

THE GENETICS OF *CORCHORUS* (JUTE)VI. INHERITANCE OF A NEW ANTHOCYANIN PIGMENTATION PATTERN IN *CORCHORUS CAPSULARIS*

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INTRODUCTION

Patel, Ghose & Das Gupta (1944) described seven pigmentation patterns in *Corchorus capsularis*. Later on, Ghose, Rao & Ghosh (1947) added to the list a new anthocyanin pigmentation type bringing the total of such types to eight.

The eight anthocyanin pigmentation types are classed into three groups, viz. (1) full-green, (2) green-pigmented and (3) red. Three loci are involved in the production of these patterns and the genes concerned are:

C-c=chromogen gene fundamental for the production of colour. **C**=pigmented; **c**=full-green.

A^R-A^L-A-a=a multiple allelomorphous series controlling the intensity and distribution of pigment. **A^R**=red; **A^L**=light red; **A**=coppery red and **a**=no colour effect on the stem but delayed light coppery red pigment on petiole, veins, etc.

R-r=pigment reducer factor pair, the effect of which is most markedly seen on the stem. **R**=reduction of colour; **r**=no reduction of colour.

All the members of the full-green group are phenotypically alike and carry the recessive chromogen gene **c**. Between members of the group, the difference lies in the combinations of the particular alleles of the multiple allelomorphous series and the pigment reducer factor pair. They can be identified by test-crosses with coppery red (**CCAarr**).

While the dominant chromogen gene **C** is basic to all the pigmented types, the different pigmentation patterns met with in *C. capsularis* are results of interaction between the members of the multiple allelomorphous series and the pigment reducer factor pair.

Interaction between **A^R-A^L-A-a** and **R** produces green-pigmented types with their characteristic uneven distribution of the pigment on the stem. The members of the red group carry the recessive reducer gene **r**, and in these there is no reduction of pigment on the stem, the intensity and pattern of the type being determined solely by the allele of the anthocyanin multiple allelomorphs present.

Between members within a particular pigmented group, there is always a single gene difference. Between members of the green-pigmented and the red groups, the difference may be monogenic or digenic; while between members of the full-green group and either of the pigmented groups, the difference may be monogenic, digenic or trigenic.

Ghose, Rao & Kundu (1948), while studying the linkage relations of bitter taste, anther and corolla colour, mentioned yet another pigmentation type in *C. capsularis* that may be considered to be the highest member of the red group. The studies reported here deal with this new pigmentation type and its relationship with the known ones.

MATERIALS

The new *capsularis* pigmentation type, hereafter called dark-red, was isolated from a material called Maniksari, collected from Chittagong hill tracts. Besides being dark-red, this new type is characterized by the absence of the bitter glucoside peculiar to *C. capsularis* and also by its dwarf bushy habit, early maturity and pale yellow corolla.

As already mentioned, the dark-red type is marked by the more intense development of red pigment on the different parts of the plant body such as stem and branches, stipule, upper and lower surfaces of the petiole, leaf margin, veins, outer surface of calyx and capsules. In fact, it represents the maximum development of the anthocyanin pigmentation in *C. capsularis* at present known.

The known pigmentation patterns employed in the investigation are given below:

Pigmentation pattern	Genotype	Strain
	Full green type	
1. Full-green (2)	ccAArr	Kalichar
	Green-pigmented types	
2. Green-coppery-red	CCAARR	D 154
3. Green-red	CCA^RA^RRR	Assam 16
	Red types	
4. Coppery-red	CCAArr	Kajla Comilla
5. Light-red	CCA^LA^Lrr	Rangamati Wild
6. Red	CCA^RA^Rrr	Lalpat Meghal

The intensity of anthocyanin pigmentation of the dark-red type, Maniksari, which is higher than that of the red, suggests that it carries a higher allele than **A^R** which may be designated as **A^D** (dark-red).

Relationship of dark-red with full-green (2)

The full-green types are marked by total absence of pigmentation on all the parts of the plant body. Full-green (2), with the genetic constitution **ccAArr**, was used in the cross, as it is known to be a tester for both the recessive reducer gene **r** and the recessive chromogen gene **c**.

Crosses of the full-green (2) with dark-red gave F_1 's that were dark-red though slightly diluted.

The F_2 's segregated into dark-red, coppery-red and full-green phenotypes:

Family	Dark-red	Coppery-red	Full-green	Total
8037/50	316	101	150	567
8054/50	263	85	126	474
8055/50	166	48	74	288
8159/50	252	85	94	431
Total	997	319	444	1760
Expected (9:3:4)	990	330	440	1760

$$\chi^2 = 0.45. \quad P = 0.80.$$

All the backcrosses of the F_1 's with the dominant red parent gave only dark-red progenies as expected.

The data show incomplete dominance of the dark-red over full-green (2) and a digenic difference between the two parents. The dark-red parent must, therefore, carry the dominant chromogen gene **C**. The absence of green-pigmented types and the presence of coppery-red among the F_2 segregates indicate that the dark-red type is homozygous with

the full-green (2) in respect of the recessive reducer gene *r*. The digenic ratio obtained in the present cross suggests that the heterozygosity involves the alleles of the multiple allelomorph series in addition to the chromogen gene. The slight dilution observed in the F_1 may be explained as a result of the heterozygote $A^D A$.

Relationship with members of the green-pigmented group

Between the corresponding members of the green-pigmented and the red groups, e.g. green-coppery-red and coppery-red, green-red and red, there is hardly any difference in the pigmentation of the petiole, veins and leaf margins. The uneven distribution of the pigment on the stem of the green-pigmented types is due to the presence of the reducer gene *R*, which hardly affects the pigment on the other parts of the plant. Thus, as pointed out earlier, between corresponding members of the two groups the difference is monogenic, and between the other members it is digenic.

The studies involved crosses with two of the members of the group, viz. green-coppery-red ($CCAARR$) and green-red ($CCA^R A^R RR$).

Green-coppery-red, D 154 × Dark-red, Maniksari

The F_1 's were green-dark-red with marked reduction of pigment on the stem as compared to the dark-red parent. The flush on the stem was, however, darker than that on the normal green-red plants. The stipules, petioles, veins and leaf margins were more prominently coloured red, the reduction of pigment on these parts being less pronounced. The F_2 segregated into four classes, Green-dark-red, Dark-red, Green-coppery-red and Coppery-red.

Family	Green-dark-red	Dark-red	Green-coppery-red	Coppery-red	Total
8041/50	135	45	44	15	239
8042/50	364	133	139	32	668
8043/50	316	95	119	30	560
Total	815	273	302	77	1467
Expected (9:3:3:1)	825.19	275.06	275.06	91.69	1467

$$\chi^2 = 5.135. \quad P = 0.20-0.10$$

The F_1 was backcrossed to both the parents. The population in the backcrosses with the dark-red parent was rather poor and segregated into green-dark-red and dark-red types as shown under:

Family	Green-dark-red	Dark-red	Total
S89/50	4	1	5
S91/50	2	5	7
Total	6	6	12
Expected 1:1			

However, an adequate population was obtained in the backcrosses with the green-coppery-red. They segregated into green-dark-red and green-coppery-red types:

Family	Green-dark-red	Green-coppery-red	Total
S148/50	5	6	11
S150/50	5	7	12
S212/50	10	8	18
Total	20	21	41
Expected 1:1	20.5	20.5	41

The reduction of pigment on the stem in the F_1 , and the appearance of green-dark-red and coppery-red in addition to the parental types in the F_2 segregates in a dihybrid ratio, indicate that the interaction involves the multiple alleles and the reducer gene, while the chromogen gene, C, is common to both. The production of coppery-red among the F_2 segregates shows that r is present in the dark-red, which in interaction with A from the green-coppery-red produces the coppery-red types. On the other hand, the production of the green-dark-red phenotype among the F_2 's implies that the dark-red carries A^p , a higher multiple allele than A, but its action is modified by interaction with R from green-coppery-red, thus giving rise to an entirely new phenotype.

Green-red, Assam 16 × Dark-red, Maniksari

The F_1 was green-dark-red, the intensity of the pigmentation being markedly more than that of the normal green-red. Compared with the F_1 of the previous cross, the coloration was found to be more intense. The F_2 segregated into green-dark-red, green-red, dark-red and red phenotypes. In the first scoring, some difficulty was experienced in distinguishing between green-dark-red and green-red, as also between red and dark-red types. Close observation of the intensity and distribution of the pigment on the petiole, veins and leaf margin, besides the stem colour, revealed that the pigment on the petiole, veins, etc., of the dark-red and the green-dark-red was different from that of the other two patterns. The scoring was repeated and the reclassification was satisfactory:

Family	Green-dark-red	Green-red	Dark-red	Red	Total
8183/50	66	30	32	5	133
8184/50	438	143	140	36	757
8185/50	89	17	29	8	143
Total	593	190	201	49	1033
Expected (9:3:3:1)	581.06	193.69	193.69	64.56	1033

$\chi^2 = 4.341. \quad P = 0.30-0.20.$

Backcrosses of the F_1 with the dark-red parent segregated into green-dark-red and dark-red types:

Family	Green-dark-red	Dark-red	Total
S52/50	0	3	3
S53/50	2	5	7
S58/50	5	2	7
S59/50	0	1	1
S61/50	2	0	2
Total	9	11	20
Expected 1:1	10	10	20

Backcrosses with the green-red parent segregated into green-dark-red and dark-red types as under:

Family	Green-dark-red	Green-red	Total
S101/50	3	2	5
S240/50	7	11	18
S242/50	3	6	9
S243/50	6	4	10
Total	19	23	42
Expected 1:1	21	21	42

$\chi^2 = 0.21. \quad P = 0.66.$

The data presented show a two-factor difference between the two parents, involving clearly the members of the allelomorphous series and the pigment reducer gene as in the previous cross. The appearance of red as a phenotype among the F_2 segregates points again to the presence of the recessive **r** in the dark-red which in interaction with **A^R** from the green-red produces the red pattern; while the production of green-dark-red is explained by the interaction of **A^D** with **R** from the green-red.

Relationship of dark-red with members of the red group

Between the members of the red group, the difference is one of the intensity of anthocyanin pigmentation only. From the lowest to the highest member there is a gradation in the pigmentation, and they are in the order of dominance determined by the particular allele of the multiple allelomorphous series carried by the type. Thus the difference between any two members within the group is monogenic. Dark-red was crossed to all the known members of the red group, viz. coppery-red (**CCA^{rr}**), light-red (**CCA^LA^Lrr**) and red (**CCA^RA^Rrr**). In general, the F_1 's approached more closely the dark-red parent. A gradual increase in the intensity of the pigmentation in the F_1 was, however, observed in crosses with correspondingly higher members of the series, e.g. the F_1 of the cross light-red \times dark-red was found to be more intensely pigmented than that of the cross coppery-red \times dark-red. The F_1 of red \times dark-red was, again, more intensely pigmented than that of light-red \times dark-red. All the three types of F_1 's were, however, darker than the normal red type.

Coppery-red, Kajla Comilla \times Dark-red, Maniksari

The F_1 was dark-red, but the pigment was found to be diluted. The F_2 segregated into the two parental types:

Family	Dark-red	Coppery-red	Total
1881/50	353	142	495
1882/50	765	252	1017
8156/50	545	189	734
Total	1663	583	2246
Expected 3:1	1684.5	561.5	2246

$$\chi^2 = 1.097. \quad P = 0.28.$$

Backcrosses to the dark-red parent did not show any segregation and gave only dark-red progenies. Unfortunately, no backcrosses could be obtained with the recessive coppery-red parent.

The F_2 segregations show a monogenic difference between the two parents and incomplete dominance of the dark-red which is explained by the heterozygote **A^DA**.

Light-red, Rangamati wild \times Dark-red, Maniksari

The F_1 was dark-red. The pigment was diluted as compared to the dark-red parent, though it was distinctly deeper than the normal light-red as also the F_1 of the previous cross. The F_2 segregated into the parental types:

Family	Dark-red	Light-red	Total
1868/50	360	124	484
1871/50	167	62	229
8188/50	419	132	551
Total	946	318	1264
Expected 3:1	948	316	1264

As expected, the backcrosses to the dark-red parent gave only dark-red progenies. Backcrosses to the light-red segregated into dark-red and light-red types as under:

Family	Dark-red	Light-red	Total
S32/50	7	9	16
S33/50	4	4	8
S34/50	8	4	12
S82/50	2	3	5
S83/50	5	4	9
Total	26	24	50
Expected 1:1	25	25	50

The data show a monogenic difference between the two types. The higher intensity of pigmentation of the F_1 in this case as compared to that of the previous cross is due to the presence of the higher allele A^L in the heterozygote $A^D A^L$.

Red, Lalpat Megnal × Dark-red, Maniksari

The F_1 was dark-red and very slightly diluted. The intensity of pigmentation of the F_1 in this case was markedly higher than that of the F_1 's of the two previous crosses. As in the classification of the F_2 families in the cross between green-red and dark-red, some difficulty was experienced in the initial scoring of the dark-red and red segregates in the F_2 's of the present cross. This difficulty was solved by taking into consideration the intensity and distribution of pigmentation on the petiole, veins, etc., in addition to the stem. Subsequent scoring on this basis proved satisfactory:

Family	Dark-red	Red	Total
8170/50	100	32	132
8197/50	375	115	490
8198/50	659	220	879
Total	1134	367	1501
Expected 3:1	1125.75	375.25	1501

$\chi^2=0.241. P=0.62.$

Progenies of backcrosses of the F_1 's to the dark-red were all dark-red; those to the red parent segregated into the two parental types as shown below:

Family	Dark-red	Red	Total
S 6/50	3	3	6
S 7/50	5	4	9
S17/50	2	5	7
S18/50	1	5	6
Total	11	17	28
Expected 1:1	14	14	28

$\chi^2=0.893. P=0.35.$

The data again show a monogenic difference between the parents. The slight dilution observed in the F_1 is due to the heterozygote $A^D A^R$, and the higher intensity of pigmentation of the F_1 in the present case is due to the presence of the allele A^R which is dominant over A^L and A .

DISCUSSION

The foregoing evidence shows the relationship of the new dark-red type with full-green (2), the green-pigmented and the red types.

Full-green (2) ($ccAArr$), in a cross with the dark-red, gave F_1 's that were dark-red though slightly diluted and in F_2 gave a digenic ratio. The dominance of the dark-red

over full-green (2), the absence of green-pigmented types among the F_2 segregates and the production of coppery-red as one of the phenotypes in F_2 , point to the presence of dominant chromogen gene **C** and the recessive reducer gene **r** in the dark-red.

In crosses with green-coppery-red (**CCAARR**) and green-red (**CCAR^RARR**) the new type gave F_1 's that were green-pigmented. The intensity of pigment in the F_1 's was distinctly higher than that of the typical green-red type, and as such may be designated as green-dark-red, hitherto unknown in nature. The F_1 of the green-red cross was, however, more intensely pigmented than the other. In F_2 both gave a digenic ratio. In addition to the two parental types, two non-parental phenotypes were obtained in both the combinations, the new green-pigmented type, viz. green-dark-red being common to both and dominant. The dark-red parent must, therefore, carry a new allele of the allelomorphous series higher than **A^R**. This has been designated earlier as **A^D**. The appearance of the non-parental phenotypes, coppery-red (**CCAarr**) and red (**CCAR^RARR**) in F_2 's of crosses with green-coppery-red (**CCAARR**) and green-red (**CCAR^RARR**) respectively further confirm that the new type carries the recessive reducer gene **r**. Thus the constitution of the new dark-red type is **CCAD^DARR** and that of the synthetic green-dark-red **CCAD^DARR**.

A limited number of selections of the green-dark-red were carried forward to the F_3 generation. Two of the progenies bred true to the green-dark-red type, while the rest segregated. This type is being maintained in our collection of pigmentation types.

Green-dark-red type produced by the interaction of **R** with **A^D** is characterized by stem, stipules, petioles, veins and leaf margins which are more intensely pigmented than green-red, but the pattern of flush on the stem is the same in both. Compared with the dark-red there is hardly any difference in the intensity and distribution of pigment on the stipules, petioles, veins and leaf margins. The intensity of pigment on the stem is also the same in both, but the distribution of pigment on the stem differs; in the dark-red type, the stem is uniformly dark-red, while in the green-dark-red type the pigment is flushed, showing some green which is characteristic of the green-pigmented group. The green-red type has, so far, been considered the highest member of the green-pigmented group. But the new green-dark-red type, which belongs to the same group, is more intensely pigmented than green-red and as such it is the highest member of the green-pigmented group.

The F_1 's of the crosses of the dark-red with members of the red group, viz. light-red and coppery-red, were dark-red with some dilution, the degree of dilution being noticeably higher with lower members of the group. The F_2 's in all cases segregated into the parental types in a monogenic ratio, the dark-red being always dominant. This further confirms that the dark-red parent carries the dominant allele **A^D** in the multiple allelomorphous series and has, in common with the other members of the red group, the recessive reducer gene **r**. Thus it is established that three loci are involved in the production of the new dark-red pigmentation pattern, which in addition to the basic chromogen gene **C** carries a new allele **A^D**, which is the highest allele in the allelomorphous series and the recessive reducer gene **r** giving the constitution **CCAD^DARR**. It, therefore, belongs to the red group of which it is the highest member known at present.

It has been pointed out that in crosses of dark-red which carries the allele **A^D** with types involving any one of the other alleles of the multiple allelomorphous series, there is noticeable dilution of pigment in the heterozygote. This points to the partial dominance

between the alleles $A^D-A^R-A^L-A$ which were under investigation. The greatest intensity of the anthocyanin pigmentation known in *C. capsularis* is thus associated with A^D and not A^R of the multiple allelomorphous series as reported by Ghose *et al.* (1947).

Isolation of full-green genotypes

The possible full-green genotypes involving the multiple alleles in interaction with the reducer factor pair $R-r$ are as under:

- | | |
|-----------------|-------------------|
| †1. $ccaRR$ | *6. ccA^LA^Lrr |
| 2. $ccaarr$ | *7. ccA^RA^RRR |
| *3. $ccAARR$ | *8. ccA^RA^Rrr |
| *4. $ccAArr$ | 9. ccA^DA^DRR |
| 5. ccA^LA^LRR | *10. ccA^DA^Drr |

Of these ten possible genotypes, $ccAARR$ and $ccAArr$, which occur freely in nature, were the first to be identified: a third genotype, ccA^RA^Rrr , was isolated from the F_2 progenies of a cross between $ccAArr$ and the red type CCA^RA^Rrr (1944) and $ccaRR$ was isolated from the full-green progenies of a cross between a material collected from Orissa and green-coppery-red type $CCAARR$ (1947). Using the coppery-red type ($CCAArr$) as a tester, it was possible to isolate ccA^DA^Drr from the full-green F_2 progenies of the cross between $ccAArr$ and dark-red, CCA^DA^Drr . Besides the five genotypes listed above, two more types, ccA^RA^RRR and ccA^LA^Lrr , were also isolated from planned crosses carried out for the purpose. Thus, of the ten possible full-green genotypes, the following three remain to be isolated:

$ccaarr$
 ccA^LA^LRR
 ccA^DA^DRR

The isolation of full-green genotypes is nearing completion. The addition of the lowest and the highest alleles of the anthocyanin allelomorphous series and their interaction with the reducer factor pair $R-r$ added four possible genotypes to the first list of six (1944).

Being the earliest genotypes to be isolated, $ccAARR$ and $ccAArr$ were designated in that list as full-green (1) and full-green (2) respectively.

As allele A of the allelomorphous series, $A^D-A^R-A^L-A-a$, is dominant over the allele a , any system of enumeration should begin with the lowest allele a and not with the allele A , which comes next in sequence, while dealing with pigmented types. It has been the practice to place the green-pigmented types first and follow them with red types. Keeping these two considerations in view, the following system of numbering the pigmented and full-green types is adopted for the convenience of handling the materials. In Table 1 it may be seen that when the order of types representing the different patterns of pigmentation is fixed, that of the corresponding green genotypes is automatically fixed.

* Indicates full-green genotypes isolated.

† This genotype was isolated but lost at Dacca at the time of partition of India.

SUMMARY

1. As a result of this investigation, a new allele A^D of the anthocyanin multiple allelomorphic series in *Corchorus capsularis* is brought to light. This brings the number of known alleles to five, viz. $A^D-A^R-A^L-A-a$.
2. The highest intensity and distribution of the anthocyanin pigmentation known in *C. capsularis* has been found to be associated with the new allele A^D and not with A^R as reported by earlier workers.
3. The dark-red pigmentation pattern in *C. capsularis* has been identified as $CCA^D A^D rr$. It is the highest member of the red group.
4. A new synthetic green-pigmented type, called green-dark-red, $CCA^D A^D RR$, has been isolated. It is the highest member of the green-pigmented group.
5. A new full-green genotype, $ccA^D A^D rr$, has also been isolated. Occurrence of another full-green genotype, $ccA^D A^D RR$, is predicted. This brings the total number of full-green genotypes to ten. Isolation of two more full-green genotypes, $ccA^R A^R RR$ and $ccA^L A^L rr$, is also reported.
6. A system of enumeration of the different anthocyanin pigmentation patterns in *C. capsularis* and their full-green derivatives has been evolved.

Table 1. *Anthocyanin pigmentation patterns and their corresponding full-green genotypes in Corchorus capsularis*

Pigmentation grade no.	Pigmented type		Corresponding full-green type		Remarks
	Pattern	Genotype	Nomenclature	Genotype	
Green-pigmented group					
1	Green-light-coppery-red*	$CCaaRR$	Full-green (1)*	$ccaaRR$	Isolated
2	Green-coppery-red	$CCAARR$	Full-green (2)	$ccAARR$	Isolated
3	Green-light-red	$CCA^L A^L RR$	Full-green (3)	$ccA^L A^L RR$	Not isolated
4	Green-red	$CCA^R A^R RR$	Full-green (4)	$ccA^R A^R RR$	Isolated
5	Green-dark-red	$CCA^D A^D RR$	Full-green (5)	$ccA^D A^D RR$	Not isolated
Red group					
6	Light-coppery-red	$CCaarr$	Full-green (6)	$ccaarr$	Not isolated
7	Coppery-red	$CCAarr$	Full-green (7)	$ccAarr$	Isolated
8	Light-red	$CCA^L A^L rr$	Full-green (8)	$ccA^L A^L rr$	Isolated
9	Red	$CCA^R A^R rr$	Full-green (9)	$ccA^R A^R rr$	Isolated
10	Dark-red	$CCA^D A^D rr$	Full-green (10)	$ccA^D A^D rr$	Isolated

* Lost at Dacca at the time of partition of India.

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