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In vitro Screening of Drought Tolerant Hararghie Coffee Genotypes *(Coffea arabica* L.) using Polyethylene Glycol in Ethiopia

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College Of Natural Sciences

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DECLARATION

I, the undersigned, declare that this thesis entitled **"***In vitro* screening of drought tolerant Harar Coffee *(Coffea arabica* L.) Using Polyethylene glycol"is my original work and has not been presented for Degree in any other University, and that all sources of materials used for the thesis have been fully acknowledged.

…….... **……**

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BIOGRAPHICAL SKETCH

The author was born at Bale Robe (Bale Zone), Oromia regional state on 5th June, 1989 G.C. She attended her primary education at Bale Robe primary school from 1996-2003 G.C and secondary and preparatory school from 2004-2007 G.C at Bale Robe Secondary and preparatory School. Then she joined Mada Walabu University in 2008 G.C. and was awarded B.Sc degree in Applied Biology in 2010 G.C. Then, in 2011, she has got her MSc chance and joined Jimma University, Department of Biology to study Botanical science sponsored by Ethiopian ministry of Education.

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LIST OF ABBREVIATIONS

CRD: Completely Randomized Design

DW: Dry weight

EIAR: Ethiopian Institute of Agricultural Research

FAO: Food and Agricultural Organization

FW: Fresh Weight

GNP: Gross national product

gs: Stomatal conductance

JARC: Jimma Agricultural Research Center

mm: Millimeter

MS: Murashige and Skoog medium

MoA: Ministry of Agriculture

OA: Osmotic Adjustment

RWC: Relative Water Content

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In vitro Screening of Drought Tolerant Hararghie Coffee Genotypes *(Coffea arabica* L.) using Polyethylene Glycol in Ethiopia

Eyerus Dadi

Abstract

Drought stress is a major abiotic factor affecting crop production worldwide. Coffee cultivation in Ethiopia has been highly affected by drought stress. However, very limited research has been conducted on Screening Arabica Coffee genotypes in the country in general, and in vitro screening of Hararghie Coffee (Coffea arabica L.) accessions for drought stress tolerance using Polyethylene Glycol (PEG) solution in particular. Hence, this study was conducted in Agricultural Biotechnology Research Laboratory at Jimma Agricultural Research Center (JARC), Southwest Ethiopia, to see the response of 13 Hararghie Coffee genotypes (Coffea arabica L.) to various levels of PEG concentrations (0, 2, 4, 6, 8 and 10%) under in vitro condition at an interval of 15 days and, thus, to understand drought stress tolerance capacity of the accessions in MS medium. Seeds of the accessionswere obtained from JARC and growth parameters like number of leaves(NL), number of roots(NR), root length(RL), shoot length(SL) and leaf area(LA) of in vitro grown plantlets were measured. Completely Randomized Design (CRD) with two replications was used to run the experiment. It was observed that differences among accessions were significant for NL, NR, RL, SL and LA. Significant differences between 15 and 45 days of stress were observed for majority of the measurements in 6, 8, and 10% PEG concentrations. Particularly, LA, SL, NL and NR of all accessions significantly decreased with increasing concentrations of PEG. The highest values for NL, NR, RL and SL were recorded during the stress time for accession H-674/98, followed by accession H-618/98, H-822/98 and H-739/98, whereas the lowest values were for accession H-980/98. The reduction was mainly caused at PEG concentration of 8 and 10%. On the basis of the present study, it was concluded that drought stress at earlier stages severely reduced growth attributes of Hararghie coffee genotypes and some accessions (H-674/98, H-618/98, H-822/98 and H-739/98) have shown the capacity of tolerance to drought stress.

Key words: Harar coffee, Stress, PEG concentration

1. INTRODUCTION

Coffee is a perennial dicot in the Rubiaceae family and genus *Coffea*. Among some 103 species of the genus *Coffea*; Arabica coffee (*Coffea arabica L.)* and Robusta coffee (*Coffea canephoraPierre ex Froehner*) economically dominate the world coffee trade, contributing to about 99% of the world bean production (Davies *et al*., 2006). However, out of the overall world coffee production, 70% of the consumption is from Arabica coffee, while the rest comes from Robusta coffee (Vieira *et al*., 2006). Arabica coffee also plays a significant role in Ethiopia's economy, accounting for 5% of GNP, more than 60% of foreign exchange earnings and 25% of the population's employment opportunity (Taye, 2006).However, the national average yield is very low (250 to 475 kg ha-^{1}annum-^{1}), which is attributed to several production constraints including adverse climatic factors (MoA, 2003).

In its center of origin and genetic diversity in Ethiopia, *Coffea arabica L.*evolved as an understory shrub under the deeply shaded forest canopy at attitudes of 1400 to 1800 meters a.s.l and with annual precipitation ranges from 1,200 to 2,000 mm and mean temperatures varying from 18 to 22^{0}C (Righi *et al*., 2007). Ethiopia is currently the third largest*Coffea arabica L*. producer after Brazil and Colombia (DaMatta, 2004). In Brazil, total coffee yield was about 48,500 million bags of green beans in 2002/2003 (41% of the world production). It is predicted to decrease to around 28,000 million bags in 2003/2004 due to largely unfavorable climatic conditions, particularly limited water supply. Not only in Brazil, but also in several coffee growing countries, drought is considered to be the major environmental stress affecting coffee production (DaMatta *et al*., 2002). In Ethiopia 321,000 tons of *Coffea arabica* was produced during the 2006 crop year. During the same year, coffee accounted for about 35% of total exports of the country, which has in previous decades been as high as 65% (Belachew *et al*., 2008).

Plant cell and tissue culture has been useful tool to study stress tolerance mechanism under *in vitro* conditions. *In vitro* techniques can make it possible to screen large number of genotypes rapidly for stress tolerance since *in vitro* plant cultures even at different stage of development may exhibit the capacity of plants to withstand stress (Tewary *et al*., 2000). Drought is one of the physical factors that have profound impact to limit coffee production by inhibiting growth, yield

and quality. Drought impairs mitosis, cell elongation and expansion, resulting in a reduction in plant length, leaf area and crop growth (Kaya *et al*., 2006; Hussain *et al*., 2008). Reduction of leaf size is the first morphological manifestation of drought and associated with reduced light interception and leads to a reduction in dry matter accumulation (Deblonde *et al.*, 1999). Drought reduces number of leaves, leaf area, stem length, number of tubers, tuber growth and yield (Schittenhelma *et al*., 2006). This problem is expected to become more challenging due to the changes in global climate (DaMatta and Ramalho, 2006).

Studies on tolerance to drought stresses have been done on various crops, such as rice, maize, potato, cassava and bean (Bartels and Sunkar, 2005; Hirasawa *et al*., 2006). The existence of high genetic diversity among Arabica coffee for disease resistance and other traits related to drought tolerance has also been reported in Ethiopia. Varietal differences in biomass allocation to the stems and leaves, and leaf area were reported for coffee (Tesfaye, 2005), but studies on Hararghie coffee types for drought tolerance have not been reported. For drought stress induction, one of the most popular approaches is to use high molecular weight osmotic substances, like polyethylene glycol (PEG) (Turkan *et al*., 2005; Landjeva *et al*., 2008). Polyethylene glycol molecules are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain uniform water potential throughout the experimental period (Muhammad *et al*., 2010). They are also used for rapid screening and give more consistent result than other osmoticum and easily absorbed and disintegrated by plants (Nepomuceno *et al*., 1998). In the current study, PEG was used for drought stress induction in coffee. The limitation of this study could be the fact that coffee is perennial crop which has a long generation time. Moreover, screening for tolerance to drought stresses is complicated due to differences in field management practice, phenotypic variations and unexpected events (Lafitte *et al*., 2004). Indeed, new techniques to identify coffee accessions that are tolerant to drought stresses are needed. Therefore, this study was designed to develop an *in vitro*technique to screen Hararghie coffee accessions for drought tolerance by applying Polyethylene glycol at different levels of concentration.

1.1. OBJECTIVES

1.1.1. General Objective*:*

- To develop an *in vitro* technique to screen Hararghie coffee accessions (*Coffea arabica L.*) for drought toleranceby applying Polyethylene glycol (PEG)

1.1.2. Specific Objectives:

- To determine suitable concentration of PEG to induce osmotic stress for *in vitro* plantlets of Hararghie coffee genotypes
- To determine the interaction effect of accessions, time of exposure and PEG concentration on *invitro* grown plantlets
- To determine the fresh weight and dry weight of the plantlets under different PEG concentrations for various periods

2. LITERATURE REVIEW

2.1. Botanical Description

Arabica coffee is an evergreen shrub of variable size. It grows up to 8-10 m in height and 7cm in diameter at breast height (Dias *et al*., 2007). The species is well known by its two types of branches; orthogeotropic which grow vertically and is commonly called suckers, and plagiogeotropic branches which have different orientation angles in relation to the main stem and is commonly called primaries (Taye, 2006). Leaves are petiolate, sometimes bearing interpetiolar stipules; and arranged oppositely on stems and decussate, with successive pairs of leaves arising at 90^{o} angles from each other around the stem. Flowers produced in dense clusters along reproductive branches in the axils of the leaves. Branches are semi erect when young and spreading or pendulous when old (Coste, 1992).

Fruit is a drupe; pericarp composed of shiny exocarp, fleshy mesocarp and relatively thin but tough endocarp, in which the seeds are enclosed. Immature berries are dull green; on ripening the skin colour changes through yellow to bright red. The coffee plant takes approximately three years to bear fruit. A well-managed coffee tree can be productive for up to 80 years or more, but the economic life span of coffee plantation is rarely more than 30 years (Wintgens, 2004).

Seeds consist mainly of green corneous endosperm, folded in a peculiar manner, and a small embryo near the base. The size and shape of beans differ depending upon the variety, environmental conditions and management practices. Bean shape can be sub- globular, ovoid, linear, oblong, either rounded at both ends or pointed at one end and rounded at the other (FAO, 2008). On average, beans are 10 mm long, 6-7 mm wide, 3-4 mm thick and weigh between 0.15-0.20 g. Bean color can be yellowish- grey to slate- grey, bluish or grey green, depending upon the variety, method of preparation and storage condition (Dias *et al*., 2007).

2.2. Importance of *Coffea arabica L.*

2.2.1. Medicinal Value

Coffee is a potent source of healthful antioxidants: Coffee shows more antioxidant activity than green tea and cocoa. Antioxidants fight inflammation, an underlying cause of many chronic conditions, including arthritis, atherosclerosis and many types of cancer.

Coffee may help protect against cognitive decline: Regular coffee consumption may help prevent cognitive decline associated with Alzheimer's disease and other types of dementia. Drinking three to five cups of coffee daily at midlife was associated with a 65 percent decreased risk of Alzheimer's and dementia in later life**.**

Coffee is healthy for your heart: Moderate coffee drinkers had a 20 percent lower risk of heart disease as compared to heavy or light coffee drinkers, and nondrinkers.

Lowered risk of Type 2 Diabetes: Those who consumed 6 or more cups per day had a 22% lower risk of diabetes.

Reduces suicide risk and Depression: women who drink 4 or more cups of coffee were 20% less likely to suffer from depression.

Coffee may help curb certain cancers: Men who drink coffee may be at a lower risk of developing aggressive prostate cancer. Drinking four or more cups of coffee daily decreased the risk of endometrial cancer in women by 25 percent as compared to women who drank less than one cup a day.

2.2.2. Food Value

Dried seeds (beans) are roasted, ground and brewed to make most popular beverages in the world. Cooked in butter, it can be used to make rich flat cakes. Coffee is widely used as flavorant in ice cream, candies and liqueurs. In Arabia, a fermented drink from the pulp is consumed; pulp and parchment are occasionally fed to cattle in India (Mekuria *et al*., 2004).

2.2.3. Economic Value in Ethiopia

The estimated coffee production area (2% of total cultivated land) in Ethiopia is in the range 320,000-700,000 ha (FAO, 2008), althoughthere are a potential of 6 million ha of cultivable land suitable for coffee production (Mekuria *et al*., 2004). The economy of Ethiopia is based on agriculture, and coffee is the central agricultural export product (Mohammed and Astatkie, 2010). Historically, Ethiopia is the oldest exporter of coffee in the world and it is the largest coffee producer and exporter in Africa. Coffee is a means of subsistence for the rapidly growing population of the country as a complement or even sole source of income, and it plays a fundamental role in both the cultural and socio-economic life of the nation (Taye and Burkhardt, 2011). LMC(2003) estimated that 15 million peoples are dependent on coffee for at least a significant part of their livelihood. Ethiopian coffee (Arabica coffee) ranks highly in intrinsic quality of the bean and it is the principal economic species, contributing over 70% of the world's commercial coffee (Gole *et al*., 2002). Ethiopian farmers normally produce nine spectra of the finest single-origin or specialty coffees (Harar, Nekemte, Illubabor, Limu, Tepi, Bebeka, Yirga Chefe, Sidamo and Jimma), which are now well diffused into the trade circuits of the coffee industry (Mekuria *et al*., 2004).

2.3. Coffee Production in Ethiopia

The production of coffee is an enormous relevance for Ethiopia, playing a dominant role in economic, social, cultural and environmental terms (Grades, 2006). In contrast to other coffee producing countries, the Ethiopian coffee production is dominated by smallholder agriculture, contributing with more than 90-95 percent to the total harvest with an average farm size of 0.5 ha (Woldemariam and Senbeta, 2008). The production system of coffee in Ethiopia is generally grouped into four (Table 1).There are variations in genotypes, eco-physiology and the biosphere of coffee under different production systems. Plantation coffee can be regarded as an intensively technified system.

Table 1. Coffee production systems in Ethiopia

Coffee production system	Area cover (%)
Forest coffee	10
Semi- forest coffee	35
Cottage (garden coffee)	50
Plantation coffee	5

source: (Aga *et al*., 2003).

2.4. Drought and Coffee

Moisture deficit is one of the main problems affecting the expansion of coffee in drought prone coffee growing areas of Ethiopia. This problem is expected to become more challenging due to the changes in global climate and also because coffee cultivation has spread towards marginal lands, where water shortage and unfavorable temperatures constitute major constraints to coffee yield (DaMatta, 2004).Drought is considered as the main environmental stress greatly affecting coffee populations in different coffee growing countries (Miller and Conko, 2004). In Ethiopia, the recurrent drought becomes the main threat to the coffee gene pool (together with other human impacts).During water stress the water content of the plant decreases, which causes the cells to lose turgor pressure and shrink. The loss of turgor pressure in the cells inhibits turgor dependent activities such as cell expansion, which affects the growth of the whole plant. Reduced cell growth during water stress has e.g. been found to decrease the stem length in *Arabidopsis thaliana,* soybean (*Glycine max*), potato (*Solanum tuberosum*), ocra (*Abelmoschus esculentus)* and parsley (*Petroselinum crispum*) (Park *et al*., 2007; Sankar *et al*., 2008). Similarly reduced cell enlargement reduces the leaf expansion in *Populus* (Ren *et al*., 2007). By reducing the leaf expansion the leaves become smaller and therefore transpire less. In some cases water stress can even lead to leaf abscission. This has e.g. been seen in *Populus* and paper birch (*Betulapapyrifera)* (Gu *et al*., 2007). To increase water uptake and maintain a minimum osmotic pressure during drought many plants increase their root growth, either deeper or laterally. By increasing the root growth the area for water uptake becomes larger and water further away and deeper in the soil may be reached. This growth response has been found in Maize (Z*ea mays*),

Madagascar periwinkle (*Catharanthus roseus)* and Date palm (*Phoenix dactylifera)* (Trachsel *et al*., 2010).

2.4.1. Impacts of Drought and High Temperature on Coffee Production

Coffee is highly environmentally dependent crop and an increase of a few degrees of average temperature and/or short periods of drought in coffee growing regions can substantially decrease yields and quality of coffee (DaMatta, 2004).Taking into account the global warming phenomena, severe reduction of adequate coffee growing areas are to be expected DaMatta and Ramalho (2006) thus, sustainability of coffee productivity and quality may become more difficult to maintain.

Extreme temperatures, depending on their intensity and duration, impair cell metabolic processes (e.g. photosynthesis), growth and survival of plants, as well as their economic exploitation (DaMatta and Ramalho, 2006). In fact, temperature may limit the successful economic exploitation of the coffee crop, because coffee growth is particularly affected by both high and low temperatures (Barros *et al*., 1997; Silva *et al*., 2004). The optimum mean annual temperature range for *Coffea arabica* is 15-24ºC. Above 24ºC, development and ripening of fruits are accelerated, often leading to loss of quality (Camargo, 2009). Continuous exposure to temperatures as high as 30ºC could result in not only depressed growth but also in abnormalities such as yellowing of leaves and growth of tumors at the base of the stem (DaMatta and Ramalho, 2006). A relatively high temperature during blossoming, especially if associated with a prolonged dry season, may cause abortion of flowers (Camargo, 2009). In addition, large variations in temperature also increase bean defects, modify bean biochemical composition and the final quality of the beverage (Carr, 2001; Silva *et al*., 2005).

2.4.2. Drought and Heat Tolerance Mechanisms in Crop Plants

Drought is a multidimensional stress affecting plant at various levels of their organization. Jones (1993) has pointed out drought-tolerant genotypes of most crop plants are those giving some yield in a particular water-limited environment.Like many plant species, coffee have mechanisms to avoid and endure drought and heat stresses developed within the genetic bounds of the plant/species (Mohammed and Astatkie, 2010). Root characteristics and growth play a crucial role in maintaining the water supply to the plant, and drought adapted plants are often characterized by deep and vigorous root systems (Taye and Burkhardt, 2011). However, Burkhardt *et al*., (2006) observed coffee plants with fibrous root system but vulnerable to drought due to their hydraulic system and Stomatal behavior.

Drought tolerant coffee genotypes are able to maintain higher tissue water potential and water use efficiency than drought sensitive ones under water deficit conditions (DaMatta, 2004). Such differences are even more evident in the field, where the development of the root system is much less restricted (DaMatta *et al*., 2003). When comparing the yields of drought tolerant and drought sensitive clones, the better crop yield of the drought tolerant clone was associated with maintenance of leaf area and higher tissue water potentials, as a consequence of smaller Stomatal conductance, which would result in less carbon isotope discrimination. Combining traits associated with a favorable water status and suitable biochemical characteristics, which enable some degree of tissue tolerance to desiccation, should improve coffee yields over a range of drought conditions (Dias *et al.,* 2007).

Different species of coffee (e.g. Arabica and Robusta) may also differ in morphological and/or physiological mechanisms that allow them to produce considerably well under limited water supply (DaMatta, 2004). *Coffea arabica* genotypes have been found to differ in drought adaptation mechanisms such as stomata control and soil water extraction efficiency, plant water use and biomass allocation to the stems and leaves (Dias *et al*., 2007) and tissue water potential (DaMatta, 2004). On the other hand, studies on Robusta coffee showed deeper root system and larger root dry mass in drought tolerant clones than in drought sensitive ones (Pinheiro *et al*., 2005).

In general, plants adapt one of the following three strategies to endure drought. These are (i)Drought Escape: mechanisms which enable plants to complete vegetative and reproductive phases of their life cycle during favorable moisture conditions before the occurrence of meteorological drought (e.g. desert ephemerals) ((Dias *et al*., 2007). (ii)Dehydration Avoidance: when the rate of water loss by transpiration exceeds that of absorption, a state of dehydration is caused to the conducting tissues. Plants postpone this injurious dehydration by physiological mechanisms to sustain balance between loss and uptake of water. Such a balance is achieved in the short term period mainly by controlling water loss through Stomatal closure. In long term, cuticle thickness, changes in leaf angle and small canopy leaf area through reduced growth and shedding of older leaves are potential features in minimizing water loss (Kramer and Boyer, 1995).(iii) Dehydration Tolerance: the 'desiccation-tolerant' plants are the most dramatic examples of dehydration tolerance that can recover even from below 10% relative water contents. When fully dehydrated, these plants are in a metabolically dormant state (Vicre *et al*., 2004).

2.5. Crop Responses to Water Deficit Stress

2.5.1. Physiological Responses (Photosynthesis)

Water stress reduces photosynthesis by decreasing both leaf area and photosynthetic rate per unit leaf area**(**Yordanov *et al.,* 2003*).* Studies have shown that the first morphological effect of drought is leaf size reduction which results in lessened photosynthesis and reduction in dry matter accumulation in tubers (Taiz and Zeiger, 2006). Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth, or increasing senescence of leaves under drought conditions, may also inhibit photosynthesis in existing leaves (DaMatta, 2003). Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis, and interference with many other basic metabolic processes (Kramer and Boyer, 1995).

In the field, plants are normally not deprived of water rapidly. During slowly increasing water stress, photosynthesis and transpiration usually decrease at similar rates(Yordanov *et al*.,

2003).The two main factors causing Stomatal closure are usually an increase in the concentration of gaseous carbon within leaves and a decrease in water potential of leaf cells (DaMatta, 2003).

The simplest explanation for the inhibition of photosynthesis during water stress would be that the stomata close and the internal CO_2 concentration decreases, since Stomatal limitation is more severe when a plant is stressed than when it is not. Therefore, it is rather surprising that photosynthesis often decreases in parallel with, or more than, Stomatal conductance (Cornic *et al.,* 1989).

2.5.2. Biochemical Responses to Water Deficit Stress

Water is essential in the maintenance of the turgor which is essential for cell enlargement, growth and for maintaining the form of herbaceous plants. Turgor is also important in the opening of stomata and the movements of leaves, flower petals, and various specialized plant structures (Kramer and Boyer, 1995).Although turgor measurements on segments the non-growing lamina have often appeared to show declining rates of leaf growth with decreasing turgor, turgor measurement in regions of leaves and stems, where cell enlargement usually occurs, often show little or no decrease, even when cell enlargement is largely inhibited due to soil drying. This is believed to be due to osmotic adjustment, the process in which solutes accumulate in growing cells as their water potential falls of osmotic potential arising from the net accumulation of solutes in response to by maintaining turgor in tissues, osmotic adjustment may allow growth to continue at low water potential (Fan *et al*.,1994).

Osmotic adjustment (OA), defined as a net increased solute concentration, may be perceived as an important survival mechanism to drought stress in addition to being frequently correlated with yield stability in dry environments. According to Meinzer *et al*., (1990a) in *Coffee arabica* grown in the field, OA is not a general trait observed in genotypes grown under rainfed conditions. Furthermore, development of increased leaf water deficits upon discontinuing irrigation may circumstantially be more rapid (or severe) in genotypes having greater amplitude of OA (Meinzer *et al*., 1990b). These observations imply that OA may not be an effective mechanism of drought tolerance in coffee, as has also been reported for several other woody species (Fan *et al*., 1994).

Turner and Jones (1980) have defined osmotic adjustment as "the lowering water deficits or salinity". Osmotic adjustment usually depends mainly on photosynthesis to supply compatible solute. As dehydration becomes more severe, photosynthesis is inhibited, resulting in a smaller solute supply for osmotic adjustment. With continued water limitation, osmotic adjustment delays, but cannot completely prevent dehydration (Kramer and Boyer, 1995). In leaves and stems at least, solute accumulation does not fully compensate for the effects of limited water supply on cell enlargement. Osmotic adjustment has been found in many species and has been implicated in the maintenance of Stomatal conductance, photosynthesis, leaf water volume and growth (Morgan, 1984).

Osmotic adjustment is usually not permanent and plants often respond rapidly to increased availability of water. Munns *et al*., (1979) found that the change in osmotic potential in the apex and enclosed developing leaves of wheat seedlings under rapidly developing water stress was mainly due to the accumulation of soluble sugars, amino acids (particularly asparagine and proline) and K^+ ions. Morgan and Condon (1986) showed that such increase in solute concentration gives tissues a temporary advantage, enabling turgor to be maintained at low water potentials by decreasing their osmotic potentials. According to Mazzafera and Teixeira (1989), proline accumulation in drought stressed plants of arabica coffee might be more directly related to injury imposed by water limitation rather than being a defense mechanism against drought stress. When dehydration occurs in the absence of high external salinities, there can also be rapid increases in solute content of cells. The growing tissues throughout the plant may show osmotic adjustment when the soil loses water (Fan *et al*., 1994).

2.5.3. Growth and Development in Response to Drought

From a meteorological point of view, drought refers only to a period in which rainfall fails to keep up with potential evapotranspiration. However, particularly in the tropics, drought episodes are remarkably aggravated by both high solar radiation and high temperature, so drought should be considered as a multidimensional stress (DaMatta 2003).As for other stresses, the slow imposition of drought allows the development of a range of time-dependent morphological and physiological acclimation responses. Despite this, many studies concerning drought effects on

coffee physiology have been carried out on plants grown in small containers under greenhouse conditions. This has been done in order to rapidly understand the plant's behavior without the complicating effects of environmental variability. However, such an approach provides only a limited model of natural conditions since (i) growth, particularly of roots, is restricted in potted trees, with observations largely dependent on the rate of stress progression; (ii) soil substrates in pot experiments usually have lower unsaturated hydraulic conductivity than natural soils, thus enhancing the probability of short and local water deficit i n the roots; (iii) the air surrounding the plant within a greenhouse is usually isolated from the external atmosphere, thus fully decoupling transpiration; and (iv) if humidity and temperature control within a greenhouse is lacking, evaporative demand may strongly rise due mainly to increased temperature which can reach values as high as 45 °C or more in the afternoon on sunny summer days (DaMatta, 2003).

2.6. Plant Water Relations

Water relations of coffee have been studied by several investigators over the last decades, and some reviews have been published (Carr, 2001; DaMatta and Rena, 2002). In the short term soil water deficit, yield reduction may be related to reduce Stomatal conductance (gs) and concomitantly lower rate of net carbon assimilation (*A*) (DaMatta, 2003).

Coffee retains high leaf relative water content (RWC) under dehydrating conditions, being considered water saving rather than dehydration tolerant species (DaMatta *et al*., 1993). This may be attributed to (i) an efficient Stomatal control on transpiration, and/or (ii) low cell-wall elasticity (Pinheiro, 2004). A small water loss should therefore cause a shift in turgor so that leaves tend to maintain high leaf relative water content. Thus, it appears that under water deficit the maintenance of a high RWC is more important than OA in conferring drought tolerance to the coffee plant (DaMatta *et al*., 1993). Conservation of a high symplast volume may be crucial for maintaining gas exchange, as shown by Meinzer *et al*. (1990b) working with five arabica coffee cultivars subjected to drought. They showed that *g*s were associated with changes in relative symplast volume rather than with changes in leaf turgor. In arabica coffee, *g*s have been reported to be an early indicator of water shortage, showing decreases as soon as one-third of the available soil water has been depleted (DaMatta, 2004). Also, *g*s declines sharply with increasing evaporative demand irrespective of the leaf water status (Tausend *et al*., 2000a).

In *Coffea arabica*, hydraulic conductance was found to be positively correlated with total daily transpiration (Tausend *et al*., 2000b). Thus, genotypes with higher hydraulic conductance should have higher rates of water use. This means that they may deplete accessible soil water more rapidly and have a deeper root system than genotypes with lower hydraulic conductance. The strong association of total transpiration with hydraulic conductance might reduce variation in water potential with variation in water availability, which may help to avoid non-stomatal limitation to photosynthesis and xylem cavitations (Tausend *et al.*, 2000a). This would be advantageous with non-limiting soil water or with brief periods of water deficit, but disadvantageous with longer-term drought since a high hydraulic conductivity would accelerate the development of severe plant water deficit. Hence, knowledge of the nature of the target environment to which cultivars are bred is crucial for the success of coffee breeding program (Tausend *et al.*, 2000b).

3. MATERIALS AND METHODS

3.1. Description of the Study Site

The study was carried out in the Laboratory of Biotechnology at Jimma Agricultural Research Center, South-west Ethiopia. The Center is coordinating the national coffee research program in the country and located at 365 km away from Addis Ababa and 12 km from Jimma town. The center is found at an altitude of 1750 m above sea level, with geographical coordinates of 7^{o} 46' North latitude and 36^{o} 47' East longitude in the sub humid tropical belt of southwestern Ethiopia. The average annual rainfall of the area is 1594 mm with mean minimum and maximum temperatures of 11.6^{o}c and 26.3^{o}c, respectively (Taye, 2006).

3.2. Culture Media Preparation

For this experiment, MS (Murashige and Skoog medium; 1962) basal media containing macro and micro nutrients, organic supplements, vitamins and sucrose (3%), were used for embryo germination. Then, different concentrations of PEG (2%, 4%, 6%, 8% and 10%) were directly added to the MS media as water stress triggering agent. The pH of the solution was adjusted at 5.75 using PH meter. Thereafter,7g/l agar was added, melted by heating and dispensed into glass jars (40ml/jar) before autoclaving at 121ºC for 20 minutes. The culture media were removed from the autoclave and kept until they cooled prior to starting the inoculation.

Table: 2. MS basal media composition (Murashige and Skoog, 1962)

Ingredients	**Amount (mg/1l medium)**	**500 ml stock Solution (20x)**
Major salts (Stock 1)		
NH_4NO_3	1650 mg	16.5 g
KNO_3	1900 mg	19 g
$CaCl_2.2H_2O$	440 mg	4.4 g
$MgSO_4.\ 7H_2O$	370 mg	3.7 g
KH_2PO_4	170 mg	1.7 g
Minor salts (Stock II)		**500 ml stock (200x)**
KI	0.83 mg	83 mg
H_3BO_3	6.2mg	620 mg
$MnSO_4.4H_2O$	22.3mg	2230 mg
$ZnSO_4.7H_2O$	8.6mg	860 mg
$Na_2MoO_4.2H_2O$	0.25mg	25 mg
$CuSO_4.5H_2O$	0.025mg	2.5 mg
$CoCl_2$	0.025mg	2.5 mg
Iron stock (Stock III)		**500 ml stock (200x)**
$FeSO_4.7H_2O$	55.7 mg	5,570 mg
$NA_2.EDTA.2H_2O$	74.5 mg	7,450 mg
B5 Vitamins (Stock – IV)		
Thiamine HCl	0.1 mg	10 mg
Nicotinic acid	0.5 mg	50 mg
Glycine	2.0 mg	200 mg
Pyridoxine HCl	0.5 mg	50 mg
Sucrose (g)	30	-----
Agar (g)	7.00	------

3.3. Plant Materials and Culture Conditions

Seed samples of thirteen Hararghie coffee accessions were used. The seeds were selected based on vigor and color. The selected seeds were immersed in a solution of formic acid (1.6%) for 30 minutes after being washed in distilled water. Thereafter, the seeds were washed three times with sterile distilled water and soaked into 0.5 ml/100 ml solution of boric acid (5%) for 72 hours. Then, Seeds were surface sterilized in 20% (v/v) sodium hypochlorite for 20 minutes, followed by three washes with sterile distilled water under aseptic conditions (Ramos *et al*., 1999). Finally, the seeds were immersed in 25 mg/l of cysteine for 10 minutes to trap the excess chlorine and washed three times with sterile distilled water. Then embryos were aseptically excised and four to five embryos were inoculated into each glass jar containing the MS medium. Cultures were maintained under 2480 Lux light intensity at 21-37°C using photometer. After four weeks, well developed plantlets with true leaves were selected.

Of the 13 Coffee genotypes, nine (H-618/98, H-674/98, H- 739/98, H-822/98, H- 823/98, H-858/98, H-929/98, H- 968/98, H-980/98) have given true plantlets, while the remaining four accessions (H-622/98, H-856/98, H-857/98, H-958/98, H -981/98) failed to fully develop true leaved plantlets due to probably their inherent genetic potential. Accordingly, 24 six-week old plantlets from each of the nine accessions were selected and sub- cultured on MS medium supplemented with PEG and on MS medium without PEG to determine suitable concentration of PEG and to test the effect of PEG concentrations on their growth performance. Thereafter, for each accession, two plantlets per jar were inoculated on both the control (0%) and the stressing MS medium treated with (2%, 4%, 6%, 8% and 10%) PEG and kept in growth room for six weeks with a refreshing of culture medium every two weeks.

3.4. Experimental Design and Data Analysis

The experiment was conducted in Completely Randomized Design (CRD) with six PEG treatments in two replicates, making 54 treatment combinations (nine accessions x six levels of PEG) per replication, and two explants per jar for each treatment. Statistical analysis for the collected data was done using the SAS statistical software (version 9.2) and, after ANOVA, mean separation was carried out using Duncan's multiple range test at α=5 %.

3.5. Data Collection

Different growth parameters, including number of leaves (NL) and number of roots(NR), shoot length (SL) and root length(RL), leaf area (LA), fresh weight (FW) and dry weight (DW) of the plantlets were measured. Accordingly, number of leaves and roots were measured by counting and root and shoot length were measured using ruler at 15^{th}, 30^{th}, and 45^{th} days of culture. On the other hand, fresh weight (FW) and dry weight (DW) were determined at the end of the experiment (after 45 days of culture), when total leaf area was also determined by excising each leaf and drawing its surface on square paper. Plantlets were removed from the tubes and fresh weight (FW) was immediately determined with analytical balance. To determine the dry weight (DW), plantlets were oven dried at 70°C for 72 hr.

4. RESULTS AND DISCUSSION

4.1. RESULTS

The analysis of variance results (Table 3) showed a highly significant (P<_ 0.01) interaction between accessions, time (duration of culture) and PEG concentrations. The nine Hararghie coffee (*Coffee arabica* L.) accessions responded differently to the drought stress induced by different PEG concentrations. The accessions also showed different growth responses to different days after PEG treatment.

In general, there was a highly significant (P<_0.01) difference between the accessions for LN, RN, RL, SL, FW and DW, while the difference for LA was not significant. Similarly, almost all the parameters were highly significantly affected by PEG concentration, duration of culture and their interaction. The interaction between accession and PEG concentration and duration (time) of culture was also significant especially for RN, FW and DW (Table 3).

Table 3. Mean squares for growth parameters of plantlets of nine Hararghie coffee genotypes.

	LN	RN	RL	SL	LA	FW	DW
A	17.3**	5.51**	2.3**	0.66**	0.16^{ns}	3.21**	1.11**
C	19.9**	10.4**	2.6**	2.2**	16.9**	0.74**	0.33*
T	3.64^{ns}	7.96**	1.10*	0.06^{ns}	2.34**	1.73*	0.23**
A x C	1.68^{ns}	0.72*	0.63*	0.20^{ns}	0.15^{ns}	0.66**	0.72*
A x T	6.28**	1.75**	0.4^{ns}	0.39**	0.13^{ns}	2.11**	0.51**
C x T	13.5**	8.02**	3.0**	2.46**	0.95**	0.72**	0.71**
Ax C x T	2.64**	1.02**	0.7**	0.26**	0.14*	0.12**	0.34*
$LSD_{0.05}$	1.28	0.56	0.51	0.35	0.26	0.01	0.03
CV (%)	1.97	1.97	1.97	1.97	1.97	1.45	0.68

Where, A= accessions, C= PEG concentrations, T= time, ns= non-significance, *= significance difference at 0.05, **= significance at 0.01, LN= leaf number, RN=root number, RL=root length, SL=shoot length, LA= leaf area, FW= fresh weight, DW= dry weight

4.1.1. Shoot Growth

The interaction of accession and day of treatment was significant for number of leaves (NL) and shoot length (SL) and nonsignificant for leaf area (LA). In terms of growth, the nine Hararghie coffee accessions did not show any difference for evaluated growth parameters on the control medium and showed the highest average values for number of leaves, shoot length after 15 days of stress (Table 4 and Table 5). Significant decreases in growth rate of shoot were observed for 15 days of PEG stress period.

The effects of stressing media on all investigated growth parameters were significantly different, whereas all the coffee accessions grown on the control medium performed similarly and showed the highest average number of leaves, shoot length and leaf area (Table 4, 5, and 6). However, it was observed that as the concentration of PEG increased, the plantlet growth decreased. After 15 days, significant decreases in number of leaves shoot length and leaf area with increasing concentrations of PEG and duration of culture was observed in all accessions. Similarly, after 30 and 45 days, the mean number of leaves, length of shoot and leaf area of accessions was significantly decreases with increasing concentration of PEG and duration of culture.

In general, the accessions showed a decrease in morphological parameters (particularly leaf area) as PEG concentration in the MS medium increased with prolonged time. At 2% PEG concentration, plants produced new leaves and roots. Differences in morphological parameters occurred only at 4% PEG concentration. Hence, results of 4% PEG concentration was taken as a critical threshold level for screening these accessions for drought tolerance. At the higher PEG concentrations (6, 8 and 10%), plants did not develop any new leaves and roots.Drought decreased shoot length in all accessions and variation in number of leaves per plant was also observed. Accordingly, number of leaves, length of shoot and total leaf area decreased in all accessions due to drought.

Table 4.Mean values of number of leaves of plantlets of Hararghie coffee accessions as affected by different levels of PEG induced osmotic stress after various periods (Days).

Coffee accession	Days	PEG concentration (%)						$LSD_{0.05}$	CV (%)
		0	2	4	6	8	10		
H-618/98	15	7.0^{gh}	8.0^{ef}	8.5^{de}	6.0^{ij}	11.0^{a}	8.5^{de}	2.85	17.3
H-674/98		8.0^{ef}	9.0^{cd}	7.5^{fg}	9.5^{bc}	11.0^{a}	9.0^{cd}		
H-739/98		7.5^{fg}	8.0^{ef}	8.0^{ef}	7.0^{gh}	7.0^{gh}	7.5^{fg}		
H-822/98		6.5^{hi}	8.5^{de}	9.0^{cd}	9.0^{cd}	10.5^{b}	7.5^{fg}		
H-823/98		5.5^{jk}	6.5^{hi}	7.5^{fg}	7.0^{gh}	9.5^{bc}	9.0^{cd}		
H-858/98		6.5^{hi}	7.5^{fg}	5.5^{jk}	7.5^{fg}	6.0^{hi}	9.0^{cd}		
H-929/98		4.0^{n}	7.0^{gh}	7.0^{gh}	6.0^{hi}	7.0^{gh}	10.5^{b}		
H-968/98		4.5^{lm}	5.5^{jk}	6.5^{ij}	6.5^{hi}	8.0^{ef}	6.5^{hi}		
H-980/98		5.0^{kl}	4.0^{n}	6.0^{ij}	8.0^{ef}	6.5^{hi}	7.5^{fg}		
H-618/98	30	8.0^{ef}	8.5^{de}	8.0^{ef}	5.5^{jk}	9.5^{bc}	6.0^{ij}	2.98	21.4
H-674/98		9.5^{bc}	9.5^{bc}	7.0^{gh}	8.0^{ef}	7.5^{fg}	7.5^{fg}		
H-739/98		8.5^{de}	8.0^{ef}	7.5^{fg}	5.5^{jk}	6.0^{ij}	5.0^{kl}		
H-822/98		8.5^{de}	8.5^{de}	8.0^{ef}	8.0^{ef}	7.0^{gh}	6.5^{hi}		
H-823/98		6.5^{hi}	6.5^{hi}	7.0^{gh}	6.0^{ij}	6.5^{hi}	7.0^{gh}		
H-858/98		7.0^{gh}	8.0^{ef}	4.5^{lm}	5.5^{jk}	5.0^{kl}	7.0^{gh}		
H-929/98		5.5^{jk}	7.5^{fg}	7.0^{gh}	8.0^{ef}	5.5^{jk}	6.0^{ij}		
H-968/98		6.0^{ij}	6.0^{ij}	6.0^{ij}	5.0^{kl}	6.0^{ij}	5.0^{kl}		
H-980/98		6.0^{ij}	6.0^{ij}	6.0^{ij}	7.0^{gh}	6.0^{ij}	5.5^{jk}		
H-618/98	45	10.5^{b}	9.0^{cd}	8.0^{ef}	$5.0^{hijklmn}$	7.0^{gh}	5.0^{kl}	3.13	29.5
H-674/98		11.0^{a}	10.5^{b}	7.0^{gh}	$7.0^{defghijkl}$	5.0^{kl}	5.5^{jk}		
H-739/98		9.0^{cd}	8.5^{de}	7.0^{gh}	4.0^{lmn}	5.0^{kl}	4.0^{lmn}		
H-822/98		9.0^{cd}	9.0^{cd}	7.5^{fg}	7.0^{gh}	5.0^{kl}	4.0^{lmn}		
H-823/98		7.5^{fg}	7^{gh}	6.0^{ij}	5.0^{kl}	5.0^{kl}	4.0^{lmn}		
H-858/98		7.5^{fg}	8.0^{ef}	4.5^{lm}	4.5^{lm}	4.0^{lmn}	5.5^{jk}		
H-929/98		7.5^{fg}	7.5^{fg}	6.0^{ij}	4.5^{lm}	4.5^{lm}	4.0^{lmn}		
H-968/98		7.5^{fg}	6.0^{ij}	6.0^{ij}	4.5^{lm}	4^{lmn}	4.5^{lm}		
H-980/98		7.0^{gh}	5.0^{kl}	5.0^{kl}	5.0^{kl}	4.5^{lm}	4.0^{lmn}		

Figures followed by same letter(s) are not significantly different at p<_0.05.

Table 5.Mean values of shoot length of plantlets of Hararghie coffee accessions as affected by different levels of PEG induced osmotic stress after various periods (Days).

Coffee accessions	Days	PEG concentration (%)						$LSD_{0.05}$	CV (%)
		0	2	4	6	8	10		
H-618/98	15	1.45^{vw}	7.3^{de}	6.75^{fg}	7.25^{de}	3.65^{op}	3.5^{op}	0.77	6.68
H-674/98		6.5^{gh}	8.0^{bc}	5.0^{kl}	5.75^{ij}	7.5^{de}	5.0^{kl}		
H-739/98		5.5^{ij}	6.0^{hi}	7.0^{ef}	2.5^{tu}	3.15^{qr}	4.25^{mn}		
H-822/98		4.25^{mn}	5.5^{ij}	7.0^{ef}	3.0^{rs}	7.5^{de}	5.5^{ij}		
H-823/98		6.5^{gh}	6.5^{gh}	8.0^{bc}	4.0^{no}	4.5^{lm}	4.0^{no}		
H-858/98		6.5^{gh}	2.75^{st}	6.0^{hi}	5.5^{ij}	4.0^{no}	5.0^{kl}		
H-929/98		5.5^{ij}	5.5^{ij}	6.5^{hi}	6.5^{gh}	5.25^{jk}	3.0^{rs}		
H-968/98		1.5^{vw}	5.5^{ij}	4.0^{no}	6.5^{gh}	7.5^{de}	3.0^{rs}		
H-980/98		6.0^{hi}	5.25^{jk}	6.0^{hi}	3.0^{rs}	7.5^{de}	3.5^{op}		
H-618/98	30	1.5^{vw}	7.5^{de}	6.5^{gh}	6.0^{hi}	2.0^{uv}	3.0^{rs}	0.83	7.19
H-674/98		6.55^{gh}	8.5^{ab}	4.25^{mn}	4.45^{lm}	5.5^{ij}	4.45^{lm}		
H-739/98		6.0^{hi}	6.0^{hi}	6.5^{gh}	1.5^{vw}	2.0^{uv}	3.0^{rs}		
H-822/98		4.45^{lm}	5.5^{ij}	6.55^{gh}	2.5^{tu}	5.5^{ij}	4.5^{lm}		
H-823/98		7.0^{ef}	7.0^{ef}	7.5^{de}	3.0^{rs}	3.0^{rs}	3.0^{rs}		
H-858/98		7.0^{ef}	3.0^{rs}	5.25^{jk}	4.0^{no}	2.5^{tu}	3.0^{rs}		
H-929/98		6.0^{hi}	5.75^{ij}	6.0^{hi}	5.25^{jk}	2.5^{tu}	1.5^{vw}		
H-968/98		2.5^{tu}	6.0^{hi}	4.0^{no}	6.0^{hi}	5.5^{ij}	2.5^{tu}		
H-980/98		6.5^{gh}	5.75^{ij}	5.5^{ij}	2.5^{tu}	5.25^{jk}	1.5^{vw}		
H-618/98	45	2.5^{tu}	7.55^{cd}	5.5^{ij}	4.25^{mn}	0.5^{x}	2.5^{tu}	0.85	6.65
H-674/98		6.75^{fg}	9.0^{a}	3.5^{op}	3.0^{rs}	2.75^{st}	2.75^{st}		
H-739/98		7.0^{ef}	6.5^{gh}	5.5^{ij}	0.5^{x}	1.5^{vw}	2.25^{tu}		
H-822/98		5.5^{ij}	5.75^{ij}	6.5^{gh}	1.5^{vw}	3.0^{rs}	2.5^{tu}		
H-823/98		7.75^{cd}	7.75^{cd}	6.55^{gh}	2.0^{uv}	1.5^{vw}	1.5^{vw}		
H-858/98		7.75^{cd}	4.0^{no}	4.0^{no}	3.0^{rs}	0.5^{x}	0.5^{x}		
H-929/98		6.5^{gh}	6.0^{hi}	5.5^{ij}	4.0^{no}	0.5^{x}	0.5^{x}		
H-968/98		3.0^{rs}	6.5^{gh}	3.5^{op}	5.5^{ij}	3.0^{rs}	1.5^{vw}		
H-980/98		7.0^{ef}	6.0^{hi}	5.25^{jk}	1.5^{vw}	2.5^{tu}	0.5^{x}		

Figures followed by same letter(s) are not significantly different at p<_0.05.

Table 6.Mean values of total leaf area of plantlets of Hararghie coffee accessions as affected by different levels of PEG induced osmotic stress after various periods (Days).

Coffee accessions.	Days	PEG concentration (%)						$LSD_{0.05}$	CV (%)
		0	2	4	6	8	10		
H-618/98	15	0.88^{gh}	1.5^{bc}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.52	7.19
H-674/98		1.28^{cd}	0.6^{lm}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-739/98		0.53^{kl}	1.18^{de}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-822/98		0.73^{ij}	0.123^{op}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-823/98		0.58^{kl}	0.18^{no}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-858/98		0.53^{kl}	0.15^{op}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-929/98		0.53^{kl}	0.13^{op}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-968/98		0.73^{ij}	0.29^{mn}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-980/98		0.09^{qr}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-618/98	30	1.05^{fg}	0.10^{pq}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.56	5.13
H-674/98		2.0^{b}	0.09^{qr}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-739/98		0.6^{lm}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-822/98		0.83^{hi}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-823/98		0.6^{lm}	0.29^{mn}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-858/98		0.65^{lm}	0.10^{pq}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-929/98		0.65^{lm}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-968/98		0.85^{gh}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-980/98		0.13^{op}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-618/98	45	1.10^{ef}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.64	5.25
H-674/98		2.83^{a}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-739/98		0.78^{ij}	0.05^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-822/98		1.05^{fg}	0.03^{rs}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-823/98		0.78^{ij}	0.004^{t}	0.004^{t}	0.003^{rs}	0.003^{rs}	0.003^{rs}		
H-858/98		0.68^{jk}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-929/98		$0..68^{jk}$	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-968/98		0.65^{jk}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		
H-980/98		0.15^{op}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}	0.004^{t}		

Figures followed by same letter(s) are not significantly different at p<_0.05.

4.1.2. Root Growth

The interaction of accession and day of treatment was significant for number of roots and root length. In all Hararghie coffee accessions grown in the control medium performed similarly and showed the highest average number of root and root length after 15, 30 and 45 days. However, it was observed that as the concentration of PEG increased the plantlet growth decreased. A significant decrease in root length with increasing concentration of PEG was observed.Analysis of variance also showed a significant effect of duration of PEG treatment on all responses, except for root length. This indicated that the growth of coffee was affected differently by 15, 30 and 45 days of PEG stress (Table 7 and 8). Under PEG induced waterdeficit conditions H- 674/98 and H-8222/98 performed better root length compared to accessionH-858/98, H-929/98 and H-980/98which showed drastic reduction in biomass. The ability of plants to recover completely after stress is crucial to survive and complete their life cycle.

Table 7.Mean values of number of roots of plantlets of Hararghie coffee accessions as affected by different levels of PEG induced osmotic stress after various periods (Days).

Coffee accessions	Days	PEG concentration (%)						$LSD_{0.05}$	CV (%)
		0	2	4	6	8	10		
H-618/98	15	0.5^{j}	2.5^{ef}	3.0^{de}	3.0^{de}	5.5^{a}	3.0^{de}	1.13	14.8
H-674/98		3.0^{de}	3.0^{de}	2.5^{ef}	3.5^{cd}	4.0^{bc}	3.0^{de}		
H-739/98		1.0^{hi}	2.0^{fg}	2.0^{fg}	3.0^{de}	4.5^{b}	2.0^{fg}		
H-822/98		1.0^{hi}	2.0^{fg}	3.0^{de}	2.5^{ef}	5.5^{a}	2.0^{fg}		
H-823/98		1.0^{hi}	1.0^{hi}	1.5^{gh}	1.5^{gh}	3.0^{de}	4.0^{bc}		
H-858/98		0.5^{j}	1.5^{gh}	2.0^{fg}	2.5^{ef}	4.5^{b}	1.0^{hi}		
H-929/98		0.5^{j}	1.0^{hi}	1.5^{gh}	2.0^{fg}	3.5^{cd}	1.5^{gh}		
H-968/98		1.0^{hi}	1.0^{hi}	1.5^{gh}	3.5^{cd}	1.5^{gh}	1.5^{gh}		
H-980/98		1.0^{hi}	1.0^{hi}	1.5^{gh}	1.5^{gh}	2.5^{ef}	1.5^{gh}		
H-618/98	30	1.0^{hi}	2.5^{ef}	2.5^{ef}	2.5^{ef}	3.0^{de}	2.0^{fg}	1.22	15.6
H-674/98		3.5^{cd}	3.5^{cd}	2.0^{fg}	3.0^{de}	2.5^{ef}	2.5^{ef}		
H-739/98		1.5^{gh}	2.5^{ef}	2.0^{fg}	2.0^{fg}	2.5^{ef}	1.0^{hi}		
H-822/98		1.5^{gh}	2.5^{ef}	2.5^{ef}	2.0^{fg}	3.5^{cd}	1.5^{gh}		
H-823/98		1.5^{gh}	2.0^{fg}	1.0^{hi}	1.0^{hi}	2.0^{fg}	2.0^{fg}		
H-858/98		1.0^{hi}	1.5^{gh}	1.5^{gh}	2.0^{fg}	2.0^{fg}	0.5^{j}		
H-929/98		1.0^{hi}	1.0^{hi}	1.5^{gh}	2.0^{fg}	2.0^{fg}	1.0^{hi}		
H-968/98		1.5^{gh}	1.0^{hi}	1.5^{gh}	2.5^{ef}	1.0^{hi}	1.0^{hi}		
H-980/98		2.0^{fg}	1.0^{hi}	1.5^{gh}	1.0^{hi}	1.5^{gh}	0.5^{j}		
H-618/98	45	2.0^{fg}	3.0^{de}	2.5^{ef}	1.0^{hi}	1.5^{gh}	1.0^{hi}	1.38	19.4
H-674/98		4.5^{b}	3.5^{cd}	2.0^{fg}	2.5^{ef}	1.0^{hi}	1.5^{gh}		
H-739/98		2.0^{fg}	2.5^{ef}	1.5^{gh}	1.5^{gh}	1.0^{hi}	1.0^{hi}		
H-822/98		2.5^{ef}	3.5^{cd}	2.5^{ef}	1.5^{gh}	1.5^{gh}	1.0^{hi}		
H-823/98		2.0^{fg}	2.0^{fg}	1.0^{hi}	1.0^{hi}	1.0^{hi}	1.0^{hi}		
H-858/98		1.0^{hi}	2.0^{fg}	1.5^{gh}	1.0^{hi}	1.0^{hi}	0.5^{j}		
H-929/98		1.5^{gh}	1.0^{hi}	1.0^{hi}	1.0^{hi}	1.5^{gh}	0.5^{j}		
H-968/98		2.0^{fg}	1.5^{gh}	1.0^{hi}	2.0^{fg}	1.0^{hi}	0.5^{j}		
H-980/98		3.0^{de}	1.0^{hi}	1.0^{hi}	0.5^{j}	0.5^{j}	0.5^{j}		

Figures followed by same letter(s) are not significantly different at p<_0.05.

Table 8.Mean values of root length of plantlets of Hararghie coffee accessions as affected by different levels of PEG induced osmotic stress after various periods (Days).

Coffee accessions.	Days	PEG concentration (%)						$LSD_{0.05}$	CV (%)
		0	2	4	6	8	10		
H-618/98	15	5.5^{mno}	7.75^{de}	3.5^{qrs}	3.5^{qrs}	7.5^{efg}	5.5^{mno}	0.98	6.71
H-674/98		7.0^{hi}	7.5^{efg}	7.85^{de}	9^{b}	8.75^{b}	7.0^{hi}		
H-739/98		7.5^{efg}	5.5^{mno}	7.75^{de}	7.0^{hi}	9.5^{a}	7.0^{hi}		
H-822/98		6.5^{jk}	4.75^{opq}	8.5^{bc}	4.75^{opq}	6.0^{klm}	8.0^{cd}		
H-823/98		7.25^{gh}	5.5^{mno}	6.0^{klm}	6.5^{jk}	6.5^{jk}	8.0^{cd}		
H-858/98		6.5^{jk}	5.5^{mno}	5.5^{mno}	5.5^{mno}	4.75^{opq}	2.0^{stu}		
H-929/98		6.75^{ij}	6.5^{jk}	4.75^{opq}	7.5^{efg}	6.0^{klm}	6.5^{jk}		
H-968/98		6.75^{ij}	7.5^{efg}	6.0^{klm}	8.3^{cd}	7.0^{hi}	7.5^{efg}		
H-980/98		5.5^{mno}	6.5^{jk}	5.5^{mno}	5.5^{mno}	6.5^{jk}	3.5^{qrs}		
H-618/98	30	5.58^{mno}	8.5^{bc}	2.75^{st}	2.75^{st}	4.65^{opq}	5.0^{op}	1.13	6.75
H-674/98		7.5^{efg}	8.5^{bc}	7.5^{efg}	8.3^{cd}	7.5^{efg}	5.0^{op}		
H-739/98		7.75^{de}	5.65^{mn}	7.65^{efg}	6.0^{klm}	7.0^{hi}	5.5^{mno}		
H-822/98		7.0^{hi}	5.0^{op}	8.0^{cd}	4.25^{qr}	4.75^{opq}	6.0^{klm}		
H-823/98		7.55^{efg}	6.0^{klm}	5.5^{mno}	5.5^{mno}	4.75^{opq}	6.0^{klm}		
H-858/98		7.0^{hi}	6.0^{klm}	4.75^{opq}	4.75^{opq}	2.15^{stu}	1.5^{v}		
H-929/98		7.0^{hi}	6.25^{klm}	3.55^{qrs}	6.5^{jk}	4.25^{qr}	4.75^{opq}		
H-968/98		7.0^{hi}	7.55^{efg}	5.5^{mno}	7.5^{efg}	6.0^{klm}	4.25^{qr}		
H-980/98		6.0^{klm}	6.75^{ij}	5.0^{op}	4.25^{qr}	5.0^{op}	4.75^{opq}		
H-618/98	45	6.5^{jk}	8.55^{bc}	2.15^{stu}	1.5^{v}	2.0^{stu}	4.25^{qr}	1.25	9.18
H-674/98		8.0^{cd}	6.75^{ij}	6.75^{ij}	7.55^{efg}	6.0^{klm}	$4.75q^{op}$		
H-739/98		8.0^{cd}	6.0^{klm}	7.35^{gh}	5.5^{mno}	4.25^{qr}	4.25^{qr}		
H-822/98		7.55^{efg}	6.25^{klm}	7.75^{de}	3.5^{qrs}	2.0^{stu}	4.75^{opq}		
H-823/98		8.0^{cd}	6.25^{klm}	4.75^{opq}	4.75^{opq}	3.5^{qrs}	3.55^{e}		
H-858/98		7.55^{efg}	6.25^{klm}	3.55^{qrs}	3.5^{qrs}	1.5^{v}	1.5^{v}		
H-929/98		7.75^{de}	6.5^{jk}	3.5^{qrs}	5.5^{mno}	2.0^{stu}	1.5^{v}		
H-968/98		$7.0.^{hi}$	8.0^{cd}	5.0^{op}	6.0^{klm}	4.25^{qr}	2.0^{stu}		
H-980/98		6.5^{jk}	7.0^{hi}	4.75^{opq}	3.5^{qrs}	3.5^{qrs}	1.5^{v}		

Figures followed by same letter(s) are not significantly different at p<_0.05.

4.1.3. Fresh and Dry Weight

Results of this experiment generally showed that plantlets grown on the MS without PEG (control) had high growth rate than did grown on culture media with different concentrations of PEG. The comparison of fresh weight (FW) and dry weights (DW) (Table 9 and 10) also revealed significant differences between plantlets grown on culture medium with different levels of PEG. In general, plantlets grown on culture medium without PEG showed significantly higher FW and DW than did those grown on media treated with PEG, and the values generally decreased with increasing concentration of PEG. At the higher PEG concentrations (8 and 10%), the plantlets did not show any significant difference.

Table: 9. Mean values of fresh weight (FW) of plantlets in response to stressing culture media after 45 days.

Coffee accessions	PEG Concentration (%)						$LSD_{0.05}$	CV (%)
	0	2	4	6	8	10		
H-618/98	0.035^{bc}	0.015^{fg}	0.03^{cd}	0.02^{ef}	0.001^{i}	0.001^{i}	0.006	2.006
H-674/98	0.04^{b}	0.025^{de}	0.025^{de}	0.01^{gh}	0.001^{i}	0.001^{i}		
H-739/98	0.025^{de}	0.04^{b}	0.01^{gh}	0.01^{gh}	0.001^{i}	0.001^{i}		
H-822/98	0.05^{a}	0.03^{cd}	0.015^{fg}	0.025^{de}	0.001^{i}	0.001^{i}		
H-823/98	0.03^{cd}	0.035^{bc}	0.035^{bc}	0.01^{gh}	0.001^{i}	0.001^{i}		
H-858/98	0.035^{bc}	0.015^{fg}	0.01^{gh}	0.006^{hi}	0.001^{i}	0.001^{i}		
H-929/98	0.02^{ef}	0.03^{cd}	0.01^{gh}	0.006^{hi}	0.001^{i}	0.001^{i}		
H-968/98	0.02^{ef}	0.03^{cd}	0.01^{gh}	0.01^{gh}	0.001^{i}	0.001^{i}		
H-980/98	0.015^{fg}	0.006^{hi}	0.01^{gh}	0.01^{gh}	0.001^{i}	0.001^{i}		

Figures followed by same letter(s) are not significantly different at p<_ 0.05.

Table: 10. Mean values of dry weight (DW) of plantlets in response to stressing culture media after 45 days.

Coffee accessions	PEG Concentration (%)						$LSD_{0.05}$	CV (%)
	0	2	4	6	8	10		
H-618/98	0.001[c]	0.001[c]	0.001[c]	0.001[c]			0.0043	2.006
H-674/98	0.01[a]	0.001[c]	0.01[a]	0.001[c]	0.001[c]	0.001[c]		
H-739/98	0.01[a]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		
H-822/98	0.001[c]	0.001[c]	0.006[b]	0.001[c]	0.001[c]	0.006[b]		
H-823/98	0.006[b]	0.006[b]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		
H-858/98	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		
H-929/98	0.006[b]	0.006[b]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		
H-968/98	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		
H-980/98	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]		

Figures followed by same letter(s) are not significantly different at p<_ 0.05.

4.1.4. Suitable Concentration of PEG to Induce Osmotic Stress

The aim of this experiment was to identify suitable concentration of PEG to induce osmotic stress for *in vitro* screening plantlets of Hararghie coffee genotypes for drought tolerance. Accordingly, it was observed that all the nine accessions did not show any significant difference for all evaluated growth parameters on the control medium. But as the concentration of PEG increased, growthof the plantlets decreased. The effects of PEG treated media on all the growth parameters were significantly different. Plantlets grown on the control medium showed the highest values of NL, NR, RL, SL, LA, FW and DW, while plantlets of all the nine accessions grown on MS medium supplemented with 6%, 8% and 10% PEG did not develop any new leaves and roots. Therefore, 2% PEG concentration found to be the suitable concentration that Coffee plantlets can tolerate and show different growth responses for drought screening under *in vitro* conditions (Fig.1).

Figure: 1. Growth of plantlets of nine Hararghie coffee genotypes on suitable concentration (2%) culture media after 45 days.

4.2. DISCUSSION

Screening a large number of genotypes for drought tolerance in the field is difficult due to spatial heterogeneity of soil chemical and physical properties and seasonal fluctuations. Polyethylene glycol (PEG) is used for rapid screening and gives more consistent result than other osmoticum (Muhammad *et al*., 2010). In the current study, PEG was used for drought stress induction in Hararghie coffee genotypes. In vitro screening of coffee varieties for water deficit stress tolerance has been proposed as a rapid method and an alternative approach to costly, labor-intensive and sometimes problematic field-based screening (Rahman *et al*., 2008).Therefore, the significance of this study was to recognize the drought tolerance capacity of Hararghie Coffee accessions,to get evidential knowledge about the extent of drought tolerance of Hararghie Coffee is important for coffee producers as well as for economic development of countries,to provide awareness for coffee producers in Hararghie as well as in Ethiopia to give attention for climatic factors that affect the yield and quality of Hararghie Coffee accessions and also serves as a documented information for other people who are interested to further conduct their research in the similar issue in another area.

The effect of water or salinity stress on *in vitro* potato growth has been reported to be similar to that observed under field conditions (Zhang and Donnelly 1997; Aghaei *et al*., 2008). But, there has been limited information on *in vitro* screening of Coffee Arabica with no report at all on Hararghie coffee genotypes for drought tolerance using PEG induced media. In the present study**,** the addition of PEG to MS medium mostly decreased root length of the accessions. In this respect, Mozafar and Goodin (1981) reported that addition of PEG to MS medium decreased water potential; inducing drought stress and affecting shoot and root growth of plants.

Growth performances of coffee plants were generally influenced by 45 days of drought stress period. In line with this, Guridi*et al*., (1987) reported that every growth characteristic in coffee seedlings is affected by prolonged drought stress. Different authors (Wrigley, 1988; Dias *et al*., 2007; Tesfaye *et al*., 2008) have also reported that reduction in the growth of coffee under field and/or greenhouse conditions due to prolonged drought stress or water deficit.

Generally, the marked reduction in leaf area observed in this study was attributed to water deficit. In agreement with this, many researchers (Wrigley, 1988; Ludlow, 1989; Tesfaye *et al*., 2008) underlined that a reduction in total leaf area of coffee plants due to drought stress, slower leaf growth with narrower leaves during dry season and slower leaf elongation rate after 35 days of deficit irrigation. In the present study, leaf area increased initially, but it was decreasing when the PEG concentration increases. On the other hand, the effect of duration of stress on root length was not significant for coffee accessions; though, in other studies (Tschaplinski and Blake, 1989; Burckhardt *et al*., 2007), drought stress was found to have profound and inexhaustible effect on root growth of coffee and Populus trees grown in the field under dry conditions. As it was reported by Le Roux *et al*. (1996), deep and extensive root system is a very important morphological characteristic for plants particularly in drier or water deficit stress environments. Another study (Dias *et al*., 2007) conducted using pot grown coffee varieties under greenhouse showed a greater absolute length of the root system for a drought stress-tolerant variety than for a less tolerant one. The long root system helped a drought stress-tolerant variety to gain greater access to water towards the bottom of the pots, therefore, maintaining a more favorable internal water status than the less tolerant variety. Likewise, Pinheiro *et al*., (2005) have also reported a similar result for clones of *Coffea canephora*. In this respect, in the present study, accession H-674/98 has shown the longest root and best growth performance. This condition better suited the accession to drought stress through its extensive root system. The higher leaf number and other growth characteristics of accession H-674/98 and H-618/98 also showed that they are relatively more tolerant to drought than the other accessions. Conversely, Dias *et al*. (2007) have reported that leaf area of drought stress tolerant variety decreased to a greater extent than less tolerant variety under water stressed condition. This shows that coffee varieties have different mechanisms to withstand the effects of water deficit stress (Burckhardt *et al*., 2006). The smaller leaf number, root number and root length in accession H-980/98 suggest that the accession is less tolerant to drought stress.

The significant difference between accessions and days of stress for root number, leaf number, leaf area and root length shows that the tested accessions responded differently to different levels of drought stress, supporting the initial hypothesis of this study. This result also agrees with

some previous findings on drought stress responses of different coffee accessions (Carr, 2001; Burckhardt *et al*., 2006).

Plant morphological characteristics considered in this study seem among the important traits for selecting drought tolerant coffee accessions. Significantly higher values of most growth characteristics, except leaf area and root number, for coffee accessions in this study confirm previous reports (Silva *et al*., 2004). It was shown that growth of coffee is well recovered and faster after a short period of drought stress (Tesfaye, 2005). In general, the responses of coffee accessions to water stress were manifested by reductions in leaf area and growth rate of shoot part and by increment in girth, root volume and total dry matter. This study also showed varietal differences in some morphological responses to drought stress, which indicates the potential for selection of drought tolerant coffee varieties in Ethiopia.

4.3. CONCLUSION

This is the first attempt whereby tissue cultures of Hararghie coffee (*Coffea arabica* L.) genotypes have been subjected to drought stress. Therefore, based on the present study, it could be suggested that *in vitro* screening with the induction of chemical drought stress using PEG is proper track to select drought-tolerant accessions of coffee within shorter period of time. In line with this, culture medium with 2% concentration of PEG is a suitable concentration to screen drought tolerant coffee genotypes under *in vitro* condition. Moreover, growth parameters mainly, number of leaves and roots, root and shoot length and leaf area was proved to be relevant in identifying drought tolerant coffee genotypes under *in vitro* condition.

After 45 days, the mean number of leaves and roots, length of roots andshoots and leaf area of accessions significantly decreased with increasing concentration of PEG. It was observed that, in the control medium (0% PEG), the highest mean count of leaves (11.00) was recorded for accession H-674/98 and the lowest (7.0) was recorded for H-980/98. Similarly, in highest concentration of PEG (10%), the highest number of leaves (5.5) was recorded for H-674/98 and H-858/98, but the lowest (4.0) was recorded for H-739/98, H-929/98, H-822/98 and H-823/98.The highest mean value of shoot length was recorded (7.75cm) for accession H-823/98 and H-858/98 and the lowest was recorded (2.5cm) for accession H-618/98 in the control medium. While, the highest mean value (2.75cm) was recorded for accessionH-674/98 and the lowest (0.5cm) was for accession H-858/98, H-929/98 and H- 980/98 at higher concentrations of PEG.The highest mean value of leaf area (2.83cm^2) was recorded for accessionH-674/98 and the lowest (0.15cm^2) was for accession H-980/98 in the control treatment, while total leaf area decreased with increasing concentration of PEG for all accessions.The highest mean count of roots (4.5) was recorded for accession H-674/98 and the smallest (1.0) was for accession H-858/98 in the control (0%),in 10% PEG concentration, H-674/98 also showed the highest mean value (1.5), while the lowest (0.5) was recorded for H-858/98, H-929/98, H-968/98 and H-980/98. The highest mean root length (8.0cm) was recorded for accessions H- 674/98, H- 823/98 and H- 739/98 and at higher concentrations of PEG; the highest mean root length (4.75cm) was also recorded for accessionH-674/98 and H-822/98, while the lowest mean value (1.5cm) was for accession H-858/98, H-929/98 and H- 980/98.

Generally, as the concentrations of PEG and duration of culture increases, growth parameters of accessions decreased and better growth performanceswere observedfor accession H-674/98, H-618/98, H-822/98 and H-739/98. The most sensitive genotypes identified in this study was H-980/98.

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5. **RECOMMENDATIONS**

- It is recommended that future research work should include physiological (e.g stomatal conductance and rate of photosynthesis etc.) and morphological traits (e.g. leaf rolling, cuticle deposition and leaf surface reflectance) that have not been explored in this study.

- Besides, drought stress tolerant genotypes identified in this study should be further checked for their tolerance level under field conditions

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