Inheritance of purple pericarp colour in maize (*Zea mays* L.)

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Abstract

Purple maize has high content of beneficial anthocyanin. Kernel colours and their intensity are associated with the concentration of phytochemicals and minerals, which could benefit human health. Breeding for purple-kernelled maize requires an understanding of its mode of inheritance. This study was conducted to investigate the mode of inheritance of purple pericarp colour in maize. A cross and its reciprocal was carried out between two maize lines: UIp01 with purple kernels and TZi3 which has white kernels. Ears bearing kernels of the six basic generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2) and F_3 derived from UIp01 × TZi3 (F_1) and TZi3 × UIp01 (RF_1) were sorted by colour, and grouped into purple and non-purple. Data were summarized for the distribution of kernel colour. Chi-square analysis with 95% confidence interval was carried out on the segregation ratio. The F_1 and RF_1 differed in pericarp colour. The F_1 had the purple maternal phenotype for pericarp colour, while the RF_1 had kernels with yellow pericarp. Irrespective of the direction of the cross, the F_2 segregated in the ratio 9:7 purple to non-purple, for complementary gene action. Segregation ratio of purple-kernelled to non-purple-kernelled ears in the F_3 had a good fit with ratio 1:3. Maternal effect was present in the inheritance of purple kernel colour in maize, with a complementary gene action in which the purple kernel colour was not completely dominant over the transparent phenotype, but the expression of purple kernel colour determined by the genotype of the parents.

Keywords: Complementary gene action, Inheritance pattern, Kernel colour, Maternal effect, Purple maize.

Introduction

Maize (*Zea mays* L.) is a cereal crop well adapted to a wide range of environments with a global total production of 1.24 billion tonnes with an average yield of 5.96 tonnes per hectare (FAOSTAT, 2023). It serves as an important food security crop in regions such as sub-Saharan Africa, Latin America and Southern Asia (Grote *et al*., 2021; Soto-Gomez and Perez-Rodrıguez, 2022; Bhushan *et al*., 2024). In many parts of Africa, maize is an important staple food consumed in a wide variety of forms and as feed for animals. Apart from being rich in starch and protein, maize possesses the capacity to synthesize coloured pigments, particularly anthocyanins and carotenoids which are known to possess nutritional and functional roles (Nuss *et al*., 2012; Rouf Shah *et al*., 2016; Graham and Rosser, 2000; Bhushan *et al*., 2024).

Maize has a vast genetic variation for kernel colour, which ranged from white through a succession of diverse shades of yellow, red, pink, purple, blue to black. However, the white and yellow variants are the most common commercial types (Ron-Parra *et al*., 2016; Mendoza-Mendoza *et al*., 2019). The diversity in maize types and the use to which it is put is closely associated with the culture of a given community and production system (Gomez *et al*., 2000; Louette and Smale, 1998).

Purple maize is a flint maize type with its origin in the Andean region of South America and widely cultivated in countries like Peru, Mexico, Ecuador, Bolivia, Argentina, India and China, and distributed in the markets of Asia, United States of America and Europe (Colombo *et al*., 2021; Kim *et al*., 2023). Purple maize, like every other maize type, can be used as food, feed and raw materials for industries. However, it differs from the more common white and yellow maize due to its rich content of anthocyanins and phenolic compounds which are known to possess health-promoting effects (Lao and Giusti, 2016; Lao *et al*., 2017; Wang *et al*., 2024). These compounds are mainly located in pericarp or aleurone of purple maize (Bridle and Timberlake, 1997; Colombo *et al*., 2021; Barba *et al*., 2022). Studies have demonstrated that pigments in purple maize have anti-diabetic, anti-obesity, anti-hypertensive, anti-proliferative, anti-microbial, and anti-inflammatory properties (PenichePavía *et al*., 2022; Kim *et al*., 2023).

As a result of the distinct colour and antioxidant contents of purple maize, it is utilized as a natural functional food colorant in South America, Asia and Europe in substitution for unhealthy synthetic dyes (Moreno *et al*., 2005; Jing *et al*., 2007; Montilla *et al*., 2011 and Lao et al., 2017). Reports from previous studies (Cevallos-Casals and Cisneros-Zevallos, 2003; Wu et al., 2006 and Li et al., 2008) have shown that the average anthocyanin contents in purple maize kernels (16.4 mg g^{-1}) are considerably higher than those of known anthocyanin-rich plants like blueberries (1.3 to 3.8 mg g^{-1}), strawberries (0.18 to 0.24 mg g^{-1}), red cabbage (2.81 to 3.63 mg g^{-1}), egg plants (8.57 mg g^{-1}) and chokeberries (14.8 mg g^{-1}). Despite the benefitial effects and utility of purple maize, there islimited awareness on its value in Africa. In Nigeria, purple maize is usually found as 'spots' in local farmers' maize fields. There are no known purple maize varieties in Nigeria, and limited research is devoted to its improvement and applications.

Elsewhere, studies are being conducted to increase the contents of phytochemicals in purple and red maize. Also, due to the association of the intensities of these colours with important phytochemicals and minerals, several efforts are being directed at transfering purple and red colours to adapted commercial varieties for the promotion of human health (Hong et al., 2020; Öztürk and Uzun, 2024). There is the need to develop and promote purple maize as a specialty corn with high contents of phenolic compounds and nutritional quality in Nigeria. Breeding for purple maize requires an understanding of its mode of inheritance and the genetic factors underlying the expression of the trait. A key component of such breeding goal is the investigation of patterns of inheritance of the purple pericarp trait. Outcomes from such studies could facilitate successful selection of parents, and the development of promising hybrids (Melchinger et al., 1986; Khamphasan et al., 2018). Therefore, this study was conducted to investigate the mode of inheritance of purple pericarp colour in maize.

Materials and Methods

Experimental site

The study was conducted at the experimental field of the Department of Crop and Horticultural Sciences, Faculty of Agriculture, University of Ibadan (N07.45192°, E003.88994°, and 203 masl), Ibadan, Oyo State, Nigeria. The experimental field was cleared, and minimally tilled.

Genetic Materials, Crosses and Pericarp Colour Evaluation

Two maize inbred lines: TZ$_I$3 (formerly 1368) and UIp01 differing in kernel colour were used as genetic materials in this study. The line UIp01, a purple-kernelled flint maize inbred, was developed at the Department of Crop and Horticultural Sciences, University of Ibadan, Nigeria. The purple-kernelled maize was originally identified and collected from an open pollinated maize field and subjected to cycles of self-pollination and selection for purple kernels. Line TZ$_I$3, a white-kernelled flint maize inbred developed by the International Institute of Tropical Agriculture (IITA), Ibadan, is one of the parental lines of the commercial white-kernelled hybrid Oba Super-1.

The two maize lines were planted at different dates so as to synchronize flowering. The line UIp01 (P$_1$) was crossed as female parent to line TZ$_I$3 (P$_2$) to generate F$_1$. The reciprocal cross using TZ$_I$3 as female parent was also carried out to generate RF$_1$. Plants of the F$_1$ generations (including RF$_1$) were self-pollinated to produce F$_2$ kernels, and backcrossed to both parental lines to generate backcross populations. All pollinations were controlled and done by hand. Standard agronomic and field management practices for the cultivation of maize were followed. Harvesting was carried out $\geq$35 days after pollination when physiological maturity must been attained and air dried to about 12% moisture. Ears of the F$_2$ and backcross populations were harvested and processed individually. Ears of parental lines, F$_1$ and RF$_1$, F$_2$ and backcross populations were visually assessed for kernel colour, sorted by colour and counted. Thereafter, ears segregating for kernels were hand shelled, sorted by colour and counted to determine the inheritance pattern of the trait. The purple ears from the F$_2$ generations of both populations were then advanced to F$_3$. The ears were harvested at physiological maturity, processed individually, dried to 12% moisture content, assessed visually for kernel colour, sorted by kernel colour and counted.

Data Analysis

Data were summarized for the distribution of colours. Chi-square analysis was carried out on the segregation ratios of different kernel colours with a 95% confidence interval.

Results and Discussion

All the F$_1$ ears resulting from the cross between UIp01 (P$_1$) and TZ$_I$3 (P$_2$) were purple-kernelled, while the reciprocal cross (P$_2$ × P$_1$) yielded ears that were all yellow-kernelled (Figure 1). A Comparison of the F$_1$ and RF$_1$ showed the presence of reciprocal difference in the phenotypic expression for purple pericarp kernel colour. The finding is consistent with previous reports in maize (Hossain *et al*., 2019), and sesame (Laurentin and Benitez, 2014) on the existence of reciprocal differences. Differences in the pericarp phenotype of the F$_1$ and its reciprocal had been attributed to the influence of maternal genotype (Wilson and Hudson, 1979; Pandey et al., 2013) in sesame, and Ron-Parra et al. (2016) in red pericarp grain of maize. The findings also show that nuclear genes may have no role in the inheritance of purple kernel colour in maize. However, in the present study, the purple and yellow pericarp colour of the F$_1$

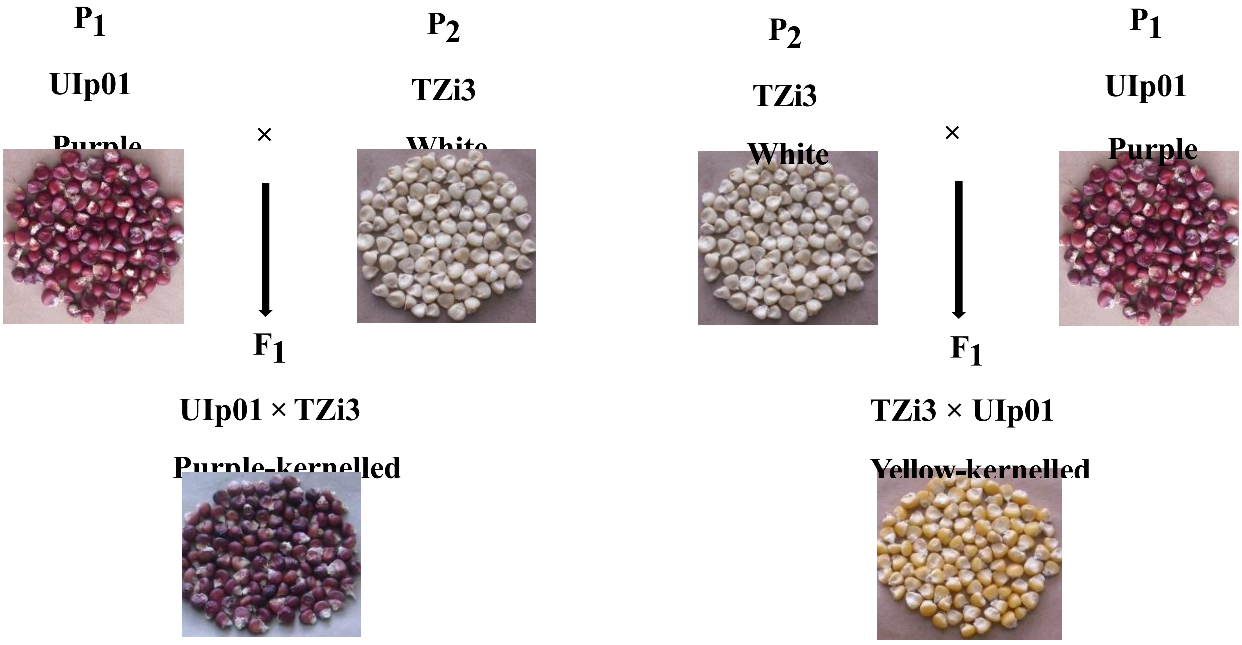

Figure 1. Distribution of kernel colours in the kernels of the F_1 hybrids arising from a cross between a purple (UIp01) and a white (TZi3) kernelled maize and RF_1, respectively suggested that purple pericarp may not be dominant over transparent pericarp. Van-Deynze and Pauls (1994) had shown that the expression of seed coat colour in *Brassica*, though primarily controlled by the maternal genotype, is determined by the genetic constitution of the parents. The expression of yellow pericarp colour in the reciprocal cross in this study could be a consequence of complementary gene action. Our findings contradicts the results of Ron-Para *et al.* (2016) who had earlier reported maternal effect with complete dominanace of the red phenotype over the transparent phenotype in the inheritance of red pericarp colour in maize.

Irrespective of the direction of the cross that generated the F_1 seeds, on selfing, the ears on the F_1 plants bearing F_2 kernels yielded cobs that were either all purple-kernelled or segregated for yellow and white kernels. No ear segregated for purple kernels on a cob (Figures 2 and 3). This shows that F_2 kernels on a cob do not segregate for purple pericarp. The findings from the present study however, contradicts the reports of Hossain et al. (2019) on the inheritance of red pericarp colour in maize, and Laurentin and Benitez (2014) on the inheritance of seed coat colour in sesame. The authors had reported that all the F_2 seeds independent of the direction of cross expressed the same phenotype. The proportion of purple kernelled ears to non-purple kernelled ears (ears that segregated for yellow and white kernels on a cob) had a good fit with 9:7 ratio (Table 1). This supports the digenic control of purple kernel colour in maize (Schoemaker *et al.*, 2024). Additionally, the segregation ratio of the ears segregating for yellow and white kernels on a cob was in conformity with the 3:1 ratio (Table 1). The direction of cross of the F_1 notwithstanding, backcrosses to the purple parental line yielded ears with purple kernels, while those to the white parental line yielded ears that segregated for yellow and white kernels (Table 1). The segregation of the yellow and white kernels arising from backcrosses to the white parental line had a good fit with the 1:1 ratio (Table 2).

On selfing the purple F_2 seeds derived from the different crosses, the ears on F_2 plants bearing F_3 kernels yielded four categories of ears. There were ears that were all purple-kernelled, those that segregated for yellow and white kernels on the cob, all yellow-kernelled or all white-kernelled. Similar to the observation for the F_2 seeds ears, no ear segregated for purple kernels on a cob. The ratio of purple-kernelled ears to non-purple kernelled ears (sum of ears that segregated for yellow and white kernels on a cob, all yellow-kernelled and all white-kernelled ears) had a good fit with 3:1 ratio expected for plant producing purple and non purple kernels (Table 2). Similar results were reported by Laurentin and Benitez (2014) for the inheritance of brown seed coat in sesame.

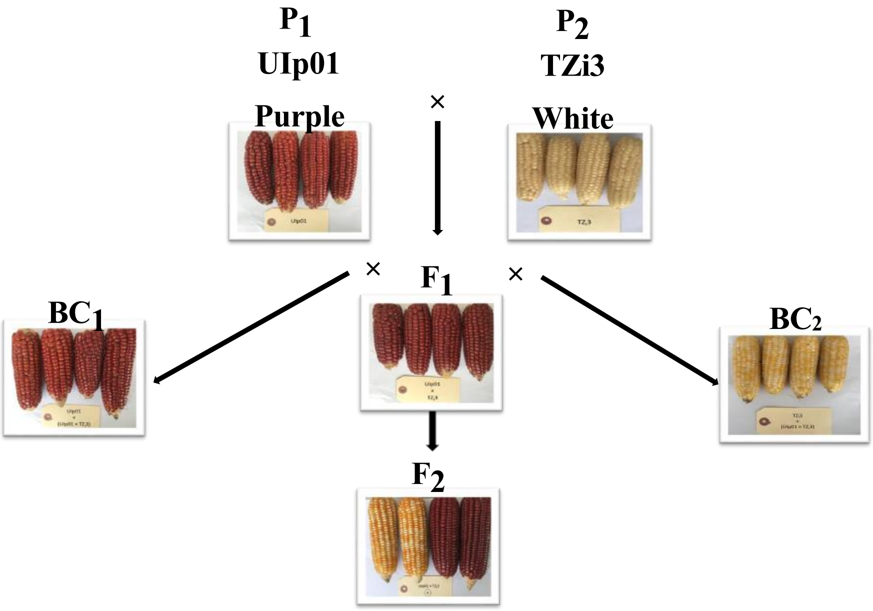

Figure 2. Distribution of kernel colour in the kernels of the F_2 and backcrosses of the cross UIp01 × TZi3

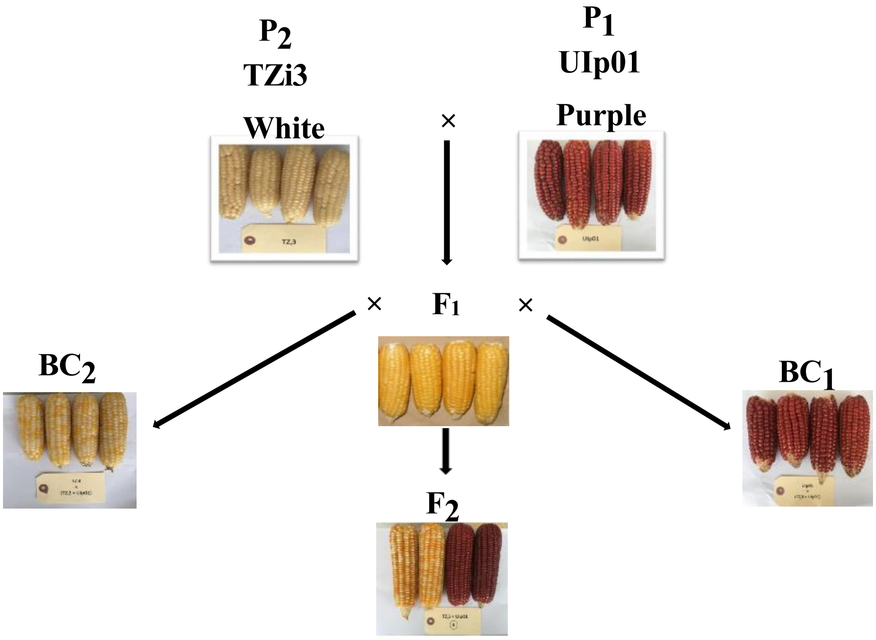

Figure 3: Distribution of kernel colour in the kernels of the F_2 and backcrosses of the cross TZi3 × UIp01

Table 1. Summary statistics of kernel phenotype and segregation ratios for purple pericarp in ears of the six basic generations and F_3 of reciprocal crosses between a purple maize line (UIp01) and a white maize line (TZ_I3) in Ibadan, Nigeria

Generation		Total number of ears	Number of ears with colour				Ratio (Purple:non purple	χ^2 calculate	χ^2 tabulated
			Purple	White	Yellow	Segregants			
UIp01	(P_1)	30	all						
TZ_I3	(P_2)	30		all					
$P_1 \times P_2$	(F_1)	60	all						
$P_2 \times P_1$	(RF_1)	60			all				
F_2		322	197			125	9:7	3.23	3.84
RF_2		320	175			145	9:7	0.32	3.84
$P_1 \times F_1$		68	all						
$P_2 \times F_1$		88				all			
$P_1 \times RF_1$		64	all						
$P_2 \times RF_1$		111				all			
F_3		300	230	13	14	43	3:1	0.44	3.84
RF_3		366	279	13	23	51	3:1	0.30	3.84

Pp : Purple; W : White; Y : Yellow

Table 2: Segregation ratios of yellow to white on cobs of segregant ears of the F_2, RF_2, $P_2 \times F_1$, $P_2 \times RF_1$ generations of crosses between purple maize line UIp01 (P_1) and white maize line TZi3 (P_2)

Population	Number of segregant ears	Number of kernels per cob	Number kernels with colour		Ratio (Yellow:White)	χ^2	
			Yellow	White		calculated	tabulated
F_2	125	417	309	108	3:1	0.205	3.84
RF_2	145	408	302	106	3:1	0.209	3.84
$P_2 \times F_2$	88	260	138	122	1:1	0.984	3.84
$P_2 \times RF_2$	111	260	141	119	1:1	0.862	3.84

Conclusions and Recommendation

The inheritance of purple pericarp colour in maize was investigated by generating a cross and its reciprocal using a purple kernelled maize line Up01 and white kernelled maize line TZi3. Maternal effect was present in the inheritance of purple kernel colour in maize, with a complementary gene action in which the purple pericarp colour was not completely dominant over the transparent phenotype. The genetic constitution of the parental lines determined the expression of purple pericarp colour in maize. Our findings, which contradicts reports on the inheritance patterns of other kernel colours in maize, further demonstrates the considerable variability that exists in the expression of pericarp colours in maize. It is therefore important for the researcher to determine the inheritance pattern of the pericarp colour in the parents to be used.

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Conflict of interest

The authors declare no conflict of interest.

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