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Morphogenetic and biochemical basis for stem borer *(Conogethes punctiferalis* guen) resistance in turmeric *(Curuma longa* L.) genotypes

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Abstract : Field trial was conducted in Turmeric *(Curcuma longa* L.) in the Department of Spices and Plantation crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University in a randomized block design with twenty six genotypes in two replications during the year 2000-2003. Observations were recorded on yield, stem borer *(Conogethes punctiferalis* Guen) incidence and various biochemical traits such as total phenols, poly phenol oxidase (PPO), peroxidase and catalase. The results revealed that the genotypes with higher tolerance limit showed higher peroxidase, PPO, catalase and phenol content. The genotype CL 101 exhibited lower incidence (10 per cent) coupled with higher yield, total phenols, PPO and catalase activity. The genotypes such as CL2, CL22, CL47, CL101 and CL153 exerted resistance to this pest and hence, these genotypes are suitable for growing under the conditions where the pest incidence is predominant.

Key words: Conogethes punctiferalis Guen., total phenols, catalase, polyphenol oxidase, tannins and biochemical resistance.

Introduction

Turmeric *(Curcuma longa* L. Syn. *C.domestica* Val.) of commerce is the dried rhizome, it belongs to the family Zingiberaceae that traces its origin to tropical rain forests of South East Asia. Host plant resistance is an environmentally benign, low input method for managing insects or other pests. Past work has indicated that plant resistance is often related to levels of secondary metabolites present in the plants. More recently, protein-based defensive factors have been recognized as important host defensive compounds. Majority of the identified proteins act directly and potentially including lectins and protease or other enzyme inhibitors (Gatehouse, 1999). Other enzymes, in the presence of appropriate colactors and substrates, can potentially generate chemicals that are more toxic to pests than the original substrates. These enzymes include polyphenol oxidases, catalase and peroxidases. Turmeric is vegetatively propagated and hence systematic efforts on introduction and evaluation of stem borer resistant varieties have not been undertaken consistently. The shoot borer *(Conogethes punctiferalis* Guen) (Pyralidae:Lepidoplera) is the most widespread and serious insect pest of turmeric *(Curcuma tonga* L) in India (Devasahayam, 2002). The larvae of the pest bore into shoots and feed on internal tissues resulting in yellowing and drying of infested shoot. The shoot borer is highly polyphagous and has been recorded on many economically important crops. Yield loss caused by the pest indicated that when 50 per cent of the pseudostem in a clump was affected there was reduction of 38g of yield per clump (Koya *et al.*, 1986). Inspite of the serious nature of the pest, very few reports are available regarding the pest resistance studies in turmeric. Hence a preliminary screening was undertaken, based on the morphogenetic and biochemical

Table 1. Biometrical observations on twenty six turmeric genotypes

Genotypes	Maturity (days)	Weight of mother rhizome (g)	Weight of primary rhizome(g)	Girth of primary rhizome(cm)	Rhizome to core ratio	Curcumin content (%)	Oleoresin content (%)
CL2	242.50	49.77	132.74	7.05	1.58	4.40	5.24
CL5	243.00	25.40	20.30	2.93	2.87	3.53	4.57
CL19	258.00	43.40	2.99	2.47	1.74	4.29	3.96
CL22	254.50	55.44	125.82	8.69	1.58	4.65	4.51
CL47	257.25	65.19	83.64	6.52	1.59	4.58	4.07
CL58	252.50	40.60	24.13	4.92	3.25	3.77	3.96
CL63	255.00	21.85	9.15	2.68	1.44	3.77	4.10
CL101	253.50	58.75	172.25	7.84	2.17	4.04	3.87
CL110	252.50	9.75	2.99	2.22	1.93	3.76	4.07
CL112	253.00	71.36	128.65	6.87	1.46	3.02	4.23
CL113	253.50	59.65	179.54	7.55	1.51	3.27	3.65
CL115	257.25	61.21	127.36	7.31	0.77	4.76	4.03
CL118	252.50	56.88	88.31	4.71	1.00	3.82	3.78
CL119	253.50	53.08	134.04	7.42	1.18	5.09	3.83
CL122	230.00	58.75	56.20	4.98	1.37	4.51	4.37
CL127	231.00	63.55	108.80	7.75	1.74	4.76	4.11
CL128	230.25	76.44	114.10	7.86	1.79	4.42	3.98
CL137	232.50	85.34	50.80	4.97	1.99	4.04	3.78
CL138	232.00	67.46	103.60	4.91	1.90	5.00	4.14
CL153	240.50	99.08	154.77	7.97	1.72	4.49	4.03
CL173	241.00	51.27	101.26	5.22	2.74	2.95	3.97
CL195	256.00	71.57	78.64	5.97	1.75	3.97	3.65
CL196	229.50	67.26	112.48	6.97	2.21	3.66	3.96
CL200	238.25	39.57	54.67	4.26	2.18	3.92	4.26
CL209	250.50	49.67	107.83	5.22	2.35	3.35	4.27
CL223	259.50	39.54	68.31	4.49	1.93	3.57	3.98
Mean	246.52	55.45	90.13	5.76	1.84	4.05	4.09
SE(d)	1.74	2.54	2.96	0.22	0.10	0.12	0.18
C.D (0.05)	3.52	5.22	6.09	0.54	0.20	0.24	0.37

properties of both susceptible and tolerant genotypes in turmeric.

Materials and Methods

Field trial was conducted with an objective to investigate the various mechanisms of stem borer resistance/ tolerance in turmeric genotypes in a randomized block design with twenty-six genotypes in two replications. Observations were taken on maturity, rhizome characters, yield (kg/plot), cucumin (Manjunath *et al.* 1991), oleoresin (Anon., 1975), stem borer incidence (Heinrichs *et al.* 1985) and various biochemical traits such as total phenols (Malik and Singh, 1980), poly phenol oxidase (Bateman and Daly, 1967), peroxidase (Anglini *et al.* 1990), catalase (Gopalachari, 1963) and tannins (Schandrel, 1970) in three stages *viz.*, Stage I (3rd month), Stage II (5th Month) and Stage III (8th month) after planting. The

Table 2. Per se performance of twenty six turmeric genotypes for curing per cent, shoot borer incidence, yield/3m^2, total phenol content and polyphenol oxidase content

Genotypes	Curing (%)	Shoot borer incidence (%)	Yield (kg/3m^2)	Total phenols (µg g^{-1})			Poly phenol oxidase (AOD 490 nm g^{-1}hr^{-1})		
				Stage1	Stage2	Stage3	Stage1	Stage2	Stage3
CL2	16.09	10.00 (18.44)	7.00	95.115	87.825	91.235	0.646	0.779	0.645
CL5	14.85	65.00(53.78)	0.41	55.080	27.580	31.235	0.353	0.366	0.357
CL19	12.75	60.00 (50.77)	0.36	57.000	29.475	31.965	0.393	0.399	0.398
CL22	14.08	15.00 (22.50)	8.49	118.820	114.785	116.515	0.755	0.848	0.686
CL47	12.88	10.00 (18.44)	11.69	119.835	115.780	118.805	0.762	0.825	0.831
CL58	15.81	65.00(53.78)	0.19	53.280	30.280	34.560	0.411	0.419	0.446
CL63	12.80	70.00 (56.79)	0.19	55.485	28.800	31.430	0.358	0.360	0.360
CL101	13.61	10.00(18.44)	12.42	117.605	113.765	116.155	0.709	0.818	0.754
CL110	16.02	70.00 (56.79)	0.15	58.630	31.420	34.205	0.400	0.404	0.401
CL112	18.07	35.00 (3622)	5.94	88.235	78.805	82.970	0.524	0.538	0.528
CL113	20.36	40.00 (39.23)	3.61	71.335	57.525	59.770	0.513	0.518	0.516
CL115	15.11	25.00(29.89)	6.68	90.550	83.775	87.320	0.608	0.609	0.636
CL118	12.63	55.00(47.89)	1.02	59.795	40.295	44.635	0.408	0.414	0.409
CL119	14.79	20.00(25.82)	6.35	89.235	85.230	86.265	0.540	0.524	0.542
CL122	20.55	55.00(47.89)	0.56	63.190	37.285	41.735	0.410	0.428	0.413
CL127	17.90	55.00(47.89)	1.96	64.805	41.605	44.520	0.411	0.424	0.416
CL128	17.63	50.00(45.00)	1.04	62.870	36.280	42.560	0.418	0.432	0.441
CL137	12.39	50.00 (45.00)	0.49	53.280	26.310	32.190	0.412	0.423	0.461
CL138	15.96	65.00(53.78)	0.52	55.940	27.457	33.217	0.412	0.398	0.433
CL153	19.97	15.00 (22.50)	12.06	116.240	115.270	114.290	0.812	0.814	0.816
CL173	17.65	25.00(29.89)	3.70	90.715	80.765	83.155	0.607	0.612	0.629
CL195	14.61	55.00 (47.89)	1.03	54.890	27.290	33.620	0.405	0.409	0.452
CL196	21.64	45.00(42.12)	4.66	69.615	52.290	54.590	0.434	0.450	0.436
CL200	14.27	60.00 (50.90)	0.89	57.140	29.310	32.240	0.407	0.411	0.442
CL209	16.35	45.00(41.99)	5.94	74.585	59.340	60.230	0.513	0.541	0.526
CL223	22.47	55.00(47.88)	0.58	66.590	53.195	53.475	0.429	0.436	0.430
Mean	16.20	46.44	3.76	75.379	58.142	61.264	0.501	0.522	0.515
SE(d)	0.95	5.47	0.31	2.098	1.415	1.965	0.010	0.014	0.012
C.D (0.05)	1.95	11.27	0.65	4.321	2.915	4.048	0.020	0.030	0.025

biochemical's were quantified by spectrophotometer method. The results pertaining to the study were presented in the Table 1,2 and 3.

Results and Discussion

The longest maturity was observed in the genotype CL223 (259.50 days) and the genotypes CL19 (258.00 days), CL47 (257.25 days), CL115 (257.25 days) and CL195 (256.00 days) were on par. The genotype, which recorded the shortest maturity, was CL196 (229.50 days). Generally, the genotypes with longer duration had higher incidence than short and medium duration genotypes, which might be due to the extended exposure of the host to the pest

Table 3. *Per se* performance of turmeric genotypes for catalase activity, peroxidase activity and tannin content

Genotype	Catalase activity (AOD 430 nm $g^{-1}hr^{-1}$)			Peroxidase (AOD 430 nm $g^{-1}hr^{-1}$)			Tannin content (ug g^{-1})		
	Stage1	Stage2	Stage3	Stage1	Stage2	Stage3	Stage1	Stage2	Stage3
CL2	0.989	1.155	1.121	0.052	0.060	0.058	50.267	56.324	53.648
CL5	0.576	0.673	0.652	0.033	0.038	0.037	23.167	26.316	25.649
CL19	0.578	0.676	0.655	0.034	0.040	0.038	22.395	26.319	24.146
CL22	1.306	1.525	1.480	0.056	0.065	0.063	61261	66.247	63.297
CL47	1.560	1.822	1.768	0.064	0.075	0.072	59.349	65.209	62.178
CL58	0.523	0.593	0.637	0.032	0.042	0.037	19.236	22.311	19.547
CL63	0.558	0.665	0.629	0.033	0.038	0.038	21.541	25.692	23.154
CL101	1.180	1.378	1.337	0.055	0.065	0.063	61.234	66.974	64.267
CL110	0.583	0.680	0.660	0.034	0.040	0.039	22.319	23.649	24.618
CL112	0.789	0.922	0.894	0.050	0.058	0.056	54.231	57.219	56.298
CL113	0.663	0.774	0.751	0.042	0.050	0.048	49.357	53.141	51.018
CL115	0.860	1.004	0.974	0.051	0.060	0.058	52.319	56.228	54.267
CL118	0.612	0.715	0.694	0.035	0.041	0.040	36.326	40.219	38.224
CL119	0.757	0.884	0.858	0.051	0.059	0.058	51.230	54.119	52.547
CL122	0.615	0.718	0.696	0.039	0.046	0.045	32.169	36.118	35.219
CL127	0.628	0.734	0.712	0.037	0.043	0.042	34.292	37.229	36.219
CL128	0.639	0.697	0.794	0.034	0.043	0.039	27.661	30.265	29.317
CL137	0.564	0.611	0.679	0.033	0.037	0.042	24.361	27.231	25.678
CL138	0.516	0.558	0.681	0.036	0.044	0.038	21.316	25.462	23.179
CL153	1.618	1.651	1.662	0.069	0.070	0.072	63.219	66.648	65.149
CL173	0.849	0.990	1.010	0.052	0.062	0.031	51.264	54.164	53.216
CL195	0.584	0.591	0.651	0.034	0.042	0.037	26.319	30.224	29.315
CL196	0.662	0.773	0.750	0.044	0.051	*0.049*	25.366	28.668	27.264
CL200	0.516	0.543	0.681	0.034	0.042	0.038	24.168	27.222	26.319
CL209	0.695	0.812	0.788	0.044	0.051	0.049	39.218	43.641	42.658
CL223	0.699	0.816	0.792	0.040	0.047	0.045	24.333	29.613	26.114
Mean	0.773	0.882	0.884	0.043	0.050	0.048	37.612	41.402	39.711
SE(d)	0.032	0.033	0.049	0.008	0.001	0.002	1.168	1.262	1.835
C.D(0.05)	0.066	0.069	0.101	0.003	0.003	0.005	2.406	2.600	3.781

(Table 1). Plants that evade insect attack by this mechanism are in reality susceptible, provided the pest incidence occurrs at the right time. As the crop duration of the genotypes is under genetic control, the technique of breeding by selection in this clonally propagated crop for early and medium duration crops from the shoot borer incidence is only by the escape mechanisms. This could be well justified from the fact that population dynamics for the shoot borer is very low in the short and medium duration as compared to long duration genotypes,

which favours the host susceptibility in long maturing clones. The lower pest incidence in the short and medium duration genotypes might be attributed by multivariate changes in the biochemical and morphological in the plant might have increased the resistance of plants to further incidence of the pest.

The genotype CL113 (179.54g) recorded the highest primary rhizome weight, which significantly differed from other genotypes (Table 1) and it was closely followed by the genotype CL101 (172.25g). The lowest weight of 2.99g was observed in two genotypes *viz.*, CL19 and CL110. The primary rhizome girth ranged between 2.22 cm (CL110) and 8.69 cm (CL22). The genotypes varied significantly for rhizome to core ratio among the genotypes evaluated. The highest ratio was observed in the genotype CL58 (3.25) that varied significantly from other genotypes and the lowest ratio was registered in the genotype CL115 (0.77)

The curing per cent ranged between 12.39 and 22.47 per cent (Table 2). The highest per cent was observed in the genotype CL223 (2.47 per cent) and the genotypes CL196, (21.64) was on par. The oleoresin content significantly varied from 3.65 per cent in CL195 to 5.24 per cent in CL2 (Table 1). The highest curcumin content was observed in the genotype (CL119 (5.09 per cent) which differed significantly from other genotypes and it was closely followed by CL138 (5.00 per cent). The lowest curcumin content was observed in the genotype CL173 (2.95 per cent) and it was on par with CL112 (3.02 per cent).

The genotype CL101 (12.42 Kg) was superior in yield of fingers and it was closely followed by the genotype CL153 (12.06 Kg), CL47 (11.69 Kg) and CL153 (12.06 Kg). Poor yield of 0.15 Kg was observed in the genotype CL110 (Table 2). Generally genotypes with lower incidence of pest recorded higher yield. It would have been due to the fact that, defence genes are activated which may function as deterrents or anti-feedants or elicited defence related products. Induced responses to herbivory are "immune-like" responses that reduce the performance or preference of herbivore (or both). The action of induced systemic resistance is based on the defense mechanisms that are activated by inducing agents and once expressed it activates multiple potential defense mechanisms that include increased activity of chitinases, β-3-glucanases and peroxidases. In the present investigation the lower level of incidence in the resistance genotypes could have been due to the production of certain PR III proteins *(i.e.* chitinases) involved in octadecanoid pathway. A separate signalling pathway with jasmonic acid (JA) is involved in systemic responses to wounding and insect herbivory.

In the present investigation, the pest incidence varied from 10.00 per cent to 70.00 per cent (Table 2). Out of twenty-six genotypes studied for the shoot borer incidence, the resistant genotypes were CL2, CL22, CL47, CL101 and CL153 indicate that these genotypes are amenable for growing under the conditions where the pest incidence is predominant. The higher incidence of shoot borer was recorded in the genotypes *viz.*, CL63 and CL110.

Enzymes in the presence of appropriate cofactors and substrates can potentially generate chemicals that were more toxic to pest than the original substrates. These enzymes include polyphenol oxidase, catalase and peroxidase and anti-nutritional factors like phenols and tannins. Deposition of more structural proteins and phenolics in the cell wall regions would directly influence the resistance mechanisms. In the present investigation, there were significant variations among the genotypes evaluated for acessine the total phenol content at third, fifth and eighth month after sowing (Table 2). Under all the stages of crop growth, the genotypes

with higher phenol content expressed lower incidence of the pest (Table 2). Similar observations were recorded by Umamaheswari (2001). Similarly, it was exhibited that the level of phenols is greatly increased in response to cutting injury in all kinds of plant tissues and resistance offered to the pest might be due to the production of chlorogenic acid and isochlorogenic acid. In the present investigation, the tolerant genotypes expressed lower phenol content than the resistant cultivars which might be due to the enhanced enzyme activity such as polyphenol oxidase on phenols to produce toxic substances like quinines. This is in agreement with the results of Odabasoglu and Kufervioglu(2001).

In the present study, there were significant variations among the genotypes for polyphenol oxidase content (Table 2). Generally, the genotypes with lower incidence expressed high polyphenol oxidase content. The increased polyphenol oxidase activity in the resistant genotypes might also be due to the production of chemically active substances which are produced by orthohydroxylation of monophenols by polyphenol oxidase to yield corresponding ortho quinones and ortho diphenols. The ortho quinones may act as feeding deterrent to shoot borer and triggered increase in this compound would restrict the feeding behaviour. Among the different stages, Stage 2 exhibited higher polyphenol oxidase activity than other stages, which could have been due to the induced defence triggered signalling of shoot borer incidence (Table 2). This is in consonance with the earlier reports of Mohhamadi and Kazemi (2002). Polyphenol oxidase usually accumulates upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult because the quinonoid reaction products of PPO covalently modify and cross-link the enzyme. In the present investigation, polyphenol oxidase activity was decreased at Stage 3, but the total phenol content increased irrespective of all the genotypes. It may be due to the block in natural reductive mechanism, which caused an abnormal activity of polyphenol oxidase, so that quinines accumulate to the extent of becoming detrimental to the entire cellular metabolism. So, there was increased phenol content and decreased polyphenol oxidase activity in Stage 3.

The genotypes with lower incidence expressed higher catalase activity irrespective to the various stages studied (Table 3). It might be due to removal of excessive hydrogen peroxide by catalase, which would have caused tissue damage, thus making more susceptible to insect pest. Among the three different stages evaluated, the highest mean activity of catalase was observed in Stage 3 and the least was in Stage 1. The peroxidase activity had increased with lower shoot borer incidence of genotypes under the study irrespective to various stages. It would have been due to the fact that peroxidase increased the toxic substances such as quinines by oxidation of phenols and thus induced insect resistance. This is in concomitant with the previous works of Dowd and Lagrimini (1996).

Although peroxidase alone has little effect on insect (Dowd and Vega, 1996), there was some evidence that peroxidases can contribute to insect resistance. The increased resistance in the genotypes may also be attributed due to the increased stiffness and toughness in plant tissues through lignification or other polymerization or cross linking reactions which was catalysed by peroxidase. At Stage 3, there was reduction in peroxidase activity than Stage 2 (Table 3), which may be due to breakdown of peroxidase by phenols with subsequent production of hydrogen peroxide necessary for lignification. Peroxidase reduces H_2O_2 to water at the expense of electron from coniferyl alcohol and the process initiates the lignification chain reaction in plants. The early and increased expression of the peroxidase enzymes would have involved

n the biochemical reaction necessary for lignification which protected the plants after wounding. Lignified plant tissues were generally not preferred for feeding by the soft bodied larval stage of the borers and hence the incidence of shoot borer will be lower in the genotypes where the lignification process was hastened. This is in corroboration with the earlier reports of Moder *et al.* (1975).

Significant variations were observed among the genotypes for tannin content in all the three stages and among them Stage 2 expressed the highest content followed by Stage 3 and the least in Stage 1 (Table 3). Irrespective of the stages, the genotypes with lower incidence exhibited higher tannin content. It might be attributed by the fact that tannins combine with protein to form indigestible complexes. The generalized nature of interaction between tannins and protein involved extensive formation of hydrogen and perhaps covalent bonds, probably rendered it difficult for insects to develop specific detoxification mechanisms like those known for alkaloids and other poisonous plant constituents. This is in accordance with the findings of Martin *et al.* (2002). The resistance might also be due to the fact that, when tannic acid was ingested inside the mid gut lumen of insect, it generated significant quantities of peroxides, including hydrogen peroxide, a powerful cytotoxin, readily diffused across cell membranes which would had ultimately collapsed the alimentary tract of the pest. Thus, the genotypes with higher tannin content expressed lower incidence of pest compared to the susceptible genotypes. This is in agreement with the findings of Raymond *et al.* (2001).

An understanding of" biochemical nature and knowledge of the specificity and compatibility of the signalling systems that regulate the expression of inducible responses could optimize the utilization of these responses in crop protection. The presence of induced resistance for a longer time extends the possibility of any direct or indirect effects on natural enemies and other non-target pests. Thus, these possible long-term effects of induced resistance should be studied at a tritrophic and possibly multitrophic level of plant insect interactions.

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