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GENETIC ANALYSIS OF STOMATAL DENSITY AND LEAF AREA IN ACID LIME (*CITRUS AURANTIFOLIA* SWING.)

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ABSTRACT

Highly significant variation for stomatal density, leaf area and stomatal number per leaf was observed in 27 acid lime (*Citrus aurantifolia* Swing.) clones. They were grouped into low, medium and high density categories based on their stomatal density. No appreciable differences among the clones for stomatal size were observed. The genotypic coefficient of variation for all the characteristics were higher than the respectively phenotypic coefficient of variation. High heritability was observed for stomatal density, indicating lower influence of environmental factors on this characteristic. The lower GA estimated for stomatal density indicated that the heritability for the characteristic may be of nonadditive nature. Correlation studies revealed negative association between stomatal density and leaf area. The role of stomata determining several properties in plants, such as, tolerance to diseases and drought, plant vigour and yield, indicated scope for selection and breeding in acid lime by applying selection pressure for stomatal density.

Key words: Acid lime, stomatal density, leaf area, genetic variability, heritability, correlations.

Stomata play an important role in the response of plants to several factors like tolerance to diseases [1–3], drought tolerance [4–6], plant vigour [7–9], yield [10, 11], and winter hardiness [12]. Stomatal density and size, epidermal cell density and leaf area are reported to be associated with each other [6, 13, 14].

Acid lime (*Citrus aurantifolia* Swing.) is the third most important Citrus crop of India producing about 0.45 million metric tonne fruits annually. Large scale variation among different strains for yield and fruit characteristics has been reported [15–17], but no information is available on the genetic variability and association for stomatal density and size, and leaf area, which may play important role in yield and tolerance to diseases and drought in acid lime also [1]. Hence the study was conducted to assess the clonal and genetic variability and association of stomatal density and size with leaf area in acid lime.

MATERIALS AND METHODS

The study was conducted to determine stomatal density per mm^2, leaf area, stomata number per leaf, and stomatal size (length and breadth) on 10-year-old plants of 27 acid lime genotypes/clones, henceforth referred to as accessions, collected from different parts of India. Five mature leaves (fifth from terminal) per plant per replication in each accession were collected randomly in the forenoon of sunny days in three replications. In each accession three plants were used for sampling leaves and were considered as replicates. Leaf area was recorded on five leaves per replication through the LICOR automatic leaf area meter. Stomatal density on the abaxial side of leaves was recorded as per the technique suggested by Beakbane and Majumdar [7] under a high power (1x10 magnification) microscope for each mm^2 area. Stomatal length and breadth were also recorded on the same material with a micrometer. Stomata number per leaf was calculated based on the stomatal density mm^2 and leaf area. The data were analysed for randomized block design for variation among the different clones. The genotypic and phenotypic variability (GCV, PCV), heritability (h^2), and genetic advance (GA) as percentage of mean were estimated as per Burton and De Vane [18]. The genotypic and phenotypic correlations were estimated by suggested by Johnson et al. [19].

RESULTS AND DISCUSSION

Highly significant variation among the clones was observed for stomatal density, leaf area and stomata number per leaf (Table 1). There was no appreciable variation for stomatal size among the accessions. Stomatal length and breadth were generally about 23 µm and 22 µm, respectively. Stomatal density (No./mm^2) varied from 177 to 315 among the different accessions. The Accession-13 recorded minimum and Ac-19 recorded maximum stomatal density.

Table 1. Stomatal density and leaf area in acid lime

Accession No.	Stomatal density per mm^2	Leaf area (cm^2)	Stomata No./leaf (x10^5)
13	177	14.35	4.01
1	221	9.24	2.02
8	222	18.07	4.02
7	227	15.36	3.48
12	247	18.00	4.45
11	250	12.95	3.24
14	253	14.41	3.58
29	256	12.25	3.14
9	258	14.14	3.71
15	268	12.82	3.43
3	280	8.78	2.46
21	282	14.88	4.17
10	284	14.23	3.98
28	289	14.77	4.25
16	289	14.82	4.26
23	289	15.75	4.54
26	293	11.44	3.34
17	290	17.42	5.05
24	296	16.62	4.92
5	302	11.44	3.45
25	302	15.62	4.72
27	301	16.93	5.09
4	305	11.64	3.55
18	305	13.89	4.27
22	311	13.38	4.17
20	312	14.31	4.49
19	315	16.57	5.22
SEm (±)	8.66	1.19	0.33
C.V. (%)	5.36	14.43	14.51
C.D. (P = 0.01)	32.81	4.49	1.26

Leaf area varied from 8.78 to 18.00 cm^2 among the accessions. Ac-26 recorded minimum and Ac-12 maximum leaf area. Stomata number per leaf varied from 2.02 x 10^5 to 5.22 x 10^5 in the accessions 27 and 19, respectively.

The genetic variability for stomatal density, leaf area and stomata number per leaf (Table 2) showed that GCV for all the characters were generally higher than PCV. Heritability was high for stomatal density and also moderately high for leaf area and stomata number per leaf. Genetic advance was low in case of stomatal density and moderate for leaf area and stomata number per leaf.

Table 2. Genetic variability for stomatal density and leaf area in acid lime

Character	PCV	GCV	Herit-ability	GA (% of mean)
Stomatal density/mm^2	5.357	9.283	0.7718	17.84
Leaf area	14.459	14.658	0.5068	21.50
Stomata No./leaf	14.753	16.955	0.5691	26.33

Genotypic and phenotypic correlations (Table 3) between stomatal density and leaf area (i.e. leaf size) were negative, but highly significant positive correlations were obtained between stomatal density and stomata number per leaf as well as between leaf area and stomata number per leaf. This is quite expected.

Table 3. Correlation coefficients between stomataldensity and leaf area in acid lime

Character pair	PCV	GCV
Stomatal density/mm^2 vs. leaf area	- 0.11	- 0.07
Stomatal density/mm^2 vs. stomata no./leaf	0.41**	0.80**
Leaf area vs. stomatal density/leaf	0.82**	0.83**

**Significant at P = 0.01.

The study of variation for stomatal density and leaf area revealed large variation among genotypes/accessions for these characters. Among the characters studied, stomatal density is the most important trait affecting performance of the plant. Stomatal density per unit leaf area is reported to be responsible for stomatal conductance which directly regulates water loss [13] and under certain circumstances may even influence the ratio of photosynthesis to transpiration, both of which are likely to be advantageous in drought conditions [14] as well as in increasing yield [10]. The stomata are also the channel for direct entry of certain pathogens into the plants [1, 2], including *Xanthomonas campestris* in acid lime. Therefore, based on the results on stomatal density, the accessions were categorised into three groups, viz., low, medium and high stomatal density groups. Eight accessions (AC-13, 1, 8, 7, 12, 11, 14 and 29) were grouped under the low density category. Another set of eight accessions (Ac-9, 15, 3, 21, 10, 28, 16 and 23) constituted the medium density category, and the remaining eleven accessions (Ac- 26, 17, 24, 5, 25, 27, 4, 18, 22, 20 and 19) formed high density category. The variation for leaf area and stomata number per leaf was also highly significant.

The accessions with higher leaf area generally had higher stomata number per leaf in spite of lower stomatal density. The variations in leaf area and stomata number per leaf are mostly due to the response of plants to environmental conditions [14]. Thus, in the absence of appreciable variation for stomatal size, the most important characteristic appears to be the stomatal density, which may be exploited in selecting genotypes tolerant to diseases and drought. Simultaneous selection for characters like low stomatal frequency, smaller stomata and leaf area etc. was suggested to improve drought tolerance in plants [6]. Such selection could result in improved tolerance to *Citrus* bacterial canker also.

The studies on genetic variability revealed that the characters studied are amenable to improvement as is evident from higher GCV than PCV. High heritability for stomatal density indicates less influence of environment on this character. The lower genetic advance for stomatal density indicates nonadditive nature of gene effects for this character.

The study shows a definite scope for selections and breeding in acid lime based on stomatal density and leaf area with specific objectives. Several workers [5, 10, 20–23] had suggested genetic manipulation in crops through stomatal characteristics.

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