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Article

Evaluation of the popping capacity of amaranth genotypes and genetic parameters

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Abstract

Currently, there is worldwide interest in taking advantage of the agronomic and nutritional characteristics of superfoods such as amaranth, in Mexico it is consumed popped so it is important to elucidate the factors that influence this capacity for its better use. Therefore, the objective was to evaluate the popping capacity of 12 amaranth genotypes, genetic variability, heritability and the association of industrial quality variables with the popping volume. The experiment was conducted in the localities of Santa Lucía de Prías and Boyeros, State of Mexico and Cuapiaxtla, Tlaxcala in the years 2019 and 2020. The most outstanding genotype in popping volume was Tlahuicole, followed by AGIM, both had the largest increase (6 and 5.93), respectively. It was observed that the variation due to environmental effects was the main source in 4 of the 5 variables studied, such variation is not capitalizable in selection schemes; on the other hand, weight of one thousand grains was the variable that showed the highest variation due to genetic effects (65.96%), which is capitalizable in a selection scheme. The variables seed diameter, popping volume and volume increase presented a variation due to genetic effects between 4.46 and 6.5%, if there is intention to start a selection scheme, germplasm with greater variability in these traits must be included. The significant association between seed diameter and weight of non-popped seed indicates that these traits can be used as selection criteria for popping volume.

Keywords:

Amaranthus, correlations, genetic variability, heritability, popping.

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Introduction

As global food demand is constantly increasing, minor cereals and pseudocereal proteins are gaining increasing attention (Guerrieri and Cavaletto, 2018). Amaranth is a pseudocereal of multipurpose cultivation with good potential for exploitation of grain, vegetable and fodder, the crop has high levels of proteins (14-17%) and minerals, compared to other cereals such as sorghum, rice, wheat and corn (Mustafa *et al*., 2011).

Currently, 70 species of *Amaranthus* have been documented, among these are *Amaranthus hypochondriacus* L., *Amaranthus cruentus* L. and *Amaranthus caudatus* L., which are the main grain-producing species (Trucco and Tranel, 2011). The amaranth grain is widely used for food in the country, the best known is as popped grain for the preparation of traditional candies known as 'alegría', brittles, granola, cookies and flakes.

It is rich in essential amino acids such as lysine and methionine (Januszewska and Synowiecki, 2008), gluten-free (Aderibigbe *et al*., 2022), and contains squalene, a terpenoid that helps reduce cholesterol levels in the body (Shin *et al*., 2004). Popping is a process that is carried out under vapor pressure. The micropores present in the grain structure cause the vapor to be expelled at high pressure because they expand or inflate. Inflating the grain results in a porous structure that increases their flavor and aroma (Nath *et al*., 2007).

However, the quality of the popping is at risk when working during the process with inadequate temperatures and with immature seeds derived from indeterminate inflorescences and plants susceptible to lodging. Therefore, it is necessary to carry out research focused on studying the effect of genotype and environment on the popping of amaranth grain, in order to determine the best genotypes for these environments and the respective quality characteristics of the harvested grain and its processing.

Popping is influenced by the genotype, by the size of the seed and by the method of popping (Vázquez *et al*., 1988), within the genotype there is the variant of varieties with binding and non-binding starch, determined by type of perisperm, which is a function of the composition of the starch and influences its physicochemical properties and the popping capacity of the amaranth seed, opaque perisperm genotypes popped better than crystalline ones (Aguilar *et al*., 2022).

Conventional improvement methods for autogamous species, in a general way, lead to a progressive and intense reduction of opportunities for genetic recombination, resulting in a reduction of genetic variability and restriction of selection gains. In addition to this, an excessive narrowing of the genetic base of the populations used has been verified (Servellón, 1996). Heritability and genetic advancement are important selection parameters, which, when studied, allow the evaluation and identification of superior genotypes (Tasiguano *et al*., 2019).

Heritability values help in the selection of outstanding genotypes from various genetic populations. Genetic advancement measures the amount of progress that might be expected with the selection. In population improvement, only the additive genetic component is passed on to the next generation. The level of improvement also depends on the rigor of selection and the genetic advancement obtained from the population (Lovely and Vijayaraghava, 2017).

In contrast, the selection of suitable varieties is difficult due to their different levels of response to the environment (Ferreira *et al*., 2006). Nevertheless, it is necessary to identify which varieties have high grain yield, agronomic characteristics suitable for a certain environment and high expansion volume of popped grain (Ortiz *et al*., 2018). Therefore, the objective of the present research was to evaluate amaranth genotypes for their popping capacity, in addition to genetic variability, heritability and the association of quality variables that favor the popping volume.

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Materials and methods

Biological material

The research used the late genotypes Tlahuicole, L-4, Areli, Determinada and L-145, the intermediate genotypes Nutrisol and AGIM and the early genotypes Revancha, Chichiltic of *A. hypochondriacus*; the genotypes Benito and Amaranteca of the species *A. cruentus*, in addition to the early genotype Huitzilin, obtained from the *hypochondriacus*/ *hybridus*/ *hypochondriacus* cross.

Evaluation environments

The experiment was evaluated in the localities of Santa Lucía de Prías and Boyeros in the State of Mexico, as well as in Cuapiaxtla, Tlaxcala. In the first locality, it was established in two years (2019 and 2020) and two sowing dates, which gives a total of six environments (Table 1). The sowings were carried out with the recommendations of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in Spanish) for the crop. Once physiological maturity was reached, the plants were cut and tied in sheaves until they dried. They were then harvested with a Pulman thresher. The seed from each useful plot was kept in a paper bag until it was used in popping tests in 2021.

Table 1. Evaluation environments of 12 amaranth genotypes.

Environment	Sowing date	Altitude (m)	Type of soil	Precipitation (mm)
Santa Lucía 16 1F, St. Mex.	June 7	2 259	Sandy-loam	344.5
Santa Lucía 16 2F, St. Mex.	June 29	2 259	Sandy-loam	270.4
Cuapiaxtla, Tlax.	May 18	2 466	Sandy-loam	446.3
Santa Lucía 17 1F, St. Mex.	May 28	2 259	Sandy-loam	352.4
Santa Lucía 17 2F, St. Mex.	June 30	2 259	Sandy-loam	299.8
Boyeros, St. Mex.	June 29	2 250	Clayey-loam	350.2

Sample cleaning and preparing

The grain was cleaned using two sieves (No. 10 and 12) and then a blower, which, through air flow, eliminates the impurities of lower weight, in this way the amaranth seeds were almost clean, then they are passed through sieve No.16 to eliminate the remaining impurities. For grain preparing, 30 g of amaranth seed was taken from each experimental unit, the volume was measured and they were placed in a glass bottle with a lid. The moisture content was determined and enough water was added to bring the grain to 14% moisture. They were left to stand for 14 h, after this time they were left in the air for two hours to remove excess moisture and avoid crowds in the popping machine.

Popping process

To carry out this process, an electric mini-popping machine for amaranth grain of the brand Amaranta® M15 (San Miguel de Proyectos Agropecuarios, Huichapan, Hidalgo) was used, which was located in the oven room of the quality laboratories of the Valle de México Experimental Field. This equipment has as its principle of operation a fluidized bed system with a high temperature air flow. The amaranth grain was dosed directly into the hot air flow.

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Initially, the motor of the mini-popping machine is turned on in the control box, it was left on for 15-30 s to verify that there was no air leakage, then the potentiometer was turned to 7.5% air flow, then the temperature was programmed in the pyrometer to 240 ºC, and the resistor was turned on to reach the desired temperature. Next, the grains were added in the grain hopper and the dosing valve was opened to 65%, until all the seed finished passing through the fluidization cones. The popped seeds were collected in a container, left to cool in paper bags, to then take samples of the volume and weight of the popped grain, as well as the weight of the loss to record and obtain the yields of each experimental unit.

Variables studied

The variables studied were weight of one thousand grains, which was evaluated by counting five sets of 100 grains and they were weighed on an analytical balance and the weight was multiplied by 10. The seed diameter was determined through the measurement in mm of 10 seeds at random from each experimental unit with a digital vernier (Whitworth). The weight in g of non-popped seed of the grain that passed through sieve No. 16 at the exit of the popping machine was calculated. The popping volume of the popped grain that was retained in a No. 16 sieve was measured in a graduated burette (Kimax Kimble), and the volume increase was estimated, which is the number of times the popping volume increased compared to the initial volume of 30 g of non-popped seed.

Estimation of genetic parameters and data analysis

In the statistical analysis model, genotypes and environments were considered random effects. The analysis of variance was performed with the GLM procedure of SAS (SAS Institute, 2012) and the comparison of means by the Tukey method (*p*# 0.05). The variances for the estimation of genetic parameters were obtained by the Varcom procedure of SAS using the REML method (SAS, 2012). A correlation analysis of yield and its components was performed through the CORR procedure of SAS (SAS Institute, 2012).

The coefficient of genetic variation was calculated by means of the quotient of the genetic standard deviation between the mean. Coefficients greater than 20 were classified as having high genetic variability, from 12 to 20, intermediate and less than 10, low (Villaseñor *et al*., 2017). Amaranth is an autogamous plant, which is why the genetic variance of the material evaluated was between homozygous genotypes. Heritability was calculated in a narrow sense, which was obtained by dividing the genetic variance by the phenotypic variance.

Results and discussion

Table 2 shows the mean squares of the combined analysis of variance for the variables under study. Highly significant differences ($p\leq 0.1$) were detected for environments in all the variables analyzed. For genotypes, there were only highly significant differences ($p\leq 0.1$) in weight of one thousand grains, popping volume and volume increase. For the genotype-environment interaction, the variables weight of one thousand grains, seed diameter, popping volume and volume increase were highly significant ($p\leq 0.1$).

Table 2. Mean squares for 12 amaranth genotypes evaluated in six environments of the High Valleys of Central Mexico.

Source of variation	df	Weight of one thousand grains (g)	Seed diameter (mm)	Weight of non-popped seed (g)	Popping volume (cm^3)	Volume increase
Environment (E)	5	0.015**	0.123**	22.786**	10143.6**	11.935**
REP(A)	15	0.005	0.019	13.365	1292.7	1.177

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Source of variation	df	Weight of one thousand grains (g)	Seed diameter (mm)	Weight of non-popped seed (g)	Popping volume (cm^3)	Volume increase
Genotype (G)	11	0.108**	0.019 ns	3.594 ns	2999.3**	2.702**
E*G	46	0.004**	0.008**	3.495 ns	2016.4*	1.894**
Error	140	0.002	0.004	3.225	1290.7	1.022

*, ** = differences significant at *p*# 0.05 and *p*# 0.01, respectively; ns= non-significant differences.

Genotype, environment and their interaction play an important role in the final expression of grain yield and quality attributes (Kaya and Akcura, 2014). Such interaction indicated that genotypes performed differently in different environments and that genotypes should be selected to adapt to specific environments.

Table 3 shows the comparison of means between localities in each of the environments. When analyzing the influence of the environment of the different localities, it is observed that it influenced the weight of one thousand grains, seed diameter and popping volume. The environment that resulted with the greatest weight of one thousand grains was that of Cuapiaxtla (0.813 g) and those of the lowest weight were Santa Lucía 2017 in the two sowing dates (0.753 with and 0.746 without), the seeds with the largest diameter were found in the environments of Santa Lucía 16 2F and Santa Lucía 17.

Table 3. Comparison of means by environment for 12 amaranth genotypes evaluated in the High Valleys of central Mexico.

Environment	Weight of one thousand grains (g)	Seed diameter (mm)	Weight of non-popped seed (g)	Popping volume (cm^3)	Volume increase
Santa Lucía 16 1F	0.771 bc	1.213 cd	4.632 b	210.6 a	6.28 ab
Santa Lucía 16 2F	0.77 bc	1.321 a	6.194 a	169.9 c	5.01 cd
Cuapiaxtla, Tlax.	0.813 a	1.238 bc	6.513 a	168.4 c	4.9 d
Santa Lucía 17 with	0.753 c	1.168 d	4.622 b	214 a	6.45 a
Santa Lucía 17 without	0.746 c	1.357 a	6.225 a	171.3 c	4.99 cd
Boyeros, Mex	0.786 ab	1.27 b	4.757 b	186.9 bc	5.67 bc
hmd	0.03	0.049	1.32	26.8	0.75

Means with the same letter by column are statistically equal (Tukey, *p*= 0.05).

The environment that presented the highest popping volume was Santa Lucía 2017 with (214 cm^3). The least favorable locality for amaranth popping volume were Cuapiaxtla and Santa Lucía 2017 without, while Santa Lucía 16 1F was the most favorable environment for volume increase (6.45). According to Guy (2001), popped grains must generally contain more than 60%starch to ensure the desired expansion, so that any structural damage to the perisperm affects the expansion process and this can produce two effects: the non-popping of the grain or the reduction of the final volume of the expanded product (Tandjung *et al*., 2005), this information coincides with the results of this research.

Therefore, the relationship between the environment and the effect on the variables studied may be due to the shortening of the carbon assimilation cycle and the grain filling period (Awasthi *et al*., 2014), which produces smaller seeds. Table 4 shows the comparison of means by genotype. Tlahuicole, Revancha, Areli, Determinada and L145 presented the highest weight of one thousand grains (0.871 to 0.838 g), contrary to the Chichiltic genotype which was the one with the lowest weight (0.661 g), followed by AGIM (0.685 g).

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Table 4. Comparison of means by genotype for 12 amaranth genotypes.

Genotype/variable	Weight of one thousand grains (g)	Seed diameter (mm)	Weight of non-popped seed (g)	Popping volume (cm^3)	Volume increase
Tlahuicole	0.838 a	1.293 ab	5.214 a	203.3 a	6.007 a
AGIM	0.685 de	1.246 bc	5.456 a	202.8 a	5.933 a
L-4	0.716 cd	1.279 abc	5.563 a	198 a	5.958 a
Revancha	0.868 a	1.312 ab	4.809 a	194.1 a	5.831 a
Areli	0.879 a	1.347 a	4.124 a	193.4 a	5.841 a
Nutrisol	0.714 cd	1.241 bc	5.618 a	190.2 a	5.651 ab
Huitzilin	0.738 bc	1.287 abc	5.802 a	181.5 a	5.437 ab
Determinada	0.879 a	1.291 abc	5.713 a	181 a	5.365 ab
Chichiltic	0.661 e	1.21 c	6.247 a	177.9 a	5.265 ab
Benito	0.781 b	1.234 bc	5.948 a	173.4 a	5.218 ab
Amaranteca	0.731 bcd	1.255 bc	5.898 a	164.2 a	4.969 ab
L145	0.871 a	1.295 ab	5.62 a	160.6 b	4.507 b
hmd	0.051	0.084	2.26	41	1.288

Means in rows with the same letters are statistically equal, hmd honest minimum significant difference.

The genotype with the largest seed diameter was Areli (1.347 mm) and those with the smallest diameter were Chichiltic, Benito and Amaranteca. In the variable of popping volume, all varieties showed a good yield, except for the genotype L145, although all are within the acceptable range. In volume increase, the most outstanding genotype was Tlahuicole, followed by AGIM, both had the largest increase in popping volume (6 and 5.93), respectively.

The analysis of different genotypes in different environments allows determining the agronomic potential for each of them in order to obtain the best yields and industrialization characteristics. In this sense, according to Bishaw *et al*. (2007), seeds obtained from different geographical areas are modified in their viability by the conditions present at the time of their formation, development and maturation.

Deficiency in water (Dornbos *et al*., 1989; Ghassemi *et al*., 1997), minerals and extreme temperatures (Grass and Burris, 1995) are the most common and have the greatest effect on seed quality. In contrast, genotype is not associated with seed diameter and weight of non-popped seed. Only this last variable does not show significance in the genotype-environment interaction. Therefore, it is important to determine and quantify how environmental factors and the genotype-environment interaction influence the variation in each grain quality parameter (Kaya and Akcura, 2014).

Figure 1 shows the origin of the variation of the characteristics studied. It can be observed that variation due to environmental effects was the main source of 4 of the 5 variables studied; this coincides with studies reported in other crops (Papastylianou *et al*., 2021). Weight of one thousand grains was the one that showed the highest variation due to genetic effects (65.96%), which means that this genetic variability could be capitalized in a selection scheme.

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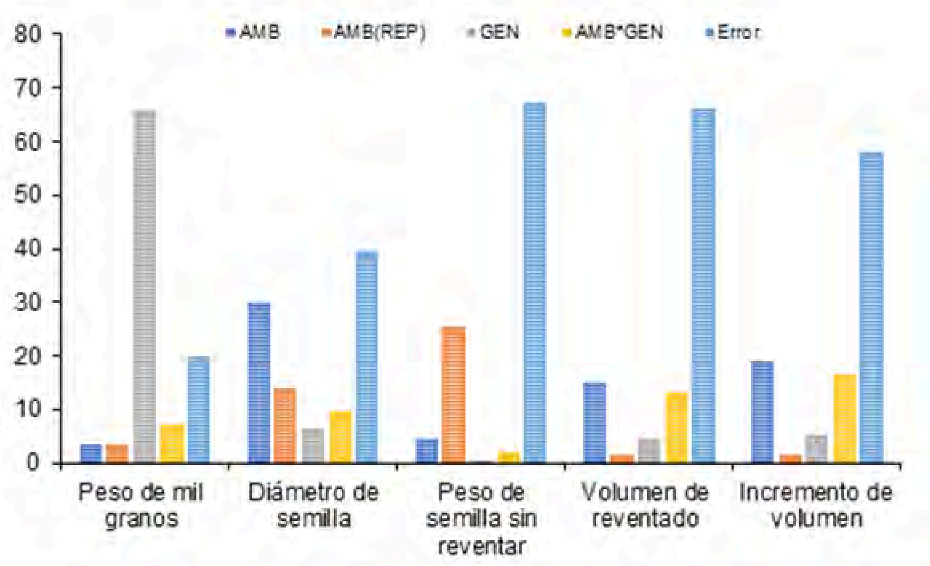
Figure 1. Proportion of variability by source of variation in 12 amaranth genotypes evaluated in six rainfed environments in the Central Plateau. AMB= environment; AMB(REP)= repetitions within the environment; GEN= genotypes; AMB*GEN= environment by genotype interaction.



This variable is important because it is related to heavier grains, that is, grains with greater storage of starch, which represents about 63% of the seed content, and it is associated with grain yield, this trait is closely linked to solar radiation and accumulation of dry matter in the plant, that is, there is also an environmental effect. On the other hand, some studies show that the starch content is connected to the popping volume, so the physicochemical characteristics have an important function in the quality of the popping, therefore, this also causes some grains not to pop (Núñez Limón, 2018).

For the variables seed diameter, popping volume and volume increase, they presented a variation due to genetic effects, between 4.46 and 6.5%, while the variable of weight of non-popped grain showed only a variation consequence of the genetic effects of 0.55%. Therefore, the variation of these four variables was predominantly due to environmental effects; where the largest was due to environments in seed diameter (29.87%) and volume increase (18.99%).

The magnitude of the variation due to the experimental error is remarkable, this could occur due to the low genetic variability in the traits studied. This can be explained by the high percentage of self-pollination that occurs in the plant (Agong and Ayiecho, 1991) and because gene flow between species is very limited (Ruiz *et al*., 2018).

In relation to the effects due to the genotype-environment interaction, this was higher in volume increase (16.4%) and popping volume (13%), for seed diameter it was medium (9.8%), while for weight of one thousand grains (7.3%) and weight of non-popped seed (1.9%) it was low. The variation due to interaction was less than that of the two main sources and less than 20%. The similarity in the environments, characterized by similar precipitation, and the inclusion of only two localities of the High Valleys of Mexico with little longitudinal variation, altitude and latitude, could be the cause to understand the low variation of the genotype-environment interaction.

Table 5 shows the results obtained from the coefficient of genetic variation and heritability for the variables evaluated. The variables seed diameter (0.065), weight of non-popped seed (0.006), popping volume (0.045) and volume increase (0.053) presented low heritability values, which indicates that there is an influence of the environment as they present values close to zero. Joshi

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and Rana (1992) found high values (0.60 to 0.76) for different genotypes of *A. hypochondriacus*, for variables such as plant height and inflorescence length and intermediate values (0.26 to 0.47) for yield (Pandey, 1982).

Table 5. Means and range for variables by genotype of 12 amaranth varieties grown in the High Valleys of Mexico.

Variable	Mean	Minimum	Maximum	Coefficient of genetic variation	Heritability
Weight of one thousand grains (g)	0.775	0.502	0.98	9.9	0.66
Seed diameter (mm)	1.267	1.058	1.471	2.1	0.065
Weight of non-popped seed (g)	5.586	2.01	13.84	2.9	0.006
Popping volume (cm^3)	184.912	68	298	5	0.045
Volume increase	5.48	1.5	8.765	5.5	0.053

Plant breeders use heritability estimates to determine the influence of environmental and genetic factors on the trait of interest and choose the selection procedure that should be implemented to make improvements to crops (Kaya and Akcura, 2014).

Knowing the correlations between the variables and their heritability allows improving the efficiency of selection programs. Correlations often depend on heritabilities, so that, if both correlated traits have low heritabilities, the phenotypic correlation will be determined mainly by the environmental correlation; but if they have high heritabilities, then the genetic correlation is the most important (Falconer, 1984).

Therefore, for the variables evaluated, only the weight of one thousand grains is determined by the genotype (Table 5) and it can be used to carry out genetic improvement and increase the efficiency of selection programs. Table 6 shows low negative correlations between the different variables, except for seed diameter and weight of one thousand grains, which indicates that the diameter influences the weight of one thousand grains and the volume of popping favors the increase in volume.

Table 6. Pearson correlations for 12 amaranth genotypes evaluated in the High Valleys of Central Mexico.

Variables	Seed diameter	Weight of non-popped seed	Popping volume (cm^3)	Volume increase
Weight of one thousand grains (g)	0.214**	-0.05	-0.13	-0.14
Seed diameter		-0.16**	-0.22**	-0.22**
Weight of non-popped seed			-0.54**	-0.55**
Popping volume (cm^3)				0.98**

According to Mishra *et al*. (2014), the variety is a factor that significantly affects the volume of popped seeds, this agrees with the results obtained in this study, which was also corroborated by Ortiz *et al*. (2018); likewise, the type of starch of the genotypes influences the popping capacity (Aguilar *et al*., 2022).

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This may be related to the moisture content of the seed at the time of popping (Haught *et al*., 1976) or the method of popping (Vázquez *et al*., 1988; Dofing *et al*., 1990). It is clear that the genotype has a slight effect on the popping capacity, which is not comparable with the effect of the environment; this indicates that the popping capacity in amaranth is not an easy trait to improve.

Conclusions

The genotype component was the main cause of variation for weight of one thousand grains, in addition to presenting the highest coefficient of genetic variability and the highest heritability. Weight of one thousand grains was the variable that showed the highest variation due to genetic effects, so it is a characteristic that can be used in plant breeding.

The environment factor was the most important component for seed diameter, weight of non-popped seed, popping volume and volume increase, so they are traits difficult to improve, at least for this group of genotypes. The positive correlation between seed diameter and weight of non-popped seed with popping volume and volume increase indicate that these characteristics can be used as selection criteria to improve popping volume.

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