Ex-situ Performance Evaluation of Coffee (*Coffea arabica)* Seedlings under Different Management Conditions: II. Root Growth Characteristics of Accessions

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Abstract

Root characteristics of coffee seedlings were studied with the main objective to compare the variations among twelve Coffea arabica germplasm accessions under contrasting nursery environments at Jimma Research Center, southwest Ethiopia. Coffee seedlings from four wild coffee populations, namely, Harenna, Bonga, Berhane-Kontir and Yayu were ex-situ established under common nursery settings. The treatments included coffee germplasm accessions, shadings (moderate shade and full sunlight) and irrigation levels (well-watered and water-stressed). One-year-old coffee seedlings were used to record root growth traits from five central seedlings per plot and the data were analyzed using SAS software. Coffee accessions significantly differed in most root characteristics. The longest and shortest lateral roots were obtained from Yayu and Harenna seedlings, respectively. Berhane-Kontir accessions had significantly the lowest root volume as opposed to the highest value for the Harenna seedlings. Significantly higher root dry biomass was obtained from unshaded than from shaded seedlings. The difference between watering regimes was also significant for root dry biomass and it was higher for water-stressed than for well-irrigated seedlings. Coffee accessions were significantly differed in root proliferation and dry biomass and consequently, the lowest and highest average values were obtained from Berhane-Kontir and Harenna seedlings, respectively. The Harenna seedlings had a higher root mass than the others, particularly the Berhane-Kontir accessions. The ratios of root to shoot dry biomass of the seedlings were significantly differed among coffee accessions, but not between shade and irrigation levels. The significantly lowest and highest root to shoot values was determined for the Berhane-Kontir and Harenna accessions, respectively. Hence, Harenna genotypes can be considered as parents in coffee improvement programs under limiting water conditions.

Key Words: Coffee forest ecology; coffee diversity; moisture stress; natural resource conservation; root growth characteristics

Introduction

The montane rainforests of Ethiopia are the only known primary center of origin and genetic diversity for the highland arabica coffee (*Coffea arabica* L.) species. Arabica coffee is thus a shade adapted plant in the natural multi-strata forest ecosystems with the occurrence of wild Arabica coffee populations (Wrigley 1988; Wintgens 2004). Ethiopia is endowed with wide ecological suitability and genetic potentials for sustainable production and export of the finest quality specialty coffee varieties and natural forest grown coffees, while conserving healthy forest environments and promoting the green economy development strategy. According to Gole (2003) and Bellachew and Sacko (2009), however, coffee genetic resources of Ethiopia are under continuous threat of genetic erosions due to deforestation and land degradation. This coupled with the increasing patterns of climate change are endangering coffee environments and diversity (Taye, 2006), requiring urgent collaborative actions before the situations reach an irreversible stage to the global coffee sector.

In Ethiopia, there is immense genetic diversity among and within wild Arabica coffee populations, farmers' domesticated coffee landraces and released coffee varieties for improvement of any desirable traits such as high yielding, desirable quality standard, disease resistance, drought tolerance, low caffeine content, etc. To date, a total of 6385 coffee germplasm collections are *ex-situ* maintained and conserved at the field genebanks of the Jimma Research Center and its sub-centres and sub-stations for research works. Besides, there are also substantial number of Arabica coffee collections being conserved at the Institute of Biodiversity field genebanks. This is a unique genetic opportunity for the future development of the coffee sector and calls for strong national and international cooperation to support the complementary use of both the *in-situ* and *ex-situ* conservation approaches. This would promote sustainable production and marketing of best coffees, while maintaining traceable quality profile. Bellachew and Sacko (2006) pointed out significant genotype by environment interactions and the need to adopt local landrace coffee variety development program in Ethiopia.

Root is the hidden plant growth part that is equally important to the above ground growth in the adaptation and distribution of plant species, though it is little studied and used in perennial crops like coffee. Hence, identificantion of ideal coffee genotypes with desirable root traits is important against the increasing multifaceted challenges on arabica coffee genetic resources in Ethiopia, its birth place. The typical root system of a mature Arabica coffee tree consists of a taproot, axial vertical roots; lateral roots, some of which are more or less parallel to the soil surface (surface plate roots) and other deeper roots that ramify evenly in the soil and sometimes become vertical, feeder bearers evenly distributed, and feeder-borne roots at all depths (Wintgens, 2004). The horizontal and vertical growth of coffee roots can be influenced by plant, environmental and soil factors. This characteristic enables it to exploit a considerable volume of land and to thus offset a relative lack of soil fertility condition. Coffee plant requires an effective depth of greater than 150 cm. Coffee trees can root deeply in a normal soil although about 90% of the roots develop in the upper 30 cm soil layer. These roots are sensitive to climatic variations (temperature, drought and moisture), but can be protected by shade and mulch conditions (Wrigley, 1988). Thus, root characteristics are considered to play an important role with regard to survival and better performance of perennial crops like coffee. Drought-adapted plants are often characterized by deep and vigorous root systems and according to Daniel et al. (2004) total transpiration was high in coffee genotypes with relatively high root biomass.

In Ethiopia, previous coffee research focused on *ex-situ* characterization and evalaution of the diverse coffee collections, by considering largely the shoot growth system. These include yield and yield components, resistance to diseases and insects, quality attributes and tolerance to drought environments (Girma et al., 2008). Therefore, identification of efficient accessions with respect to ideal root traits is essential. The existences of substantial inter- and intra-genetic molecular diversity (Kassahun, 2006) and morpho-physiological growth natures (Taye, 2006) of wild arabica coffee populations have been reported in the natural coffee forests of Ethiopia. The interplay between root and shoot system depends on environmental and plant factors, and the physiological root to shoot behaviors vis-à-vis plant age and environmental influences remains to be investigated. The impact of changing climatic pattern and declining soil fertility status can be evidenced from the poor performances of coffee trees, though research information is scanty especially on root growth natures of coffee genetic resources under different agro-ecologies and production systems in Ethiopia.

In view of the huge genetic and environmental opportunities and progressive climate changes, knowledge of below ground growth traits is important in characterization, evaluation and utilization of suitable coffee cultivars. To this end, investigations are necessary to assess the underlying mechanisms and identify drought-tolerant accessions along varying amplitudes of environmental stresses. This study would provide insights into understanding and identifying the best genotype-environment relationships and targeting management options that contibute to sustainable conservation and use of coffee genetic resources in the country. Thus, seedlings of arabica coffee accessions were assessed under varying sunlight and watering conditions with the aim of comparing the extent of variations in root growth characteristics so that future breeding program will be targeted to identify drought tolerant arabica coffee varieties for specific agro-ecologies.

Material and Methods

The study area

The *ex-situ* experiment was conducted in southwest Ethiopia, at Jimma Research Center (JRC) (7°46′N and 36° E). The center coordinates the coffee research in the country where national and international coffee collections of about 6385 are maintained with which various breeding, agronomy and-soil-related studies are undertaken. The center is situated within the temperate to cool humid highland agro-ecological zone at an altitude of 1753 m.a.s.l. The area receives a high amount of mean annual rainfall (1556.88 mm) with average maximum and minimum air temperatures of 26.7 and 12.8°C, respectively.

Experimental design and treatments

Fully ripe red cherries were selectively 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 coffee forests are located in the more humid southwest Ethiopia (Table 1). 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 common nursery settings at Jimma Research Centre in 2005. The potting medium was prepared from blends of topsoil and decomposed coffee husk compost at the respective ratio of 3:1 (v/v) as recommended by Taye et al. (2004). Black plastic pots (volume = 5.8 liter) were perforated at the bottom, firmly filled with the growing medium and arranged on seedbeds. Then, 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). The coffee seedlings were grown under optimal nursery environments for about one-year period until they attain desirable growth stage to commence the treatments.

Table 1. Characteristics of the four montane rainforests where the study arabica coffee accessions collected in Ethiopia

Variable	Harenna	Bonga	Berhane-Kontir	Yayu
Latitude (N)	6°23′-6°29′	7°17′-7°19′	7°04′-7°07′	8°23′
Longitude (E)	39°44′-39°45′	36°03′-36°13′	35°25′-35°26′	35°47′
Altitude (m a.s.l)	1420-1490	1520-1780	1040-1180	1400
Slope (%)	2-3	3-6	4-18	1-8
Rainfall (mm/year)	950	1700	2100	1900
Max temperature (°C)	34.4	29.9	31.4	34.7
Min temperature (°C)	10.4	8.7	13.8	7.6
Mean temperature (°C)	22.2	18.2	20.3	19.7
Minimum RH (%)	37.9	45.0	50.8	41.8
Maximum RH (%)	84.3	95.2	85.4	98.5
Mean RH (%)	63.2	80.4	68.9	80.9
Wind speed (m/ h)	0.93	0.64	0.43	0.35

Factorial combination of treatments in a randomized complete block design with three replications was used to arrange the three main treatments (shading, irrigation and accessions). The experimental plots were oriented in east to west direction. The treatments included shadings (moderate shade and full sunlight), irrigation (well-watered and water-stressed) and 12 coffee germplasm accessions of varying collection areas. The coffee seedlings were maintained under controlled contrasting shade environments for eight months. Each treatment consisted of 25 seedlings per plot. Moderate (50% light interception) overhead shade (2 m height) and side shades were constructed from uniform bamboo slants. Maximum care was taken to avoid side-shading effects between the treatments. For this, the shade plots were far apart from each other (12 m), while the spacing between irrigation and coffee accession plots was 2 m and 1 m, respectively. The spacing between coffee seedlings was increased with ageing of the seedlings. At the beginning, it was 10 cm x 10 cm and later arranged at 20 cm x 20 cm spacing. The water withheld plots were covered with white plastic sheet, whenever there is rain during day and night times throughout the study period. The diurnal microclimate variables were monitored during the study period and the results depicted significant differences between the shade regimes (Taye, 2006). This depicts that the treatment is enough to see the variability among coffee genetic accessions in root growth natures due to the induced sub-optimal water and sunlight stress environments.

Root measurements

One-year-old seedlings were used to record root growth traits of the different arabica coffee accessions under varying nursery management conditions. For this, five central seedlings per plot were used for root measurements and the roots were immersed and washed in water to remove adhering soil. The most important root growth parameters (lateral root number, lateral root length, taproot length, root volume) were recorded for each independent varaible. Subsequent to recording root fresh weigt, each root part was oven-dried at 105°C for 24 h and weighed using a high sensitive balance and other root growth derivatives were determined.

Data analysis

Two-way analysis of variance (ANOVA) was carried out using SAS system for Windows version 8.1 (SAS Institute Inc., Cary, NC) to see the variations in seedling root growth characters among arabica coffee accessions under contrasting shade and irrigation conditions. For any significant results, the treatemt means were compared according to the Tukey's test at p = 5%. Finally, the Pearson correlation matrix was run between the most relevant root triats.

Results

The number and volume of lateral roots was comparable between shade treatments, though the values were higher for unshaded than for shaded seedlings. Taproot and lateral roots were slightly longer in the shade, though not significantly different from those of unshaded seedlings. On the other hand, coffee accessions significantly differed in total root volume ($P<0.001$), taproot length ($P<0.05$) and length of lateral roots ($P<0.01$). The accessions, however, did not differ in the number of lateral roots, though the respective maximum and minimum counts were obtained from the Harenna and Berhane-Kontir accessions. The longest (19.19±0.45 cm) and shortest (17.03±0.99 cm) lateral roots were obtained from Yayu (IV-1) and Harenna (I-3) seedlings, respectively. Berhane-Kontir accessions had significantly lowest root volume (28.80±4.67 g cm^{-3}) as opposed to the highest value (48.50±2.78 g cm^{-3}) for the Harenna seedlings (Table 2).

A significantly ($P<0.01$) higher root dry biomass was obtained from unshaded than from partially shaded seedlings, where a reduction of about 12% was noted. Similarly, higher dry mass of leaf and total dry matter were recorded for seedlings exposed to direct sunlight than shaded plots (Figure 1). The difference between watering regimes was also significant ($P<0.01$) for root dry mass and was higher for water-stressed than for well-irrigated seedlings. In the same manner, coffee accessions significantly differed ($P<0.01$) in root dry mass and consequently, the lowest (III-1 = 6.48 g) and highest (I-2 = 10.43 g) average values were obtained from Berhane-Kontir and Harenna seedlings, respectively (Table 2).

Table 2. Root growth parameters (means ± SD) in Arabica coffee seedlings according to shade, irrigation and accession treatments

Treatment	RDW (g)	RV (g cm^{-3})	TRL (cm)	LRN	LRL (cm)	R:S	RP (%)
Shading	**	*	*	Ns	Ns	Ns	Ns
Full sunlight	8.93±1.64	38.52±7.32	29.57±5.91	38.73±4.17	17.97±1.05	0.29±0.03	22.22±1.89
Partial shade	7.89±1.52	36.03±7.09	32.03±4.20	37.84±4.64	18.42±1.46	0.28±0.04	21.45±2.81
Irrigation	**	Ns	***	*	Ns	Ns	Ns
Water stressed	8.90±1.52	36.36±6.03	33.68±4.90	39.52±4.00	18.42±1.19	0.29±0.03	22.35±2.02
Well-watered	7.92±1.66	38.18±8.31	27.91±3.80	37.05±4.48	17.98±1.35	0.27±0.04	21.33±2.68
Accession	**	***	*	Ns	*	**	**
I-1	10.02±1.11a	46.25±9.19ab	34.45±5.00	41.10±2.68	19.62±1.35	0.31±0.04ab	23.68±1.89ab
I-2	10.43±1.23a	48.50±2.78a	35.42±6.13	43.20±5.60	19.45±0.63	0.33±0.01a	24.82±0.87a
I-3	9.22±0.95ab	42.70±2.55abc	34.06±6.35	38.43±0.97	19.19±0.45	0.31±0.02ab	23.33±0.66ab
II-1	8.30±1.44abc	34.95±4.99cd	28.78±3.76	39.25±7.93	17.07±0.97	0.28±0.02abc	21.48±1.19abc
II-2	7.97±1.11abc	35.25±5.54bcd	31.74±5.06	39.70±3.50	17.77±0.77	0.28±0.03abc	22.16±1.45abc
II-3	8.15±1.34abc	37.55±4.35a-d	30.71±6.20	36.58±1.83	19.16±0.61	0.29±0.04abc	22.37±2.04abc
III-1	6.48±1.09c	32.70±5.13cd	27.38±4.15	33.08±3.74	17.99±1.18	0.25±0.04 bc	19.50±3.15bc
III-2	7.22±1.66bc	31.20±4.98d	28.73±4.53	36.50±4.89	18.11±1.41	0.27±0.04abc	20.75±2.89abc
III-3	6.86±1.20bc	28.80±4.67d	27.39±6.56	36.60±2.15	17.31±0.91	0.23±0.03c	18.34±1.86c
IV-1	8.67±0.30abc	37.25±4.33a-d	27.14±2.74	38.15±4.83	17.03±0.99	0.28±0.02abc	21.88±1.32abc
IV-2	9.39±1.00ab	38.65±3.22a-d	30.58±1.25	39.75±2.71	18.38±1.34	0.29±0.01abc	22.39±0.92abc
IV-3	8.23±2.43abc	33.45±4.89cd	33.21±5.18	37.10±3.19	17.32±1.03	0.27±0.05abc	21.38±2.81abc
Mean	**8.41**	**37.27**	**30.80**	**38.29**	**18.20**	**0.28**	**21.84**
CV (%)	**10.72**	**10.61**	**10.38**	**9.77**	**5.05**	**9.31**	**7.67**

Ns = Not significant; *, ** and *** = significant at P<0.05, P<0.01 and P<0.001, respectively. Means followed by same letter with in a column are not different from each other (Tukey‘s test at P = 0.05). Abbreviations: RDW= root dry weight, RV = root volume, TRL = taproot length, LRL = lateral root length, R:S = root to shoot ratio, RP = root partitioning.

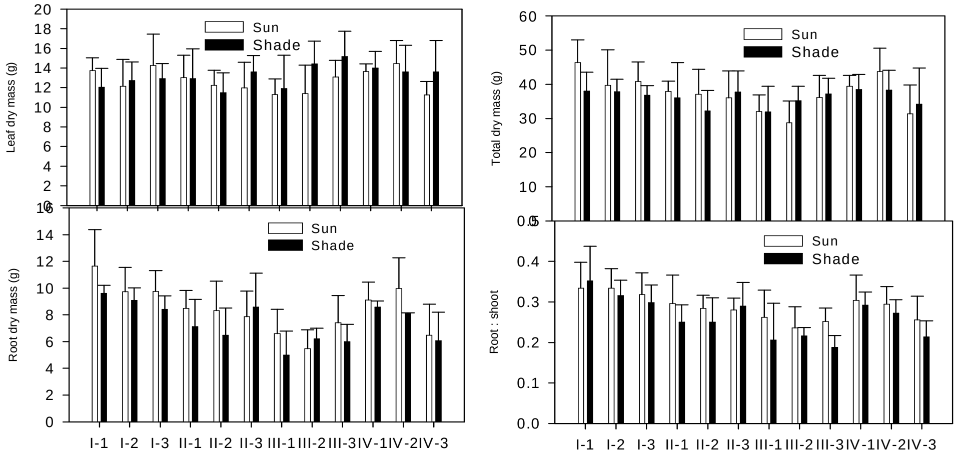

Figure 1.Biomass yield and root to shoot ratio of Arabica coffee accessions under full sun and shaded conditions

In general, the Harenna seedlings had a higher root mass than the other coffee genotypes, particularly the Berhane-Kontir accessions, which had a low root biomass ranging between 6.48 and 7.22 g. This was less than the overall average root dry biomass of 8.41 g. The ratios of root to shoot dry mass of the seedlings were also significantly differed among the accessions, but not between shade and irrigation levels. Though insignificant, the amount of total dry mass partitioned to the root part was higher in full sunlight and water-stressed seedlings as compared to the lower shares in partial shading and well-watered seedlings. The coffee accessions from Berhane-Kontir had significantly (P<0.01) the lowest (18.3%) root partitioning as compared to the Harenna seedlings, which had the highest (38.6%) root share. However, root to shoot ratio of some seedlings surpassed those in shadow conditions. The significantly lowest (III-3 = 0.23) and highest (I-2 = 0.33) root to shoot values were determined for the Berhane-Kontir and Harenna accessions, respectively (Figure 2). As a whole, root to shoot ratio was higher for the other accessions from Harenna than those from Berhane-Kontir. In addition, there were positive and significant associations between most root and shoot growth characteristics of coffee seedlings (Table 3), and stem and root dry weights were equally and significantly (r = 0.96**) correlated to total biomass yield. Moreover, leaf dry matter was significantly associated with root biomass (r = 0.71**) and total dry matter (r = 0.85**), indicating the role of leaves in carbon assimilation and distribution to different plant parts in coffee seedlings.

Table 3. Pearson correlation between root parameters in seedlings of arabica coffee genotypes

Parameter	RFW	RDW	RV	TRL	LRN	LRL	RS
RDW	0.94**						
RV	0.99**	0.92*					
TRL	0.51	0.53	0.51				
LRN	0.74**	0.79**	0.73**	0.73**			
LRL	0.81**	0.73**	0.81**	0.32	0.51		
RS	0.82**	0.73**	0.84**	0.46	0.75**	0.70*	

*, ** = Correlations are significant at 0.05 and 0.01 levels, respectively (2-tailed). For abbreviation see Table 1 above.

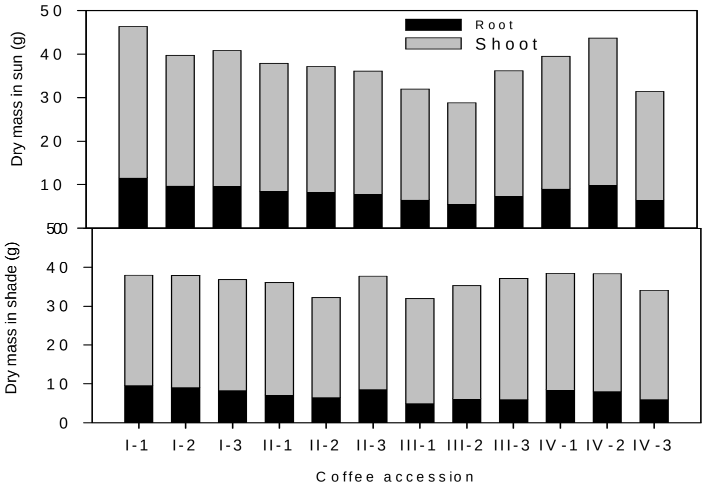

Figure 2. Root and shoot dry mass for seedlings of arabica coffee accessions under (a) full sunlight and (b) shaded nursery conditions

Discussion

The longest and shortest lateral roots were obtained from Yayu and Harenna seedlings, respectively. Berhane-Kontir accessions had significantly lowest root volume as opposed to the highest value for the Harenna seedlings. This reflects that coffee saplings can adapt to drought situations by extending the root system into deeper soil layers. The correlations between root growth parameters and root hydraulic resistance were strong and indirect in well-irrigated coffee seedlings, i.e., seedlings with better root systems showed significantly higher root hydraulic conductance and were more productive in terms of biomass production (Burkhardt et al., 2006) and hydraulic conductance patterns under field conditions (Taye and Burkhardt, 2010). This was observed in the direct sunlight exposed seedlings and Harenna accessions. Thus, the results obtained provide information on the closeness of shoot and root physiological events under drought-stress environments, which ultimately lead to the control of transpiration water loss and total dry matter distribution patterns. To this end, several studies (Yacob et al., 1996; Pinheiro et al., 2005) showed correlations between morphological and yield components. The knowledge of the correlation is invaluable to the breeder in selecting desirable morphological traits. In this study, simple correlation was computed between morphological parameters, which showed a different magnitude of relationships.

The influence of irradiance was not significant on root growth response, perhaps due to the limited time for the shade treatment to bring about differences. Nevertheless, full sunlight exposed seedlings had a slightly higher number and volume of lateral roots. In contrast, a longer taproot and lateral roots were observed for shaded seedlings than unshaded ones. This indicates the influence of short-term drought stress in affecting root growth. On the other hand, there were significant differences among the coffee accessions in total root volume, taproot length and length of lateral roots. Root morphological parameters were significantly highest for Harenna as opposed to the lowest values obtained from the Berhane-Kontir accessions. Similar to shoot, the Yayu and Bonga accessions were intermediate in root growth. The higher lateral root count and volume in full sun seedlings were in line with the root dry weight and root to shoot ratio recorded on the same coffee seedlings. This could be an indicative of high root access to maximize soil water uptake in limited soil-water conditions. Hence, the significantly better root growth in the Harenna accessions could be related to their soil moisture-stress avoidance mechanism as compared to the southwest origin coffee accessions. The amount of total dry mass partitioned to the root part was higher in full sunlight and water stressed seedlings as compared to those under shading and well-watered seedlings. This corresponds with the more luxurious shoot growth of coffee seedlings at resource rich environments as opposed to deep root systems in drought situations, indicating the influence of genetic, climate and soil conditions.

The coffee accessions from Berhane-Kontir had highly significantly lowest root partitioning as compared to the Harenna seedlings, which had the highest root share (Taye, 2006). The present root growth performance is in consistent with the variations in the shoot growth response of the same coffee accessions (Taye et al., 2004), indicating the interplay between root and shoot growth system. According to Taye (2006), the coffee accessions were grouped into three broad dissimilarity classes. The first cluster consisted of a mixture of accessions from Yayu, Bonga, Berhane-Kontir and Harenna, whereas the Bonga and Harenna accessions were classified into the second and the third cluster, reflecting the similarity within the two wild coffee forest units. Kassahun (2006) also found inter- and intra-genetic molecular diversity in the same wild coffee populations. Coffee accessions from the driest site, Harenna, showed highest conductance, largely due to their extensive root system, high transpiration and biomass production (Burkhardt et al., 2006). However, these were most vulnerable and they failed to withstand under persisting moisture deficits as opposed to others, especially the Bonga and Berhane-Kontir accessions. The same findings demonstrated that the southeastern and southwestern coffee germplasm accessions were found to follow opportunistic and conservative ways of water use strategies, respectively. The substantial variations in root growth characteristics are in agreement with the findings under field conditions (Kufa and Burkhardt, 2011), indicating the consistency of root traits and in part underlined the diversity in adaptation mechanisms as pointed out by Sobrado (2003).

Conclusion

The present study assessed the influence of varying nursery management practices on seedling root growth response of wild coffee genotypes. Early stage root traits were significantly different due to coffee accessions. This may demonstrate the more influence of genetic as compared with environmental factors. Harenna accessions had ideal root characteristics and performed better under limited soil moisture conditions and these genotypes could be used as desirable parents in coffee improvement programs. Root characteristics can be used to identify suitable coffee genotypes for specific climate and soil conditions. In view of the current climate variability and change, it is also essential to understand drought tolerant coffee cultivars with efficient water use efficiency and to target effective management options under varying agro-ecologies and production systems. The variation in root growth response was substantial among accessions of distant geographical areas, possibly due to coffee genetic diversity and water use efficiency in arabica coffee genotypes. The findings also contribute to promote sustainable conservation, management and use of arabica coffee genetic resources and its shade environments in Ethiopia.

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