Industrial Crops and Products 31 (2010) 192–196

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Biochemical basis of resistance to leafminer in castor (*Ricinus communis* L.)

K. Anjani*, M. Pallavi, S.N. Sudhakara Babu

Directorate of Oilseeds Research, Hyderabad 500030, India

ARTICLE INFO

Article history:
Received 29 July 2009
Received in revised form 1 October 2009
Accepted 5 October 2009

Keywords:
Castor
Dark purple leaf
Leafminer
Phenols
Resistance

ABSTRACT

The present study was conducted to establish relation between total phenol concentration and resistance against leafminer, *Liriomyza trifolii* (Burgess), Diptera Agromyzidae, which causes severe damage to castor (*Ricinus communis* L.) foliage. Crosses were made between leafminer resistant dark purple (RG 1930) and susceptible green leaf (RG 2788) parents and vice-versa. Parents and F_1, F_2 and F_3 generations were screened against leafminer. Total phenol concentrations in them were estimated. High positive significant correlation was observed between leafminer resistance and total phenol concentration. Dark purple leaf progenies possessed high concentrations of total phenol and were resistant to leafminer whereas green leaf type progenies had low total phenol concentrations and were susceptible. The study clearly established the significant relation between high total phenol concentrations and leafminer resistance in castor and projected that selecting dark purple leaf phenotype in breeding programmes is an effective visual and reliable approach for indirect selection of leafminer resistance.

1. Introduction

India is the major castor growing country in the world with 0.8 m ha of area under castor and 1.0 m tonnes of production (Damodaram and Hegde, 2007). Leafminer, *Liriomyza trifolii* (Burgess), Diptera Agromyzidae, causes severe damage to castor (*Ricinus communis* L.) foliage right from cotyledenary stage to 120–150 days after planting. It is widespread in all castor growing regions of the country (Lakshminarayana et al., 1992). Adult female leafminer punctures the upper surface of leaf for feeding and oviposition. Larvae eat on mesophyll tissue and form serpentine mines on leaf surface, thus reducing the photosynthetic area and cause premature leaf senescence (DOR, 2003; Parrella et al., 1985). Insecticides are not effective against leafminer. Developing host plant resistance is a viable solution for leafminer management. Leafminer resistant castor cultivars are not presently available. In the entire castor repository of 3384 accessions being maintained at the Directorate of Oilseeds Research, Hyderabad, India (17.366°N and 78.478°E), two accessions (RG 1930 and RG 2008) collected from northeastern India (Anjani et al., 1994) were found to be free from leafminer infestation. These are dark purple colour morphotype where the entire plant including leaves is dark purple in colour (Anjani, 2005). Castor plants with green or red stem, green leaves and green or red capsules are common in India. Dark purple colour morphotype is a very rare type and localized to northeastern part of India. All the green leaf type castor germplasm accessions screened at the Directorate were found to be susceptible to leafminer. RG 1930 and RG 2008 stably expressed dark purple colour in the entire plant and resistance to leafminer over years. When high concentrations of total phenol were estimated in these accessions (Prasad and Anjani, 2001), the present study was undertaken to establish systematically the role of total phenol in resistance against leafminer. Total phenol concentration in a resistant dark purple leaf type accession (RG 1930) and a susceptible green leaf type accession (RG 2788) as parents as well as in various leaf colour types in F_1, F_2 and F_3 generations of resistant × susceptible and susceptible × resistant crosses was estimated in order to assess the role of total phenol in leafminer resistance and association with leaf colour. The findings would be useful in planning for leafminer resistance breeding.

2. Materials and methods

2.1. Plant material

RG 1930 is a leafminer resistant dark purple morphotype where the entire plant including leaves, stem, petioles and capsules is dark purple in colour (Fig. 1). RG 2788 is a leafminer susceptible green colour morphotype where the entire plant is green in colour (Fig. 2). The resistant × susceptible (RG 1930 × RG 2788) and susceptible × resistant (RG 2788 × RG 1930) crosses and their respective F_2 and F_3 generations were developed under controlled pollination conditions to avoid pollen contamination. Castor inflorescence is monoecious; it produces male flowers at the bottom and female flowers at the top. Proportion to male and female flowers in an inflorescence varies with genotype

* Corresponding author. Tel.: +91 040 24015345; fax: +91 040 24017969.
E-mail address: anjani_kammili@rediffmail.com (K. Anjani).

0926-6690/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2009.10.005

Fig. 1. Dark purple leaf type accession, RG 1930.

and growing environment. All male flowers in the first and second order racemes of the female parent plant were manually removed. Opened male flowers from male parent, collected in a closed Petri dish, were used for pollinating receptive styles of female flowers on female parent for cross-pollination. The cross-pollinated racemes of the female parent were covered with butter paper bags till harvesting. Similarly, self-generations of parents as well of F_2 and F_3 generations of both crosses were generated by covering the racemes with butter paper bags. The resistant × susceptible cross produced dark purple and purple-green leaf type progenies (Fig. 2) whereas susceptible × resistant cross produced only green leaf type progenies. Dark purple leaf type plants had absolute dark purple colour leaves whereas purple-green leaf type had combination of purple and green pigments with varying intensities. Green leaf type had absolute green colour leaves.

2.2. Screening against leafminer

Parents and F_1, F_2 and F_3 generations of resistant × susceptible and susceptible × resistant crosses were screened simultaneously against leafminer under natural heavy infestation in field at the Directorate of Oilseeds Research, Hyderabad, India (17.366°N and 78.478°E). Each parent was planted in six rows and F_1, F_2 and F_3 generations of resistant × susceptible cross were planted in 33, 60 and 18 rows, respectively. Whereas F_1, F_2 and F_3 generations of susceptible × resistant cross were planted in 23, 57 and 17 rows, respectively. Each row was of 5 m long. Row-to-row spacing was 90 cm while plant-to-plant spacing was 45 cm. The number of mines/leaf and heavily infested leaves/plant (%) were taken as indicators to score the reaction of each plant against leafminer. Data on number of mines/leaf were recorded on 45–55 days old plants coinciding with the peak leafminer infestation in August and September months. As leafminer infestation on a plant progresses from bottom leaf upwards, number of mines/leaf were counted on six bottom leaves and mean of six lower leaves was taken in each plant. Data on heavily infested leaves/plant (%) were recorded at the end of leafminer incidence when the plants were around 120 days old. Leaf was considered as heavily infested when more than 50% of lamina was damaged by leafminer. The number of such heavily infested leaves per plant were counted and expressed as percentage of heavily infested leaves/plant. Based on varying reaction of test entries against leafminer, plants recording zero to 10 mines per leaf and 0% heavily infested leaves/plant were scored as resistant and those recording higher than these values were scored as susceptible. Thus, each plant was characterized as either resistant or susceptible type. Arc sine transformation of data on heavily infested leaves/plant (%) and square-root transformation of data on number of mines/leaf were applied as applicable. Mean differences of resistant and susceptible parents as well as of dark purple, purple-green and green leaf types within each generation and combined over generations were compared using paired *t*-test.

Fig. 2. Purple-green leaf type (first row) and green leaf type (second row).

2.3. Estimation of total phenol concentration

The method outlined by Price and Butler (1977) standardized for this study, was followed for spectophotometric determination of total phenol concentration. Total phenol concentration in leaf tissue was estimated during peak infestation period of leafminer. One hundred milligrams of each of fresh leaf tissue in 5 ml of methanol was heated on water bath at 60 °C for 20 min. The contents were filtered through Whatman No. 1 filter paper, by washing with an additional 5 ml of methanol. The filtrate was mixed with 25 ml of distilled water and analysed within an hour. For analysis, 0.5 ml of aliquot was transferred to 5 ml of absolute methanol and then 25 ml of water was added. To this added 0.2 ml of 0.1 M $FeCl_3$ in 0.1N HCl, immediately followed by 0.2 ml of 0.008 M $K_3Fe(CN)_6$. The optical density of the solution was read after 10 min at 720 nm on a Sanyo SP 75 UV/vis spectrophotometer. A blank of identical composition without leaf extract was run and the value subtracted from sample readings. Total phenol concentrations were expressed as catechin equivalents from fresh solutions of commercial D-catechin. Catechin equivalents (C.E.) are expressed as milligrams of catechin/100 mg of leaf tissue. Samples included 50 plants in each parent, dark purple and purple-green phenotypes in F_1; 70 plants from each dark purple and purple-green phenotypes in F_2; 50 dark purple and 9 purple-green types in F_3 generation of resistant × susceptible cross. Additionally, samples from 50 F_1, 70 F_2 and 50 F_3 plants of susceptible × resistant cross were also analysed. Each sample was replicated twice and analysis of variance was done using completely randomized design. Resistant parent, susceptible parent and dark purple, purple-green and green leaf types in each generation as well as combined over generations were taken as sources of variation. When the variance due to these treatments was found significant ($P = 0.05$ and 0.01) for total phenol concentration, mean differences of each pair of treatments were compared with $LSD_{0.05}$ value. The relationship between leafminer infestation and total phenol concentration was determined in each generation using simple linear correlation coefficients. Statistical analysis was done using MSTAT-C software.

Table 1
Means and ranges of number of mines/leaf and heavily infested leaves/plant (%) in parents and different phenotypes in resistant × susceptible and susceptible × resistant crosses.

Parents and different phenotypes in of R × S and S × R crosses	Number of plants	Number of mines/leaf		Heavily infested leaves/plant (%)		Reaction against leafminer
		Mean[a]	Range	Mean[a]	Range	
Parents						
RG 1930	50	0.6 (1.0)	0–4 (0.7–2.1)	0 (0)	–	R
RG 2788	50	103 (10.2)	85–157 (9.2–12.5)	87 (68.8)	82–90 (65–71.5)	S
R × S cross						
Dark purple	832	1.8 (1.4)	0–8 (0.7–2.9)	0 (0)	–	R
Purple-green	257	52 (7.4)	31–54 (5.6–7.3)	55 (48.2)	51–57 (45.5–49.6)	S
S × R cross						
Green	957	104 (10.3)	85–156 (9.2–12.5)	86 (68)	76–95 (60.6–77.0)	S

[a] ($P < 0.001$). Figures in parentheses are transformed values. R: Resistant; S: Susceptible. The data from F_1, F_2 and F_3 generations were pooled to derive mean values and ranges in both crosses.

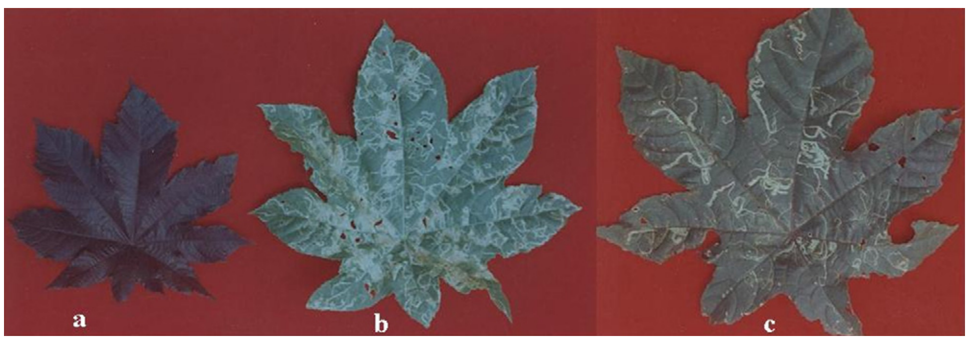

Fig. 3. Leafminer infestation on dark purple (a), green (b) and purple-green (c) leaves.

3. Results

3.1. Leafminer infestation

The mean and range of number of mines/leaf and heavily infested leaves/plant (%) recorded on different leaf colour progenies of resistant × susceptible and susceptible × resistant crosses and their parents are given in Table 1. The resistant and susceptible parents varied significantly ($P < 0.001$) with respect to number of mines/leaf and heavily infested leaves/plant (%). Similarly, significant ($P < 0.001$) differences were observed between different leaf colour types (Fig. 3) in each generation. Dark purple and purple-green leaf type plants were observed only in F_1, F_2 and F_3 generations of resistant × susceptible cross where the female parent was a dark purple leaf type. Susceptible × resistant cross produced only green leaf type plants in all generations. The dark purple leaf type in resistant × susceptible cross exhibited resistance against leafminer with very few mean number of mines/leaf (0, 3 and 1.5 in F_1, F_2 and F_3, respectively) and no heavily infested leaves/plant while the co-segregated purple-green leaf type showed comparatively higher mean number of mines/leaf (52, 53 and 52 in F_1, F_2 and F_3, respectively) and heavily infested leaves/plant (53%, 54% and 57% in F_1, F_2 and F_3, respectively) than dark purple leaf type. The green leaf plants observed in reciprocal cross exhibited susceptibility to leafminer with very high mean number of mines/leaf (102, 105 and 103 in F_1, F_2 and F_3, respectively) and high percent of heavily infested leaves/plant (87%, 86% and 87% in F_1, F_2 and F_3, respectively). Purple-green leaf plants exhibited comparatively lesser leafminer infestation than the green leaf plants.

3.2. Total phenol concentration

Highly significant treatment variance and non-significant replication variance were revealed for total phenol concentration. The total phenol concentration in parents and different colour offspring observed in resistant × susceptible and susceptible × resistant crosses are given in Table 2. Great difference was observed between between leafminer resistant dark purple leaf (RG 1930) and susceptible green leaf (RG 2788) parents for total

Table 2
Total phenol concentrations in parents and different leaf colour offspring observed in resistant × susceptible and susceptible × resistant crosses.

Parents and crosses	Leaf colour	Reaction against leafminer	Number of plants analysed	Total phenol concentration[a] (C.E.)	
				Mean[b]	Range
RG 1930	DP	R	50	2.31	2.22–2.41
RG 2788	G	S	50	0.23	0.21–0.24
R × S cross	DP	R	170	2.17	2.10–2.22
	PG	S	129	0.54	0.45–0.64
S × R cross	G	S	170	0.21	0.14–0.26

[a] Total phenol concentration was expressed as catechin equivalents (C.E.); C.E. is milligrams of catechin/100 mg of leaf tissue; R; Resistant; S: Susceptible; DP: Dark purple; PG: Purple-green; G: Green. The data from F_1, F_2 and F_3 generations were pooled in each leaf color type in both crosses.
[b] LSD (0.05) = 0.71.

Table 3
Correlation of total phenol concentration with number of mines/leaf and heavily infested leaves/plant in different generations of resistant × susceptible and susceptible × resistant crosses.

Different generations of resistant × susceptible and susceptible × resistant crosses	Correlation coefficients between	
	Total phenol concentration and number of mines/leaf	Total phenol concentration and heavily infested leaves/plant (%)
Resistant × susceptible		
F_1	$-0.98^{*,**}$	$-0.99^{*,**}$
F_2	$-0.95^{*,**}$	$-0.97^{*,**}$
F_3	$-0.92^{*,**}$	$-0.95^{*,**}$
Susceptible × resistant		
F_1	$-0.97^{*,**}$	$-0.99^{*,**}$
F_2	$-0.96^{*,**}$	$-0.96^{*,**}$
F_3	$-0.98^{*,**}$	$-0.98^{*,**}$

(*,**) Significant at 0.05 and 0.01 level of probability.

phenol concentration. The resistant and susceptible offspring also exhibited significant difference ($LSD_{0.05}$ = 0.71) for total phenol concentration. The leafminer resistant dark purple leaf type in various generations as well as combined over generations had significantly ($LSD_{0.05}$ = 0.71) higher concentrations of total phenol (F_1 = 2.15, F_2 = 2.18, F_3 = 2.16 C.E.) as compared to susceptible green (F_1 = 0.22, F_2 = 0.20, F_3 = 0.21 C.E.) and purple-green (F_1 = 0.58, F_2 = 0.52, F_3 = 0.49 C.E.) leaf types. Significant ($LSD_{0.05}$ = 0.71) differences were not observed between leaf miner resistant parent RG 1930 and dark purple leaf type offspring with regard to total phenol concentrations and leafminer infestation. Both had high concentrations of total phenol and were resistant to leafminer. Similarly, green leaf type offspring did not significantly vary from susceptible green leaf parent for total phenol concentrations. Both were susceptible and had low concentrations of total phenol. The susceptible purple-green offspring exhibited non-significantly higher total phenol concentrations than green leaf type but significantly much lower concentrations than resistant dark purple leaf type.

Total phenol concentration showed significant negative associations with number of mines/leaf (pooled $^{*,**}r = -0.97$) and percent heavily infested leaves/plant (pooled $^{*,**}r = -0.98$). The correlation coefficients between total phenol concentration and number of mines/leaf and percent heavily infested leaves/plant in F_1, F_2 and F_3 generations of resistant × susceptible and susceptible × resistant crosses are presented in Table 3. Negative associations were observed in each generation between concentration of total phenol and number of mines/leaf and percent heavily infested leaves/plant.

4. Discussion

The results established a significant positive association between total phenol concentration and leafminer resistance. Phenols are known to play an important role in plant defense against insects. Such association has been established in several crops (Panda and Khush, 1995). For example, in rapeseed (*Brassica campestris* L.), the plants with low phenols concentration were more vulnerable to *Liriomyza brassicae* Riley (Ipe and Sadaruddin, 1984). Negative correlation between total phenol concentration and whitefly population densities (Butter et al., 1992) and survival of bollworm (Zummo and Segers, 1984) was observed in cotton. Resistance level against stem borer (Santiago et al., 2005) and weevil (Classen et al., 1990) in maize was correlated with the amount of phenols. Gall midge *Orseolia oryzae* (Wood-Mason) resistant rice genotypes revealed higher levels of total phenol (Amudhan et al., 1999; Vijaykumar et al., 2009). In eggplant (*Solanum melongena* L.), high purple-pigmented cultivars exhibited resistance to shoot and fruit borer (Bajaj et al., 1989; Prabhu et al., 2007). These studies showed total phenol concentration as a leading and reliable indicator of resistance against insects.

Purple pigment conferring anthocyanins are chemically categorized under phenols (Brouillard et al., 1997). High purple pigmentation in dark purple offspring might have resulted into high concentration of total phenol. The purple-green leaf type had relatively higher leafminer infestation than dark purple leaf type and lower infestation than green leaf type. Leafminer susceptibility of purple-green leaf type indicates that the concentrations of total phenol in purple-green leaves were sub-optimal to resist leafminer infestation. High quantity of total phenol in dark purple leaf type is a factor of inherent host resistance. It rendered dark purple type toxic to leafminer; this was evident from formation of very few mines (1–2) of less than 1 cm size by maggots on 818 plants out of total 882 dark purple leaf plants screened in the present study.

5. Conclusions

Total phenol concentration based leafminer resistance in castor was established evidently over generations. The significantly high concentrations of total phenol in leafminer resistant dark purple leaf plants projected that selection of dark purple leaf phenotype is an effective visual and reliable approach for indirect selection of leafminer resistant genotypes in breeding programmes and also the need for estimating total phenol concentration can be avoided.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.indcrop.2009.10.005.

References

Amudhan, S., Rao, P.U., Bentur, J.S., 1999. Total phenol profile in some rice varieties in relation to infestation by Asian rice gall midge *Orseolia oryzae* (Wood-Mason). Curr. Sci. 76, 1577–1580.

Anjani, K., 2005. Purple-coloured castor (*Ricinus communis* L.)—a rare multiple resistant morphotype. Curr. Sci. 88, 215–216.

Anjani, K., Chakravarty, S.K., Prasad, M.V.R., 1994. Collecting castor (*Ricinus communis* L.) germplasm in North-Eastern Hill Province of India. IBPGR Newslett. APO 17, 13.

Bajaj, K.L., Singh, D., Kaur, G., 1989. Biochemical basis of relative field resistance of eggplant (*Solanum melongena* L.) to the shoot and fruit borer (*Leucinodes orbondalis* Guen.). Veg. Sci. 16, 145–149.

Brouillard, R., Figueiredo, P., Elhabiri, M., Dangles, O., 1997. Molecular interactions of phenolic compounds in relation to the colour of fruit and vegetables. In: Tomas-Barberan, F.A., Robins, R.J. (Eds.), Proceedings of the Phytochemical Society of Europe. Phytochemistry of Fruits and Vegetables. Clarendon Press, Oxford, pp. 29–49.

Butter, N.S., Vir, B.K., Kaur, T.H., 1992. Biochemical basis of resistance to whitefly *Bemisia tabaci* Genn. (Aleyrodidae. Hemiptera) in cotton. Trop. Agric. (Trindad) 69, 119–122.

Classen, D., Arnason, J.T., Serratos, J.A., Lambert, J.D.H., Nozzolillo, C., Philogene, B.J.R., 1990. Correlation of phenols acid content of maize to resistance to *Sitophilus*

zeamais, the maize weevil, in CIMMYT'S collections. J. Chem. Ecol. 16, 301–315.
Damodaram, T., Hegde, D.M., 2007. Oilseeds Situation: A Statistical Compendium. Directorate of Oilseeds Research, Hyderabad, India.
DOR, 2003. Castor in India. Directorate of Oilseeds Research, Hyderabad, India.
Ipe, I., Sadaruddin, M., 1984. Infestation and host specificity of *Liriomyza brassicae* Riley and the role of phenols compounds in host plant resistance. Entomon 9, 265–269.
Lakshminarayana, M., Basappa, H., Vijay, Singh, Singh, V., 1992. Report on the incidence of hitherto unknown leafminer *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) on castor. J. Oilseeds Res. 9, 175–176.
Panda, N., Khush, G.S., 1995. Host Plant Resistance to Insects'. CAB International, Wallingford, Oxon, UK.
Parrella, M.P., Jones, V.P., Youngman, R.R., Lebeck, L.M., 1985. Effect of leaf mining and leaf stripping of *Lyriomyza* spp. on photosynthetic rate of chrysanthemum. Ann. Entomol. Soc. Am. 78, 90–93.
Prabhu, M., Natarajan, S., Pugalendhi, L., 2007. Biochemical basis of fruit and shoot borer (*Leucinodes orbonalis* G.) resistance in bringal (*Solanum melanogena* L.). In: Kesavchandran, R., Nazeem, P.A., Girija, D., John, P.S., Peter, K.V. (Eds.), Recent Trends in Horticultural Biotechnology. New Delhi Publishing Agency, New Delhi, pp. 829–837.
Prasad, Y.G., Anjani, K., 2001. Resistance to serpentine leafminer *Liriomyza trifolii* (Burgess) in castor (*Ricinus communis* L.). Indian J. Agric. Sci. 71, 351–352.
Price, M., Butler, L.G., 1977. Rapid visual estimation and spectorphotometric determination of tannin content of sorghum grain. J. Agric. Food Chem. 55, 1268–1273.
Santiago, R., Malvar, R.A., Baamonde, M.D., Revilla, P., Souto, X.C., 2005. Free phenols in maize pith and their relationship with resistance to *Sesamia nonagriodes* (Lepidoptera: Noctuidae) attack. J. Econ. Ent. 98, 1349–1356.
Vijaykumar, L., Chakravarthy, A.K., Patil, S.U., Rajanna, D., 2009. Resistance mechanism in rice to the midge *Orseolia oryzae* (Diptera: Cecidomyiidae). J. Econ. Ent. 102, 1628–1639.
Zummo, B.J.H., Segers, J.C., 1984. Effect of the plant growth regulator Mepiquate chloride on host plant resistance in cotton to bollworm (Lepidoptera: Noctuidae). J. Econ. Ent. 77, 922–924.