding programs is to obtain tropical lettuce cultivars, characterized by resistance to bolting, allowing plants to adapt to the high temperatures and rainfall common in the tropical regions of Brazil (Sala & Costa, 2012). In this respect, genotypes UFU 66#4, UFU 215#1 and UFU 215#7 as well as cv. 'Purpurita' showed the greatest tolerance to early bolting, taking around 46 to 50 days to bolting in spring/summer. All the cultivars exhibited the same behavior in the fall/winter growing season (Figure 2C) and required 40 or more days for flowering to occur in spring/summer (Figure 2C), indicating that all of the genotypes are tolerant to early bolting, given that the mini lettuce crop cycle is less than 40 days in total. Bolting involves premature emergence of a flowering stem, which occurs when days are long and temperatures high, making them unfit for commercialization due to the production of latex, which is associated with a bitter taste (Aquino, Seabra Junior, Camilli, Diamante, & Pinto, 2014).

The SPAD index was used to indirectly measure leaf chlorophyll content (Cassetari et al., 2015). UFU 215#2 and UFU MC MINIBIOFORT2 cultivated in fall/winter and UFU 215#1 and UFU 215#2 cultivated in spring/summer had a significantly higher chlorophyll content compared to the remaining genotypes (Figure 2B), therefore these genotypes are promising in terms of biofortification. The biofortification of these genotypes has been confirmed in other studies (Jacinto et al., 2019; Maciel et al., 2019). Additionally, UFU MC MINIBIOFORT2 has been registered with the Ministry of Agriculture and Livestock (Process number 35967) as a mini lettuce cultivar biofortified in carotenoids.

However, all the genotypes studied here can be considered biofortified since their SPAD indices ranged from 25.48 (UFU 66#8) to 44.06 (UFU MC MINIBIOFORT2) in fall/winter and 17.91 (UFU 66#8) to 30.04 (UFU 215#1) in spring/summer (Figure 2B), which was higher than those recorded for the UDI 10,000 line, which is used as a standard for carotenoid content in a number of studies (Jacinto et al., 2019; Maciel et al., 2019). Given the correlation between total chlorophyll and total carotenoids (Klooster, Cregg, Fernandez, & Nzokou, 2012), all of the genotypes can be selected for breeding programs targeting carotenoid-rich lettuce.

The agronomic performance of the genotypes in the fall/winter crop was found to be higher to that of the spring/summer counterparts. This finding can be attributed to the Mediterranean origin of lettuce, as they are adapted to temperate climates. Since temperatures during the fall/winter season varied between 14 – 23°C with no rainfall, and those in spring/summer ranged between 23 – 29°C with rain, the climate conditions in fall/winter are more similar to the Mediterranean environment, resulting in better development despite the smaller size of the lettuce.

However, it is important to emphasize that in both growing seasons, all genotypes performed better than cv. 'Purpurita', especially UFU 66#3, UFU 66#7, UFU 215#7 and UFU 215#13. This is related to the fact that these genotypes were previously bred and selected by the Federal University of Uberlândia (UFU) for their high carotenoid content and in order to adapt them to tropical conditions. However, to add more value to these genotypes and to commercialize them as cultivars, they should also exhibit high resistance to *P. carotovorum* subsp. *carotovorum*, which is one of the main diseases in the summer lettuce crops of Brazil, providing the benefit of requiring less pesticide.

The assessment of resistance to *P. carotovorum* subsp. *carotovorum* indicated no significant statistical difference between treatments ($p > 0.05$) for AUDPC in either growing season (Table 1).

Although mean AUDPC values did not differ between treatments, the average results were between 24.00 and 29.11 (fall/winter) and 27.55 and 29.56 (spring/summer) (Table 1), considered promising when compared to the findings of Silva et al. (2012), who obtained AUDPC values from 68.10 to 132.30 for *P. carotovorum* subsp. *carotovorum* in curly lettuce.

Disease quantification based on the AUDPC has been used for several pathosystems, such as *Capsicum* × *Sclerotium rolfsii* (Silva, Carvalho, Silva, Lins, & Oliveira, 2014) and onion × *Peronospora destructor* (Alves et al., 2018), with low values indicating possible resistance.

Soft rot is considered one of the most destructive diseases in lettuce crops and is most prevalent in summer under high temperatures (close to 30°C) and soil moisture content (Nazerian, Sijan, Meor Ahmad, & Vadamalai, 2013). This is evident in the results obtained here, since the disease caused greater damage in the spring/summer crop, with UFU 215#2, UFU 215#7, and UFU 215#10 displaying resistance in fall/winter, but moderate resistance in spring/summer (Table 1).

**Table 1.** Average values of sum of the scores in the area under the disease progress curve (AUDPC) for all the assessment days, severity (SEV), and disease resistance class (DRC) of 11 biofortified mini lettuce genotypes and two lettuce cultivars in two growing seasons. Monte Carmelo, Minas Geris State, Brazil, UFU, 2019–2020.

| | Fall/Winter (GS1) | | | Spring/Summer (GS2) | | |
|---|---|---|---|---|---|---|
| Genotype | AUDPC | SEV | DRC | AUDPC | SEV | DRC |
| UFU 66#3 | 26.00 a | 1.89 b | R | 28.89 a | 2.00 b | R |
| UFU 66#4 | 26.89 a | 2.33 a | MR | 29.56 a | 2.78 a | MR |
| UFU 66#7 | 26.45 a | 1.89 b | R | 27.55 a | 2.00 b | R |
| UFU 66#8 | 28.00 a | 2.20 a | MR | 29.11 a | 2.11 b | MR |
| UFU 215#1 | 26.00 a | 1.67 b | R | 28.44 a | 2.00 b | R |
| UFU 215#2 | 24.00 a | 2.00 b | R | 28.67 a | 2.11 b | MR |
| UFU 215#6 | 28.67 a | 2.33 a | MR | 27.56 a | 2.00 b | R |
| UFU 215#7 | 27.56 a | 2.00 b | R | 28.44 a | 2.22 b | MR |
| UFU 215#10 | 27.33 a | 1.89 b | R | 28.66 a | 2.56 a | MR |
| UFU 215#13 | 29.11 a | 2.11 a | MR | 28.22 a | 2.11 b | MR |
| UFU MC MINIBIOFORT2 | 24.45 a | 2.00 b | R | 27.56 a | 2.00 b | R |
| Purpurita | 26.89 a | 2.11 a | MR | 29.55 a | 2.78 a | MR |
| Vitória de Santo Antão | 27.78 a | 1.89 b | R | 28.00 a | 2.00 b | R |
| CV(%) | 8.44 | 8.52 | - | 5.58 | 10.77 | - |

Means followed by the same letter are not significantly different according to the Scott- Knott test at 5% probability.

Genotypes UFU 66#3, UFU 66#7, UFU 215#1, and 'UFU MC MINIBIOFORT2' and cv. 'Vitória de Santo Antão' exhibited resistance in both growing seasons, with average severity values between 1.67 and 2.00 (Table 1). Félix et al. (2014) also found that cv. 'Vitória de Santo Antão' was a promising source of stable and durable resistance to soft rot, despite being classified as moderately resistant.

All the genotypes studied obtained severity scores from 1.67 to 2.33 (fall/winter) and 2.00 to 2.78 (spring/summer) (Table 1), that is, values lower than those reported by Félix et al. (2014), who found average values between 2 and 4. The average severity values were higher in spring/summer than in fall/winter because temperature and humidity are higher, promoting the emergence of soft rot symptoms.

In general, the disease evolved significantly between four and eight days after inoculation and stabilized thereafter for UFU 66#7, UFU 215#7, and UFU 215#10 in fall/winter (Figure 3A) and UFU 66#3, UFU 66#7, UFU 66#8, and UFU 215#6 in spring/summer (Figure 3B).

Figures 4 and 5 show the relative differences in AUDPC for the genotypes studied on each assessment day, based on the reference value for AUDPC obtained for cv. 'Purpurita', considered susceptible to Feltrin® (Figure 4) and cv. 'Vitória de Santo Antão', considered moderately resistant according to Félix et al. (2014) (Figure 5).

In both the fall/winter and spring/summer experiments, only the genotype UFU MC MINIBIOFORT2 exhibited values less than or equal to those of the Purpurita cultivar on all of the assessment days (Figure 5A and B), which may have caused this genotype to be classified as resistant in both experiments (Table 1).

When compared to cv. 'Vitória de Santo Antão' (considered moderately resistant according to Félix et al., 2014), the genotypes UFU 66#3, UFU 215#1, UFU 215#2, and UFU MC MINIBIOFORT2 were considered promising for their low AUDPC values in fall/winter in relation to the Vitória de Santo Antão cultivar (Figure 5A and B).

In the spring/summer experiment at 16 DAI, all genotypes displayed AUDPC values lower than or equal to cv. 'Vitória de Santo Antão', with the exception of UFU 66#4 and UFU 215#10.

Although reports of soft rot-resistant lettuce genotypes and cultivars are rare (Silva, Mariano, Michereff, Silveira, & Medeiros, 2007), UFU 66#3, UFU 66#7, UFU 215#1, UFU 215#6, and UFU MC MINIBIOFORT2 showed potential for use in lettuce breeding programs because of their resistance to *Pectobacterium carotovorum* subsp. *carotovorum* (Table 1) and were superior to cv. 'Purpurita' and 'Vitória de Santo Antão' (Figures 4 and 5).

These data corroborate those of Félix et al. (2014), who reported four lettuce cultivars as a source of resistance against a moderately virulent *P. carotovorum* subsp. *carotovorum* isolate, while Silva et al. (2007) observed variety-based resistance in cv. 'Verdinha' and 'Salad Bowl,' which exhibited a low disease incidence.

As such, soft-rot resistant genotypes are promising for future breeding programs. Additionally, investigating sources of resistance may be the most viable control method, particularly for leafy vegetables.

Given that all the genotypes developed by the Federal University of Uberlândia and those assessed in this study are considered biofortified, the most promising genotypes for commercialization showed better agronomic performance in both growing seasons and high resistance to *P. carotovorum*, mainly in summer. In this respect, genotypes UFU 66#3, UFU 66#7, and UFU MC MINIBIOFORT2 stand out because they display all three characteristics of the same genotype.

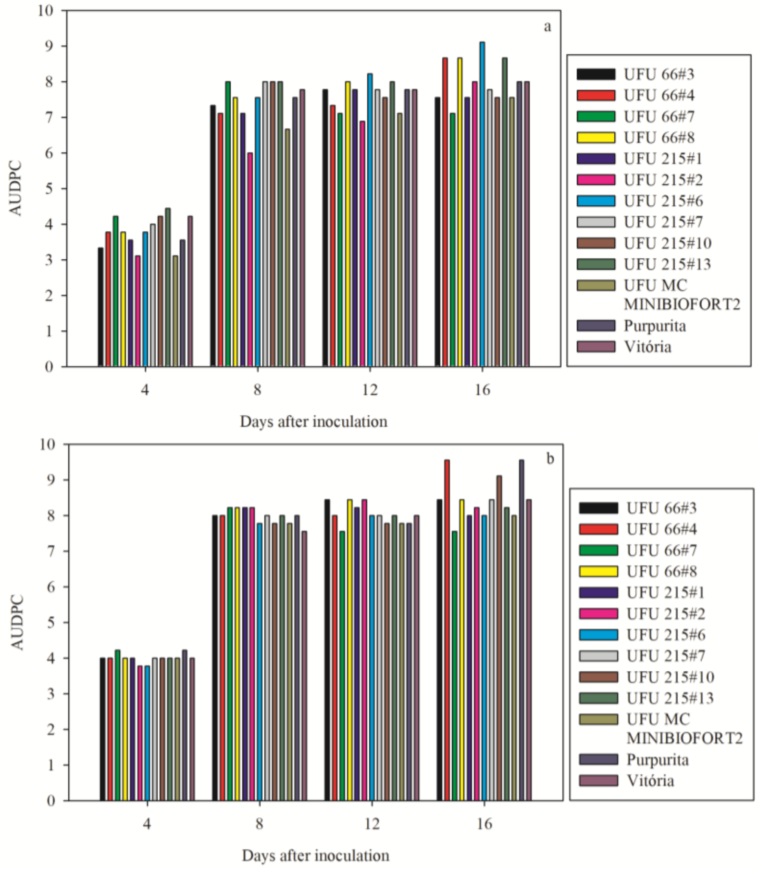


**Figure 3.** Disease progression, demonstrated by the area under the disease progress curve (AUDPC) on each of the assessment days, for 11 genotypes and two mini lettuce cultivars. (a) Experiment conducted between May 2019 and July 2019 (fall/winter); (b) Experiment conducted between November 2019 and March 2020 (spring/summer).

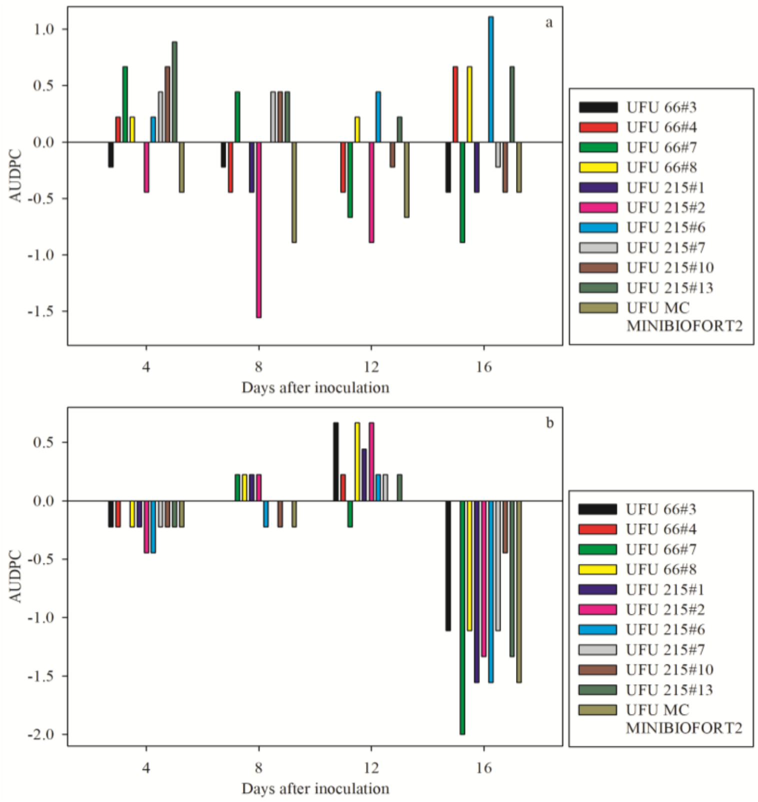


**Figure 4.** Bidirected graph comparing all the genotypes with the susceptible Purpurita cultivar for area under the disease progress curve (AUDPC) across the assessment days. (a) Experiment conducted from May 2019 to July 2019 (fall/winter); (b) Experiment conducted from November 2019 to March 2020 (spring/summer).

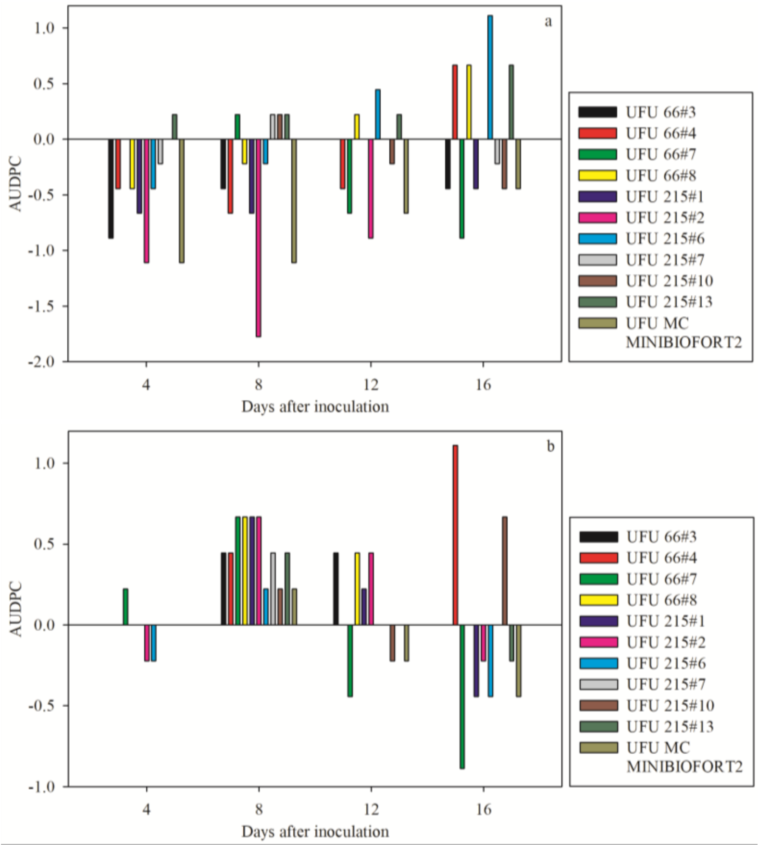


**Figure 5.** Bidirected graph comparing all the genotypes with the moderately resistant Vitória de Santo Antão cultivar for area under the disease progress curve (AUDPC) over the assessment days. (a) Experiment conducted from May 2019 to July 2019 (fall/winter); (b) Experiment conducted from November 2019 to March 2020 (spring/summer).

## Conclusion

All the genotypes studied were biofortified, however the genotypes UFU 215#1 and UFU 215#2 had the highest levels of carotenoid. The genotypes UFU 66#4, UFU 215#1, and UFU 215#7 showed high bolting resistance and so could be recommended for spring/summer crops. All the genotypes performed best in the fall/winter season and could, therefore, be cultivated in this growing season. Genotypes UFU 66#3, UFU 66#7, UFU 215#1, UFU 215#6, and UFU MC MINIBIOFORT2 and cv. 'Vitória de Santo Antão' were resistant to *P. carotovorum* subsp. *carotovorum* isolate UFU A7.

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