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CROP PRODUCTION

Radiographic images and relationship of the internal morphology and physiological potential of broccoli seeds

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ABSTRACT. Image analysis using X-rays is an efficient technique for assessing the seed quality of several species, presenting itself as a rapid response method that is simple to execute, reproducible and non-destructive. Thus, this research adjusted a methodology that aims to relate the internal morphology of broccoli seeds to their physiological potential through radiographic analysis of seeds and computerized images of seedlings. The broccoli cultivars used were *Piracicaba Precoce* and *Ramoso Santana* represented by ten lots of each one. The study was conducted using X-ray imaging for seed analysis, in which the area, density and circularity measures were obtained as internal morphology characteristics of seeds. After a germination test, the seedling length was obtained 5 days after sowing. Multivariate analysis was carried out using principal components analysis. It was concluded that the X-ray test is efficient for evaluating the internal morphology of broccoli seeds and associating it with seedling length, thereby classifying seed lots at different levels of vigor.

Keywords: internal morphology, X-ray, vigor, seedling length.

Imagens radiográficas e a relação da morfologia interna com o potencial fisiológico de sementes de brócolis

RESUMO. A análise de imagens de raios X é uma técnica eficiente para avaliação da qualidade de sementes de várias espécies, apresentando-se como método de resposta rápida, com metodologia de simples execução e reprodução, além de ser não destrutivo. Com isso, esta pesquisa adequou metodologia que visa relacionar a morfologia interna de sementes de brócolis com seu potencial fisiológico por meio de análises de imagens radiográficas de sementes e imagens computadorizadas de plântulas. Para tanto, foram utilizadas sementes de dois cultivares de brócolis, Piracicaba Precoce e Ramoso Santana, representados por dez lotes de cada. O estudo foi realizado por meio da análise de imagens radiográficas das sementes, em que foram obtidas as medidas de área, densidade e circularidade como sendo as características de morfologia interna das sementes após cinco dias de germinação, foram determinados os comprimentos de plântulas de forma individual. Realizou-se estatística multivariada utilizando-se análise de componentes principais. Conclui-se que o teste de raios X é eficiente em avaliar a morfologia interna de sementes de brócolis e associá-la ao comprimento de plântulas e, com isso, classificar lotes de sementes em diferentes níveis de vigor.

Palavras chave: morfologia interna, raios X, vigor, comprimento de plântulas.

Introduction

Brassica oleraceae L. var. *italic* Plenk, is a member of the Brassicaceae family and is popularly known as broccolis or brócolos. Brassicaceae is one of the main families from the economic point of view. It includes a great variety of vegetable species, the main being *Brassica oleraceae*, and from this, several horticultural varieties were obtained through breeding, including broccoli (Souza & Lourenzi, 2008). Brassicaceae has flowering, fruiting and seed maturation progressively throughout its cycle. It is difficult to determine the physiological maturity of seeds that present indefinite growth extended to the flowering period, which is the case for brassica species, such as broccoli (Still & Bradford, 1998). Therefore, several maturation stages can be found in the same broccoli seed lot after harvest (Lewis & Welbaum, 1996), resulting in lower seeds vigor in view of the non uniform characteristics related to internal seed morphology. Moreover, the same authors reported that the development of broccoli seeds is highly sensitive to variations in temperature and air relative humidity, promoting large differences in the maturity stage of the seeds, which was verified using vigor testing.

The use of efficient techniques to evaluate the vigor of seeds that reliably estimate physiological potential and identify significant differences among lots with similar germination rates is very important for agriculture. Several studies with crops of agronomic interest have been carried out using physical, physiological, biochemical and resistance tests, according to the vigor rating tests proposed by McDonald (1975). However, even with increased investment in studies on vegetable seeds, it is still necessary to develop research to evaluate seeds vigor tests, especially tests for broccoli crops.

In recent years, research on the evaluation of the internal morphology of seeds has been based on image analysis techniques. The inclusion of new approaches depends on this research; therefore, image analysis is a recent alternative for understanding several aspects of seeds development, allowing the establishment of a relationship between internal morphology and structural integrity and allowing the physiological potential of seed lots to be determined (Marcos Filho, 2010).

Radiographic images of seeds combined with techniques of computer image analysis from seedlings indicate new research guidelines towards the development of non-destructive seed analysis methods, enabling integration with quality control programs of production companies (Gomes Junior, 2010). Radiographic image analysis is effective for determining the internal morphology and parameters associated with physiological quality of several species such as maize (Cícero, Van Der Heijden, Van Der Burg, & Bino, 1998; Cícero & Banzatto Junior, 2003), soybean (Flor, Cicero, França-Neto, & Krzyzanowski, 2004), rice (Menezes, Cicero, Villela, & Bortolotto, 2012), papaya (Santos et al., 2009; Dias, Dias, Gomes Junior, & Cicero, 2014), pepper (Dell' Aquila, 2007), bell pepper (Gagliardi & Marcos Filho, 2011), eggplant (Silva, Cicero, & Bennett, 2012), cucumber (Gomes Junior, Chiquito, & Marcos Filho, 2013), jatropha (Pinto, Marcos Filho, Forti, Carvalho, & Gomes Junior, 2009), among other species.

As for studies related to seed development as identified by X-rays, one of the current research concerns is the automation of these determinations in order to establish greater accuracy of assessments and therefore eliminate subjective interpretations. In this sense, evaluations of such as pixel density, which is measured in gray scale, RGB (Red-Green-Blue), and free space characteristics related to internal morphology of seeds are used. Such characteristics can be measured using specific software for image analysis, which makes the evaluation less subjective. The use of techniques that exhibit a rapid response regarding the physiological quality of seeds and present non-destructive sampling methods that enable the subsequent use of the seeds, besides simple execution and reproduction methodology, is increasing and has helped to improve the traditional methods.

Considering the economic importance and high technology of vegetable seed production, few methodologies provide fast and secure data about seed quality. Therefore, this research aimed to adjust a methodology that relates the internal morphology of broccoli seeds to their physiological potential through radiographic image analysis of seeds and computerized images of seedlings.

Material and methods

This experiment was conducted in the Laboratory of Images Analysis and Seed Analysis in the Department of Crop Science at the "Escola Superior de Agricultura Luiz de Queiroz", São Paulo University (LPV/ESALQ/USP), in Piracicaba, São Paulo State, Brazil. Two broccoli cultivars were used, *Piracicaba Precoce* and *Ramoso Santana*, which are assigned by different companies, represented by ten lots each, with different levels of vigor based on initial germination of seed lots.

Analyses of the internal morphology of the seeds were performed using 8 replications with 25 seeds from each cultivar lot to facilitate the arrangement of seeds to take the images. The samples were distributed on transparent plastic foil containing double-sided adhesive tape to prevent seed movement, allowing them to remain in the same order until the end of the evaluations so that they could be identified in the subsequent measurements. All seeds were placed on the foil in a similar position, enabling the visualization of the internal parts. The radiographic images were taken using digital equipment: Faxitron model MX-20 DC-12 coupled to a Core 2 Duo computer (3.16 GHz, 2 GB of RAM memory, Hard Disk of 160 GB) and Multi Sync monitor (LCD1190SX with 17 inches). The generated images were saved and used in subsequent analysis. Seeds with no damage were evaluated using the ImageJ® software (*Image Processing and Analysis in Java*) to determine the development of the seeds. The variables obtained by this software are detailed below.

Area: first, the seeds selected had their area obtained in number of pixels. Then, the units were transformed to square millimeters (mm^2). Density (average of gray scale value): the average gray scale value within a selected area of every seed, and the sum of the gray values of all pixels in the selected area divided by the number of pixels. Circularity:

measured using the following formula: Circ = $4\pi \times \frac{(\text{Area})}{(\text{Perimeter})^2}$. In this equation, values equal to 1.0 indicates a perfect circle and values close to 0 suggest an elongated shape.

The seed water content was determined by the oven method at 105 ± 3°C for 24 hours based on the *Regras para Análises de Sementes* (Brasil, 2009). Two replications of 0.50 g were evaluated from every seed lot, and the results were expressed as percentages (wet basis).

Germination tests were performed for all lots, maintaining the same arrangement of seeds originally used in the X-ray test. To carry out the germination tests, the seeds were distributed on the germination paper (Germitest) previously moistened with distilled water at a ratio of 2.5 times the dry weight of the paper and under alternating temperature conditions (20 - 30°C) (Brasil, 2009). The previously identified seeds were distributed on top of the paper to allow individualized development. The evaluation of seed germination was performed on the fifth day after sowing, which is the recommended date for the first count (Brasil, 2009). The normal, abnormal seedlings and non-germinated seeds were scanned using a device (HP, model Scanjet 200) set in the inverted position inside a custom scanning box (60 x 50 x 12 cm) constructed of a metal sheet and an aluminum bed attached to the computer.

The length of the seedlings was measured using the software Seed Vigor Imaging System (SVIS®) (Sako, McDonald, Fujimura, Evans, & Bennett, 2001). The results were interpreted using parallel analysis of radiographic images of the seeds and images of their respective normal and abnormal seedlings and non-germinated seeds. Descriptive analysis was performed to characterize the density of broccoli seeds for both cultivars. The results were reported using box and whiskers plots.

Seed density was classified as a function of the normal frequency distribution (Figure 1) for each cultivar to correlate the morphological characteristics from radiographic images with the seedlings' performance and allow us to infer seed lot vigor. The seed density value observed for the cultivars *Piracicaba Precoce* and *Ramoso Santana* were similar; therefore, the same rating was adopted for both cultivars.

Four seed categories were adopted based on the frequency distribution from the gray scale pixel density for both cultivars as it follows: Category I – seed density lower than 75.9; Category II – seed density from 76 to 79.9; Category III - seed density from 80 to 83.9; Category IV - seed density higher than 84.

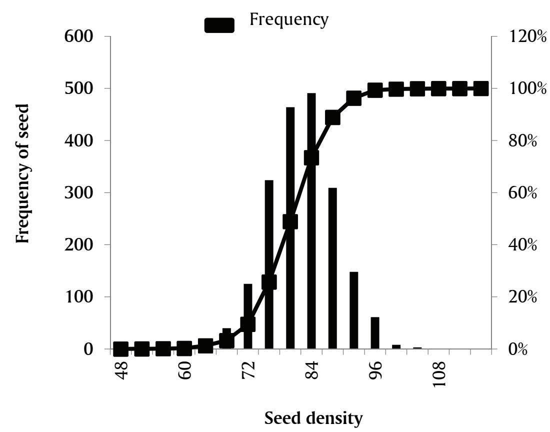

Figure 1. Distribution of gray scale pixel density from broccoli seeds of the cultivars *Piracicaba Precoce* and *Ramoso Santana*.

Multivariate statistics using principal components analysis (PCA) were used to analyze the data. Averages from every seed lot were calculated and transformed to mean zero and variance one. The implementation of this transformation aims to avoid overestimating or underestimating a variable due to differences in scale measures. An “n x p” matrix was obtained, in which "n" is the number of categories (n = 4) and "p" is the number of variables analyzed (p = 4), as follows: seed area (mm^2), density, circularity and seedlings length (mm).

Eigen values (representative values of the variability retained for each new component) and eigenvectors (representative values of the point’s location in the graph) were calculated from the covariance matrices and plotted on two-dimensional graphs (*biplot*). They were adjusted using the Microsoft Excel macro proposed by Lipkovich and Smith (2002). The ideal number of principal components was considered when the sum of the eigen values exceeded 80% of the data variability, following Jollife (2002).

Results and discussion

The water contents in the seed lots ranged from 5.0 to 5.7% for the cultivar *Piracicaba Precoce* and from 4.2 to 5.2% for the cultivar *Ramoso Santana* (Table 1). Water content is an important factor that significantly affects the physiological quality of seed lots, the harvesting moment during seed production, the seed storage potential, and the interpretation of some seed vigor tests. Based on reports from Simak (1991) and Gagliardi and Marcos Filho (2011) about techniques useful for images analysis such as X-rays, water content affects the optical density of seeds. Therefore, the lower the water content, the higher the optical density of seeds is allowing a better view of their internal parts. When seeds have lower water content it is possible to detect results from physiological tests with greater reliability.

Furthermore, during the imbibition process of *Brassica oleracea* seeds, Dell'Aquila, Van Eck, and Van Der Heijden, (2000) observed linear increases in seed length, width and thickness with high moisture. Thus, to compare vigor among seed lots, the variation in water content cannot be high, for the non-uniform development because of differences in the vigor of lots.

Table 1. Mean water content of broccoli seed lots from the cultivars *Piracicaba Precoce* and *Ramoso Santana*.

Lot	*Piracicaba Precoce*	*Ramoso Santana*
	Watercontent (%)	
1	5.6	5.2
2	5.5	4.8
3	5.4	4.5
4	5.0	4.2
5	5.2	4.9
6	5.3	4.3
7	5.7	4.3
8	5.4	4.6
9	5.0	4.2
10	5.6	4.6

Figure 2 shows an overview of the seeds density obtained using Image J software. The box plot graph can be used as a quick way for analyzing one or more characteristics of a sample, allowing to inferences about minimum and maximum values observed and a margin of 50% (from 25 to 75%) of the data included in the box. It is noted that several of the *Piracicaba Precoce* lots showed more uniform densities than the *Ramoso Santana* lots. It is also observed that density levels will vary among lots within each cultivar.

For the cultivar *Piracicaba Precoce*, more lots presented low density, such as lots 2, 4, and 10. Lot 6 presented a single seed with high density. The distance between the first and third quartile varied among lots, among which those with smaller distances between quartiles are lots that have 50% of the seeds with lower density variation and can be classified as more uniform. The seed density ranged from 72 to 92 (Figure 2A).

For the cultivar *Ramoso Santana* lot 2 had few seeds with lower density and lot 5 presented the maximum density, however lower values prevailed among lots. The distance between the first and third quartile varied among lots and was less uniform when compared to the cultivar *Piracicaba Precoce*. The density of seeds from this cultivar presented higher values, ranging from 75 to 95 (Figure 2B).

The principal component analyses are shown in Figures 3 and 4 as biplot graphs for cultivars *Piracicaba Precoce* and *Ramoso Santana*, respectively. Both cultivars presented similar behavior.

Piracicaba Precoce had an eigenvalue of 3.69 in the first component (PC1), with a total of 4, generated by the combination of the variable area, density, circularity and length of seedlings, and explaining 92% of the total variability of the data. The second component presented an eigenvalue of 0.3, representing 7.7% of the total variability. According to Jollife (2002), the sum of the two components is enough, since they explain more than 80% of the total variability (Figure 3).

Also, according to Figure 3, the results for the cultivar *Piracicaba Precoce* indicated in the principal component analysis represented by the *biplot* graph indicated that the first principal component (PC1) was comprised of the linear sum of the results of area, density and length of seedlings with subtraction of circularity results. Thus, principal component 1 (PC1) represents the results of the 10 lots according to the mathematical model assigned to this component, as follows:

PC1 = 0.51*(standardized area) + 0.51*(standardized density) + 0.50*(standardized length seedlings) - 0.45*(circularity).

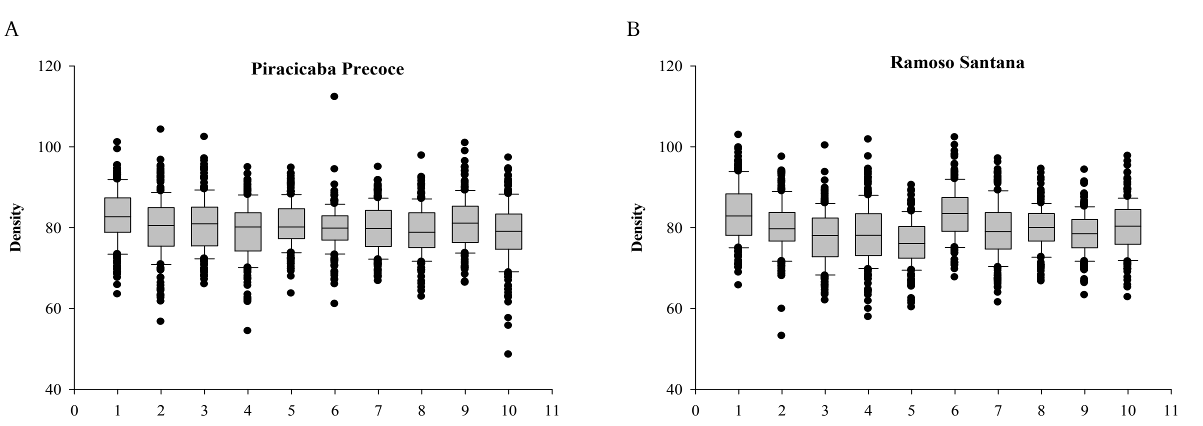

Figure2. Gray scale density distribution of broccoli seed lots, cultivar *Piracicaba Precoce* (A) and *Ramoso Santana* (B) obtained using the software ImageJ.

According to the mathematical model proposed for the principal component 1 (PC1), all variables showed similar weight in the calculation of this component.

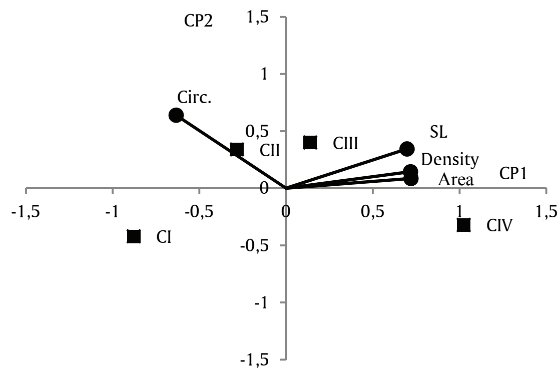

Figure 3. *Biplot* graph obtained by grouping variables related to characteristics of seeds and seedlings, classified into different categories for the cultivar *Piracicaba Precoce*. Subtitles: PC1 - Principal Component 1, PC2 - Principal Component 2. Circ - circularity; SL - seedling length; Density - Gray scale; Area - mm^2.CI - Category I; CII - Category II; CIII - Category III; CIV - Category IV.

Principal component 2 (PC2) represents the results from 10 lots of the cultivar *Piracicaba Precoce* according to the following mathematical model:

PC2 = 0.11*(standardized area) + 0.19*(standardized density) + 0.46*(standardized length seedlings) + 0.85*(circularity).

According to the mathematical model of PC2 the more important variable in the calculation is the circularity (0.85).

In this research, morphological characters and vigor were attributed to the evaluated parameters. Thus, circularity is a morphological parameter. However, area can be both a morphological parameter and a vigor parameter, with greater areas coinciding with higher amounts of available reserves for seed germination with good formation when completing the ripening process. The seed density and seedling length showed vigor characteristics and seedling performance.

Cultivar *Ramoso Santana* presented an eigenvalue of 3.75 for PC1 that was generated by the combination of area, density, circularity and seedlings length and represented 93% of the total data variability. The second component had an eigenvalue of 0.23, representing 5.7% of the total variability. The sum of these two values was 98.7%, which met the minimum adequate amount proposed by Jollife (2002) (Figure 4).

According to Figure 4, the *biplot* graph indicates that the first principal component (PC1) was formed by the linear sum of the results of area, density and seedlings length, with subtraction of circularity. Thus, PC1 represents the results of the 10 lots, according to the following mathematical model:

PC1 = 0.51*(standardized area) + 0.51*(standardized density) + 0.49*(standardized length seedlings) - 0.47*(circularity).

According to this all variables showed similar weight in the calculation of this component.

Principal component 2 (PC2) represents the results from 10 lots of the cultivar *Ramoso Santana*, according to the following mathematical model:

PC2 = 0.11*(standardized area) + 0.07*(standardized density) + 0.58*(standardized length seedlings) + 0.80*(circularity).

As observed for the cultivar *Piracicaba Precoce*, circularity (0.80) was the most relevant variable for PC2.

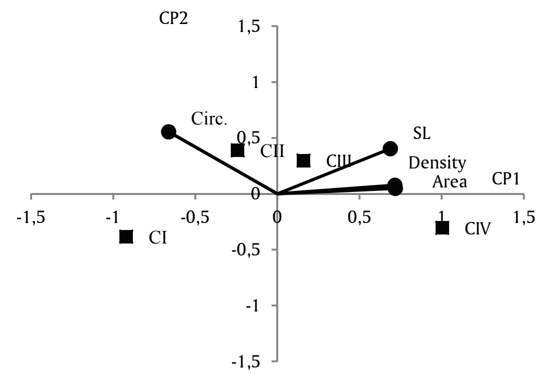

Figure 4. *Biplot* graph obtained by grouping variables related to characteristics of seeds and seedlings, classified into different categories for the cultivar *Ramoso Santana*. Subtitles: PC1 - Principal Component 1, PC2 - Principal Component 2. Circ - circularity; SL - seedling length; Density - Gray scale; Area - mm^2.CI - Category I; CII - Category II; CIII - Category III; CIV - Category IV.

In front of these proposed equations for the two cultivars, after replacing values in the mathematical model of principal component 1 (PC1), the results indicated that the seeds from class IV presented the highest values for area, density and length of seedlings and the lowest values for circularity. Class III showed behavior similar to class IV, however a lower PC1 value was assigned to this class. In addition, categories I and II showed lower values for PC1, indicating that seeds from these classes presented worse performance regarding the parameters analyzed.

According to the proposed model for principal component 2 (PC2), the class I seeds from both cultivars showed higher circularity, while category IV seeds presented lower circularity.

Therefore, it can be inferred that the most vigorous broccoli seeds from both cultivars presented larger area, higher density, and larger and more elongated seedlings than the less vigorous seeds.

According to the *biplot* graphs of each cultivar, it is possible to verify the relationship of each category with the variables analyzed in this study through perpendicular projections from these points (categories) in relation to the eigenvectors. For example, the perpendicular projection of category IV on the eigenvector representative of seedling length was longer than category III. These associations can be performed to estimate seed quality through projections between categories and variables (eigenvectors), facilitating the understanding and interpretation of the results.

A correlation matrix of the analyzed variables from cultivars *Piracicaba Precoce* and *Ramoso Santana* is in Table 2. For both cultivars, positive correlations were observed between area and density, area and seedling length, and density and seedling length. On the other hand, circularity showed high negative correlations with all analyzed variables.

The high correlation between seed density and seedling length is relevant information in order to adapt a methodology using X-ray image analysis techniques to broccoli seeds. This result suggests that X-ray testing is efficient for estimating seedling performance from seed density data. Morphological characters of seeds, such as area and circularity, were also good indicators of seedling performance, wherein the larger the area and the lower the circularity, the better was the seedling's performance.

Table 2. Correlation matrix obtained from broccoli seeds and seedling characteristics using cultivars *Piracicaba Precoce* and *Ramoso Santana*. Subtitles: Area (mm^2); Density (gray scale); SL - seedling length (mm).

Cultivars	Variables	Area	Density	Circularity	CP
Piracicaba Precoce	Area	1.00			
	Density	0.99	1.00		
	Circularity	-0.85	-0.82	1.00	
	SL	0.98	0.99	-0.73	1.00
Ramoso Santana	Area	1.00			
	Density	0.99	1.00		
	Circularity	-0.89	-0.91	1.00	
	SL	0.96	0.96	-0.78	1.00

Radiographic images of other species such as cucumber (Gomes Junior et al., 2013) and squash (Silva, Freitas, Cicero, Marcos Filho, & Nascimento, 2014, Antonio et al., 2016) have efficiently related the internal morphology of seeds with their physiological quality. However, different evaluation parameters were used to characterize the internal morphology of the seeds such as internal free space and seed categories.

X-ray testing can also be used for another purposes, e.g., Marcos Filho (2010) showed that it is possible to measure the embryo size of cucurbit, cucumber and melon seeds and, therefore, determine the development degree. Some research tries to validate the use of different software, such as Tomato Analyzer and Image J, to define specific characteristics of seeds. Additionally, in several situations, considering major crops and forest species, this technique has been used to detect mechanical damage, insect attack, and other seed features.

Since principal component analyses were performed using 10 lots of each cultivar, totaling 2,000 seeds per cultivar; in Table 3 it is possible to verify the percentage of seeds distributed in each lot in 4 categories adopted as a function of the values obtained for density.

The representativeness of seeds in each category may be indicative of the maturation degree. Therefore, the quality of broccoli seeds in lots that exhibit a large amount of seeds in the first category can be considered low, as these seeds will result in shorter seedlings when compared to the other categories. Additionally, seeds with high physiological quality can be found in category IV. However, even when a lot does not present most of its seeds within category IV, category II also presented a good distribution of seeds with suitable conditions.

This information may also be relevant to guide the processing activities of broccoli seeds, wherein the seeds classified in category I can be eliminated, assigning to these lots low vigor because the seeds are less dense due to any factor that interfered in the seed formation process or to any damage that caused malformation.

For cultivar *Piracicaba Precoce* lot 1 showed the lowest amount of seeds in the first category (16%), and continually increased in the following categories, with higher amount of seeds in category IV (38%). Lots 5 and 6 showed less than 20% of seeds in the first category and over 80% of seeds distributed within other categories, which is a suitable high quality characteristic, as observed in lot 1. In contrast, lots 4, 8 and 10 had the highest amount of seeds in category I, which is undesirable since these seeds are characterized as less effective (Table 3).

Table 3. Representativeness of the density data on broccoli seeds in different categories adopted for all lots of the cultivars *Piracicaba Precoce* and *Ramoso Santana*. CI - Category I; CII - Category II; CIII - Category III; CIV - Category IV.

Cultivars	Categories	Lots (%)									
		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Piracicaba Precoce	CI	16.0	27.5	28.0	31.5	18.5	19.5	28.0	31.5	20.0	31.5
	CII	17.0	20.0	16.0	18.0	31.0	31.0	22.5	25.5	25.0	26.0
	CIII	29.0	22.5	24.5	27.5	23.5	32.0	22.0	20.0	23.0	19.5
	CIV	38.0	30.0	31.5	23.0	27.0	17.5	27.5	23.0	32.0	23.0
Ramoso Santana	CI	11.0	18.5	39.5	34.0	45.5	11.5	30.0	19.0	28.0	22.0
	CII	20.0	29.0	17.5	25.5	26.5	15.5	23.0	28.5	29.0	24.0
	CIII	25.5	28.5	25.5	16.5	18.0	26.0	22.5	31.5	29.5	27.0
	CIV	43.5	24.0	17.5	24.0	10.0	47.0	24.5	21.0	13.5	27.0

Cultivar *Ramoso Santana* presented lots 1 and 6 with the lowest amount of seeds in the first category (11% and 11.5%), and continually increasing distribution among other categories, with higher amounts of seeds in category IV (43.5% and 47%). Lots 3, 4, 5 and 7 had higher amount of seeds in category I, and among these, lots 3 and 5 showed the lowest amounts of seeds in category IV, suggesting that they present lower vigor. Lots 2 and 8 showed less than 20% of seeds in the first category and over 80% of seeds distributed in other categories. This is a suitable characteristic of high quality lots, as observed for lots 1 and 6. Lot 9 showed peculiar behavior, with uniform distribution among categories I, II and III and a low amount of seeds in category IV.

It is possible to observe in Figure 5 the X-ray images of broccoli seeds from each category adopted in this study and their seedlings at five days after sowing. Regarding the radiographic images of the seeds, it is possible to observe differences in coloration, such as the darkest part in the first category of seeds from *Piracicaba Precoce* (Figure 5AI). This difference in gray scale value of images can be attributed to non-uniform maturation of seeds from the same lot, where lighter gray scale values indicate higher tissue density and therefore better physiological quality. Only large differences can be visually detected; hence, categories II, III and IV look similar in the images. .

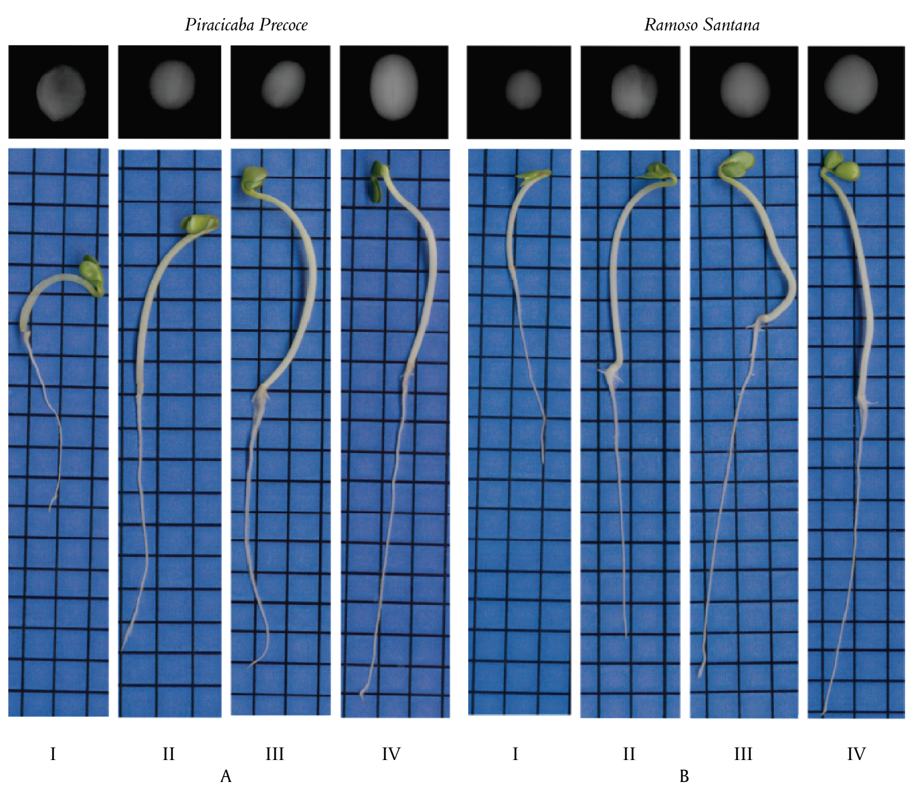

Figure 5. Radiographic images of broccoli seeds from two cultivars and their respective seedlings at 5 days after sowing. Seeds and seedlings classified in four categories according to their gray scale density levels. A – Cultivar *Piracicaba Precoce*; B –Cultivar *Ramoso Santana*. AI and BI - Category I (lower than 75.9); AII and BII - Category II (from 76 to 79.9); AIII and BIII - Category III (from 80 to 83.9) and AIV and BIV - Category IV (higher than 84).

Regarding the internal characteristics of broccoli seeds and based on the radiographic images a lack of free space is noted in the structure. Therefore, considering the weather conditions and the X-ray intensity used, the embryo could not be detected in the images due to optical density, probably because of its similarities with other parts of the seed. The seeds' shape was not uniform, and there were seeds ranging from circular shape to oblong for both cultivars (Figure 5)

Seeds from different categories of density generated seedlings with distinct lengths. Figure 5 shows their behavior by multivariate analysis, in which there was an increase in the seedling's length due to an increase in seed density. Therefore, the seeds classified in category I, with density lower than 75.9, generated seedlings with smaller length, while seeds in category IV, with density higher than 84, generated seedlings with larger length (Figure 5). Henceforth, higher seed density values can be assigned greater vigor and better seedling performance.

Moreover, the results allow concluding that small variations in seed density generate differences in seedling length for both cultivars. This result can be easily seen in Figure 5, suggesting that X-ray image analysis is efficient in the evaluation of broccoli seed and their classification into different levels of vigor.

Conclusion

The X-ray test is efficient for evaluating the internal morphology of broccoli seeds.

It is possible to associate seed gray scale density with seedling length when evaluating the internal morphology of seeds and therefore to classify seed lots into different levels of vigor.

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