BIOCHEMICAL AND CUTICULAR VARIATION IN TEAK CLONE LEAVES AND RESISTANCE TO TEAK DEFOLIATOR

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Introduction

Clonal forestry is now well recognized as a method for mass production of desired trees to get maximum economic benefits since it enables the capture of genetic gain generated by skillful selection. During the last two decades species like teak, poplar, eucalypts and casuarinas have been extensively planted commercially under agroforestry as well as in many other afforestation programmes. Large scale defoliation by *Hyblaea puera* (Lepidoptera : Hyblaeidae) in *Tectona grandis* Linn. f (Verbenaceae) is a growing problem in plantations and in areas covered under agroforestry with teak. Approximately 44% of the potential increment in volume is lost due to defoliation (Nair *et al*., 1985). Mass migrating behaviour of defoliators limits the use of natural enemies or pesticides against them. To overcome such limitations, an ecological approach to make the environment unfavourable to pests and less harmful to the natural enemies is needed. There are innumerable possibilities for an unfavorable condition to exist in a plant that would reduce the possibility of its successful utilization by an insect. This unfavorable condition that could be of chemical or physical basis adversely affects the insect's feeding, growth and reproduction leading to insect pest resistance in plants. Limited works have been carried out to identify and evaluate resistant phenotypes in teak. Identification of a resistant crop/tree variety, which forms the basic foundation for an integrated pest management programme, holds promise for effective suppression of defoliators in teak. While comparing the incidence and extend of damage by teak defoliator on various teak clones, a significant difference was observed in the extent of feeding damage on certain clones in the field. An attempt has been made therefore to analyse and correlate the physical and chemical nature of the leaves of selective teak clones with the extent of feeding damage done by teak defoliator, *H. puera* on individual clones.

Material and Methods

Selected clones with negligible feeding under field conditions (MYSA-3, APKKA-1, APAKB-1, APKKP-1, APKZB-1, APNBB-1, APKKC-2, TNT-16, APNPL-6), moderately susceptible (BHA-30) and highly susceptible (APKKR-4) to defoliator larvae were selected from an earlier study (Prasanth Jacob *et al*., 2000). Clone identity and origin are given in Table 1. Fresh cut leaves were provided for fixed number of equal aged and starved final instar larvae of *H. puera* for feeding under choice and no choice conditions for a period of 24 hours. Area fed on each clone was calculated and compared. Five replicates were kept for each clone.

6-7 μ thickness sections of fresh leaves of the studied clones were taken in a Cryostat and histological details were studied with the help of Lietz DMRB (E) Microscope. One cm^2 bits of fresh leaves were cut and boiled in glass vial with distilled water at 80°C for 15 minutes. Leaf bits were further boiled with Ethyl alcohol followed by Lactic acid and then washed with water, mounted on a slide and observed under microscope for assessing the types of trichomes and their density. Biochemical estimation of clone leaves was done for total protein (Lowry *et al.*, 1951), carbohydrates (Dubois *et al.*, 1956) and lipids (Folch *et al.*, 1957). For the detection of phenols, flavanoids and anthocyanidins, the method of Harborne (1973) was adopted.

Results

Table 1 shows the comparison of feeding area by *H. puera* on teak clone leaves under no choice and choice tests. The area fed showed that there was no significant variation (CD at 5% 2.35) in the extent of feeding by *H. puera* among the clones viz. MYSA-3, APKKA-1, APAKB-1 and APKKP-1 under no choice condition. Again under choice test, no significant difference was noticed among these clones except APKKP-1. Though the extend of feeding under choice test condition in clones APKZB-1, APNBB-1 and TNT-16 were also not significant there

Table 1

Comparison of feeding area by H. puera on teak clone leaves under no choice and choice tests

Category	Clone Origin	Clone Identity	No choice test			Choice test		
			Area Fed (cm^2/insect)		Grade	Grade	Area Fed (cm^2/insect)	
Unattacked/ resistant	Karnataka	MYSA-3	7.80	0.38	1	3	4.08	0.17
	Andhra Pradesh	APKKA-1	8.00	0.39	2	1	2.83	0.12
	Andhra Pradesh	APAKB-1	9.26	0.55	3	2	3.58	0.20
	Andhra Pradesh	APKKP-1	9.60	0.49	4	9	5.64	0.24
	Andhra Pradesh	APKZB-1	10.25	0.46	5	4	4.14	0.18
	Andhra Pradesh	APNBB-1	10.62	0.40	6	5	4.28	0.10
	Andhra Pradesh	APKKC-2	21.30	1.64	7	8	5.46	0.20
	Karnataka	TNT-16	47.25	2.12	8	6	4.41	0.22
	Andhra Pradesh	APNPL-6	48.25	2.84	9	7	4.91	0.27
Moderately susceptible	Karnataka	BHA-30	50.45	2.97	10	10	32.87	1.81
Highly susceptible	Andhra Pradesh	APKKR-4	69.55	3.30	11	11	45.58	2.63

CD at 5% 2.35

was a shift in the grade or status of these clones compared to that under no choice situation. However, it was interesting to note that the first, second and third grades remained within the three clones viz. MYSA-3, APKKA-1 and APAKB-1 under choice and no choice tests. Observations on the feeding behaviour of *H. puera* larvae showed that feeding was mostly attempted at the margins and patches of veins were left in the lamina with only the tissues scrapped and fed in resistant clones while the whole leaf is consumed in susceptible clone.

Histological studies of leaves showed marked variation in leaf surface cuticular layer among clones. Cross section of leaves showed that most susceptible and moderately susceptible clones have a smooth or even cuticular layer whereas in resistant clones it is rough (Fig. 1). The adaxial surface of resistant clones is rough with numerous convex projections with a diameter of 400-600μ, topped with a single, pointed, hard trichome (Type I) (Fig. 2E). The supporting cuticular cells towards the apex region of these convex projections are columnar with sharp edges on the free outer surface. In addition to this numerous long, sharp (Type II) and short, blunt spines (Type III) are also distributed on the adaxial surface. The abaxial surface of these unattacked clones also has numerous long (240 μ) and short (60 μ) soft hairs with a density of 5460-5500/cm^2 (Table 2), (Fig-2 F). In the case of most susceptible clone, the upper cuticular surface is smooth without any spines. Very few diffused type of cuticular thickenings of only 100 microns in diameter were observed. Lower surface of these clones had isolated, multicellular long hairs (200-240 μ) (Fig. 2 A and B). Moderately susceptible clones also had a similar pattern as that of most susceptible clones. But the number of cuticular thickenings on the adaxial surface was comparatively more than that in most susceptible clones (Fig. 2 C and D).

Results show that the total protein content of resistant clones was comparatively lesser than the clones of most and moderately susceptible category. Further, the total carbohydrate and lipid contents in the leaves of unattacked or resistant group were higher than the most and moderately susceptible clones (Fig. 3).

About 8 phenolic and 7 flavanoid fractions were detected and identified with the clones subjected to the test. Of which the phenols such as Orcinol, and Pyrogallol were found common in the clones of relatively resistant category as compared to the susceptible clones, where these compounds were either absent or in trace amounts (Table 3). Clone MYSA-3 showed the presence of additional fractions such as Protocatechuic acid and 2-Methyl resorcinol (Table 3). Hesperidin, naringenin and naringin formed the common flavanoids in all the tested clones, which include both the susceptible and unattacked groups. However, presence of some additional flavanoids such as Aureusidin 6-glucoside and Aureusidin 4-glucoside were detected with few of the relatively resistant clones. Analysis of anthocyanidins expressed trace amount of anthocyanidin compounds with almost all the clones studied except in APKKR-4 and APKKP-1, where two specific fractions could be detected.

Discussion

Insects respond to various feeding stimuli when selecting their host plants. The plant surface is embedded with

Fig. 1

Cross section of Teak clone leaves

A. APKKR-4 clone leaf; B. BHA-30 clone leaf; C. APKKA-1 clone leaf

Cu- Cuticle; Ct- Cuticular thickening; Tr II – Trichome Type II; Tr III – Trichome Type III

Bar represents 40µ

Table 2

Trichome type and density in teak clone leaves

Category	Clone	No. of trichomes/cm^2				Remarks
		Adaxial surface		Abaxial surface		
Most susceptible	APKKR-4	180	10.44	240	14.16	Very few diffused type of upper cuticular thickenings. No spines. Isolated, multicellular long hairs on lower surface.
Moderately susceptible	BHA-30	600	29.94	200	8.78	Diffused type of upper cuticular thickenings. No spines. Isolated, multicellular long hairs on lower surface.
Unattacked / Resistant	MYSA-3	1075	63.42	5500	302.50	Numerous convex projections topped with a single, pointed, hard trichome (Type I) numerous sharp spines (Type II) and blunt spines (Type III) distributed on the adaxial surface. High density of soft, long and short trichomes on the abaxial surface.
	APKKA-1	825	48.67	5460	322.10	
	APAKB-1	1000	48.90	5550	327.41	
	APNBB-1	800	47.20	5520	269.92	

physical and chemical factors like trichomes, leaf surface chemicals, tissue toughness, nutrient deficiency and repellant and deterrent chemicals responsible for antixenosis to feeding insects (Southwood, 1986). Observations on the feeding and reproductive behavior of the defoliators in the field revealed that the adults did not exhibit any ovipositional preference among the clones. In the field eggs were also laid on resistant clones. The larvae hatched out cut small leaf flaps and fold it over and fed form inside. However no further feeding was observed in clones like MYSA-3 and APAKB-1. The larvae fed initially died or subsequently left the leaf flaps. Trichomes frequently impart resistance by providing an effective barrier that prevents insects from landing on the surface and prevents movements and feeding (Goertzen and Small, 1993). In the present study the abaxial surface trichomes tends to impede movement and prevent the early instars of *H. puera* from reaching the leaf surface to feed on. A similar phenomenon was encountered by the newly hatched Heliothis virescens on cotton with pubescence (Ramalho *et al.*, 1984). Rate of travel by fifth instar larvae of pink bollworm Pectinophora gossypiella was faster on smooth leaves than on those with pubescence (Smith *et al.*, 1975). Leaf texture of clones varied from soft and smooth as in APKKR-4 to tough and spiny as in MYSA-3 and APKKA-1. The thick cuticular structures and spines on the adaxial surface of resistant teak clones tends to reduce the rate of feeding as well as food utilization by *H. puera* larvae. Leaf toughness and increased fibrous materials

Fig. 2

Upper and lower surfaces of various Teak clone leaves

A. Upper leaf surface of APKKR-4 clone; B. Lower leaf surface of APKKR-4 clone; C. Upper leaf surface of BHA-30 clone; D. Lower leaf surface of BHA-30 clone; E. Upper leaf surface of APKKA-1 clone; F. Lower leaf surface of APKKA-1 clone.

Ct - Cuticular thickening; Tr I - Trichome Type I; Tr II - Trichome Type II; Tr III - Trichome Type III

Bar represents 200µ

Table 3

Phenols, flavanoids and anthocyanidins in teak clones

Clones	Phenols*	Flavanoids*	Anthocyanidins*
APKKR-4	p-Hydroxybenzoic acid Vanillic acid Resorcinol	Naringenin (Flavanone) Apigenin (Flavone)	Pellargonidin Delphinidin
BHA-30	Vanillic acid Orcinol Salicylic acid Resorcinol	Phloridzin (Dihydrochalcone) Naringenin (Flavanone) Naringin, Hesperidin (Favanone)	Anthocyanin groups were expressed in the chromatogram but not specified by any anthocyanidins.
MYSA-3	Orcinol Protocatechnic acid Pyrogallol Salicylic acid 2-Methyl resorcinol	Aureusidin 6-glucoside Aureusidin 4-glucoside	Anthocyanin groups were expressed in the chromatogram but not specified by any anthocyanidins.
APKKA-1	Oricinol Pyrogallol	Hesperidin	Anthocyanin groups were expressed in the chromatogram but not specified by any anthocyanidins
APAKB-1	Oricinol Pyrogallol	Aureusidin 4-glucoside Naringin	Anthocyanin groups were expressed in the chromatogram but not specified by any anthocyanidins

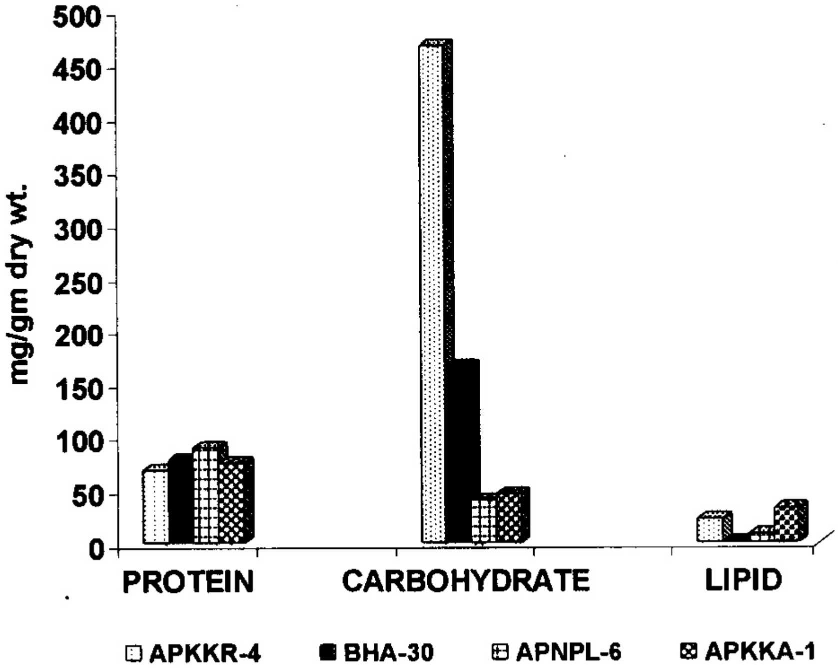
* Fractions with best separations were eluted.

may reduce the suitability of leaves as food source for phytophagous insects leading to decreased consumption and egg out put (Raupp, 1985). Such non-nutritional bulk cellulose and lignin pass through the insect gut without being digested (Smith and Kreitner, 1983) and the young larvae die from the unbalanced diet of fibrous materials. In the present study histological profiles of APKKP-1 and APNPL-6 showed comparatively thicker cuticular layer. In addition to this is the density and distribution of cuticular structures on upper surface of teak leaves which may deter *H. puera* larvae from continued feeding on resistant clones.

Conclusion

The present study shows that phytochemical and trichome mediated resistance influencing the growth and distribution of *H. puera* is prevalent in certain teak clones. Teak is a species now widely used in clonal forestry and new selections should continue to provide sufficient genetic diversity required for inhibiting the spread of pests. Genetic

Fig. 3



Primary metabolites in teak clone leaves

variation in physical and chemical properties is far less studied and should be exploited to develop resistant strains of trees against key pests through breeding as well as genetic engineering/ transformation.

Acknowledgements

The authors are thankful to the Director and Coordinator (Research), IFGTB for providing all the facilities and encouragement during the course of this work. Financial assistance through a World Bank funded Forestry Research Education and Extension Project is also acknowledged.

SUMMARY

Nine resistant teak clones identified based on a four-year field survey were taken for confirmation of its resistant nature against teak defoliator, *Hyblaea puera*, by comparing the feeding preference with most susceptible and moderately susceptible teak clones under controlled conditions in a choice and no-choice situation. Three clones showed minimum or negligible feeding damage without significant variation under choice and no-choice situation. The study also demonstrated the physical and chemical variations in resistant and susceptible clone leaves, which may interfere with the feeding behaviour of *H. puera*. Observed results call for a detailed study on genetic variation in physical and chemical properties in tree clones of economic importance for exploitation to develop resistant strains of trees against key pests through breeding as well as genetic engineering/transformation.

सागौन कृन्तकों की पत्तियों में मिलता जैव रसायनिक और उत्स्तरीय अन्तर और सागौन निष्पत्रक कीट के प्रति प्रतिरोधिता

जे० प्रशांत जैकब व ए० बालू

सारांश

नौ कीट–प्रतिरोधी सागौन कृन्तक, जिन्हें चार वर्षीय क्षेत्र सर्वेक्षण के आधार पर पहचाना गया था, नियंत्रित दशाओं के अंतर्गत चुनो या मत चुनो स्थिति में सर्वाधिक सुप्रभाव्य और मध्यम प्रभाव्य सागौन कृन्तकों के साथ सागौन निष्पत्रक कीट, *हाइब्लिया प्यूरा* की भोजन पसन्दगी की तुलना करके उनकी प्रतिरोधिता संपुष्टि के लिए, लिए गए। तीन कृन्तकों ने चुनो या मत चुनो स्थिति में बिना कोई खास अन्तर दिखे पोषण से न्यूनतम अथवा नगण्य हानि पहुंची प्रदर्शित की। इस अध्ययन ने प्रतिरोधी और सुप्रभाव्य कृन्तकों की पत्तियों में भौतिक और रासायनिक अन्तर रहता भी प्रदर्शित किया जो *हाइ० प्यूरा* के भोजन करने के व्यवहार में विघ्न डाल सकता है। प्रेक्षित किए गए ये परिणाम समुपयोजन के लिए आर्थिक महत्व वाले वृक्ष कृन्तकों की भौतिक और रासायनिक विशेषताओं में पाए जाने वाले आनुवंशिक अन्तर का विस्तृत अध्ययन करना आवश्यक सुझाते हैं ताकि प्रजनन एवं आनुवंशिक अभियांत्रिकी/रूपान्तरण दोनों के द्वारा प्रधान नाशिकीटों की प्रतिरोधी वृक्ष किस्मों को विकसित किया जा सके।

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