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Nutritional Enhancement of Polimaize Lines: Integrating Native Mexican Maize Alleles into High-Yield Varieties

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Abstract: In this study, we evaluated Polimaize lines, named Polimaize, through the integration of alleles from the Native Blue Corn (NBC-JIQ), a local Mexican maize variety indigenous to the northeast region of Michoacán, Mexico, into elite maize lines curated by the International Maize and Wheat Improvement Center (CIMMYT). This crossbreeding aimed to enhance the nutritional profile of maize, particularly in terms of antioxidants and anthocyanins, which are scarce in elite lines. Our results demonstrated a significant increase in these compounds in the Polimaize lines, with variations due to heterosis. Despite these nutritional improvements, some traits showed decreased concentrations compared to parent lines, notably in sucrose and tryptophan, suggesting a potential trade-off. The study also found significant heritability in amino acids and tryptophan, while hexose sugars showed no substantial heritability. The Polimaize variety exhibited high starch content heritability, comparable to elite lines. Field trials confirmed Polimaize's promising yield and agronomic traits, highlighting its potential for enhancing consumer health and contributing to sustainable agriculture through enriched crop nutritional quality. This project underscores the value of integrating local race alleles into elite lines, offering genetic diversity in maize cultivation.

Keywords: anthocyanins; maize breeding; nutritional enhancement; genetic diversity; agronomic performance

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1. Introduction

Maize is the world's most significant crop, as highlighted by the Food and Agriculture Organization in 2023 [1]. Notably, it serves as a primary carbohydrate source in developing nations, as indicated by Kiran et al. [2]. In Mexico, where maize consumption per capita reaches approximately 125 kg annually [3], the crop is deeply rooted in history and culture. Mexico is not only the center of origin but also the hub of maize diversification, boasting around 64 distinct landraces cultivated throughout the country [4,5]. However, it is important to note that these figures might vary based on the classification methodology employed, be it morphological, molecular marker, or sequencing [6]. These landraces, cultivated and selectively bred over hundreds of generations, have adapted to a variety of environmental challenges such as drought, pest resistance, and soil fertility. They have also been selected for specific organoleptic qualities like color, size, and flavor [7]. This has made

them a valuable genetic reservoir, offering traits that could enhance new maize lines [8]. Their appeal extends beyond Mexico, with colored maize varieties gaining appreciation in Europe and other regions [8,9].

Modern genetic improvement efforts are increasingly focused on enhancing nutritional value, such as incorporating higher amino acid levels [10,11]. In this context, quality protein corn (QPM) varieties have been developed to enrich maize with essential amino acids like lysine and tryptophan, traditionally present in low concentrations in corn kernels [12]. However, these amino acids correlate with challenges like softer kernel textures, reduced yields, storage difficulties, and increased pest vulnerability [13].

Another recent focus has been the integration of anthocyanins and antioxidants in maize kernels [14]. Anthocyanins, the compounds imparting a blue–red hue to kernels, have been recognized for their health benefits in various studies [15,16]. Similar selection processes for high anthocyanin content have been observed in other crops like barley [17]. In Mexico, landraces rich in anthocyanins are widespread, presenting potential for exploitation [18,19]. Yet, challenges remain with these blue landraces, such as low yields, secondary stem issues, lodging, and a floury kernel texture, leading to yields as low as 3 tons per hectare in some areas [20], limiting their industrial application.

Genetic improvement programs have successfully developed high-yield maize varieties adapted to diverse environmental conditions [21]. These elite maize strains, rich in starch, tend to be deficient in other nutrients like proteins, lipids, and amino acids. A promising approach to boost nutritional quality is the introduction of alleles from landraces into the genetic background of these varieties. However, such integration can alter genetic correlations within the new lines.

Polimaize is an innovative project launched in 2018 at National Polytechnic Institute, specifically within the Interdisciplinary Research Center for Regional Development, Michoacán Unit (CIIDIR-IPN Unidad Michoacán), situated in the northeast region of Michoacán, Mexico. This pioneering initiative aims to merge the nutritional attributes of local blue maize landraces with the high-yield maize lines developed by the International Maize and Wheat Improvement Center (CIMMYT). The project represents a significant stride in combination with the nutritional benefits of traditional maize varieties with the advanced agricultural productivity of modern breeding techniques. After nine cycles of selection and crossbreeding, the first Polimaize lines are now prepared for distribution to farmers. However, the biochemical properties of these derivative lines may differ from their parent strains. Initial field tests show that Polimaize yields about 9 tons per hectare. A critical question remains, i.e., whether the Polimaize lines retain the starch, lipids, amino acids content of the elite lines while also incorporating the anthocyanins and antioxidant activity from the landraces.

This study aims to evaluate the grain quality, focusing on biochemical parameters, and to measure the heritability of these traits in the Polimaize program lines. We anticipate that these derived lines will integrate the high-quality characteristics of blue corn with the enhanced anthocyanins, total amino acids, tryptophan, and lipid content, relative to their parent lines. This research not only bridges traditional landrace qualities with modern agricultural advancements but also promises to elevate the nutritional profile of one of the world's most vital crops.

2. Materials and Methods

We selected thirteen elite maize lines from CIMMYT based on criteria that included yield performance, disease resistance, and superior grain quality, each identified by a CML suffix. These lines were specifically developed for growth in subtropical and high valley regions. To incorporate anthocyanin production into these lines, we crossed them with a locally renowned variety, which we refer to as "Native Blue Corn (NBC-JIQ)". This variety is well known for its cultivation in the northeast region of Michoacán, Mexico, and is distinguished by its dark blue kernel coloration, a result of its high anthocyanin and antioxidant content. The creation of the Polimaize lines involved strategic crosses,

as detailed in Table 1. After nine cycles of selection within the Polimaize program, we stabilized new lines, designated by their stock IDs. Notably, lines 59 and 69 were crossed with CML lines 491 and 492, respectively, both characterized by their quality protein corn (QPM) traits. We planted these genotypes in the northeast region of Michoacán, Mexico (coordinates: 20.163105805792316, −102.70524966296367), considering the local humid subtropical climate (Köppen classification Cwa) and an altitude of approximately 1700 m. The plants received optimal agronomic care, including irrigation, fertilization, and pest management, to maximize their yield. At the end of the growth cycle, we harvested cobs from the most agronomically successful plants and stored them at 4 °C. The kernels from these cobs were later used for biochemical analysis.

Table 1. Pedigree of the Polimaize lines.

ID Lines	Pedigree
IPN-007	[(NBC-JIQ × LPSC7-F103-2-6-1-1-BB)//LPSC7-F103-2-6-1-1-BB//LPSC7-F103-2-6-1-1-BBB]//LPSC7-F103-2-6-1-1-BBB//LPSC7-F103-2-6-1-1-B*4]-B-B-B-B
IPN111-031	[(CML254xNBC-JIQ)//CML254//CML254]//CML254//CML254//CML254//CML254//CML254]-B
IPN111-047	[(NBC-JIQ × HLBP)-B//CML460]//CML460//CML460//CML460//CML460//CML460]-B
IPN111-055	[(NBC-JIQ × CML343)//CML384//CML384]//CML384//CML384//CML384]//CML384//CML384]-B
IPN111-057	[(NBC-JIQ × CML343)//CML321//CML321]//CML321//CML321//CML321]//CML321//CML321]-B
IPN111-059	[(NBC-JIQ × CML343)//CML492//CML492//CML492//CML492//CML492]//CML492//CML492]-B
IPN111-061	[(NBC-JIQ × CML343)//CML444//CML444//CML444//CML444//CML444]//CML444//CML444]-B
IPN111-065	[(NBC-JIQ × LPSC7-F103-2-6-1-1-BB)//CML311//CML311//CML311//CML311//CML311]//CML311//CML311]-B
IPN111-067	[(NBC-JIQ × LPSC7-F103-2-6-1-1-BB)//CML312//CML312//CML312//CML312//CML312//CML312//CML312]-B
IPN111-069	[(NBC-JIQ × LPSC7-F103-2-6-1-1-BB)//CML491//CML491]//CML491//CML491//CML491//CML491//CML491]-B
IPN111-071	[(NBC-JIQ × LPSC7-F103-2-6-1-1-BB)//CML494//CML494//CML494//CML494//CML494//CML494//CML494]-B
IPN111-077	[[(NBC-JIQ × CML216)//CML216]//CML216//CML216//CML216]//CML216//CML216//CML216]-B
IPN111-079	[(Pool17 × NBC-JIQ)//Pool17-f4//Pool17-f4//Pool17-f4//Pool17-f4//Pool17-f4//Pool17-f4//Pool17-f4]-B

"BB" denotes the specific alleles in the genetic background. "B" indicates selection for blue kernel coloration, the primary phenotypic marker in Polimaize line development.

2.1. Metabolite Determination

We dried the harvested maize cobs of the derived lines and stored the kernels at 4 °C. These kernels were ground into a fine powder, ensuring particle sizes of less than 500 µm, using a Retsch Post Mill grinder. We then stored the resulting flour in paper bags in a low humidity environment (below 10%).

2.2. Total Anthocyanin Determination

We followed Panda et al. [22] to determine total anthocyanin content. This involved weighing 20 mg of flour and dissolving it in 1.3 mL of 1% trifluoroacetic acid. We mixed the samples for 90 min at 4 °C at 150 rpm, then centrifuged them at 14,000 rpm for 5 min. We measured the absorbance of the supernatant (100 µL) at 520 nm using a microplate. Cyanidin-3-glucoside was the standard for the calibration curve, as suggested by Abdel-Aal et al. [23]. We expressed the results in molar equivalents of cyanidin-3-glucoside, with each sample undergoing three technical replicates.

2.3. Antioxidant Capacity

We assessed kernel antioxidant capacity using the Sigma® Antioxidant Assay Kit (Merck KGaA, Darmstadt, Germany). We used a standard Trolox® curve for concentration determination, with levels ranging from 0 mM to 0.42 mM (0 mM, 0.015 mM, 0.045 mM, 0.105 mM, 0.21 mM, and 0.42 mM). Results were expressed in Trolox® equivalent moles.

2.4. Relative Abundance of Anthocyanins

We quantified anthocyanin abundance following Chirinos et al. [24]. We dissolved 50 mg of flour in 1 mL of 80% ethanol and incubated for two hours, and then centrifuged the samples at 14,000 rpm for 5 min. We dried the supernatant in a vacuum chamber at −50 °C for 24 h, and then re-suspended the residues in 100 µL of 80% ethanol. We stored the resulting extracts in amber vials for HPLC analysis using an Agilent Technology 1200® (Agilent, Waldbronn, Germany) with a binary pump and a diode-array detector (DAD). Post-analysis, we re-equilibrated the column. Standards included cyanidin chloride, pelargonidin chloride, and peonidin 3-O-glucoside chloride.

2.5. Soluble Sugars (Glucose, Fructose, Sucrose)

We extracted soluble sugars from 20 mg of seed flour diluted in 1 mL of distilled water. We mixed the samples at 80 °C for 10 min, and then centrifuged them at 13,000 rpm and 4 °C for 15 min. We used the supernatant for sugar determination and the pellet for starch analysis. Sugar quantification was performed using a coupled enzyme system, and this method involves a series of enzymatic reactions that specifically target the sugars present in the supernatant, converting them into measurable products [25,26].

2.6. Starch Analysis

We dissolved 20 mg of flour in 1 mL of deionized water, and mixed and incubated it at 80 °C for 10 min with stirring. After centrifuging at 13,000 rpm and 4 °C for 15 min, we re-suspended the pellet in 1 mL of 80% ethanol and incubated again at 80 °C for 10 min at 900 rpm. Post-centrifugation, we washed the pellet twice with distilled water, followed by re-suspension in 500 µL of 10 mM potassium hydroxide solution, and incubated at 99 °C for 1 h at 900 rpm. We then mixed the samples with 500 µL of 50 mM sodium acetate, pH 5, and 200 µL of starch degradation solution, and incubated them at 37 °C for 16 h. After a final centrifugation, we used 5 µL of the supernatant for spectrophotometric analysis via the coupled enzyme method. Each biological sample was evaluated in three biological replicates, and further analyzed in three technical replicates.

2.7. Total Amino Acids

The quantification of total amino acids in the kernels was conducted in accordance with the protocol established by Nurit et al. [27]. For this, 20 mg of powdered sample was mixed vigorously with 1 mL of 80% ethanol at room temperature for 5 min. After centrifuging the samples at 13,000 rpm and 4 °C for 15 min, we discarded the pellets and utilized 10 µL of the ethanolic supernatant. This extract was then mixed with 200 µL of a specially prepared amino acid determination solution. This solution comprised 50 µL of 1 M citric acid (pH 5.2), 0.2% ascorbic acid, 1% ninhydrin in 70% ethanol, and 100 µL of a 50% ethanol solution. After gentle mixing, the microplate, covered with foil, was incubated at 70 °C for 30 min. Subsequently, the reaction was halted by cooling the microplate on ice, followed by acclimatization at room temperature, before reading at 570 nm. The necessary calculations were performed to express the results in µmol mg^{-1} DW. A standard curve for amino acid determination was established using Leucine, known for its consistent reaction with ninhydrin.

2.8. Tryptophan

For tryptophan determination, we modified the glyoxylic acid method as per Nurit, Tiessen, Pixley, and Palacios-Rojas [27]. We utilized the remaining flour from samples previously used for lipid determination. Each sample, consisting of 80 mg of flour, was combined with 3 mL of a 1 mg/mL crude papain solution and incubated at 64 °C for 16 h. After the centrifugation of the hydrolysate at 13,000 rpm for 5 min, 3 mL of colorimetric reagent was added. This reagent was prepared by combining Reagent "A" (0.9205 g of glyoxylic acid in 100 mL of 7 N sulfuric acid), Reagent "B" (0.05 g of ferric chloride in 100 mL of Reagent "A"), and Reagent "C" (30 N sulfuric acid). After incubating the mixture

at 64 °C for 30 min and allowing it to cool, we analyzed it using a plate spectrophotometer at 560 nm. Concentrations were obtained by comparing the data against a standard curve of known L-tryptophan concentrations. Each sample was analyzed in biological triplicates.

2.9. Total Lipids

Total lipids were quantified using a direct gravimetric method on lipid extracts. Each 20 mg flour sample was mixed with 1 mL of hexane and shaken at 60 °C for 20 min. Subsequently, it was centrifuged at 13,000 rpm and 4 °C for 15 min, and the supernatant was collected in a glass vial. This extraction process was repeated three times to ensure complete lipid extraction. The hexane was then evaporated using a vacuum pump over 5 h. The vials were weighed before and after the process to determine the lipid content, based on the weight difference between the vial with the extract and the empty vial, in relation to the weight of the original sample. The total lipid content was expressed as μg mg DW.

2.10. Statistical Analyses

Statistical analyses compared each parental CML line with its converted line using a Student's t-test at a significance level of $P < 0.05$. Before conducting the statistical analyses, we verified the normality of the data using the Shapiro–Wilk test. These comparisons are depicted in all figures, where, on the right side of each one, we included both the B73 line and the original value of NBC-JIQ. This provides a reference to a widely used line (B73) and the original value observed in local maize (NBC-JIQ). For the heritability analyses, we used the averages of each CML line and derived line, displaying the standard deviation at each point in the figures. A total of eight replicates were used in all determinations, including the biochemical analyses. To examine the effect of allele introduction from native varieties on heritability in elite lines, we estimated narrow-sense heritability. This was based on the parent–offspring (parental-derived) regression, multiplying the slope by 2, following the Falconer's method [28]. Specifically, we used the formula "$h2 = 2 \times bPO$" for our calculations, where "h2" denotes the narrow-sense heritability of the trait, and "bPO" represents the regression slope of offspring on parents. This approach allowed us to effectively capture the genetic transmission between generations and quantify the proportion of phenotypic variance attributable to additive genetic variance. Additionally, we conducted a correlation analysis in the converted lines to determine if the incorporation of anthocyanins from native varieties influenced other biochemical traits.

3. Results

3.1. Anthocyanins

We observed a notable increase in anthocyanin presence in all converted lines, contrasting with the elite lines that generally lacked anthocyanins due to their grain coloration. This enhancement is attributed to the introduction of alleles from NBC-JIQ. While the anthocyanin levels in converted lines (47, 55, 57, 61, 65, 67, 69, 71, and 79) were lower than those in NBC-JIQ, line 31 exhibited an exceptional overexpression of anthocyanins, potentially due to heterosis, as its value surpassed both parent lines (Figure 1).

3.2. Antioxidant Activity

The converted lines showed an increase in antioxidant activity compared to that of their elite counterparts, a result of introducing alleles from NBC-JIQ. Converted lines 7, 67, 77, and 79 matched the antioxidant concentrations of NBC-JIQ. However, lines 47, 55, 57, 61, 65, and 71 displayed lower antioxidant levels than the original variety. Notably, lines 31 and 59 demonstrated a heterosis effect, enhancing their antioxidant activity beyond both parent lines (Figure 2).

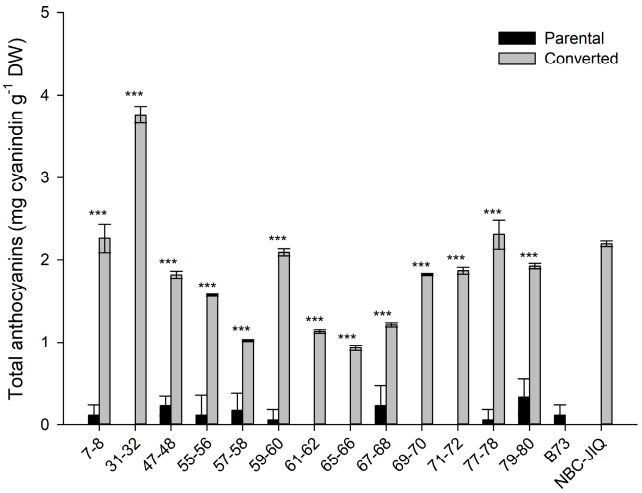


Figure 1. Total anthocyanin concentration in parent-derived Polimaize kernels (mg cyanindin g^{-1} DW). Bars indicate standard error relative to the mean. Statistical differences are denoted as ***, corresponding to significance levels of 0.001, respectively.

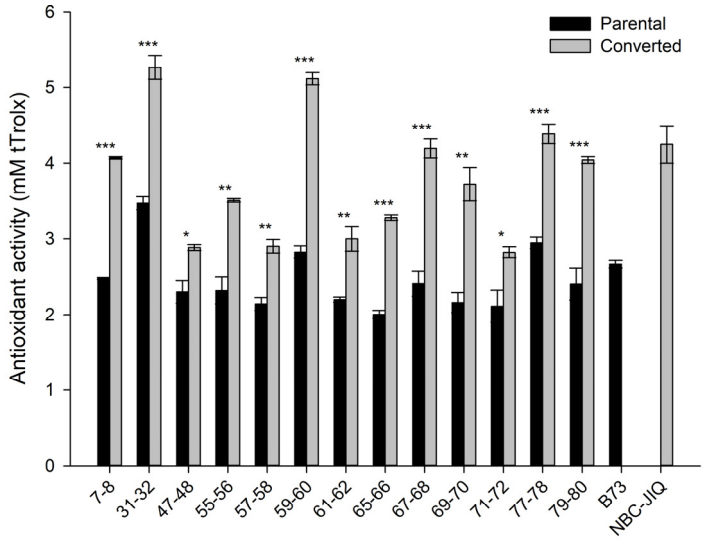


Figure 2. Antioxidant activity in parent-derived Polimaize kernels (mMt Trolox). Bars indicate standard error relative to the mean. Statistical differences are denoted as *, **, and ***, corresponding to significance levels of 0.05, 0.01, and 0.001, respectively.

3.3. Relative Abundance of Anthocyanins

Predominantly, NBC-JIQ contains cyanidin and pelargonidin anthocyanins. Interestingly, certain converted lines also presented peonidin in their grains. The predominant anthocyanins in these lines were cyanidin 3G and pelargonidin 3G. In lines 69 and 79, peonidin 3G was more abundant than pelargonidin 3G. It is important to note that not all anthocyanins were detected in every line; for example, pelargonidin 3G was absent in lines 65 and 71, while line 77 only contained peonidin 3G (Figure 3).

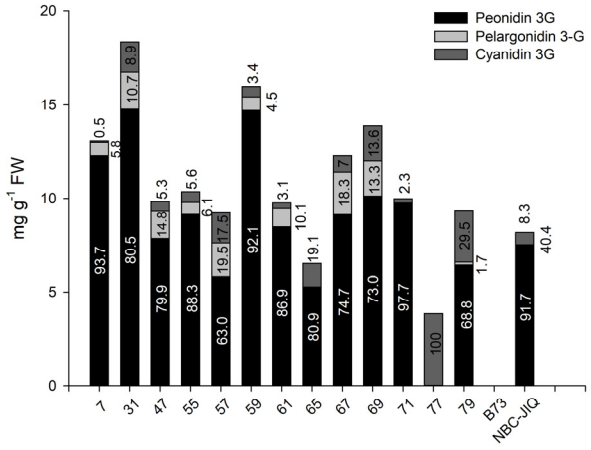
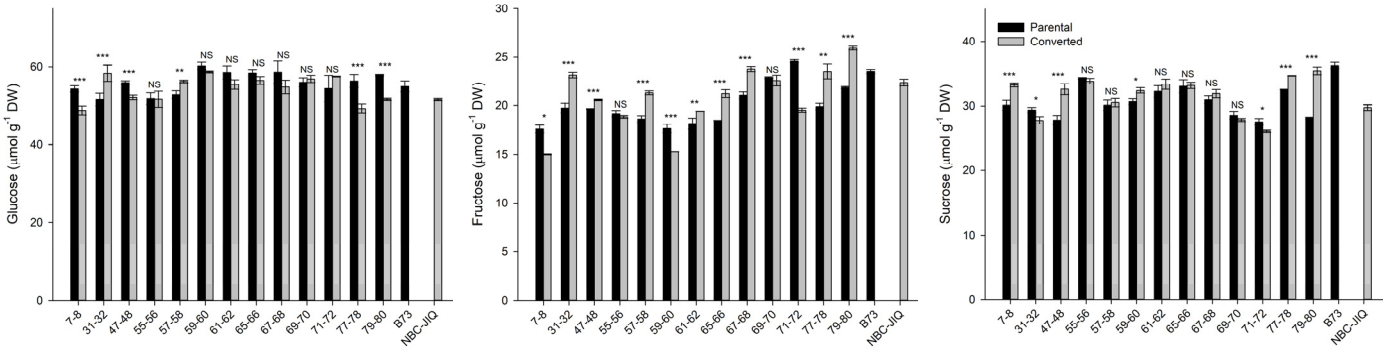


Figure 3. Anthocyanin profile in parent-derived Polimaize kernels: concentration and percentage of peonidin 3–glucoside, pelargonidin 3–glucoside, and cyanidin 3–glucoside (mg g-1 FW and %).

3.4. Soluble Sugars (Glucose, Fructose, Sucrose)

The glucose content in the converted lines varied minimally compared to the elite lines, with no significant differences in many instances. Lines 7, 47, 77, and 79 had lower glucose concentrations than their respective elite counterparts. In contrast, the converted line 31–32 showed an increased glucose concentration, potentially due to a heterosis effect (Figure 4a). Fructose concentration varied relative to the local race; typically, the elite lines had lower fructose levels. The hybridization process reduced fructose levels in converted lines 7, 59, and 71. Conversely, lines 31, 67, 77, and 79 exhibited a heterosis effect, increasing their fructose concentration above that of both parent lines (Figure 4b). The sucrose content in corn grains was generally consistent between the elite and their respective converted lines. Many elite lines had higher sucrose levels compared to the local race, leading to similar results in both parental and converted lines. Lines 47, 77, and 79 showed a heterosis effect, elevating their sucrose content above that of both parents (Figure 4c).



(**a**) (**b**) (**c**)

Figure 4. Soluble sugar content in parent-derived Polimaize kernels: (**a**) glucose (µmol g -1 DW); (**b**) fructose (µmol g-1 DW); and (**c**) sucrose (µmol g^{-1} DW). Bars indicate standard error relative to the mean. Statistical differences are denoted as NS (not significant) and *, **, and ***, corresponding to significance levels of 0.05, 0.01, and 0.001, respectively.

3.5. Starch

The starch content remained consistent across the elite lines, the local race, and the parent-converted lines, with only a few exceptions. Lines 7, 31, 77, and 79 experienced a decrease in starch concentration (Figure 5).

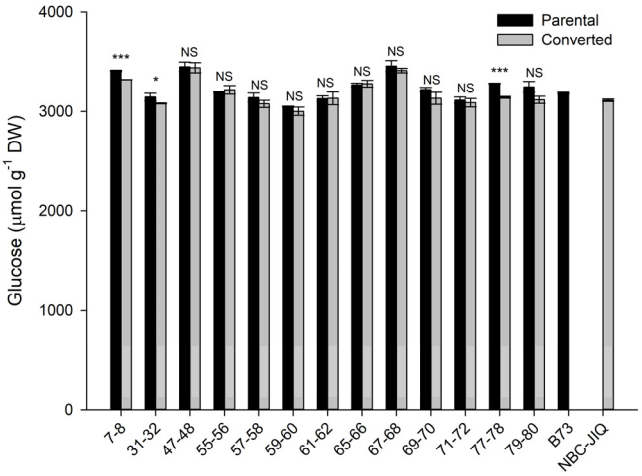


Figure 5. Starch content in parent-derived Polimaize kernels (µmol g^{-1} DW). Bars indicate standard error relative to the mean. Statistical differences are denoted as NS (not significant) and * and ***, corresponding to significance levels of 0.05 and 0.001, respectively.

3.6. Amino Acids

The integration of blue alleles led to a reduction in amino acid concentration in converted lines (7, 31, 47, 55, 59, 61, 67, 77, 79) compared to their respective elite counterparts. Nonetheless, some converted lines saw an increase in amino acid concentration compared to that of NBC-JIQ. An exception was line 57, where the converted line had even lower amino acid levels than the local race. Lines 47, 55, 59, and 77, with the highest amino acid content, emerged as prime candidates for ongoing genetic enhancement (Figure 6).

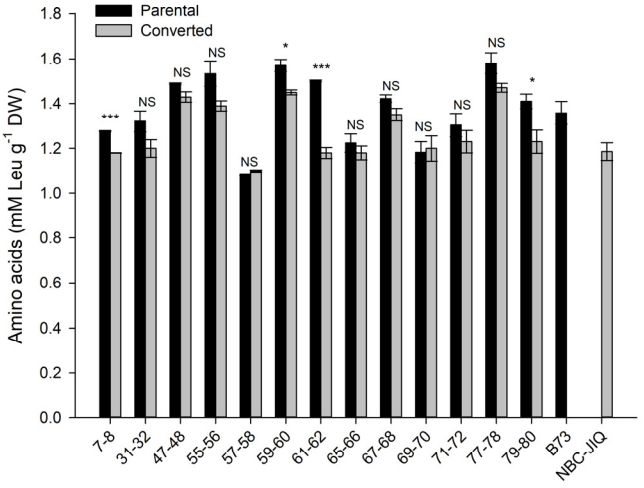


Figure 6. Amino acid content in parent-derived Polimaize kernels (mM Leu g^{-1} DW). Bars indicate standard error relative to the mean. Statistical differences are denoted as NS (not significant) and * and ***, corresponding to significance levels of 0.05 and 0.001, respectively.

3.7. Tryptophan

This essential amino acid, vital for human nutrition, was found in lower concentrations in NBC-JIQ (1.55 mg g^{-1} DW) compared to the elite lines (2.03 mg g^{-1} DW). The converted lines, particularly 7, 59, 61, 67, 69, and 71, exhibited a heterosis effect, boosting tryptophan levels. For instance, line 59 achieved a concentration of 3.14 mg g^{-1} DW (Figure 7).

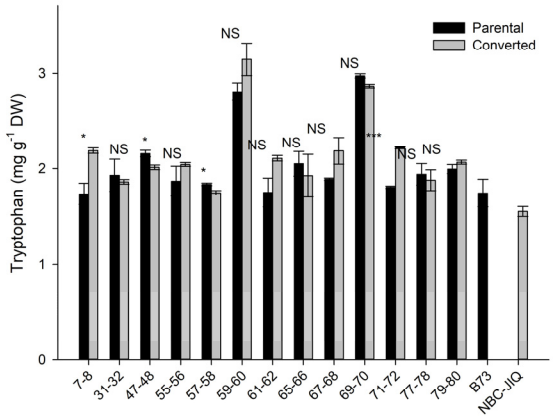


Figure 7. Tryptophan content in parent-derived Polimaize kernels (mg g^{-1} DW). Bars indicate standard error relative to the mean. Statistical differences are denoted as NS (not significant) and * and ***, corresponding to significance levels of 0.05 and 0.001, respectively.

3.8. Lipids

We noted variations in lipid content between the local race and the elite lines. Some converted lines (57, 65, 67, 71, 77) showed a reduction in lipid percentage, whereas others (7, 47, 55, 61, 69, 79) demonstrated heterosis, enhancing their lipid content (Figure 8).

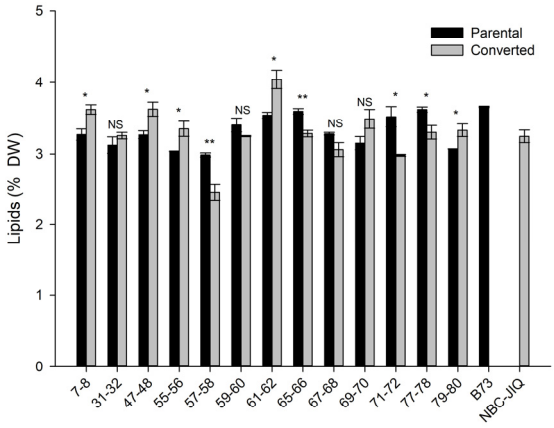


Figure 8. Lipids content in parent-derived Polimaize kernels (%). Bars indicate standard error relative to the mean. Statistical differences are denoted as NS (not significant) and *, **, corresponding to significance levels of 0.05, 0.01 respectively.

3.9. Heritability and Correlation of Biochemical Traits

We observed a notable genetic influence on the variation in certain biochemical traits in the Polimaize lines. Specifically, traits such as antioxidants, starch, amino acids, and tryptophan demonstrated a high heritability value of one (Table 2). This high heritability indicates that the genetic contribution from local race alleles did not significantly impact the concentrations of these compounds compared to those of the parental lines. This finding is a compelling insight into the genetic stability of these traits despite crossbreeding. In contrast, for other traits where significant heritability was not detected, the phenomenon of heterosis (hybrid vigor) might have played a role, potentially overshadowing genetic contributions. This variance in heritability across different biochemical traits underscores the complex interplay between genetic inheritance and hybridization effects in plant breeding.

Table 2. Heritability of traits among CML and Polimaize lines.

Trait	Slope	Intercept	r^2	h^2	Significance
Antioxidant Activity	1.72	−0.43	0.74	1	***
Anthocyanins	−11.99	19.45	0.03	0	NS
Sucrose	0.70	10.47	0.27	1	NS
Lipids	0.53	1.58	0.10	1	NS
Glucose	0.14	46.64	0.01	0.27	NS
Fructose	0.76	5.65	0.25	1	NS
Starch	0.96	86.04	0.86	1	***
Amino acids	0.65	0.38	0.70	1	***
Tryptophan	0.85	0.41	0.70	1	***

Statistical differences are denoted as NS (not significant) and ***, corresponding to significance levels of 0.001, respectively.

Furthermore, our correlation analysis revealed a significant positive association between the production of anthocyanins and the levels of antioxidants and lipids in Polimaize lines (Table 3). This relationship underscores the synergy between the biochemical mechanisms regulating these substances. Conversely, we identified a negative correlation between sucrose and tryptophan content, reflecting the complexity and intricate nature of the interactions among these biochemical traits. These findings provide deeper insight into the biochemical tapestry underlying the grain composition in Polimaize, highlighting how different components can be interconnected in unexpected and significant ways.

Table 3. Correlation among anthocyanin content and other kernel traits.

	Correlation Coefficient	Standard Error	*p* Value	Significance
Intercept	0.406	0.120	0.002	**
Antioxidant	0.381	0.059	0.001	***
Glucose	−0.002	0.001	0.077	NS
Fructose	−0.001	0.001	0.719	NS
Sucrose	−0.008	0.002	0.001	***
Starch	−0.001	0.001	0.068	NS
Lipids	0.049	0.011	0.001	***
Amino Acids	0.060	0.034	0.083	NS
Tryptophan	−0.054	0.011	0.001	***

Statistical differences are denoted as NS (not significant) and **, and ***, corresponding to significance levels of 0.01, and 0.001, respectively.

Additionally, regression analyses on parent–offspring Polimaize lines were conducted to assess grain heritability and nutritional quality. These analyses have uncovered significant correlations between the parental and converted lines, emphasizing the effective transmission of desirable traits across generations. Strong positive correlations were noted in antioxidant activity and lipid and sucrose contents, indicating a pattern of favorable and consistent genetic inheritance (Figure 9). While the selection based on anthocyanin and tryptophan contents from the parental lines appears complex, due to the negative correlations and moderate predictive power, the amino acid content demonstrated significant positive heritability. This finding underscores the substantial potential for the nutritional enhancement of maize within the Polimaize breeding program.

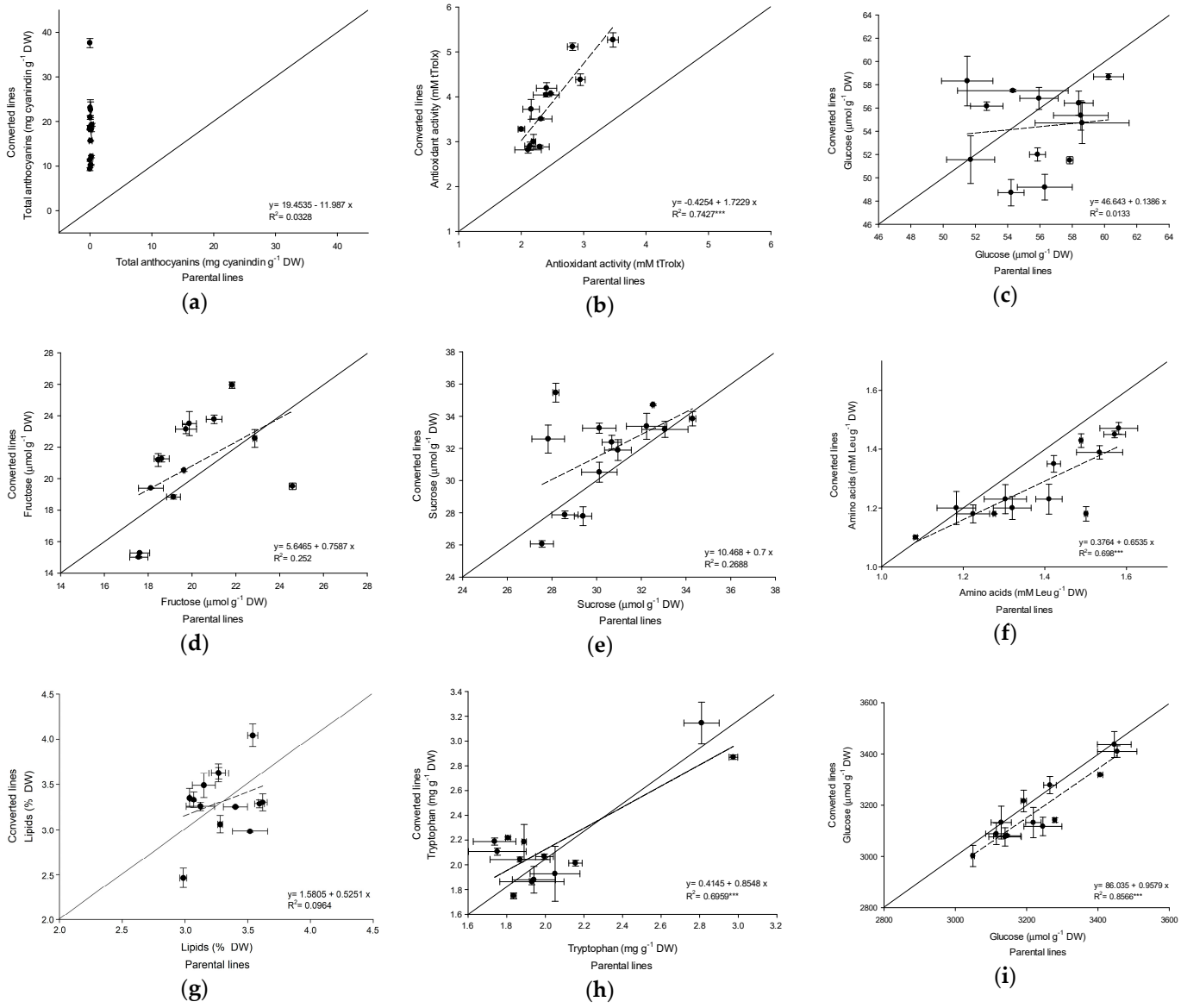


Figure 9. Regression analyses for parent–sibling lines of Polimaize, assessing the following: (**a**) total anthocyanins (mg cyanindin g^{-1} DW), (**b**) antioxidant activity (mMt Trolox), (**c**) glucose (µmol g^{-1} DW), (**d**) fructose (µmol g^{-1} DW), (**e**) sucrose (µmol g^{-1} DW), (**f**) amino acids (mM Leu g^{-1} DW), (**g**) lipids (%), (**h**) tryptophan (mg g^{-1} DW), and (**i**) starch (µmol g^{-1} DW). The bars represent standard errors relative to the mean. ***, corresponding to significance levels of 0.001.

4. Discussion

The inclusion of alleles from the landrace NBC-JIQ has markedly increased the accumulation of antioxidants and anthocyanins in the converted lines. These compounds were nearly absent in elite lines. Overall, the nutritional values in converted lines surpassed those of the elite lines. Nonetheless, some traits exhibited variations relative to the parental traits, often due to heterosis. The breeding program employed in developing Polimaize has successfully preserved the nutritional characteristics of elite lines while enhancing them with additional antioxidants and anthocyanins.

A notable achievement of Polimaize is its significantly higher antioxidant capacity and anthocyanin content compared to other hybrids in the same region [29,30]. The anthocyanin concentration in NBC-JIQ, the donor landrace, is substantially higher (around 220 mg per 100 g) than that reported in other landraces globally, such as in Bolivia (63.73 mg per 100 g) and Italy (161 mg per 100 g) [9,31]. This trait even surpasses those in improved lines from

the USA, Serbia, and the Netherlands [32]. Given Mexico's status as the center of origin and diversification for this species, a vast array of landraces with diverse traits, including color, flavor, and texture, adapted to various conditions, are found here. These colored maize varieties represent an underexplored yet valuable genetic resource [33,34].

However, not all evaluated nutrients showed an increase in concentration compared to both parental lines. The introduction of anthocyanins demonstrated a negative phenotypic correlation with sucrose and tryptophan levels. For tryptophan, in particular, while some lines exhibited increased concentrations compared to NBC-JIQ, the converted lines generally showed reduced concentrations relative to CML lines. This reduction is attributable to either a negative genetic correlation or a trade-off due to the anthocyanin and antioxidant accumulation in the kernels, as corroborated by correlation analyses. In this sense, the incorporation of new biochemical characteristics in the kernel can modify the biochemical proportions, for example, Menkir et al. [35,36] found that the incorporation of carotenoid traits in maize kernel showed positive and negative correlations among distinct carotenoids. Genetic correlations among β-cryptoxanthin, α-carotene, and β-carotene were positive. Zeaxanthin had a positive genetic correlation with β-cryptoxanthin and α-carotene. On the other hand, this carotenoid had a negative correlation with β-carotene.

Regarding heritability in certain metabolites, hexose sugars (glucose, fructose, sucrose) exhibited no significant heritability in converted lines. Conversely, other metabolites crucial for nutrition, such as amino acids and tryptophan, maintained significant heritability. Notably, NBC-JIQ is deficient in tryptophan, as is generally the case with maize kernels in essential amino acids [37]. Some CML lines used in the improvement, like lines 491 and 492, were quality protein corn (QPM). In lines 59 and 69, the tryptophan concentration was statistically comparable to that of the parental lines. Despite QPM maize's disadvantage of floury kernels, which hinders its use in derivative products like tortillas and increases susceptibility to insect damage, Polimaize kernels exhibit desirable physical properties for traditional product preparation. Lago et al. [9] reported a similar case with the introduction of anthocyanins in a maize variety for popcorn production without altering popcorn properties.

The heritability for anthocyanin content in converted lines was notably zero, reflecting the reduced anthocyanin levels in these lines. Conversely, the starch content showed high heritability among parental-converted lines, with NBC-JIQ exhibiting similar starch concentrations to those of elite lines. The high starch content in kernels could be attributed to low genetic variance in this trait, as maize kernels have historically been selected for increased yield, closely linked to kernel weight. Bearing this fact in mind, selecting higher starch content in these lines may be challenging due to their limited genetic variance. Liu et al. [38] found in a WGAS that at least 77 candidate genes are associated with starch production in maize. Even new loci related to starch accumulation are still being reported [39].

The successful introgression of alleles from NBC-JIQ and subsequent recurrent selection have effectively fixed alleles associated with the production of antioxidants and anthocyanins, maintained even after nine generations of selection. Some converted lines continue to exhibit heterosis, enhancing anthocyanin concentration and kernel color intensity. Preliminary genotyping by sequencing (GBS) indicates that Polimaize shares approximately 80% of its genetic makeup with CML parentals, underlining the effectiveness of this breeding strategy (research in progress). Similar values in introgression are reported by Meseka et al. [40], who found 25% introgression in the genetic improvement of drought-resistant maize.

Despite this, many studies highlight the inferior nutritional and agronomical performance of landraces compared to that of elite varieties. Consequently, contemporary breeding programs are increasingly focused on incorporating alleles linked to nutraceutical traits into elite line backgrounds [41,42].

Polimaize represents a significant endeavor to improve consumer health by integrating a natural source of anthocyanins and antioxidants into diets, eliminating the need for

costlier supplementary products. This project could offer farmers an opportunity to cultivate high-yield varieties with the added value of nutritionally enhanced kernels. Generally, colored maize commands a higher market value and is favored in gourmet markets. Our findings suggest that Polimaize is a viable option for farmers. Furthermore, the inclusion of alleles from landraces serves as an effective form of in situ conservation, countering the decline in landraces due to economic or yield-related factors [43–45]. Utilizing these alleles enhances the value of genetic diversity and can stimulate interest in research focused on functional genetic diversity.

5. Conclusions

The study successfully demonstrated that the Polimaize program lines, developed through the integration of alleles from the local race NBC-JIQ into elite maize lines, significantly enhanced the nutritional profile of maize, with a notable increase in anthocyanin concentration and antioxidant activity, surpassing elite lines by up to 220 mg per 100 g. Additionally, some Polimaize hybrids have shown a heterosis effect, improving tryptophan concentration up to 3.14 mg g^{-1} DW, and enhancing lipid quality. This enhancement of grain quality, particularly in biochemical parameters, underlines the potential of Polimaize in bridging traditional landrace qualities with modern agricultural advancements. Notably, the research highlights the value of genetic diversity in crop improvement, offering a promising approach to elevate the nutritional profile of maize, a crucial global crop. This integration of enhanced nutritional traits and the maintenance of agronomic performance in Polimaize signify a significant step forward in the field of agricultural biotechnology and crop improvement.

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