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BIPLOT ANALYSIS IN BIOFORTIFIED LETTUCE LINES

ANÁLISE DE BIPLOT EM LINHAGENS BIOFORTIFICADAS DE ALFACE

ANÁLISIS DE BIPLOT EN LÍNEAS DE LECHUGA BIOFORTIFICADA

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ABSTRACT
Lettuce breeding programs have focused on developing genotypes tolerant to biotic and abiotic stress, with few programs aiming at biofortification to increase the nutritional quality of this leafy vegetable. Biplot analysis remains an important tool in breeding programs, allowing for a clear and integrated visualization of both genotype performance and the importance of the evaluated variables. Thus, this work aimed to evaluate, through biplot analysis, biofortified green and purple lettuce lines in summer and winter. Two experiments were conducted at the Vegetable Experimental Station of the Federal University of Uberlândia, Monte Carmelo campus. Seven lines from the Biofortified and Tropicalized Lettuce Genetic Improvement Program at UFU were evaluated. The experimental design adopted was randomized blocks with three replications, totaling 21 plots. The quantification of chlorophyll a (CoA), chlorophyll b (CoB), total chlorophyll (CoT), carotenoids (CaR), and anthocyanins (AT) was performed using the Multiskan™ FC Microplate Photometer at wavelengths of 645, 652, 663, 470, and 535 nm. The analysis showed that PC1 and PC2 explained 72.44% and 22.57% of the variance, respectively. This study demonstrated that lines L2, L6, and L9 have high potential as candidates in genetic improvement programs focused on biofortification. It also showed that chlorophyll, carotenoid, and anthocyanin levels are strongly influenced by edaphoclimatic conditions, highlighting the importance of genotype-environment interactions.

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Keywords: *Lactuca Sativa L*. Biofortification. Chlorophylls. Carotenoids. Anthocyanin.

RESUMO
Os programas de melhoramento genético em alface têm se concentrado no desenvolvimento de genótipos tolerantes ao estresse biótico e abiótico, havendo poucos programas objetivando a biofortificação para aumentar a qualidade nutricional desta folhosa. A análise biplot continua sendo uma importante ferramenta em programas de melhoramento genético, permitindo visualizar de forma clara e integrada tanto o desempenho dos genótipos quanto a importância das variáveis avaliadas. Assim, este trabalho teve como objetivo avaliar por meio de análise biplot linhagens de alface biofortificadas verdes e roxas no verão e inverno. Dois experimentos foram conduzidos na Estação Experimental de Hortaliças da Universidade Federal de Uberlândia, campus Monte Carmelo. Foram avaliadas sete linhagens do Programa de Melhoramento Genético de Alface Biofortificada e Tropicalizada da UFU. O delineamento experimental adotado foi em blocos casualizados com três repetições, totalizando 21 parcelas. A quantificação dos teores de clorofila a (CoA), clorofila b (CoB), clorofila total (CoT), carotenoides (CaR) e antocianinas (AT) foi realizada utilizando o Multiskan™ FC Microplate Photometer nos comprimentos de 645, 652 e 663, 470 e 535 nm. A análise demonstrou que o PC1 e o PC2 explicaram 72,44% e 22,57% da variância, respectivamente. Este estudo demonstrou que as linhagens L2, L6 e L9 apresentam elevado potencial como candidatas em programas de melhoramento genético voltados para biofortificação. Também evidenciou que os níveis de clorofilas, carotenoides e antocianinas são fortemente influenciados pelas condições edafoclimáticas, ressaltando a importância das interações genótipo–ambiente.

Palavras-chave: *Lactuca Sativa L*. Biofortificação. Clorofilas. Carotenoides. Antocianina.

RESUMEN
Los programas de mejoramiento de lechuga se han centrado en el desarrollo de genotipos tolerantes al estrés biótico y abiótico, mientras que pocos programas se han enfocado en la biofortificación para aumentar la calidad nutricional de esta hortaliza de hoja. El análisis de biplot sigue siendo una herramienta importante en los programas de mejoramiento, ya que permite una visualización clara e integrada tanto del rendimiento del genotipo como de la importancia de las variables evaluadas. Por lo tanto, este trabajo tuvo como objetivo evaluar, mediante análisis de biplot, líneas de lechuga verde y morada biofortificadas en verano e invierno. Se realizaron dos experimentos en la Estación Experimental de Hortalizas de la Universidad Federal de Uberlândia, campus Monte Carmelo. Se evaluaron siete líneas del Programa de Mejoramiento Genético de Lechuga Biofortificada y Tropicalizada de la UFU. El diseño experimental adoptado fue de bloques al azar con tres réplicas, totalizando 21 parcelas. La cuantificación de clorofila a (CoA), clorofila b (CoB), clorofila total (CoT), carotenoides (CaR) y antocianinas (AT) se realizó con el Fotómetro de Microplacas Multiskan™ FC a longitudes de onda de 645, 652, 663, 470 y 535 nm. El análisis mostró que PC1 y PC2 explicaron el 72,44% y el 22,57% de la varianza, respectivamente. Este estudio demostró que las líneas L2, L6 y L9 tienen un alto potencial como candidatas en programas de mejoramiento genético enfocados en la biofortificación. También se demostró que los niveles de clorofila, carotenoides y antocianinas están fuertemente influenciados por las condiciones edafoclimáticas, lo que resalta la importancia de las interacciones genotipo-ambiente.

Palabras clave: *Lactuca Sativa L*. Biofortificación. Clorofilas. Carotenoides. Antocianinas.

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1 INTRODUCTION

The lettuce (*Lactuca sativa* L.) is a leafy vegetable cultivated and consumed worldwide (MEDINA-LOZANO et al., 2021). Kuwait, Belgium and Democratic Republic of Congo are the main lettuce producers worlwide, with a production over 41 tons (OUR WORLD IN DATA, 2026). São Paulo is the largest producing state in Brazil, with 7.701 hectares of production (CEPEA, 2024).

Genetic improvement programs have aimed at developing genotypes tolerant to biotic and abiotic stresses, as well as with desirable morphological traits. To this end, quantitative trait loci (QTLs) have been identified (SALEM et al., 2023). Plant pigments such as chlorophylls, carotenoids, and anthocyanins provide significant benefits to human health. Due to their neuroprotective, antioxidant, and antiangiogenic properties, they have been used in the prevention of cardiovascular diseases, cancer, and diabetes, as well as for improvements in visual health, anti-obesity, and antibacterial activity (MEDINA-LOZANO et al., 2021; ANUM et al., 2024).Nowadays nutraceutical foods are leading the public interest, because many clinical trials and studies showed a correlation between bioactive compounds and human health (VIGNESH et al., 2024).

Studies have shown that increases of bioactive compounds could be obtained by hybridization (OLIVEIRA et al., 2021, SANCHES et al., 2025). Biplot and principal component analysis are methods used to measure stability and compatibility of genotypes (STANLUOS et al., 2023). Also, the environmental and genotypes effect on traits are betters analyzed using these methods (SHOJAEI et al., 2022). Thus, to identify the best genotype in relation to environmental factors and bioactive compounds is to use biplot and principal component analysis.

Thus, this work aimed to evaluate, through biplot analysis, biofortified green and purple lettuce lines during summer and winter.

2 METHODOLOGY

Two experiments were conducted at the Vegetable Experimental Station of the Federal University of Uberlândia, Monte Carmelo campus (18º42’43.19” S; 47º29’55.8” W; 873 m altitude), during two periods. The region's climate is Aw-tropical, characterized by hot and humid summers and cold and dry winters, according to the Köppen classification (1948).

The soil is classified as a dystrophic Red Latosol and presented the following characteristics in the 0–20 cm layer for experiment 1: clayey texture 86 (> 50%); pH in CaCl2

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= 4.9; Ca = 3.3 cmolc dm-3; Mg = 1.3 cmolc dm-3; H + Al = 4.9 cmolc dm-3; SB = 4.89 cmolc dm-3; OM = 3.9 dag kg-1; For experiment 1: P(rem) = 7.91 mg dm-3; K = 0.29 cmolc dm-3, CEC = 9.79 cmolc dm-3; and V% = 50. For experiment 2: clayey texture 86 (> 50%); pH in CaCl2 = 5.2; Ca = 3.5 cmolc dm-3; Mg = 1.7 cmolc dm-3; H + Al = 4.2 cmolc dm-3; SB = 5.52 cmolc dm-3; OM = 3.9 dag kg-1; P(rem) = 8.01 mg dm-3; K = 0.32 cmolc dm-3, CEC = 9.72 cmolc dm-3; and V% = 43.

The lines used in both experiments are part of the UFU's Biofortified and Tropicalized Lettuce Genetic Improvement Program, registered in the BG α BIOFORT (Table 1).

Table 1

Description of the seven biofortified lettuce lines registered in the BG α BIOFORT

ID	Lines	Color of leave
L1	UFU - 189#2#2#1	Green
L2	UFU - 215#2#2	Purple
L4	UFU - 66#4#2	Green
L6	UFU - MCBiofort 2	Purple
L8	UFU - Biofort189E43	Purple
L9	UFU - 206#1#3#1	Purple
L10	UFU - 75#2#2#1	Purple

The sowing of the lines (Table 1) was carried out in expanded polystyrene trays with 200 cells filled with commercial substrate based on coconut fiber. After sowing, the trays remained in an arch-type greenhouse covered with 150-micron transparent polyethylene film, treated against ultraviolet rays.

For both experiments, the soil of the experimental area was previously prepared with harrowing and subsoiling, then 1,3 meter beds were built using a rotary tiller. Transplanting was carried out 35 days after sowing (DAS) in experiment 1 and 45 DAS in experiment 2. Maximum and minimum temperatures, and precipitation were monitored during the study period for experiment 1 (Figure 1) and experiment 2 (Figure 2).

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Figure 1

Climatic conditions (minimum temperature, maximum temperature and precipitation) in the months of December 2023 to March 2024 - period for experiment 1

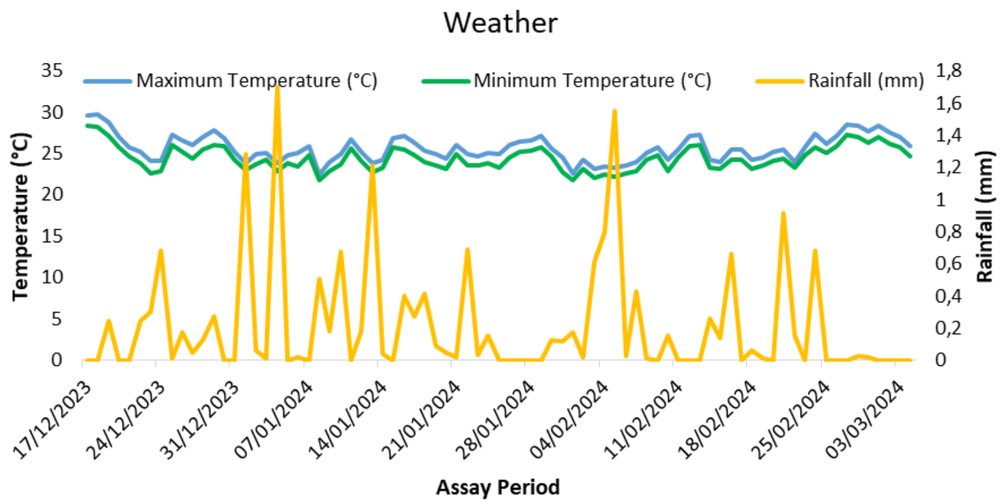
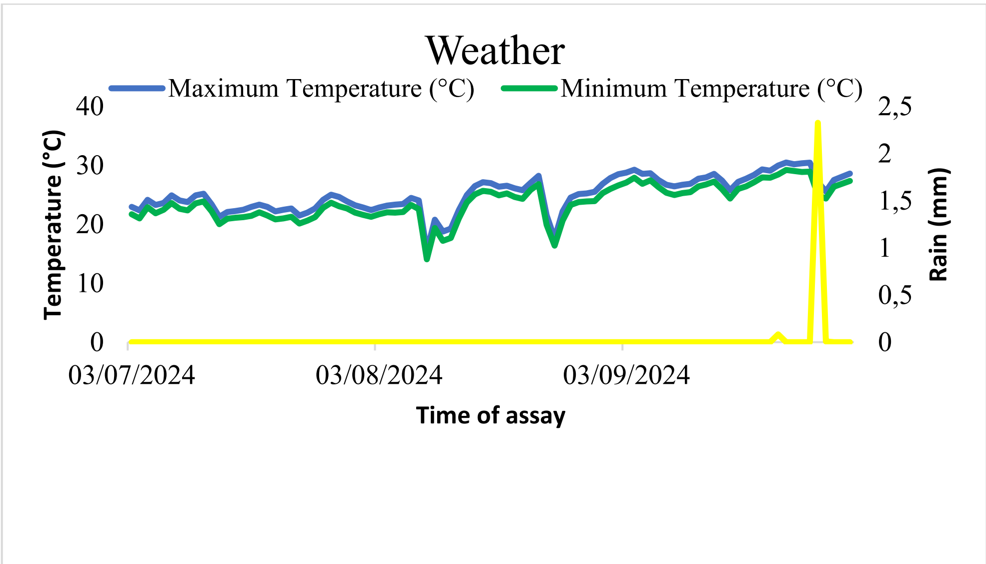


Figure 2

Climatic conditions (minimum temperature, maximum temperature and precipitation) in the months of July/2024 to October/2024 - period for experiment 2



For both experiments, each plot consisted of fifty plants, arranged in two central rows per bed, with a spacing of 0.25 x 0.25 meters. The ten central plants of the plot were evaluated. The experimental design adopted was randomized blocks with three replications, totaling 21 plots and 1,050 plants. Cultivation was carried out as recommended for lettuce cultivation (FILGUEIRA, 2013). Irrigation was carried out using drip hoses according to the

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plants' requirements. Forty-three days after transplanting (DAT) in both experiments 1 and 2, the plants were harvested and evaluated.

The quantification of Chlorophyll a (CoA), Chlorophyll b (CoB), Total Chlorophyll (CoT), carotenoids (CaR), and anthocyanin (AT) levels was performed using 0.5 g of live tissue from the central plants, with 3 repetitions per analysis. After extraction of the compounds, the absorbance was measured using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific Inc., MA, USA) at wavelengths of 645, 652, and 663 nm for CoA, CoB, and CoT; 470 nm for CaR; and 535 nm for AT. From the absorbances, the leaf pigment contents (mg.100g-1 of fresh tissue) were calculated (Francis, 1982; Witham et al., 1971).

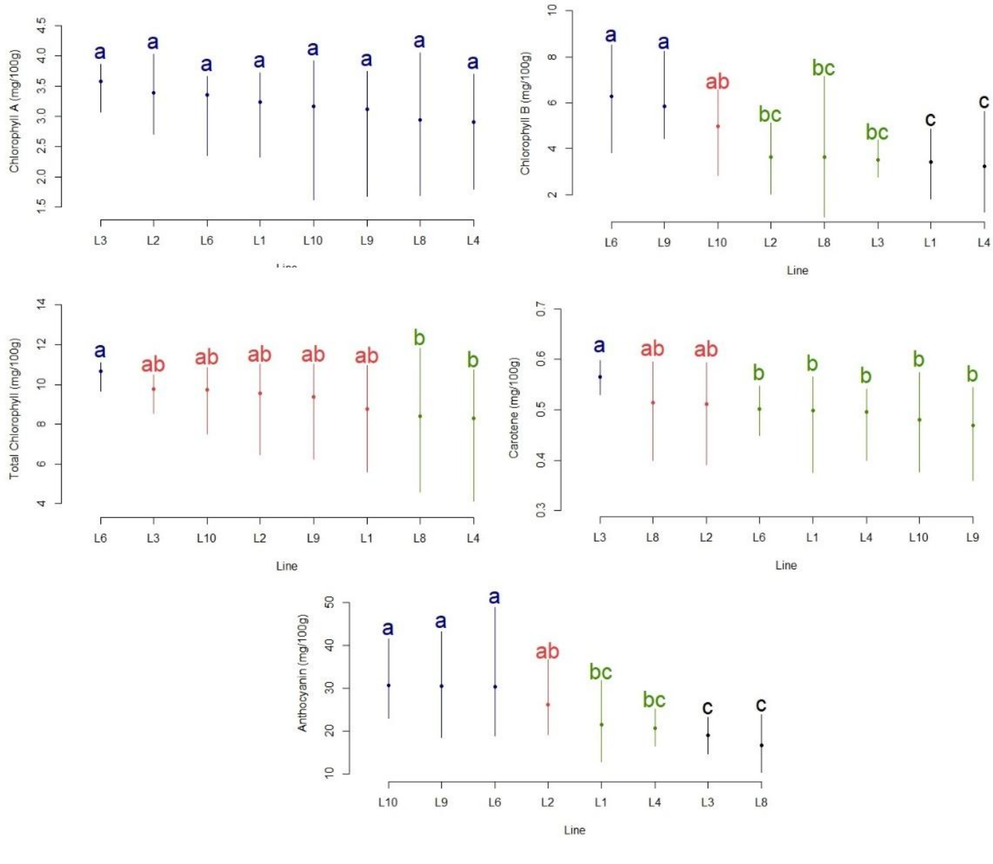
The data were submitted to ShapiroWilk, and all the data were all significant, thus did not need to be transformed. Mean comparison used Least Significance Difference (LSD) with bonffering probability adjusting. Biplot and principal component analysis were used to classify the lines in two experiments (summer and winter). The analyses were performed using software R version 4.4.0 (R CORE TEAM, 2025), with the packages metan and agrciolae.

3 RESULTS AND DISCUSSION

In experiment 1, the lines showed significant differences in the levels of chlorophyll b, total chlorophyll, carotenoids, and anthocyanins, except for chlorophyll a. The lines L6 and L9 had the best result for chlorophyll b, the line L6 had the best result for total chlorophyll, the line L3 had the best result for carotenoide, and the lines L6, L9 and L10 had the best result for anthocyanin (Figure 3).

Figure 3

Least Square Distance (LSD) mean comparison of chlorophyll a, chlorophyll b, total chlorophyll, carotene and anthocyanin from experiment 1



In experiment 2, significant differences were observed across all evaluated bioactive compounds. Line L2 stood out for chlorophyll a and carotenoids, while both L6 and L2 were prominent for total chlorophyll. For anthocyanins, line L9 exhibited the highest levels (Figure 4).

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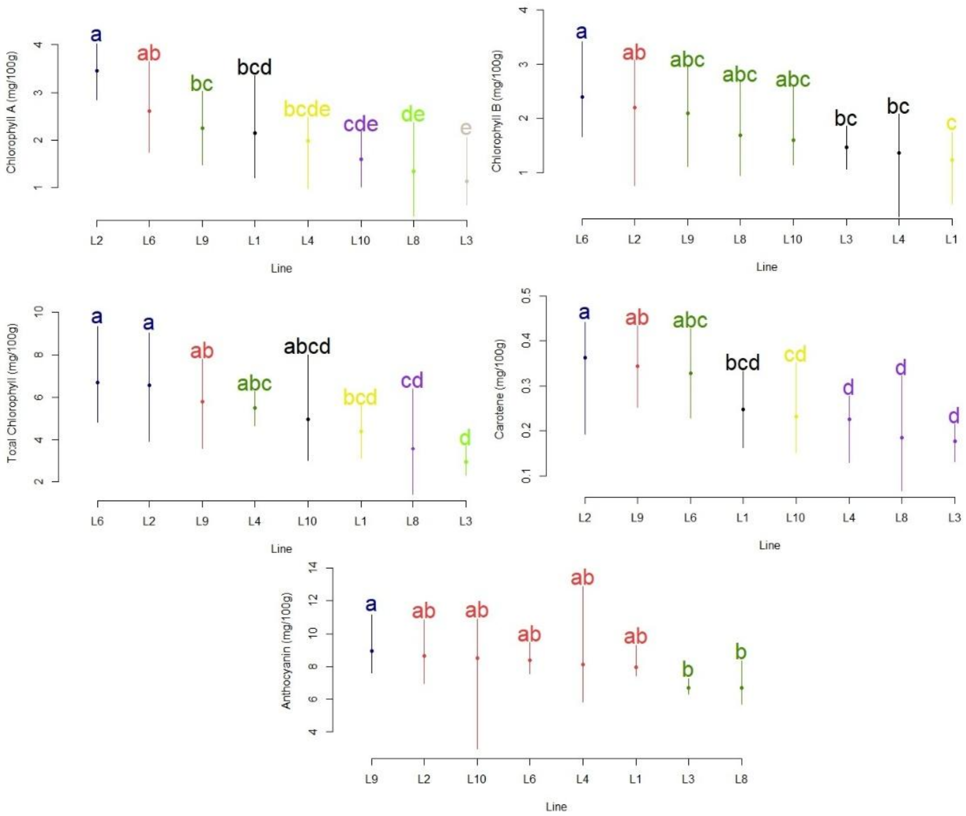
Figure 4

Least Square Distance (LSD) mean comparison of chlorophyll a, chlorophyll b, total chlorophyll, carotene and anthocyanin from experiment 2



Lower levels of chlorophyll, carotenoids, and anthocyanins were observed in experiment 2, conducted during the winter, compared to experiment 1 in the summer. Environmental conditions, particularly temperature, can negatively impact plant physiological processes such as photosynthesis by altering the accumulation of photoassimilates (HASANUZZAMAN et al., 2013). Leaf pigments, including anthocyanins, carotenoids, and chlorophylls, have been shown to increase significantly at temperatures up to 35 °C (LEVINE et al., 2023). Under stress conditions, plants typically accumulate reactive oxygen species, and stress tolerance is often associated with enhanced activity of antioxidant enzymes (SAIRAM and SAXENA, 2000). Thus, the increase in anthocyanins and carotenoids observed in experiment 1 (summer – Figure 1) can be attributed to the generation of reactive oxygen species induced by high temperatures and the consequent activation of antioxidant mechanisms for their removal.

Lines L6 and L9 stood out in both experiments. However, line L3 exhibited high carotenoid content exclusively in the summer, whereas line L2 showed enrichment in

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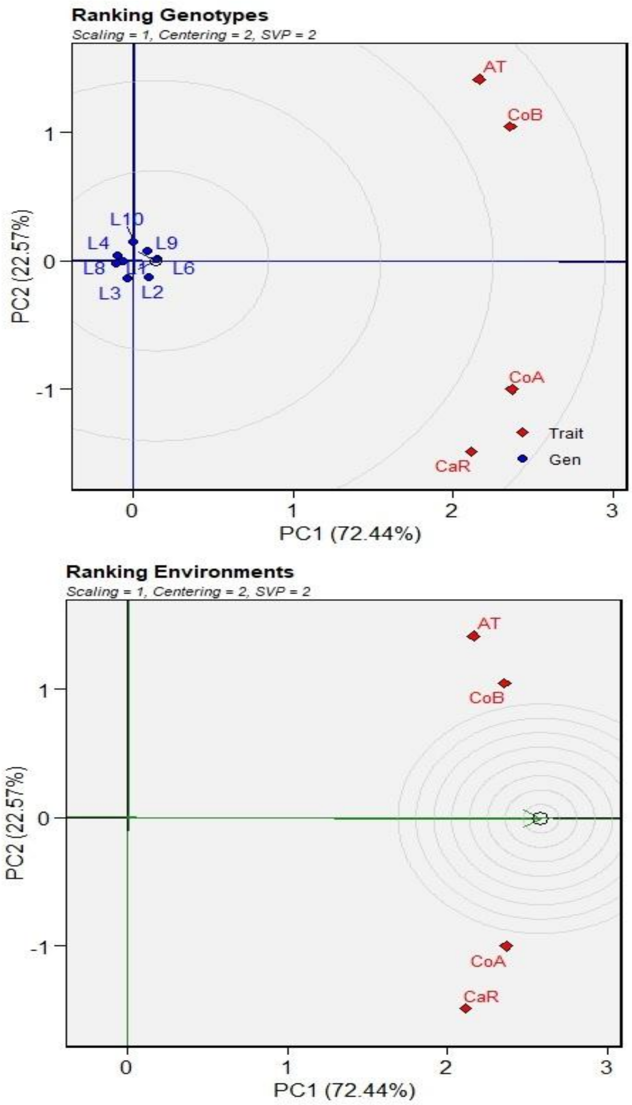
chlorophyll a, total chlorophyll, and carotenoids only in the winter. These findings are relevant for genetic improvement programs aimed at developing cultivars adapted to different edaphoclimatic conditions.

The genotype–trait analysis through biplot revealed a clear differentiation among the evaluated genotypes, with principal components PC1 and PC2 explaining 72.44% and 22.57% of the variance, respectively, together accounting for 95.01% of the observed variance (Figure 5).

Figure 5

Biplot anaylisis of bioactive pigments from experiment 1 and 2. Ranking genotypes and ranking environments



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Genotypes L2, L6, and L9 stood out due to their proximity to the pigment vectors, indicating high levels of chlorophyll a, total chlorophyll, carotenoids, and anthocyanins. Specifically, L2 was associated with chlorophyll a and carotenoids, L6 with total chlorophyll, and L9 with anthocyanins, highlighting distinct and complementary profiles. These findings reinforce the potential of these lines as promising candidates in breeding programs aimed at biofortification and adaptation to different edaphoclimatic conditions.

Environments associated with higher temperatures, such as those in the summer season, were positioned closer to the vectors of carotenoids and anthocyanins, indicating increased accumulation of these pigments under heat stress. In contrast, winter environments were more closely related to chlorophyll a and total chlorophyll, reflecting enhanced photosynthetic pigment stability under cooler conditions. These findings highlight the strong influence of edaphoclimatic factors on pigment expression in lettuce and underscore the importance of considering seasonal environments in breeding programs aimed at developing cultivars with improved nutritional quality and stress resilience.

Studies conducted by Clemente et al. (2023) and Siquieroli et al. (2025), which evaluated lettuce lines for biofortification and tropicalization, also highlighted lines L2 and L6, thereby confirming the potential of these genotypes.

4 CONCLUSION

This study demonstrated that the lines L2, L6, and L19 exhibit high potential as candidates for genetic improvement programs. Moreover, it was shown that the levels of chlorophylls, carotenoids, and anthocyanins are strongly influenced by light intensity and edaphoclimatic conditions, underscoring the importance of genotype–environment interactions. Nevertheless, further studies are required to deepen breeding strategies aimed at developing biofortified cultivars adapted to diverse cultivation scenarios.

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