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MICROSTRUCTURE OF NUÑAS: ANDEAN POPPING BEANS
(PHASEOLUS VULGARIS L.)

Stephen C. Spaeth, 1* Daniel G. Debouck, 2
Joseph Tohme, 2 and Janny van Beem 2

Grain Legume Genetics and Physiology Research Unit
Agricultural Research Service, U. S. Department of Agriculture
Washington State Univ., Pullman, Washington 99164-6421, 1 and
Centro Internacional Agricultura Tropical
Apartado Aéreo 6713, Cali, Colombia. 2

Abstract

Nuñas, popping beans (Phaseolus vulgaris L.), burst and expand when heated rapidly. Differences in seed microstructure between popping and conventional (non-popping) bean genotypes conceivably contribute to popping in nuñas. However, the microstructural characteristics which contribute to the popping attribute and sites of expansion have not been identified. Seeds and excised cotyledons of unpopped and popped nuñas were examined using scanning electron microscopy (SEM). Protoplasts of unpopped nuñas were similar to protoplasts of conventional beans. Intercellular spaces of unpopped nuñas were occluded by schizogenous cell walls. The occluded form of intercellular spaces differed distinctively from the open form in popped nuñas and untreated conventional beans. The expansion of cotyledon mesophyll in popped nuñas came primarily from expansion of cell walls and secondarily by expansion of the intercellular spaces. Cell wall thickness and dimensions of protoplasts were not changed during popping. Expansion of cell walls away from protoplasts created intracellular voids. SEM images indicated that starch granules (grains) in popped nuñas were generally not altered by popping. Starch granules did not gelatinize or melt during popping as indicated by retention of birefringence. In contrast to popcorn (Zea mays L.), starch granules did not contribute to expansion of popped nuñas cotyledons.

Introduction

Nuñas are a type of common bean (Phaseolus vulgaris L.) which burst and expand during rapid heating (Hyland, 1968; Zimmerer, 1985; National Research Council, 1989). Popping beans have been and still are grown and consumed almost exclusively at high elevations in the Andes of Peru and Bolivia. In traditional Andean cooking, nuñas are roasted in sand or oil (Zimmerer, 1985, 1987). Nuñas can also be popped in hot air or microwave ovens (National Research Council, 1989; van Beem and Spaeth, 1989). The advantage of nuñas over conventional (non-popping) bean genotypes is a shorter cooking time. This difference is especially important at fuel-scarce high elevations. Popping of nuñas requires less than 5 min, while cooking of beans in boiling water may require more than an hour for cotyledons to become soft. Nuñas do not require additional cooking after popping.

While nuñas are an ancient crop (Zimmerer, 1987; National Research Council, 1989), they are relatively unknown outside of the Andean Region and have received little scientific study. The mechanism of popping and sites which contribute to volumetric expansion during popping of nuñas are unknown. In contrast, the mechanism and sites which contribute to expansion of popcorn have been studied (Hoseney et al., 1983). The expansion of popped tissue relative to unpopped tissue is substantially greater for popcorn (Hoseney et al., 1983) than the doubling of volume observed for nuñas (van Beem and Spaeth, 1989). In popcorn, the starch granules (grains) expand and contribute substantially to bulk expansion of the popped tissue (Hoseney et al., 1983). Large numbers of small fractures in popped corn endosperm also contribute to volumetric expansion (Hoseney et al., 1983). While the microstructure of conventional common bean cultivars has been studied (Varriano-Marston and Jackson, 1981; Swanson et al., 1985; Hughes and Swanson, 1985; Spaeth, 1987a, 1989), no information on the microstructure of nuñas was found in the literature.

Characteristics which allow nuñas to pop are also unknown. Zimmerer (1985) suggested that differences in density between nuñas and conventional beans might be partially responsible for popping. A National Research Council report (1989) derived from an informal survey of researchers familiar with nuñas indicated that the quality or quantity of starch in nuñas may differ from conventional non-popping beans and be responsible for expansion of nuñas during popping.

The mechanism of popping is of interest for analysis of popping and because characteristics which permit popping may also influence physiological processes in seeds during maturation, storage, imbibition and germination. Scanning electron microscopy (SEM) is an excellent tool for examining
structural characteristics which may contribute to popping of *nuña* seeds before and after popping. *Nuña* microstructure was compared with published descriptions of popcorn microstructure (Hoseney et al., 1983) to identify features which may allow *nuña* to burst and expand. SEM was used to examine starch granules, protein bodies, protoplast, cell walls and intercellular spaces of unpopped and popped cotyledons of *Nuña* Pava and unpopped cotyledons of five additional *nuña* cultivars and three conventional non-popping bean cultivars.

**Materials and Methods**

Sources of the beans (*Phaseolus vulgaris* L.) examined in this study are given in Table 1. *Nuña* were field-grown in the highlands of Peru where cultural practices and environmental conditions are conducive to production of *nuñas* which pop well. *Nuñas* were held at ambient temperature and relative humidity before popping and preparation for SEM. *Nuñas* and conventional beans had water contents of 0.05 to 0.06 g H₂O/g seed (dry wt. basis) before popping. Seeds were dried in an oven for 24 h at 100°C for determination of water content.

Individual beans were popped by heating for 2.5 to 4 min at full power in a 1600 watt, 2450 MHz Kenmore microwave oven (Sears Roebuck and Co.). Beans were observed visually during heating and removed from the oven after complete expansion. Microwave preparation of beans for SEM avoided contamination with oil (Hoseney et al., 1983). Microwave preparation also minimized artifacts caused by agitation or redistribution of material by air currents during hot-air popping.

Beans studied with SEM were mature air-dry or popped oven-dry tissues, therefore, fixation, freeze fracture, and critical-point drying were not required (Spaeth, 1987a). Cotyledons were fractured by hand using single-edge razor blades. Beans were sputter coated with gold and observed and photographed at 20 kV on an Hitachi S-570 Scanning Electron Microscope.

Birefringence of starch granules was examined using a Zeiss Photomicroscope II.

Cotyledons from six *Nuña* Pava seeds and two seeds each of the five remaining *nuña* and three conventional cultivars were examined.

**Results**

The cotyledons of *nuñas* heated in a microwave oven not only burst the testa and expanded, but also cracked so that the shape of beans was altered as a result of the heating treatment (Fig. 1). The expansion of microwave popped mesophyll excluding the large cracks was similar to that observed for *nuñas* heated in oil or hot air (van Beem and Spaeth, 1989).

The cracks in microwave popped cotyledons ranged from large, easily visible cracks to small checks. The large cracks were generally transverse with respect to the long axis of the bean (Fig. 1). The epidermis near the large cracks exhibited many small checks roughly parallel to the larger cracks (Fig. 2a). Outside this region, the epidermis was not checked. The mesophyll tissue fractured through cell walls rather than separating between cells at the middle lamella (Fig. 2b). Protoplasts adhered to the fracture cell walls or were pulled away and left empty cell walls. Fractures also pulled vascular bundles apart.

Cotyledon tissue of unpopped *nuñas* fractured through protoplasts of mesophyll cells (Fig. 3a). Cell walls surrounding protoplasts that contained starch granules, protein bodies...
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and protoplasm. Cotyledons of un popped nuñás exhibited only a small volume of intracellular voids. Large concave depressions in the fracture surfaces of protoplasts (Fig. 3a) were left by starch granules in the complementary fracture surface. Intercellular spaces were small.

Cotyledon tissue of popped nuñás was fractured through cell walls but was not fractured through protoplasts (Fig. 3b). During popping, cell walls expanded, separated from protoplasts, and created intracellular voids. The thicknesses of cell walls were not changed. The separation of walls from protoplasts exposed the exterior surfaces of protoplasts and interior surfaces of cell walls. Popped tissue contained many more voids than un popped tissue. The largest voids opened between the surfaces of protoplasts and the interiors of cell walls. The distances between the exterior surfaces of protoplasts and interior surfaces of cell walls differed within and among cells but the maximum separation ranged from 10 to 20 μm.

The second contribution to expansion in popped tissue came from the opening of intercellular spaces (Fig. 3b). Schizogenous wall cells occluded intercellular spaces in un popped nuñás (Fig. 4a). Schizogenous walls are the parts of cell walls which separate during seed growth and development, and surround intercellular spaces in mature seeds (Kollöffel and Linssen, 1984, Spaeth, 1989). In contrast, schizogenous cell walls did not occlude intercellular spaces in popped nuñás (Fig. 4b). Longitudinal sections through intercellular spaces of un popped nuñás show that the spaces were occluded along much of the length of the spaces (Fig. 5a). Bent or folded segments of schizogenous wall filled the lumens of intercellular spaces. Longitudinal sections through intercellular spaces of popped nuñás show that the spaces were open from end to end (Fig. 5b).

The open, continuous network of intercellular spaces in popped nuñás (Fig. 5b) was also observed in untreated conventional cotyledons (Fig. 5c). Intercellular spaces in un popped cotyledons of the additional five nuñás cultivars exhibited the occluded form of intercellular spaces while the three conventional (non-popping) cultivars exhibited the open form as observed in popped nuñás. The epidermal layer of un popped nuñás had fewer voids than the mesophyll tissue of un popped nuñás (Fig. 6). While occasional intercellular spaces in mesophyll of nuñás remained closed or partially closed after popping (Fig. 7a), most intercellular spaces in popped tissue formed an interconnecting network of open spaces. Cracks intermediate in size between macroscopic cracks (Figs. 1,2) and intracellular voids (Figs. 3b, 7a) were not observed in popped Nuñá Pava other than the checks in the epidermis (Fig. 2a).

Protein bodies were not distinguishable from protoplasm in popped nuñás (Fig. 7a). Numerous projections were observed on the surfaces of protoplasts in popped Nuñá Pava. Depressions in the interior surfaces of contact cell walls (Figs. 4b, 7a) corresponded to the sites where the projections were observed on the surfaces of protoplasts.

Starch granules were not directly visible in popped nuñás, because cells of popped beans did not fracture through protoplasts. Starch granules in popped nuñás were some-times evident as bumps beneath the surfaces of protoplasts (Fig. 7a). Size and shape of most starch granules beneath the surface of protoplasts fell within the range of sizes and shapes of starch granules in un popped nuñás (Fig. 7a,b). We observed only one starch grain in the beans studied with SEM that changed markedly during popping (Fig. 7b). The outside diameter of the altered starch granule was larger than the diameters of other starch granules in popped or un popped nuñás. A hole in the surface of the altered starch grain revealed an internal cavity.

The failure of the popping process to alter the external appearance of starch granules prompted an examination of birefringence of starch granules in un popped and popped nuñás. Starch granules in popped nuñás exhibited birefringence patterns similar to birefringence patterns of starch granules from un popped nuñás and conventional beans.

Discussion

The volumes of intracellular and extracellular voids in cotyledon mesophyll and epidermis of un popped nuñás were small. The major difference between un popped nuñás (Figs. 3a,4a,5a,6) and conventional beans (Fig. 5c; Spaeth, 1989) was the smaller volume of intercellular spaces in nuñás due to occlusion of the spaces by schizogenous cell walls. The relative absence of voids in nuñá cotyledons was similar to the absence of voids in vitreous endospem of un popped corn (Hosney et al., 1983).

Popping in corn requires steam pressure which builds in the kernel during heating (Hosney et al., 1983). The occlusion of intercellular spaces in un popped nuñás (Figs. 3a,4a,5a,6) may contribute to popping of nuñás by increasing the resistance to flow of steam out of cotyledons, or by absence of space into which steam may expand. Intercellular spaces in popped nuñás (Figs. 3b,4b,5b) were more similar to the open, continuous network of intercellular spaces observed in conventional cultivars (Fig. 5c; Hughes and Swanson, 1985; Spaeth, 1987b, 1989) than they were to the intercellular spaces of un popped nuñás. The open network of intercellular spaces of popped nuñá cotyledons may be pathways for escape of steam under pressure. The observation that some intercellular spaces were still occluded after popping (Fig. 7a) indicated that occluded intercellular spaces were present before popping. Cell walls may also constitute barriers to flow of steam during rapid expansion. Resistance to steam flow by occluded intercellular spaces in nuñás is a plausible mechanism of popping. Tests of the resistance hypothesis will require nuñás and conventional beans produced in one location at one time.

Protoplasts of un popped nuñás (Figs. 3a,6) were similar to protoplasts of conventional non-popping beans (Fig. 5c; Varriano-Marston and Jackson, 1981; Hughes and Swanson, 1985; Spaeth, 1989). The appearance of nuñá protoplasts was altered by popping as the appearance of conventional bean protoplasts is during imbibition (Varriano-Marston and Jackson, 1981; Hughes and Swanson, 1985; Spaeth, 1989). Projections from the surfaces of protoplasts and depressions in cell walls of popped nuñás were similar to

Fig. 5. Longitudinal fractures through intercellular spaces (IS) in bean cotyledons. Unpopped Nuñá Pava (5a) with occluded sections of intercellular spaces (between arrows). Bar = 10 μm. Popped Nuñá Pava (5b) with contact cell wall (CW), interior surface of contact wall (ICW), schizogenous cell wall (SW), exterior surface of schizogenous wall (ESW). Bar = 50 μm. Calima (5c), a conventional non-popping cultivar of Andean bean with open section of intercellular space (between arrows). Bar = 50 μm.

Fig. 6. Fracture through unpopped Nuñá Pava near the abaxial surface (AS) with mesophyll tissue (M) and epidermal layer (E) lacking air-filled voids. Bar = 50 μm.

Fig. 7. Fractures through popped Nuñá Pava cotyledons showing starch granules (arrows) beneath the exterior surface of protoplasts (EP). Figure 7a shows open (O), partially open (P) and closed (C) intercellular spaces. Bar = 50 μm. Figure 7b shows starch grain (SG) and expanded starch grain (ESG) with a cavity (Ca) inside it. Bar = 50 μm.
those observed in beans after imbibition. The sizes of protoplasts did not change during popping (Figs. 3a, 5a, 7b).

The expansion of nutias during popping came primarily from changes in the space between cell walls. Cell walls expanded and created intracellular voids around protoplasts (Figs. 3b, 5b, 7a, b). Schizogenous cell walls of the occluded form in unpopped nutias (Figs. 4a, 5a) separated to the open form in popped nutias (Figs. 4b, 5b). Intracellular voids contributed more to cotyledon expansion of popped nutias than the open form of intracellular spaces.

The smaller expansion of nutias (van Beem and Spaeth, 1989) relative to the expansion of popcorn (Hoseney et al., 1983) was primarily due to differences in starch granules and the frequency of small cracks. Most starch granules in nutias were not visibly changed during popping of nutias (Figs. 5, 6, 7) while most of the starch granules in the vitreous endosperm of popcorn expand during popping (Hoseney et al., 1983). The structure of the altered starch granule in Nutila Pava (Fig. 7b) was similar to starch granules of popped corn (Hoseney et al., 1983) than to other starch granules of either unpopped (Fig. 3a) or popped (Fig. 7) nutias.

The loss of birefringence by starch granules of popcorn indicates that the starch granules have gelatinized during heating (Hoseney et al., 1983). The retention of birefringence in starch granules of popped nutias indicated that the granules did not gelatinize during the popping process. Starch granules in nutias, therefore could not expand in the way starch granules in popcorn expand during popping (Hoseney et al., 1983). Gelatinization and expansion are important determinants of starch digestion rate in the gastro-intestinal tract (Thorne et al., 1983; Würsch et al., 1986). Since starch granules did not gelatinize during popping, starch in popped nutias is probably digested slowly. Reductions in rates of starch digestion are an important consideration for dietary management of diabetes (Thorne et al., 1983).

The reason starch granules did not gelatinize during popping of nutias is not clear. The temperature inside of nutias at time of popping may be lower than the temperature inside of popcorn, or the starch granules in nutias may have a higher temperature for gelatinization than starch granules in the vitreous endosperm of popcorn. Since gelatinization of starch granules depends on temperature and moisture content of the starch, analyses will require measurements of both.

The large cracks in popped nutias were generally transverse with respect to the long axis of the bean (Fig. 2a) similar to transverse cracks formed during imbibition (Spaeth, 1986). Cotyledon mesophyll tissue of popped nutias generally lacked the small cracks (Figs. 2, 3, 7) which make a substantial contribution to the expansion of popped corn endosperm (Hoseney et al., 1983). The epidermal surface of popped nutia cotyledons exhibited small checks (Fig. 2a) which contributed little to expansion.

Nutias which pop immediately after harvest sometimes fall to pop after a period of aging (National Research Council, 1989). Another aging process of common bean cultivars is the development of the hard-to-cook phenomenon (Varriano-Marston and Jackson, 1981; Swanson, Hughes and Rasmussen, 1985; Hincks and Stanley, 1986). Several mechanisms are reported to contribute to development of hard-to-cook beans including changes in cell wall properties (Hincks and Stanley, 1986) after storage at high temperatures and high humidities. Changes in cell wall properties may also affect the popping process and could explain why popping is sometimes incomplete and variable, even within a nutia line.

The occluded intercellular spaces in nutia cotyledons constitute an interesting modification of cotyledon tissue from the perspective of seed physiology. The diffusion of gases through dry nutia tissue may differ from diffusion of gases through cotyledons of conventional beans because of the differences in pathways for diffusion. The condition of intercellular spaces in nutias before and during dehydration, and during and after rehydration is not known. The occluded intercellular spaces may also alter the hydraulic conductivity and consequently transport of liquid water into cotyledons.

**Conclusions**

SEM analysis of nutias indicated that the primary sites of expansion were the intracellular spaces between the protoplast and cell wall, and intercellular spaces which changed from an occluded form in unpopped nutias to an open form in popped nutias. The expansion differed from popcorn in that neither starch granules nor small multicellular cracks contributed significantly to the expansion of nutias. The occluded form of intercellular spaces in unpopped nutias differed distinctively from the open form of intercellular spaces in popped nutias and unpopped conventional beans.

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Discussion with Reviewers

W. J. Wolf: If the cell wall expands during popping, wouldn’t you expect a narrowing of the cell wall? Have you looked for evidence of changes in the structure of the cell wall as a result of the expansion?

Authors: For a homogeneous, isotropic material, one would expect a decrease in thickness after expansion. Since cell walls are neither homogeneous, nor isotropic, we did not know what to anticipate. We have not observed marked changes in thickness of cell walls. We have not studied changes in cell wall structure as a result of popping in greater detail.

R. W. Yaklich: Do you think the differences you observed in microstructure of popped nuñas would be the same at a higher elevation?

Authors: The cooking time required for popping of nuñas is reported to increase at high elevations (K. S. Zimmerer, personal communication). Since elevation can influence popping time, elevation may also influence microstructure of popped nuñas.

R. C. Hoseney: What is the significance of the fact that popping only takes 4 min to produce a soft bean while cooking in water takes a much longer time?

Authors: Beans become soft during boiling because cells in cotyledons separate from each other along the middle lamella (Hincks and Stanley, 1986). We observed no evidence of cell separation along the middle lamella in popped nuñas. Therefore, the process by which nuñas become soft during popping differs from the process during boiling. Two different processes of softening apparently develop at substantially different rates.

B. G. Swanson: Is the moisture content of nuñas prior to popping similar to un popped corn?

Authors: The water content of nuñas before popping in the current research was below the range of water contents conducive to optimal popping of popcorn (Hoseney et al., 1983). We have not determined the range of initial water contents conducive to popping of nuñas.

B. G. Swanson: Is moisture expansion, as steam, responsible for changes in intercellular spaces?

Authors: We hypothesize that steam pressure is responsible for the observed expansion of cells and intercellular spaces, however, tests of this hypothesis need to be conducted.

B. G. Swanson: Is moisture or structure primarily responsible for popping of nuñas?

Authors: Moisture and structure both seem to be essential for popping. We suspect that constrained intercellular spaces limit the escape of moisture and cause the nuñas to pop after pressure increases in the cotyledons.

B. G. Swanson: Do nuñas have a shorter cooking time than conventional beans in water?

Authors: When nuñas and conventional beans are both boiled, nuñas do not cook more quickly than conventional beans (CIAT, 1989). Softening of nuñas during boiling probably takes place by the cell separation mechanism, so the popping mechanism confers no advantage during boiling.

Additional References