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BIOLOGY

Amylose content and micromorphology of popcorn progenies with different popping expansion volumes

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ABSTRACT: *Popcorn (Zea mays var. everta) has a higher commercial value than common maize, in addition to being a popular food among consumers. Today, there is a constant search for cultivars with superior performance for several traits of interest in the case of popcorn, yield and popping expansion. On this basis, this project proposes to characterize progenies of popcorn with different values of expansion capacity regarding chemical composition and micromorphology. Kernels from the fifth cycle (C5) of intrapopulation recurrent selection were evaluated. The progenies were selected based on the popping expansion volume of their kernels. The kernels were quantified for amylose and analyzed for starch granule arrangement and pericarp thickness by scanning electron microscopy. Progenies with low popping expansion volume (0 and 7 mL g-1) showed amylose contents of 21.24 and 20.18%, respectively; a less compact endosperm, with individual starch granules interspaced with empty spaces; and pericarp thickness between 40.94 and 38.99 µm, respectively. By contrast, progenies with high popping expansion volume (30 and 35 mL g-1) showed amylose contents of 23.92 and 26.10%; a vitreous endosperm; more-compact starch granules without empty spaces in between; and pericarp thickness between 107.66 and 107.84 µm. Progenies with higher popping expansion volume exhibited a thicker pericarp, a high amylose percentage and a more-compact endosperm, whereas those with the lower expansion volumes showed a thinner pericarp, a lower amylose percentage and individual starch granules.*
Key words: *Zea mays var. everta, endosperm, pericarp, starch granule, thickness.*

Teores de amilose e micromorfologia de progênies de milho-pipoca com diferentes volumes de expansão

RESUMO: *O milho-pipoca (Zea mays var. everta) tem um valor comercial mais elevado do que o milho comum e é um alimento popular entre os consumidores. Hoje, existe uma constante busca por cultivares com desempenho superior para os diversos caracteres de interesse, no caso da pipoca, produtividade e capacidade de expansão. O trabalho propõe caracterizar progênies de milho-pipoca com diferentes valores de capacidade de expansão quanto à composição química e micromorfologia. Foram estudados grãos do quinto ciclo (C5) de seleção recorrente intrapopulacional. As progênies foram selecionadas com base nos valores de capacidade de expansão de seus grãos. Os grãos foram quantificados para amilose e analisados quanto ao arranjo dos grânulos de amido e espessura do pericarpo por microscopia eletrônica de varredura. Progênies com baixa capacidade de expansão (0 e 7 mL g-1) apresentaram teor de amilose de 21,24 e 20,18% respectivamente; endosperma menos compacto, com grânulos de amido individualizados e intercalados com espaços vazios; e espessura do pericarpo medindo entre 40,94 e 38,99 µm, respectivamente. Por outro lado, progênies com alta capacidade de expansão (30 e 35 mL g-1) apresentaram teor de amilose de 23,92 e 26,10%, endosperma vítreo, sendo os grânulos de amido mais compactos e sem espaços vazios entre eles; e espessura do pericarpo medindo entre 107,66 e 107,84 µm. Progênies com altos valores de capacidade de expansão apresentam maior espessura de pericarpo, alta porcentagem de amilose e endosperma mais compactado, enquanto as com menores valores de expansão, apresentam menor espessura do pericarpo, menor porcentagem de amilose e grânulos de amido individualizados.*
Palavras-chave: *Zea mays var. everta, endosperma, pericarpo, grânulos de amido, espessura.*

INTRODUCTION

Popcorn (*Zea mays* var. *everta*) has a higher commercial value than common maize and is a popular food among consumers all over the world. Popcorn consumption has increased over the years, which has made it an economically attractive crop to producers (RIBEIRO et al., 2016). The development and release of new popcorn cultivars are essential to reducing the dependence on genotypes from foreign countries and meeting the demand of producers and consumers by offering

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them cultivars and products with the traits of interest (GUIMARÃES et al., 2018).

Popping expansion is the main feature that indicates quality in popcorn (ARNHOLD et al., 2010). This parameter is defined as the ratio between the volume of popped popcorn and the weight of the kernel used for popping (VIEIRA et al., 2009).

Breeding programs constantly search for cultivars with superior performance for the various traits of interest to meet the demand of both producers and consumers; in the case of popcorn, these traits are yield and popping expansion, respectively. The recurrent selection methodology is used repeatedly, whereby the best genotypes in each cycle are selected to obtain gains at the end of each selection process (HALLAUER et al., 2010).

In popcorn, the endosperm alone represents more than 80% of the dry kernel and is composed of two regions: one hard and crystalline (vitreous endosperm) and one soft and opaque (floury endosperm), each having specific physicochemical features (PIOVESAN et al., 2011). The importance of evaluating the endosperm of popcorn kernels lies in that there seems to be a strong correlation between popping expansion and kernel density, which in turn is correlated with the amount of hard starch in the kernel (TANDJUNG et al., 2005).

In addition to representing a protection for the embryo and the endosperm, pericarp thickness plays important roles in determining grain quality, especially for popcorn varieties (DONG et al., 2015). When the pericarp ruptures, the superheated water within it vaporizes and rapidly diffuses through nucleation sites, creating a driving force for popcorn expansion (SWELEY et al., 2013). Pericarp thickness has been positively correlated with expansion volume (MOHAMED et al., 1993).

Popping expansion may be affected by several factors. Several studies have reported the effects of genotype (PHUMELELE et al., 2014), starch composition and structure (BORRAS et al., 2006), physical properties (PORDESIMO et al., 1990), yield components (LI et al., 2008), chemical composition (PARAGINSKI et al., 2017), agronomic characteristics (SOUSA et al., 2016) and kernel water content. However, none of those studies investigated the potential relationship between micromorphology and popping expansion in popcorn.

The use of scanning electron microscopy has been proven to be of great importance to characterize the microstructure of various materials. On this basis, this project proposes to characterize progenies of popcorn with different values of expansion capacity regarding chemical composition and micromorphology.

MATERIALS AND METHODS

Sampling

Ten half-sib popcorn progenies from the fifth cycle (C5) of intra-population recurrent selection with different expansion volumes (0, 3, 7, 10, 20, 23, 30, 31, 33 and 35 mL g^{-1}) were sampled. These values were calculated as the ratio between the volume of popped popcorn and kernel weight. For popping, 10 g samples with 13% moisture were collected from each treatment and put into a hot-air popcorn popper for 10 s (ARNHOLD et al., 2009).

Kernel amylose content

Amylose content was determined in all ten progenies, by the iodine colorimetric method (MCGRANCE et al., 1998). Approximately 20 mg of defatted starch were added to 8 mL of 90% dimethyl sulfoxide (DMSO), shaken for 20 min and subjected to a water bath at 85 °C for 2 h. After cooling, the content was transferred to a volumetric flask, completed to 25 mL with distilled water and homogenized. A 1-mL aliquot of the solution was added to 5 mL of I_2/KI solution (0.0025 mol L^{-1} of I_2 and 0.0065 mol L^{-1} of KI) and the volume was completed to 50 mL. The resulting solution was homogenized and left to rest for 15 min, after which time its absorbance was read at 600 nm. To obtain the standard curve, 20 mg of pure potato amylose (Sigma-Aldrich) were subjected to the same process described above, and aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 mL had their UV - absorbances determined using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

Kernel micromorphology

For the micromorphological analysis of starch granule arrangement, kernels were sampled from progenies with expansion volumes of 0 and 7 (low popping expansion) and 30 and 35 mL g^{-1} (high popping expansion). For pericarp thickness measurement, kernels from progenies with expansion volumes of 0, 7, 10, 20, 30 and 35 mL g^{-1} were sampled. Cross-sections were obtained from the mid region of kernels, mounted on aluminum stubs with a double-sided adhesive carbon tape and sputter-coated with gold (sputter coated equipment model SCD 050). Sampling was standardized so that the micrographs were obtained from the same region in all kernels. Photographic documentation was carried out using a scanning electron microscope (LEO Evo 40) at the

Laboratory of Electron Microscopy and Ultrastructural Analysis at Federal University of Lavras, Brazil.

Statistical analysis

A randomized block design with five replicates was used for amylose percentage quantification and a completely randomized design with five replicates was used for pericarp thickness assessment**.** Data were subjected to analysis of variance and means were grouped by the Scott and Knott test ($P<0.05$). Subsequently, the Spearman correlation (STEEL et al., 1980) between popping expansion and amylose content was estimated (This comment was answered in the reviewer's word document 1). Data were analyzed using packages of SAS (SAS Institute, 2000) and Sisvar (FERREIRA 2011) software.

RESULTS AND DISCUSSION

Amylose content

The studied popcorn progenies differed significantly for amylose content in the kernel endosperm. Kernels form progenies with high popping expansion volumes (30 and 35 mL g^{-1}) showed higher amylose percentages: 23.92% and 26.10%, respectively (Table 1). Kernels from progenies with popping expansion volumes of 20 and 23 mL g^{-1} showed intermediate amylose percentages (Table 1). Kernels from progenies with lower popping expansion volumes (0, 3, 7 and 10 mL g^{-1}) showed the lowest amylose percentages (Table 1).

The amylose content of starch granules may determine endosperm hardness (DOMBRINK-KURTZMAN et al.,, 1997). In hard endosperms, because the peripheral amorphous region of starch is larger, compression in this endosperm type results in a higher amylose content (BEMILLER et al., 2011). Regarding the chemical basis of kernels with a hard endosperm, variations may exist in starch chemical composition, structural organization of starch granule components, structure of the protein layer among granules, presence of intercellular spaces and degree of compaction of starch granules (LEE et al., 2014).

Conversely, starch granules in the floury endosperm are subjected to less compression and have a higher proportion of amylopectin. SWELEY et al (2012) studied three hybrids from three different locations in the USA and reported a negative correlation between the percentage of amylopectin and kernel popping expansion. In our study on the popcorn kernel, the estimated correlation between popping expansion and amylose percentage was 0.95 ($P\leq 0.01$), which thus reveals that higher popping expansion is associated with higher amylose percentages.

Starch granule arrangement

Kernels of progenies having low popping expansion volumes (0 and 7 mL g^{-1}) showed a less compact endosperm (Figure 1A and B) and (Figure 2A and B). In the endosperm, an individual arrangement of granules interspersed with voids is observed. In this endosperm type, starch granules are dispersed and not surrounded by a protein matrix, which results in the formation of voids during the grain-drying process from spaces that had been previously filled with water throughout grain development (PAES et al., 2011).

The progenies with high popping expansion volumes (30 and 35 mL g^{-1}); conversely, had more compact endosperms with aggregated

Table 1 - Amylose content and pericarp thickness in kernels of popcorn progenies with different popping expansion volumes.

Popping expansion (mL g^{-1})	Amylose content (%)	Pericarp thickness (µm)
0	21.24c	40.94c
3	21.20c	-
7	20.18c	38.99c
10	21.98c	46.43c
20	23.69b	82.22b
23	23.82b	-
30	23.92b	107.66a
31	25.78a	-
33	24.39b	-
35	26.10a	107.84a

*Means followed by different letters in a column differ by the Scott-Knott test ($P\leq 0.05$).

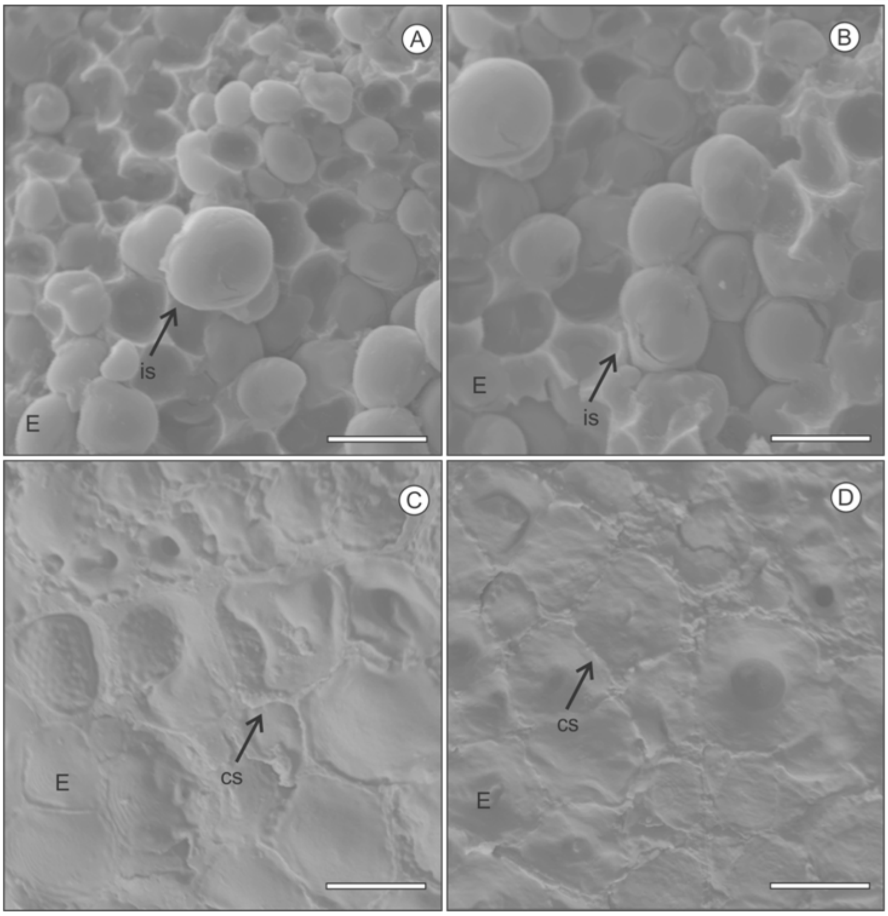

Figure 1 - Scanning electron micrographs of the endosperm in kernels of popcorn progenies. (A) and (B) Progenies with low popping expansion volumes (0 and 7, respectively) showing individual starch granules (is). (C) and (D) Progenies with high popping expansion volumes (30 and 35, respectively) showing compact starch granules (cs). Bars= 10 μm.

starch granules without voids interspersed among them (Figure 1C and D). In the vitreous endosperm, there is a dense protein matrix with well-structured protein bodies, without voids among them (PAES et al., 2011). The kernels of popcorn cultivars with hard and semi-hard endosperm have higher density values, more compact starch granule arrangement, endosperm cell walls with firmer appearance and polygon-shaped starch granules (PEREIRA et al., 2008). It is possible to observe the more angular shape of the granules (Figure 2C and D), which is characteristic of vitreous endosperm.

The ideal popcorn kernel has a smaller amount of floury endosperm and a higher proportion of vitreous endosperm, as the latter endosperm type is the one that most contributes to kernel expansion during popping (BORRAS et al., 2006). As a result of increasing vapor pressure and temperature within the kernel, water vapor in the translucent endosperm is forced into the starch granules (VAN DER SMAN et al., 2017), which soften and become a cohesive, viscoelastic mass. Pereira et al. (2008) stated that starch granules of the translucent endosperm undergo a process of gelatinization and crystalline melting. Conversely to what occurs in translucent endosperm during heating, in the opaque endosperm, water vapor apparently does not penetrate the starch granules, but rather enters the numerous voids among them. As a result, starch granules in the opaque endosperm do not undergo gelatinization during popping (VAN DER SMAN et al., 2017). While starch granules in the translucent endosperm are highly expanded and

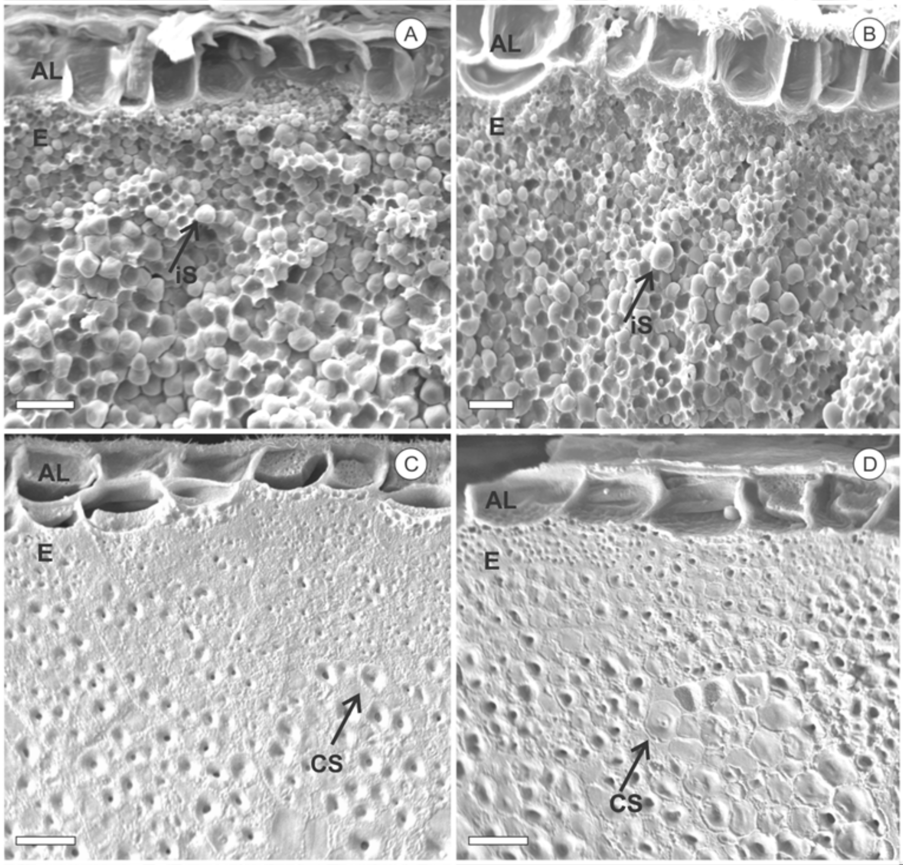

Figure 2 - Scanning electron micrographs of the endosperm in kernels of popcorn progenies. (A) and (B) Progenies with low popping expansion volumes (0 and 7, respectively) showing individual starch granules (is). (C) and (D) Progenies with high popping expansion volumes (30 and 35, respectively) showing compact starch granules (cs). Bars= 20 µm. AL= aleurone layer, E= endosperm.

responsible for flake formation, starch granules in the opaque endosperm appear to undergo little change during popping other than moving apart from each other (PEREIRA et al., 2008). Floury endosperms contribute little to the bulk of the starch foam (PARKER et al., 1999). The endosperm contributes to expansion volume (SANTOS et al., 2008). Higher amounts of opaque endosperm decrease expansion volume (WILLIER & BRUNSON et al. 1927).

Pericarp thickness

Significant differences in pericarp thickness were observed between the analyzed popcorn progenies (Table 1). The progenies with high popping expansion volumes (30 and 35 mL g^{-1}) showed higher pericarp thickness (107.66 and 107.84 µm, respectively) **(**Figure 3C and D), while the progenies with low popping expansion volumes (0 and 7 mL g^{-1}) showed lower values of this variable (40.94 and 38.99 µm, respectively) (Figure 3A and B).

Some of the phenotypic traits known to affect popped volume include physical characteristics such as pericarp thickness and kernel size, shape, density and hardness (BORRAS et al., 2006). Popcorn kernels with high popping expansion volumes usually have not only a predominantly vitreous endosperm, but also a thicker pericarp (WILLIER et al., 1927).

The popcorn pericarp thickness varies from 40 to 120 µm depending on the maize variety, location of kernel on the ear and position of the pericarp on the kernel (PEREIRA et al., 2008). This fact highlighted the importance of standardizing the

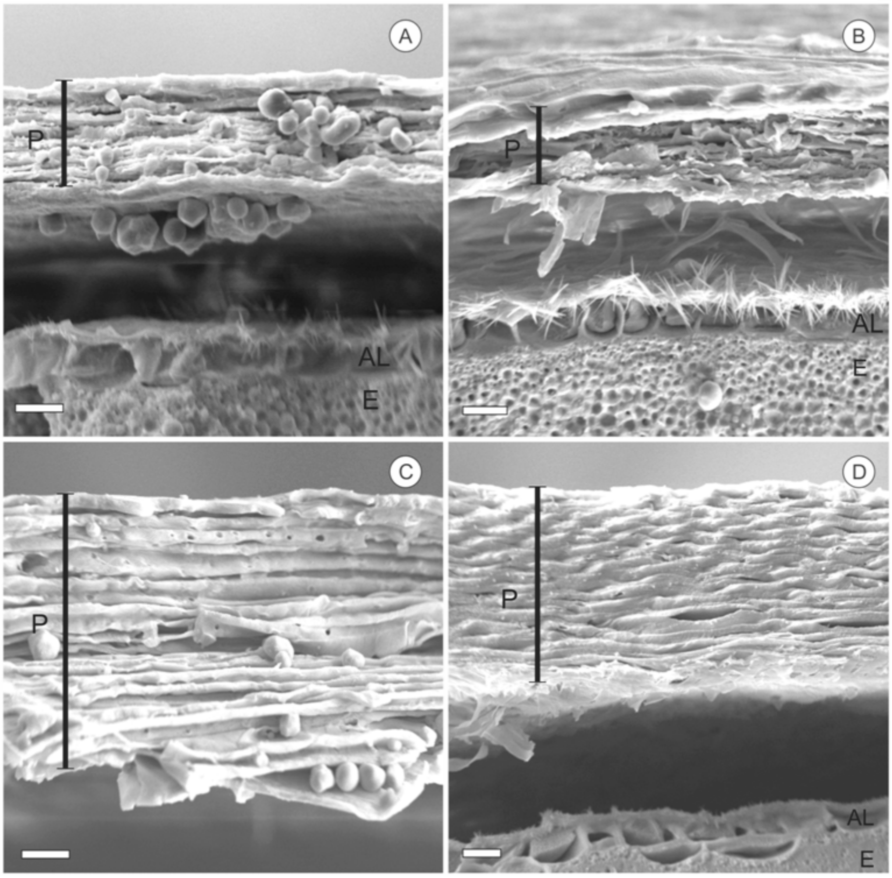

Figure 3 - Scanning electron micrographs of the endosperm of the pericarp, in a longitudinal section, in kernels of popcorn progenies. (A) and (B) Progenies with low popping expansion volumes (0 and 7, respectively). (C) and (D) Progenies with high popping expansion volumes (30 and 35, respectively). Bars= 20 µm. AL= aleurone layer, P= pericarp.

region in studies evaluating pericarp thickness, as was done in the present work.

Pericarp rupture occurs when the inner pressure in the kernel exceeds the combined burst pressure of the pericarp and atmospheric pressure. The popcorn kernel pericarp not only provides protection and containment to the endosperm, but also acts as a pressure vessel during heating, which gives popcorn its characteristic popping ability (PEREIRA et al., 2008). As heat is applied to popcorn, the pericarp limits kernel moisture loss and facilitates vapor pressure build-up and water superheating within the kernels (RAKESH et al., 2013). SWELEY et al. (2013) suggested the use of pericarp thickness as an indicator of popping quality.

It should be emphasized that the use of complementary techniques is very important for breeding programs, since their adoption increases selection accuracy and reliability. In this sense, by using micromorphology studies in progenies of popcorn, the correlation between amylose content, pericarp thickness and starch granule density in the endosperm with popping expansion was reported to be of great importance for the development of cultivars in popcorn breeding programs.

CONCLUSION

The kernels of popcorn progenies with higher popping expansion volumes have a thicker pericarp, higher amylose percentage and a more compact endosperm, whereas progenies with lower popping expansion volumes showed a thinner pericarp, lower amylose percentage and individual starch granules.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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