

Original Research Article

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Determination of Correlation between Stomatal Density and Gas Exchange Traits in Guava

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ABSTRACT

The control of gaseous exchange between the leaf and bulk atmosphere by stomata governs CO₂ uptake for photosynthesis and transpiration, determining plant productivity and water use efficiency. The balance between these two processes depends on stomatal responses to environmental and internal cues and the synchrony of stomatal behavior relative to mesophyll demands for CO₂. Stomatal conductance (Gs) is an important trait responsible for the genotypic difference in gas diffusion for photosynthesis and transpiration in guava (*Psidium guajava* L.). Correlation studies among different horticultural traits indicated that leaf stomatal density was positively correlated with stomatal conductance (Gs), to net photosynthesis rate (Pn, 0.110), and leaf net CO₂ assimilation rate (An, 0.177) (Table 1). Similarly, number of stomata is negatively correlated with Transpiration (E, 0.193). Further our results indicated that there is highly significant positive correlation among net photosynthesis rate, transpiration rate, stomatal conductance and internal CO₂ concentration (Ci). The differences in leaf stomatal numbers and gas exchange values that existed among the genotypes can be taken into consideration as selection criteria for guava to be grown in regions with varying environmental conditions. These findings also add new insights into the correlations among traits involving carbon and water economy in guava.

Keywords

Guava, Correlation analysis, Stomatal number and gas exchange apparatus.

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Introduction

In order for plants to function efficiently, they must balance gaseous exchange between inside and outside the leaf to maximize CO₂ uptake for photosynthetic carbon assimilation (A) and to minimize water loss through transpiration (Nilson and Assmann, 2007). Stomata are the “gatekeepers” responsible for all gaseous diffusion, and they adjust to both internal and external environmental stimuli governing CO₂ uptake and water loss. The pathway for CO₂ uptake from the bulk atmosphere to the site of fixation is

determined by a series of diffusional resistances, which start with the layer of air immediately surrounding the leaf (the boundary layer). Stomatal conductance for gas diffusion (gs) and transpiration is closely correlated with leaf photosynthesis in plants (Kuroda and Kumura, 1990; Miah *et al.*, 1997; Kanemura *et al.*, 2005). Strong indications have been obtained for a major influence of gs on crop growth rate during reproductive period and yield of some crop species in previous studies (Lu *et al.*, 1994;

Fischer *et al.*, 1998; Horie *et al.*, 2006). Therefore, it is expected that crops would be improved by manipulating the morphological characteristics of stomata (Jones, 1992; Ishimaru *et al.*, 2001), but the effect of the improvement on crop growth rate or yield is not evident. Many researchers have reported stomatal density responses to various environmental factors, such as elevated CO₂ concentration (Woodward, 1987), heat stress (Beerling and Chaloner, 1993), salt stress (Zhao *et al.*, 2006), drought (Galme's *et al.*, 2007), precipitation change (Yang *et al.*, 2007), and plant density (Zhang *et al.*, 2003). Sahin and Soyulu (1991) showed the effect of drought on the stomata frequency of selected chestnut types. In annual plants stomatal conductance was found to be related to yield in some instances. Ulloa *et al.*, (2000) confirmed that high stomatal conductance was associated with high cotton lint yields at supra-optimal temperatures for the cotton crop under irrigated environments.

The purposes of this study were to determine the response patterns of stomatal density to different gas exchange characteristics, and to develop the relationship of stomatal density with gas exchange. An understanding of the relationship between stomatal features and environmental factors would be of considerable importance for the better fruit tree growth on the warming globe.

Materials and Methods

Plant materials

The present investigation was carried out during the month of May 2013 on 22 indigenous guava cultivars of 6 years age maintained by the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute (IARI), New Delhi, India located at the altitude of 228 m above mean sea level with 28° 40' N latitude and 77° 13'

E longitudes. The average maximum temperature in summer is about ~32.8°C and minimum temperature about ~21.7°C and the average annual relative humidity of this area is about 64% with rainfall of about 680 mm. The trees were maintained under uniform cultural practices. The experiment was laid out in Randomized Block Design (RBD) with three replications as per the procedure outlined by Panse and Sukhatme (1955).

Gas exchanges

Gas exchange measurements were made once in a day (from 9.00 to 11.00 AM) on south facing mature leaves by using a portable infrared gas analyzer system (IRGA) (Li-Cor 6200, Li-Cor Biosciences, Lincoln, NE, USA) with an 11 cm² window leaf chamber at CO₂ concentration of 360 cm³/m³ and 21% O₂ or 2% O₂ air conditions (to inhibit the apparent photosynthesis) for net photosynthesis rate, transpiration rate, stomatal conductance and internal CO₂ concentration (C_i). The gas exchange rates were determined at each step after maintaining the leaf for 5–10 min at the new CO₂ concentration. All measurements were done in fully expanded leaves (usually the sixth to eighth from the top) of four different plants just before midday to reduce diurnal variation interference (Cartechini and Pallioti, 1995). The data was stored in the data logger in the equipment and analyzed statistically.

Stomatal determinations

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area (Radoglou and Jarvis, 1990). The leaves selected were those for which gas exchange was measured. The abaxial epidermis of the leaf was cleaned first using a degreased cotton ball, and then carefully smeared with nail varnish in the mid-area

between the central vein and the leaf edge, for approximately 20 min. The thin film (approximately 5 mm x 315 mm) was peeled off from the leaf surface, mounted on a glass slide, immediately covered with a cover slip, and then lightly pressured with fine point tweezers. Numbers of stomata (s) for each film strip were counted under a photomicroscope system with a computer attachment (Olympus, CX40RF200). Impressions were taken from the six youngest, fully expanded leaves for each plant and stomata number was calculated per mm².

Statistical analysis

Observations on physiological traits were subjected to analysis using standard statistical package and mean the difference between treatments will be separated using Randomized Block Design (RBD) with the experimental unit consisted of a six leaf sample taken from one selected plant. Four plants per treatment were employed as replications. All these leaves were at the same physiological age of those used for the gas exchange measurements. The data on different parameters were analyzed by using analysis of variance (ANOVA) by using Statistical Package for Agricultural Workers (STAT OP Sheoran <http://14.139.232.166/opstat/default.asp>). Valid conclusions were drawn only on significant differences between the treatment mean at 0.05 level of probability.

Results and Discussion

The correlation studies among different traits of guava genotypes would certainly provide an idea, which might be utilized for selection of desirable parameters for future breeding programs in guava. The highly significant positive correlation between desirable characters is favorable to a plant breeder because it might help in simultaneous

improvement of both the characters. On the other hand, the negative correlation would hinder the synchronized expression of both the characters. In such a situation, it would require to making some compromise including economic ones. Photosynthetic capacity of crop plants has been widely used as most important physiological attribute to evaluate plant vigor, biomass and ultimately economic yield.

The correlation between E, Gs, Pn were analyzed and showed in table 1. Among the genotypes, differences in the functioning of the photosynthetic apparatus were found. Leaf stomatal density was positively correlated with stomatal conductance (Gs) to net photosynthesis rate (Pn, 0.110), and leaf net CO₂ assimilation rate (An, 0.177) (Table 1). Transpiration is another important parameter, which is directly linked with temperature sensitivity vis a vis water availability for a plant. The number of stomata is negatively correlated with Transpiration (E, -0.193). However, the relationship between stomatal index and gas exchange parameters was remarkably scattered with no significant association found (data not shown). Further our results indicated that there is highly significant positive correlation among net photosynthesis rate, transpiration rate, stomatal conductance and internal CO₂ concentration (Ci).

Photosynthetic capacity is closely linked to SD (Xu and Zhou, 2008). Leaf A was negatively correlated with SD when plants were exposed to elevated CO₂ (Woodward, 1987; Ainsworth and Rogers, 2007), whereas a positive correlation occurred when grass was subjected to a water status gradient (Xu and Zhou, 2008). Moreover, photosynthetic potential might be enhanced with increased SD in Arabidopsis by a modulating gas diffusion function, as was recently reported by Tanaka *et al.*, (2013).

Table.1 Correlation among stomatal number and gas exchange characteristics

S. No.	Parameter	Stomatal Density	Intercellular CO ₂ (An)	Transpiration (E)	Stomatal conductance (Gs)	Net Photosynthesis (Pn)
1	Stomatal Density	1.000	0.177	-0.193	0.198	0.110
2	Intercellular CO ₂		1.000	0.984*	0.943*	0.466*
3	Transpiration			1.000	0.971*	0.502*
4	Stomatal conductance				1.000	0.569*
5	Net Photosynthesis					1.000

Critical values for Pearson's correlation coefficient of two tailed test: *, significant at 5% level of significance (0.404).

In this case, the A increase at elevated CO₂ is tightly associated with increased SD. When stomatal density increases independently of stomatal size, improvement of gs is expected (Schlüter *et al.*, 2003), but there are a number of experimental reports showing that increased stomatal density does not enhance gs (Jones, 1977; Kawamitsu *et al.*, 1996). This is because stomatal density negatively correlates with stomatal size. Our study showed that genotypic difference of stomatal density was not related to that of gs, but suggested two different ways to achieve highs. One is to have high specific stomatal conductance, as observed in the varieties in Mridula showing high gs despite with low stomatal density (data not shown). The other is to have high stomatal density.

Many reports showed an increase in stomatal density and a decrease in cell size under water deficit, indicating that an adaptation to drought could occur (Martinez *et al.*, 2007). The compromise between stomatal size and density is related to the limitation of the leaf area allocated to stomata. Thus, it was suggested (Franks *et al.*, 2009) that taking into account the leaf area limitation, there is a point when the only way to increase stomatal conductance is by decreasing stomatal size and increasing density, since increasing stomatal conductance by increasing size will require a greater percentage of leaf surface allocated to stomata and a higher change in the leaf area. Several researchers

(Hetherington and Woodward, 2003; Russo *et al.*, 2010) had reported that smaller stomata are better at improving WUE, due to their more rapid response to changes in environmental conditions such as humidity (Drake *et al.*, 2013). In a recent study, it was revealed (Dow *et al.*, 2014) that high stomatal density restricted CO₂ diffusion and lowered photosynthesis. These findings were explained by the reduced functional capabilities of the guard cells from reduced availability of ions required for driving membrane processes, as well as the influence of turgor pressure for adjacent guard cells and the disruption of signalling processes due to the close proximity of the guard cells.

Some studies (Zhao *et al.*, 2015) had also established significant negative correlations of stomatal density with photosynthesis and demonstrated that higher stomatal density reduced leaf photosynthesis. They argued that small stomata could maintain the pores opening with lower guard cell turgor pressures compared with larger stomata. The higher stomatal density and reduced stomatal size responding to drought can effectively inhibit transpirative water loss and better ensure water balance (Bosabalidis and Kofidis, 2002).

In apples the stomatal behaviour of the leaves appears to be correlated with photosynthetic rate (Lakso, 1994). Several factors are known to control stomatal conductance, such as light,

soil water potential, internal CO₂ concentration as well as sink strength in trees. But, genotypical differences in stomatal conductance were often neglected. The results of this study showed that stomatal conductance values of the guava genotypes grown in the same conditions greatly varied. Also, a low stomatal sensitivity to drought in benefit of an increase in growth would probably be a more successful strategy under the competitive conditions during tree establishment (De Lucia *et al.*, 1991). The stomatal conductance seems to be independent of stomatal frequency or stomata size, since there was no significant relation between them in all genotypes tested (data not shown). In cotton, high stomatal conductance might confer some adaptive advantage to genotypes that experience supra-optimum temperatures (Ulloa *et al.*, 2000). However, unlike cotton, fruit trees are perennial plants with more complex physiological features that are difficult to be related. Using shading nets in apricots resulted in higher leaf stomatal conductance values than in exposed plants (Nicolas *et al.*, 2005). Thus, climatological differences should be taken into consideration when stomatal conductance values are to be discussed. The result of this study provided evidence that there are cultivar differences with respect to stomatal frequency and stomatal conductance. Therefore, this information is important in the development of adaptation studies, particularly concerning heat stress to which trees experience in summer. Improving regulation capacity in stomatal traits by breeding selection and/or genetic methods would enable plants to acclimate to environmental stresses, such as drought. While genetic variation in intrinsic TE for any given level of conductance may be only one of a number of sources of variation in crop TE in the field, this study indicates that it could provide a useful target for breeding programmes if reliable and cost-effective screening can be implemented.

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