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Effects of Mutant Cytoplasmic Factors on the Expression of Agronomic Traits in Cowpea, *Vigna unguiculata* (L.) Walp.

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Abstract

Nuclear genes have to a large extent been exploited in genetic cowpea improvement, but little is known about the effects of cytoplasmic mutations on agronomic traits. This study investigated the effect of cytoplasmic factors agronomic traits in crosses between two cytoplasmic mutants and some nuclear cowpea lines. The experiment was conducted at Department of Crop Protection Environmental Biology, University of Ibadan. A yellow variant derived from the sorting-out of mutant plastids of mixed cells lineage (IB-Y-Cyt.) was crossed to four normal green lines and a variegated mutant in all possible combinations. F_1 and reciprocal-F_1 progenies from the crosses and six generations comprising parents, F_1, F_2 and their reciprocals from four selected crosses were evaluated in pots using Randomized Complete Block Design with four replicates. Data were collected on the following quantitative traits; days to flowering and ripe pod, pod length and seed traits. Data were analyzed using t-test and correlation coefficient analysis. Crosses involving the cytoplasmic mutants as female parents had negative effects on most of the traits studied. The mutant cytoplasms had negative effects on traits affecting the productive capacity of the plants as well as related traits in the F_1 generation, these effects in turn transcend to most of the quantitative traits leading to poor seed formation especially in the yellow cytoplasmic mutant.

Keywords: Cytoplasmic mutation, Agronomic traits, Cowpea, Uniparental, Reciprocal differences.
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Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. - a leguminous crop, is a self pollinating herbaceous annual, believed to have originated from Africa due to the great genetic diversity of its germplasm found in this region (Ng and Maréchal, 1985). It is an important source of protein for most people in the tropical and subtropical regions of the world. Optimum cowpea production is limited by many factors such as, pests and diseases throughout its life cycle, problems associated with soil such as water lodging and soil acidity, inadequate cultural practices, yield barriers which arise from the uncertainty about the definition of high-yield ideotypes. Breeding varieties to overcome or ameliorate many of these constraints has been a major goal of many cowpea breeding programmes (Rachie, 1985).

Most agriculturally important traits are controlled by genes outside the nucleus. Consequently, major efforts of plant breeders and geneticists have been focused on nuclear genes. Cytoplasmically inherited traits (traits outside the nucleus or extrachromosomal traits) however are transmitted via the cytoplasm as distinct from nuclear inheritance (traits within the nucleus or chromosomal inheritance). Cytoplasmic traits can be detected by differing contributions of male and female parents in during reciprocal crosses.

Interactions between normal nuclear and cytoplasmic factors (leaf colour mutation) may result in pigment deficiencies (Stubbe, 1964; Wu *et al.*, 2014). However, most pigment deficiencies are the direct result of either nuclear or cytoplasmic mutations (Shoemaker *et al*., 1985). The combinations of nuclear and cytoplasmic components can result in abnormal plastid development and reduction in leaf pigment content (Schotz, 1970; Zhang *et al*., 2017). Cytoplasmically transmitted traits had been reported in crops such as; *Zea mays* (Bosacchhi et al., 2015); *Glycine max* (Hatfield *et al*., 1985); *Vigna unguiculata* (Porbeni and Fawole, 2012); *Gossypium hirsutum* (Han *et al.*, 2008; Zhang *et al*., 2009).

Plastids associated with morphological or biochemical markers had been used with varying successes to describe the sexual transmission of extrachromosomal organelles (Tilney-Bassett and Birky, 1981). Cytoplasmic male sterile mutants in maize have greatly facilitated hybrid seed production (Allard, 1960; Allen, 2005). Resistance to herbicides, for example atrazine, due to modifications in the plastid membrane that prevents atriazine binding thereby conferring resistance had been documented in *Brassica campestris* (Wu et al., 2006); *Oryza sativa* (Harisprasauna et al., 2004). Cytoplasmic effect had both positive and negative for days to flowering, plant height and grain yield in rice (*Oryza sativa*) [Young and Virmani, 1990]. Information on cytoplasmic and nuclear gene interaction and their consequent effects on yield related traits in cowpea however are limited. In order to utilize both nuclear and cytoplasmic mutants effectively during crop improvement, a thorough understanding of how they affect agronomic and yield related traits is necessary. The objective of this study therefore was to determine the effect of cytoplasmic and nuclear mutants interaction on some agronomic traits of cowpea.

MATERIALS AND METHODS

Origin of normal and cytoplasmic mutant lines.

The origin and descriptions of normal parents (green foliage); Ife-BPC, Ife Brown Crinkled (IF-BR-Cr) and IBS 4474 were reported by Fawole and Afolabi, 1983; Fawole (1997 and 2000); and the cytoplasmic mutants (variegated and yellow) by (Fawole, 2001; Porbeni and Fawole, 2012) respectively.

Cross-compatibility test

In order to test for reciprocal differences among crosses of normal and cytoplasmic mutants of cowpea and effect on agronomic traits, hybridization was achieved using the method of Rachie et al., (1975), to obtain crosses in all possible combinations with reciprocals for the six parental lines. A preliminary cross-compatibility test was carried out on the cytoplasmic mutants, IB-Y-Cyt. and IB-Var-3 (yellow and variegated mutants), crossed to each of the lines, that is; Ife –BPC, IBS 447, IF-BR-Cr (nuclear mutants) and G10603 (breeding line- normal). Crosses involving IB-Y-Cyt. as female parent and Ife BPC, IBS 4474 led to partial sterility (fewer seeds), while reciprocal crosses with the cytoplasmic mutants produced well filled seeded pods (adequate seeds for the inter-character relationship studies). Ife BPC and IBS 4474 crosses with the cytoplasmic variant however, could not be used for further studies due insufficient number of seeds.

The cross population comprising the F_1 crosses, their reciprocals (RF_1) and the parental lines were evaluated on the rooftop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria, in a Randomized Complete Block Design, with four replicates. Three seeds of each line were planted in a 35cm diameter pot filled with garden soil. A replicate consisted of 15 pots planted to the F_1, 15 pots to the reciprocal F_1 and six to the parental lines, to give a total of 144 pots. Two seeds per buckets were planted to 100 pots each for the F_2 and its reciprocals to give a total of 200 pots per replicate. Cultural practices including daily watering and pest management were carried out. Data were collected on the following quantitative traits: number of days from sowing to first flower and to first ripe pod, pod length (cm), number of seeds per pod, and 100-seed weight (g).

Data were subjected to analysis of variance to test for genotypic differences among the lines using the General Linear Model (GLM) procedure of the Statistical Analysis (SAS 2008) programme, after which pair-wise comparison of each F_1 mean and its reciprocal was carried out using the t-test. Inter-character relationships among traits in the F_2 generation was carried with using correlation coefficient analysis.

Results

Effects of cytoplasmic factors on some agronomic traits of cowpea.

Comparison of reciprocal F_1 progenies for mean number of days from sowing to first flower and ripe pod is presented on Table 1. Number of days to first flower ranged from 45.75 days (IF-BR Cr x IBS 4474) and 72.00 days (IB-Y-Cyt. x G10603), while IB-Y-Cyt. x IB-VAR-3 and IB-Y-Cyt. x IBS 4474 (93.00 and 59.25 days) to first ripe pod respectively. The cytoplasmic mutants as female parents recorded longer number of days to first flower and consequently longer days to first ripe pod for all crosses except, IB-Y-Cyt. x IF-BR-Cr (0.222) and IB-Y-Cyt. x Ife-BPC (0.117) where no significant effect was observed. Crosses involving all the green x green lines did not show significant reciprocal difference for both number of days to first flower and ripe pod.

Table 1: Comparison by t-test of reciprocal F_1 progenies for mean number of days to first flower and first ripe pod in crosses involving two cytoplasmic mutants and four normal cowpea lines.

Crosses [a]	Number of days to first flower (days)				Number of days to first ripe pod (days)			
	$\bar{F}_1$	$\overline{RF}_1$	$\bar{F}_1 - \overline{RF}_1$ [b]	P-value	$\bar{F}_1$	$\overline{RF}_1$	$\bar{F}_1 - \overline{RF}_1$ [b]	P-value
IB-Y-Cyt. x G10603	72.00	67.50	4.50	0.023**	87.00	76.25	10.25	0.054**
IB-Y-Cyt x IF-BR-Cr	55.50	45.50	10.00	0.001***	78.50	63.25	15.25	0.222
IB-Y-Cyt. x IB-Var-3	68.25	56.25	12.00	0.002**	93.00	74.00	19.00	<0.001***
IB-Y-Cyt x IBS 4474	57.75	45.50	12.25	0.001**	80.00	59.25	20.75	<0.001***
IB-Y-Cyt x Ife-BPC	62.25	54.00	8.25	0.028*	83.00	70.00	13.00	0.117
IB-Var-3 x G10603	63.00	55.00	8.00	0.033**	81.25	74.50	6.75	0.095**
IB-Var-3 x IF-BR-Cr	54.00	41.00	13.00	0.020**	68.25	58.00	10.25	0.006**
IB-Var-3 x IBS 4474	60.50	45.00	15.50	0.001***	77.00	65.00	12.00	<0.001***
IB-Var-3 x Ife-BPC	71.25	51.75	19.50	<0.001***	92.25	66.00	26.25	0.383
G10603 x IF-BR-Cr	50.75	48.00	2.75	0.459	70.25	66.50	3.75	0.726
G10603 x Ife-BPC	50.75	53.75	-3.00	0.420	69.25	70.75	-1.50	0.383
G10603 x IBS 4474	54.00	49.00	5.00	0.180	70.25	66.50	3.75	0.641
Ife-BPC x IBS 4474	50.25	49.25	1.00	0.788	69.25	67.25	2.00	0.954
Ife-BPC x IF-BR-Cr	47.25	47.00	0.25	0.946	64.75	64.50	0.25	0.954
IF-BR-Cr x IBS 4474	45.75	45.75	0.00	1.000	64.00	64.75	-0.75	0.861

a First named parent = female, second named parent = male
b F_1 means compared with RF_1 means
*** significant at 0.1%, ** at 1%, * at 5% probability levels by t-test,

All crosses involving the cytoplasmic mutants as male parents produced longer pods; except IB-Y-Cyt. x IB-Var-3 and IB-Y-Cyt. x Ife-BPC which showed no significant reciprocal difference for pod length (0.163 and 0.117) respectively. However, the cytoplasmic mutants as female parents led to a decrease in pod length, except IB-VAR-3 x Ife- BPC (11.15cm). All crosses involving the IB-VAR-3 x green lines showed significant reciprocal effect except IB-Var-3 x Ife-BPC, for number of seeds per pod. All F_1 crosses involving the IB-Y-Cyt. mutants were reciprocally different, while other F_1 progenies except IB-Var-3 x IF-BR-Cr, IB-Var-3 x IBS 4474 and IB-Var-3 x Ife-BPC showed no significant reciprocal difference. IF-BR-Cr x IBS 4474 was the only green x green cross that showed significant reciprocal difference for number of seeds per pod (Table 2).

Table 2: Comparison by t-test of reciprocal F_1 progenies for mean pod length (cm) and number of seeds per pod in crosses involving two cytoplasmic mutants and four normal cowpea lines.

	Pod length (cm)				Number of seeds per pod			
Crosses [a]	$\bar{F}_1$	$\overline{RF}_1$	$\bar{F}_1 - \overline{RF}_1$ [b]	P-value	$\bar{F}_1$	$\overline{RF}_1$	$\bar{F}_1 - \overline{RF}_1$ [b]	P-value
IB-Y-Cyt. x 10603	11.41	16.95	-5.54	<0.001***	5.56	12.28	-6.72	<0.001***
IB-Y-Cyt x IF-BR-Cr	11.70	15.06	-3.36	0.026*	6.93	9.47	-2.54	0.004**
IB-Y-Cyt. x IB-Var-3	10.70	14.46	-3.76	0.163	4.23	8.91	-4.68	<0.001***
IB-Y-Cyt x IBS 4474	11.94	15.24	-3.30	0.026*	5.48	8.37	-2.89	0.001***
IB-Y-Cyt x Ife-BPC	12.49	14.29	-1.80	0.117	5.83	8.75	-2.92	<0.001***
IB-Var-3 x G10603	13.51	17.99	-4.48	0.008***	8.29	11.49	-3.20	<0.001***
IB-Var-3 x IF-BR-Cr	14.03	14.38	-0.35	0.125*	9.66	10.63	-0.97	0.117
IB-Var-3 x IBS 4474	10.59	14.35	-3.76	0.003**	6.41	8.36	-1.95	0.024
IB-Var-3 x Ife-BPC	11.15	11.04	0.11	0.759	7.78	7.71	0.07	0.953
G10603 x IF-BR-Cr	15.68	15.18	0.50	0.941	11.98	12.66	-0.68	0.423
G10603 x Ife-BPC	14.91	14.98	-0.07	0.616	10.40	10.51	-0.11	0.900
G10603 x IBS 4474	15.64	15.85	-0.21	0.735	9.08	9.60	-0.52	0.540
Ife-BPC x IBS 4474	11.19	12.26	-1.07	0.274	6.11	9.94	-3.83	0.336
Ife-BPC x IF-BR-Cr	12.71	12.41	0.30	0.813	9.34	10.29	-0.95	0.269
IF-BR-Cr x IBS 4474	12.79	11.89	0.90	0.603	8.21	7.13	1.08	0.024*

a First named parent = female, second named parent = male
b
F_1 means compared with RF_1 means
*** significant at 0.1%, ** at 1%, * at 5% probability levels by t-test,

A comparison of reciprocal F_1 progenies for mean 100 -seed weight is presented in Table 3. Six reciprocal F_1 progenies, four involving the IB-Y-Cyt. as a parent and the other two involving IB-Var-3 as a parent, showed significant reciprocal effects. All other crosses did not exhibit significant reciprocal difference.

Table 3: Comparison by t-test of reciprocal F_1 progenies for mean 100- seed weight (g) of crosses involving two cytoplasmic mutants and four normal cowpea lines

Crosses [a]	$\bar{F_1}$	$\bar{RF_1}$	$\bar{F_1}$ - $\bar{RF_1}$ [b]	P-value
IB-Y-Cyt. x G10603	13.90	19.89	-5.99	<0.001***
IB-Y-Cyt x IF-BR-Cr	10.45	16.22	-5.77	0.004***
IB-Y-Cyt. x IB-Var-3	7.53	15.71	-8.18	<0.001***
IB-Y-Cyt x IBS 4474	13.63	19.44	-5.81	<0.001***
IB-Y-Cyt x Ife-BPC	18.79	16.88	1.91	0.172
IB-Var-3 x G10603	12.83	13.33	-0.50	0.757
IB-Var-3 x IF-BR-Cr	9.57	13.48	-3.91	0.752
IB-Var-3 x IBS 4474	10.83	14.36	-3.53	0.031*
IB-Var-3 x Ife-BPC	12.16	8.77	-3.39	0.038*
G10603 x IF-BR-Cr	12.24	12.80	-0.56	0.723
G10603 x Ife-BPC	13.10	15.31	-2.21	0.174
G10603 x IBS 4474	13.99	15.13	-1.14	0.483
Ife-BPC x IBS 4474	11.98	13.13	-1.15	0.476
Ife-BPC x IF-BR-Cr	15.62	16.29	-0.67	0.675
IF-BR-Cr x IBS 4474	14.45	11.49	2.96	0.069

a First named parent = female, second named parent = male
b
F_1 means compared with RF_1 means
*** significant at 0.1%, ** at 1%, * at 5% probability levels by t-test

Correlation coefficients of some agronomic traits in four cowpea crosses.

Inter-character correlation coefficients between some agronomic traits studied in the F_2 generation of four crosses are shown on (Tables 4 and 5). Number of days to first flower was highly significant and positively correlated with number of days to first ripe pod in all the crosses, a similar trend was observed for pod length with number of seeds per pod.

Plant height was negatively correlated with most of the traits studied except number of seeds per pod (r= 0.259) and 100-seed weight (r= 0.246). Number of days to first ripe pod also had a negative relationship with all the characters studied except number of days to first flower. Pod length was strongly and positively correlated with number of seeds per pod and 100-seed weight (r= 0.551 and 0.290 respectively) Table 4.

A positive relationship was recorded among all the traits except, number of days to first flower and number of seeds per pod (r= -0.015) and number of seeds per pod with 100-seed weight (r= -0.013) in the cross IB-VAR-3 x IF –BR-Cr. In the cross between IB-VAR-3 x G10603, number of days to first flower was negatively correlated with pod length (r= -0.043) and number of seeds per pod (r= -0.019), number of days first ripe pod was also negatively correlated with pod length (r= -0.071) and number of seeds per pod (r= -0.025). A positive association was however observed among all the traits studied.

Table 4: Correlation coefficient of some quantitative traits studied in crosses between the cytoplasmic mutant and two normal green lines.

IB-Y-Cyt. x IF-BR-Cr	**Plant hgt @ 6wks (cm)**	**Days to 1st flower**	**Days 1st ripe pod**	**Pod length (cm)**	**Seeds per pod**	**100-seed weight (g)**
Plant hgt @ 6wks (cm)	-					
Days to 1st flower	-0.332*	-				
Days 1st ripe pod	-0.432*	0.772***	-			
Pod length (cm)	0.259	-0.277	-0.336*	-		
Seeds per pod	-0.090	-0.056	-0.264	0.551***	-	
100- seed weight (g)	0.246	-0.150	-0.003	0.290	0.024	-
IB-Y-Cyt x G10603						
Plant hgt@ 6wks	-					
Days to1st flower	-0.292	-				
Days 1st ripe pod	-0.484**	0.650***	-			
Pod length (cm)	0.516**	-0.273	-0.330*	-		
Seeds per pod	0.375*	-0.239	-0.512**	0.662***	-	
100- seed weight (g)	0.348*	0.068	0.006	0.447*	0.010	-

P $\leq$0.001 (***) , 0.01 (**) , 0.05 (*).

Plant hgt @ 6wks: Plant height at 6 weeks; Days to 1st flower: days to first flower; Days to 1st ripe: days to first ripe pod.

Table 5: Correlation coefficient of some quantitative traits studied in crosses between a variegated cytoplasmic mutant and two normal green lines.

IB-VAR-3 x IF-BR-Cr	**Days to 1st flower**	**Days 1st ripe pod**	**Pod length (cm)**	**Seeds per pod**	**100-seed weight (g)**
Days to 1st flower	-				
Days 1st ripe pod	0.946***	-			
Pod length (cm)	0.056	0.004	-		
Seeds per pod	-0.015	0.033	0.675***	-	
100- seed weight (g)	0.148	0.090	0.078	-0.013	-
IB-VAR-3 x G10603					
Days to 1st flower	-				
Days 1st ripe pod	0.9843**	-			
Pod length (cm)	-0.043	-0.071	-		
Seeds per pod	-0.019	-0.025	0.719**	-	
100- seed weight (g)	0.023	0.021	0.072	0.158	-

P $\leq$0.001 (***) , 0.01 (**).

Plant hgt @ 6wks: Plant height at 6 weeks; Days to 1st flower: days to first flower; Days to 1st ripe: days to first ripe pod.

Discussion

The most common class of mutants in higher plants are lethal or sublethal mutants that are white, yellow or pale green which often arise as a result of blocks in either the pathway of chlorophyll synthesis or of the synthesis of accessory pigments (Nelson, 1967; Zhang, 2017). Bernard and Weiss (1973), and Palmer and Klein (1987) however documented many examples of mutants with various degrees of chlorophyll deficiency that are viable and fertile.

In soybean, at least 36 single-gene recessive nuclear mutants and 8 cytoplasmic mutants with various levels of chlorophyll deficiency have been reported (Sanders, 1960; Fawole, 2003). The cytoplasmic yellow foliage mutant in this study is probably the first of its kind and the second cytoplasmically inherited mutant to be reported in cowpea.

This mutant could possible be used in physiological and biochemical studies, the cowpea cytoplasmic mutants may also be useful in assessing variability in chloroplast genome of cowpea.

Reciprocal differences in quantitatively inherited agronomic traits observed in the F_1 generation of crosses between the two cytoplasmic foliage colour mutants and normal lines derived their cytoplasm from the seed parent and thus, the cytoplasm may exert some influence on phenotypic expression and possibly agronomic traits. The effect of the mutant cytoplasm increased the number of days from planting to first flower and to first ripe pod while phenotypic values for pod and seed characters were decreased. The most conspicuous effects of the mutant cytoplasm was found in the number of days from planting to first flower and the mean number seeds per pod. Effect of the source of cytoplasm of the F_1 varies for number of days from planting to first ripe pod, pod length and 100-seed weight. The varied influence of the two mutant cytoplasms in reciprocal F_1 crosses probably result from the differences in cytoplasmic source and the nuclear background of the mutant lines as well as the contributory parent (Bosacchi et al., 2015). The mutant cytoplasms had negative effects on traits affecting the productive capacity of the plant as well as related traits of the F_1 hybrids compared with the normal cytoplasm of the normal cowpea lines.

The effects of mutant cytoplasm have been reported in several crop species including maize (Bosacchi et al., 2015), and rice (Hariprasanna et al., 2004). These effects may be favourable or unfavourable depending on the type of cytoplasmic factors involved and the traits under consideration Hariprasanna et al., 2004). The cytoplasmic factors reported in this study have their primary effects on cowpea foliage colour which in turn affected negatively the expression of quantitatively inherited traits.

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