horticultura brasileira

Research

CHAVES JÚNIOR, OJ; ZEFFA, DM; CONSTANTINO, LV; GIACOMIN, RM; SIQUEIRA, RGB; SILVA, GAB; NOGUEIRA, AF; GONÇALVES, LSA; VENTURA, MU. 2021. Genetic variability among broccoli genotypes based on biochemical and molecular traits. *Horticultura Brasileira* 39: 288-293. DOI: http://dx.doi.org/10.1590/s0102-0536-20210307.

Genetic variability among broccoli genotypes based on biochemical and molecular traits

Osmar J Chaves Júnior[1]; Douglas M Zeffa [2]; Leonel V Constantino [1]; Renata M Giacomin [1]; Rafael GB Siqueira [3]; Gabriel AB Silva [3]; Alison F Nogueira [1]; Leandro SA Gonçalves [1]; Maurício U Ventura [1]

[1]Universidade Estadual de Londrina, Departamento de Agronomia (UEL), Londrina-PR, Brasil; osmarjcj@gmail.com; leonel@uel.br; giacomin.rm@gmail.com; alison.fernando@uel.br; leandrosag@uel.br; mventura@uel.br; [2]Universidade Estadual de Maringá, Departamento de Agronomia, (UEM) Maringá-PR, Brasil; douglas.mz@hotmail.com; [3]Universidade Estadual de Londrina, Departamento de Química (UEL), Londrina-PR, Brasil; rafa.beluce@gmail.com; gabrielbacinello110@gmail.com

ABSTRACT

Broccoli (*Brassica oleracea* var. *italica*) is one of the most important vegetables in the world and also shows important functional properties. The present study aimed to characterize 19 broccoli genotypes using biochemical traits (content of total phenolic compounds, total flavonoids and antioxidant activity) and AFLP (Amplified Fragment Length Polymorphism) molecular markers. The experiment was carried out in a greenhouse using a complete randomized block design with three replicates. The phenotypic data were subjected to analysis of variance ($p \leq 0.05$), Scott-Knott average clustering method ($p \leq 0.05$) and principal component analysis (PCA). Using molecular data, Ward's hierarchical clustering, Bayesian clustering and principal coordinate analysis (PCoA) were performed. Molecular data showed genetic diversity among the genotypes (three groups), also a wide variability in the total phenolic compounds, total flavonoids content and antioxidant activity using FRAP method. HT3010, Hanabi and Bonanza genotypes showed desirable biochemical traits for the demanding functional food consumers, in addition to being promising genotypes to be exploited in plant breeding programs.

Keywords: *Brassica oleracea* var. *itálica,* phenolic compounds, breeding, horticulture, post-harvest.

RESUMO

Variabilidade genética entre genótipos de brócolis baseada em características bioquímicas e moleculares

Brócolis (*Brassica oleracea* var. *italica*) é umas das principais hortaliças do mundo e apresenta importantes propriedades funcionais. O presente estudo teve como objetivo caracterizar 19 genótipos de brócolis por meio de caracteres bioquímicos (teor de compostos fenólicos totais, flavonoides totais e atividade antioxidante) e marcadores moleculares do tipo AFLP (*Amplified Fragment Length Polymorphism*). O experimento foi realizado em casa de vegetação usando delineamento em blocos completos ao acaso com três repetições. Os dados fenotípicos foram submetidos à análise de variância ($p \leq 0,05$), agrupamento de média de Scott-Knott ($p \leq 0,05$) e análise de componentes principais (PCA). A partir dos dados moleculares, foram realizados os agrupamentos hierárquicos de Ward, agrupamento Bayesiano e análise de coordenadas principais (PCoA). Os dados moleculares indicaram diversidade genética entre os genótipos (três grupos), além de ampla variabilidade para o teor de compostos fenólicos totais, flavonoides totais e atividade antioxidante pelo método FRAP. Os genótipos HT3010, Hanabi e Bonanza apresentam características bioquímicas desejáveis aos consumidores exigentes em alimentos funcionais, além de serem genótipos promissores para serem explorados em programas de melhoramento da cultura.

Palavras-chave: *Brassica oleracea* var. *itálica*, compostos fenólicos, melhoramento genético, horticultura, pós-colheita.

Received on March 5, 2021; accepted on May 27, 2021

Broccoli (*Brassica oleracea* var. *italica*) is one of the most produced and consumed vegetables worldwide (Owis, 2015). This species is the result of the selection and accumulation of mutations during the domestication process of *B. sylvestris*. Its center of origin is the Mediterranean region (Stansell & Björkman, 2020). Broccoli germplasm is divided into two main commercial groups: ramoso, which presents several small lateral inflorescences, and single-head broccoli, showing inflorescences concentrated at the shoot tip, also known as Japanese broccoli (Ordiales *et al*., 2017).

Broccoli consumption has been increasing in Brazil in the latest years, not only due to its reasonable price, making it affordable for different social classes, but also for its high nutritional value (Thomas *et al*., 2017). It is an important source of calcium, magnesium and essential amino acids (Melo, 2015; Chen *et al*., 2018). In addition, this vegetable presents antioxidants, mainly phenolic compounds, which contribute to the prevention of premature aging and various types of cancer and neurodegenerative diseases (Liu *et al*., 2018). Moreover, the phenolic compounds are part of several biochemical plant defense mechanisms

related to biotic and abiotic stresses (Melo *et al*., 2017).

Phenotypic characterization is of great importance in order to indentify agronomic and/or superior nutritional traits (Swarup *et al*., 2021). In addition, molecular markers have been widely used to characterize genotypes, mainly to provide information on DNA genetic variability, as also avoiding influences concerning environment and/or plant development stage (Ahmad & Anjum, 2018). Considering the molecular markers, the AFLP (*Amplified Fragment Length Polymorphism*) shows important characteristics, such as a large genome coverage, good reproducibility, low cost and high efficiency (Adhikari *et al*., 2017). Given the above, the aim of this study is to characterize broccoli genotypes using biochemical and molecular traits using AFLP markers.

MATERIAL AND METHODS

We evaluated 19 genotypes of broccoli, being 12 single-head broccoli genotypes and 7 ramoso broccoli genotypes (Table 1). The genotypes were sown on plastic trays with 128 cells and transplanted into 5-liter pots containing soil, organic compound and sand (3:1:1). The seedlings were transplanted 30 days after sowing, which means, when the plants reached 10-cm in length and at least five definitive leaves. The experiment was carried out in a greenhouse in the experimental area of Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil (23°19'31"S, 51°11'60"W) during the winter harvest, 2019. The experiment was carried out in a completely randomized design, with three replicates, consisting the plots of four plants.

Molecular characterization was carried out using AFLP technique, following the protocol proposed by Vos *et al*. (1995). DNA was extracted from a bulk of leaves of each cultivar using CTAB method (*cetyltrimethylammonium bromide*, Sigma-Aldrich, Missouri-USA). Approximately 500 ng of this DNA was double digested with 1 U MseI and 5 U EcoRI (Thermo Scientific, Califórnia, USA) and linked to EcoRI (0.5 µM) and MseI (5 µM) adapters in a reaction containing: T4 DNA ligase (2U); 1X T4 DNA ligase buffer; NaCl (0.05M); BSA (50 µg μL^{-1}); DTT (0.25 mM) to a final volume of 10 µL. The established program for the digestion-binding step consisted of: 37°C for 4 h, 22°C for 1 h and 70°C for 10 min. The digestion/binding pattern was visualized on a 1% agarose gel. After confirmation of digestion, the amplified product was diluted 1:4 with ultrapure water. Pre-selective amplification was performed using 3.5 µL of the GoTaq® Green Master Mix (Promega, Winchester-USA), 0.58 µL of the pre-selective primers EcoRI + A and MseI + C (4.75 µM), 3.0 µL of the dilution of the restriction-binding reaction and ultrapure water to complete the 10 µL volume. The pre-selective amplification program consisted of 1 cycle of 72ºC for 2 min, 20 cycles of 94ºC for 1 s, 56ºC for 30 s, 72ºC for 2 min and a final cycle of 60ºC for 30 min. Pre-selective PCR confirmation was confirmed on a 2% agarose gel and the amplified product was diluted 1:8 in ultrapure water.

Four primer combinations were selected for the selective amplification assay: a) EcoRI (FAM)/-ATC/MseI-CTCG, b) EcoRI (NED) – AGC/MseI-CAA, c) EcoRI (VIC) – ACT/MseI-GAG and d) EcoRI (PET) – AGC/MseI -CAC. The selective reactions were performed in a 10 µL volume containing: 3.5 µL of PCR master mix (GoTaq® Green Master Mix, Promega, Winchester-USA), 0.54 µL of each MseI primer (5 µM) and EcoRI (1µM), 2.5 µL of diluted pre-amplification reaction and 2.92 µL of ultrapure water. The amplification program consisted of 1 cycle of 94°C for 2 min, 65°C for 30 s and 72°C for 2 min; 8 cycles of 94°C for 1 s, 64°C for 30 s and 72°C for 2 min; 23 cycles of 94°C for 1 s, 56°C for 30 s and 72°C for 2 min and 1 final cycle of 60°C for 30 min. Fragment resolution was performed by capillary electrophoresis using the automatic DNA analyzer model 3500xL (Applied Biosystems, California-USA) and the fragment electrophoresis results were combined in a binary matrix by the GeneMapper® v.4.1 software (Applied Biosystems, California, USA).

The characterization of bioactive compounds was performed by quantifying the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity using the FRAP (ferric reducing antioxidant power) assay (Benzie & Strain, 1999). The samples consisted of harvesting the broccoli inflorescences, which were immediately frozen in liquid nitrogen and then lyophilized. After drying, the samples were ground in a WSG30 mill (Waring, USA), sieved in 80 mesh and stored in polyethylene pots protected from light and heat. For the extraction of TPC, TFC and FRAP, a suspension was prepared from 1.00 g of the lyophilized sample in 10.0 mL of 70% ethanol (v/v) with five replicates, according to the protocol adapted by Vázquez *et al.* (2008).

TPC quantification was performed according to Swain & Hillis (1959) using gallic acid as analytical standard, ranging from 10 to 100 mg L^{-1} (r= 0.9960), expressed as milligrams of gallic acid equivalent per 100 grams (mg GAE 100 g^{-1}). TFC quantification was based on Gurnani *et al*. (2016) using quercetin as analytical standard, ranging from 50 to 500 mg L^{-1} (r=0.9942), expressed as equivalent milligrams of quercetin 100 g^{-1} (mg QE 100g^{-1}). FRAP was quantified based on Benzie & Strain (1999) using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as analytical standard, ranging from 0.20 to 1.00 mmol L^{-1} (r= 0.9992), expressing as µmol of antioxidant capacity equivalent to trolox per 100 g (µmol TEAC 100 g^{-1}).

After being verified the normality and homogeneity of variances, the biochemical characterization data were submitted to analysis of variance (ANOVA) and when significant ($p \leq 0.05$), the averages were clustered using Scott-Knott test ($p \leq 0.05$). Principal Component Analysis (PCA) was performed using the standardized average Euclidean distance. These analyses were performed using R software (https://www.r-project.org/) version 3.6.0 using 'Expdes.pt' and 'FactoMiner' packages.

Using the AFLP markers, population

genetics structure was verified by Bayesian clustering using Structure software version 2.3.4 (Pritchard *et al.*, 2000), based on the method described by Evanno *et al.* (2005). We used 500,000 MCMC iterations (Monte Carlo Markov chain), a burn-in period of 100,000 iterations, with admixture model and correlated allelic frequencies. Subgroups values (ΔK) from 1 to 19 were tested, with ten independent interactions for each value of K. The ideal number of K was determined using Structure Harvester software version 0.6.92 (Earl, 2012). The analysis of principal coordinates (PCoA) was performed using the R software version 3.6.0 through the 'ape' and 'plot3D' packages. Additionally, the Jaccard distance between cultivars was calculated and Ward's hierarchical grouping was performed using the 'philentropy', 'factoextra' and 'ggplot2' packages.

RESULTS AND DISCUSSION

The authors verified that AFLP markers are efficient to detect the genetic variability among the broccoli genotypes evaluated in this study. The four primer combinations resulted in 409 amplified bands, all of which are polymorphic. These results are superior to the ones observed by Lin *et al.* (2013), studying the genetic diversity among broccoli accessions based on nine primer combinations AFLP; these authors observed a total of 334 amplified bands, being 208 (62.3%) polymorphic. AFLP methodology associated with automated capillary electrophoresis system has been considered an important tool for exploring the genetic divergence in several vegetable species (Cardoso *et al.*, 2018; Constantino *et al.*, 2020; Massucato *et al.*, 2020).

Ward's hierarchical grouping, Bayesian clustering and the analysis of principal coordinates (PCoA) of the 19 broccoli genotypes are observed in Figure 1. The average similarity (*d*') among these evaluated genotypes was 0.560, being the cultivars Maraton and Domador genetically closest (*d*' = 0,289), whereas the genotypes Seminis002 and Hanabi are the most distant (*d*' = 0,774). Considering the simulations performed using ΔK value method (Evano *et al.*, 2005), we observed an optimal number of K= 3, indicating the formation of three distinct groups.

Group 1 (green) was formed by nine genotypes (Crioulo 2, Seminis002, Avenger, AF1125, Domador, Maraton, Seminis001, HT9631F1 and HT3010). Groups 2 (red) and 3 (blue) consisted of four genotypes (Bonanza, HT1607, Calabres and AF1957) and six genotypes [Piracicaba Precoce (Horticeres), Piracicaba Precoce (Sakata), Hanapon, Santana, Akemi ande Hanabi], respectively. The genotypes Crioulo 2, AF1125, Bonanza and AF1957 were classified as *admixture*, since none of them showed ancestry coefficient greater than 0.6 for no group.

The first three main coordinates (PCoA1, PCoA2 and PCoA3) explained 45.09% of the total variation among the evaluated genotypes. Using PCoA, it is possible to observe that the genotype clustering was similar to the one observed through Bayesian clustering and Ward's hierarchical grouping, showing consistency among these three clustering approaches with molecular data. The results of molecular characterization are consistent with traits related to the types of inflorescences of the studied genotypes, since the genotypes in group 2 (red) are single-headed inflorescence genotypes, whereas genotypes in group 3 (blue) are ramoso inflorescence genotypes. The genotypes in group 1 (green) are all single-headed, except Crioulo 2. However, this accession was considered mixed by Bayesian clustering, indicating that it shares alleles from groups 1 and 3.

The averages of the evaluated biochemical traits are shown in Table 2. The antioxidant activity determined by FRAP assay ranged between 16.80 (Seminis001) and 56.24 μmol TEAC 100 g^{-1} (HT3010), whereas the contents of phenolic compounds ranged between 22.97 (Seminis001 and AF1957) and 68.91 mg GAE 100 g^{-1} (HT3010). The total flavonoid contents ranged between 41.06 (Hanapon) and 61.67 mg QE 100 g^{-1} (HT3010). In general, the contents of total phenolic compounds and the antioxidant activity determined by FRAP assay were higher in single-headed inflorescence genotypes when

Table 1. Traits of 19 broccoli genotypes evaluated in this study. Londrina, UEL. 2019.

Genotype	Company	Inflorescence	Type
Marathon	Sakata	Single head	Cultivar
Hanapon	Sakata	Ramoso	Cultivar
Hanabi	Sakata	Ramoso	Cultivar
AF1125	Sakata	Single head	Inbred line
Avenger	Sakata	Single head	Cultivar
AF1957	Sakata	Single head	Inbred line
Piracicaba Precoce	Sakata	Ramoso	Cultivar
HT3010	Bioseeds	Single head	Inbred line
HT9631F1	Bioseeds	Single head	Inbred line
HT1607	Biosseds	Single head	Inbred line
Calabres	Feltrin	Single head	Cultivar
Akemi	Horticeres	Ramoso	Cultivar
Piracicaba Precoce	Hoticeres	Ramoso	Cultivar
Domador	Horticeres	Single head	Cultivar
Seminis001	Seminis	Single head	Inbred line
Seminis002	Seminis	Single head	Inbred line
Bonanza	Isla	Single head	Cultivar
Santana	Isla	Ramoso	Cultivar
Crioulo 2	–	Ramoso	Landrace

compared to the ramoso-type genotypes. None of the commercial groups showed a greater differentiation in terms of flavonoid contents.

Studying the bioactive compounds in *Brassica oleracea* L. var. *acephala,* Rigueira *et al*. (2016) observed high contents of phenolic compounds (173 and 244 mg GAE 100 g^{-1} g in leaves and 86 and 180 mg GAE 100 g^{-1} in stems) and the average antioxidant activity was 62.5 and 34.5% in leaves and stalks, respectively. We concluded that, in addition to the genetic variability existing among genotypes, the cultivation system also influences the concentration of bioactive compounds. Moreover, other factors such as part of the plant, phenological stage and crop management can also influence the concentration of these bioactive compounds (Rigueira *et al.*, 2016; Batista *et al.*, 2017; Nasser *et al.*, 2020; Zanzini *et al.*, 2020).

The two first main components (PC1 and PC2) showed a total of 70.7 and 21.8% of total variation, respectively (Figure 2). Using PCA, we could observe that cultivars Hanabi and AF1125 showed interesting accessions for studies related to flavonoids, since they showed high averages for this trait (Figure 2, Table 2). Cultivars Akemi and Bonanza were associated with the antioxidant activity trait vector determined by FRAP method, presenting

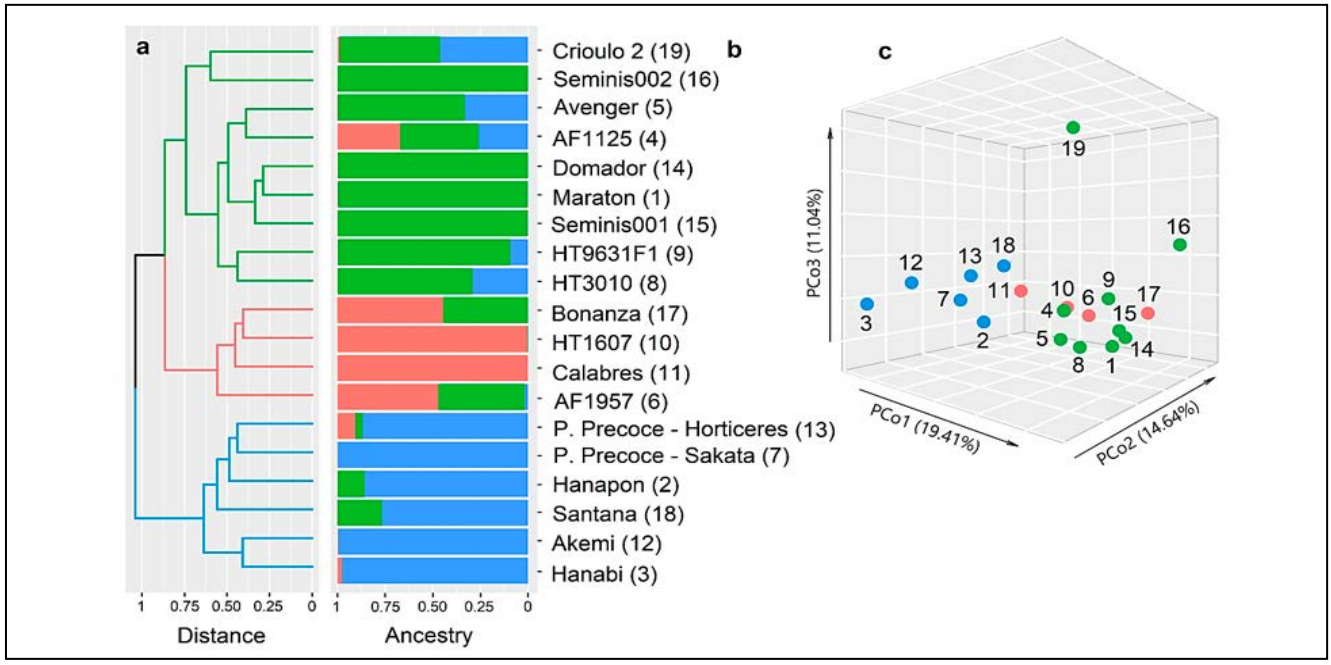

Figure 1. Ward's hierarchical grouping (a), Bayesian clustering considering K=3 (b) and the analysis of principal coordinates (c) of 19 broccoli cultivars. Londrina, UEL, 2019.

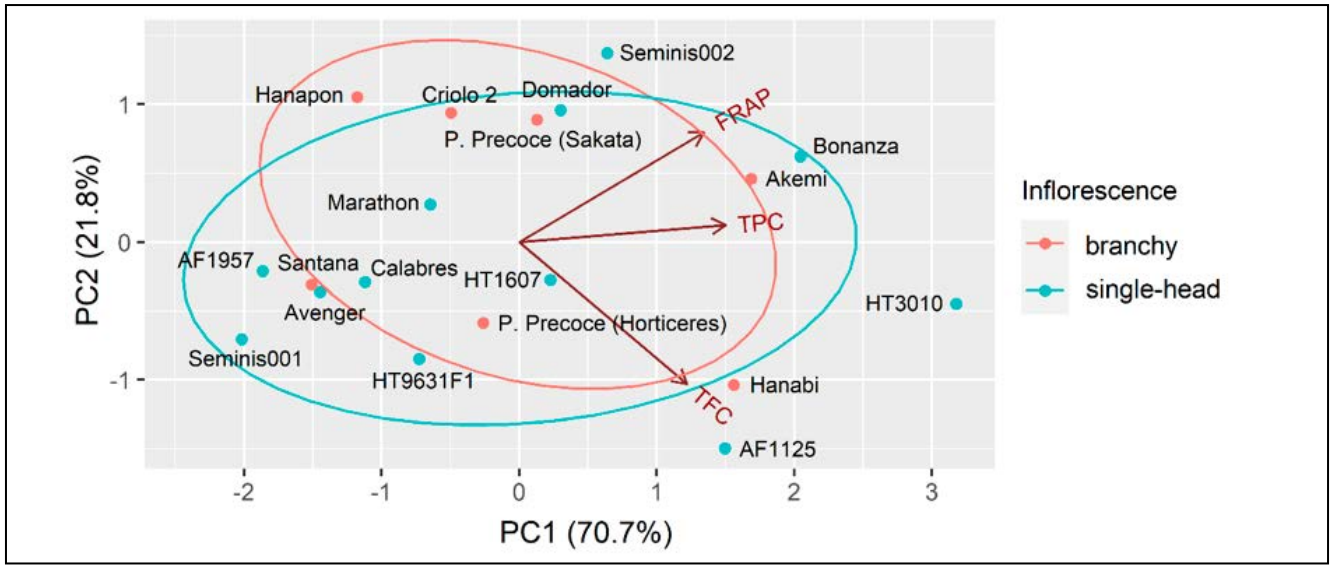

Figure 2. Principal Component Analysis (ACP) of 19 broccoli genotypes evaluated for antioxidant activity (FRAP), content of total phenolic compounds (TPC) and total flavonoids (TFC). Londrina, UEL, 2019.

Table 2. Averages and standard deviation of antioxidant activity (FRAP), content of total phenolic compounds (TPC) and total flavonoids (TFC) of 19 broccoli genotypes. Londrina, UEL. 2019.

Genotypes	FRAP	TPC	TFC
Maraton	29.77 ± 2.52 c	42.86 ± 2.41 d	45.00 ± 4.08 c
Hanapon	37.33 ± 3.03 c	31.93 ± 1.39 f	41.06 ± 3.37 c
Hanabi	40.03 ± 3.66 b	53.78 ± 5.45 b	59.55 ± 2.81 a
AF1125	34.09 ± 0.97 c	55.18 ± 2.10 b	61.06 ± 2.48 a
Avenger	21.12 ± 3.33 e	31.93 ± 5.55 f	46.21 ± 0.94 c
AF1957	23.28 ± 2.43 d	22.97 ± 2.62 g	45.30 ± 0.94 c
P, Precoce (Sakata)	51.92 ± 1.75 a	34.17 ± 3.28 f	47.73 ± 4.08 c
HT3010	56.24 ± 4.45 a	68.91 ± 2.92 a	61.67 ± 4.29 a
HT9631F1	26.53 ± 1.46 d	31.93 ± 3.67 f	51.97 ± 2.48 b
HT1607	42.73 ± 0.84 b	34.45 ± 1.05 f	53.48 ± 2.48 b
Calabres	25.99 ± 0.49 d	32.21 ± 4.10 f	47.42 ± 2.48 c
Akemi	52.46 ± 4.31 a	57.42 ± 1.39 b	52.58 ± 2.48 b
P,Precoce (Hoticeres)	30.31 ± 2.22 c	38.66 ± 2.92 e	51.67 ± 2.81 b
Domador	47.60 ± 2.70 b	44.26 ± 3.19 d	46.21 ± 3.37 c
Seminis001	16.80 ± 1.75 e	22.97 ± 3.96 g	46.82 ± 2.48 c
Seminis002	52.46 ± 2.70 a	49.02 ± 1.82 c	45.00 ± 3.37 c
Bonanza	54.08 ± 3.66 a	63.87 ± 1.82 a	52.27 ± 0.94 b
Santana	17.34 ± 2.95 e	36.69 ± 3.67 e	44.70 ± 4.29 c
Crioulo 2	34.09 ± 4.20 c	47.90 ± 1.05 c	41.67 ± 3.37 c
CV (%)	8.21	6.61	6.35

Averages followed by the same letter in the lines do not statistically differ from each other by the Scott-Knott test at 5% significance.

high averages for these traits. Among the evaluated genotypes, HT3010 was the genotype which presented the highest averages for the antioxidant activity using FRAP assay, content of total phenolic compounds and content of total flavonoids. No difference between ramoso or single-headed inflorescence genotypes was observed through PCA.

The genetic variability shown in the studied broccoli genotypes observed using AFLP markers and biochemical traits can be explored in broccoli breeding programs. Among the evaluated genotypes, the genotypes HT3010, Hanabi, Bonanza, Akemi and AF1125 stood out as they presented the highest contents of antioxidant activity using FRAP assay, total phenolic compounds and total flavonoids. These genotypes can be used as parents in breeding programs aiming to develop broccoli cultivars with high contents of active compounds.

REFERENCES

ADHIKARI, S; SAHA, S; BISWAS, A; RANA, TS; BANDYOPADHYAY, TK; GHOSH, P. 2017. Application of molecular markers in plant genome analysis: a review. *The Nucleus*, 60: 283-297. https://doi.org/10.1007/s13237-017-0214-7

AHMAD, R; ANJUM, MA. 2018. Applications of molecular markers to assess genetic diversity in vegetable and ornamental crops - a review. *Journal of Horticultural Science and Technology*, 1: 1-7. https://doi.org/10.46653/jhst180101001

BATISTA, CFT; SILVA, CO; MELO, CMT; TASSI, EMM; PASCOAL, GB. 2017. Alterações nutricionais e físico-químicas em repolho branco (*Brassica oleracea*) após o processamento mínimo e durante o armazenamento. *Demetra*. 12: 305-318. https://doi.org/10.12957/demetra.2017.22115

BENZIE, IF; STRAIN, JJ. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology* 299: 15-27. https://doi.org/10.1016/S0076-6879(99)99005-5

CARDOSO, R; RUAS, CF; GIACOMIN, RM; RUAS, PM; RUAS, EA; BARBIERI, RL; GONÇALVES, LSA. 2018. Genetic variability in Brazilian *Capsicum baccatum* germplasm collection assessed by morphological fruit traits and AFLP markers. *PloS one* 13: e0196468. https://doi.org/10.1371/journal.pone.0196468

CHEN, L; ZHAO, B; FAN, Z; LIU, X., WU, Q; LI, H; WANG, H. 2018. Synthesis of novel 3, 4-chloroisothiazole-based imidazoles as fungicides and evaluation of their mode of action. *Journal of Agricultural and Food Chemistry* 66: 7319-7327.

CONSTANTINO, LV; FUKUJI, AYS; ZEFFA, DM; BABA, VY; CORTE, ED; GIACOMIN, RM; GONÇALVES, LSA. 2020. Genetic variability in peppers accessions based on morphological, biochemical and molecular traits. *Bragantia* 79: 443-446. https://doi.org/10.1590/1678-4499.20190525

EARL, DA. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359-361. DOI:10.1007/s12686-011-9548-7

EVANNO, G; REGNAUT, S; GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

GURNANI, N; GUPTA, M; MEHTA, D; MEHTA, BK. 2016. Chemical composition, total phenolic and flavonoid contents, and in vitro antimicrobial and antioxidant activities of crude extracts from red chilli seeds (Capsicum frutescens L.). Journal of Taibah University for Science 10: 462-470. https://doi.org/10.1016/j.jtusci.2015.06.011

LIN, KH; CHANG, LC; LAI, CD; LO, HF. 2013. AFLP mapping of quantitative trait loci influencing seven head-related traits in broccoli (Brassica oleracea var. italica). The Journal of Horticultural Science and Biotechnology 88: 257-268. https://doi.org/10.1080/14620316.2013.11512964

LIU, M; ZHANG, L; SER, SL; CUMMING, JR; KU, KM. 2018. Comparative phytonutrient analysis of broccoli by-products: The potentials for broccoli by-product utilization. Molecules 23: 900. https://doi.org/10.3390/molecules23040900

MASSUCATO, LR; NAKAMURA, KK; RUAS, PM; ZEFFA, DM; SILVA, DJHD; GONÇALVES, LSA. 2020. Genetic diversity among Brazilian okra landraces detected by morphoagronomic and molecular descriptors. Acta Scientiarum. Agronomy 42. http://dx.doi.org/10.4025/actasciagron.v42i1.43426

MELO, RAC. 2015. A cultura do brócolis.

Brasília, DF: Embrapa, 153p.

MELO, TA; ARAUJO, MUP; SERRA, IMRS; PASCHOLATI, SF. 2017. Produtos naturais disponíveis comercialmente induzem o acúmulo de fitoalexinas em cotilédones de soja e mesocótilos de sorgo. Summa Phytopathologica, 43: 205-211. https://doi.org/10.1590/0100-5405/167358

NASSER, MD; CARDOSO, AII; VIEITES, RL; NASSER, FADCM; FURLANETO, KA; RAMOS, JA; SANTOS, JA. 2020. Compostos bioativos e caracterização físico-química da couve-de-folha sob composto orgânico, gessagem e doses de enxofre em cobertura. Scientia Plena 16. https://doi.org/10.14808/sci.plena.2020.090201

ORDIALES, E; IGLESIAS, DJ; ALARCÓN, MV; ZAJARA, L, GIL, J; GUTIÉRREZ, JI; SALGUERO, J. 2017. Characteristics defining broccoli cultivars from different seed producers. International Journal of Agronomy. https://doi.org/10.1155/2017/8216390

OWIS, AI. 2015. "Broccoli; the green beauty: a review." Journal of Pharmaceutical Sciences and Research: 7: 696.

PRITCHARD, JK; STEPHENS, M; DONNELLY, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959. https://doi.org/10.1093/genetics/155.2.945

RIGUEIRA, GDJ; BANDEIRA, AVM; CHAGAS, CGO; MILAGRES RCRM. 2016. Atividade antioxidante e teor de fenólicos em couve-manteiga (Brassica oleracea L. var. acephala) submetida a diferentes sistemas de cultivo e métodos de preparo. Semina: Ciencias Biológicas e da Saúde. 37: 3-2. http://dx.doi.org/10.5433/1679-0367.2016v37n2p3

STANSELL, Z; BJÖRKMAN, T. 2020. From landrace to modern hybrid broccoli: the genomic and morphological domestication syndrome within a diverse B. oleracea collection. Horticulture Research 7: 1-17. https://doi.org/10.1038/s41438-020-00375-0

SWAIN, T; HILLIS, WE. 1959. The phenolic constituents of Prunus domestica. I.-The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture 10: 63-68. https://doi.org/10.1002/jsfa.2740100110

SWARUP, S; CARGILL, EJ; CROSBY, K; FLAGEL, L; KNISKERN, J; GLENN, KC. 2021. Genetic diversity is indispensable for plant breeding to improve crops. Crop Science 61: 839-852. https://doi.org/10.1002/csc2.20377

THOMAS, M; BADR, A; DESJARDINS, Y; GOSSELIN, A; ANGERS, P. 2017. Characterization of industrial broccoli discards (Brassica oleracea var. italica) for their glucosinolate, polyphenol and flavonoid contents using UPLC MS/MS and spectrophotometric methods. Food Chemistry 245: 1204-1211. https://doi.org/10.1016/j.foodchem.2017.11.021

VÁZQUEZ, G; FONTENLA, E; SANTOS, J; FREIRE, MS; GONZÁLEZ-ÁLVAREZ, J; ANTORRENA, G. 2008. Antioxidant activity and phenolic content of chestnut (Castanea sativa) shell and eucalyptus (Eucalyptus globulus) bark extracts. Industrial Crops and Products 28: 279-285. https://doi.org/10.1016/j.indcrop.2008.03.003

VOS, P; HOGERS, R; BLEEKER, M; REIJANS, M; LEE, T; VAN, DE; HORNES, M; FRITERS, A; POT, J; PALEMAN, J; KUIPER, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407–4414. https://doi.org/10.1093/nar/23.21.4407

ZANZINI, AP; OLIVEIRA, JAC; COUTINHO, GSM; ARAUJO, ABS; BARROS, HEA. 2020. Bioactive compounds present in kale (Brassica oleracea L.) at three stages of development and comparison of their antioxidant capacities. Research, Society and Develo*pment*. 9: e391974242. https://doi.org/10.33448/rsd-v9i7.4242