

Identification of radiation induced mutants of cassava (*Manihot esculenta* Crantz) using morphological and physicochemical descriptors¹

Identificación de mutantes radio-inducidos de yuca (*Manihot esculenta* Crantz) mediante descriptores morfológicos y fisicoquímicos

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ABSTRACT

For mutants of starch and other desirable traits of interest in cassava, we evaluated the physical-chemical morphology of the root and paste in inbred populations of six M2 families, derived from irradiated seeds. 1,097 plants were established, 829 of Gamma treatment and 268 of neutrons. In the morphological description, most chimeras recorded in M1 were identified except hermaphrodite flowers and bark of different colors on the same plant, new abnormalities in M2 were found, such as root without suberification, no anthesis, early flowering, meristems and changes in the supply-demand relationship. However, mortality, reduced vigor, yield and anthesis loss have also been identified as effects of inbreeding. Simple techniques such as optical microscopy and iodine staining were effective for the identification of mutants of the granule and the results were confirmed with electron microscopy analysis and pulp properties, except in mutant granule hole, in which no abnormalities of the granule were correlated with significant variations in the paste. However, Pearson correlations associated the hot paste viscosity with consistency and reorganization. Variability was found in the granule characteristics and properties of the paste between years, suggesting significant influence of genotype-environment interaction. Similarly, there were differences in paste properties associated with the type of radiation. In addition, promising mutants were identified with post-harvest physiological deterioration tolerance associated mainly to gamma.

Key words: inbreeding, optical microscopy, viscosity of the starch, root quality, morphological, mutagenesis.

RESUMEN

En busca de mutantes deseables del almidón y otros caracteres de interés en yuca, se evaluó la morfología, físico química de raíz y la pasta, en poblaciones de seis familias endocriadas M2, derivadas de semillas irradiadas. Se establecieron 1.097 plantas, 829 del tratamiento Gamma y 268 de neutrones. En la descripción morfológica, se identificaron, la mayoría de las quimeras registradas en M1, excepto flores hermafroditas y corteza de diferente color en la misma planta; se encontraron nuevas anomalías en M2, como raíces sin suberificación, ausencia de apertura floral, floración anticipada, alteraciones en meristemas y relación fuente demanda. Sin embargo, la mortalidad, reducción del vigor, rendimiento y pérdida de la apertura floral, también se han identificado como efectos de endocria. Técnicas sencillas como microscopía óptica y tinción por yodo fueron efectivas para la identificación de mutantes del gránulo y los resultados se ratificaron con análisis de microscopía electrónica y propiedades de la pasta, excepto en los mutantes de gránulo hueco, en los que no se correlacionaron anomalías del gránulo con variaciones significativas en la pasta. Sin embargo, correlaciones de Pearson asociaron viscosidad de pasta caliente con reorganización y consistencia. Se encontró variabilidad en características del gránulo y propiedades de la pasta entre años, lo cual sugiere influencia significativa de la interacción genotipo ambiente. De igual forma, se presentaron diferencias en las propiedades de la pasta asociadas con el tipo de radiación. Además, se identificaron mutantes promisorios con tolerancia al deterioro fisiológico poscosecha asociados principalmente, a gamma.

Palabras clave: endogamia, microscopía óptica, viscosidad del almidón, calidad de raíces, caracterización morfológica, mutagénesis.

Introduction

Cassava (*Manihot esculenta* Crantz), is a key component in the diet of one billion people, thanks to its high content of

starch in the roots, with values up to 85% (FAO and IFAD, 2000). To consolidate the industrial expansion of cassava, one is required to find value-added features with high yields. Other challenges in crop improvement include: decreasing

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post-harvest physiological deterioration (PPD), tolerance to major biotic and abiotic constraints endemic in every area of cultivation, manipulation of numbers and floral synchrony between cultivars. To achieve these objectives, we have modified the conventional breeding methods and biotechnology tools are being implemented (Tofiño *et al.*, 2007).

Mutagenesis is a strategy to create variability in agronomically important characters and quality of agricultural products (Liu *et al.*, 2004). We have identified mutant populations, large numbers of morphological flowering and fruiting distortions (Masuda *et al.*, 2004; McManus *et al.*, 2006); morphological and physiological alterations in vegetative states (Wu *et al.*, 2005; Xin *et al.*, 2009); and increased tolerance to biotic and abiotic factors (Anderson *et al.*, 1996; Kowalski and Cassels, 1999; Nagata *et al.*, 1999; Rai, 2001; Dita *et al.*, 2006).

Specifically, starchy species have been obtained by mutation-induced changes in the characteristics of starch such as complete deletions in the Waxy locus (Kawakami *et al.*, 2007), decreased size of rice granule accumulation and modification in the amylopectin structure (Satoh *et al.*, 2008), differences in the rate of starch digestibility (Groth *et al.*, 2008) and series of point mutations in the Waxy gene in potatoes with high amylopectin content (Muth *et al.*, 2008).

Additionally, the number of mutations that occur in a mutant population depends on the interaction of multiple factors and the likelihood that the mutation produces an altered phenotype (Wu *et al.*, 2005; Nag and Smerdon, 2009). CIAT, the research group of the cassava breeding improvement program, reported the discovery of the mutation of small granule in the M2 population (Ceballos *et al.*, 2008). However, other atypical characteristics in the plants or quality of roots have not been reported in these populations. According to the above, this article proposes to confirm M2 inbred populations, the occurrence of atypical morphological features of interest, previously identified in generating chimeric M1 (Tofiño *et al.*, 2008) and advance the understanding of the effect of ionizing radiation on cassava germplasm, especially on some morphological characteristics and quality of the plant roots.

Materials and methods

Irradiation was done with γ rays 200 Gy (Cobalt 60) and fast neutrons 0.2 MeV (235 U), on 1,400 seeds of six families with full and half brothers (CM9331, GM155, SM3015, SM3045, C4 and C127). We performed self-pollination on M1 plants, to obtain seeds and M2 plants.

Evaluation -M2 plants

Phenotypic evaluations were performed according to the descriptors of stem, leaves and roots of Fukuda and Guevara (1998), to screen potential mutants, identified in M2, M1 registered chimeras (Micke and Donini, 1993). Within populations, survival was evaluated at 180, 240 and 300 days after seeding. Vigor and flowering were also evaluated. Physico-chemical, microscopic and viscosity analyses were carried out to identify individuals with unusual characteristics in the roots, with respect to limit values recorded in the cassava germplasm bank, including non-irradiated parents (Ceballos *et al.*, 2007). Analyses were conducted in two crops (sexual and clonal seed), amylase, dry matter, cyanide and PPD. The PPD was evaluated by measuring the level of damage to perimeter roots at 8 and 15 days after harvest (dah).

Determination of the morphology of starch granules by light and electron microscopy. Trans-sectional root cuttings were rubbed on slides with a drop of 0.2% iodine subsequently added, and observed by light microscopy (Olympus CX41) at 40X. Individuals with abnormalities in the granule were evaluated by electron microscopy. Starch granules were dusted on double-sided tape mounted on circular aluminum, covered with 35 nm of gold-aluminum, photographed and measured in a Scanning Electron Microscope (Jeol JSM 820, Tokyo, Japan) with an accelerating voltage of 20 kV.

Amylase content by iodine staining

The harvested roots were cut crosswise and sprayed with 2% iodine (2 g KI and 0.2 g I₂ in 100 cm³ of distilled water).

Determination of dry matter content of cassava roots.

Five g of grated cassava was placed in a crucible of known weight and placed in an oven at 60°C. The crucible was removed and placed in a drying hood to reach room temperature. It was weighed and the difference settled with the fresh weight and taken as a percentage.

Determination of cyanide content of cassava roots

Forty g cassava had 50 mL of extraction buffer added and liquefied. The liquefied sample was centrifuged at 6,000 rpm. 0.4 mL of 0.1 M phosphate buffer pH 7.0 was added and a 0.1 mL sample was taken and placed in a water bath at 30°C. 0.1 mL of linamarase was taken, centrifuged, bathed at 30°C. 0.6 mL of 0.2 N NaOH was added as well as 2.8 mL of 0.1 M buffer pH 6.0 and 0.1 mL of chloramine T 1%. 0.6 mL of color reagent was added as well as 0.1 mL of chloramine T, and 0.6 mL of color reagent, and read by spectrophotometry at 605 nm (Aristizabal *et al.*, 2007).

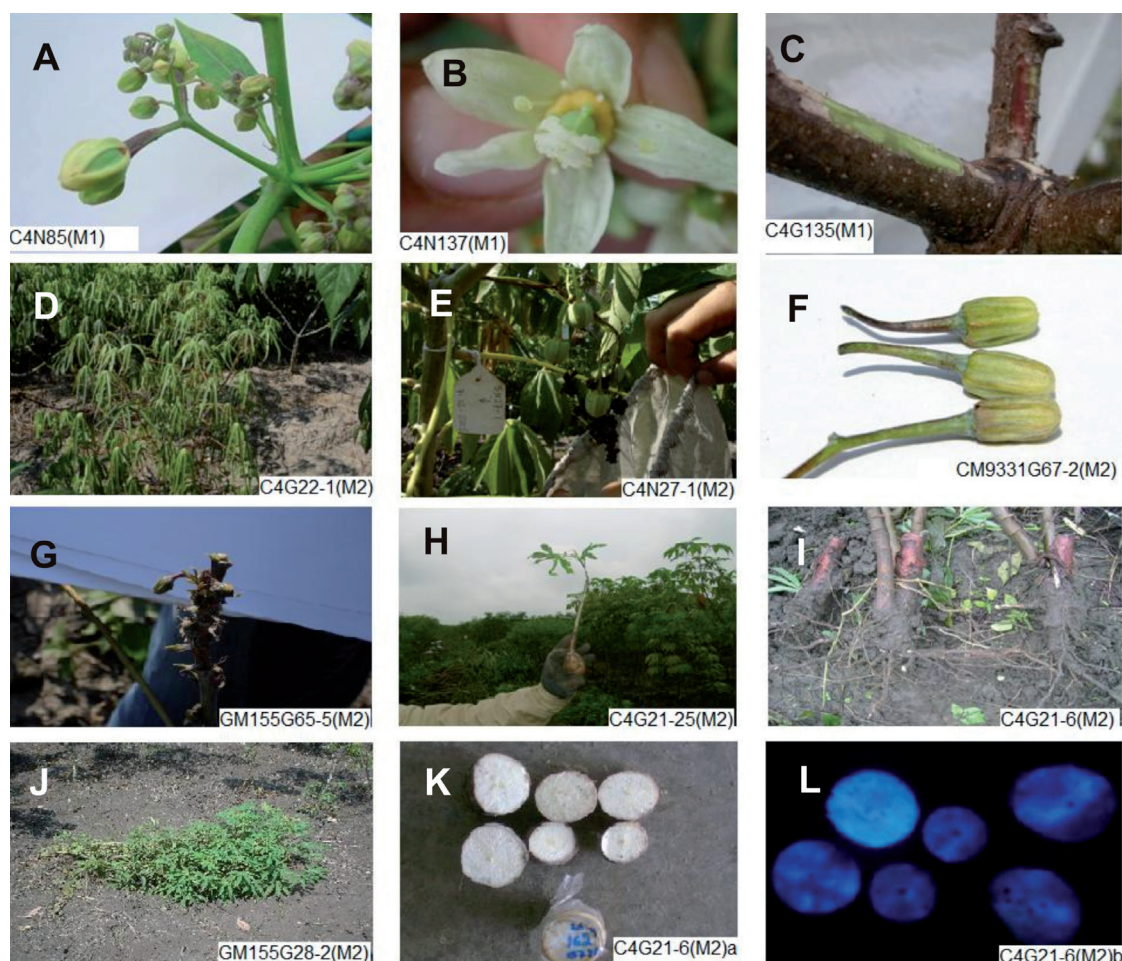


FIGURE 1. Atypical individuals identified in the M1 and M2 generations cassava germplasm subjected to ionizing radiation. (A) flower with thickening of the ovary without anthesis, M1, (B) hermaphrodite flowers, M1 (C): Contrasting colored stem bark, M1, (D): plants with a minimum leaf area index due to the narrowness of the leaflets, (E): thickening of the ovary without anthesis (M2), (F): loss of the ability of mature flowers to open (M2), (G): early floral development detached from the branching process in plants with low vigor, (H): change in the supply-demand relationship (M2), (I): 10 month old plants with non-tuberized roots (M2), (J): cassava plant with decumbent habit (M2), (K): cassava root cuttings with 15 days after harvest PPD incipient damage, (L): determination of scopoletin content visualized with a UV lamp, which here shows a low concentration of the substance, a state associated with PPD damage.

Determination of amylase

For 0.1 g of starch was gathered in 100 mL balls and 1 mL of absolute ethanol at 95% was added and homogenized. Nine mL of 1N sodium hydroxide was added. A volume of 100 mL was reached with distilled water. One mL of acetic acid and 2 mL of 2% iodine were added. A volume of 100 mL was reached with distilled water, reacted in the dark and read at 620 nm (Aristizabal *et al.*, 2007).

Analysis of the viscosity of the paste by RVA

The profiles of hot paste viscosity were obtained by RVA4 Series model RVA (Newport Scientific, Australia). 5% suspension (w/v) dry basis of each sample was added to aluminum viscoamilografic containers and subjected to the temperature program (Ceballos *et al.*, 2007).

Data analysis

Statistical analysis of the variables evaluated in the field and laboratory was performed using the SAS® statistical package version 9.1 and frequency tables were made with the qualitative variables. Analysis of variance was performed with untransformed data, using the Tukey test to determine differences between families, treatments and years. Similarly, we applied the Pearson correlation test between the variables measured. The coefficient of variation and the coefficient of model determination were 12% and 0.9, respectively.

Results and discussion

The 1,097 M2 plants were established, of which 829 received Gamma treatment and 268 neutron treatment. Ionizing radiation has largely contributed in the production of

varieties in crops of economic importance (Tofiño *et al.*, 2007; Xin *et al.*, 2009).

Morphological characterization of mutants, individual seedling is commonly performed on cereals in later generations, M3 and M4, which has been identified as alterations in the color of the organs, leaf characteristics and reproductive abnormalities (Wu *et al.*, 2005; Xin *et al.*, 2009). In the case of rice and *Lotus japonicus*, the assessment of an average of 40,000 M2 plants was required to detect mutant genotypes in genes involved in a wide range of metabolic and developmental processes (Wu *et al.*, 2005).

The verification of the identified abnormalities in M1, was performed in M2 without observing hermaphrodite flowers or stem bark of different color in the same plant. In contrast, apomictic plants are identified with weak form, state of descriptor form of leaflets and atypical leaf area, not recorded in cassava. Similarly, new abnormalities were identified such as root without suberification, no anthesis, early flowering, changes in demand and supply ratio, and meristems distortion. In the populations tested, at least one atypical feature was identified in 62.2% of individuals (Fig. 1, Tab. 1). Overall, the heterogeneity of radiation sensitivity was evident in the population, the SM3045 family with gamma treatment showed 13.5% of individuals with at least one abnormality while the same

family under neutron treatment presented 100% atypical individuals. In contrast, the C4 family had more than 80% atypical individuals under both ionizing radiations (Tab. 1). Also, 17% of plants were weak, smaller than 30 cm, (Tab. 1). However, this feature, or the decrease in root production, is associated with inbreeding depression. Therefore, these criteria should not be interpreted as indicators of the effect of the mutation in M2 (Rojas *et al.*, 2009). Evolutionary studies of cassava and population genetic models indicate that the size of the plants is correlated with multilocus heterozygosity (Pujol and McKey, 2006) and possible causes of inbreeding depression on the characteristics of agronomic importance in open or mixed pollinated plants, associated mainly to the expression of deleterious recessive alleles (Charlesworth and Charlesworth, 1999).

High rates of mortality were identified in M2 populations, 20%, as well as overall decline in the production of roots, less than 5 kg per plant, (Tab. 1). The above features cannot be associated exclusively with the effects of radiation because inbreeding depression effects mortality in the field and in surviving plants. Studies on cassava suggest that the rate is not uniform among inbred families (Rojas *et al.*, 2009), which may become apparent between the mutant inbred populations, especially in the SM3015 and C4 families which showed mortality and presence of atypical individuals as highly variable between sources of radiation.

TABLE 1. Identification of atypical individuals in assessing individual M2 plants from seed.

Family	Treatment	Individual seed evaluation	Plants with mutation	Atypical individuals in the population (%)	Leaves		Stem			Root	Flowering				Vigor		
					Forml 11	Forml 13	TIPP 2	TIPP 6	DM	RNS	NF	TIPF 8	TIPF 2	TIPF 7	VIG 0	VIG 1	VIG 1*
CM9331	G	60	50	83,3	1	0	7	0	9	0	15	3	0	0	11	4	0
	N	5	4	80,0	0	0	0	2	0	0	2	0	0	0	0	0	0
GM155	G	123	103	83,7	11	1	15	0	14	0	30	0	0	1	10	20	1
	N	0	0	0,0	0	0	0	0	0	0	0	0	0	0	0	0	0
SM3015	G	77	66	85,7	0	0	0	1	4	0	51	0	0	0	5	5	0
	N	14	11	78,6	0	0	0	0	0	0	10	0	0	0	0	1	0
SM3045	G	37	5	13,5	0	0	0	0	0	0	1	0	0	0	4	0	0
	N	2	2	100,0	0	0	0	0	0	0	2	0	0	0	0	0	0
C4	G	521	451	86,6	3	0	3	0	1	4	170	0	0	0	153	115	2
	N	247	200	81,0	0	0	0	0	1	0	126	0	3	0	35	35	0
C127	G	11	6	54,5	0	0	0	0	0	0	4	0	0	0	0	2	0
	N	0	0	0,0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		1097	898	62,2	15	1	25	3	29	4	411	3	3	1	218	182	3

G = Gamma, N = neutron; forml 11 = minimum leaf area, forml 13 = increased leaf area, TIPP 2 = No. branching, TIPP 6 = poor habitat ; DM = meristem distortion, RNS = roots without suberification, NF = no flowering ; TIPF 8 = no anthesis; TIPF 2 = enlargement of the ovary (pseudo apomictic); TIPF 7 = premature flowering, VIG 0 = were planted but died during the study; VIG 1 = height <30 cm and indeterminate morphological characteristics; VIG 1* = high harvest index.

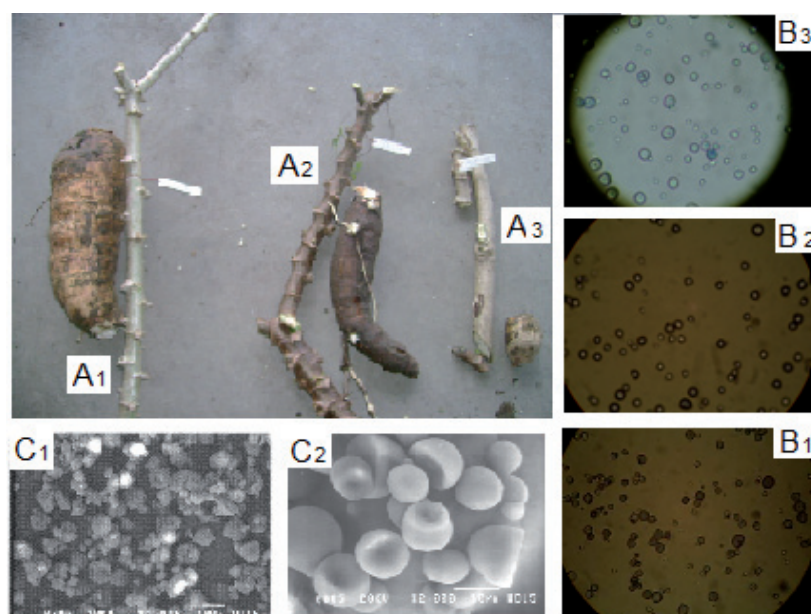


FIGURE 2. Changes in plant morphology, root size and starch granule between irradiated and non-irradiated individuals in cassava family C4. (A1) ascending non-irradiated, (A2) parental M1 and (A3) small granule genotype M2. (B) photographs of light microscope (40X), show the characteristics of the starch granule for each individual listed in A with the same numbering. (C1) photographs of hollow granules and fractured granules (C2), electron scanning microscopy. The bar at the bottom corresponds to 10 μ m.

On the other hand, despite the inclusion of at least one inbred generation in new models for improving cassava, the effect of inbreeding on granule size and other characteristics of the starch has not yet been registered. The decrease in root size and performance observed among related genotypes, non-irradiated grandfather, M1 parent and the M2 mutant with small granule is accompanied by changes in size, shape and density of starch granules, detectable by microscopy (Fig. 2).

However, the combination of microscopic techniques, root staining with iodine (Carvalho *et al.*, 2004) and morphological description (Singh *et al.*, 2006), have been used successfully in the characterization of variability and identification of mutant cassava starch and different crops. Iodine staining has been useful in identifying natural mutants in cassava such as Waxy and Sugary; in both studies the preliminary observation of the results of this simple, low cost technique was confirmed with biochemical and molecular analysis (Ceballos *et al.*, 2007).

However, staining with iodine only allows discrimination because extreme genotypes with thick, reddish-brown coloration is typical of amylase-free starches, while low starch or high sugar content does not cause visible changes in the initial coloring of the solution. The starch content of 16.6 to 23.6% of amylase stains dark blue root (Ceballos *et al.*, 2007). A scope with a finer discrimination is required for

quantification of amylase by spectrophotometer or *Differential scanning calorimeter* (DSC) (Ceballos *et al.*, 2008). After iodine staining, an individual was identified without appreciable changes in the initial coloration of the solution that could be associated with real sugar (GM155N35-1). Similarly, optical microscopy allowed the identification of small granules of M2 genotypes, with a predominance of 5-8 μ m diameter (C4G160-13, C4G160-16, C4G160-18, SM3015-1), granule holes (C4N83-9, and C4G28 GM155G28-9-10) and fractured granules (GM155G75-1) (Fig. 2). The combination of both techniques allowed the identification of individuals with little starch (C4G190-11, C4N14-5, C4N14-14, CM9331, G67-36), no change in the color of the solution and a low number of granules. In rice, induced mutants were identified with features that correspond to defects in the gene *Pho1* such as variability in wrinkling, mature seed size and starch content (Wu *et al.*, 2005).

One of the main objectives of the establishment of a clonal test with sexual M2 seed, was to verify the persistence of the detected abnormalities and thus rule out a possible masking effect in the interaction between the environment genotype and the M2 mutant population. Especially in the expression of granule abnormalities, rheological properties of starch, amylase (Am), dry matter (DM) and cyanide (HCN). With respect to behavior in the two assessments, analysis of variance and Tukey's test suggest that C4 diffe-

TABLE 2. Identification of individuals with atypical characteristics of starch granules by light microscopy.

Family	Treatment	Population	No. atypical genotypes evaluated clone	No. atypical genotypes individual seed
CM 9331	G	43	9	4
	N	2	0	0
GM 155	G	83	23	3
	N	0	0	0
SM 3015	G	29	6	3
	N	3	0	0
SM 3045	G	11	1	2
	N	2	1	0
C 4	G	210	37	48
	N	138	18	33
C 127	G	6	3	0
	N	0	0	0
Total	G	382	79	60
	N	145	19	33
Total		527	98	93

G = gamma, N = neutron.

red statistically from the other families in the accumulation of MS and HCN; gamma treatment showed a general trend toward the accumulation of DM and HCN, and the neutron treatment showed a significant effect between years in the production of Am, MS, and HCN. The C4 family presented greater variability in the distribution of data for the gamma treatment and the lowest and highest value of MS and Am with 14.8, 71.6 and 17.2, 30.67, respectively.

Similarly, in the C4 neutron treatment, the highest value of HCN was found while the lowest value was recorded in GM155, 1,643 and 45 ppm, respectively. In studies of starches from different sources: cereals, legumes, roots

and tubers, the correlation between thermal, rheological, Am and granule size has been proven (Li and Yeh, 2001). In this vein, it is suggested that the characterization of starch mutants must be made from the integrated analysis of these characteristics over time and for greater reliability, in various locations.

The evaluation of the morphology of starch granules by light microscopy showed the effect of genotype-environment interaction because there were differences in the number of individuals with abnormalities between years, in every family of gamma treatment. In the treatment with neutrons, they found abnormalities in the morphology of

TABLE 3. Evaluation of tolerance to PPD in evaluating M2 clonal population derived from seeds subjected to ionizing radiation.

Family	Treatment	Individuals with postharvest deterioration			
		High	Medium	Low dry matter	*Low/high dry matter
CM9331	G	19	11	4	0
	N	1	0	0	0
GM155	G	61	16	1	1
	N	0	0	0	0
SM3015	G	21	5	0	1
	N	1	0	0	0
SM3045	G	7	1	0	3
	N	2	0	0	0
C4	G	78	56	11	12
	N	46	15	5	5
C127	G	2	0	0	1
	N	0	0	0	0
Total	386	238	104	21	23

* Individuals with dry matter content greater than 33% evaluated in two years.

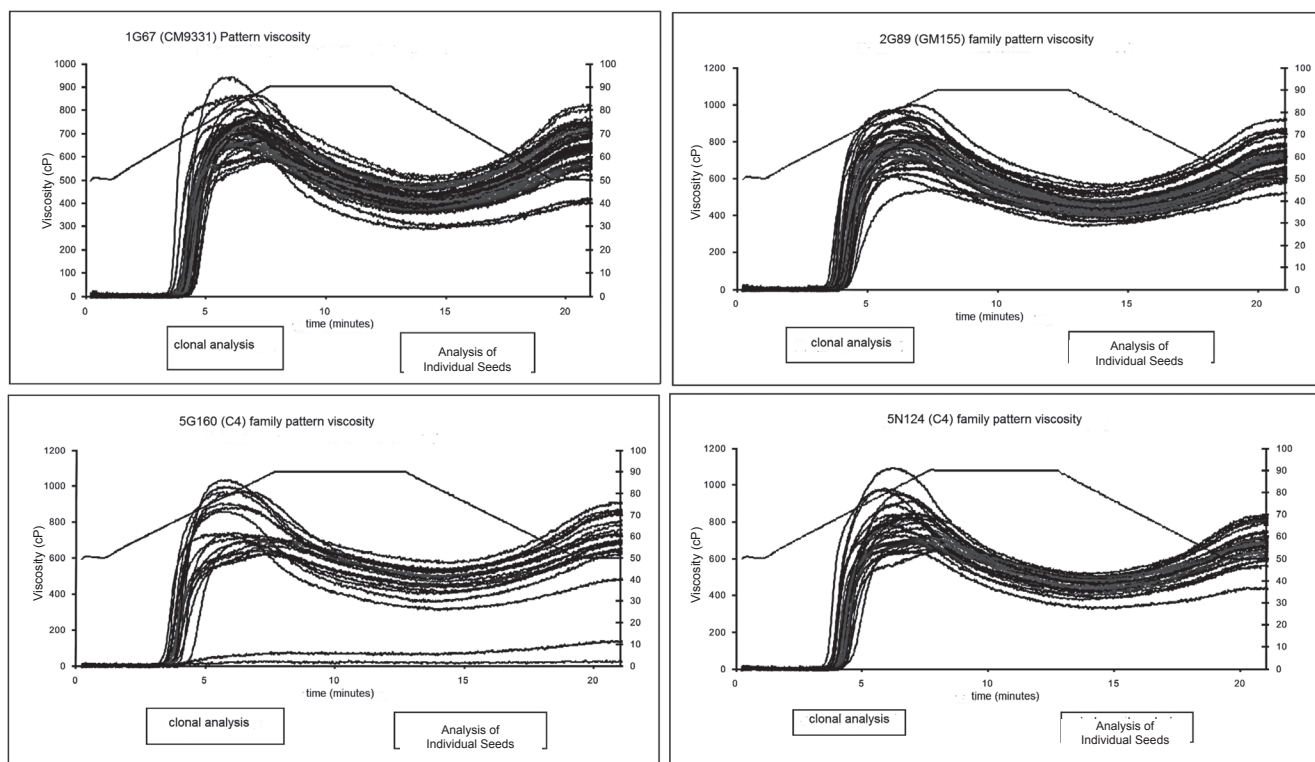


FIGURE 4. Effect of type of plant material used such as seed on the characteristics of starch in M2 individuals of different families in both treatments. In the X axis, we see the time in minutes, up to 20 and in Y, the viscosity value, maximum value 900 cP. Figures obtained from the RVA analysis.

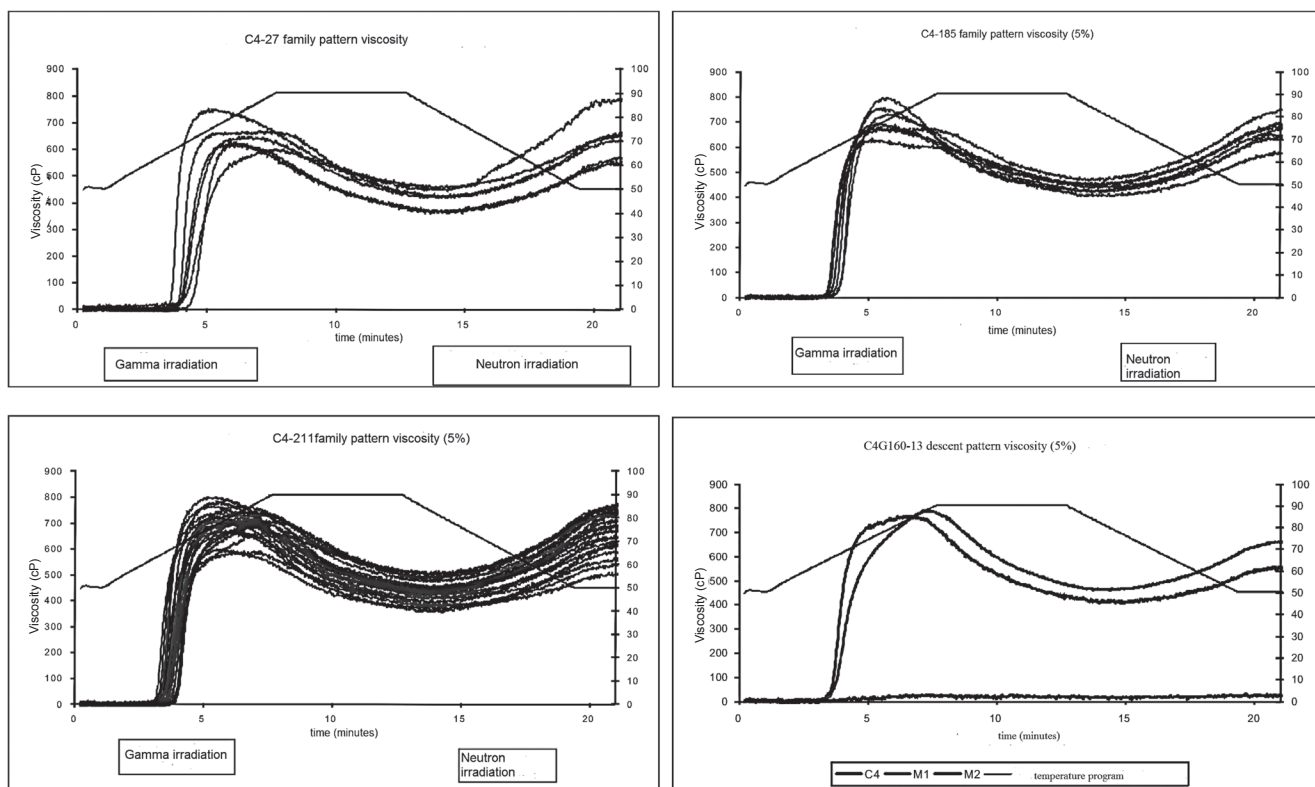


FIGURE 5. Effect of type of radiation on the rheological properties of starch in families derived from seeds subjected to ionizing radiation.

the granule in only the families SM3045 and C4 in both evaluations (Tab. 2).

For the evaluation of physiological deterioration (PPD), we performed two readings from 8 to 15 dah. The analysis of variance and Tukey's test indicate that family SM3045 was more prone to deterioration and the treatment presented significant effect on the intensity of damage, neutron showed higher vascular obstruction while gamma higher rotting. Materials with low damage after 15 d, were associated with dry matter content as there is an undesirable inverse relationship between PPD and MS in clones with moderate content of carotenoids (El-Sharkawy and Cadavid, 2002; Sánchez *et al.*, 2006). We identified 23 individuals in families C4, SM3045, SM3015 and GM155 of gamma, in descending order according to the number of individuals identified. In the neutron treatment promising individuals were identified only in C4 and SM3045 (Tab. 3). It is recommended that individuals with high promise in PPD and dry matter be evaluated in areas with contrasting rainfall since differences in the expression of PPD have been recorded due to sowing environment (El-Sharkawy and Cadavid, 2002).

Several studies of wild starches (Blazek and Copeland, 2008; Kong *et al.*, 2009) and mutants have correlated differences in the characteristics of the starch granule, chemical and physical properties, and content of amylase and amylopectin, with changes in the pattern viscosity. This is true in the case of rice, a mutant resistant starch exhibited reduced viscosity of the paste, low peak temperature and final temperature, and enthalpy of gelatinization (Shu *et al.*, 2006). However, in hollow granule mutants C4N83-9, GM155G28-9-10 and C4G28, there is no correlation between granule abnormalities and significant variations in the properties of the paste. Contrary to what was observed in the small granule mutant, in which granule analysis, quality of roots and critical values of RVA show changes between the generations: non-irradiated grandfather, M1 parent and M2 mutant with respect to gradual decline in performance, granule size, instability and clarity of the gel, and dry matter content of Am (Tab. 4, Figs. 2 and 5).

The RVA analysis has been useful in the identification and characterization of yucca mutants such as Waxy and small granule, in which the values of peak viscosity, breakdown and reorganization of the gel are drastically different from normal starch (Ceballos *et al.*, 2007; Ceballos *et al.*, 2008). Quality variables in roots and RVA, analysis of variance and Tukey test indicate significant differences between GM155 and the other families in hot paste viscosity and consistency. The hot paste viscosity is also affected differentially by the treatment, and the neutron treatment which showed the highest values for paste temperature, while gamma showed the highest values in gel consistency. On the other hand, the viscosity was very sensitive to the effect of seed type as assessed for two years, there were statistical differences in the variables: maximum viscosity (Vm), hot paste viscosity (VPC), cold paste viscosity (Vpf), instability of the gel (Ig), reorganization (Rg) and consistency (Cg). Differences in the evaluation for two years indicate influence of genotype-environment interaction in the expression of these characters, cassava M2. In this sense, there was more variability in the rates of RVA in the clonal evaluation and a strong tendency to produce more viscous starches (Fig. 4). Concerning the effect of radiation on the pulp properties in C4 family, the neutron treatment produced more viscous starches than gamma due to higher values in Vm and Ig while gamma produced starches more prone to retrogradation due to elevated Rg and Cg (Fig. 5).

In the potato and *Amaranthus*, differences have also been identified between varieties in Am, swelling power and solubility, as well as peak temperature, and a tendency for retrogradation Ig (Singh *et al.*, 2006; Kong *et al.*, 2009). Location research in the potato reveals the influence of the genotype-environment interaction on granule size and paste temperature, and temperature-dependent tuber development (Kaur *et al.*, 2007).

The relationships between different properties of starch determined by the Pearson correlation analysis showed positive correlation between Vpc, Rg ($r^2 = 0.673$, $P \leq 0.001$) and Cg ($r^2 = 0.311$, $P \leq 0.001$) but negatively correlation with Ig ($r^2 = -0.446$, $P \leq 0.001$). The Vm showed positive correlation with Vpf ($r^2 =$

TABLE 4. Comparison of the characteristics of roots and starch quality between the non-irradiated descent and two related mutant generations (C4G160-13, small graule).

	Tpasting (°C)	Vmax (cP)	Vpasthot (cP)	Vpastcold (cP)	Instability of the gel (cP)	Reorganization (cP)	Consistency (cP)	Clarity (%)	Amylase	Ms	Cyanide
C4	63,50	766	422	510	344	-344,00	88	56	21,19	36,48	210
M1	64,08	799	483	610	316	-316,00	127	50	22,47	34,69	61
M2	63,15	418	19	21	399	-399,00	2	15	30,67	16,67	1167

C4 non-irradiated grandfather, M1 parent derived from irradiated seeds; M2 small granule mutant, product of self-pollination of the M1 parent.

0.0.809, $P \leq 0.001$), Ig ($r^2 = 0.738$, $P \leq 0.001$) and Cg ($r^2 = 0.755$, $P \leq 0.001$), while Rg showed negative correlation ($r^2 = -0.380$, $P \leq 0.001$). These correlations differ from those reported in potato germplasm and localities in which Vpc was positively correlated with Vpf and Te (pasting temperature), while Rg was negatively correlated with Te (Kaur *et al.*, 2007).

Am's correlation with some variables such as viscosity has been reported in cassava and other starch (Li and Yeh, 2001, Kong *et al.*, 2009). In the integrated analysis of the two M2 assessments, Am and Vpc were negatively correlated ($r^2 = -0.44$, $P \leq 0.001$), Vm ($r^2 = -0.25$, $P \leq 0.010$); Vpf ($r^2 = -0.285$, $P \leq 0.0038$) and R ($r^2 = -0.30$, $P \leq 0.002$). These correlations are inverse to those recorded in the potato where Am was positively correlated with VPC (Kaur *et al.*, 2007) but are consistent with the behavior of cassava starch type Waxy, enriched with amylopectin, peak viscosity is higher while in the small granule starch with unusually high content of amylase for cassava, the peak viscosity is low (Ceballos *et al.*, 2007, 2008). In addition to Amylase content, variation in the content and characteristics of amylopectin affects the properties of the paste, especially Te and in some cases Vm (Shu *et al.*, 2006).

Conclusions

In M2, 80% of the M1 chimeras and new abnormalities in M2 were identified from radiation similar in mutagenic efficiency (82%). However, fast neutrons, further limits the survival and reproductive capacity of M1 plants, which reduced the number of M2 plants derived from such treatment. However, mortality, reduced vigor, yield and anthesis loss are associated with effects of inbreeding in cassava. Variability in the characteristics of the granule and pulp properties was found in assessments of two years, suggesting significant influence of genotype-environment interaction in these characteristics.

The phenotypic evaluation of inbred M2 populations of mutant cassava germplasm allows the identification of atypical individuals but does not clarify the genetic origin since changes in the starch may be associated with gene expression of an intricate network of genes, where the posttranscriptional relationship is equally significant. Similarly, in M2 populations, effects of inbreeding depression come together, increasing homozygosity of the genetic background and possible fixation of mutations induced by ionizing radiation in sexual seeds. So, it is necessary to apply molecular techniques such as TILLING and *In situ* expression to identify the origin of the phenotypic abnormalities identified in the M2 population.

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