Phenotypic stability of traits associated with fruit quality in butternut squash (*Cucurbita moschata* Duch.)

Estabilidad fenotípica de caracteres asociados con la calidad del fruto del zapallo (*Cucurbita moschata* Duch.)

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ABSTRACT

The aim of this study was to identify and select genotypes with high fruit dry matter, represented by high starches and carotenes, to obtain a cultivar apt for agroindustrial processes. The model of Eberhart and Russell was used to measure the stability parameter and evaluate the phenotypic stability of traits associated with fruit quality in four populations of butternut squash at three localities in the department of Valle del Cauca (Colombia): Candelaria, Palmira and El Cerrito. Highly significant differences were detected for the genotype x location (P≤0.001) interaction for the traits of dry matter, carotene and starch. Except for population 1 in El Cerrito, there was a negative effect on the accumulation of photosynthates at 1,536 m a.s.l., probably due to the low quality of the heliophany. The populations 1, 2 and 3 were stable and predictable, complying with the parameters β = 1 and d = 0 of the Eberhart and Russell methodology. Population 1 was notable in terms of high fruit production, high dry matter content and good phenotypic stability.

Key words: population, dry matter content, carotenes, starch, yields.

RESUMEN

El objetivo de este trabajo fue identificar y seleccionar genotipos de zapallo con alta materia seca en el fruto, representada en alto contenido de almidón y carotenos, con el fin de obtener un cultivar para procesos agroindustriales. Se utilizó el modelo de Eberhart y Russell para medir los parámetros de estabilidad y evaluó la estabilidad fenotípica de los caracteres asociados con la calidad del fruto en cuatro poblaciones de zapallo en tres localidades del departamento del Valle del Cauca (Colombia): Candelaria, Palmira y El Cerrito. Se detectaron diferencias altamente significativas en la interacción genotipo x localidad (P≤0,001) para los caracteres de materia seca, carotenos y almidón. Excepto para la población 1 en El Cerrito, hubo efecto negativo para la acumulación de fotosintatos a 1.536 msnm, probablemente debido a baja calidad de la heliofanía. Las poblaciones 1, 2 y 3 fueron estables y predecibles, al cumplir con los parámetros β = 1 y d = 0, de la metodología de Eberhart y Russell. La población 1 se destacó por su elevada producción de frutos, alto contenido de materia seca y buena estabilidad fenotípica.

Palabras clave: población, contenido de materia seca, carotenos, almidón, rendimiento.

Introduction

The increasing importance of butternut squash (*Cucurbita moschata* Duch.) in Colombia's food security and the growing demand for new raw materials for agroindustrial purposes have been fundamental in promoting research on this crop.

Increased productionof this crop can be attributed to its versatility—it can be consumed directly in soups, creams, sweets, purees, juices, pastries, and preserves or used as raw material for the agroindustrial processing of flours and dehydrated products—as well as its high nutritional value related to its contents of carotene (provitamin A), ascorbic acid (vitamin C), minerals (calcium, iron, phosphorus) and amino acids such as thiamin and niacin (Vallejo and Mosquera, 1998).

The attractiveness of the fruit of the butternut squash lies in its nutritive value as a health food for human consumption, both fresh and processed. However, because its moisture content is high (90%), it is highly perishable with a short shelf life and diluted nutrients. Therefore, to include butternut squash fruit in agroindustrial processes, it is necessary to dehydrate the fruit—a postharvest processing operation that is costly in terms of capital and loss of raw material (González and Prado, 2003; Ortiz *et al.*, 2008). To use the integral biomass of the butternut squash fruit in animal feed, genotypes containing at least 20% dry matter (DM) in the fruit are required (Ortiz *et al.*, 2013).

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The butternut squash fruit contains 5.5–25.0% starch (Ortiz *et al.*, 2013), 490–1365 μg g^{-1} pro-vitamin A in the form of total carotenes (Hopp *et al.*, 1960; Arima and Rodríguez-Amaya, 1990; Maynard *et al.*, 2004; Ortiz *et al.*, 2008; Ortiz *et al.*, 2013) and >80% DM digestibility (Maynard *et al.*, 2004; Ortiz *et al.*, 2010).

β-carotene is one of the natural pigments widely distributed in nature, and is responsible for the yellow-to-intense-orange color in plants. This pigment is found in the chromoplasts of higher plants, but its color may be masked by chlorophyll (Zaccari *et al.*, 2007). β-carotene, abundant in several varieties of butternut squash, is a principal precursor of vitamin A, which plays a role in many bodily functions: it enhances the immune system and decreases the risk of degenerative diseases such as cellular aging, cancer, cardio vascular diseases, arteriosclerosis, age-related muscular degeneration and cataract formation. It is a powerful antioxidant that deactivates free radicals and captures singlet oxygen which causes the aforementioned adverse effects (Astorg, 1997; Clevidence *et al.*, 2000; Rodríguez-Amaya, 2001; Bramley, 2003; Southon and Faulks, 2003).

This study therefore aimed to identify and select genotypes with high fruit dry matter, represented by high starches and carotenes, to obtain a stable cultivar apt for agroindustrial processes.

Materials and methods

Location

The study was conducted at three localities in the department of Valle del Cauca, Colombia:

The Mario González Aranda (MGA) farm, located in Palmira, at 03º30'26.8" N and 76º18'47.6" W, 998 m a.s.l., with a sub-humid climate, classified based on precipitation and sunshine (Mejía, 1983).

The Experimental Center of the Universidad Nacional de Colombia–Palmira campus (CEUNP), located in the municipality of Candelaria, at 03º25'25.3" N and 76º25'47.8" W, 972 m a.s.l., with 26°C average annual temperature, 1,100 mm annual precipitation and 76% relative humidity (Ortiz *et al.*, 2013).

Alto Castillo, located in the municipality of El Cerrito, at 03º39'49.3" N and 76º11'15.2" W, 1,536 m a.s.l. (Valdés *et al.*, 2010).

Genetic material

Four butternut squash populations, selected for high fruit dry matter (DM), were selected from the Cucurbits collection of the germplasm bank of the Universidad Nacional de Colombia–Palmira campus. The four populations were derived from populations 79, 28 and 6 in generations S_2, S_3 and S_4, where population 1 ($79S_3x79S_3$) was full sib; population 2 ($79S_3x(79S_2x6S_2)$) a triple hybrid; population 3 ($79S_2x(6S_2x28S_2)$) a triple hybrid; and population 5 ($79S_4x$ $79S_4$) selfed (Valdés *et al.*, 2010).

Traits evaluated

The following traits were evaluated:

Production per plant (kg): average fruit weight per plant multiplied by the number of fruits per plant.

Fruit dry matter (DM) (%): 5 g of pulp were weighed on a Toledo XS64 analytical balance scale and then placed in a Binder 960555 oven at 105°C for 24 h; both the initial and final weights of the samples were recorded.

Dry matter (DM) per plant (%): fruit DM multiplied by the number of fruits per plant.

Dry matter (DM) per hectare (%): DM per plant multiplied by the number of plants/ha.

Total carotenoids (TC) in fruit (μg g^{-1} DM): obtained by the petroleum ether-acetone extraction technique, washed with decanted water and submitted to spectrophotometry at 450 nm (Rodríguez-Amaya and Kimura, 2004).

Total fruit starch (μg g^{-1}): gelatinization and dispersion of starch in KOH 4M, followed by hydrolysis with a heat-resistant amyloglucosidase and specific determination of glucose using a glucoseoxidasere agent and spectrophotometry at 500 nm (Cumarazamy *et al.*, 2002).

A randomized complete block experimental design with six replicates was used with the four abovementioned experimental populations and a UNAPAL-BoloVerde commercial control. Experimental units consisted of five plants, planted at a distance of 3 m between and within furrows. Combined analysis of variance was performed for each of the traits associated with the butternut squash fruit for all three localities.

The methodology proposed by Eberhart and Russell (1966) was used, which is based on the following model:

$$X_{ij} = \mu_i + b_i + I_j + d_{ij} \quad (1)$$

where:

X_{ij}= performance of variety i in environment j.

μ_i= mean of variety i across all environments.

b_i= regression coefficient, which measures the response of variety i to environments.

I_j= environmental index obtained as the difference between the mean of all varieties in environment j minus the overall mean.

d_{ij}= deviation of the regression of variety i in environment j.

A stable variety is one that shows a regression coefficient close to one (bi=1) and the sum of its deviations is proximate to zero ($\Sigma d_{ij}^2=0$).

Statistical analysis was performed using the SAS® 9.1 software package (Windows version) (SAS Institute, 2000), using the protocol proposed by Cadena *et al.* (1998).

Results and discussion

Location analysis

In the combined analysis of variance, differences were highly significant ($P\leq0.001$) among populations and locations (Tab. 1). Since the population x location interaction was significant for most of the variables assessed: plant dry matter, dry matter per hectare, total carotenes; a location analysis was performed.

Highly significant differences ($P\leq0.001$) were found between populations (Tab. 1). These differences can be attributed, on the one hand, to the advances made in selection and recombination in previous cycles, which led to experimental populations (Valdés *et al.*, 2010), and, on the other hand, to the fact that the control is a variety with low DM content, mainly used for fresh consumption.

Palmira test site

Although the four experimental populations showed superior fruit quality, population 3 was notable with a high DM production per plant (2.57 kg), higher average fruit weight (3.14 kg) and high number of fruits per plant (5.46) (Tab. 2).

The control presented high carotene content (521.26 μg g^{-1}) and low dry matter content in fruit (8.47%). A different situation was observed in the experimental populations, which presented high fruit dry matter (DM) and low carotene content, confirming the findings of (Ortiz *et al.*, 2013) regarding the negative correlation between fruit dry matter and total carotenes. Population 3, however, had a high carotene content (359.75 mg g^{-1}) and high fruit dry matter (15.97%), which, combined, is a factor of quality and important for developing a cultivar for agroindustrial purposes.

TABLE 1. Mean squares of combined analysis of variance for traits associated with the butternut squash fruit.

Source of variation	DF	FDM (%)	PDM (kg)	DMH (kg)	TC (μg g^{-1} DM)	ST (μg g^{-1} DM)
Location	2	1013.82**	42.07**	51942910.60**	518720.05**	656.37**
Block	15	31.51**	3.68**	4552195.20**	16883.28 NS	45.23**
Population	4	1391.60**	14.77**	18257236.40**	983040.81**	1093.69**
Location x Population	8	1.15 NS	9.23**	11405397.90**	47281.45**	11.89 NS
Experimental error	60	12.31 NS	1.32 NS	1630699.30NS	18236.01 NS	13.42 NS
Mean		16.02	2.74	3045.90	347.24	8.71
CV		21.89	39.36	39.30	36.89	36.66

FDM: fruit dry matter; PDM: plant dry matter; DMH: dry matter/ ha; TC: total carotenes; ST: starch.
** Highly significant difference ($P\leq0.001$). NS = Non-significant difference at a level of significance of $P\leq0.05$. CV = Coefficient of variation.

TABLE 2. Means of the traits associated with the butternut squash fruit for each population at the Palmira test site.

Population	FDM (%)	PDM (kg)	DMH (kg)	TC (μg g^{-1} DM)	ST (μg g^{-1} DM)
1	18.56**	2.37**	2632.90**	298.73**	9.72**
2	16.10**	2.30**	2560.00**	281.45**	8.30**
3	15.97**	2.57**	2856.00**	359.75**	8.59**
5	18.27**	2.41**	2678.70**	341.77**	11.40**
Control	8.47	1.18	1316.00	521,26	2.70
Mean	15.44	2.16	2396.55	361.29	8.11
LSD	1.63	0.40	442.70	71.46	1.43

FDM: fruit dry matter; PDM: plant dry matter; DMH: dry matter/ha; TC: total carotenes; ST: starch.
** Highly significant difference ($P\leq0.001$).

Candelaria test site

Populations 1 and 5 presented the highest fruit dry matter (DM), but population 3 had the highest dry matter yield/ha because of its higher average fruit weight (4.29 kg) and number of fruits per plant (4.68). Similarly, the control UNAPAL-Bolo Verde, followed by population 2, showed a high carotenoid content (427 μg g^{-1}) and low starch content (4.02 μg g^{-1}) (Tab. 3).

Alto Castillo test site

In general, there was an adverse effect on populations planted at this test site regarding the study variables. The traits of fruit DM, plant DM and DM per hectare yielded negative values when compared with the control, except in the case of population 2 (Tab. 4), indicating that the locality had a negative effect on the experimental populations evaluated but proved favorable for the control UNAPAL-Bolo Verde.

Analysis of adaptability

This analysis was performed according to the Eberhart and Russell model (1966). Tab. 5 presents the stability parameters $\hat{b}$i and $\hat{S}di^2$ for each experimental population and the control UNAPAL-Bolo Verde, as well as the means of production variables per plant and fruit DM content. Except for the regression coefficient for fruit DM in population 2, the other coefficients are statistically equal to one (1.0), concluding that the performance of all populations was stable in both favorable and unfavorable environments.

TABLE 3. Means of the traits associated with the butternut squash fruit for each population at the Candelaria test site.

Population	FDM (%)	PDM (kg)	DMH (kg)	TC (μg g^{-1} DM)	ST (μg g^{-1} DM)
1	22.61**	3.55**	3954.00**	233.95**	14.13**
2	19.81**	3.24**	3605.70**	275.76**	11.40**
3	19.24**	3.74**	4159.50**	262.56**	10.91**
5	21.44**	3.59**	3989.30**	208.80**	15.05**
Control	11.49	1.99	2214.10	427.00	4.02
Mean	18.85	3.21	3572.75	282.89	11.03
LSD	2.16	0.58	650.30	54.80	1.94

FDM: fruit dry matter; PDM: plant dry matter; DMH: dry matter per hectare; TC: total carotenes; ST: starch.
** Highly significant difference ($P \leq 0.001$).

TABLE 4. Means of the traits associated with the butternut squash fruit for each population at the Alto Castillo test site.

Population	FDM (%)	PDM (kg)	DMH (kg)	TC (μg g^{-1} DM)	ST (μg g^{-1} DM)
1	17.02**	2.93**	3263.00 NS	339.15**	9.31**
2	14.17**	3.73**	4155.20**	367.38**	7.29**
3	14.47**	2.25**	2508.20**	333.65**	7.37**
5	14.93**	2.37 NS	2642.00NS	284.40**	8.96**
Control	8.00	2.94	3271.10	644.95	2.03
Mean	13.56	2.85	3174.64	402.17	6.84
LSD	1.65	0.70	782.80	75.99	1.62

FDM: fruit dry matter; PDM: plant dry matter; DMH: dry matter per hectare; TC: total carotenes; ST: starch.
** Highly significant difference ($P \leq 0.001$). NS = Non-significant difference at a level of significance of $P \leq 0.05$.

TABLE 5. Means and adaptability parameters of Eberhart and Russell (1966) for production per plantand fruit DM for different populations of butternut squash in different environments.

Population	PPL (kg)			DMF (%)		
	Mean	$\hat{b}$i	$\hat{S}di^2$	Mean	$\hat{b}$i	$\hat{S}di^2$
1	15.72	0.59	1.67	19.40	1.09	0.07
2	19.01	1.52	2.18	16.70	1.09	0.01
3	17.45	0.17	7.08	16.57	0.92	0.00
5	15.28	0.27	4.61	18.22	1.21	0.84
Control	22.76	2.79	21.50	9.32	0.70	0.32

PPL: production per plant; FDM: fruit dry matter.

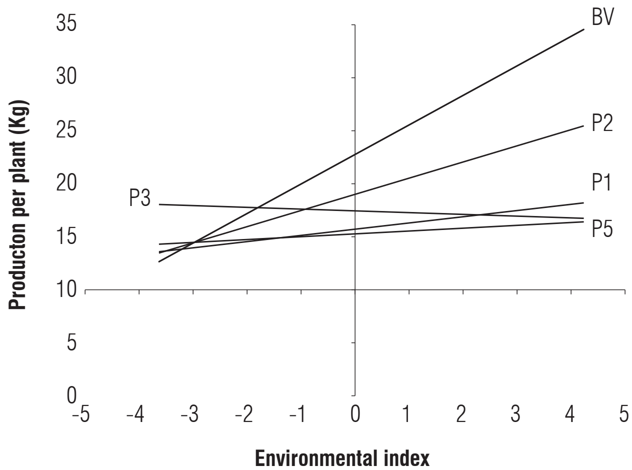

FIGURE 1. Production per plant of experimental populations in terms of environmental index. P1: population 1; P2: population 2; P3: population 3; P5: population 5; BV: UNAPAL-Bolo Verde.

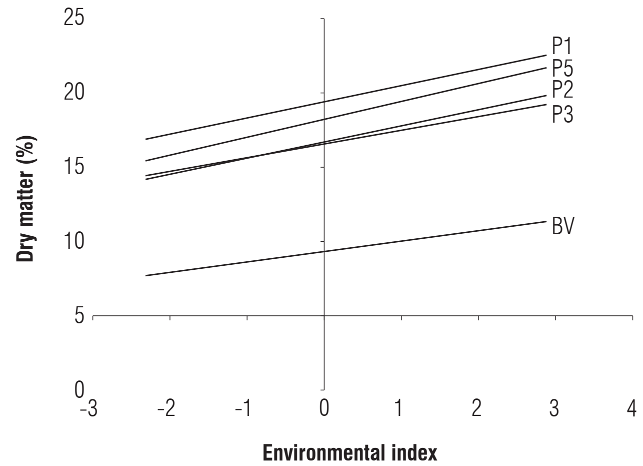

FIGURE 2. Fruit dry matter production of experimental populations in terms of the environmental index. P1: population 1; P2: population 2; P3: population 3; P5: population 5; BV: UNAPAL-Bolo Verde.

Based on the trend analysis of the line of regression (Fig. 1) and the bi values, regardless of statistical criteria, one might assume that all genotypes are unstable: some presenting a better production response to favorable environments ($\hat{b}i$>1.0), for example population 2 and the control UNAPAL-Bolo Verde, and others presenting a better response in unfavorable environments ($\hat{b}i$ <1.0), for example, populations 1, 3 and 5.

For the DM variable, populations 1, 2 and 3 can be considered relatively stable ($\hat{b}i$~1.0), whereas population 5 responded well to favorable environments (Fig. 2).

Regarding the parameter $\hat{S}di^2$, which is used as an indicator of the degree of reliability of the predictions made about the performance of a population when submitted to a certain environment, it is deduced that the predictions of performance for populations 1, 2 and 5 can be closer to the actual performance than for population 3 and the control UNAPAL-Bolo Verde, whereas, regarding fruit DM content, population performance is predictable for both favorable and unfavorable environments. This may be attributable to the greater genetic complexity of the production trait as compared to fruit DM.

Because the suitability of a raw material like butternut squash for industrialization is measured in terms of parameters such as fruit DM content, plant DM and DM per hectare, all four experimental populations showed promise (Tab. 6); however, based on their comprehensive performance regarding all the traits evaluated at the three test sites, population 1 is considered the best option for becoming a new variety of butternut squash for agroindustrial purposes.

TABLE 6. Comparison of means of experimental populations with the commercial control UNAPAL-Bolo Verde and their significance for fruit quality variables.

Population	FDM (%)	PDM (kg)	DMH (kg)	TC (μg g^{-1} DM)	ST (μg g^{-1} DM)
1	19.40**	2.94**	3268**	290**	11.05**
2	16.82**	3.08**	3417**	306**	9.09**
3	16.63**	2.88**	3198**	317**	9.01**
5	18.42**	2.82**	3132**	278**	11.98**
Control	9.32	2.04	2267	531	2.92
Mean	16.02	2.74	3045.9	347	8.72
LSD	1.06	0.33	362.00	38.7	0.97
Promax–LSD*	18.35	2.75	3055	267	8.12

FDM: fruit dry matter; PDM: plant dry matter; DMH: dry matter/ha; TC: total carotenes; ST: starch.
* Promax– LSD = Difference between the mean of the best performing population and the least significant difference determines the threshold above which the means of the populations presenting a performance similar to that of the best performing population can be found (control not considered).
** Highly significant difference ($P \leq 0.001$).

Conclusions

The correlation between fruit dry matter (DM) and starch content was high and significant, particularly in the case of population 1, indicating that not only does the potential exist for its use in agroindustrial processes because of its high dry matter content, especially in the production of animal feed concentrates, but also that the costs of selecting genotypes for dry matter (DM) content can be eventually reduced because of the guaranteed availability of materials with a high starch content.

An inverse relationship was observed between fruit DM and total carotenoids, but a direct relationship was observed between fruit DM and starch content, indicating that breeding for starch and carotene can be conducted separately by reciprocal recurrent selection or backcrossing.

Regarding the variable DM, populations 1, 2 and 3 were stable and predictable, complying with the parameters $\beta=1$ and d=0 of the Eberhart and Russell model (1966). Population 1, however, fulfilled all parameters efficiently and, based on its favorable response, can be a useful tool in future plant breeding work with butternut squash.

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