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Anthocyanins content in the kernel and corncob of Mexican purple corn populations

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Abstract

Purple corn has acquired great interest by its high content of anthocyanins and bioactive properties. Among this type of corn the Andean purple corn has been the most studied, however, in Mexico, we have the "maíces morados", which is recognized by its dark purple color. Since there is no record about its content of anthocyanins, in this study we quantified the total anthocyanins (TA) accumulated in the pericarp, aleurone layer, kernel, and corncob of 52 corn populations with different grades of pigmentation. Results showed that TA was superior in purple corn than in blue and red corn. TA ranged from 0.0044 to 0.0523 g of TA · 100 g^{-1} of biomass in the aleurone layer; in the pericarp from 0.2529 to 2.6452 g of TA · 100 g^{-1} of pericarp; in the kernel from 0.0398 to 0.2398 g of TA · 100 g^{-1} of kernel and in the corncob from 0.1004 to 1.1022 g of TA · 100 g^{-1} of corncob. Although a dark color of the kernel and corncob indicated a high concentration of anthocyanins, we observed that the distribution, hue, and color intensity in the aleurone layer, pericarp, and corncob, influenced the concentration of anthocyanins in each structure.

Introduction

Purple corn is a type of corn that is pigmented by anthocyanins. This variant along with the blue corn have earned great interest in science, particularly due to their anthocyanin content and bioactive properties (Lao *et al.*, 2017). This type of corn has the darkest purple tones within the plant kingdom (Lao and Giusti, 2015) and it has been pointed out that the productive potential of anthocyanins is greater than other corns pigmented by this flavonoid. Purple corn has a higher concentration of this compound and it is synthesized and stored in significant quantities in the aleurone layer, in the pericarp and other organs of the plant such as the corncob and corn husks.

Several studies indicate that purple corn is native to Peru and that its varieties come from the "Kculli" race (Guillén-Sánchez *et al.*, 2014). However, since Mexico is the center of origin, domestication, and diversification of corn, it has its variants derived from Mexican corn races. Quite recently, Mexican purple corn is being studied,so there is still not enough information about it in the scientific community. Although, the Andean and other pigmented corns, together with the purple corn have been important since pre-Hispanic times; we have knowledge about several culinary uses and also about its power to color traditional foods and beverages ("chicha morada", "atole agrio", tortillas, snacks, etc.). It has been cherished as a sacred and ornamental kernel and despite the displacement and genetic erosion suffered by the pigmented corns in Mexico, a wide diversity with vast potential is still conserved *in situ*.

Countries such as Peru, Mexico, and Bolivia already have an appreciable background in the study and use of native pigmented crops (like corn and purple potatoes) and countries like China, Turkey, and Italy, where pigmented corn was introduced, they are also under examination (Quiñones and Coy-Barrera, 2015). Given nowadays scientific and social importance of antioxidant-rich foods, a wide range of researchers are motivated to know about and to take advantage of the potential of this source of anthocyanins.

Although studies in this kernel (especially in the Andean corn) have been conducted on the concentration and type of pigment, there are few investigations car-

ried out in purple corn and particularly, in corn from San Juan Ixtenco, Tlaxcala, Mexico, in which a wide exploitation potential has been identified (Mendoza-Mendoza *et al.*, 2017), but precedents are lacking on the productive potential of the anthocyanins of this variant.For this reason,in this research, we explored 52 populations (most of them from Mexico) with different levels of pigmentation of the kernel and corncob to assess the productive potential of total anthocyanins of the corncob, kernel and two particular structures of the kernel (the pericarp and the aleurone layer).

Material and methods

Germplasm

We studied 52 corn populations to assess the anthocyanin production potential in some Mexican purple corns (most of them from the Conical race). The kernel and the corncob showed different levels of pigmentation, reason why we classified the populations in **Group I**: 35 populations with purple kernel and purple corncob (Pop 1 to Pop 35); **Group II**: four populations with blue kernel (Pop 36 to Pop 39) and one with pink kernel (Pop 40), none of pigmented corncob; **Group III**: six populations from the Cacahuacintle race with white kernel and purple corncob (Pop 41 to Pop 46); **Group IV**: three populations with red kernel and pigmented corncob (Pop 47 to Pop 49), and **Group V**: three populations with non-pigmented corncob but with a different color of the kernel: yellow (Pop 50), creamy white kernel (trigueño) (Pop 51), and white kernel (Pop 52).

Populations were obtained from corn producers of Ixtenco and the genetic improvement program of pigmented corn from the Colegio de Postgraduados. We sowed the seeds at San Juan Ixtenco, Tlaxcala, Mexico, under a population density of 50,000 plants ha^{-1} during the rainfall season with no fertilization. We carried out controlled pollinations for each population to guarantee the expression of their particular inherent traits in the kernel and the corncob. A representative sample for each population consisted of three healthy ears.

Sample preparation, extraction, and quantification of total anthocyanin content

To determine the total anthocyanin content stored in the pericarp (TAper) and the aleurone layer (TAal), 25 kernels were taken from the central part of the ear and they were immersed in distilled water for 3.5 h to separate the pericarp from the rest of the kernel (a single piece formed by the aleurone layer, endosperm, and embryo). These structures were dehydrated in a drying oven at 40 °C for 48 h. and then each sample was weighed (Wper = weight of pericarp and Wal = weight of aleurone + endosperm + embryo; both in g). To obtain the total kernel biomass (TWkrnl) we added both the Wper and the Wal. We determined the percentage of the pericarp (% per) using the formula:

$$\%\ per = \frac{(Wper * 100)}{TWkrnl}$$

and to obtain the percentage of the rest of the kernel (% al) we used:

$$\%\ al = \frac{(Wal * 100)}{TWkrnl}$$

We pulverized each sample of the structures to a particle size of 0.5 mm. Since the aleurone is a thin layer of tissue and it is very difficult to separate it from the rest of the kernel, it was grounded together with the endosperm and the embryo.To determine the content of total anthocyanins in the corncob (TAcob),we milled a fraction of the central part of the corncob from each ear to a particle size of 0.5 mm.

The extraction and quantification of anthocyanins were performed in the laboratories of the Colegio de Postgraduados, Campus Puebla. The extraction was done by ultrasound, adding 25 mg of the sample (Per = pericarp, Al = aleurone, and Cob = corncob) to a 2 mL Eppendorf tube, each sample was studied by triplicate. We added 96% ethanol and 1.5 N hydrochloric acid (85:15 v/v) to each tube using solid-liquid ratios of 1:25 in the Al samples and 1:80 in the Per and Cob samples. The tubes were immediately placed in an ultrasound bath for 15 minutes, centrifuged in a micro-centrifuge at 11,510 *g* for 5 min and the supernatant was collected. A second extraction was carried out using the same procedure and both extracts were concentrated.

The quantification of anthocyanins was performed at 535 nm, using a Thermo Scientific® Varioskan Flash microplate spectrophotometer and three aliquots of 200 µL were taken from each extract. Total anthocyanins (TA) were calculated using the following formula:

$$TA = \frac{(A \cdot Vext \cdot MW \cdot DF)}{(\varepsilon \cdot Swt)}$$

where TA = Total anthocyanins, expressed in mg · g^{-1} of kernel for total anthocyanin content in the kernel (TAkrnl); in mg · g^{-1} of pericarp for TAper; in mg · g^{-1} of biomass (is the grain without the pericarp) for TAal, and in mg · g^{-1} of corncob for TAcob; A = absorbance, Vext = volume of extraction in mL, MW = 449.2 g mol^{-1}, DF = dilution factor, Ɛ = 26900 L cm^{-1} mg^{-1} and Swt = sample weight in g. MW and Ɛ corresponded to cyanidin 3-glycoside.

The results of TAper, TAal, and TAcob were transformed

into g of TA · 100 g^{-1}. The total anthocyanin content in the kernel (TAkrnl) was obtained with the formula:

$$TAkrnl=\left(\frac{\%\ per * TAper}{100}\right)+\left(\frac{\%\ al * TAal}{100}\right)$$

Statistical analysis

We performed ANOVA under a randomized complete block experimental design, as well as the corresponding comparison of means with the Tukey test ($P \leq 0.05$) between groups and populations within each group. The statistical analysis of the data was computed with the SAS 9.0 Statistics Software (SAS, 2009).

Results and discussion

ANOVA

The analysis of variance showed that for every trait there was a statistical difference of $P \leq 0.01$ between groups and populations within each group (Table 1).Differences between groups were mainly attributed to the contrast in the color intensity of the kernel and the corncob (Figure 1 to Figure 4), while differences detected within populations of each group were associated with the genetic diversity existing in each group. We consider that the factors that influenced the concentration of anthocyanins in these structures were: the distribution of the pigment in the tissue or in the organ, the dark shades of color, and the color intensity.

Total anthocyanin content in the aleurone layer (TAal)

The content of total anthocyanins in the aleurone layer (TAal) was different in the five groups.The Group I had the highest average of TAal (populations with a purple kernel, 0.0364 g of TA · 100 g^{-1} of biomass), and the Group II (populations with a blue and pink kernel) was the second-highest average of TAal (0.0306 g of TA · 100 g of biomass). We observed that Groups I and II were outstanding by their ability to accumulate anthocyanins in this tissue, while Groups III, IV, and V had a very low TAal average (Table 2), they had a low quantity of pigment (Figure 1, i and j), a different type of pigment (carotenoids) (Figure 1l) or they lacked it (Figure 1, k, m, and n).

Although all the populations from Group I synthesized and accumulated anthocyanins in the aleurone layer (Alyr), we identified that they had different concentrations (Table 2). The top ten populations by TAal, ordered from the highest to the lowest concentration were: Pop 31, 25, 1, 23, 32, 35, 28, 14, 33, and 29, with values from 0.0402 to 0.0523 g of TA · 100 g^{-1} of biomass. Pop 31 differed statistically from the rest of the populations of this group (Table 2, Figure 1a).

We observed that most of the 35 purple corn populations produced more anthocyanins or a similar ratio of TAal than the blue kernel populations from Group II, which had from 0.0237 to 0.0491 g of TA · 100 g^{-1} of biomass (data not shown). Since 0.03 g of TA · 100 g^{-1} of biomass is the most reported total anthocyanin content (TAC) in blue kernel genotypes (Abdel-Aal *et al.*, 2006; del Pozo-Insfran *et al.*, 2006; Espinosa *et al.*, 2009), when compared to the TAal average of Group I, it is clear that they are similar, and that only in three populations (Pop 13, Pop 27 and Pop 19) the capacity to accumulate anthocyanins in the Alyr was less than blue corn capacity. They had a higher TAal than the pigmented accessions 707G and 707B that Paulsmeyer *et al.* (2017) reported as outstanding (0.0133 g and 0.0128 of TA · 100 g^{-1} of biomass, respectively).Furthermore, Pop 13 and Pop 27 accumulated more anthocyanins than these accessions, reason why it should be emphasized that even the populations of purple corn with a low TAal possess a promising potential to produce this pigment.

Although the color of the anthocyanin-pigmented kernels was intense and uniform, we perceived that when the pericarp was removed, the coloration of the Alyr was variable in hue and / or intensity (Figure 1, a - j).

Table 1 -Mean squares of ANOVA for anthocyanin content in the aleurone layer, pericarp, kernel, and corncob of 52 corn populations with different pigmentation levels obtained at San Juan Ixtenco, Tlaxcala, Mexico.

SV	Df	TAal	TAper	TAkrnl	TAcob
Replications	24•	16.84 x 10^{-6}ns	63.83 x 10^{-3}ns	38.78 x 10^{-5}ns	31.19 x 10^{-3}ns
Group	4	36.98 x 10^{-3}**	641263769**	32.64 x 10^{-2}**	650748494**
Pop(Group)	47	14.48 x 10^{-4}**	44330511**	22.02 x 10^{-3}**	136005289**
Error		33.81 x 10^{-6}	18.66 x 10^{-2}	77.39 x 10^{-5}	75.13 x 10^{-3}

SV = source of variation; df = degrees of freedom; TAal = total anthocyanin content in the aleurone layer; TAper = total anthocyanin content in the pericarp; TAkrnl = total anthocyanin content in the kernel; TAcob = total anthocyanin content in the corncob; ** = $P \leq 0.01$; • = df of replications for TAper and TAcob = 25.

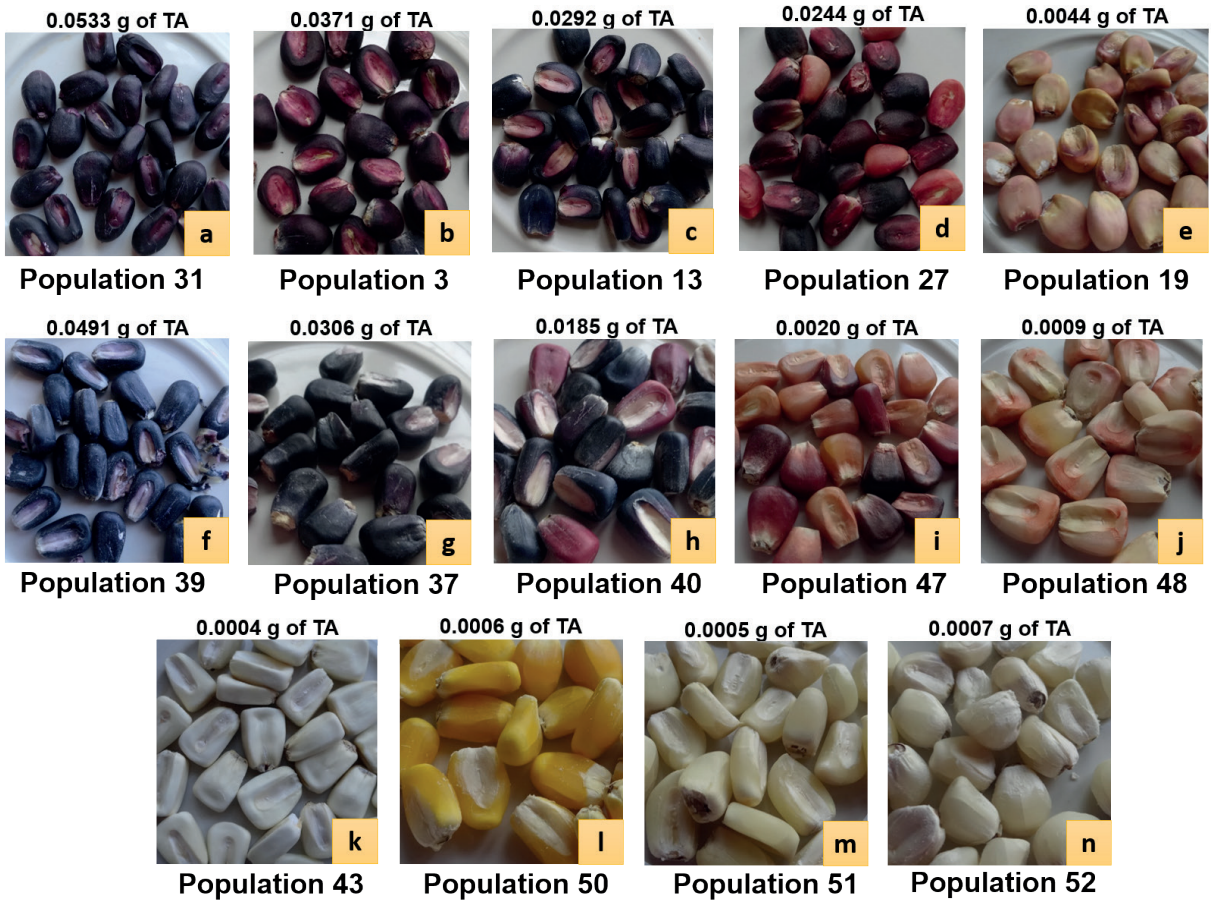

Fig. 1 - Expression of the pigment in the aleurone layer of corn kernels. Group I = populations with a purple kernel (a - e); Group II = populations with a blue kernel (f - g) and a pink kernel (h); Group III = white kernel populations from the Cacahuacintle race (k); Group IV = red kernel populations (i - j); Group V = populations without anthocyanin pigment in the kernel (l - n). TA = Total anthocyanin content, g of TA · 100 g^{-1} of biomass.

This might explain why we observed a variation of the TAal between groups and within the populations of each group (Table 1and Table 2).

In general, the color of the Alyr from Group I (Figure 1, a - e) was darker and more intense than the color of the Alyr from Group II (Figure 1, f - h) and Group IV (Figure 1, i - j). The Alyr of the Pop 31 (highest TAal) had the darkest, most intense and uniform color throughout the aleurone layer surface (Figure 1a) and although the Pop 39 (Figure 1f, blue kernel) did not have a color of Alyr as intense as the Pop 31, it had a similar TAal (it was the second-highest TAal from the 52 populations), so we considered that the Pop 39 was of interest within Group II. Figures 1b and 1g illustrate the aspect of the Alyr from populations with a similar TAal to the TAal average from Group I and Group II, respectively. Figures 1c, 1d, and 1e correspond to the Alyr of the purple corn populations with the lower TAal.In these figures,we observe that the low TAC could be associated to a lower intensity of the color of the Alyr, to the variation in the Alyr pattern of pigmentation detected in each kernel, or to the reduced synthesis and accumulation of anthocyanins in the Alyr (low pigmentation), respectively.

Through the examination of the color of the Alyr and the quantification of the TAal, we corroborated that kernels with greater darkness and color intensity have a greater TAC, as can be verified by contrasting the Alyr of the Pop 31 and Pop 3 (Figure 1a and Figure 1b, respectively) from purple kernel populations; the Pop 39 (Figure 1f) with Pop 37 (Figure 1g) and Pop 40 (Figure 1h) from Group II;the Pop 47 and Pop 48 with a red kernel (Figure 1i and 1j, respectively). In the populations from Group III and Group V (Figure 1, k - n), no pigmentation evidenced the presence of anthocyanins; however, an almost imperceptible TAC was recorded (Table 2).

Total anthocyanin content in the pericarp (TAper)

We observed that between groups, the Group I had

Table 2 -Content of total anthocyanins in the aleurone layer, pericarp, kernel and corncob of 10 outstanding populations from Group I, and averages of each group of corn populations produced at San Juan Ixtenco, Tlaxcala, Mexico.

OutPop GI	TAal	TAper	TAkrnl	TAcob	Group	TAal	TAper	TAkrnl	TAcob
1	0.0474 $^{(3)}_{bc}$	1.0255 $^{(21)}_{f}$	0.1102 $^{(15)}_{e}$	0.2207 $^{(26)}_{fg}$	I	0.0364_a	1.2280_a	0.1051_a	0.4306_a
5	0.0347 $^{(19)}_{e}$	2.3489 $^{(2)}_{b}$	0.1566 $^{(4)}_{c}$	0.1763 $^{(29)}_{g}$	II	0.0306_b	0.0313_b	0.0307_b	0.0070_b
7	0.0344 $^{(20)}_{ef}$	1.6661 $^{(8)}_{d}$	0.1263 $^{(11)}_{d}$	0.6783 $^{(5)}_{d}$	III	0.0010_d	0.0041_b	0.0011_c	0.5248_a
12	0.0311 $^{(28)}_{fg}$	2.3330 $^{(3)}_{b}$	0.1769 $^{(2)}_{b}$	0.9984 $^{(3)}_{b}$	IV	0.0137_c	0.0161_b	0.0144_c	0.0099_b
14	0.0420 $^{(8)}_{d}$	1.6292 $^{(10)}_{d}$	0.1465 $^{(6)}_{c}$	0.4413 $^{(15)}_{e}$	V	0.0007_d	0.0007_b	0.0006_c	0.0054_b
25	0.0495 $^{(2)}_{ab}$	2.6452 $^{(1)}_{a}$	0.2398 $^{(1)}_{a}$	1.0339 $^{(2)}_{ab}$	MSD	0.0026	0.2032	0.0143	0.1240
31	0.0523 $^{(1)}_{a}$	1.9592 $^{(5)}_{c}$	0.1725 $^{(3)}_{b}$	0.9019 $^{(4)}_{c}$					
33	0.0410 $^{(9)}_{d}$	1.9769 $^{(4)}_{c}$	0.1562 $^{(5)}_{c}$	0.4886 $^{(13)}_{e}$					
34	0.0307 $^{(30)}_{g}$	0.9182 $^{(23)}_{f}$	0.0780 $^{(25)}_{f}$	1.1022 $^{(1)}_{a}$					
35	0.0445 $^{(6)}_{cd}$	1.4480 $^{(12)}_{e}$	0.1306 $^{(9)}_{d}$	0.2561 $^{(25)}_{f}$					
Min	0.0044	0.2529	0.0398	0.1004					
Max	0.0523	2.6452	0.2398	1.1022					
MSD	0.0035	0.1776	0.0118	0.0729					

OutPop GI = Outstanding populations from Group I; $^{()}$ = Ranking of the population among 35 purple corn populations, the position was assigned based on its total anthocyanin content; Values followed by different lowercase letters within a column are significantly different ($P \leq 0.05$); Min = Lowest value of total anthocyanin content in Group I; Max = Highest value of total anthocyanin content in Group I; TAal = Total anthocyanin content in the aleurone layer, g of TA · 100 g^{-1} of biomass; TAper = Total anthocyanin content in the pericarp, g of TA · 100 g^{-1} of pericarp; TAkrnl = Total anthocyanin content in the kernel, g of TA · 100 g^{-1} of kernel; TAcob = Total anthocyanin content in the corncob, g of TA · 100 g^{-1} of corncob. MSD = Minimum Significant Difference ($\alpha = 0.05$).

the highest total anthocyanin content in the pericarp (TAper) (purple kernel, 1.228 g of TA · 100 g^{-1} of pericarp); it was statistically different from the remaining groups that barely accumulated anthocyanins in this structure (Table 2, Figure 2).

Among the populations of Group I, we noticed that the potential to produce anthocyanins in the pericarp (Per) was contrasting (Table 2). The ten populations with the higher TAper (mentioned from the highest to the lowest concentration) were: Pop 25, 5, 12, 33, 31, 26, 2, 7, 3, and 14; which had from 1.6292 to 2.6452 g of TA · 100 g^{-1} of pericarp. We detected that from these, the Pop 25, Pop 5 and Pop 12 stood out by producing more than 2 g of TA · 100 g^{-1} of pericarp and that the Pop 25 was statistically different from the rest of the populations from the group (Table 2).

In comparison to the scarce available information on TAper, we found that only Mendoza-Mendoza *et al*. (2017) reported higher concentrations than the TAper of the Pop 25. They quantified more than 3 g of TA · 100 g^{-1} of pericarp in S_2 inbred lines derived from Mexican purple corn. We identified that the ten purple corn populations with the higher TAper accumulated more anthocyanins than the Peruvian and Arrocillo corn races studied by Salinas *et al*. (2005) (1.524 and 1.473 g of TA · 100 g^{-1} of pericarp, respectively). Likewise, we observed that 20 populations of Group I exceeded the TAper from the Peruvian purple corn studied by Monroy *et al*. (2016) (TAper = 1.06 g of TA · 100 g^{-1} pericarp).

Moreover, we discovered that the color of the Per differed between populations (Figure 2). The Pop 25 (Figure 2a) had the darkest and the most intense color among the 52 populations, as well as the maximum TAper. A similar coloration was observed in the Per of the Pop 14 (Figure 2b); however, the difference in TAper between them was of 38.4 % (Table 2, $P \leq 0.05$). In Group I (Figure 2, a - e), the coloration of the Per was variable in intensity and distribution, this affected the magnitude of the TAper. Figure 2c shows the Per of the Pop 32 which had a similar TAper to the TAper average from Group I, while Figure 2d corresponds to the Pop 19, which contains Peruvian purple corn germplasm and that only stored 0.6058 g of TA · 100 g^{-1} of pericarp. It is known that in the Peruvian corn the TAper could be higher (Salinas *et al*., 2005; Monroy *et al*., 2016).

The TAper was significantly lower in the populations that did not belong to Group I (Table 2, Figure 2). The Pop 40 (Figure 2f, pink kernel) just accumulated 0.0251

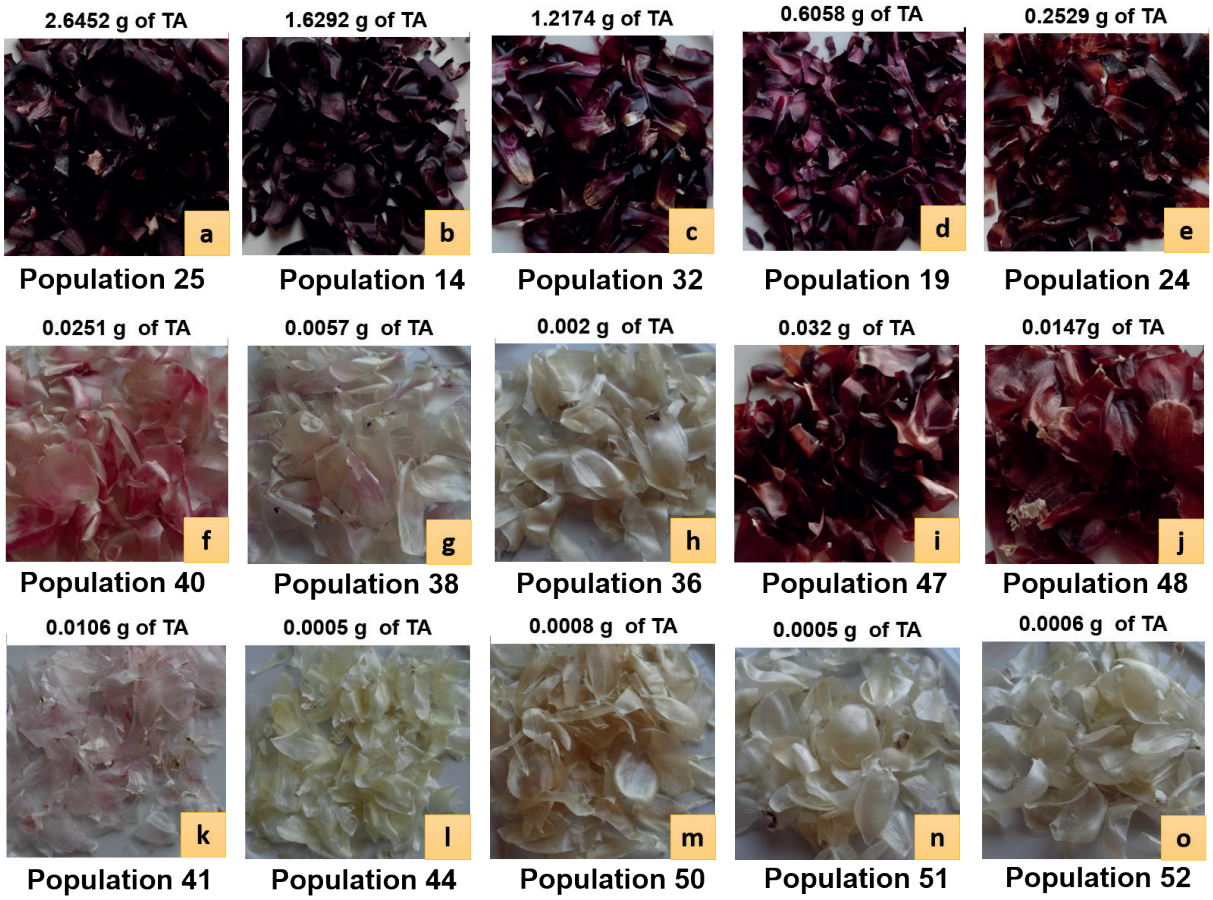

Fig.2 - Expression of the pigment in the pericarp of corn kernels. Group I = populations with a purple kernel (a - e); Group II = populations with a pink kernel (f) and a blue kernel (g and h); Group III = white kernel populations from the Cacahuacintle race (k and l); Group IV = red kernel populations (i and j); Group V = populations without anthocyanin pigment in the kernel (l - o); TA = Total anthocyanin content, g of TA · 100 g^{-1} of pericarp.

g of TA · 100 g^{-1} of pericarp, and since blue corn does not accumulate anthocyanins in the Per (Salinas *et al.*, 2013) and we quantified anthocyanins in two blue kernel populations (Figure 2 g – h), we suggest that this was related to the presence of small portions of the Alyr that remained attached to the Per (Pop 36) and in other cases, it was associated to the color segregation of the Per, since these native populations are daily planted around purple corn, which favors cross-pollination, as happened in the Pop 38. The same performance was observed in the Pop 41 with a white kernel (Figure 2k; Group III), which Per was slightly pigmented (it accumulated 0.0106 g of TA · 100 g^{-1} of pericarp). In Figure 2l we can appreciate the typical color of the Per from a white corn population from the Cacahuacintle race, which appearance and low TAper were similar to the populations from Group V (Figure 2, m - o; Table 2).

The comparison of the Per and the TAper average between Group I (purple kernel) and Group IV (red kernel),evidenced that the color of the Per from the second group was reddish and of a duller hue (Figure 2, i - j), as well we observed that the TAper was 76.3 times smaller (Table 2).However, in contrast with the results from other researchers, the red kernel populations had a similar TAper to the content of the Apache Red 9 corn inbred line (0.021 g of TA · 100 g^{-1} of pericarp) studied by Chatham *et al.* (2018), who also reported a TAper of 0.2702 g of TA · 100 g^{-1} of pericarp for the Apache Red 2 inbred line, that in their study was the inbred line of best performance. It surpassed the TAper of the Pop 9 and Pop 24 with a purple kernel (0.2642 and 0.2529 g of TA · 100 g^{-1} of pericarp, respectively).The previous data evidence that purple corn (including populations of low TAal) had a greater potential to accumulate anthocyanins than the red corn. Also, it suggests that the populations from Group IV might increase their potential to synthesize and accumulate anthocyanins in the Per.

Total anthocyanin content in the kernel (TAkrnl)

Although there is a high positive correlation between the color of the kernel and the anthocyanin content (to

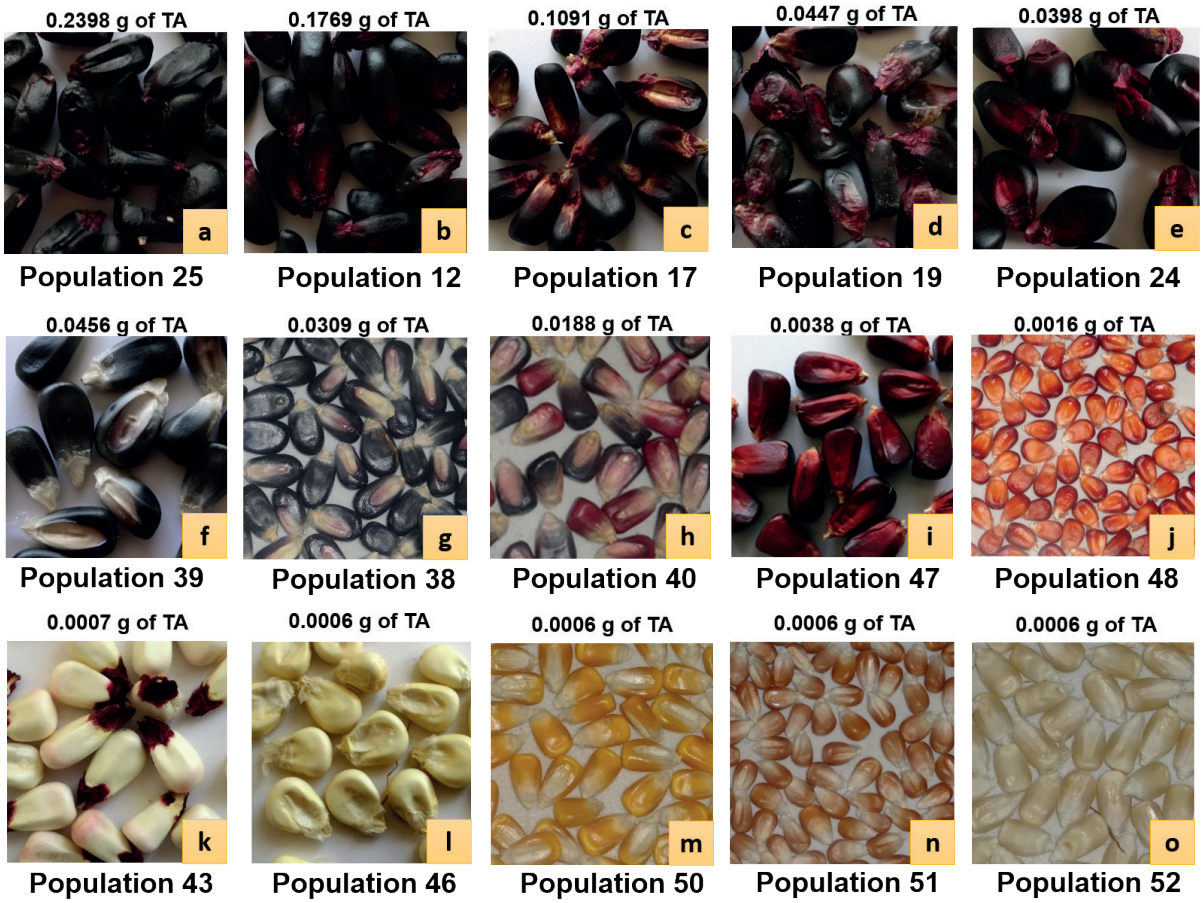

Fig.3 - Expression of the pigment in corn kernels. Group I = populations with a purple kernel (a - e); Group II = populations with a blue kernel (f and g) and a pink kernel (h); Group III = white kernel populations from the Cacahuacintle race (k and l); Group IV = red kernel populations (i and j); Group V = populations without anthocyanin pigment in the kernel (m - o). TA = Total anthocyanin content, g · 100 g^{-1} of kernel.

a greater darkness and color intensity, the TAC will be greater), we observed that the ability of purple corn to synthesize anthocyanins both in the Per and the Alyr, gives them a greater potential to accumulate anthocyanins in the kernel (Group I = 0.1051 g of TA · 100 g^{-1} of kernel). Even the Group II (blue and pink kernel; Figure 3, f - h) had 3.4 times less total anthocyanin content in the kernel (TAkrnl) than Group I, we found that it was superior and statistically different from Groups III, IV, and V (Table 2).

The TAkrnl showed that some purple corn populations that stood out by their high content of TAper and / or TAal were not among the ten most productive populations by TAkrnl, as happened with Pop 1, 2, 7, 23, 28, 29 and 32 (data not shown). The ten populations of the Group I with the higher TAkrnl, ordered from the highest to the lowest concentration were: Pop 25, 12, 31, 5, 33, 14, 26, 30, 35, and 3, with 0.1280 to 0.2398 g of TA · 100 g^{-1} of kernel. It can be observed that Group I was outstanding by TAkrnl, including the population withthe lowest TAkrnl (Pop 24 = 0.0398 g of AT · 100 g^{-1} of kernel) that surpassed the TAkrnl average from Group II (0.0307 g of TA · 100 g^{-1} of kernel).

Although the Pop 25 had the maximum TAkrnl, purple kernel genotypes with higher anthocyanin yields have been reported, they have around 1.26 and 7.42 times more anthocyanins, with a TAkrnl from 0.3045 to 1.78 g of TA · 100 g^{-1} of kernel (Li *et al.*, 2008; Zhao *et al.*, 2008; Lopez-Martinez *et al.*, 2009; Yánez *et al.*, 2016). Pop 25, Pop 12, and Pop 31 defeated the TAkrnl of a variety of purple corn studied by Khampas *et al.* (2013) that had 0.165 g of TA · 100 g^{-1} of kernel. Besides, another 22 purple corn populations produced more TAkrnl than the purple corn populations reported by Harakotr *et al.* (2014), Cuevas *et al.* (2011), and Salinas *et al.* (2005), which had values from 0.0716 to 0.0765 g of TA · 100 $g^{-1}$of kernel. This indicates that the Group I populations are a rich source of anthocyanins and that they are outstanding compared to purple corn variants from different national and foreign origin.

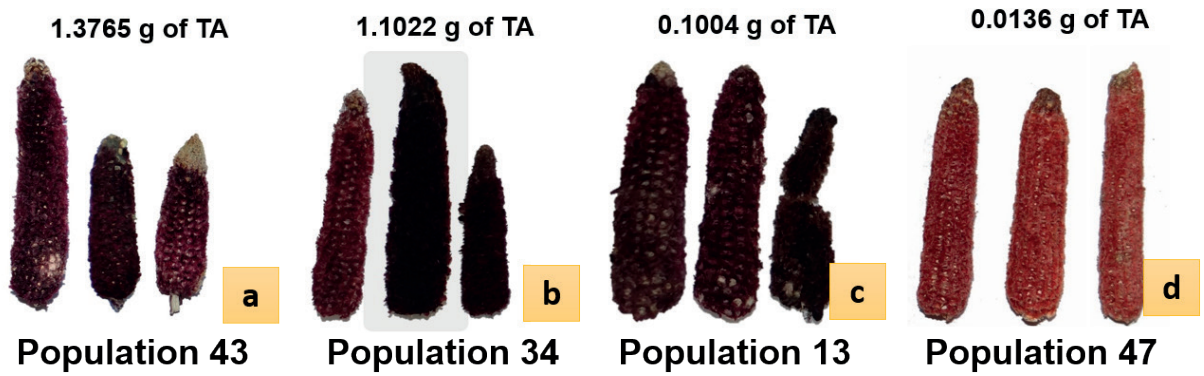

Fig.4 - Expression of the pigment in the corncob. Group I = population with a purple kernel (b and c); Group III = white kernel population from the Cacahuacintle race (a); Group IV = red kernel populations (d). TA= Total anthocyanin content, g of TA· 100 g^{-1} of corncob.

In Figure 3 we can see the color of the kernel of some populations. Figure 3a corresponds to the kernel of the Pop 25, which had the highest TAkrnl.The color of this kernel did not differ greatly from the kernel of the Pop 12 (Figure 1b); however, its TAkrnl was different ($P \leq 0.05$, Table 2). Figure 3c shows the kernel of the Pop 17 that had a similar TAkrnl to the TAkrnl average from Group I, while Figures 3d and 3e correspond to the Pop 19 (which contains Peruvian purple corn germplasm) and the Pop 24 (lowest TAkrnl from Group I), respectively. The color of the red kernel populations (Figure 3, i - j) is easily differentiated from the Group I populations. In Figure 3 from k - o, we show the kernels of some populations from Group III and Group V that did not have a coloration by anthocyanins, including yellow, creamy white,and white kernels that recorded almost an imperceptible trace of anthocyanins (Table 2).

Total anthocyanin content in the corncob (TAcob)

We noted that the Group III had the maximum content of total anthocyanins in the corncob (TAcob) and that it was statistically similar to the Group I (TAcob = 0.5248 and 0.4306 g of TA · 100 g^{-1} of corncob, respectively). Both groups differed from Groups II, IV, and V ($P \leq 0.05$, Table 2). Within the populations from Group I, we found that the ten populations of higher TAcob (ordered from the highest to the lowest TAcob) were: Pop 34, 25, 12, 31, 7, 32, 21, 26, 24 and 10, with contents from 0.5744 to 1.1022 g of TA · 100 g^{-1} of corncob.

We observed that Mendoza *et al*. (2016), Monroy *et al*. (2016), and Yánez *et al*. (2016) reported a higher TAcob than the TAcob averages from Group I and Group III,they quantified from 1.05 to 10.4 g of TA · 100 g^{-1} of corncob. In our study, there were populations with a higher or a similar TAcob to the range of 0.0307 to 0.728 to g of TA · 100 g^{-1} of corncob, found by Yang *et al*. (2008), Wang and Zeng (2009), Yang and Zhai (2010) and Piyapanrungrueang *et al*. (2016). This was the case of the Pop 43 (Figure 4a) and Pop 34 (Figure 4b, Table 2) that had the highest TAcob in the Group III and Group I, respectively.We noticed that the color of the corncob of the Pop 13 (Figure 4c),which had the lowest TAcob from Group I, did not differ markedly from the color of the corncob from the populations with a greater TAcob (Figure 4, a - b) and that there were differences in the color intensity. Also, we sighted that the TAcob average from Group IV (populations with reddish corncobs, Figure 4d) was lower than the TAcob average from Group I (Figure 4, Table 2).

Conclusions

Mexican purple corn shelters and represents an important genetic resource that has a broad potential to be used as a bioactive compound or as a natural dye. We found that this type of corn has a high capacity to synthesize and to accumulate anthocyanins both in the corncob and the kernel. Some populations surpassed the quantity of anthocyanins accumulated by the Peruvian purple corn, the nowadays best-known variant of purple corn. Also, we identified that the pericarp had the highest content of anthocyanins followed by the-corncob, kernel, and the aleurone layer.

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