

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

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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 \times purple (RG2788 \times 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 F₁ of the cross between purple \times green and not in the reciprocal cross. Further, F₁ 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 \times green (RG1930 \times RG2788), the reciprocal cross between green \times purple (RG2788 \times RG1930) and their respective F₂, F₃ 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.

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

Cross and phenotype of parents	Backcross	Leaf colour of parents in backcross		Total number of plants	Number of plants with different leaf colour phenotypes and their reaction against leafminer			Leafminer infestation on different leaf colour phenotypes					
		F	M		DP	G	PG	Number of mines/leaf			Heavily infested leaves/ plant (%)		
								DP	G	PG	DP	G	PG
Purple × green (RG1930 × RG2788)	F ₁ × RG1930	DP	DP	189	189 (R)	0	0	0–8	–	–	0	–	–
	RG1930 × F ₁	DP	DP	202	202 (R)	0	0	0–3	–	–	0	–	–
	F ₁ × RG2788	DP	G	192	192 (R)	0	6 (S)	0–8	–	50–56	0	–	52–55
Green × purple (RG2788 × RG1930)	RG2788 × F ₁	G	DP	178	0	178 (S)	0	–	75 to >100	–	–	82–95	–
	F ₁ × RG2788	G	G	181	0	181 (S)	0	–	78 to >100	–	–	80–94	–
	F ₁ × RG1930	G	DP	186	0	186 (S)	0	–	70 to >100	–	–	80–92	–
	RG1930 × F ₁	DP	G	185	185 (R)	0	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 F₁, F₂ and F₃ 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 self-pollination. 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 F₂, F₃ 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 anthocyanoplasts in vacuoles (Pickett 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 F₁ of a purple × green cross. Cosegregation of dark purple and purple-green types in F₁, F₂ and F₃ 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 F₁ of purple \times green, F₁ of reciprocal cross \times RG2788, F₁ 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, F₁ of purple \times green \times RG1930, RG1930 \times F₁ of purple \times green, RG1930 \times F₁ 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 F₁ \times RG2788 backcross where the female was a dark purple F₁ 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 cytoplasm 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 *PI-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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