

## Studies on stomatal frequency and its relationship with leaf biomass and rhizome yield of turmeric (*Curcuma longa* L.) genotypes

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### Abstract

Stomatal frequencies of twelve turmeric (*Curcuma longa* L.) genotypes were recorded from different locations of both surfaces of the fourth, sixth and eighth nodal leaves. Turmeric leaves were amphistomatous having lower stomatal frequency in the upper surface (426.6 - 1773.7 sq. cm<sup>-1</sup>) and higher in the lower surface (7131.5 - 12,270.9 sq. cm<sup>-1</sup>) of the leaves. With a few exceptions, stomatal frequency of the leaves of the genotypes tested increased from fourth to eighth nodal position. The stomatal frequency of the genotypes either at the same location within leaf, at different locations within leaf or both leaf surfaces taken together did not vary significantly. The genotype TCP-9 had the highest stomatal frequency. However, stomatal frequency increased from base towards tip on both surfaces of the leaf, irrespective of the position of the leaf in all the genotypes. The stomatal frequency appeared significant and more positively correlated with rhizome yield than leaf dry weight in genotypes resistant to leaf blotch disease while in susceptible types it exhibited significant and negative correlation with leaf dry weight and rhizome yield.

**Key words :** *Curcuma longa*, genotypes, stomatal frequency, turmeric.

### Introduction

Size, frequency and distribution of stomata on the epidermal layer of leaf surface influence several physiological processes. Stomatal frequency or density can vary significantly within leaf, between leaves of the same plant or between plants (Datta 1968). It can be modified by environmental factors, leaf morphology and genetic composition (Willmer & Fricker 1996). Information regarding the stomatal frequency of leaves at different nodal positions, leaf surfaces and locations within the leaf in turmeric is lacking, which could be utilized in

characterizing the genotypes. The relationships of total and individual leaf stomatal frequency with total and individual leaf dry weight as well as fresh rhizome yield are also wanting. The study on this relationship may point out the role of stomata on the accumulation of photosynthate in leaf and its subsequent translocation to the underground rhizome. The present investigation was conducted with a view to find out differences if any, in leaf stomatal frequency of turmeric genotypes and to study its role in accumulation of leaf dry matter and photosynthates in the rhizomes.

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## Materials and methods

Twelve turmeric (*Curcuma longa* L.) genotypes were grown at the research farm of Bidhan Chandra Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal in a randomized block design with three replications. The plot size was 3 m x 1 m with a spacing of 30 cm x 20 cm. Seed rhizome pieces weighing 20 g each @ 45 pieces per plot were sown during the first week of May after applying recommended doses of manures and fertilizers and mulched with wheat straw. Intercultural operations were done as and when necessary.

Fully unfurled leaves at fourth, sixth and eighth nodal positions of three plants, randomly selected from each genotype, and from each replication were harvested. Four peelings from each location of leaf lamina from both upper and lower surfaces were taken and observed under microscope at lower (X 10) and higher (X 40) magnifications. Number of stomata in one square centimetre were recorded from four microscopic fields of each peel and number of stomata for entire area of the leaf was calculated. Leaves were dried in hot air oven at 70°C and dry biomass was recorded. The average rhizome yield of each genotype was recorded from five randomly selected plants in each replication. Data on stomatal frequency were analyzed statistically following four factor analysis. The four factors were genotype, location within leaf, nodal position and leaf surface. Simple correlations were worked out separately between base, middle and tip locations on both surfaces, two surfaces taken together as well as base, middle and tip locations of the upper surface to the corresponding locations at the lower surface. Correlations were also worked out separately between nodal positions at the upper, lower and both the surfaces taken together. Total stomatal frequencies of each nodal leaf and all the three nodal leaves taken together were calculated. The percent disease index (PDI) for leaf blotch disease (*Taphrina maculans*) of turmeric genotypes under study was also recorded. The genotypes were grouped under

two categories namely, susceptible and resistant genotypes. Simple correlations between stomatal frequency of fourth, sixth and eighth nodal leaf individually or taking together with their respective and total dry weight and fresh rhizome yield per plant were computed separately for susceptible and resistant genotypes.

## Results and discussion

The mean stomatal frequencies of turmeric genotypes were found to vary with regard to leaf surface and nodal position (Table 1). The genotypes (irrespective of leaf surface, nodal position and location in a leaf), TCP-9, TCP-4 and TCP-10 differed significantly from the rest of the genotypes for stomatal frequency but insignificant difference was observed among TCP-5, TCP-52, TCP-62, Sonajuli local and Meghalaya local; between Nagaland local and TCP-7 and also between TCP-11 and TCP-8. Results indicated that the turmeric genotypes have different stomatal frequencies and they can be placed in different groups as per stomatal spacing with 'low', 'close' with 'medium' and 'compact' (densely) with high stomatal frequency per unit area of the leaf. Such variations in stomatal frequencies in genotypes of soybean were observed earlier by Ciha & Brun (1975).

The stomata in turmeric are present on both the upper (adaxial) and the lower (abaxial) surfaces and hence turmeric leaves are amphistomatous. Usually stomatal frequency is lower (996.3 sq. cm<sup>-1</sup>) at the adaxial surface compared to abaxial surface (8113.5 sq. cm<sup>-1</sup>) (Tables 1 & 3). Stomatal frequency in the genotypes ranged from 426.6 - 1773.7 and 7131.5 - 12270.9 sq. cm<sup>-1</sup> in the adaxial and abaxial surfaces, respectively (Table 1). The variations of stomatal frequencies at the adaxial (81-170 sq. cm<sup>-1</sup>) and abaxial (242-385 sq. cm<sup>-1</sup>) surfaces were recorded earlier (Ciha & Brun 1975) in soybean genotypes. The results of the present investigation corroborate the above findings.

The stomatal frequency on both surfaces of the leaf in the genotypes varied significantly with the nodal position. The lowest frequency being

Table 1. Leaf surface x nodal position x genotype interaction, irrespective of location within leaf for stomatal frequency

Genotype	Stomatal frequency at nodal position											
	Upper leaf surface				Lower leaf surface				Irrespective of leaf surface			
	Fourth	Sixth	Eighth	Mean	Fourth	Sixth	Eighth	Mean	Fourth	Sixth	Eighth	Mean
TCP-4	1118.3	1276.3	1639.7	1344.8	7979.6	9032.6	10587.9	9200.0	4548.9	5154.2	6113.8	5272.4
TCP-5	714.5	1062.1	1009.4	928.7	7122.4	7040.4	8677.8	7613.6	3918.5	4051.3	4843.6	4271.4
TCP-7	1167.4	1062.5	1109.5	1097.8	6931.3	6931.4	7531.7	7131.5	4049.4	3974.0	4320.6	4114.6
TCP-8	581.1	661.8	653.1	632.0	6604.0	7204.4	7995.8	7268.1	3592.5	3933.1	4324.4	3950.0
TCP-9	1601.1	1785.4	1934.6	1773.7	10997.4	12252.7	13562.7	12270.9	6299.3	7019.0	7748.6	7022.3
TCP-10	798.8	883.1	983.1	888.3	8732.4	7176.9	9196.4	8368.6	4765.6	4030.0	5089.8	4628.5
TCP-11	391.5	359.9	528.4	426.6	6958.8	7832.0	7859.3	7550.0	3675.1	4095.9	4193.9	3988.3
Meghalaya local	979.6	1104.2	1158.7	1080.8	7313.3	7286.1	7558.9	7386.1	4146.5	4195.2	4358.8	4233.5
Nagaland local	919.9	1009.4	1336.0	1088.4	6576.6	7204.2	7995.7	7258.8	3748.2	4104.8	4665.8	4173.6
TCP-52	1009.5	1141.1	1234.2	1128.3	6904.2	7231.7	8787.0	7641.0	3956.8	4186.4	5010.6	4384.6
Sonajuli local	993.6	1088.4	955.0	1012.4	6822.2	7804.7	7913.8	7513.6	3907.9	4446.6	4434.4	4263.0
TCP-62	347.6	510.9	802.3	553.6	6685.8	8514.4	9251.0	8159.4	3516.7	4526.2	5026.6	4356.5
Mean	885.2	991.6	1112.0	996.3	7469.0	7961.5	8909.8	8113.5	4177.1	4476.6	5010.9	4554.9

	Genotypes (G)	Nodal Positions (NP)	Leaf surfaces (LS)	G x NP	G x LS	NP x LS	G x NP x LS
SEM ±	66.5	24.2	69.6	115.2	94.0	34.2	162.9
CD at 0.05	184.3	78.9	423.8	319.3	260.7	111.5	451.5

at the fourth and the highest at the eighth nodal position on both surfaces together or separately. Similar views have been put forward by Datta (1968) who stated that the younger leaves in the top of the plant have greater number of stomata per unit area than those situated at lower position. Nodal position  $\times$  genotype interaction was found significant, irrespective of leaf surface (Table 1). Stomatal frequency on both leaf surfaces increased from fourth to eighth nodal position, except in TCP-5, TCP-7, TCP-8, TCP-11 and Sonajuli local at the upper surface and in all the cultivars with the exception of TCP-5, TCP-7, TCP-10 and Meghalaya local on the lower surface. But taking both surfaces together all genotypes showed the increasing trend of stomatal frequencies from fourth to eighth nodal position, except in TCP-7, TCP-10 and Sonajuli local. Such variation in stomatal frequency, may be due their difference in the state of maturity. Cole & Dobrenz (1970) observed the highest stomatal frequency in alfalfa (*Medicago sativa* L.) at the higher insertion levels as compared to lower insertion and stated the reason of such variation might be due to the difference in leaf maturity. The discrepancy in stomatal frequency per unit area between younger and older leaves might be due to difference in density of stomata between these two types of leaves caused by the difference in size of plant cells. This was also corroborated the view of Salisbury (1927) that the younger leaves remain at the top of the plant and higher stomatal frequency of the top leaves was due to younger and smaller cells.

The interaction of genotype  $\times$  location within leaf  $\times$  nodal position (taking both surfaces together) was found significant (Table 2). An increasing trend of stomatal frequency was observed from base towards the tip of the leaf for all the three nodal positions, irrespective of leaf surface and genotype (Tables 2 & 3). The same trend in stomatal frequency from base to the tip was noticed in genotypes TCP-4, TCP-8, TCP-9, TCP-11, Meghalaya local and Nagaland local at the fourth nodal leaf ; but for

sixth nodal leaf it was true in all except TCP-5, TCP-11, TCP-52 and for eighth nodal leaf it was observed also in all excepting TCP-9, Nagaland local and TCP-52. Interestingly, TCP-52 did not show any increasing trend of stomatal frequency from base towards tip in any of the nodal leaves. Datta (1968) also stated that the greater number of stomata per unit area was at top, the lowest towards the base and the middle frequency was in the centre of the leaf.

Considering all genotypes together, the stomatal frequency at the base, middle and tip locations of the leaf showed significant variation according to nodal position and leaf surface (Table 3). The frequency increased from base towards the tip in each nodal position for both upper and lower surfaces. The same trend was noticed even at the fourth, sixth and eighth nodal positions when considered both the leaf surfaces together.

Stomatal frequency has been found to vary within leaf often in a specific manner. In some plants with broad leaves, stomatal frequency was greater at the leaf margin than near the midrib, while in some monocotyledonous plants, this frequency increased from the base, reaching maximum value in the middle of the lamina before decreasing towards the tip (Salisbury 1927). In the present investigation, stomatal frequency, irrespective of the genotype, nodal position and leaf surface was found to increase from base towards the tip (Table 3). While the observations made by Salisbury (1927) in the location wise variations of stomatal frequency on the leaf lamina in monocotyledons affirmed partially the findings of the present investigation. Salisbury (1927) noted the decreasing frequency towards leaf tip whereas it was increasing in the present study. However, such increasing trend of stomatal frequencies towards leaf tip was earlier observed by Miranda *et al.* (1981) and Heichel (1971) in young developing and relatively matured maize leaves .

Considering simultaneously the locations within leaf, nodal positions and leaf surfaces i.e. the

Table 2. Nodal position x location within leaf x genotype interaction (irrespective of leaf surface) for stomatal frequency

Genotype	Stomatal frequency at nodal position of leaf															
	Fourth				Sixth				Eighth				Irrespective of nodal position			
	Base*	Middle	Tip	Mean	Base	Middle	Tip	Mean	Base	Middle	Tip	Mean	Base	Middle	Tip	Mean
TCP-4	4126.1	4515.4	5005.2	4548.9	4900.0	5192.6	5370.6	5154.4	5261.1	6282.9	6797.4	6113.8	4762.4	5330.3	5724.4	5272.4
TCP-5	3762.6	4005.1	3987.8	3918.5	3490.0	4345.0	4318.8	4051.3	4308.9	5086.0	5136.0	4843.6	3853.8	4478.7	4480.9	4271.1
TCP-7	3667.0	4288.2	4193.0	4049.4	3639.4	4015.6	4266.9	3974.0	3487.2	4529.9	4944.7	4320.6	3597.9	4277.9	4468.2	4114.6
TCP-8	3337.5	3667.2	3772.9	3592.5	3692.6	4014.5	4092.3	3933.1	3576.4	4520.3	4876.6	4324.4	3535.5	4067.3	4247.3	3950.0
TCP-9	5581.4	6476.8	6839.5	6299.3	6249.2	7239.8	7568.1	7019.0	7640.3	7508.1	8097.6	7748.6	6490.3	7074.9	7501.7	7022.3
TCP-10	4439.8	5085.0	4772.0	4765.6	3613.1	4195.1	4281.8	4030.0	4850.3	4889.4	5529.7	5089.8	4301.0	4723.2	4861.2	4628.5
TCP-11	3374.7	3795.7	3855.0	3675.1	3460.5	4517.9	4309.5	4095.9	3670.4	4307.7	4603.5	4193.9	3501.9	4207.1	4256.0	3988.3
Meghalaya local	3804.4	4165.9	4469.1	4146.5	3178.7	4531.4	4875.4	4195.2	3617.0	4298.9	5160.4	4358.8	3533.4	4332.1	4835.0	4233.5
Nagaland local	3406.5	3566.4	4191.8	3748.2	3397.7	4135.4	4787.4	4104.8	4222.5	4952.4	4822.6	4665.8	3702.2	4218.1	4600.6	4173.6
TCP-52	3549.9	4397.1	3923.6	3956.8	3508.3	4534.2	4516.7	4186.4	5023.1	4952.5	5056.2	5010.6	4027.1	4627.9	4498.8	4384.6
Sonajuli local	3515.2	4124.7	4083.9	3907.9	4274.6	4428.5	4636.5	4446.6	3907.8	4455.2	4940.1	4434.4	3899.2	4336.1	4553.5	4263.0
TCP-62	3278.2	3632.1	3639.8	3516.7	3937.2	4589.2	5052.1	4526.2	4586.5	5101.0	5392.4	5026.6	3934.0	4440.7	4694.8	4356.5
Mean	3826.9	4310.0	4394.5	4177.1	3945.1	4644.9	4839.7	4476.6	4512.6	5073.7	5446.4	5010.9	4094.9	4676.2	4893.5	4195.4

\* Location within leaf

	Genotypes (G)	Locations within leaf (LL)	Nodal positions (NL)	G x LL	G x NP	LL x NP	G x LL x NP
SEM ±	66.5	33.2	24.2	NS	115.2	57.6	199.5
CD at 0.05	184.3	92.2	78.9	-	319.3	159.6	553.0

Table 3. Leaf surface x nodal position x location within leaf interaction (irrespective of genotypes) for stomatal frequency.

Location within leaf	Stomatal frequency											
	Upper leaf surface				Lower leaf surface				Irrespective of leaf surface			
	Fourth*	Sixth	Eighth	Mean	Fourth	Sixth	Eighth	Mean	Fourth	Sixth	Eighth	Mean
Base	596.9	686.0	818.1	700.3	7057.0	7204.3	8207.2	7489.5	3826.9	3945.1	4512.6	4094.9
Middle	944.9	1034.9	1114.8	1031.5	7675.0	8254.9	9032.6	8320.8	4310.0	4644.9	5073.7	4676.2
Tip	1113.9	1253.9	1403.1	1257.0	7675.0	8425.4	9489.2	8530.1	4394.5	4839.7	5446.4	4893.5
Mean	885.2	991.6	1112.0	996.3	7469.0	7961.5	8909.8	8113.5	4177.1	4476.6	5010.9	4554.9

\* Nodal position

	Locations within leaf (LL)	Nodal positions (NP)	Leaf surface (LS)	LL x NP	LL x LS	NP x LS
SEM ±	33.2	24.2	69.6	57.6	47.0	34.2
CD at 0.05	92.2	78.9	423.8	159.6	130.3	111.5

genotype x location within leaf x nodal position x leaf surface interaction with regard to stomatal frequency was found significant.

Any variation in the frequency and distribution of stomata within leaf and between leaves of the same plant (Datta 1968) may influence some vital physiological processes substantially (Wilkins 1993; Epstein 1972). Simple correlations for leaf stomatal frequency of the twelve turmeric genotypes in relation to base, middle and tip locations of fourth, sixth and eighth nodal leaves showed high positive correlations in stomatal frequency between any two locations of the leaf lamina from three nodal positions on upper (upper off diagonal values without bracket), lower (lower off diagonal values without bracket) and even irrespective of leaf surfaces (upper off diagonal value within first bracket) (Table 4). Correlation remained among base, middle and tip locations under particular nodal position of the upper surface to the corresponding locations at the lower surface, except between base locations of sixth nodal position under upper and lower surfaces (correlation values indicated within third bracket). Highly significant and positive correlation also existed among nodal positions at the upper (Fourth vs Sixth=0.945, Fourth vs Eighth= 0.883 and Sixth vs Eighth=0.924), lower (Fourth vs Sixth=0.817, Fourth vs

Eighth=0.883, Sixth vs Eighth=0.925) and even when both surfaces were taken together (Fourth vs Sixth=0.860; Fourth vs Eighth=0.886 and Sixth vs Eighth=0.943). Significant correlation ( $r = 0.686$ ) was found between stomatal frequencies of leaf surfaces. Therefore, it is evident from the results that though the frequencies of stomata are changing within leaf, leaf surface and leaf to leaf still strong positive interrelationship was found to exist among stomatal frequency of different locations within leaf, leaf surfaces and nodal position.

The simple correlations between stomatal frequencies with leaf dry weight and fresh rhizome yield were negative in genotypes susceptible to leaf blotch disease but almost positive in resistant genotypes (Table 5). The stomatal frequency appears to be more positively related to the rhizome yield than dry weight of leaves. The correlation between the total stomatal frequency and the frequency of individual fourth and sixth nodal leaf with rhizome yield was found to be positive and significant in resistant genotypes. However, the total and individual stomatal frequency of fourth, sixth and eighth nodal leaves had no significant correlation with total dry weight of leaves as well as with the dry weight of respective leaf in the resistant types. But the same exhibited significant negative correlation when

**Table 4.** Simple correlations among base, middle and tip locations of leaf on both surfaces and irrespective of leaf surface from the leaf at different nodal positions.

Nodal position	Location within leaf	Fourth*			Sixth			Eighth		
		Base	Middle	Tip	Base	Middle	Tip	Base	Middle	Tip
Fourth	Base	-	0.959 (0.973)	0.909 (0.969)	0.818 (0.782)	0.802 (0.838)	0.968 (0.802)	0.722 (0.856)	0.735 (0.821)	0.797 (0.892)
	Middle	0.954	-	0.952 (0.930)	0.872 (0.774)	0.841 (0.819)	0.936 (0.769)	0.804 (0.854)	0.793 (0.773)	0.837 (0.849)
	Tip	0.970	0.902	-	0.894 (0.847)	0.820 (0.891)	0.940 (0.885)	0.822 (0.856)	0.887 (0.859)	0.889 (0.922)
Sixth	Base	0.733	0.708	0.824	-	0.930 (0.910)	0.852 (0.893)	0.855 (0.851)	0.874 (0.906)	0.911 (0.924)
	Middle	0.799	0.783	0.861	0.890	-	0.833 (0.969)	0.818 (0.906)	0.802 (0.898)	0.867 (0.923)
	Tip	0.747	0.684	0.824	0.862	0.972	-	0.759 (0.885)	0.813 (0.898)	0.890 (0.913)
Eighth	Base	0.851	0.858	0.819	0.792	0.874	0.852	-	0.933 (0.945)	0.819 (0.916)
	Middle	0.808	0.736	0.840	0.899	0.877	0.879	0.905	-	0.861 (0.962)
	Tip	0.879 [0.609]	0.826 [0.623]	0.918 [0.629]	0.900 [0.538]	0.885 [0.642]	0.856 [0.441]	0.855 [0.679]	0.949 [0.696]	- [0.578]

\*Nodal position

Correlation co-efficient for 10 df at 5% level=0.576 at 1% level = 0.708

Upper off diagonal values without brackets are the correlation values of locations within leaf at the upper leaf surface

Lower off diagonal values without brackets are the correlation values of locations within leaf at the lower leaf surface

Upper off diagonal values within first bracket are the correlation values of location within leaf irrespective of leaf surface.

The values within third bracket indicate the correlation among base-, middle- and tip- locations of the upper surface to the corresponding locations of the lower surface.

compared with the dry weight of sixth and eighth nodal leaves individually and with total dry weight in susceptible genotypes. The differential relationship between stomatal frequency with leaf dry biomass and rhizome yield in resistant and susceptible genotypes may be due to the differential production and accumulation of photosynthate. In resistant genotypes, the leaf tissues and stomata were less affected and or unaffected by leaf blotch disease. Such leaf produced and simultaneously translocated higher photosynthate to the rhizomes and thereby the yield was increased. Therefore, it was quite predictable that the correlation between stomatal frequency and the

rhizome yield in resistant genotypes would be positive. While in susceptible genotypes, leaf tissues and stomata were severely damaged by the disease and consequently the leaf dry biomass and rhizome yield were considerably affected. So, it was quite obvious that correlation of stomatal frequency with leaf dry biomass and rhizome yield would be negative in susceptible genotypes.

The present study conclusively indicated that the turmeric genotypes differed in stomatal frequency. Turmeric leaf was amphistomatous and its leaf stomatal frequency increased from base towards the tip of the leaf, from upper to

**Table 5.** Simple correlations between total and individual frequency of fourth, sixth and eighth nodal leaves with their respective leaf dry weight and rhizome yield in resistant and susceptible genotypes.

Stomatal frequency	Dry weight of leaf		Yield plant <sup>-1</sup>	
	vs Susceptible	vs Resistant	vs Susceptible	vs Resistant
Fourth nodal leaf	(-) 0.443	0.161	(-) 0.483*	0.599*
Sixth nodal leaf	(-) 0.614*	0.011	(-) 0.227	0.658*
Eighth nodal leaf	(-) 0.666*	(-) 0.135	(-) 0.451*	0.328
Total	(-) 0.633*	(-) 0.039	(-) 0.509*	0.614*

lower surface and from fourth to eighth nodal leaf. Strong positive correlation was found to exist between stomatal frequencies of any two locations, surfaces and nodal positions. Stomatal frequency showed positive correlation with rhizome yield in resistant genotypes, on the contrary, it showed negative correlation with rhizome yield in susceptible genotypes. Therefore, stomatal frequency can be considered as an useful tool for characterizing turmeric genotypes and to study the impact on leaf dry matter production and on rhizome yield in resistant and susceptible genotypes.

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