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Do the Geo-Climatic Variables shape Morphological Plasticity Pattern in *Melia dubia* Cav. Natural Populations in Gujarat?

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Abstract: This work presents the morphological variations in foliar and fruit traits among four natural populations of *Melia dubia* in Gujarat, India. Results showed considerable but significant variations exist among and within populations for seven leaf and four fruit traits. *Sagai (SG)* population had maximum values for most foliar traits, whereas the *Kaparada (KP)* and *Nanapondha (NP)* populations had higher dimensions of fruit traits. The intraclass correlation exhibited a strong positive relationship between leaf length, petiole length, and leaf base diameter. Similarly, pulp weight had strong positive correlations with fruit dimensions (length, width, and weight). Multivariate analysis of morphological data reduced it into three principal components (PCs), capturing a cumulative variability of 80.7%. Correlation and regression analysis of PC scores with geo-climatic variables revealed a strong direct relationship between habitat latitude, altitude, and mean annual temperature. The mean annual rainfall also had a strong but inverse influence, whereas longitude did not record any significant association with PC1. The populations occupying niches of higher altitude, higher MAT, and lower precipitation were characterised by longer and narrower leaves with a maximum number of pinnae and leaflets and smaller fruits.

Keywords: Geoclimate, Correlation, Morphological Traits, *Melia dubia*

Morphological characteristics of any organism are thought to exist and develop as a result of inherited genetic information, and it is also an accepted fact that changing environmental and geographical conditions play an important role. In fact, geographic variation leads to variability in the environmental conditions that any species experiences. The stress or stimulation exerted by the environment motivates the plants to adapt. *Cacti*, for example, adjust their leaf morphology to cope with a resource-poor and harsh environment. In wild tree species, many common garden experiments have apparently established the influence of geo-climatic variables of origin in forming morphological variations (Warren et al 2005, Vitasse et al., 2009, Akalushi et al., 2018). The morphological variability in foliar and fruit traits of *M. dubia* were invested at the population level and attempted to understand how the geo-climatic gradients play a role in shaping these traits.

Malabar neem (*Melia dubia* Cav.) is an indigenous species of moist localities and tropical forests that grows naturally in most parts of India. Due to its fast-growing nature, broader industrial uses, and thus economic prospects, this species has caught the interest of researchers in recent years for domestication and productivity improvement (Thakur et al., 2017, Parmar et al., 2019, Chauhan et al., 2021, Parmar et al., 2019).

M. dubia is a large deciduous tree with bark that is dark brown in colour and peels in flimsy, restricted strips with expansive, shallow, longitudinal breaks. The leaves are typically bi- or tri-pinnate and range in length from 30 to 90 cm. Pinnae: 3-7 pairs, 10-20 cm long; rachis: 10–30 m long. The leaflets are ovate or oblong-lanceolate, 4 to 8 cm long, and come in 2 to 11 pairs. The violet and white flowers, which are about 8 mm long and fragrant, are borne on the upper axils of the leaves. When young, the fruit is drupaceous, ellipsoid, 1.5 cm long, smooth and shiny, and yellowish when fully ripe (https://indiabiodiversity.org/species/show/31551).

MATERIAL AND METHODS

Population details and habitat characteristics: For this study, chose four natural populations of *M. dubia* from the hilly regions of South Gujarat (Chauhan et al., 2018). The Kaprada (KP) and Nanapondha (NP) populations belong to the Valsad district, while Waghai (WG) from the Dangs and Sagai (SG) from the Narmada district. The population's geographic situations (latitude, longitude and altitude) are depicted in the Figure 1. The climate of population sites is typically dry sub humid, characterised by a fairly hot summer, a moderately cold winter, and a humid and warm monsoon. Most of the precipitation is received from the south west monsoon, concentrated during the months of July and August. Edapho-climatic details of the sites were taken from World Weather Online (https://www.worldweatheronline.com), Gujarat State Disaster Management Authority (https://www.gsdma.org), and The Commission of

Agriculture (http://www.gujenvis.nic.in) and given in Table 1.

Morphological variability assessment: This investigation includes ten individuals from each population who had a good phenotype and were at least 100 metres apart. Fresh leaves were examined in the field for morphological variations. With the help of a tree pruner, six small branches were carefully removed from each tree, covering all directions (E-W-N-S). One fully developed and intact leaf was chosen from each branch for measurement. Figure 2 shows a diagram illustrating leaf measurements, and the methodology adopted is provided in Table 2. Assessment of variation in fruit traits was carried out in the Seed Technology Laboratory, College of Forestry, NAU, Navsari. A sufficient quantity of mature fruits was collected from each individual in the population while maintaining proper identity. For evaluation of morphometric variations, 60 uniform drupes per individual were used in a population as per standard procedure (Table 2).

Statistical analysis: Shapiro-Wilk's Test was used to check for normality in the morphological data of leaf and fruit traits. Analysis of variance and multiple comparison tests were done to analyse the differences among the populations. Morphological differences within the population were also presented using descriptive statistics. Simple correlation (Pearson) analysis was done in order to check the intra- and inter-class relations between foliar and fruit traits. After transforming the data (Box-Cox), Principal Component Analysis (PCA) was done, which reduced the morphological dataset to 3 meaningful principal components (PCs). The decision for the inclusion of PCs for interpretation was taken on the basis of the scree plot (eigenvalues > 1). Further

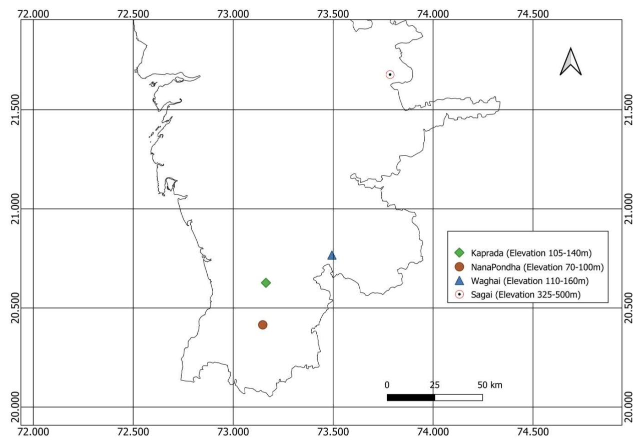

Fig. 1. Geographic positions of the selected natural population of *M. dubia* in Gujarat, India

Table 1. Edapho-climatic details of selected *M. dubia* populations in Gujarat, India

Population	Abbreviation	Soil type	Max. Temp. (^oC)	Min. Temp. (^oC)	M.A. Temp. (^oC)	M.A. rainfall (cm)
Kaprada	KP	Lateritic medium black	32.3	24.2	28.2	274.4
Nanapondha	NP		34.5	22.6	28.6	244.1
Waghai	WG	Lateritic deep black clayey and sandy soil	33.4	21.3	27.3	241.2
Sagai	SG	Black to loamy soil	35.3	25.8	30.6	113.3

Table 2. Details of the morphological traits characterized in natural populations of *M. dubia*

Morphological traits	Abbreviation	Unit	Procedure
Leaf length	LL	cm	Measured from the base of the petiole to the apex with the help of a ruler scale (stainless steel)
Leaf width	LW	cm	Measured at its widest part, with the help of a ruler scale
Petiole length	PL	cm	Using a ruler scale, measure from the base to the point of attachment of the first pair of pinnae.
Rachis length	RL	cm	Measured from the point of attachment of first pair of pinnae to the leaf apex, with the help of ruler scale
Base diameter	BD	mm	Measured with the help of a digital calliper
Number of pinnae (pair) per leaf	NP/L	Nos.	Counted in the half side of the leaf and expressed as pair.
Number of leaflets per pinnae	NL/P	Nos.	Counted in each pair of pinnae and took averages.
Fruit length	FL	mm	Measured between the end of the vertical axis with the help of a digital calliper,
Fruit width	FW	mm	Measured at the site of the maximum circumference with the help of a digital calliper
Fruit weight	FWt	g	Weighed Individual fresh fruits on an electronic weighing balance
Pulp weight	PWt	g	Individual fruit was de-pulped and weighed using an electronic weighing balance.

choose the first principal component scores from the correlation matrix for correlation and regression analysis with geo-climatic variables. Statistical analysis was performed with the online resources OPSTAT (Sheoran et al., 1998) and PAST (Hammer et al., 2001).

RESULTS AND DISCUSSION

Morphological variations in leaf and fruit traits: The mean values recorded for these characters varied significantly among the four natural populations of *M. dubia* (Table 3).

Leaf variables like leaf length (102.77 cm), petiole length (23.09 cm), rachis length (64.73), and base diameter (9.24 mm) were recorded as highest in the *SG* population. Although these traits did not differ significantly between the other populations, lower values were in the *KP* or *WG* populations (Table 3). In contrast, significantly wider leaves were attributes of the *NP, KP,* and *WG* populations, whereas the narrowest leaf (54.74 cm) was observed in the *SG* population. Additionally, the *SG* population had the maximum pair of pinnae (6.93) as well as the highest number of leaflets per pinnae (9.54).

Leaf morphology is believed to vary among different genotypes of the same species due to genomic differences or environmental changes in the habitat (Bussotti and Pollastrini 2015). In the present investigation, all the leaf traits recorded significantly higher in the *SG* population, except leaf width, which was significantly narrower. It is a well-established fact that smaller leaves are adaptations to higher elevations and high temperatures and the opposite is true for adjustments in humid and cold areas (Cordell et al., 1998). Similarly, large leaf area is a prerequisite in fast-growing species, and narrow leaves with large petiole sizes are adaptations in resource poor habitats, particularly in drier environments (Pyakurel and Wang 2013). Because the *SG* population is a fast-growing species that lives in relatively hot, drier conditions at high elevation (Table 1, Fig. 1), trees with the longest leaf length and narrowest width may have indirectly reduced leaf area in order to withstand transpiration loss. The greatest number of pinnae and leaflets in the *SG* population, on the other hand, are attributed to the system's pressure to manage the high pull of food resources in order to sustain its rapid growth. Longer petiole length, longer rachis length, and a thicker base diameter might have reinforced the longer leaf, as evident with their intra-class correlations (Table 4). Variability in foliar morphology due to varying environmental conditions is well documented in other species as well. Atchaya et al. (2019) recorded a significant influence of temperature on changes in the number of leaves, number of leaflets, and leaf area in *M. dubia*. Similarly, Ramchandran and Vasudeva (2020) opined that variation in the leaf morphology of *Pyranacantha volubilis* was due to environmental variables at different geographic origins of the seeds. There was significant variation in fruit traits among the four natural populations of *M. dubia* (Table 3). In variance analysis, the KP population had the same maximum fruit length (28.34 mm) and width (23.20 mm) as the *NP* population (28.31 mm and 23.16 mm, respectively). Heaviest fruits (9.10 g/fruit) were measured in *NP,* not significantly different from the *KP* population (8.39 g/fruit), whereas, lightest fruits were observed in the *SG* and *WG* populations. The maximum pulp weight (7.26 g/fruit) in the *NP* population was significantly *at par* to the *KP* population (6.83 g/fruit) and the minimum 5.70 g/fruit in the SG population did not significantly differ from the WG population.

In the present study, fruit traits differ significantly among the populations. The *KP* and *NP* populations were found to be equally superior for all of the investigated fruit traits, whereas the SG population had the lowest trait values. Fruit morphology similarities and differences may be due to genotype or ecological similarity and dissimilarity between populations (Table 1, Fig. 1). A range of coefficients of variability for fruit characters (CV = 5.13-14.80) indicated that the genotypic contribution to variability was limited.

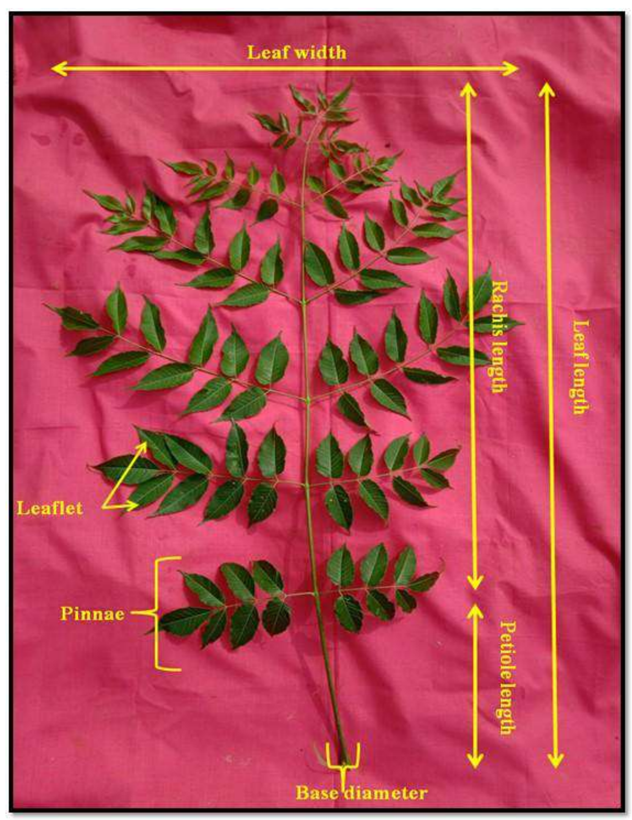

Fig. 2. Illustration of measurements of leaf morphology in *M. dubia*

Differences in biotic stress levels experienced by the mother tree during the fruit development stage, on the other hand, could have also resulted in such variations. Moisture availability and mineral nutrition influence fruit morphology (Gutterman 2000). Variability in fruit morphology due to differences in moisture and nutrition is well documented in other woody perennials like *Melia azedarach* (Irmayanti et al 2015), *Tamarindus indica* (Okello et al., 2018) and *Terminalia chebula* (Sharma et al 2016).

Inter and Intra correlations among leaf & fruit traits: There were significant positive intra-class correlations observed for most leaf traits (Table 4). Leaf length exhibited a very strong positive correlation (r=0.838) with petiole length. Base diameter was a strong positive correlation with the number of pinnae (r=0.768), leaf length (r=0.669) and petiole length (r=0.635). The number of pinnae was positively and significantly correlated with the number of leaflets or pinnae (r=0.739). In contrast, except for the number of pinnae, which was found to be negatively correlated in moderate magnitude (r = -0.425), leaf width showed either insignificant or a weak correlation with other interclass morphological characters. Inter-character correlations are of major interest in tree improvement programmes as the improvement of a character may lead to synchronised changes in the linked character. Interpretation of the correlations among leaf traits revealed the crucial role of the petiole, rachis length, and base diameter in determining the leaf length and strength. Similarly, a greater number of pinnae could have increased the number of leaflets in a leaf. The possible explanation for the direct positive relationship of these traits with leaf size might be the provisioning of structural reinforcement for better interception of light resources. Similar conclusions were drawn by Eichelmann et al. (2004) in *Betula pendula*; in addition, positive relations between leaf length and petiole length and negative leaf width were in *Fagus orientalis* by Bayramzadeh et al. (2012).

Table 3. Descriptive statistics and analysis of variance for 11 morphological traits within and among the population of *M. dubia* in Gujarat

Character		Descriptive statistics				Analysis of variance	
		Populations				Mean	C.V. %
		Kaprada	Nanapondha	Waghai	Sagai		
Leaf length (cm)	Mean	94.56[b]	97.17[b]	94.64[b]	102.77[a]	97.29	5.27
	C.V.	7.07	5.37	5.49	2.39		
Leaf width (cm)	Mean	65.58[a]	63.10[a]	62.66[a]	54.74[b]	61.52	9.61
	C.V.	9.70	9.22	9.42	10.1		
Petiole length (cm)	Mean	19.69[b]	20.69[b]	19.47[b]	23.09[a]	20.74	9.64
	C.V.	14.89	6.67	10.37	5.15		
Rachis length (cm)	Mean	56.33[b]	58.42[b]	58.36[b]	64.73[a]	59.46	9.71
	C.V.	6.71	13.84	10.42	6.36		
Base diameter(mm)	Mean	7.21[b]	7.33[b]	7.03[b]	9.24[a]	7.7	7.86
	C.V.	7.35	8.87	5.41	8.55		
Number of leaflets/pinnae	Mean	8.11[c]	8.57[b]	8.68[b]	9.54[a]	5.76	4.38
	C.V.	5.53	4.74	3.42	6.28		
Number of pinnae (pair)	Mean	5.48[b]	5.07[c]	5.57[b]	6.93[a]	8.73	5.17
	C.V.	3.64	0.29	5.50	2.82		
Fruit length (mm)	Mean	28.34[a]	28.31[ab]	25.57[c]	25.08[bc]	26.83	6.54
	C.V.	6.61	5.62	5.94	7.97		
Fruit width (mm)	Mean	23.20[a]	23.16[ab]	21.75[bc]	21.21[c]	22.33	5.91
	C.V.	3.35	4.36	6.02	7.26		
Fruit weight (g)	Mean	8.39[ab]	9.10[a]	7.37[c]	7.26[c]	8.03	14.8
	C.V.	10.61	10.66	19.54	18.73		
Pulp weight (g)	Mean	6.83[ab]	7.26[a]	5.88[bc]	5.70[c]	7.35	13.73
	C.V.	11.70	14.20	22.11	19.30		

Figures in the same letter (s) did not differ significantly at $p < 0.05$

Fruit traits showed strong to very strong positive relationships among themselves (Table 4). Fruit weight was significantly and positively correlated with fruit width (r=0.940) and fruit length (r=0.867), whereas fruit width had a strong positive correlation with fruit length (r=0.782). Fruit pulp weight had a very strong correlation with fruit weight (r = 0.986). As far as inter-class correlations of leaf and fruit traits are concerned, none of the fruit traits demonstrated a significantly strong relationship with foliar traits (Table 4). Stronger correlations between fruit weight, length, and width indicated the crucial role of these traits in deciding fruit dimensions. In addition, strong relationships between physical dimensions (length, width, and weight) and pulp weight indicate that the more fruit dimensions, the greater the pulp mass. Given that the fruit pulp of *M. dubia* is regarded as an alternate source of feedstock (Sukhadiya et al 2021) and large dimensional fruits may be a selection criteria for improvement in pulp mass, as recommended in *Tamarindus indica* by Algabal et al. (2012) and in *Trichoscypha acuminata* by Tsobeng et al. (2020).

Association between geo-climatic variables and morphological traits: The association of the geographic and climatic variables of the habitat with the morphological variability shown by individual trees in the population. To reduce the dataset of morphological variables into a meaningfully small set, principal component analysis (PCA) was done. PCA resolved the morphological variables into three PCs (Eigenvalues > 1), explaining a total variability of 80.7%. The PC 1 elucidated maximum variability (46.4 %) with positive loadings for most of the leaf characteristics except its width and negative loadings for fruit traits (Table 5). With higher loadings for leaf length and petiole length, PC 2 explained 24.0% of the total variation, while PC 3 accounted for 10.4 % of the variation with the highest loadings for leaf width. Furthermore, the strength of the correlation (person) coefficient (r) revealed that the PC1 scores had the strongest direct relationship with geo-climatic variables except for longitude (Table 6). Morphological traits (PC1) had a significant positive relationship with latitude (r = 0.725), altitude (r = 0.778), mean temperature (r = 0.639), and an inverse relationship with mean rainfall (-0.818) of the habitation. The regression equation revealed that latitude (R^2 = 0.525), altitude (R^2 = 0.605), mean temperature (R^2 = 0.408), and mean rainfall (R^2 = 0.669) explained 52.5% of the variability in PC1 scores (Fig. 3). The regression analysis further revealed that when latitude increased by 1 degree, the value of PC1 increased by 1.35 (b_1=1.3528). Similarly, every unit increase in altitude (m) and mean temperature (0 °C) increased PC1 scores by 0.06 and 0.52 times, respectively. In contrast, a reduction in PC1 scores by 0.01 with an increment of rainfall of 1 cm is explained (b_1= -0.01308) (Fig. 3).

The relationship between morphological traits and their surroundings has been advocated as the result of plants' evolutionary response to changing environmental conditions (Westoby et al., 2002). Earlier reports have confirmed the role of geo-climatic factors in the expression of dissimilarity among plant populations in terms of leaf morphology (Danquah 2010, Atchaya et al., 2019, Liu et al., 2020). In this study, latitude, altitude, and mean annual temperature showed a linear relationship with foliar morphology except, leaf width (negative vector loadings in PC1), whereas, precipitation showed an inverse relation except, leaf width (PC1). To be specific, longer (leaf length and associated characters like petiole and rachis length), narrower (leaf width), more sturdy leaves (petiole base diameter), and leaves with more leaflets and pinnae, are attributes of higher altitude and a higher MAT zone. The population inhabiting

Table 4. Intra and inter-character correlations (Pearson) matrix of 11 morphological traits in*M. dubia* populations

Characters	LL	LW	PL	RL	BD	NP/L	NL/P	FL	FW	FWt	PWt
LL	1										
LW	X	1									
PL	0.838**	X	1								
RL	0.599**	X	0.458**	1							
BD	0.669**	X	0.635**	0.432**	1						
NP/L	0.487**	-0.425**	0.471**	0.476**	0.768**	1					
NL/P	0.537**	X	0.497**	0.424**	0.595**	0.739**	1				
FL	X	X	X	X	X	-0.464**	-X	1			
FW	X	X	X	X	X	-0.439**	-0.413**	0.775**	1		
FWt	X	X	X	X	X	-0.416**	-0.446**	0.835**	0.779**	1	
PWt	X	X	X	-X	X	-0.415**	-0.450**	0.809**	0.731**	0.986**	1

Non-significant and correlations (r < 0.39) are shown with 'X',** Significant at p<0.01, * Significant at p<0.05

along the rainfall gradient, on the other hand, was wider, sturdier, and had a greater number of leaflets. In distinction to the fact that shorter and wider leaves are often at higher elevations and temperature (Guo et al., 2018), the present study uncovered a linear relation of leaf length and number of leaflets and inverse relation of width with elevation and mean annual temperature. Given that the angle of radiation is smaller at higher elevations, energy is forced to be adjacent to a larger surface area (CSE http://www.ces.fau.edu/nasa/module-3/why-does-temperature-vary/angle-of/the-sun.php).This could probably be seen as a survival strategy, during which plants evolved longer, more sturdy leaves with a greater number of leaflets so as to capture the maximum quantity of sunlight energy. In the same way, inverse relationships between rainfall and leaf traits are thought to balance water use efficiency., Liu et al. (2020) observed an inverse relation of leaf width with altitude in three species; Warren et al. (2005) noted decreased petiole length with

Table 5. Eigenvector loadings in Principal Component analysis (PCA) of 11 morphological traits of *M. dubia*. (Strong loadings in respective principal components are bold faced)

Characters	Eigenvectors		
	PC1	PC2	PC3
LL	0.259	0.417	0.287
LW	-0.184	0.091	0.758
PL	0.237	0.426	0.012
RL	0.263	0.204	0.403
BD	0.305	0.321	-0.226
NP (Pair)	0.365	0.137	-0.246
NL/P	0.348	0.131	-0.093
FL	-0.332	0.323	-0.06
FW	-0.322	0.293	-0.068
PW	-0.326	0.355	-0.157
FW	-0.326	0.374	-0.167
Eigenvalue	5.102	2.636	1.143
Proportion	0.464	0.24	0.104
Cumulative proportion	0.464	0.703	0.807

Table 6. Strength of Correlations (r) between principal components matrix of morphological traits and geo-climatic variables (Strong relations of r>0.6 are boldfaced)

Geo-climatic variables	PC1	PC2	PC3
Latitude	0.725^{**}	0.085^{NS}	-0.453^{**}
Longitude	0.090^{NS}	0.147^{NS}	-0.056^{NS}
Altitude	0.778^{**}	0.287^{NS}	-0.296^{NS}
Mean annual temperature	0.639^{**}	0.436^{**}	-0.358^{*}
Mean annual rainfall	-0.818^{**}	-0.272^{NS}	0.344^{*}

*Significant at p<0.05), ** Significant at p<0.01, NS -Non-significant

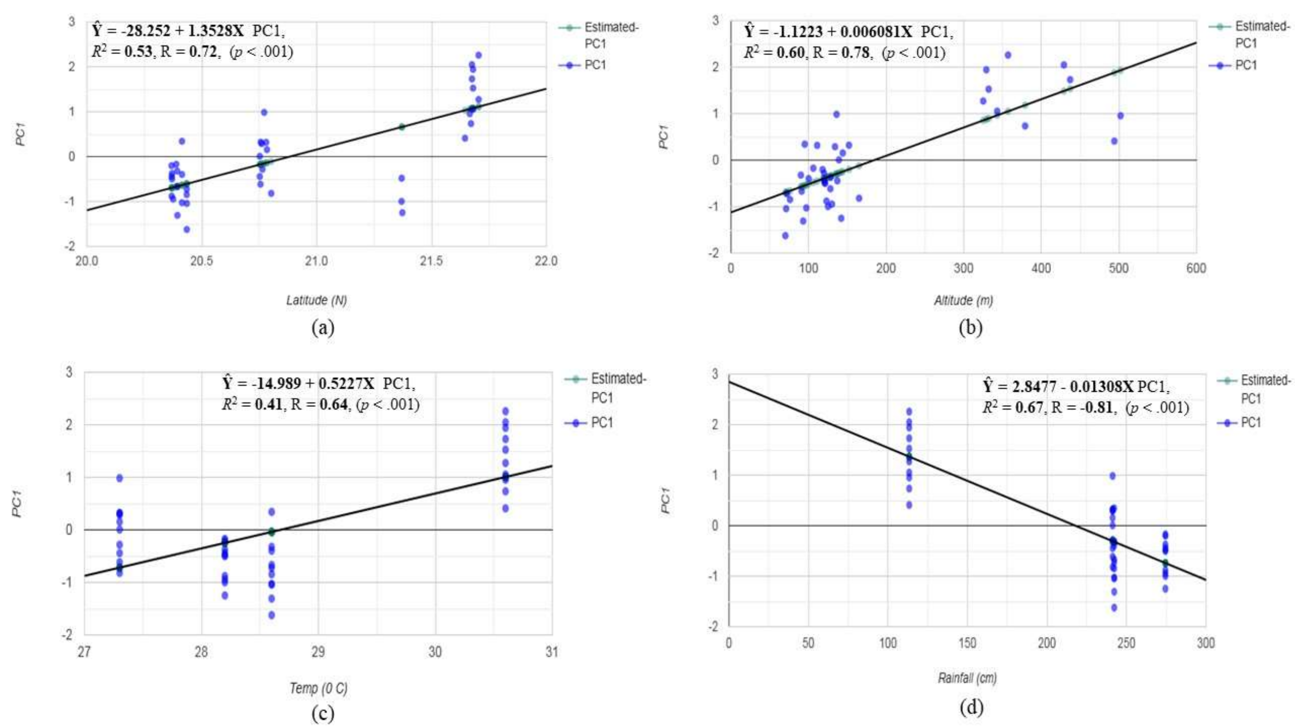

Fig. 3. Good fit plots of correlations between geo-climatic variables and PC1 scores

increasing seed source rainfall in *Eucalyptus sideroxylon;* Alcanatra-Ayala et al (2020) found longer and narrower leaves in lower rainfall regions and longer leaves in higher temperature regions in *Ternstroemia lineata.*

The relationship between fruit size (length and width) and geo-climatic gradient in PC1 (negative vector loadings) is interpreted as fruit size decreasing with latitudinal and altitudinal gradient and increasing with precipitation availability. The influence of geographic trends on fruit dimensions is also documented in *M. azedarach* by Chen et al (2015). Ramchandran and Vasudeva, (2020) noted an inverse relation between latitude and fruit length and width. Additionally, Rawat and Bakshi (2011) observed an inverse correlation of cone and seed traits with altitude and latitude in blue pine. Sudrajat et al (2016) discovered a negative relationship between fruit and seed traits and latitude and a positive relationship between fruit and seed traits and precipitation in *Anthocephalus cadamba* populations.

CONCLUSION

The study quantified the variations in foliar and fruit traits among selected natural populations of *M. dubia* in Gujarat, India. The population of *Sagai* region (*SG*) was superior for foliar traits, and the rest of the population did not significantly differ from each other. Populations from *Kaparada (KP)* and *Nanapondha (NP)* had *at par* finer fruit traits. The observed differences in morphological traits might be adaptations to geo-climatic differences. A good fit plot established that morphological variations exist among the population along latitudinal and altitudinal gradients. Furthermore, the study population's foliar and fruit traits were found to be strongly influenced by mean temperature and rainfall. Out of all the geo-climatic variables, rainfall explained the highest morphological variability in PC1, making it the most important selection pressure for shaping morphological elasticity. Moreover, the possibility of a partial role for genetic differences in phenotypic plasticity cannot be ruled out; hence, a common garden experiment is needed.

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