Characteristics and Inheritance of Xantha Terminalis in Pearl Millet

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A new mutant phenotype designated xantha terminalis was identified in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. The mutant was indistinguishable from the normal plants until internode elongation, about 50 days after emergence. The top three to five leaves of the mutant were then yellow, while the basal five to seven older leaves remained green. The occurrence of lethal albinos in the selfed progenies is due to the assortment of normal and mutant plastids during gamete formation. The trait was found to be governed by a single recessive nuclear gene, designated as *xt*. The mutant is a good genetic marker and a useful tool for studies on biochemical genetics.

A variety of chlorophyll-deficient mutants have been reported in a number of crop plants, including pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Burton and Powell 1965; Hanna et al. 1978; Koduru and Krishna Rao 1983; Mengesha and Appa Rao 1989). Chlorophyll-deficient mutants in pearl millet have been used to elucidate the structure and function of plastids (Reddy et al. 1988). During the course of evaluating the world collection of more than 21,000 accessions of pearl millet, we identified a new mutant phenotype that has not been previously reported in any crop. This paper describes the morphology and the mode of inheritance of this new mutant in pearl millet.

Materials and Methods

A pearl millet germplasm accession 'IP 8169' collected in Niger produced two mutant plants in a progeny of 85 plants grown at ICRISAT, Patancheru, India. To ascertain whether the mutant condition was due to any mineral deficiency, we grew the mutant plants under controlled conditions with different nutrient solutions. To study the mode of inheritance, we made reciprocal crosses between the normal and the mutant plants during the postrainy season of 1988 by taking advantage of protogyny (Burton 1979). The F_1 was crossed to the normal and to the mutant. We calculated segregation ratios from the F_2 and backcross populations and tested the goodness of fit with chi square.

Results and Discussion

At seedling emergence, the mutant plants were green and were indistinguishable from normal plants for the first 50 days of growth. By that time the plants had produced five to seven normal leaves on the main shoot. During internode elongation, the newly emerging leaves were light green to greenish yellow. The leaves that emerged subsequently tended to be more yellow, and the flag leaf was completely yellow (Figure 1). In mutant plants the inflorescence was yellow at emergence and remained yellow, whereas in normal plants the glumes and involucral bristles that were yellowish green at emergence turned green. The top three to five leaves of the tillers of the mutant plants also emerged as yellow as those in the main shoot. Though the terminal leaves of all the tillers tended to turn green at their leaf base and apex, the middle two-thirds of the leaf blades of the top three to five leaves remained yellow, and the mutant plants were distinguishable until maturity. Seed mass of 1,000 grains of the mutant was reduced to 6 g compared to 8 g in the normal plant. The mutant condition was not due to any mineral deficiency.

The progenies of selfed mutant plants from S_1 to S_{13} produced 0.8% to 4.1% albino seedlings, whereas the rest were mutant type. All the F_1 plants from crosses between normal and mutant plants produced normal green plants. However, in reciprocal crosses where the mutant was used as female, we found 1.8% albino seedlings, which might be due to the maternal transmission of the mutant plastids. Plants in the F_2 generation were easily classified as normal or mutant. Segregation in F_2 was 3 normal : 1 mutant (Table 1), suggesting that the mutant phenotype was governed by a single recessive gene. When the F_1 was backcrossed to the mutant, the backcross F_1 segregated into 1 normal : 1 mutant (Table 1), confirming that the mutant trait was controlled by a single recessive gene. Several reports and summaries of the inheritance of qualitative characters in pearl millet (Burton and Powell 1965; Hanna et al. 1978; Koduru and Krishna Rao 1983; Mengesha and Appa Rao 1989) did not report such a mutant. As the terminal three to five leaves are characteristically yellow with green basal leaves (Figure 1), we propose that the mutant should be designated xantha terminalis with the gene symbol *xt*. In maize, several virescent mutants in which the plants are yellow at emergence and subsequently turn green are known to occur (Neuffer et al. 1968). However, instances in which normal green plants turn yellow at floral initiation appear to be rare.

Even after 13 generations of selfing, xantha terminalis produces 0.8% to 4.1% of albino seedlings. The remaining green plants subsequently change to xantha terminalis. A nuclear gene that controls plastid development therefore seems to be involved in the expression of xantha terminalis. It appears that the embryo of the mutant plant contains both normal and mutant plastids that assort during the formation of gametes: female gametes that

contain exclusively mutant plastids develop into albino seedlings, whereas those that contain both normal and mutant or exclusively normal plastids develop into green plants, which subsequently turn to xantha terminalis. In pearl millet, stem elongation coincides with floral initiation. Since the leaves emerging after floral initiation did not turn green, it is presumed that the plastids lost their ability to develop normally. Some substance whose action coincides with the time of floral initiation appears to govern or block subsequent development of normal plastids. This needs to be investigated further.

Xantha terminalis is a clearly expressed genetic marker that should prove useful in linkage studies. It appears to involve a developmental abnormality of plastids, which is triggered at floral initiation. It should be useful to study the structure, function, and biogenesis of plastids. It could be a possible tool in the study of biochemical genetics, especially the origin of specific proteins.

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Figure 1. Morphology of the xantha terminalis mutant in which the older leaves are green while the terminal three to five leaves are yellow. Green sectors can be seen toward the leaf base and leaf tip.

Table 1. Inheritance of xantha terminalis mutant in pearl millet

Cross	Generation	No. of plants		Ratio	χ^2	*P*
		Normal	Mutant			
Mutant × Normal	F_1	58	0			
Mutant × Normal	F_2	662	204	3:1	1.21	.2–.3
(Mutant × Normal) Mutant	BC_1	178	164	1:1	0.57	.3–.5
(Mutant × Normal) Normal	BC_1	196	0	1:0		
Normal × Mutant	F_1	76	0			
Normal × Mutant	F_2	510	159	3:1	0.54	.3–.5
(Normal × Mutant) Mutant	BC_1	138	149	1:1	0.42	.5–.7
(Normal × Mutant) Normal	BC_1	157	0	1:0		

Melanin Concentrations in Feathers from Wild and Domestic Pigeons

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Vanes from secondary remiges and greater coverts (i.e., feathers that form the wing bars of the wild coloration type) were analyzed for their eumelanin and pheomelanin contents. The samples were taken from wild rock pigeons and from domestic breeds representing different coloration types of known genetic background. The bars in the wild rock pigeons and in similarly colored domestic birds are eumelanic but also contain some pheomelanin. In ash red (B^A) pigeons the bars are pheomelanic with drastically reduced eumelanin concentrations, and in brown (*b*) specimens they are of a pheomelanic to mixed type. Mutant *S* (spread) eliminates the differences in the melanin concentrations between the bars and the adjacent areas, resulting in a uniform coloration. Its effects on eumelanin and pheomelanin contents vary in dependence from the alleles at the color locus and from other genes. Recessive red (*e e*) pigeons are pheomelanic. The effects of the dilution factor (*d*) cannot be described by a single dilution index but depend on combination with other genes. Our findings are consistent with the idea that the greater variation of coloration in domesticated animals than in their wild ancestors is caused by mutations that affect the distribution, amounts, and proportions of pigments but not their chemistry.

Under domestication many internal and external characteristics of plants and animals show a greater variation than in the state of nature (Darwin 1868). The high variation of coloration in domestic animals is the result of mutations and recombination of mutations that have been favored by human selection. The mutations seem to affect the distribution, amounts, and proportions of pigments but not their chemistry (Herre and Röhrs 1970). Sossinka (1982) claimed that "no really new color has been 'invented' by domesticated birds."

The visual impression of feather pigmentation in wild rock pigeons (*Columba livia*) and their domestic counterparts apparently contradicts these statements. As in mammals, there are two types of melanin in birds: eumelanin and pheomelanin (Durrer 1986). The plumage coloration of the wild rock pigeon is bluish-gray with a subterminal black band in the tail and two black bars across the wings (for detail see Goodwin 1970). According to Lloyd-Jones (1915) and Hollander (1938) only one type of pigment, black, is present in the wild *C. livia*. The appearance of "blue" portions of the plumage resembles a half-tone print with each barbule showing striations or clumping of black granules under the microscope. The black portions of the plumage reveal only traces of the clumping or striation, if any; the clumps are so wide as to coalesce These two phases of the blackish pigment have been called "clumping" and "spreading," respectively. "Spreading" can be further subdivided into the "smooth" and the "coarse" type. There is apparently no microscopic difference between them, but they are affected in a different way by several mutants. "Smooth" spreading is found in the black tail band and in the dark ends of the flights, whereas the "coarse" type is shown by the black wing bars.

On the other hand, many domestic breeds exhibit yellowish, reddish, and brownish pigmentations that indicate the existence of pheomelanin which, thus, should have been "invented" during domestication of this species. However, such speculations must be checked by chemical analyses as it is known from mammalian studies that eumelanin and pheomelanin can sometimes be confused phenotypically (Sponenberg et al. 1988b).

Microscopic findings from the beginning of this century are contradictory with respect to the pigment granule types that form the wild rock pigeon's color. In addition to black granules, Spöttel (1914) described and illustrated pigment granules that vary from chocolate color to golden yellow, which Lloyd-Jones (1915) never observed in rock pigeon-colored birds of his colony. Lloyd-Jones suggested that this difference could be due to the fact that Spöttel apparently did not restrict himself to feathers from birds of the wild rock pigeon color. Spöttel also gave no information about the origin of his *C. livia*.

In this study, we measured the eumelanin and pheomelanin concentrations in feathers taken from wild rock pigeons and from domestic breeds representing different coloration types of known genetic background. This will contribute to the solution of the above-mentioned problems and to quantify the effects of some genes that have hitherto been identified by their actions on visual characteristics.

Materials and Methods

Feathers from wild rock pigeons (*C. livia livia*) were taken from birds that have been bred for about 15 years at the Institut für Haustierkunde, University of Kiel. The stock was originally obtained from an aviculturist who had removed squabs from nests of a wild rock pigeon colony in Turkey.

We took feathers from domestic breeds that were bred and kept by the authors (E.H. and A.S.). In all cases inner secondary remiges and greater coverts of the wing from adult pigeons—that is, feathers that form the wing bars of the wild type coloration—were used for pigment analyses. From these feathers we separated the dark and light pigmented parts using scissors and labeled them A and B, respectively (Figure 1). In genotypes with masked bars (*S* or *e e*) we separated the slightly darker distal parts of the vanes (C) from the somewhat paler proximal parts (D) (Figure 1). In specimen no. 11 a distinction of C and D was not possible due to uniformly light pigmentation. In two cases (nos. 8 and 14) the analyzed material corresponded mainly to region C.

We analyzed eumelanin and pheomelanin in feather samples by the method of Ito and Fujita (1985) using chemical degradation and high performance liquid chromatography (HPLC). Approximately 30 mg of a feather sample were homogenized in water at a concentration of 10 mg/ml. For eumelanin estimations, which were performed in duplicate, we transferred 200 μl of homogenate (2 mg feather) to a screw-capped test tube, mixed it with 800 μl of 1 M H_2SO_4, and oxidized the sample with 3% $KMnO_4$. The product, pyrrole-2,3,5-tricarboxylic acid (PTCA), was analyzed by HPLC with ultraviolet detection. For pheomelanin estimation, we transferred 200 μl of the homogenate to a screw-capped test tube and hydrolyzed it at 130°C for 24 h with 500 μl of 57% hydriodic acid in the presence of H_3PO_2. The product, aminohydroxyphenylalanine (AHP), was analyzed by HPLC with electrochemical detection. Contents of PTCA and AHP of 1 ng roughly correspond to a eumelanin content of 50 ng and a pheomelanin content of 5 ng, respectively.

Results and Discussion

The results are given in Table 1.

From sample nos. 1-7 it can be seen that the vane areas forming the bars (A) con-