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Diversity and Relationships in Key Traits for Functional and Apparent Quality in a Collection of Eggplant: Fruit Phenolics Content, Antioxidant Activity, Polyphenol Oxidase Activity, and Browning

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ABSTRACT: Eggplant (*Solanum melongena*) varieties with increased levels of phenolics in the fruit present enhanced functional quality, but may display greater fruit flesh browning. We evaluated 18 eggplant accessions for fruit total phenolics content, chlorogenic acid content, DPPH scavenging activity, polyphenol oxidase (PPO) activity, liquid extract browning, and fruit flesh browning. For all the traits we found a high diversity, with differences among accessions of up to 3.36-fold for fruit flesh browning. Variation in total content in phenolics and in chlorogenic acid content accounted only for 18.9% and 6.0% in the variation in fruit flesh browning, and PPO activity was not significantly correlated with fruit flesh browning. Liquid extract browning was highly correlated with chlorogenic acid content (r=0.852). Principal components analysis (PCA) identified four groups of accessions with different profiles for the traits studied. Results suggest that it is possible to develop new eggplant varieties with improved functional and apparent quality.

KEYWORDS: breeding, chlorogenic acid, DPPH scavenging activity, functional quality, germplasm, *Solanum melongena*

INTRODUCTION

Fruits with a high content in phenolics have been reported to present increased antioxidant activity and to prevent some chronic and degenerative diseases, including several types of cancer.[1] Eggplant (*Solanum melongena* L.) is one of the vegetables with greatest antioxidant activity,[2] and presents anti-diabetic, hypotensive, cardioprotective, and hepatoprotective effects.[3-5] These properties have been attributed to its high content in phenolics.[6] Major phenolic compounds in eggplant include hydroxycinnamic acids,[7-9] which are found both in the fruit flesh and in the skin, and anthocyanins, which are present only in the skin.[10] Given that most of the volume of the eggplant fruit is fruit flesh, hydroxycinnamic acids, of which chlorogenic acid (5-*O*-chlorogenic acid and its isomers) is the major representative in eggplant,[11] are the phenolic compounds that make a greater contribution to the functional quality of the eggplant fruit.[12] Chlorogenic acid presents many properties beneficial for human health, such as anti-oxidant, anti-carcinogenic, anti-inflammatory, analgesic, anti-microbial, neuroprotective, and cardioprotective effects.[6] Furthermore, chlorogenic acid has a low bitterness,[13] and at the concentrations found in eggplant flesh has no appreciable impact on eggplant bitterness, which is mostly caused by saponins.[14] In addition, chlorogenic acid also plays an important role in plant defence.[15]

The interest in developing new eggplant cultivars with enhanced bioactive properties has led to the development of breeding programs specifically aimed at improving the content in total phenolics, in particular of chlorogenic acid.[6,11,16] A wide diversity, has been found among eggplant cultivars for total phenolics and chlorogenic acid content.[7,11,17,18] Broad-sense heritability values for total phenolics in eggplant are intermediate,[18] which is an indication that selection and breeding for content in phenolics in eggplant will be efficient for the development of improved cultivars.

However, it is well known that in fruits and vegetables the oxidation of phenolics after the exposure of internal tissues to the air results in browning, which reduces the apparent quality.[19] The destruction of the cell compartments allows the orthodiphenolic substrates (chlorogenic acid and other hydroxycinnamic acid derivatives), mostly confined within the vacuoles, to be accessible to polyphenol oxidases (PPOs), which are found in the plastid membranes.[19,20] PPOs catalyze the oxidation of these phenolic compounds to quinones, which subsequently react non-enzymatically with O_2, and other compounds, like sulfhydryl compounds, amines, amino acids, and proteins to give brown-colored compounds. PPO activity, together with phenolics levels, play a major role in browning of cut tissues in a number of crops.[20] Eggplant PPOs have shown a great affinity for chlorogenic acid,[21,22] which might suggest that increases in chlorogenic acid content could result in increased fruit flesh browning. Also, several studies have shown that there are differences among eggplant varieties for PPO activity,[8,9,22,23] which opens the way to selecting varieties with reduced PPO activity.

Measurement of browning in eggplant has been performed in the fruit flesh either visually[24] or with a chromameter.[18,25-28] Chromameter measurements are generally considered as better than visual observations, as they allow an objective and precise measurement of browning. Also, browning in eggplant can be estimated in juice or in extracts of lyophilized tissue with a chromameter or by spectrometry.[22,28,29]

Knowledge of the relationships between content in phenolics, antioxidant activity, PPO activity, and fruit flesh browning in genetically diverse collections of eggplant may be of interest in order to develop strategies for the development of new cultivars with improved fruit quality. Massolo et al.[30] and Mishra et al.[31] studied the relationships between total phenolics, chlorogenic acid, PPO activity and browning in

eggplant. However, these authors used a single cultivar. Both studies found low variation in total phenolics and chlorogenic acid content in the different samples measured, and that samples with greatest browning also presented highest levels of PPO activity.[30,31] In a recent paper, Mishra et al.[32] used eight Asian cultivars to study the evolution of phenolics content, PPO activity, and browning during postharvest storage These authors found that evolution of fruit flesh browning during storage for two weeks was positively correlated with phenolics content and PPO activity in one group of four accessions, and positively correlated with phenolics content and negatively with PPO activity in another group of four accessions.[32] However, the examination of the results of accessions before storage reveals that both the content in phenolic acids and PPO activity presented, respectively, low and moderate correlations with fruit flesh browning.[32] In any case, the number of accessions used was quite limited (eight accessions) in order to draw generalizations. Also, given that oriental (Asian) and occidental (Mediterranean and African) eggplants are genetically differentiated[33] it would be of interest to study these relationships in Occidental type materials, which are the most important in Europe, Middle East, Africa, and America.

In a study using a wide genetic diversity of eggplant (69 accessions), Prohens et al.[18] found that the correlation between total phenolics and fruit flesh browning measured with a chromameter was moderate (r=0.388) and suggested that additional factors other than the total phenolics compounds had a major role in fruit flesh browning in eggplant. Also, Prohens et al.[16] in a study of segregating generations from an interspecific family between *S. melongena* and the wild relative *S. incanum* L. reached a similar conclusion.

Here, we evaluate the total phenolics content, chlorogenic acid content, antioxidant activity, PPO activity, liquid extract browning, and fruit flesh browning in a

collection of 18 accessions with different morphological characteristics from the region of Valencia, which is situated in the Spanish secondary center of diversity for eggplant.[34] The objective is to study the variation and relationships between these traits in order to obtain information relevant for the selection and development of eggplant varieties with improved bioactive properties and reduced fruit flesh browning.

MATERIALS AND METHODS

Plant Material. Fruits from a total of 18 eggplant germplasm accessions originating from the provinces of Alicante and Valencia, situated in the Autonomous Community of Valencia (Spain), were used for the present study (Table 1). The accessions used corresponded to landraces and included different fruit sizes, shapes, and colors, reflecting the wide morphological and molecular diversity of eggplant accessions from this region.[34-36] Plants from which the fruits were harvested were grown in the open field at the Agricultural Experimental Farm of Carcaixent (Valencia, Spain) using the standard horticultural practices.

Preparation of samples. For each accession, fifteen commercially ripe fruits, evaluated by the size, color and glossiness of the fruit, were harvested between July 25 and September 5, 2011. A total of five samples, each consisting of three fruits, were considered for each accession. Fruits were washed and cut transversally with a well-sharpened knife at the mid-point between the blossom and stem ends for the measurement of fruit flesh browning. After fruit flesh browning had been measured, a 1-cm wide longitudinal section was cut from the middle of the fruit, peeled, and immediately frozen in liquid N_2 and stored at -80 ºC until lyophilized. Powdered tissue of each sample was used for the analyses.

Traits measured. Total phenolics content was measured according to the Folin-Ciocalteu procedure.[37] For each sample, 0.25 g of the lyophilized tissue were extracted with 10 mL methanol:water (80:20, v/v) for 24 h at room temperature. An aliquot of the 1.25 mL of the extracted phenolic sample was centrifuged at 8000 rpm for 5 min and 65 μL of the supernatant were mixed with 0.5 mL diluted (10%, v/v) Folin-Ciocalteu reagent (Sigma-Aldrich Chemie, Steinheim, Germany) and allowed to stand at room temperature for 5 min. Subsequently, 0.5 mL of a sodium carbonate solution (60 g/L) was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm in a Nanodrop ND-1000 (Nanodrop Technologies, Montchain, DE, USA) spectrophotometer. Chlorogenic acid (Sigma Aldrich) was used as a standard, and total phenolics content was expressed as chlorogenic acid equivalents in g/kg of dry weight.

Chlorogenic acid was extracted basically according to Naranjo et al.[38] Lyophilized samples (0.1 g) were homogenized in 2.5 mL methanol. The total extract (2 mL) was vortexed vigorously, sonicated for 10 min, and then centrifuged at 14000 rpm for 15 min using a refrigerated (4 ºC) centrifuge and 2-mL Eppendorf tubes to remove cellular debris. The pellet was re-extracted with 1 mL of methanol and centrifuged again as above. The combined supernatants were filtered through 0.45-μm Spartan 13/0.45RC filters (Schleicher & Schuell, Keene, NH, U.S.A.), nylon filters (Waters, Milford, MA, U.S.A.), and dried under nitrogen at 40 ºC using glass tubes of 5 mL. The dried residue was dissolved in 1 mL methanol containing 0.02% H_3PO_4, vortexed, and centrifuged as above for 5 min. The supernatant (1 mL) was filtered again, and aliquots (40 μl) were injected at room temperature with a Waters 717 (Waters) autosampler into a reverse-phase Symmetry 5-μm C18 (4.6 by 150 mm; Waters) column, previously equilibrated in 99 % H_2O:1% acetic acid (solvent A). A lineal gradient starting with 100% solvent A and ending with 100 % methanol (solvent B) was applied over 20 min at a flow rate of 1

ml/min. Then, the column was washed with solvent B for 10 min, and allowed to equilibrate again with solvent A, with a total run time of 40 min. Chlorogenic acid was detected photometrically (λ=320 nm) with a Waters 996 photodiode array detector, and quantified with the Waters Empower (Waters) software using authentic chlorogenic standard.[39]

The antioxidant capacity was evaluated by measuring spectrophotometrically at 517 nm the ability to quench the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) according to Falchi et al.[40] Fifty µL of soluble-methanol phenolic fraction were diluted with 2 mL of ethanol 96º. An aliquot of 0.5 mL of the resulting solution was added to 1.5 mL of ethanol 96º, and 0.5 mL ethanolic solution containing DPPH$^{\cdot}$ 0.5 mmol/L. The blank sample was prepared using 2.0 mL ethanol and 0.5 mL of the same DPPH$^{\cdot}$ ethanolic solution in order to check the radical stability. After incubation of the mixture at 25 ◦C for 10 min, the absorbance at 517 nm was measured using a Pharmacia Biotech 1000E UV-Vis (Pharmacia Biotech, Piscataway, NJ, USA) spectrophotometer. The radical scavenging activity (S) of each extract after 10 min was expressed in percentage and calculated as $S=100-[(A_x/A_o)\times100]$, where A_x is the optical density of DPPH$^{\cdot}$ solution in presence of eggplant extract and A_o the optical density of DPPH$^{\cdot}$ solution in absence of the sample.

Polyphenol oxidase (PPO) activity was measured basically according to Bellés et al.[41] Samples (0.1 g) of lyophilized material were reduced to a fine powder with a pestle in a cooled mortar and homogenized in 4 mL of 0.1 M sodium phosphate buffer (pH 6.0) and centrifuged at 12.000 rpm for 15 min at 4 ºC. Supernatant was diluted 5-fold in buffer extraction solution, and PPO assay was carried out in a total volume of 2 mL containing 50 µL of diluted supernatant (enzyme extract), 150 µL of 0.1 M chlorogenic acid (dissolved in 50% methanol), and 1.8 mL of 0.1 M sodium phosphate

buffer (pH 6.0). The reaction blank contained 50 μL of buffer instead of enzyme extract. The enzymatic reaction was followed colorimetrically at 420 nm in a Pharmacia Biotech 1000E UV-Vis spectrophotometer. PPO activity was measured as increments in absorbance at 420 nm per min and mg of dry weight during the first 1.5 min of the reaction, period in which the enzymatic activity was lineal for all substrate concentrations. One unit of enzyme activity was defined as the increase in 0.1 absorbance units per minute per mg of dry weight.

Browning in the liquid extract of lyophilized sample was determined by spectrophotometry at 420 nm.[42] For each sample, 0.25 g of lyophilized tissue were homogenized with 2.5 mL of water and was incubated at room temperature for 10 min. Subsequently, 2.5 mL of a 4% metaphosphoric solution was added to stop the oxidizing reaction.[43] For each sample, a control was prepared in which 0.25 g of lyophilized tissue were homogenized with 2.5 mL of 4% metaphosphoric acid and incubated at room temperature for 10 min. After that 2.5 mL of water were added to the solution. Both the sample and its respective control solutions were centrifuged at 8000 rpm for 5 min. Absorbance was then determined at 420 nm in a Nanodrop ND-1000 spectrophotometer. One unit of extract browning was defined as a difference in 0.01 absorbance units between the sample and the control.

For fruit flesh browning measurement, the CIELAB 1976 color coordinates of the fruit flesh were measured with a Minolta CR-300 (Minolta, Osaka, Japan) chromameter for each of the three fruits that constitute a sample. Measurements were made in the central part of a transversal section of the fruit immediately after being cut (0 min) and 10 min later. Fruit flesh browning was measured as the difference in the degree of whiteness (DW), which is calculated as $DW=[(100-L^*)^2+a^{*2}+b^{*2}]^{0.5}$,[18] between DW at 10 min (DW_{10}) and at 0 min (DW_0). For each sample, the fruit flesh browning

was obtained as the average of the three fruits. One unit of fruit flesh browning was defined as one unit in the difference between DW_{10} and DW_0.

Data analyses. For each trait, accession means were obtained and varieties were ranked from highest to lowest value. Average standard errors (SE) for the means and coefficient of variation (CV; %) were also calculated. Phenotypic correlations between traits were calculated as the linear correlations between germplasm accession means (n=18). Environmental correlations were calculated between the residuals of individual samples (i.e., the values of individual observations of each accession after the mean of each accession has been subtracted) (n=90).[44] Pearson coefficient of correlation (r), and the coefficient of determination (r^2, expressed in percentage) were calculated for phenotypic and environmental correlations. Principal components analysis (PCA) was performed for standardized values using pairwise Euclidean distances among variety means. The results of the PCA analysis were used to establish four groups of accessions with different profile for the traits studied. Signification of differences among groups of accessions for the traits studied was evaluated by means of analyses of variance (ANOVA) using a fixed-effects model for the effect of accession. All statistics were conducted using specific software (Statgraphics Centurion XVI, StatPoint Technologies, Warrenton, VA, USA).

RESULTS

Traits evaluated. Considerable variation among accessions was found for all traits (Table 2). Differences between the lowest and highest mean value for the accessions tested ranged from 1.82-fold for DPPH scavenging activity and 3.36-fold for flesh browning. The coefficient of variation did not present large differences for the traits studied, and ranged between 39.24% for total phenolics content and 54.35% for

PPO activity (Table 2), indicating that compared to the mean value of each trait the variation observed among accessions was similar for all traits.

The total phenolics content ranged between 8.14 g/kg (V21) and 22.47 g/kg (B33), with an average value of 16.86 g/kg (Table 2). The chlorogenic acid content presented a mean value of 3.55 g/kg and varied between 2.47 g/kg (V21) and 6.27 g/kg (V17). This latter accession (V17) presented a remarkably high value of chlorogenic acid content, with a value 41% greater than the accession that ranked second (V9; 4.42 g/kg) (Table 2). Chlorogenic acid was measured at 320 nm wavelength, as this was the wavelength that provided a better resolution after diode array detector scanning from 240 to 400 nm. Chlorogenic acid was the major 320 nm UV-absorbing peak and had a retention time of 11.94 min. Chlorogenic acid represented, on average, 21.1% of the total phenolics content measured by the Folin-Ciocalteu method, although considerable differences have been found among accessions for the chlorogenic acid content to total phenolics content ratio, so that the percentage of total phenolics content accounted by chlorogenic acid varied between 13.6% (B32) and 36.2% (V19).

The DPPH scavenging activity ranged between 27.5% (B36) and 50.3% (V9), with an average value of 35.6% (Table 2). Five accessions presented DPPH scavenging activity values above 40%, while other five presented values below 30%. The PPO activity varied between 0.87 units/g (B31) and 2.49 units/g (V17), with a mean value of 1.55 units/g. Liquid extract browning ranged between 2.38 units (V7) and 7.06 units (V17), with an average value of 4.12 units. In parallel to chlorogenic acid content, accession V17 presented an extract browning value much higher (26.1%) than that of the variety ranking second (V4; 5.60 units). Finally, the fruit flesh browning varied between 2.47 units (V16) and 8.31 units (B33), with the average value being 5.15 units.

Correlations between traits. The pairwise coefficients of phenotypic linear correlation among the six traits studied presented positive values and were significant ($P<0.05$) for 11 out of the 15 correlations studied (Table 3). The highest value for the phenotypic correlation coefficient was between chlorogenic acid content and liquid extract browning (r=0.852) (Figure 1). The coefficient of determination (r^2) for this phenotypic correlation had a value of 72.5%. Values for the phenotypic coefficient of correlation above 0.6 were also found between chlorogenic acid on one side and total phenolics (r=0.633) and DPPH scavenging activity (r=0.612) on the other (Table 3). The phenotypic coefficients of determination for the correlation between these pairs of traits were of 40.0% and 37.5%, respectively. The fruit flesh browning presented low, although significant, values for the phenotypic correlation with total phenolics (r=0.434) and with chlorogenic acid content (r=0.253). The coefficient of determination for the correlation between fruit flesh browning and total phenolics and chlorogenic acid contents, was of only 18.9% and 6.4%, respectively. Fruit flesh browning was not significantly correlated with PPO activity and extract browning (Table 3).

Environmental linear correlation values were mostly in agreement with the results obtained for phenotypic correlations. In this case, all correlations were also positive and the 11 pairwise correlations that were significant for the phenotypic correlations, plus the correlation between liquid extract browning and fruit flesh browning, were significant (Table 4). Also, as occurred for the phenotypic correlations, the highest value for the environmental correlation coefficient was between chlorogenic acid content and liquid extract browning (r=0.883) (Figure 1). The coefficient of determination (r^2) in this case reached a value of 78.0%. High values for the environmental coefficient of correlation were also found between total phenolics on one side and chlorogenic acid content (r=0.834) and extract browning (r=0.792) on the

other, and also between chlorogenic acid content and DPPH scavenging activity (r=0.653). In these cases, the coefficients of determination for the correlation between these pairs of traits were of 69.6%, 62.7%, and 42.7%, respectively (Table 4). Similarly to what happened for the phenotypic correlations, the fruit flesh browning presented low, although significant, values for the environmental correlation with total phenolics (r=0.304), chlorogenic acid content (r=0.253), and extract browning (r=0.319). Therefore, the coefficient of determination for the correlation between the degree of browning and the total phenolics, chlorogenic acid contents, and extract browning was, respectively, of 9.2%, 7.35, and 10.2%. As occurred with the phenotypic correlations, the fruit flesh browning did not present a significant environmental correlation with PPO activity (Table 4).

Principal components analysis. The first and second components of the PCA accounted, respectively, for 48.5% and 21.5% of the total variation for the seven traits included in this study. The first component was positively correlated with all the traits included in the PCA analysis (Table 5). The highest values (>0.4) for the correlation of the first principal component were with chlorogenic acid content, PPO activity, and liquid extract browning. Also, moderate correlations (between 0.2 and 0.4) for this first component were found with DPPH scavenging activity, total phenolics, and fruit flesh browning. The positive correlation of this first component with CGA/TP ratio had a very low value (Table 5). The second component presented a high positive correlation with the CGA/TP ratio (0.640). A moderate positive correlation (0.291) was found with liquid extract browning. The positive correlations with chlorogenic acid content, and PPO activity were much lower (<0.2). This second component presented a high negative correlation with total phenolics (-0.541), and also a moderate negative

correlation with fruit flesh browning (-0.389) (Table 5). The negative correlation with DPPH scavenging activity was very low in absolute values (-0.129).

The projection of the individual accessions in the PCA plot shows that they are widely spread over the graph area, with four to six accessions plotting in each of the quadrants (Figure 2). Accession V17, which presents very high values for the first component is the accession that had the highest values for the three traits, i.e., chlorogenic acid content, PPO activity, and liquid extract browning (Table 2) that present a higher correlation with the first principal component. Also, accession V9, which presents the second highest value for the first component (Figure 2) is the accession that ranks second for chlorogenic acid content, third for PPO activity, and fourth for liquid extract browning (Table 2). Similarly, the three accessions with lowest values for the first component are also the ones with lowest values for chlorogenic acid content (Table 2). The two accessions with highest values for the second component (V19 and V21) are also the ones with highest values for the CGA/TP ratio (highest positive correlation with this component), and present very low values for total phenolics (highest negative correlation with this component), ranking 15 and 18 respectively (Table 2). The two accessions with lowest values for the second component (V7 and B33) are among the lowest (rank 18 and 16, respectively) for the CGA/TP ratio, and present high values (ranks 3 and 1, respectively) for the total phenolics (Table 2).

Differences between accession groups. The PCA analysis allows allocating the 18 accessions to four groups according to the PCA quadrant in which they plot. We have named these groups A (positive for both components), B (positive for the first component and negative for the second one), C (negative for both components), and D (negative for the first component and positive for the second one). Significant

differences were detected among traits for total phenolics, chlorogenic acid content, chlorogenic/total phenolics ratio, PPO activity, and liquid extract browning (Table 6). No significant differences among groups were observed for DPPH scavenging activity and fruit flesh browning.

Assignation of low (-) or high (+) levels to each of the groups for the traits for which significant differences among groups were found allowed establishing a simplified profile for each of the groups (Table 7). The results obtained for individual accessions (Table 2) are largely in agreement with the results of the PCA classification. For example, group A, which is the only one with high chlorogenic acid content and liquid extract browning values (Tables 6 and 7) includes the six accessions which rank first in chlorogenic acid content, and six of the seven accessions with highest values for liquid extract browning activity. Similarly, group D, which is the only one with low total phenolics content values (Tables 6 and 7) includes four of the accessions with lowest total phenolics content (Table 2). Groups B and C, which are classified as having have high values for CGA/total phenolics (Tables 6 and 7) ratio include the 10 accessions with highest value for the CGA/total phenolics ratio, while groups C and D, which are characterized for high PPO activity include 10 out of the 11 accessions with highest PPO activity (Table 2).

DISCUSSION

Although the accessions we have studied come from a geographically limited region, a wide variation has been found for the traits studied, which confirms the high diversity of eggplants from the Mediterranean region.[34-36,45,46] The wide variation for total phenolics and chlorogenic acid is of interest for the development of new cultivars with enhanced bioactive properties. In this respect, the inheritance of total phenolics content and

chlorogenic acid content has not been studied in depth in eggplant, although our previous results[16,18] indicate that phenotypic selection can be efficient to develop eggplant cultivars with improved phenolics content. A next step consists in the mapping of genes and QTLs involved in the pathway of chlorogenic acid synthesis, which may provide tools (molecular markers) and relevant information for the breeding of eggplant cultivars with improved chlorogenic acid content.[6]

Chlorogenic acid is the major phenolic compound in the eggplant flesh, as it typically represents between 70% to 95% of the phenolic compounds as determined by HPLC studies[11,16,47]. However, we have found that total phenolics content levels as determined by the spectrophotometric Folin-Ciocalteu method[37] were considerably higher than those of chlorogenic acid. The Folin-Ciocalteu method frequently overestimates phenolics the real phenolics content, as the Folin-Ciocalteu reagent reacts not only with phenolic compounds, but also with with other antioxidants, proteins, and some inorganic ions.[48-50] In this respect, Mennella et al.[9] found a wide range of variation for the ratio chlorogenic acid to total phenolics depending on the cultivar and stage of fruit ripening. This suggests that, in eggplant, data for total phenolics obtained with the Folin-Ciocalteu method have to be interpreted with caution, especially when their correlation with chlorogenic acid or antioxidant activity is low.

The wide variation for phenolics content has been matched by high values for variation in antioxidant activity. The moderate values obtained by us for the phenotypic correlation between total phenolics and CGA content on one side and DPPH scavenging activity on the other, are similar to those obtained by other authors between total phenolics and chlorogenic acid on one side and superoxide scavenging activity,[8,9,17] and between total phenolics and ABTS antioxidant activity.[7]

Fruit flesh browning is a major issue to be taken into account when breeding for high content in phenolics in eggplant.[6] PPO activity, liquid extract browning, and fruit flesh browning also presented a high degree of variation suggesting that eggplant materials are amenable to selection for these traits. The low phenotypic correlation values between fruit flesh browning on one side and the rest of traits measured on the other may suggest that, although eggplant PPOs show a great affinity for chlorogenic acid,[21,22] several factors other than total phenolics and/or chlorogenic acid contents and PPO activity may have an influence in fruit flesh browning after the fruit has been cut.[31] Cell and cell compartments size, morphology, and composition have been reported to have an important influence in fruit flesh browning.[19,31] In this respect, PPO enzymes are present in the plastids and phenolics in the vacuoles.[19,20] Therefore, differences in: the amount of PPO and phenolics released from these cell compartments, their diffusion through the tissue, the accessibility of O_2 to phenolic compounds, the amounts of cell compounds that are able to react with the quinones resulting from the oxidation of phenolics to produce brown compounds, intracellular pH, or the concentration of other antioxidants that are able to prevent oxidation of phenolics, like ascorbic acid, may also have a main role in fruit flesh browning in eggplant.[22,23,27-29,31] These factors would have less relevance for liquid extract browning, in which the whole tissue is disintegrated and phenolics and PPO oxidases are released in the solution.[28]

The low phenotypic correlations of fruit flesh browning with total phenolics, chlorogenic acid content, DPPH scavenging activity, and PPO activity are of interest for the development of cultivars that have a high content in total phenolics and/or chlorogenic acid and at the same time present a low or moderate fruit flesh browning. In this respect, only 18.9% of the total variation for fruit flesh browning is caused by the differences in total phenolics. The results that we have found are in agreement with

those of Prohens et al.,[18] who in a collection of 69 *S. melongena* accessions found that the variation in fruit flesh browning accounted by the content in total phenolics was 15.1%. Similarly, Prohens et al.[16] in an interspecific family between *S. melongena* and *S. incanum* found that only 6.0% of the variation in fruit flesh browning found in the F2 generation was accounted by variation in chlorogenic acid content. These results are also largely in agreement with those of Mishra et al.[32] in which when eight eggplant accessions were compared before being subjected to storage, there was a moderate correlation between PPO activity and fruit flesh browning. Low or moderate values of correlation between fruit flesh browning and PPO activity have also been reported in other crops.[51,52] In any case, several studies have shown that inhibition of PPO activity in eggplant reduces browning.[26,28-31,53] In our case, lack of correlation of PPO with fruit flesh browning may suggest that PPO activity levels in the materials we have studied are not limiting to produce fruit flesh browning. This does not preclude that selection of eggplant varieties with highly reduced PPO activity may have decreased levels of fruit flesh browning.

The high phenotypic correlation value between chlorogenic acid content and DPPH scavenging activity confirms that chlorogenic acid is a key compound for the functional activity of eggplant fruit.[6] Also, the high correlation value between chlorogenic acid content and liquid extract browning suggests that the measurement of liquid extract browning could be a rapid method for the evaluation for chlorogenic acid content in large samples of material, like collections of germplasm or segregating generations in breeding programs.

Few studies have been made to evaluate the environmental effects on the traits measured in this paper.[8,17,54] The prior work shows that the environment has considerable effect on the content in phenolics and on antioxidant activity. However, no

reports are known to us on the study of environmental correlations between these traits in eggplant. Here, we have shown that there are important environmental correlations between several traits, which suggests that environment may have a relevant role in the observed phenotypic correlations. Some of these environmental correlations were expected, as those between total phenolics, chlorogenic acid, DPPH activity, and liquid extract browning, as the traits may have a common physiological basis. However, it is remarkable that positive environmental correlations also exist between PPO and CGA, suggesting that environmentally-induced increases in CGA content may activate the expression of PPO genes in eggplant.[15,20]

The results obtained from the PCA analysis shows that multivariate studies may be of great utility for classification of eggplant accessions according to their phenolics, antioxidant activity and browning profile.[54] Mishra et al.[32] found two different profiles in the postharvest evolution of PPO activity in a set of eight eggplant accessions. In one of the groups, PPO activity increased during postharvest storage, while in the other it decreased.[32] Each of the four groups identified by us included accessions from different morphological characteristics showing that a wide diversity for the traits studied exists within cultivar groups.[7,9] This suggests that it is possible to find, within a single cultivar group, accessions with different profile for phenolics content, PPO activity and extract browning. The identification of these groups may be of interest for the study of the genetic regulation of phenolics content, PPO activity, and browning, for physiology studies, and for identifying adequate sources of variation for breeding in a specific cultivar group of eggplant.

Within the collection we have studied, we have identified some accessions for interest in selection and breeding for high content in chlorogenic acid and antioxidant activity, and low fruit flesh browning. For example, among the 18 accessions evaluated,

accession V17 presents the highest value for chlorogenic acid content and also presents high values for total phenolics, and DPPH scavenging activity; however, it also has the highest fruit flesh browning. Also, accession V9 presents a high content in chlorogenic acid, has the highest DPPH scavenging activity, and a one of the lowest values for fruit flesh browning. Inclusion of accessions presenting high values for chlorogenic acid content or an adequate combination of functional activity and apparent quality traits of interest in breeding programs could be of great interest for developing new cultivars with high added value.[6] Overall, our results indicate that there are good prospects for the selection and genetic improvement of eggplant in order to obtain new varieties with improved functional (phenolics content, in particular chlorogenic acid) and apparent (low fruit flesh browning) quality.

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Figure captions:

Figure 1. Phenotypic (left) and environmental (right) relationships between chlorogenic acid content (g/kg dw, *x*-axis) and liquid extract browning (units, *y*-axis) in a collection of 18 eggplant accessions with five samples per accession. Phenotypic relationships compare accession means (n=18) for both traits. Environmental relationships compare the residuals of individual samples (i.e., the values of individual observations of each accession after the mean of each accession has been subtracted) (n=90).The linear coefficient of correlation (r) is indicated.

Figure 2. Similarities among the 18 eggplant accessions evaluated based on seven traits (total phenolics content (TP), chlorogenic acid content (CGA), CGA/TP ratio, DPPH scavenging activity, PPO activity, liquid extract browning, and fruit flesh browning) represented on the two first components (first component, *x*-axis; second component, *y*-axis) of the principal components analysis (48.5% and 21.5% of the total variation, respectively).

Table 1. Accessions, Code Used in the Present Work, Fruit Size Measures (Length, Width, and Weight; Mean±SD), Fruit Color Class, and Geographical Origin of the Eggplant Materials Used for the Study of the Diversity and Relationships Between Phenolics Content, Polyphenol Oxidase (PPO) Activity, and Browning.

Accession[a]	Code	Fruit Length (cm)	Fruit Width (cm)	Fruit Weight (g)	Fruit Color Class	Origin
B-31	B31	10.0 ± 1.2	9.4 ± 1.4	225 ± 78	Black	Valencia province, Spain
B-32	B32	14.2 ± 2.2	8.1 ± 1.5	346 ± 148	White	Valencia province, Spain
B-33	B33	15.1 ± 1.8	5.2 ± 0.5	171 ± 36	White	Valencia province, Spain
B-36	B36	14.7 ± 2.2	5.0 ± 0.7	176 ± 74	Purple	Valencia province, Spain
V-S-4	V4	19.6 ± 3.4	5.1 ± 0.7	206 ± 80	Black	Gandía, Valencia, Spain
V-S-5	V5	21.5 ± 3.6	4.9 ± 0.6	232 ± 83	Black	Xeraco, Valencia, Spain
V-S-6	V6	20.8 ± 2.9	5.2 ± 0.7	223 ± 60	Black	Xeraco, Valencia, Spain
V-S-7	V7	13.3 ± 1.4	9.2 ± 1.5	346 ± 90	Striped purple	Xeraco, Valencia, Spain
V-S-9	V9	6.7 ± 0.9	8.2 ± 1.1	233 ± 81	Green with purple streaks	La Aparecida (Orihuela), Alicante, Spain
V-S-10	V10	10.2 ± 0.9	6.7 ± 1.1	169 ± 55	Purple	La Aparecida (Orihuela), Alicante, Spain
V-S-12	V12	25.2 ± 3.4	4.6 ± 0.4	200 ± 54	Black	Benijófar, Alicante
V-S-13	V13	7.6 ± 1.1	7.8 ± 0.9	180 ± 54	Purple	San Fulgencio, Alicante, Spain
V-S-14	V14	17.5 ± 1.8	6.1 ± 0.8	250 ± 73	Black	Rafal, Alicante, Spain
V-S-16	V16	17.9 ± 4.7	7.7 ± 1.6	290 ± 85	Black	Novelda, Alicante, Spain
V-S-17	V17	17.6 ± 4.2	5.9 ± 0.8	228 ± 91	Black	Elx, Alicante, Spain
V-S-18	V18	17.5 ± 2.9	5.1 ± 0.8	168 ± 62	Black	Elda, Alicante, Spain
V-S-19	V19	23.1 ± 2.9	5.2 ± 0.6	252 ± 67	Black	Mutxamel, Alicante, Spain
V-S-21	V21	23.6 ± 3.7	5.9 ± 1.0	301 ± 107	Black	Gandía, Valencia, Spain

[a]The B and V codes indicate, respectively, accessions originating from the Agricultural Experimental Farm of Carcaixent (Valencia, Spain) and from the COMAV germplasm bank (Valencia, Spain).

Table 2. Mean Values and Rank (from Highest to Lowest; Between Brackets, Italics) for Each Accession, Average Standard Error (SE; Obtained from the ANOVA Analyses) and Coefficient of Variation (CV; %) for Fruit Traits in a Collection of 18 Eggplant Accessions.

Accession Code	Total Phenolics (g/kg dw)	Chlorogenic Acid (g/kg g dw)	DPPH Scavenging Activity (%)	PPO Activity (units/mg dw)	Liquid Extract Browning (units)	Fruit Flesh Browning (units)
B31	18.52 (*8*)	3.11 (*13*)	34.3 (*8*)	0.87 (*18*)	2.65 (*17*)	4.28 (*13*)
B32	20.99 (*4*)	2.86 (*15*)	32.7 (*10*)	0.95 (*17*)	4.42 (*8*)	4.27 (*14*)
B33	22.47 (*1*)	3.35 (*9*)	31.3 (*12*)	1.12 (*15*)	3.71 (*12*)	8.31 (*1*)
B36	20.59 (*5*)	3.28 (*10*)	27.5 (*18*)	1.42 (*10*)	3.59 (*13*)	6.14 (*4*)
V4	16.69 (*11*)	4.24 (*4*)	29.3 (*15*)	1.82 (*7*)	5.60 (*2*)	5.57 (*8*)
V5	19.13 (*7*)	3.25 (*12*)	34.7 (*7*)	1.87 (*4*)	4.77 (*5*)	4.69 (*11*)
V6	16.35 (*12*)	3.84 (*5*)	32.6 (*11*)	1.70 (*8*)	5.22 (*3*)	4.51 (*12*)
V7	21.94 (*3*)	2.98 (*14*)	46.5 (*3*)	1.83 (*6*)	2.38 (*18*)	8.08 (*2*)
V9	17.53 (*10*)	4.42 (*2*)	50.3 (*1*)	2.15 (*3*)	4.81 (*4*)	3.49 (*16*)
V10	10.06 (*16*)	2.82 (*16*)	28.0 (*17*)	1.31 (*12*)	2.81 (*15*)	5.62 (*7*)
V12	20.06 (*6*)	3.53 (*7*)	36.2 (*6*)	1.39 (*11*)	3.99 (*10*)	5.68 (*6*)
V13	18.26 (*9*)	3.49 (*8*)	40.7 (*5*)	2.17 (*2*)	2.98 (*14*)	5.21 (*9*)
V14	16.08 (*13*)	4.34 (*3*)	34.2 (*9*)	1.47 (*9*)	4.77 (*6*)	4.88 (*10*)
V16	9.86 (*17*)	2.67 (*17*)	30.9 (*13*)	1.20 (*14*)	2.72 (*16*)	2.47 (*18*)
V17	22.00 (*2*)	6.27 (*1*)	46.8 (*2*)	2.49 (*1*)	7.06 (*1*)	6.53 (*3*)
V18	14.58 (*14*)	3.25 (*11*)	45.6 (*4*)	1.07 (*16*)	3.80 (*11*)	4.08 (*15*)
V19	10.23 (*15*)	3.71 (*6*)	29.5 (*14*)	1.86 (*5*)	4.64 (*7*)	5.93 (*5*)
V21	8.14 (*18*)	2.47 (*18*)	29.0 (*16*)	1.23 (*13*)	4.27 (*9*)	3.03 (*17*)
Mean	16.86	3.55	35.6	1.55	4.12	5.15
Average SE	2.16	0.77	8.7	0.35	0.82	0.84
CV (%)	39.24	50.47	52.51	54.35	50.34	46.10

Table 3. Phenotypic Linear Correlations (r; Above the Diagonal) and Coefficients of Determination (%, Below the Diagonal) for Fruit Traits (Total Phenolics, TP; Chlorogenic Acid, CGA; DPPH Scavenging Activity, DPPH; Polyphenol Oxidase Activity, PPO; Liquid Extract Browning, LEB; and, Fruit Flesh Browning; FFB) in a Collection of 18 Eggplant Accessions.

	TP	CGA	DPPH	PPO	LEB	FFB
TP[a]		0.633***	0.461***	0.197ns	0.512***	0.434***
CGA	40.0		0.612***	0.522***	0.852***	0.253*
DPPH	21.2	37.5		0.395***	0.453***	0.135ns
PPO	3.9	27.2	15.6		0.464***	0.185ns
LEB	26.2	72.5	20.5	21.5		0.192ns
FFB	18.9	6.4	1.8	3.4	3.7	

[a]***, **, *, ns indicate significant at P<0.001, P<0.01, P<0.05, and non-significant values for the coefficient of correlation (r).

Table 4. Environmental Linear Correlations (r; Above the Diagonal) and Coefficients of Determination (%, Below the Diagonal) for Fruit Traits (Total Phenolics, TP; Chlorogenic Acid,CGA; DPPH Scavenging Activity, DPPH; Polyphenol Oxidase Activity, PPO; Liquid Extract Browning, LEB; and, Fruit Flesh Browning; FFB) in a Collection of 18 Eggplant Accessions.

	TP	CGA	DPPH	PPO	LEB	FFB
TP[a]		0.834***	0.547***	0.203ns	0.792***	0.304**
CGA	69.6		0.653***	0.458***	0.883***	0.270*
DPPH	29.9	42.7		0.379***	0.561***	0.170ns
PPO	4.1	21.0	14.3		0.439***	0.159ns
LEB	62.7	78.0	31.5	19.3		0.319**
FFB	9.2	7.3	2.9	2.5	10.2	

[a]***, **, *, ns indicate significant at P<0.001, P<0.01, P<0.05, and non-significant values for the coefficient of correlation (r).

Table 5. Correlation Coefficients for Each Fruit Trait for the First and Second Principal Components, Eigenvalue, and Relative and Cumulative Proportion of the Total Variance Explained by These Components, in a Collection of 18 Eggplant Accessions.

	Common Principal Component Coefficients	
Traits	Component 1	Component 2
Total phenolics (TP)	0.318	-0.541
Chlorogenic acid (CGA)	0.545	0.152
CGA/TP ratio	0.048	0.640
DPPH scavenging activity	0.361	-0.129
PPO activity	0.485	0.148
Liquid extract browning	0.425	0.291
Fruit flesh browning	0.232	-0.389
Eigenvalue	3.397	1.505
Variance explained (%)	48.5	21.5
Cumulative variance explained (%)	48.5	70.0

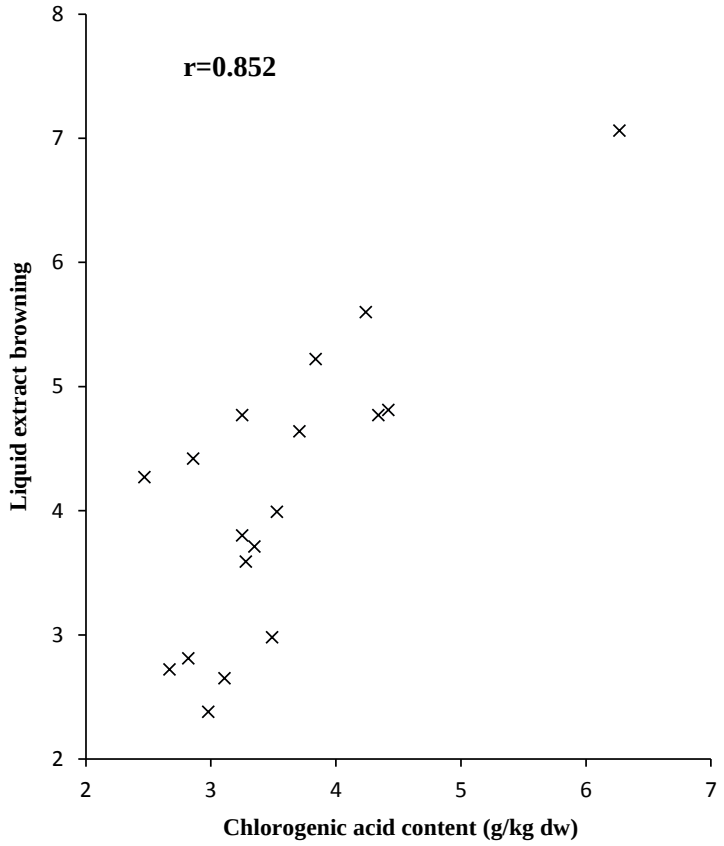
Table 6. Mean Values for Fruit Traits (Total Phenolics, TP; Chlorogenic Acid, CGA; CGA/TP Ratio, CGA/TP; DPPH Scavenging Activity, DPPH; Polyphenol Oxidase Activity, PPO; Liquid Extract Browning, LEB; and, Fruit Flesh Browning; FFB) in the Four Groups of Accessions Established (A-D) by a Multivariate Principal Components Analysis in a Collection of 18 Eggplant Accessions, and Probability of the *F*-Statistic, Obtained from ANOVA Analyses, for Differences Among Means.

	Accession Group				
	A	B	C	D	
n	6	4	4	4	Prob. *F*
TP[a]	16.48 b	19.85 b	20.64 b	10.66 a	<0.001
CGA	4.47 b	3.31 a	3.15 a	2.80 a	0.003
CGA/TP	27.64 b	16.82 a	15.31 a	26.93 b	<0.001
DPPH	37.12 a	39.53 a	31.45 a	33.38 a	0.401
PPO	1.92 b	1.82 b	1.09 a	1.20 a	0.001
LEB	5.35 b	3.53 a	3.59 a	3.40 a	0.008
FFB	5.15 a	5.92 a	5.75 a	3.80 a	0.202

[a]Means separated by different letters within a row are significantly different according to the Student-Newman-Keuls multiple range test at P<0.05.

Table 7. Simplified Representation of the Levels for the Traits for Which Significant Differences Exist Among Groups of Accessions, According to Low (-) or High (+) Levels, in the Four Groups of Accessions Established (A-D) by a Multivariate Principal Components Analysis in a Collection of 18 Eggplant Accessions.

	Accession Group			
Traits	A	B	C	D
Total phenolics (TP)	+	+	+	-
Chlorogenic acid (CGA)	+	-	-	-
CGA/TP ratio	+	-	-	+
PPO activity	+	+	-	-
Liquid extract browning	+	-	-	-


r=0.852
Liquid extract browning
Chlorogenic acid content (g/kg dw)
8
7
6
5
4
3
2
2
3
4
5
6
7

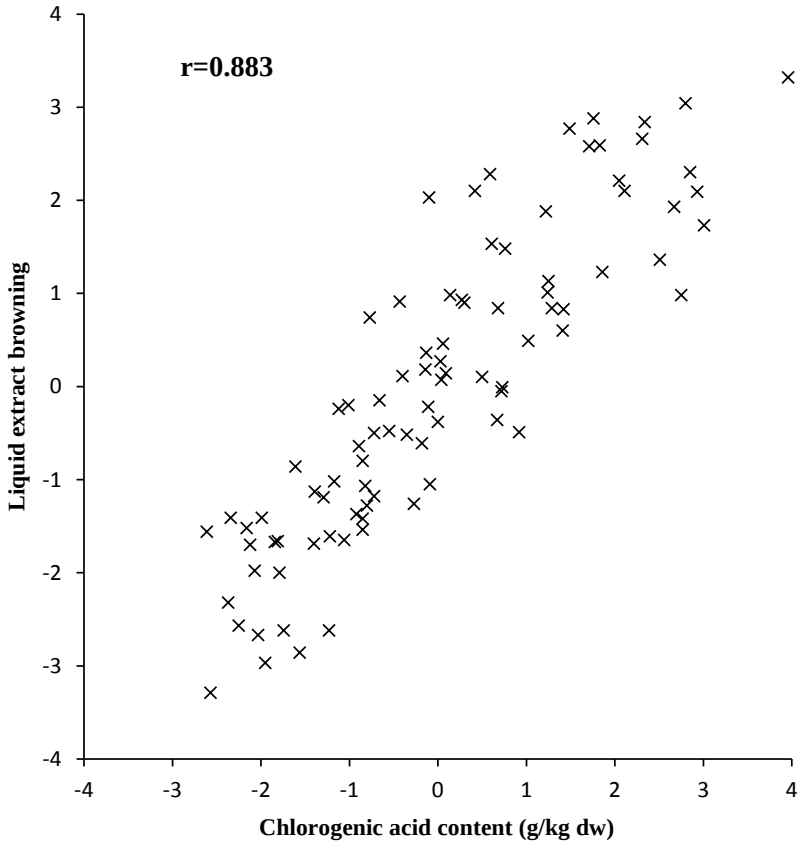

r=0.883
Liquid extract browning
Chlorogenic acid content (g/kg dw)
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4

Figure 1.

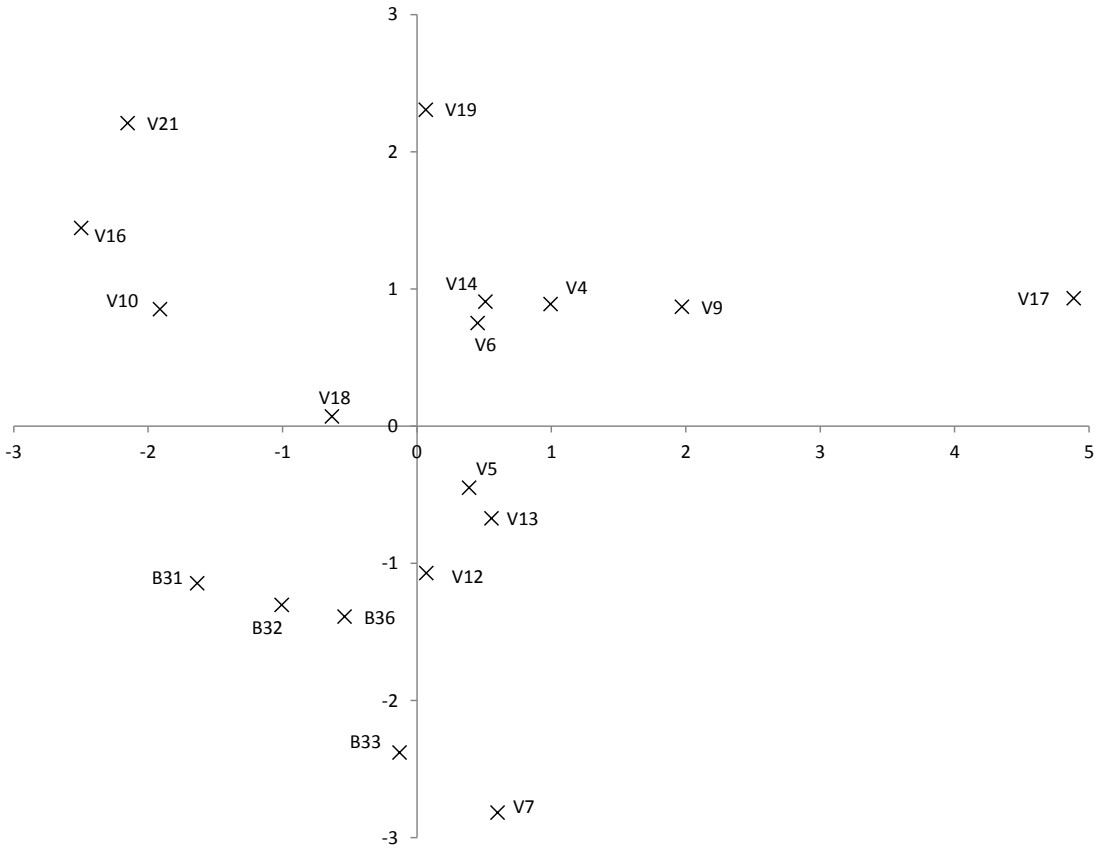

3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
4
5
V19
V21
V16
V14
V4
V10
V9
V17
V6
V18
V5
V13
V12
B31
B32
B36
B33
V7

Figure 2.

Graphic for table of contents: