CP 0124

Indian J. Bot. 7 (1) 1-5, 1984.

CHARACTERISTICS AND INHERITANCE OF VIABLE CHLOROPHYLL MUTANTS IN *PENNISETUM AMERICANUM* (L.) LEEKE

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ABSTRACT

During the course of pearl millet (Pennisetum americanum (L.) Leeke) germplasm evaluation at ICRISAT,. naturally occurring viable mutants affecting chlorophyll development were isolated and maintained by selfing. In zebra (z) mutant, yellow streaks were found only at low temperature. The zebra-virescens (zv) mutant was characterized by transverse yellow bands alternating with green bands on the third to seventh leaf blades which disappear subsequently. All the leaves of the bright-yellow (by) mutant were shiny and deep yellow from emergence to maturity. The chlorina-virescens (chv) mutant produced yellow leaves which turned pale green after flowering. The greenish-yellow (gy) mutant retained its pale-yellow colour from emergence to grain filling. In the albino-terminalis (at) mutant, the terminal 3-5 leaves remained ivory-white till maturity, while the basal leaves were green throughout. All the mutants except albino-terminalis could be identified in the seedling stage. In all the mutants, flowering was delayed compared to the respective normal genotypes except in the chlorina-virescens. From the reciprocal crosses between the normal and mutant genotypes it was established that in each case the mutant condition was governed by a single recessive gene. As most of the mutants can be identified from germination, they can be used as seedling markers for mapping chromosomes.

Keywords: Pearl millet; chlorophyll mutants; Zebra; Zebra-Virescens; Chlorina-Virescens; segregation; gene symbols.

INTRODUCTION

Pearl millet, *Pennisetum americanum* (L.) Leeke being a highly cross-pollinated crop, carries a large number of chlorophyll- deficient mutants (Harinarayana *et al.*, 1969). Such mutants are exposed in the selfed progenies as most of the mutants are recessive (Blixt, 1961). During the course of pearl millet germplasm evaluation at ICRISAT, some naturally occurring viable mutations affecting chlorophyll development were observed, isolated and maintained by selfing.

As these mutants appear to be different from those already described by Hanna *et al.*, (1978), this paper deals with the characteristics of six chlorophyll deficient mutants and their mode of inheritance in pearl millet.

MATERIAL AND METHODS

Over 15,000 pearl millet germplasm accessions from 32 countries assembled at ICRISAT are being maintained by selfing, sibbing or cluster bagging (Appa Rao, 1980) and about 500 g seed is stored in plastic containers at 4^{0} C and 35% relative humidity. A sub-sample of this seed is used for subsequent evaluations. All the mutants reported in the present study were recovered from the primitive cultivars collected from the farmers' fields and multiplied only once by cluster bagging except IP 1995 which was

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assembled by the Rockefeller Foundation. The mutants were named according to the system of nomenclature suggested by Burton and Powell (1965). The colour of the leaf blades was described with reference to the colour chart developed by the Royal Horticulural Society, London. The morphological characteristics of the mutants were studied at the ICRISAT Center, Patancheru, during the rainy season (kharif) utilising the descriptors of pearl millet (ICRISAT / Temperature sensitivity was IBPGR, 1981). studied from fortnightly sowings in sand boxes. Reciprocal crosses between the normal and the mutant were made taking advantage of protogyny as described by Burton (1980), The F2 generation was raised in sand boxes and 15 day-old seedlings were classified into normal or mutant phenotypes except the albino-terminalis which was scored at flowering stage.

RESULTS AND DISCUSSION

1. Zebra : The mutant was isolated from IP 7402 collected in 1979 from Tanzania. Transverse Lemon Yellow (14C) streaks alternating with green were found on the leaf blade. leaf sheath and internode. The characteristic streaks developed only after December (coldest month) irrespective of the planting dates. The Zebra mutant when planted in June did not produce any streaks, but the tillers developing in December produced streaks. This may be due to sensitivity to low temperature. The mutant was as vigorous as the normal plants. Such temperature sensitive mutants were reported in maize (Rumball and Grogan, 1972).

Crosses between normal and mutant produced normal F_1 plants. The F_2 plants segregated into 3:1 ratio suggesting that the mutant was monogenic-recessive to the normal. The gene symbol Z was proposed for this trait.

2. Zebra-virescens : The mutant was recovered from IP 8684 collected in 1979 from Sudan. The mutant developed Primrose Yellow (4C) transverse bands alternating with green (146C) bands giving a characteristic appearance. Though the transverse bands were seen from the third leaf onwards, the yellow bands were very clear on the sixth and seventh leaves and the subsequent leaves were normal. At this stage, the yellow bands spread on either side, decreased in intensity and tended to turn green. Though the mutant was slow growing in the beginning it was as vigorous as the normal at. maturity. The mutant produced many but short and thin spikes compared to the normal plants (Table 1).

The F_1 plants from the reciprocal crosses between the normal and mutant were normal, suggesting that the mutant was recessive. In the F_2 generation, there was segregation of normal and mutant types in 3:1 ratio (Table 2) suggesting a single recessive gene. The gene symbol zv was proposed for this character.

3. Bright-yellow : A downy mildew resistant selection IP 8990 from Samara, Nigeria segregated into bright-yellow and normal plants. The mutant developed bright-yellowish green foliage with shiny leaf surface which was observed from emergence till maturity making it distinguishable from others at all stages of growth. The leaf blade colour at its maximum expression (at head emergence) was Mimosa. Yellow (8B). The leaf sheath and stem were also yellow, though to a lesser extent. The mutant was slow growing with reduction in leaf blade length, width, plant height and spike length (Table. 1).

Reciprocal crosse between the normal and the mutant produced normal F_1 hybrid Plants in the F_2 generation were readily classified into normal and mutant in the ratio of 3 :1 (Table 2). The gene symbol by was proposed for this trait.

4. Chlorina-virescens: The mutant was isolated from IP 5335, a traditional cultivar locally called 'zongo' collected in 1977 from Niger. The mutant was distinguishable soon after germination and persisted till maturity. The leaves were yellowish-green (146B) which gradually turned to light green after flowering. The mutant flowered a week before the normal plants, accompanied by reduction in plant height (Table 1) as compared to the normal plants. Reciprocal crosses between the normal and the mutant produced normal green F_1 plants suggesting that the mutant was recessive. The F_2 plants segregated into normal and mutant in a monogenic ratio of 3 :1 (Table 2). The gene symbol *chv* has been proposed for this character.

5. Greenish-yellow: The mutant was recovered from IP 1995 introduced from Nigeria in 1964. The mutant produced Paleyellow foliage (10y) that was recognizable since plant emergence and persisted through maturity. The mutant flowered a week later accompanied by reduction in plant height and spike length (Table 1).

TABLE 1. Mor	phological	differences	between	normals	(N)	and	mutants	(M)*
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Character	Zebra- virescens	Bright- yellow	Chlorina- virescens	Greenish- yellow	Albino- terminalis
Days to 50% flowering —N —M	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrr} 60 \ \pm \ 0.3 \\ 60 \ \pm 0.3 \end{array}$	$\begin{array}{rrrr} 74 \ \pm \ 0.3 \\ 62 \ \pm \ 0.3 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Plant height (cm) -N -M	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 293 & \pm & 9.1 \\ 232 & \pm 12.7 \end{array}$	$\begin{array}{rrrr} 123 \ \pm \ 9.0 \\ 96 \ \pm \ 5.3 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Spike length (cm) —N —M	$\begin{array}{rrrr} 20 & \pm & 1.4 \\ 17 & \pm & 1.0 \end{array}$	$\begin{array}{rrrr} 27 \ \pm \ 0.9 \\ 20 \ \pm \ 0.3 \end{array}$	$\begin{array}{rrrr} 73 \ \pm \ 3.4 \\ 65 \ \pm \ 5.6 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	31 ± 4.1 19 " \pm -1.6
Spike thickness (mm) —N —M	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 24 & \pm & 1.6 \\ 21 & \pm & 1.2 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 16 \ \pm \ 1.5 \\ 15 \ \pm \ 2.6 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Stem thickness (mm) —N —M	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{cccc} 9 & \pm & 1.2 \\ 7 & \pm & 1.2 \end{array}$	$\begin{array}{rrrr} 12 \ \pm \ 1.2 \\ 12 \ \pm \ 0.1 \end{array}$	$\begin{array}{cccc} 6 & \pm & 1.2 \\ 6 & \pm & 1.0 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Leaf blade length (cm) —N —M	$\begin{array}{rrrr} 61 & \pm & 5.6 \\ 53 & \pm & 6.9 \end{array}$	$\begin{array}{rrr} 62 \ \pm \ 2.0 \\ 56 \ \pm 4.2 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	49 ± 3.5 56 ± 8.1	$\begin{array}{rrrr} 78 \ \pm \ 1.7 \\ 79 \ \pm \ 4.6 \end{array}$
Leaf blade width (mm) —N —M	$\begin{array}{rrr} 37 & \pm 3.9 \\ 28 & \pm & 2.0 \end{array}$	$\begin{array}{rrrr} 34 \ \pm \ 1.0 \\ 31 \ \pm \ 4.7 \end{array}$	$\begin{array}{rrrr} 43 & \pm & 1.7 \\ 45 & \pm 2 . 2 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 44 & \pm & 1.0 \\ 33 & \pm & 1.5 \end{array}$
Total tillers (no.) -N -M	$8 \pm 1.5 \\ 17 \pm 1.4$	$\begin{array}{ccc} 5 & \pm & 0.7 \\ 2 & \pm 0 . 7 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 4 & \pm & 1.3 \\ 4 & \pm & 0.1 \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Grain yield potential** —M	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 6 \ \pm \ 0.0 \\ 4 \ \pm \ 0.3 \end{array}$	$\begin{array}{rrrr} 6 & \pm & 0.3 \\ 6 & \pm & 0.3 \end{array}$	$\begin{array}{rrrr} 6 & \pm & 0.3 \\ 5 & \pm & 0.3. \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

* Planted on June 22, 1982 with three replications.

** Visual score on. 1-9 scale where 3 = poor, 5 = average and <math>7 = good.

The F_1 plants from the reciprocal crosses were normal indicating that the mutant was recessive. In the F_2 generation, monogenic segregation ratio of 3:1 was observed (Table 2). The gene symbol gy was proposed for. this character.

6. Albino-terminalis : The selfed progeny of IP 5440 collected in 1977 from Niger segregated for mutant and normal. The mutant resembled the normal plants till flower initiation. The top 3-5 leaves including the bristles and glumes of the inflorescence remained Amber Yellow (18C) which gradually turned light green at maturity. The mutants showed a reduction in height and spike length, and flowered 13 days later.

In the selfed progenies of the mutant, varying proportions of *albino* plants were found along with the mutant type. The F_1 plants obtained from reciprocal crosses were normal suggesting that the mutant was recessive. In the F_2 generation, there was segregation for normal and mutant in a ratio of 3 : 1 (Table 2). The gene symbol *at* was proposed for this trait.

In general, the mutants flowered later accompanied by reduction in plant and spike length except the chlorina-virescens which flowered a week earlier than the normal. The mutants were less competitive than the normal green plants when grown in thick plant population. However, in optimum specing (75 x 15cm), they were viable and produced enough seed.

Each of the mutant was determined to be controlled by a single pair of homozygous recessive genes. However a deficit of the mutant type was observed in all cases which might be due to environmental effects (Burton and Powell, 1965) or differential distribution of plastids during the first pollen mitosis (Hagemann, 1979). The occurrence

TABLE 2.	F_2	segregation	of	the	reciprocal	crosses	between	normal	and	various	chlorophyll	mutants.
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Parents	Progenies		No.	of F_2 plan	ts		P value	
Farents			Normal	Mutant	Albino*	$(3:1)^{X_2}$		
Zebra X Normal		2	230	74	0	0.070	0.70-0.80	
Normal X Zebra	_	1	110	29	0	1.269	0.20-0.30	
Zebra-virescens X Normal		2	394	98	11	2.975	0.05-0.10	
Normal X Zebra-virescens		3	105	13	5	7.049	0.02-0.05	
Bright-yellow X Normal		5	1198	389	15	0.041	0.80-0.90	
Normal X Bright-yellow		4	536	191	0	0.628	0.80-0.90	
Chlorina-virescens X Normal	_	5	538	170	21	0.560	0.30-0.50	
Normal X chlorina-virescens		4	622	169	24	0.756	0.30-0.50	
Greenish-yellow X Normal		5"	741	263	1	0:966	0.30-0.50	
Normal X Greenish-yellow	—	3	548	203	0	1.652	0.10-0.20	
Albino-terminalis X Normal	_	2'	318	118	0	0.991	0.30-0.50	
Normal X Albino-terminalis		.2	354.	124	0	0.226	0.50—0.70	

* Considered as mutant for calculating the probability.

of lethal white seedlings in the progenies of mutants may be due to the presence of completely mutant plastids. Such seedlings called *albino* were reported to be controlled by a single recessive gene (Rangaswami Ayyangar and Hariharan, 1935) or two recessive genes. (Kadam *et al.*, 1940).

Several naturally occurring chlorophyll deficient mutants controlled by a single recessive gene were reported (Burton and Powell 1965: Athwal et al., 1966: Gill, 1971: Minocha et al., 1980 and Koduru and Rao. 1980). Induced chlorophyll mutants were also controlled by a single recessive gene (Krishnaswami et al, 1942; Krishnaswamy, 1962: Chandola et al., 1963; Joshi, 1968 and Tara Mohan et al., 1973. The occurrence of longitudinal yellow stripes alternating with green stripes on the leaf blades was reported to be due to the interaction of two or three nuclear genes (Gill et al., 1969), while Rao and Koduru (1978) reported non-Mehdelian biparental plastid inheritance. Of all the mutants, only yellow and light-green mutants (Hanna et al., 1978) were non-lethal and their morphological description resembles to some extent the morphology of bright-vellow and greenish-vellow mutants described here. Hence, there is need to establish their allelic relationships. Rest of the four mutants were characterized and the mode of inheritance was established, perhaps, for the first time. As all the mutants except the albino-terminalis are distinguishable soon after germination and have normal fertility, they can be used as seedling markers.

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