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CORRELATION OF BIOCHEMICAL LEAF TRAITS AND GALL FORMATION IN SIX CULTIVARS OF MANGO, *Mangifera indica* L.

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Mango (*Mangifera indica* L.) leaves are susceptible to severe attack by insect gall formers. The study's objective was to assess how different vertical strata of the tree canopy and variation in leaf traits affect gall formation. For this, individual and temporal variation in leaf traits of different mango cultivars (Anwar ratole, Chaunsa, Dusehri, Fajri, Sindri and Siroli) and their ultimate effect on gall formation were studied in 2009-10. In addition, the approximate quantity of carbon, nitrogen, phosphorus, potassium and water content in the leaves was also analysed. Significant variation was found among all mango cultivars for leaf traits and gall formation ($p<0.05$). Within host individuals, a maximum level of gall formation (200-240 galls, in case of Fajri cultivar) was found on the foliage of the upper tree canopy followed by the lower and inner canopy with the consistent pattern over 2 years. Moreover, gall formation showed significant correlation to carbon to nitrogen (C/N) ratio, phosphorus and potassium level in the leaves of all the studied cultivars except Siroli.
Keywords: Gall formation, insect herbivores, leaf traits, *Mangifera indica,* resource heterogeneity

INTRODUCTION

The factors determining the densities of herbivores include variation in genotype, the plant's environment, differential host plant resistance and natural enemies that may act on herbivores (Stiling, 1994). Intra-population genotypic variations in resistance have been reported in several host plant species which exert selective pressures on the ability of insects for herbivory (Anderson *et al.,* 1989). Several assumptions concerning the role of morphological and physio-chemical plant characters on the infestation of herbivores have been proposed in the past (Mattson and Haack, 1987; Price, 1991). The chemistry of leaf with respect to defensive compounds and nutritional values varies among clones of a single species and such variations are expected to influence the behavior of herbivores (Hemming and Lindroth, 1995). Ultimately, these variations in leaf quality could be a source to evolve genetically isolated groups within insect species with the adaptation of individual plants (Edmunds and Alstad, 1978). There are certain prerequisites to evolve such groups including variations in plant physiology and/or leaf traits exist among host individuals, and suitability of individual host plants for several generations of insects (Ruhnke *et al.,* 2009). Of these, resource heterogeneity has been assumed an important factor, and explored in several tree species in the past (Marquis, 1988; Fortin and Mauffette, 2002; Roslin *et al.,* 2006) and most of these studies were based on nutrient analyses.

Mango (*Mangifera indica* L.) is one of the oldest cultivated and most economically significant tree for the people of the Indian subcontinent (Tharanathan *et al.,* 2006). It is heavily attacked by different insect pests, including fruit flies, hoppers, mealy bug and gall formers. (Khan *et al.,* 2013). Mango blister circular galls are now emerging threat to Asian mangoes and these are formed by still not described species of Cecidomyiidae (Diptera) in Pakistan (Khan *et al.,* 2013), but their taxonomic description is rare at Pakistan level. To date, there is a lack of basic knowledge, particularly in Pakistan, about the correlation of biochemical characteristics of leaves of different mango cultivars and gall formation. Therefore, the present study was carried out with major emphasis on to investigate the intra-specific variability in gall formation, and variation in leaf traits (nutrients) responsible for gall formation in six mango cultivars (Anwar ratole, Chaunsa, Dusehri, Fajri, Sindhri and Siroli) during 2009-2010. The findings of this research activity made it possible to explain the following research questions: a) Do leaf traits/nutrients of different mango cultivars vary among individual trees, and different zones within a tree? b) Is gall formation relate to leaf traits? c) Is variation in leaf traits/nutrients and gall abundance consistent across the study period? and d) Is gall abundance vary across different vertical strata (based on sunlight) of the mango canopy?

MATERIALS AND METHODS

The experiments were conducted in a farmer's mango orchard, Multan (30°12′0″N, 71°25′0″E), where the climate is arid sub-tropical continental with a hot summer and meek winter. The area is the central mango zone of Pakistan dominated by several mango cultivars like Anwar ratole, Chaunsa, Dusehri, Desi, Fajri, Langra, Malda, Summer behesht, Sindhri, Siroli and Totapuri.

The experiments were conducted by following the methodology of Ruhnke *et al.* (2009) with some modifications. Briefly, six mango cultivars including Anwar ratole, Chaunsa, Dusehri, Fajri, Sindri and Siroli were selected for experiments. The peak occurrence of new galls usually observed in June-July (personal communication), therefore, in June 2009 and 2010, the formation of blister circular galls was estimated in the field. Five trees of each mango cultivar, relatively of uniform age and size, were selected randomly under more or less uniform conditions of soil fertility, irrigation, interculture and other cultural operations. Within the tree canopy, an upper outer zone (full sunlight exposed leaves), a lower outer zone (semi-shade leaves) and an inner zone (shade leaves) were distinguished around the circumference. From each zone, 500 leaves were collected randomly (1500 leaves per tree) around the circumference of each tree and gall abundance (new and hatched galls) was recorded.

Water, carbon to nitrogen ratio (Schadler *et al.*, 2003), phosphorus and potassium (Goncalves-Alvim *et al.*, 2004) concentrations in the leaves are known to influence leaf palatability for herbivores. Therefore, these traits were assessed for the foliage of each tree zone of each cultivar. After collecting the data of gall formation, the leaves were dried, ground, wet digested with nitric acid, sulfuric acid and hydrogen peroxide (Plank, 1992), and phosphorus and potassium were determined by using colorimetric (Watanabe and Olsen, 1965) and flamephotometric (Jones *et al.*, 1991) methods, respectively. For the determination of total nitrogen, the organic matter destruction of the dried and ground leaves was done with sulfuric acid and digestion mixture (K_2SO_4:$FeSO_4$:$CuSO_4$ = 10:1:0.5) followed by distillation with Kjeldahl apparatus (Jackson, 1962). Total carbon contents were determined indirectly by Walkely and Black method (Walkely and Black, 1934). Leaf water contents were assessed from the difference of fresh and dry weights of leaves from each tree zone.

To meet normality and homoscedasticity assumptions of the analysis of variance (ANOVA), most of the data were square root transformed $(X+0.5)^{1/2}$. The effects of year, tree individual and tree zone were tested by three factor ANOVAs (Analytical Software, 2005). Pearson correlation was applied to check the relationship between gall formation and leaf traits.

RESULTS

***Carbon-nitrogen ratio*:** The mean C/N ratio of the leaves differed among individual trees of all the studied mango cultivars (Anwar ratole 36.95-45.07; Chaunsa 52.70-60.48; Dusehri 27.95-34.74; Fajri 154.98-167.75; Sindri 35.13-44.83; Siroli 30.02-41.98, Table I). Within a tree, the C/N ratio varied among three zones of all the cultivars and this pattern was also consistent among individual trees (Significant tree zone×tree individual interaction in all the cultivars). Across the two years, the C/N ratio of the leaves varied significantly in all the cultivars. The pattern of the C/N ratio among individual trees of all cultivars did not change across the two years (non-significant tree individual×year interaction).

***Phosphorus contents (%)*:** The mean phosphorus percentage differed significantly among the individual trees of two cultivars (Anwar ratole 0.11-0.19; Fajri 1.07-1.81, Table 1). Within the trees, P percentage varied in all the cultivars among the three tree zones, but this pattern was not consistent among the individual trees (Significant tree zone× tree individual interaction in only two cultivars, Table 1). Across

Table 1. The effect of year, tree individual and tree zone on leaf traits and gall formation.

Source of variation	df	F-values					
		Anwar ratole	**Chaunsa**	**Dusehri**	**Fajri**	**Sindri**	**Siroli**
Carbon/Nitrogen ratio							
Tree zone	2	309.63***	306.80***	156.59***	300.55***	525.65***	35.47**
Tree individual	4	21.49**	36.36**	12.62**	13.43**	51.55**	83.23***
Year	1	3.06ns	4.79*	0.39ns	1.48ns	0.13ns	1.40ns
Tree zone×Tree individual	8	6.46**	8.46**	15.38**	5.59**	11.52**	29.14**
Tree zone× Year	2	1.40ns	1.14ns	0.88ns	0.21ns	0.21ns	1.07ns
Tree individual×Year	4	0.54ns	0.14ns	0.94ns	2.22ns	0.98ns	0.59ns
Tree zone×Tree individual×Year	8	0.20ns	0.55ns	0.67ns	0.51ns	0.39ns	0.26ns
Error	60	MS=11.15	MS=9.65	MS=11.02	MS=32.36	MS=5.10	MS=5.19

Cont… Table 1

Phosphorus							
Tree zone	2	210.28***	20.34**	155.61***	122.39***	85.11***	72.40***
Tree individual	4	12.26**	2.27ns	11.17ns	14.41**	0.88ns	1.27ns
Year	1	4.31ns	0.79ns	0.01ns	0.05ns	0.86ns	0.48ns
Tree zone×Tree individual	8	4.01*	0.49ns	9.40**	2.01ns	0.67ns	1.95ns
Tree zone× Year	2	6.37*	1.58ns	1.61ns	2.80ns	3.11ns	2.32ns
Tree individual×Year	4	2.43ns	0.33ns	3.74ns	0.24ns	0.89ns	2.25ns
Tree zone×Tree individual×Year	8	0.38ns	0.27ns	0.63ns	0.20ns	0.16ns	1.01ns
Error	60	MS=0.003	MS=0.004	MS=0.001	MS=0.030	MS=0.002	MS<0.001
Potassium							
Tree zone	2	193.52***	256.93***	12.73**	29.53**	236.06***	8.03*
Tree individual	4	3.88ns	1.29*	3.01*	1.19ns	15.59ns	1.33ns
Year	1	37.21**	0.08ns	2.22ns	0.12ns	9.76*	1.01ns
Tree zone×Tree individual	8	2.05ns	0.61ns	0.41ns	0.37ns	3.28ns	0.54ns
Tree zone× Year	2	3.16ns	0.53ns	0.52ns	0.09ns	8.75*	0.40ns
Tree individual×Year	4	5.79*	4.12*	3.80*	2.64ns	2.00ns	1.66ns
Tree zone×Tree individual×Year	8	1.93ns	1.51ns	0.92ns	0.85ns	1.11ns	2.22ns
Error	60	MS=0.002	MS=0.003	MS=0.005	MS=0.371	MS=0.006	MS=0.008
Water content							
Tree zone	2	2.00ns	5.11*	4.21*	52.43***	91.00***	14.00**
Tree individual	4	8.81**	13.00**	17.35**	64.29***	810.00***	11.58**
Year	1	0.10ns	0.13ns	0.76ns	0.14ns	1.00ns	5.56*
Tree zone×Tree individual	8	15.81**	5.52*	2.85ns	49.93***	313.50***	16.50**
Tree zone× Year	2	6.95*	0.92ns	0.39ns	1.00ns	1.00ns	1.56ns
Tree individual×Year	4	4.14*	1.20ns	0.23ns	0.14ns	1.00ns	1.25ns
Tree zone×Tree individual×Year	8	2.43ns	1.03ns	0.14ns	2.31ns	0.93ns	1.37ns
Error	60	MS=0.350	MS=1.021	MS=1.100	MS=0.233	MS=0.033	MS=0.600
Gall formation							
Tree zone	2	291.55***	264.17***	80.32***	124.56***	112.05***	17.61**
Tree individual	4	9.15***	8.13***	5.71***	12.68***	8.62***	3.97**
Year	1	0.55ns	7.09**	19.36***	30.21***	13.28***	7.99**
Tree zone×Tree individual	8	3.12**	2.18*	0.53ns	3.25**	2.08ns	0.35ns
Tree zone× Year	2	5.62**	0.71ns	1.71ns	0.44ns	0.39ns	0.08ns
Tree individual×Year	4	3.57*	0.40ns	1.50ns	3.72**	0.66ns	6.95**
Tree zone×Tree individual×Year	8	1.45ns	0.55ns	0.25ns	0.95ns	1.48ns	1.12ns
Error	60	MS=26.17	MS=56.50	MS=59.89	MS=125.0	MS=231.5	MS=16.83

*P<0.05, **P<0.01, ***P<0.001

the two years, the P percentage did not change in all the cultivars.

***Potassium (%)*:** The K percentage varied significantly among the individual trees of Chaunsa and Dusehri cultivars (0.50-0.59; 0.51-0.64, respectively, Table 1). Within trees the K percentage differed in all the cultivars among the three tree zones, but there was no consistent pattern among the individual trees. Across the two years, the K percentage varied in Anwar ratole and Sindri cultivars.

***Water Contents*:** The mean water content of the leaves differed significantly among individual trees of all the cultivars (Anwar ratole 70.33-72.17; Chaunsa 70.16-73.33; Dusehri 67.83-72.33; Fajri 74.64-77.67; Sindri 70.33-75.83; Siroli 68.24-71.81, Table 1). Except Anwar ratole, it was

Table 2. Correlation between leaf traits and gall abundance.

Cultivar	C/N ratio	Phosphorus	Potassium	Water content
Anwar ratool	0.966*	0.996*	0.971*	0.814ns
Chaunsa	0.971*	0.983*	0.999*	0.552ns
Dusehri	0.982*	0.997*	0.987*	0.050ns
Fajri	0.931*	0.999**	0.952*	0.579ns
Sindri	0.989*	0.900ns	0.990*	-0.572ns
Siroli	0.024ns	-0.981ns	-0.482ns	-0.981ns

*P<0.05 ns=P>0.05

higher in shady leaves followed by semi shade and sun leaves (Chaunsa 72.00% > 71.60% > 70.60%; Dusehri 71.62% > 70.60% > 70.30%; Fajri 77.20% > 76.23% > 75.00%; Sindri 73.40% > 72.83% > 72.30; Siroli 71.00% > 70.41% > 69.20%). In contrast, this difference was varied among tree individuals except Chaunsa, and across years only in Anwar ratole.

***Level of gall abundance*:** The level of gall abundance varied among the tree individuals of all the cultivars (Fig. 1, Table 1) and this pattern was consistent across the years in Anwar ratole, Fajri and Siroli (significant tree individual× year interaction).

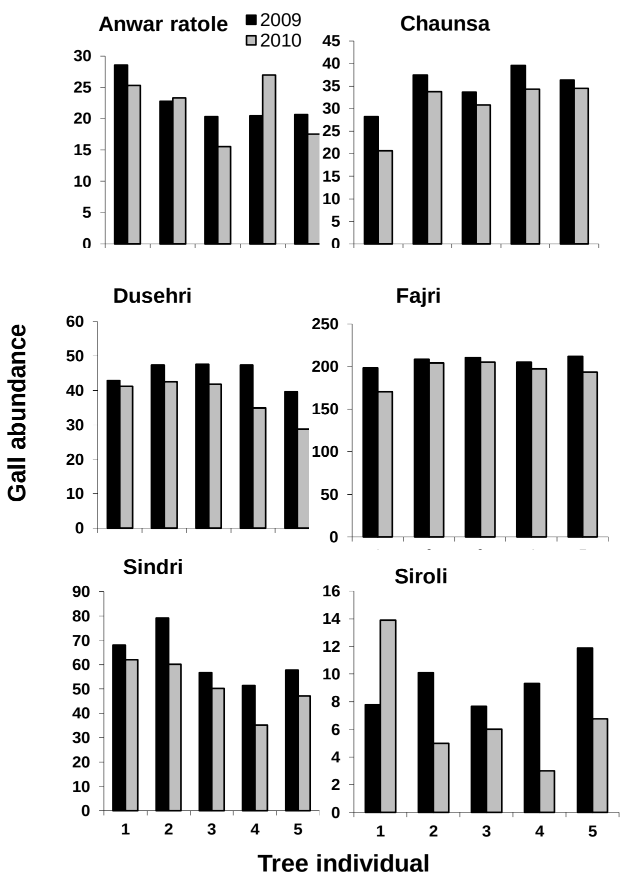

Figure 1. Effect of tree individuals on gall abundance (means±SE) in mango cultivars.

Within the tree, gall abundance varied among the three tree zones in all the cultivars and the maximum level of gall abundance was found in the upper zone followed by the lower and inner zones (Fig. 2, Table 1). But this pattern was not consistent across the years in all the cultivars except Anwar ratole (non-significant tree zone× year interaction). Across the 2 years, gall abundance also varied in all the cultivars except Anwar ratole (Table 1).

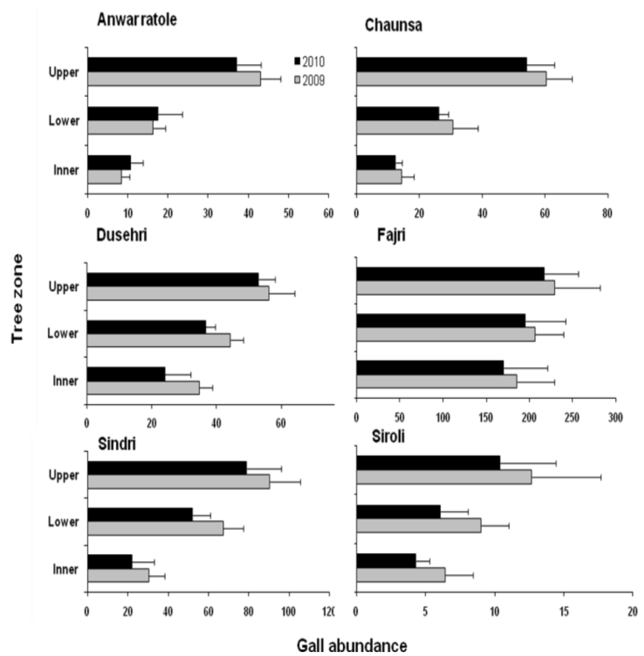

Figure 2. Effect of tree zones on gall abundance in mango cultivars. Back transformed means±SE.

C/N ratio, P and K showed positive and significant correlation (P<0.05) with gall abundance in all the cultivars except Siroli. Water contents, however, showed no correlation with gall abundance (P>0.05) (Table 2).

DISCUSSION

The chemical characteristics including concentration of nutrients (Schoonhoven *et al.,* 1998) and water content

(Cornelissen and Fernandes, 2001) in leaf tissues greatly influence the behavior of insects. The variability of these characteristics could be important in determining herbivore activity and distribution in host plants. The leaf traits studied in the present study varied among host individuals and even within the host plants. C/N ratio varied among different tree zones, might be due to light and nutrient effects, which is in accord with carbon/nutrient-balance hypothesis, but this phenomenon could strongly be under genetic control (Osier and Lindroth, 2006). Therefore, plant environment and genotype may act together to make host suitability for herbivores (Ruhnke *et al.,* 2009). The amounts of P and K in leaf tissue were positively correlated with gall abundance which is in agreement with the findings of Goncalves-Alvim *et al.* (2004) on *Qualea parviflora.* In the present study, water content had no correlation with the gall formation which could be due to the probable correlation between water content and morphological and physiological leaf traits, including secondary metabolites, carbohydrates and nutrients (Haukioja, 2005).

The level of gall abundance was considerably varied among cultivars, tree individuals and tree zones in all the investigated mango cultivars during 2009 and 2010. The galls were more abundant in the upper canopy compared to the lower and inner canopy. The most probable reason for the varied gall abundance could be due to light and nutritional effects. These factors strongly influence the distribution of herbivores within the tree canopy (Roslin *et al.,* 2006). The availability of light could influence different modes of herbivory by affecting leaf penology (Barone, 1998), herbivore preference (Niesenbaum, 1992) and the combination of these factors. Mango trees have a dense canopy and its different zones don't receive the same amount of light: the foliage of the upper canopy is fully exposed to sunlight, lower canopy foliage receives a less amount of light compared to the upper canopy while the foliage of the inner canopy usually doesn't receive sunlight. Moreover, within plant physiological and chemical characteristics are not homogeneous: sectoriality in the allocation of resources (Marquis, 1996) and induced plant defenses (Jones *et al.,* 1976) are widely documented. The complex phenomenon of within plant sectoriality is hypothesized to explain different modes of herbivory and to affect a wide range of categories of arthropod: chewers, suckers, borers and gall formers (Orians and Jones, 2001). Therefore, the variation in gall abundance within as well as among individuals of a cultivar could be due to heterogeneous resource allocation, however further studies are required to confirm the exact phenomenon.

In conclusion, the study provide some basic knowledge on the relationship between gall formation and different tree factors. However, there is a need to classify these gall formers taxonomically at Pakistan level for the purpose to devise an effective management strategy.

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