# Uniparental inheritance of purple leaf and the associated resistance to leafminer in castor bean

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With 3 figures and 2 tables

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## Abstract

Leafminer (Liriomyza trifolii Burgess), Diptera Agromyzidae, is one of the insect pests that causes economic damage to castor bean (Ricinus communis L.) foliage. Green leaf type is a common phenotype in castor bean and highly susceptible to leafminer. The rare purple leaf type germplasm accessions showed stable resistance to leafminer. Studies were carried out to understand the inheritance of purple leaf and the associated leafminer resistance. Direct and reciprocal crosses were made between a purple leaf parent RG1930 and a green leaf parent RG2788. RG1930 is resistant to leafminer while RG2788 is susceptible. Reciprocal differences were noted in segregation pattern of purple leaf colour as well as resistance to leafminer. Purple leaf phenotype was obtained only in purple  $\times$  green (RG1930  $\times$  RG2788) cross where the female parent was a purple leaf phenotype. The reciprocal cross green × purple (RG2788 × RG1930) produced only the green leaf phenotype. Uniparental inheritance was observed for purple leaf phenotype and resistance to leafminer in F1, F2, F3 and backcross generations. Progenies with a dark purple leaf were resistant to leafminer while those with a green leaf were susceptible. Visual association between a purple leaf and resistance to leafminer and their uniparental inheritance were clearly established. The role of heritable epigenetic effects are discussed in expression of purple pigment in offspring.

**Key words:** *Ricinus communis* — *Liriomyza trifolii* — purple leaf — uniparental inheritance — epigenetic effects

Castor bean (Ricinus communis L.) is a sub-tropical industrial oilseed crop. It is adapted to thrive under low rainfall and marginal soil fertility conditions. The market for castor oil is large because of its immense range of industrial uses, ranging from lubricants to medicines. Though several high-yielding commercial cultivars are available in castor bean, crop productivity is often limited by several biotic stresses. Leafminer (Liriomyza trifolii Burgess), Diptera, Agromyzidae, is one of the insect pests that causes severe damage to castor bean foliage, right from cotyledon stage to when the crop reaches 120-130 days in age. The female leafminer punctures the upper surface of leaf for feeding and oviposition. Larvae eat to mesophyll tissue and form serpentine mines on the leaf surface, which significantly reduces the photosynthetic area and cause premature dropping of leaves. Leafminer is hard to control, as it is reportedly resistant to most insecticides (Parrella 1987). Development of resistant cultivars will be the best solution for its management. None of the castor bean cultivars released are resistant to leafminer. Among 3120 germplasm accessions screened against leafminer at the Directorate of Oilseeds Research, Hyderabad, only two indigenous accessions RG1930 and RG2008 were found to be resistant to leafminer (Prasad and Anjani 2001) and so far, these are the only reported sources of leafminer resistance in castor bean. Both resistant accessions are purple colour phenotypes, where the entire plant, including stem, leaf, petiole and capsules, are dark purple in colour. This purple colour phenotype is a rare morphotype in castor bean (Anjani 2005).

Interestingly, when crosses were attempted between the purple colour morphotype as female parent and a green colour type as male parent and vice-versa, progenies with purple leaves were obtained only in the  $F_1$  of the cross between purple × green and not in the reciprocal cross. Further,  $F_1$  progenies with purple leaf exhibited resistance to leafminer while progenies with green leaf showed susceptibility. This encouraged a systematic study on the inheritance pattern of purple colour leaf and its associated leafminer resistance in castor bean, which would help in planning breeding programmes for leafminer resistance in this crop. The study was conducted at the Directorate of Oilseeds Research, Hyderabad, India (Latitude: 17°15' to 17°16' North, Longitude 78°18' to 78°19'), under endemic leafminer infestation.

## **Materials and Methods**

Plant materials: The leafminer-resistant purple leaf colour parent RG1930 of castor bean (Ricinus communis L.) is a wild collection from Assam state in India (Anjani et al. 1994). Its dark purple leaves are little preferred by the leafminer female; it makes only a few punctures for egg-laying on the leaf surface. On this plant, the larvae immediately after emergence from eggs will either die or make <10 mines no more than 1-cm length each before dying. The accession RG2788 is a green colour leaf type collected from Tamil Nadu state in India (Ashoka Vardhana Reddy et al. 2002). It is highly susceptible to leafminer. On its leaf surface, the larvae make more than 100 mines/leaf covering about 90-95% of the leaf area and total leaves. Both accessions have been maintained through self-pollination since their initial collection. The phenotypic and genotypic stability of the purple leaf trait and its associated leafminer resistance in purple leaf parent RG1930 were confirmed conclusively for more than 10 years (1995-2006). Similarly, the green leaf trait of RG2788 and its susceptibility to leafminer were confirmed continually for 5 years (2002-2006).

**Crosses:** The direct cross between purple × green (RG1930 × RG2788), the reciprocal cross between green × purple (RG2788 × RG1930) and their respective  $F_2$ ,  $F_3$  and backcross generations were developed under controlled pollination conditions, as castor bean is a cross-pollinating crop. The plants in each generation were grouped into green leaf and purple leaf (dark purple and purple green) phenotypes. Schematic presentation of the crossing programme is given in Fig. 1.

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(a)			( <b>b</b> )	
Purple ( <sup>1</sup> RG1930)	x ↓	Green ( <sup>1</sup> RG2788)	Green x (¹RG2788) ↓	Purple ( <sup>1</sup> RG1930)
♀ <sup>°</sup> RG1930 :	x <sup>2</sup> F <sub>1</sub>	x <sup>°</sup> RG2788 ∂ <sup>°</sup>	$\  \   \stackrel{_{\scriptscriptstyle 5}}{_{\scriptscriptstyle 7}} {}^{_{\scriptscriptstyle 7}} RG1930 \ x \  \   {}^{_{\scriptscriptstyle 5}} F_1 \ x$	³RG2788 ♂
♀ <sup>°</sup> RG2788 :	$x \downarrow$ ${}^{3}F_{2}$	x <sup>°</sup> RG1930 ്	°F2	<sup>™</sup> RG1930 ∂
	${}^{4}\mathrm{F}_{3}$		${}^{7}F_{3}$	

Fig. 1: Schematic presentation of crossing programme: A single plant each from RG1930 and RG2788 was used in purple x green and reverse crosses; <sup>1</sup>A single self-progeny of parental plant involved in initial crosses; <sup>2</sup>A single dark purple leaf type  $F_1$  plant backcrossed to a single self-progeny of each initial parental plant in both directions; <sup>3</sup>F<sub>2</sub> generation derived through self-pollination of a single dark purple leaf type  $F_1$  plant involved in backcrosses; <sup>4</sup>F<sub>3</sub> generation derived through self-pollination of a single dark purple leaf type  $F_2$  plant; <sup>5</sup>A single green leaf type  $F_1$  plant backcrossed to a single self-progeny of each initial parental plant in both directions; <sup>6</sup>F<sub>2</sub> generation derived through self-pollination of a single green leaf type  $F_1$  plant involved in backcrosses; <sup>7</sup>F<sub>3</sub> generation derived through self-pollination of a single green leaf type  $F_2$  plant

Screening against leafminer: Parents, F1, F2, F3 and backcross generations of purple  $\times$  green and green  $\times$  purple were screened simultaneously against leafminer under natural heavy infestations. The number of mines/leaf and heavily infested leaves/plant (%) were used as indicators to score the reaction. Data were recorded from each plant in parents, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and backcross generations of both crosses. Numbers of mines/leaf were recorded on 45- to 55-day-old plants, coinciding with the peak leafminer infestation in August and September. As the leafminer infestation progresses from the bottom leaf upwards, the mean value of six lower leaves/plant was considered for the number of mines/leaf. Due to the overlapping of mines in highlyinfested plants, 100 mines/leaf were considered as the upper limit and any higher value was recorded as more than 100 mines/leaf. Heavily infested leaves/plant (%) was taken at the end of leafminer incidence when the plants were around 120 days old. The leaf was considered as heavily infested when more than 50% of the lamina was damaged due to mining. The number of such heavily infested leaves per plant were counted and expressed as the percentage of heavily infested leaves/ plant. Only heavily infested leaves were considered because the upper leaves and a few middle leaves could not exhibit their actual reaction against leafminer because they had developed during reducing phase of leafminer incidence, and had less infestation covering <50% of lamina. Each plant was characterized as either resistant or susceptible. Based on the varying reactions of test entries against leafminer, plants recording 0-10 mines per leaf and 0% heavily infested leaves/plant were scored as resistant to leafminer and those recording higher values were scored as susceptible. The total phenols content in the leaf samples was monitored for their association with leafminer resistance. Square root transformation was applied for data on leafminer infestation. Transformed data of resistant and susceptible groups between parents and within F1, F2 and F3 generations were compared using the paired t-test.

#### Results Parents

The leafminer-resistant purple parent (RG1930) and the leafminer-susceptible green leaf parent (RG2788) bred trueto-type for their respective leaf colour and reaction against leafminer following the years of self-pollination since their collection. Inbreeding depression was not observed for these traits, in contrast to that of quantitative traits such as plant height and yield-contributing traits. The dark purple leaf trait and the intensity of purple pigment in RG1930 were consistent over generations of self-pollination. Similarly, both parents were stable in their reaction against leafminer over the years under screening. RG1930 exhibited a consistent resistance reaction with 0–2 mines/leaf and without any heavily infested leaves/plant. The susceptible parent RG2788 displayed a susceptible reaction with more than 100 mines/leaf and 90–97% heavily infested leaves/plant in the different years of screening. Paired *t*-tests revealed highly significant differences between the resistant purple parent and the susceptible green parent with regard to the number of mines/leaf and heavily infested leaves/plant (P < 0.001).

#### **F**<sub>1</sub> generation

Plants having absolutely green leaves were characterized as the green leaf phenotype while those having dark purple and purple-green leaves were characterized together as the purple leaf phenotype. Dark purple leaf type plants had absolute purple leaves (Fig. 2b), whereas purple-green leaf type had purple leaves with traces of green pigment (Fig. 3a-c). The observed number of different leaf colour phenotypes in F<sub>1</sub> generation of purple × green and of the reciprocal cross green × purple is given in Table 1. Reciprocal differences in the segregation pattern of leaf colour and resistance to leafminer were observed. In  $F_1$  of the purple  $\times$  green cross, only the purple leaf phenotype was observed. There were 320 purple leaf F<sub>1</sub> plants comprising 227 dark purple and 93 purple-green leaf plants. In the F1 of the reciprocal cross green × purple, all 226 plants were of green leaf phenotype (Fig. 3d) without any tinge of purple pigment. Distinct differences in leafminer infestation on the dark purple and green leaf phenotypes was observed (Fig. 2). Dark purple leaf types exhibited resistance against leafminer while green leaf phenotypes showed susceptible reactions (Table 1). The dark purple leaf plants in the  $F_1$  of the cross purple  $\times$  green, recorded very few mines/leaf (0-4) with no heavily infested leaves/plant. Purple-green leaf F1 plants of the same cross, exhibited comparatively few leafminer infestations (51-52 mines/leaf and 52-55% heavily infested leaves/plant) than the green leaf  $F_1$  plants of the reciprocal cross (88 to >100 mines/leaf and 82-89% heavily infested leaves/plant).

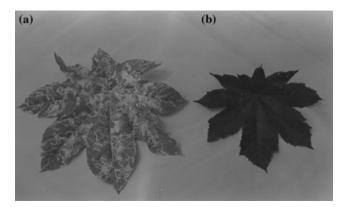


Fig. 2: Response of different leaf colour phenotypes to *Liriomyza trifolli*. (a) Susceptible – green leaf phenotype. (b) Resistant – dark purple leaf phenotype

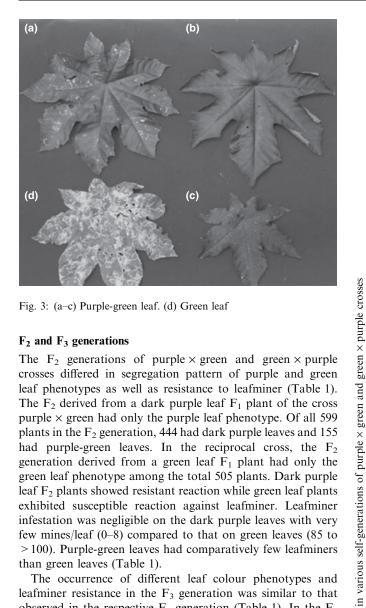


Fig. 3: (a-c) Purple-green leaf. (d) Green leaf

## F<sub>2</sub> and F<sub>3</sub> generations

The  $F_2$  generations of purple  $\times$  green and green  $\times$  purple crosses differed in segregation pattern of purple and green leaf phenotypes as well as resistance to leafminer (Table 1). The  $F_2$  derived from a dark purple leaf  $F_1$  plant of the cross purple  $\times$  green had only the purple leaf phenotype. Of all 599 plants in the F<sub>2</sub> generation, 444 had dark purple leaves and 155 had purple-green leaves. In the reciprocal cross, the F2 generation derived from a green leaf F1 plant had only the green leaf phenotype among the total 505 plants. Dark purple leaf F<sub>2</sub> plants showed resistant reaction while green leaf plants exhibited susceptible reaction against leafminer. Leafminer infestation was negligible on the dark purple leaves with very few mines/leaf (0-8) compared to that on green leaves (85 to >100). Purple-green leaves had comparatively few leafminers than green leaves (Table 1).

The occurrence of different leaf colour phenotypes and leafminer resistance in the F3 generation was similar to that observed in the respective F<sub>2</sub> generation (Table 1). In the F<sub>3</sub> generation derived from a dark purple leaf F<sub>2</sub> plant of a purple  $\times$  green cross, 161 were of dark purple leaf type and nine were of purple-green type. In the F<sub>3</sub> generation derived from a green leaf  $F_2$  plant of the reciprocal cross green  $\times$  purple, only the green leaf phenotype was observed among the total 166 plants. Leafminer infestation on various leaf colour phenotypes in F<sub>3</sub> was similar to that observed on different phenotypes in the  $F_1$  and  $F_2$  generations. Paired *t*-tests indicated highly significant differences between the mean reactions of susceptible and resistant groups for number of mines/leaf and heavily infested leaves/plant (%) among the F<sub>1</sub>,  $F_2$  and  $F_3$  generations (P < 0.001). Dark purple, purple-green and green leaf types were significantly differentiated for their reactions against leafminer in all generations (P < 0.001).

#### **Backcross generation**

The observed number of different leaf colour phenotypes and resistance to leafminer in backcrosses of purple × green and green  $\times$  purple crosses is given in Table 2. The purple leaf phenotype appeared only in those backcrosses where the mother plant was a purple leaf type. Among the seven different

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12788) (R) (R) (R) (S) (S) (S) (S) (S) (S) (S) (S) (S) (S	Purple × green	320 55	99 <sup>1</sup> 1	70 <sup>3</sup> 22	27 44	14 16	1 93	155	6	0	0	0 0	4 0	-8 0-	-3 51-	-52 5.	2-54 5	1-52		I	T	0	0 0	52-55	53-55	56-58	I	I	T	I
	$(RG1930 \times RG2/88)$ Green × purple $(RG2788 \times RG1930)$	226 5(	05 <sup>2</sup> 1	(1 66 <sup>4</sup>	± ⋧⁰	२ <sup>०</sup>	(S) 0	(x) 0	o (s)		(S)					1	I		88 to >100	85 to >100	88 to > 100		I I	I	I	Ι	82–89	84-87	82-9	-

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F<sub>2</sub> plants derived from dark purple leaf F<sub>1</sub> plant.

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from green leaf F<sub>2</sub> plant plants derived

Table 2: Segregation pattern of leaf colour and resistance to leafminer in backcross generations of purple × green and green × purple crosses

		Leaf c			with o	ber of pla different l	eaf	Lea	afminer infesta	tion on phenoty		ent leaf c	olour
Cross and		of par in backc		Total number	and t	r phenoty heir reacti ist leafmir	ion		Number of mines/leaf			eavily inf ves/ plan	
phenotype of parents	Backcross	F	М	of plants	DP	G	PG	DP	G	PG	DP	G	PG
Purple × green	$F_1 \times RG1930$	DP	DP	189	189 (R)	0	0	0–8	-	-	0	-	_
$(RG1930 \times RG2788)$	$RG1930 \times F_1$	DP	DP	202	202 (R)	0	0	0-3	_	-	0	-	-
	$F_1 \times RG2788$	DP	G	192	192 (R)	0	6 (S)	0-8	_	50-56	0	-	52-55
	$RG2788 \times F_1$	G	DP	178	0	178 (S)	0	_	75 to >100	-	_	82-95	-
Green × purple	$F_1 \times RG2788$	G	G	181	0	181 (S)	0	_	78 to >100	-	_	80-94	-
$(RG2788 \times RG1930)$	$F_1 \times RG1930$	G	DP	186	0	186 (S)	0	_	70 to >100	_	_	80-92	_
``````````````````````````````````````	$ m RG1930  imes F_1$	DP	G	185	185 (R)	Ó	0	0–3	—	—	0	—	-

F, female; M, male; DP, dark purple; G, green; PG, purple-green; R, resistant; S, susceptible.

backcrosses, three produced only dark purple leaf types while three produced only green leaf types and one produced both dark purple and purple type offspring. All the dark purple type backcross progenies exhibited resistance to leafminer while green and purple-green leaf types showed susceptibility. Leafminer infestation on different phenotypes in backcrosses was similar to that observed in the  $F_1$ ,  $F_2$  and  $F_3$  generations (Table 2).

#### Discussion

The purpose of the study was to determine the inheritance pattern of the purple leaf phenotype and the associated leafminer resistance. The true-breeding nature of parents indicates the genetically homozygous state of the purple and green leaf traits. Reaction of parents against leafminer was confirmed conclusively over the years. Inbreeding depression was not observed for these traits over the years of selfpollination. The study clearly demonstrated reciprocal differences in the segregation of purple and green leaf phenotypes and resistance to leafminer. Purple leaf phenotypes (dark purple and purple-green) were produced only through the purple leaf mother plant. The data did not fit the pattern of Mendelian segregation to explain nuclear inheritance for purple leaf phenotype and associated resistance to leafminer, the results demonstrating uniparental inheritance of these traits. Reciprocal differences in the segregation of these traits confirms the parent-specific-origin of the traits. This was further substantiated by the results of F<sub>2</sub>, F<sub>3</sub> and backcross generations.

Nuclear inheritance for purple colour leaf was reported in rice (Kadam 1974) and Ocimum basilicum L. (Phippen and Simon 2000). Similar inheritance pattern for Liriomyza trifolii resistance was reported in Lycopersicon cheesmanii Riley (Bordat et al. 1995) and melon (Dogimont et al. 1999). Uniparental inheritance of solidly red-coloured kernels was well demonstrated in maize (Kermicle 1970). Here, when a RR female (red) is crossed with a rr male (colourless), all the progenies had fully coloured kernels in the F1 and the reciprocal cross gave kernels with mottled aleurone pigmentation. It was demonstrated that the *R*-mottled phenotype was not a dosage effect but was attributed to the mode of inheritance of the R allele. Several loci that encode regulators of the anthocyanin synthesis pathway showed paramutation interactions between their different respective alleles in maize (Hollick et al. 1997, Bruce et al. 2000).

The directed and heritable inheritance of purple leaf (dark purple and purple-green) phenotype could be due to heritable epigenetic effects playing a role in the expression of purple pigmentation in leaves. The significant advancement in molecular genetics has shown that the DNA sequence alone does not carry all the information required to determine the phenotype of an offspring. In addition to the nuclear genetic contribution, a parent can also influence an offspring's phenotype through non-nuclear DNA transmitted through the cytoplasm and biochemical or structural components in the cytoplasm. Several systems of heritable epigenetic effects are known (Brink 1956, Grossniklaus et al. 1998, Chandler et al. 2000, Kakutani 2002). Epigenetic effects generate phenotypes that depend on the direction of the cross. Many epigenetic effects have been proposed to result from transcriptional imprinting, the differential expression of an allele when transmitted through the pollen or egg germline (Patterson et al. 1993, Kermicle 1996, Park et al. 1996). Purple leaf trait in castor bean in the present study was under purple female parent-of-origin control. As the switching of the direction of the cross does not change the nucleotide sequence or gene copy numbers of the parental genomes contributing to the offspring, this implies that the purple female parent-of-origin control of the purple leaf trait was derived either from maternally inherited cytoplasmic genomes or biochemical or structural components involved in the biosynthetic pathway of anthocyanin in the maternal cytoplasm. Cytosolic localization of many enzymes related to anthocyanin biosynthesis has been reported (Winkel-Shirley 2001). Anthocyanin synthesized in the cytosol is transported into the vacuole to form anthocynoplasts in vacuoles (Picket and Small 1980). Hence, it is assumed that biochemical or structural components involved in the biosynthetic pathway of anthocyanin in purple colour maternal parent cytoplasm are responsible for the expression of purple colour in the offspring. To prove the assumption further, studies are needed for the identification, characterization and exact localization of alleles controlling the biochemical or structural components.

The appearance of dark purple and purple-green leaf types in a purple × green cross and the absence of purple-green leaf type in self-generations of a purple leaf parent (RG1930) imply that the variation is not at the purple parent level, indeed this is a case of epigenetic variation. The dosage effect in a heterozygote was annulled due to the occurrence of different types in the  $F_1$  of a purple × green cross. Cosegregation of dark purple and purplegreen types in  $F_1$ ,  $F_2$  and  $F_3$  generations of a purple × green cross suggests the varying interactions between maternal cytoplasmic factors and different nuclear allele combinations in the fertilized product being responsible for differential expression of purple pigment. In other words, epigenetic alterations in the fertilized product may be regulating the expression of the purple pigment conferring nuclear alleles. The role of epigenetic modifications in regulating gene clusters by cementing silent or intermediate expression states and in controlling phenotypic variation has been clearly shown in *Arabidopsis* (Stokes et al. 2002). The role of epigenetic alterations in phenotypic variation in natural populations was reported in maize (Das and Messing 1994) and *Linaria* (Cubas et al. 1999).

In three backcrosses,  $RG2788 \times F_1$  of purple  $\times$  green,  $F_1$  of reciprocal cross  $\times$  RG2788, F<sub>1</sub> of reciprocal cross  $\times$  RG1930 (Table 2), where the female parent was a green leaf type, only green leaf type plants were produced. The backcrosses,  $F_1$  of purple  $\times$  green  $\times$  RG1930, RG1930  $\times$  F<sub>1</sub> of purple  $\times$  green,  $RG1930 \times F_1$  of reciprocal cross produced only the dark purple type. These backcross offspring possessed cytoplasm and an additional dose of nuclear factors from RG1930. The additional nuclear factors might have enhanced the interaction between the cytoplasmic and nuclear factors leading to the production of only a dark purple type. Though no trace of green colour was found in the dark purple leaf type in backcrosses and self-generations, the visual hue intensity of purple pigment in them was comparatively lower than in the purple leaf parent (RG1930). The purple-green type together with the dark purple type appeared only in the  $F_1 \times RG2788$ backcross where the female was a dark purple F<sub>1</sub> plant and the male was green leaf type. This backcross offspring, either dark purple or purple-green leaf type, received a single dose of nuclear factors from RG1930 plus cyotoplasm of the dark purple female F1 plant, while those of backcrosses which produced only dark purple types could have received an additional dose of nuclear factors of RG1930 plus the cytoplasm of the dark purple female plant. This sort of asymmetrical contributions of nuclear factors from the gametophytes or contributions of differentially expressed genes in the fertilization product was projected in a differential dosage model in Arabidopsis (Dilkes and Comai 2004). The dose disparity of nuclear factors from RG1930 together with their interaction with cytoplasmic factors in the fertilization product might be responsible for the differential expression of purple pigment. However, supplementary studies at molecular and cytochemical levels could expound these assumptions.

The data clearly established a visual association between purple leaf colour and leafminer resistance. Purple pigment in plants in general is grouped chemically under phenolics, which are known to be associated with the chemical defence of plants against insects. Such an association has been established in several crops (Panda and Khush 1995). For example, in Brassica campestris, the plants with low phenolic content were more vulnerable to Liriomyza brassicae Riley (Ipe and Sadaruddin 1984). In maize, the P1-wr allele that confers red pigmentation in the cob has been identified as a major quantitative trait locus controlling levels of silk maysin, a phenolic compound with antibiosis activity against corn earworm larvae (Byrne et al. 1996). The leafminer-resistant purple parent and dark purple type offspring in the present study had very high total phenols contents (2.11-2.18 C.E) compared to the purple-green (0.49–0.58 C.E) and green (0.17– 0.23 C.E) leaf types. The role of phenols in defence against the leafminer was proved. Disease resistance association of an epigenetic phenotypic variant *bal* was reported in *Arabidopsis* (Stokes et al. 2002). Further efforts are required to determine the molecular mechanism underlying the epigenetic control of anthocyanin expression in leaves associating with plant defence against leafminers.

Purple leaf-associated resistance to leafminer suggests direct selection of dark purple phenotypes for the selection of leafminer resistant genotypes, even in the absence of heavy infestation of leafminers. The findings would help in designing breeding programmes for leafminer resistance in castor bean.

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