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Stomatal Characteristics in Arabica Coffee Germplasm Accessions under Contrasting Environments at Jimma, Southwestern Ethiopia

[1]Taye Kufa and [2]Jürgen Burkhardt
[1]Jimma Agricultural Research Center, Ethiopian Institute of Agricultural Research,
P.O. Box 192, Jimma, Ethiopia
[2]Institute of Crop Science and Resource Conservation-Plant Nutrition, University of Bonn,
Karlrobert-Kreiten-Str.13, D-53115 Bonn, Germany

Abstract: The montane rainforests of Ethiopia are the only known centres of origin and genetic diversity for *Coffea arabica*. However, the remnant coffee forest environments with the spontaneously grown wild coffee populations are under continuous threat of genetic erosion, largely due to anthropogenic activities. The study was conducted with the objective to investigate stomatal characteristics in Arabica coffee accessions under contrasting shade regimes at Jimma (7°46' N and 36°0' E, 1750 m), southwestern Ethiopia. For this, two shade levels (full sunlight and moderate shading) and twelve-coffee germplasm accessions were arranged as main and sub-plot treatments in a split-plot design with three replicates. The results depicted that stomata were sparsely distributed and had elliptical guard cells with pores randomly oriented pattern in Arabica coffee leaf. Maximum and minimum average stomatal densities were determined in full sunlight and moderate shade conditions, respectively. In addition, the stomatal area index was significantly higher in sun-exposed than in shaded leaves. The lowest and highest values were determined for the Harenna and Yayu accessions, respectively. The interaction between season and radiation was significant ($p<0.05$) on the frequency of stomata. The contrasting shade levels had significant influence on the density of stomata in both wet ($p<0.05$) and dry ($p<0.001$) seasons. Accordingly, higher stomatal frequency was recorded in dry as compared with wet season, though the range differed among the genotypes. Overall, stomatal size values followed the rainfall gradient with the order: Harenna>Bonga>Berhane-Kontir>Yayu coffee genotypes. The results would demonstrate that coffee accessions in drier Harenna areas may be more productive under higher radiation when soil moisture is sufficient. This underlines the need to consider stomata traits in identifying and developing suitable coffee cultivars against the changing environments.

Key words: *Coffea arabica* gene pool, drought tolerant, radiation stress, stomatal characteristics, water relations

INTRODUCTION

As with most plant species, leaf stomata in coffee are usually open during the day and closed at night. Under conditions of moisture tension in the plant, widening of the stomata cannot take place as the guard cells cannot absorb water. Light, temperature, water availability, atmospheric humidity, CO_2 concentration and wind movements can directly affect stomatal movements (Wintgens, 2004; Wrigley, 1988). The first discernable response of the stomata to the onset of drought stress is to open slightly. Consequently, water will be withdrawn from the epidermis into the mesophyll tissues, resulting in a relatively temporary flaccidity which allows a turgor advantage for the guard cells and hence, stomatal opening. This opening phase, however, is only transient and as drought stress continues, water passes out of the guard cells. Therefore, the stomata close progressively with increasing drought stress (Martin *et al*., 1993) and stomatal closure occurs when the coffee leaves start to wilt (Kanechi *et al*., 1995). Furthermore, they reported that under normal conditions, a temporary decrease in stomatal aperture could be encountered, as midday stomatal closure is common in plants growing under tropical conditions.

Coffee leaves show a decrease in stomatal opening with a deficit of only 20 or 30 mg of water per dm^2 of leaf surface and complete closure with a deficit of about 80 mg per dm^2. The quantity of water evaporated per day by a non-shaded coffee tree is approximately 6 g dm^2. Moreover, high irradiance and temperature can cause a rapid increase in transpiration which in turn causes

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Corresponding Author: Taye Kufa, Jimma Agricultural Research Center, Ethiopian Institute of Agricultural Research, P.O. Box 192, Jimma, Ethiopia

enough moisture deficiency to reduce stomatal aperture. In contrast (Kanechi *et al*., 1995) reported that both turgid and wilted coffee leaves had higher transpiration rates on cloudy than on sunny days, which means greater stomatal opening on cloudy days. However, sun-exposed leaves showed no significant increase in transpiration as light intensity increased under drought conditions. This might be explained by the stomatal regulation in response to the vapor pressure deficit, which increased due to rising leaf temperature when the leaves were exposed to direct sunlight (Wintgens, 2004).

According to Coste (1992) the stomata of coffee leaves were closed when the leaf water deficit reached between 18 and 30% but the leaf dehydrated slowly and steadily and gradually lost turgidity until it reached the wilting point. Moreover, it is stated that very different drought-resistant capabilities in two cultivated varieties of *C. canephora* have manifested in the ability of the leaf tissues to withstand and resist a high degree of dehydration. The most resistant variety demonstrated swift stomatal closure and less active use of the water supply. Stomatal closure cuts off gaseous exchange. Therefore, photosynthesis and respiration are affected and hence, there is less dry matter production under conditions of drought stress (Williams, 1971). Transpiration and stomatal conductances are sometimes used almost synonymously. This however, may be problematic: low wind speed, large leaves and compact tree crowns lead to the formation of boundary layers around each leaf and around the whole crown (Grace *et al*., 1980). The resistances of these boundary layers to water vapor diffusion decouple transpiration from stomatal control (Jarvis and McNaughton, 1986). Thus, relatively high air humidity within the canopy reflects the low coupling between the inner part of the crown and the atmosphere. The importance of the boundary layer decreases with increasing wind speed. When stomatal regulation of transpiration was studied in hedgerow coffee, transpiration at the crown scale increased linearly with net radiation and tended to increase with increasing wind speed. The apparent stomatal responses to wind were partially a reflection of the stomatal response to humidity (Gutiérrez *et al*., 1994).

Additionally, the stomatal aperture of coffee leaves seems to be an adaptation of *C. arabica* to the conditions in the under storey vegetation of montane rainforests with little light and wind and well buffered against temperature change by the enveloping trees (Wrigley, 1988; Cambrony, 1992; Coste, 1992). Coffee has several times been reported to keep stomata closed at high temperatures even under conditions of sufficient water supply from the soil, thus not taking advantage of the cooling effect of transpiration. It was shown that complete elimination of shadow during a period of dry and sunny weather led to continuous stomatal closure of *C. arabica* leaves for a period of 10 days. Leaf temperatures thus reached 35-41°C, as much as 10-15°C above the ambient air temperature (Weidner *et al*., 2000). Stomata started to open only during a period of increased cloudiness and rainy weather.

Plants generally respond to acute water deficits by closing their stomata in order to match transpirational water loss through the leaf surfaces with the rate at which water can be resupplied by the roots. Stomatal frequency or density (the number of stomata per unit area of one leaf surface) can vary significantly within leaves, plants or individuals of a single species within a community and can be modified by environmental factors, leaf morphology and genetic composition. This has been reported by numerous studies (Al-Saghir and Porter, 2005; Peksen *et al*., 2006). Stomatal frequencies often vary according to cell size and smaller guard cells are usually associated with higher stomatal frequencies (Woodward and Bazzaz, 1988; Woodward, 1987).

The montane rainforests of Ethiopia are the only known centres of origin and genetic diversity for *Coffea arabica*. However, the remnant rainforests with the spontaneously grown wild coffee populations are under continuous threat, largely due to anthropogenic factors such as population pressure, indiscriminate deforestation and extensive disturbance of forest habitats (Tadesse, 2003). This would be more aggravated by the possible adverse effects of global warming and climate change on environmental sustainability and coffee diversity (Taye, 2010). The frequent occurrence of recurrent drought is becoming one of the major challenges on coffee gene pools, particularly when it occurs at moisture stressed areas and during critical growth stages. It results in poor growth and development, reducing the life span or completely drying the coffee trees and aggravating the genetic erosion of the known coffee types and brands. However, there is still immense wealth of wild and cultivated arabica coffee genetic potentials in Ethiopia (Tadesse, 2003).

There is however, information on the roles of stomata under different environmental scenarios and molecular control of stomatal initiation and spacing patterns in Arabica coffee genetic resources is scanty in Ethiopia. The mechanisms involved in determining the formation and arrangement of stomata and their ecological adjustments for adaptation calls for detail stomatal characterizations in future coffee breeding program. Hence, studies on identifying and developing coffee cultivars tolerant to environmental stresses are crucial for sustainable conservation and use of Arabica coffee gene pools at the country of origin and genetic diversity. This requires, among other, information on stomata characteristics and other physiological traits in coffee plant grown under varying environmental conditions. The aim of this study was to see variability in stomatal characteristics among arabica coffee germplasm accessions under full sunlight and partial shade environments at the Jimma Agricultural Research Center, southwestern Ethiopia.

MATERIALS AND METHODS

The study area: The study was carried out in 2005 at the Jimma Agricultural Research Center (JARC), (7°46' N and 36°0' E), southwestern Ethiopia. The area is located within the Tepid to cool humid highlands agro-ecological zone of the country at an altitude of 1750 m above sea level. The area receives adequate amount of rainfall with an average rainfall of 1595 mm per annum distributed into 173 days. The driest season lasts between December and January. The average maximum and minimum air temperatures are 25.9 and 11.2°C, respectively, the coldest month being December (Taye, 2006).

Experimental treatment and design: Fully ripe red cherries were collected from coffee trees at three sites within four wild arabica coffee populations; viz., Harenna, Bonga, Berhane-Kontir and Yayu. Except Harenna in the southeast, the other three forests are located in the more humid southwest Ethiopia. Consequently, coffee seedlings from a total of 12 accessions: Harenna (I-1, I-2, I-3), Bonga (II-1, II-2, II-3), Berhane-Kontir (III-1, III-2, III-3) and Yayu (IV-1, IV-2, IV-3), were *ex-situ* established and assessed under experimental nursery settings. The potting medium was prepared from blends of topsoil and decomposed coffee husk compost at the respective ratio of 3:1 (v/v) was used (Taye *et al*., 2001). The black plastic pots (volume = 5.8 L) were perforated at the bottom, firmly filled with the so prepared pots were arranged on experimental nursery seedbeds. Coffee seeds were sown in each plastic pot and all post-sowing nursery operations were applied according to the recommendations of the center (Yacob *et al*., 1996) until the radiation treatments were induced on coffee seedlings.

The treatments were arranged in a split-plot design with three replications. The two shade levels (full sunlight and moderate shading) and twelve-coffee germplasm accessions were assigned as a main and sub-plot factors. The coffee seedlings were maintained under controlled contrasting shade environments for a period of 8 months. Each treatment consisted of 25 seedlings per plot. Moderate (50% light interception) overhead shade (at 2 m height) and side shades were constructed from bamboo slants. The diurnal microclimate variables were monitored during the study period and the results depict significant differences between the shade regimes (Table 1). This depicts that the treatment is enough to see the variability among coffee genetic resources in stomatal nature due to natural radiation and water stress pressures.

Stomatal measurements: Irrigation was uniformly applied to maintain full leaf turgidity and stomatal measurements were recorded in the morning when leaf stomata were expected to remain open. Mature healthy leaf samples were used from the third to fourth nodes of the primary branches in the middle crown of coffee seedlings. The samples were stored in a plastic bag and immediately brought to laboratory for measurements. The leaves were cleaned and a small part of the leaf surface from the lower (abaxial) and upper (adaxial) epidermis was sampled using a thin layer of transparent nail polish. The so-prepared sample was left for about 10 min to dry and the nail polish impression was swiftly removed using a transparent adhesive tape. This was placed on an object slide and observed under a light microscope at 400 x magnification. The diameter of field of vision for the binocular was measured by stage micrometer at 40 objectives and the area was calculated as ΠR^2.

The density of stomata and dimension of guard cells were recorded within a small field of view (0.15 mm^2) as described by Weyers and Meidner (1990) and Van Duren *et al*. (1996). The density of stomata was determined in the wet (August/September) and dry (December/January) seasons. There were no clearly differentiated cells around the two guard cells for measurement. Hence, the width of guard cell pair was measured along with its maximum length and used in the estimation of other the stomatal characteristics. Hence, it was not possible to estimate the frequency of stomata relative to the other types of cells on the epidermis

Table 1: Microclimate variables in the studied full sun and moderate shade plots

		Temperature (°C)	
Variables	RH (%)	Air	Soil
Time of day	*	**	Ns
Night	80.97±9.97^{a}	16.56±1.74^{b}	19.10±7.16
Day	70.82±6.03^{b}	20.47±2.01^{a}	23.83±4.23
Irradiance	Ns	Ns	Ns
Sun	73.42±8.57	18.75±3.17	24.03±5.95
Shade	78.36±9.52	18.28±2.46	18.90±5.60
Mean	75.89	18.51	21.46
CV (%)	3.17	3.58	30.42
Time*shade	Ns	Ns	Ns

Ns = Not significant, *$p<0.05$, **$p<0.001$. Means with same letter(s) are significently different at p = 0.05

(stomatal area index). However, Stomatal Area Indexes (SAI), Stomatal Area (SA) and total number of stomata on a leaf area basis were estimated for shade treatment and coffee accession. The SAI was calculated as SAI = stomatal density×guard cell length. The total number of stomata on a leaf surface was estimated from the product of stomatal frequency by leaf area (Weyers and Meidner, 1990).

Statistical analysis: Analysis of variance (ANOVA) was carried out to assess variations in stomatal attributes in seedlings of Arabica coffee genotypes due to the main and interaction effects. The treatment means were compared according to Tukey test at $p = 0.05$ whenever the F-test showed significant differences. Data analysis was performed using the SAS system for Windows 8 software (SAS Institute, Cary, NC). Graphs of significant interactions were made with the SigmaPlot SPW 9.0 (SYSTAT Software, Inc.).

RESULTS

The results depicted that leaf stomata in Arabica coffee seedlings were sparsely distributed and had elliptical guard cells with pores randomly oriented. No stomata were found on the upper leaf surface of the coffee leaves. Maximum and minimum average stomatal densities were determined in full sun radiation and shaded seedlings, respectively (Table 1). In addition, the stomatal area index was significantly higher in sun-exposed than in shaded leaves. However, stomatal densities did not differ among the coffee accessions and the lowest and highest values were determined for the Harenna and Yayu coffees, respectively (Table 2).

In addition, stomatal frequency was insignificant due to the interactions effects arising between coffee accessions and shade levels. However, in all accessions, relatively higher value was recorded in the sun-exposed leaves as compared with shaded leaves (Table 2). Consequently, the highest values were found for the sun-exposed Harenna (I-1) and Bonga (II-3) accessions, while the lowest results were for the shaded leaves of Berhane-Kontir (III-1 = 224 mm^{-2}) and Bonga (II-1 = 226 mm^{-2} and II-2 = 226 mm^{-2}) accessions (Fig. 1).

In contrast, the coffee accessions were comparable in stomatal frequencies over seasons and seedling ageing. However, most values showed the same trend due to the combined effects of shade and coffee accessions. But, the average values for all the stomatal traits were decreased with reduced sunlight radiation, though the change was relatively small, i.e., there was an only 4% reduction in stomatal area due to variation in shade level. This was despite the significant change in leaf growth, mainly due to factors associated with shade and seedling age. This is in contrast to the significantly ($p<0.001$) higher average number of stomata in the open sun plots (271 mm^{-2}) compared with those in the shade (231 mm^{-2}) in the dry months.

Coffee accessions showed insignificantly different in some stomatal parameters studied. But, the average stomatal area ranged between 3668±253 and 5033±592 μm^2 for Berhane-Kontir (III-3) and Harenna (I-2) accessions, respectively. The average leaf stomata dimensions were 76.79 μm long and 70.58 μm wide with the density of 251 mm^{-2} and stomatal area index of 19394.63 $\mu\text{m mm}^{-2}$ (Table 2).

Table 2: Stomatal density and guard cell dimensions (Mean±SD×2.5 μm) in seedlings of coffee accessions in full sun and shade conditions

Treatment	Density (mm^{-2})	Area index ($\mu\text{m mm}^{-2}$)	Area (μm^2)	Density (mm^{-2} leaf area^{-1})	Length (μm)	Width (μm)
Shade levels	***	**	Ns	Ns	Ns	Ns
Full sun	271±48^a	22000±4169^a	4373±660	69424±12819	78±6	71±6
Shade	231±39^b	16789±2672^b	4210±629	68716±8304	73±5	71±7
Accession	Ns	Ns	Ns	Ns	Ns	Ns
I-1	290±71	24334±7260	4418±277	71985±7219	84±5	77±5
I-2	223±42	17867±3394	5033±592	63383±5348	80±0	73±0
I-3	267±90	21378±3268	4614±0	71967±14215	80±10	74±19
II-1	214±37	16445±3897	4693±1702	55717±8752	77±5	67±0
II-2	280±47	22623±6411	4035±265	76728±13165	80±10	77±5
II-3	227±28	16623±2074	4837±869	62760±1756	73±0	67±0
III-1	240±47	19423±6034	3847±0	68757±6906	80±10	70±4
III-2	270±89	19800±6568	4436±832	73375±21941	73±0	64±5
III-3	200±10	14667±692	3668±253	60028±14042	73±0	73±0
IV-1	267±47	19556±3457	4222±0	76706±3994	73±0	67±0
IV-2	274±47	20045±3457	3847±0	71271±6923	73±0	67±0
IV-3	264±90	19978±5626	3847±0	76167±12784	77±5	73±0
Mean	251.13	19394.63	4291.21	69070.04	76.79	70.58
CV (%)	12.54	16.17	15.31	16.83	7.33	9.12

Ns = Not significant,**$p<0.01$, ***$p<0.001$. Means with the same letter(s) within a column are not significantly different by Tukey test ($p = 0.05$)

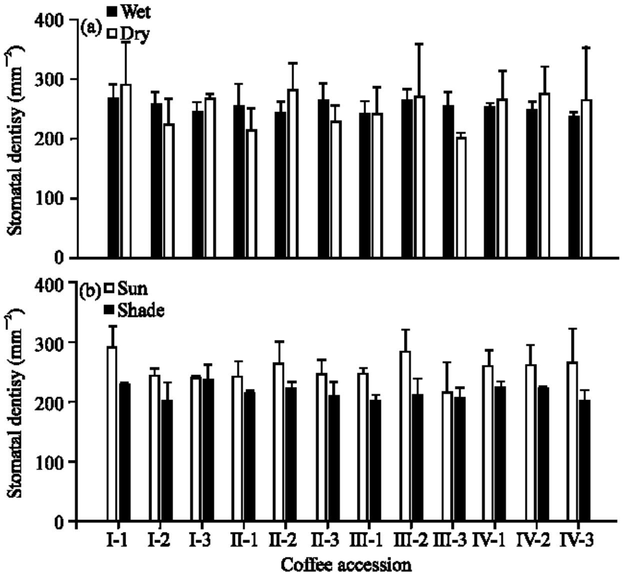

Fig. 1: Influence (a) of season and (b) shade level on leaf stomata density of seedlings coffee accessions under nursery conditions

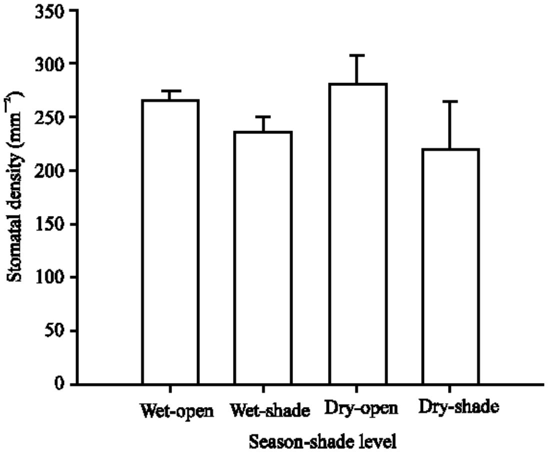

Fig. 2: Interaction effect between season and shade levels on the stomata density of Arabica coffee seedlings

With regard to interactions, the contrasting shade levels had significant influence on the density of leaf stomata in coffee in both wet ($p<0.05$) and dry ($p<0.001$) seasons. However, the revealed that stomatal density was not statistically different due to shade and accessions when compared in the wet season alone. The average density of stomata varied from 236 to 266 mm^{-2} for Yayu (IV-3) and Harenna (I-1) accessions, respectively (Fig. 1). In the dry season, the value for the Harenna accession was also the highest (290 mm^{-2}), while the lowest value was obtained for the Berhane-Kontir accession III-3 (200 mm^{-2}).

However, the interaction between seasons (wet and dry) and shade levels (sun and shade) was significant ($p<0.05$) for the frequency of leaf stomata. Consequently, the highest (282 mm^{-2}) and lowest (220 mm^{-2}) average values were recorded in the dry season under full sun and shade conditions, respectively (Fig. 2). The reduction in stomatal density in the shade plots was higher (22%) during the dry season as compared to the decline in the wet season (11%). In the open sunlight, the number of stomata increased from the wet to dry seasons, with an increment of 6% over the wet month. Conversely, there was a 6.9% reduction in the shaded environments during the dry season. The results show that neither season nor shade had significant interaction with coffee accessions in leaf stomatal characters. Nevertheless, the seasonal change in leaf stomatal frequency was in consistence for each accession. However, 67% of the coffee accessions, mainly from Harenna and Yayu, manifested an increasing number of stomata in the dry seasons. In contrast, most coffee germplasm accessions from the Bonga population revealed a reduction in stomatal density (data not shown). In general, all the studied coffee accessions had higher stomatal frequency in the dry than in the wet season, though the range differed among and within the four wild coffee populations. There were also great differences in the patterning and shape of stomata and in most cases, stomata were found in patches or clustered only in the abaxial part of coffee leaf.

DISCUSSION

The average stomatal densities and stomatal area index were significantly higher in sun-exposed than in shaded leaves. The lowest and highest values were determined for the Harenna and Yayu accessions, respectively. This is in line with the maximum and small leaf sizes recorded for the two populations, which indicates the positive relationships between mean leaf area and stomatal density in coffee leaves (Taye, 2006). This corroborates with Agbagwa and Okoli (2005) who observed variations in stomatal types and index in fruit epidermal cells and underlined the use of distinct stomatal features in systematic grouping of Abrus Adanson species.

The results depict the substantial variations in stomatal length and width due to shade regimes. The potential total area of the guard cells and stomatal index were higher in the full sun-exposed than in the shade seedlings. Stomatal pores were generally symmetrical, suggesting that there is a high degree of coordination between the daughter guard cells in coffee leaves. However, there was considerable variation between pore aperture and other stomatal characteristics over a single leaf and even between adjacent stomata.

The results of the density and sizes of stomata are consistent with the other leaf characteristics examined in reduced and maximum light intensities (Taye and Burkhardt, 2006). Besides other exogenous factors (light, water, major nutrients), the low leaf dry mass and total biomass production in the shade seedlings could be related to the modifications in stomatal characters. Therefore, the causes of variability in stomatal characteristics could be largely associated to the contrasting shade levels with significant differences in the above microclimate variables. In this case, there were considerable heterogeneity in the stomatal length and mean stomatal area in the sun-exposed coffee leaves. This is in line with the findings on stomatal of other plants (Al-Saghir and Porter, 2005; Peksen *et al*., 2006). This demonstrates the need for in-depth investigations on the patterns of variations within a leaf cell and their functional significances for planning sustainable conservation of coffee genetic potentials in Ethiopia.

The significant variation in some leaf stomatal parameters for the same coffee accession under the different shade conditions seems that environmental factors are involved in the morphogenesis of stomata. Accordingly, the higher stomatal frequency in the sun leaves could be related to the increased intensity of light throughout the study period. The decrease in stomatal frequency in the shade may be due to the expansion or greater number of epidermal cells. Besides, the relatively low stomatal frequency in the shade environments could be associated with the high leaf water potentials but low photosynthetic capacity of these coffee seedlings (Burkhardt *et al.*, 2006; Taye, 2006). This in turn could be linked to the reduced transpiration and thus vapor pressure gradients between the leaf surface and the saturated air layer above and the CO_2 concentration. Likewise, previous reports by Woodward (1987) and Woodward and Bazzaz (1988) showed that stomatal frequencies decrease with increased CO_2 concentrations. According to Woodward (1987) both stomatal index and frequency decrease with increased CO_2 concentration. Willmer and Fricker (1996) reported that water availability, light intensity, temperature and CO_2 concentrations were among the external factors affecting stomatal variables. Stomatal frequencies are usually higher in plants grown in full sunlight with high photon flux density than in plants grown in the shade with low photon flux density. (Ross-Karstens *et al*., 1998) reported similar trends of increased stomatal density in greenhouse grown coffee due to increased light intensity. They also found a significantly reduced stomatal density with the enhancement of CO_2 concentration for coffee leaves in closed vessels.

In crop plants, stomatal frequency can vary greatly among different genotypes of the same species growing under identical conditions. This was also observed in the present study, though the accessions were comparable in all the stomatal parameters. But, the average values of stomatal size followed the descending order of Harenna>Bonga>Berhane-Kontir>Yayu accessions. This is in line with the rainfall gradients, partly demonstrating their evolutionary adaptation to each geographical origin area as described by Peksen *et al.* (2006) in faba bean.

The maximum stomatal densities of the coffee accessions from the drier Harenna areas may also the suggest their adaptive capacity in hotter and water stressed situations as compared with those collection from the more humid southwestern rainforests (Bonga and Yayu) as described by Burkhardt *et al.* (2006). Hence, Harenna accessions were fast to show drought stress damage, though they had more growth performance and productive as long as soil moisture is sufficient. In contrast, the accessions from Bonga and Yayu had a suitable leaf anatomy for controlling the limited available water, partly due to stomatal natures and movements as pointed out earlier (Burkhardt *et al*., 2006). Present result supports the work done by Msogoya and Brian (2008) in that the off-type plants of banana had significantly higher stomatal density and were more vulnerable to water stress conditions. According to Paknejad *et al.* (2007) there was significant difference between different wheat varieties and irrigation levels in respect to chlorophyll content, RWC and grain yield.

This is similar to the results for leaf chlorophyll and dry matter production of the same coffee germplasm accessions under the two light regimes (Taye, 2006), indicating the influence of CO_2 concentration and light intensity on photosynthetic rate. Haniff (2006) found the inhibited diffusion of CO_2 into the leaf, decreased the intercellular CO_2 level and reduction in photosynthetic rate under water stress conditions. According to Willmer and Fricker (1996) plants growing in dry soils and low humidity generally have higher stomatal frequencies than plants growing in wet soil and in high humidity.

The decrease in most stomatal characteristics in the Berhane-Kontir accession III-3 might be related to the response of the leaves to high leaf temperature, particularly in the full sunlight plot because these seedlings were relatively younger compared to the Harenna and Yayu seedlings which showed the highest average values for most of the stomatal characteristics. This corroborates with our report (Taye and Burkhardt, 2006) on the *in situ* hydraulic conductance in wild Arabica coffee trees. In general, the results reveal that because of changing leaf area due to the difference in light intensity and leaf temperature the stomatal characteristics did not significantly differ among coffee accessions. This may be because the photon flux density was below the maximum level to bring about considerable changes as it did on other leaf growth characteristics such as leaf number and surface area. Exceptional was the accession II-2 from Bonga which had maximum values for some stomatal parameters, including the highest stomatal density per average leaf area. This corresponds with the findings of Ciha and Brun (1975) on soybean. They reported that drought stress resulted in a greater stomatal frequency, smaller leaf area and significantly lower stomatal numbers per leaflet than in non-stressed leaves.

The present study, therefore, testifies the stomatal adaptive mechanisms of coffee trees. The adaptive significance of stomata occurring on one-leaf surfaces is unclear, although hypostomaty is considered to be an evolutionary primitive character and as plants adapted to growth in more open sunlit habitats, amphistomaty may have evolved almost simultaneously (Mott *et al*., 1982). An unsubstantiated belief is that hypostomatous leaves are better adapted to dry conditions that amphistomatous leaves. The microelements between the shade levels significantly differ with appreciable effects on leaf stomatal behaviors. Certainly, the side of a leaf facing the sun may be slightly warmer than the opposite side in the shade and this would lead to a higher evaporative water loss from this side. However, amphistomaty may increase CO_2 uptake by reducing the length of the CO_2 diffusion pathway to the mesophyll (Parkhurst, 1978) and via its influence on stomatal resistances and boundary layer resistances (Mott *et al*., 1982).

Stomatal size usually decreased with increasing frequency but this feature was not observed in coffee during the dry season, indicating that stomatal movements are actively controlled in coffee seedlings. The overall seasonal average values, however, show indirect relationships between stomatal density and stomatal size due to shade and coffee accessions (Taye, 2006). The seasonal variations in the light regulation of stomatal initiation and deployment could be mediated by phytochromes (light quality) and growth hormones. The patchy stomatal opening in coffee leaves could be more pronounced as a result of sudden changes in environmental conditions and stomata within an area respond in concert apparently independent from the neighboring areas as described by Mott *et al.* (1993). It has also been suggested that a specific leaf anatomy predisposes this pattern of behavior. Terashima *et al.* (1988) observed patchy opening in heterobaric leaves. These are leaves, which have bundle sheath extensions that extend to the epidermis so that the mesophyll is separated laterally into patches as opposed to homobaric leaves which lack a bundle sheath extension (Larcher, 2003). A general view is that heterobaric leaves have stomata approximately opposite each other on both leaf surfaces between which run air-space channels. Another suggestion is that the patches of stomata are in a transitory stage in response to sudden environmental changes and eventually an optimized aperture will be reached by all stomata (Cardon *et al*., 1994). Not all species exhibit patchy responses, however and the extent to which they occur may depend critically on the severity of the imposed stress and the speed of application (Gunasekera and Berkowitz, 1992).

The significantly wider gap between shade treatments in the dry season could be explained in terms of increased air and leaf temperatures against which leaves may form more stomata with reduced sizes to reduce water loss. In other words, the minimum change during the wet season in the sun plots could be related to the reduced vapor pressure gradient due to cloud cover and high relative humidity. The increased age of the seedlings between the initial (wet) and final (dry season) measurements could also be among the possible reasons for the stomatal dynamics. The reverse relationships between stomatal attributes and water use efficiency (Burkhardt *et al*., 2006) leaf water potential and extension growth (Taye, 2006) were in harmony with present findings. Overall, seasonal climatic variables, coffee accessions, seedling age and growth characteristics in all treatments were found to affect phenotypic growth and plasticity in Arabica coffee populations (Taye, 2006). The results agree with several studies (Wrigley, 1988; Coste, 1992; Wintgens, 2004) that described a negative correlation between number and size of leaf stomata in coffee plants. According to literatures of Coste (1992), Hopkins (1995) and Wrigley (1988) transpiration and CO_2 assimilations are regulated via the stomatal opening, which is dependent on the plant water demand and environmental conditions. Ross-Karstens *et al.* (1998) pointed out the significant influence of CO_2 and light intensity on stomatal aperture and density of different plant species.

The stomatal density in the wet season was in line with the average leaf size of the coffee accessions, indicating the positive relationships between leaf surface area and number of stomata within a small field of view (Taye, 2006). He reported relationships between stomatal size and leaf water status, which was found to be different among coffee genotypes, the Harenna accessions manifesting a higher drop in leaf water potential. This may demonstrate the degree of stomatal opening and accumulation of inorganic ions with reduced availability of soil moisture deficits (Tesfaye, 2006).

Besides, the Harenna collections had high photosynthetic capacity as compared to others (Burkhardt *et al.*, 2006). The high water conductance and stomatal features could explain why the Harenna accessions experienced symptoms of soil moisture deficit earlier than the other accessions (Taye and Burkhardt, 2006) perhaps due to cavitation-induced reduction in water flow. The Berhane-Kontir and Bonga accessions were characterized by shallow roots, maintained higher leaf water potential but they seem to close stomata more efficiently than the others and hence dried more slowly (Taye, 2006). The decline in leaf water potential in the Harenna seedlings was in agreement with the increased carbon assimilation capacity and total biomass productivity of the accessions as opposed to the southwest accessions (Burkhardt *et al.*, 2006). Likewise, Nardini and Salleo (2000) reported that a high hydraulic conductance due to wide and long conduits can allow high plant growth potential, but increase the vulnerability to cavitation of plants. Hence, the results might suggest a trade-off between productivity and vulnerability to xylem embolism in Arabica coffee seedlings under sub-optimal and marginal environments as stressed by Sperry *et al.* (2002). Ahmadi and Joudi (2007) also found that leaf removal in wheat appeared to stimulate an increase of net photosynthesis rate and stomatal conductance of the remaining flag leaf under drought stress conditions. The increased remobilization of stored photoassimilate, decreased maintenance respiration by source reduction and enhanced photoassimilate partitioning toward grain and spike photosynthesis might be responsible for sustain grain growth in this condition.

CONCLUSIONS

The study depicts for the first time assessment on the stomatal natures in arabica coffee germplasm accessions under contrasting shade environments. The results outline very different in leaf stomatal characteristics in the studied Ethiopian coffees of different geographical origins. The results revealed the profound effects of direct daylight radiation stress and the need to maintain optimum environments for optimized water relations and growth of coffee seedlings. There were also remarkable variability in the pattern and shape of stomata with a direct relationship between average leaf area and stomatal density in Arabica coffee leaves. In general, leaf stomata were noticed to vary in size, shape and distribution pattern in the natural arabica coffee gene pools, possibly indicating their evolutionary adaptation strategies under natural forest habitats. The maximum stomatal densities of the coffee accessions may demonstrate their adaptive and productive capacity in hotter and drier areas as compared to those accessions from the more humid southwestern rainforests. Hence, the findings would shade a light, at a small measure, on the characterization and utilization of the immense coffee genetic resources and sustainable management of coffee environments.

As a whole, the study offers systematic baseline information on stomatal characteristics in Arabica coffee for future breeding, molecular and biotechnology works. However, there is much yet to be done about the behavior of the stomata and vascular tissues and the ecophysiological responses of arabica coffee populations to environmental stresses. Further investigations on the anatomy characterization of vascular tissues, particularly on xylem elements (trachied and vessels), contributions of phytohormones and biochemical constituents in relation to stomatal responses are required to understand the underlying mechanisms under different agro-ecologies and production systems.

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