Characterization and identification of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality

By
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(2016-21-009)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **'Characterization and identification of black pepper accessions (Piper nigrum L.) for stress tolerance and quality',** is a bonafide record of work done by me during the course of research and that the thesis has not previously formed the basis for award of any degree, diploma, fellowship or other similar title of any other University or Society.

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Certified that this thesis entitled '**Characterization and identification of black pepper accessions (*Piper nigrum* L.)**' is a record of research work done independently by Mr.Prakash K.M. (2016-21-009) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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LIST OF ABBREVIATIONS

%-Per cent

ABA-Abscisic Acid

ASTA-American Spice Traders Association

CO_2-Carbon dioxide

CSI-Chlorophyll Stability Index

DMSO-Dimethyl Sulphoxide

DSI-Disease Severity Index

EC-Electrical Conductivity

EDTA-Ethylene Diamine Tetra Acetic Acid

EST-Expressed Sequence Tag

FC-Field Capacity

GC-Gas Chromatography

H_2O_2-Hydrogen peroxide

ICAR-Indian Council of Agricultural Research

IISR-Indian Institute of Spices Research

IPGRI-International Plant Genetic Resources Institute

IRGA-Infra Red Gas Analyser

MDA - Malondialdehyde

mmol-Milli mole

µg-Micro gram

µm – Micro molar

µl – Micro litre

MSI-Membrane Stability Index

MSI-Moisture Stress Induction

NBT-Nitro Blue Tetrazolium

ºC-Degree Celsius

OD-Optical Density

P 5 – Panniyur 5

POD-Peroxidase

PPFM-Pink Pigmented Facultative Methylotrophs

PWP-Permanent Wilting Point

ROS-Reactive Oxygen Species

RWC-Relative Water Content

SOD-Superoxide Dismutase

VPD-Vapour Pressure Deficit

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Introduction

1. INTRODUCTION

Black pepper, *Piper nigrum* L., often described as the ‘ King of spices’ is the most important spice crop in the world and India (Purseglove *et al*.,1981). Indian pepper is preferred across the globe due to its intrinsic qualities. It was originated in the humid tropical forests of Western Ghats of South India and is being grown in more than 25 countries including India, Indonesia, Malaysia, Vietnam, Srilanka and China.

Domestication of black pepper started thousands of years back. Cultivar diversity is one of the principal components of diversity in pepper. About 70 cultivars have evolved in Kerala by the process of selection and rejection by man over the years from the wild and they differ greatly in morphological characters and yield potential (Mathai *et al*., 1981; Ibrahim *et al*., 1985). Most of the Indian cultivars, numbering about 100, are land races that evolved naturally and are further selected by man. Advanced cultivars have been derived mostly by clonal selections though a few are of hybrid and open pollinated background (Krishnamoorthy and Parthasarathy, 2010).

Intracultivar or inter varietal variability has been observed for morphological and qualitative characters (Ratnambal *et al*.,1985). Ravindran and Nirmalbabu (1994) reported the nature and extent of variability of morphological characters of black pepper cultivars. Eventhough geitenogamy is the rule in pepper, with the existence of viable sexual reproduction in combination with vegetative propagation, the variability present in the wild type has been conserved (Dewaard and Zeven, 1969).

Black pepper is diploid (2n=52) (Parthasarathy *et al*., 2006). Natural triploidy (2n=78) was also reported among black pepper cultivars (Nair *et al*.,1993). Polyploidy observed in few collections is also one of the reasons for the wide variability noted in the population. An induced tetraploid (2n=4x=104) of the black pepper hybrid, Panniyur-1, was developed at IISR by treating fresh seeds of Panniyur- 1 with 0.05% colchicine (Nair and Ravindran, 1992). Raju *et al*. (1983)

observed variability in quality characters among cultivars in a study involving 29 important traditional cultivars for volatile oil, piperine, oleoresin and starch content.

Indian Institute of Spices Research (ICAR-IISR), Calicut is the nodal agency in India for conserving genetic resources and persuing research on black pepper. The existing germplasm collection of ICAR- IISR consists of around 3,000 accessions of black pepper which may contain favorable traits like high yield, quality and resistance to biotic and abiotic stresses.

To ensure industrial and global demand for quality, varieties with more intrinsic properties are to be identified utilizing conserved germplasm. Essential oils, piperine and oleoresin content of berries are the quality parameters in black pepper that are important commercially.

The foot rot disease caused by the fungus *Phytophthora capsici* is a major disease affecting the pepper plantation and nurseries across the country wiping out large chunk of gardens and plantations. None of the released varieties has required level of resistance to the disease. However, a few genotypes show some degree of field tolerance (Bhai *et al*., 2007).

Drought and increasing atmospheric temperature are other important limiting factors faced by pepper farmers. According to Marschner (1995), there is increasing evidences suggesting mineral-nutrient status of plants playing a critical role in increasing plant resistance to environmental stress. Waraich *et al.* (2011), reported that potassium plays an important role in survival of plants under environmental stress. They also reported that micronutrients help the macro nutrients in drought alleviation by activation of certain physiological, biochemical and metabolic processes within the plant body. Only few works on screening for drought tolerance have been undertaken in black pepper. Attempts to identify or develop genotypes with resistance to biotic and abiotic stresses have not yielded much success.

Hence, the present study entitled ‘Characterization and identification of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality’ was taken up with the following objectives. 1. Characterization and evaluation of black pepper accessions for yield and field tolerance to biotic and abiotic stresses, 2. to screen the selected accessions for quality traits (piperine, essential oil and oleoresin content) and 3. tolerance to *Phytophthora* foot rot and drought.

Review of Literature

2. REVIEW OF LITERATURE

2.1. BLACK PEPPER AND IMPORTANCE

Black pepper (*Piper nigrum* L.) often described as black gold is the king spice in the world. Black pepper is cultivated in more than 26 countries globally and is a native spice of India due to its origin in the Western Ghats. It is one of the major export oriented and revenue generating spice for India. The uses of black pepper is increasing steadily in various fields such as food processing and pharmaceutical industry due to its acceptance as source of natural antioxidant having anti carcinogenic activity. The properties other than spices include bioavailability enhancement, carminative property, anti-inflammatory action, cholesterol lowering capacity, immune enhancer ability, anti-pyretic, antimicrobial and rubefacient activity.

India has 3rd position in the world production (55500 tonnes) after Vietnam (175000 tonnes) and Brazil (62000 tonnes) with largest area of 134280 ha, followed by Indonesia (117500 ha) and Vietnam (110000 ha). Now the share of India has gone down in the export to third (17300 tonnes), the first being Vietnam, followed by Brazil (IPC, 2017).

2.2. BRIEF DESCRIPTION OF BLACK PEPPER

2.2.1. Botanical description

The plant is a perennial climber which needs a support tree for better growth and yield. The vine is monoecious with hermaphrodite and protogynous flowers and is predominantly self pollinated. The climbing main stem is stout with leathery dark green leaves. Leaves are entire showing variability in size and shape. Pepper exhibits polymorphic branching. The main stem being orthotropic grows straight upwards acting as the back bone, climbing on the standard. The laterally growing branches known as plagiotropes bear flowers and fruits. The horizontally trailing shoots emerging from lower part of main stem is commercially used for propagation and is described as runners.

2.2.2. Floral biology

Infloroscence in black pepper is a filiform pendant spike borne opposite to leaf on fruiting branches. The spikes are up to 20 cm length bearing 5-100 minute flowers borne on axils of fleshy bracts. The cultivated types are monoecious with great variability in male, female and hermaphrodite flower composition in the spike. Self pollination is the common rule in most of the cultivars without any active pollen transfer mechanism. The presence of anthers on either side of gynoecium ensures self pollination especially in types where synchronous maturity is noticed. Protogyny is noticed in most of the cultivars. Pollination is passive, effected through gravitational descending of pollen, often aided through rain water. Geitenogamy is the major mechanism effecting pollination (Ravindran and Nirmalbabu, 1994).

2.3. CROP IMPROVEMENT

Farmers face several production constraints to achieve sustainable yield. Global climate change, limited water availability, labour scarcity, coupled with biotic stresses such as epidemic diseases and pests, are the main challenges to be addressed by scientists for crop improvement. Considering these aspects, goal of improvement of black pepper should be for bold berries with high quality and research should also be oriented to produce crop with lower levels of pesticide residues, contamination with adulterants and mycotoxins. The genetic resources of this crop in India are a great strength. This germplasm, containing local cultivars, wild forms collected from the area of origin and related species are a great wealth to be utilized for crop improvement. The main breeding objectives are high yield, with resistance to biotic and abiotic stresses, coupled with good quality parameters (Krisnamoorthy and Parthasarathy, 2010).

Domestication of black pepper started thousands of years back. Different tools like clonal selection, hybridization and open pollinated progeny selection are the major methods used for crop improvement programme in pepper (Ravindran and Sasikumar,1993; Krishnamoorthy and Parthasarathy, 2010).

The ICAR-Indian Institute of spices Research is a premier research institute of the country undertaking collection, conservation, evaluation and utilization of the spice's germplasm.

About 70 cultivars are evolved in Kerala by the process of selection and rejection by man over the years from the wild and they differ greatly in morphological characters and yield potential (Mathai *et al.,* 1981; Ibrahim *et al.*, 1985).

Eventhough geitenogamy is the rule in pepper, the existence of viable sexual reproduction in combination with vegetative propagation keeps the variability present in the wild type conserved (Dewaard and Zeven, 1969). Polyploidy observed in few collections is also a reason for wide variability noted in the population.

Intracultivar or inter varietal variability has been observed for morphological and qualitative characters (Ratnambal *et al*.,1985). Ravindran and Nirmalbabu (1994) reported the nature and extent of variability of morphological characters of black pepper cultivars.

Evaluation and selection within the germplasm has led to the isolation of many elite varieties. Evolution of most of these varieties were by clonal selection from germplasm, while a few are from seedling selection and very few are due to recombination breeding (Ravindran and Johny, 2000).

2.4. GENETIC DIVERSITY IN THE GENUS *PIPER*

The genus *Piper* belongs to the order Piperales, sub family Piperoideae of family Piperaceae which is comprised of more than thousand species and occurs throughout the tropical and subtropical regions. The distribution of *Piper* ranges from sea level to the high ranges of Andes and Sub Himalayas. Trans Gangetic region and the South Deccan are considered to be the two independent centres of origin of the genus *Piper* in India. Out of the different species of *Piper,* 110 are reported from India (Parthasarathy *et al.*, 2006). The genus includes important species like *Piper nigrum*; the black pepper, a major spice, *Piper longum*; the long pepper and *Piper betel*; the betel vine which are commonly used in the indigenous system of medicine (Ravindran *et al*., 1992d).

In a study using thirty land races of black pepper collected across Kerala using 3 AFLP primer pair combinations scorable and consistant banding pattern were obtained. Out of 173 scorable markers more than 90 per cent were polymorphic suggesting considerable variation in the available germplasm (Joy *et al*., 2007).

Assessment of genetic diversity in the germplasm of black pepper from around the world using SSR markers from EST showed agreement with the genetic and geographic distances. In addition, compared with the American accessions, the Asian wild accessions and cultivated accessions grouped together, indicated a close genetic relationship. This finding substantiated the earlier report on the origin of black pepper (Wu *et al*., 2016).

2.4.1. Morphological characterization

The application of morphological characterization is generally to derive economic and breeding achievements from germplasm collection and related families of accessions (Bekele *et al*., 2006). Several studies were conducted using biometric characters to elucidate the population structure and genetic diversity of *Piper* sp. Characterization of elite lines and varieties of high-value crops like spices is important for protection of bio wealth in the present WTO era. Conventionally, characterization of biodiversity has been done using morphological features, and visually scorable morphological markers that correspond to quantitative traits are used for morphological characterization.

Black pepper accessions and varieties have been traditionally classified based on plant characters such as leaf length and breadth, leaf shape and size, features of leaf tip and base, shoot tip colour, berry size, spike length, spike composition (bisexual, female and male), fruit set, number of fruits spike, $^{-1}$ 1000 fruit volume, 1000 fruit weight yield vine^{-1} and dry recovery. Morphological characters were earlier used to characterize the germplasm collection, but molecular markers are also being used (Sreedevi *et al.,* 2004).

Landraces, natural mutants, improved varieties and even true seedlings constitute the primary gene pool of black pepper (Sasikumar *et al*., 1999, Ravindran *et al*., 2000). Sasikumar *et al.* (2014) reported collection and characterization of two unique black pepper accessions with very long spikes, though with poor setting, from Coottanadu Estate, Wayanadu, Kerala, bordering the evergreen forest of the Western Ghats at 800m altitude from mean sea level.

A unique black pepper accession with very high dry recovery and high bulk density coupled with round, firm, bold attractive black coloured corns, hitherto unreported in world black pepper gene pool,was collected, characterized and conserved by Sasikumar *et al*. (2013).

Analysis for variations of factors contributing to yield as well as quality parameters using forty two clones of twenty year old Panniyur1 over a period of three years revealed that the plants exhibited considerable amount of variability for characters studied and the standard deviation was more for berries per spike and yield. Among the quality characteristics, oleoresin exhibited more variability than piperine (Pradeepkumar *et al*., 2003). Characterization of two inter specific hybrids of *Piper* for morphology, anatomy, isozymes, cytology and function by Sasikumar *et al*. (1999) exhibited distinct morphological and anatomical features.

Intra cultivar or inter varietal variability has been observed for morphological and qualitative characters in pepper (Ratnambal *et al*., 1985). Ibrahim *et al.* (1985) considered spike yield and spike number as the important characters in yield improvement.

2.4.2. Biochemical characterization

Black pepper (*Piper nigrum* L.) is known for its intrinsic quality. The volatile oil and pungent compounds are the two main components of black pepper. To ensure industrial and global demand for quality, varieties with more intrinsic biochemical properties are to be identified. Piperine, essential oil, and oleoresin content of berry are the quality parameters in black pepper that are important commercially. Sruthi *et al*. (2013) reported that there was profound variability in essential oil, oleoresin, piperine, total phenol, crude fibre, starch, total fat and bulk density in dried berries of black pepper variety, Panniyur-1 collected from eleven locations.

. By analysis of black pepper cultivars from Kerala *viz*. ‘Karimunda,’ ‘Kalluvally,’ ‘Arakulam munda’ and ‘Thommankody' for a period of three consecutive seasons for their oil composition, Menon and Padmakumar (2005) identified fifty five compounds in the oils by gas chromatography,using gas and mass spectrometre.

Raju *et al*. (1983), observed variability in quality characters among 29 important traditional cultivars for volatile oil, piperine, oleoresin and starch content. All the ten black pepper accessions under test, differed significantly for vegetative characters like vine column height, leaf length, leaf width and internodal length. Among them, Karimunda recorded lowest leaf length and leaf width (Preethy *et al.,* 2018).

Gopalam and Ravindran (1987) performed quality indexing of all important cultivars of black pepper by categorizing them into three classes *viz*. low, medium and high.

The biometric observation and visual assessment of plants based on qualitative traits brought intra group, inter clone and inter varietal variation of different characters among tissue cultured and conventional clones of four pepper varieties (Sujatha, 2001).

The Indian cultivars *viz*. Kottanadan, Kumbakodi, Kuthiravally and Nilgiri were rich in piperine and oleoresin, where as Balankotta, Kaniyakadan and Kumbakody were rich in essential oil (Krishnamoorthy and Parthasarathy, 2010).

2.4.3. Evaluation for tolerance to foot rot disease

Phytophthora foot rot is a major disease affecting the pepper plantation and nurseries across the country wiping out large chunk of gardens and plantations. None of the released varieties possess required level of resistance to the disease. However, a few genotypes showed some degree of field tolerance (Bhai *et al.,* 2007). Paucity of *Phytophthora capsici* tolerant cultivars is a large barrier in disease management in all black pepper producing countries (Nguyen, 2015).

According to Ristaino and Gumpertz, (2000), the oospores of the fungus *Phytophthora* can directly germinate and damage plants. Sporangia, are easy to dislodge from sporangiophore and disseminate within fields by rain, wind, irrigation water and wounds infecting both roots and aerial parts of plant.

In a screening trial of nine black pepper varieties under green house condition using soil inoculation with zoospore suspension of *Phytophthora capsici*, Divya and Sharada (2014) reported that three of the *Piper nigrum* varieties/cultivars *viz.* Panniyur-5, Aribally and Karimunda were found to be resistant to the disease, whereas Panniyur-1, Panniyur-3, Panniyur-4, Panniyur-6, and Panniyur-7 were highly susceptible and succumbed to the disease.

Inoculation of zoospore and culture discs of *Phytophthora capsici* on leaves, stems and roots in black pepper genotypes exhibited variation in per cent infection and mortality (Mammootty *et al*., 2008). Infection per cent varied from 55.00 (Panniyur-5) to 95.30 (Panniyur-7)

Two genotypes viz. Karimunda I and Panniyur-4 exhibited a high rate of mortality of 73.7 per cent while Panniyur-5 had the least mortality of 31.20 per cent. Leaf pin prick *in vitro* tests by Archana and Rajan (2013) revealed that Karimunda showed maximum infection after 48 hours, where as Panniyur varieties showed least infection.

Stem inoculation with an inoculum disc of the pathogen at the 3rd internode showed that Karimunda was resistant to *P. capsici* (Bhai *et al*., 2007).

Sarma and Nambiar (1979) developed a rapid screening technique for identifying resistant sources of black pepper using root inoculation technique. Sarma *et al.* (1982) screened 41 black pepper cultivars and 73 wild *Piper* species against *P. palmivora* adopting root dip inoculation technique and found that Narayakodi, Kalluvalli, Uthiaramkotta and Balankotta showed low percentage of infection but none of the wild species showed resistance. By adopting the stem inoculation technique a few promising lines could be isolated among hybrids, wild accessions and Kottanadan selections.

Study of Random amplified microsatellites (RAMS) and repetitive extragenic palindromic (REP) DNA fingerprinting analysis of 118 isolates of *P. capsici* from black pepper showed that the population was genetically more diverse where two mating types were found, although the overall genetic diversity was low, majority belonging to one clonal group. The low diversity among isolates suggests that the *P. capsici* population may have originated from a single source (Truong *et al.,* 2010).

2.4.4. Screening for drought tolerance

Being one of the major inherent abiotic constraints of agricultural productivity, drought stress can potentially reduce nearly 20 per cent of crop yield around the world (Scheiermeier, 2008). The plant requires water for from seed germination to maturation (Athar and Ashraf, 2005) and any type of imbalance in the uptake of water would adversely affect the yield (Wang *et al*., 2001).

Plants that are subjected to drought stress in a gradual manner accumulate solutes that maintain cell hydration and undergo complex adjustments in their morphology and photosynthetic characteristics. Many investigators explained the plant response to drought through escape, avoidance, and/or tolerance mechanisms. Substantial cooperative efforts among physiologists and breeders have resulted in understanding and manipulating such complex morpho-physiological traits for more sustainable crop performance under stress (Dhindsa *et al*.,1981).

Drought and increasing atmospheric temperature are the important limiting factors faced by pepper farmers. Stress tolerance against drought is assuming more importance in the scenario of climate change. Only few works on screening for drought tolerance have been undertaken in black pepper till date (Krishnamurthy *et al*., 2006).

The occurance of moisture deficit from March to May has been reported as a major constraint in limiting productivity of black pepper in India (Vasantha, 1996). Moisture deficit, affects the field establishment of cuttings and photosynthetic ability. Growing of drought tolerant varieties can be attempted to avoid decline in yield during water stress (Rajagopal and Balasimha, 1994).

2.4.4.1. Physiological responses

An effective screening tool in the hand of a plant breeder should be relatively simple, accurate, inexpensive, and dependable on physiological traits that are highly inherited and well correlated with crop performance under actual field-stressful conditions. Breeding for stress tolerance is very complex and strongly challenging. Stomatal resistance, transpiration rate and leaf water potential can be used to screen drought tolerant types (Vasantha and Ramadasan, 1990). Tremendous advances have been made in understanding the physiological and molecular basis of plant response to stress in the past.

2.4.4.1.1. Relative Water Content

Relative water content is considered a good measure of internal plant water status, reflecting the metabolic activity in tissues. It is used as an index for dehydration tolerance. Relative Water Content (RWC) of leaves is higher in the initial stages of leaf and reduces as the drought stress accumulates. The fall in the RWC in response to drought stress has been reported in a wide variety of plants when leaves were subjected to moisture stress (Nayyar and Gupta, 2006). To study the response of physiological parameters uniformly aged plants were subjected to moisture stress in black pepper by withholding irrigation, keeping one set as control. The stress treatment were continued starting with field capacity till the plants started showing wilting symptoms. A negative correlation between RWC and cell membrane leakage was observed.(Krishnamurthy *et al.,* 2006).

The difference in physiological parameters such as relative water content (RWC) and cell membrane leakage and also the differential expression of 11 drought responsive genes in drought tolerant and drought sensitive black pepper genotypes was reported by George *et al*. (2017).

Krishnamurthy *et al.* (1998) reported that drought tolerant black pepper accessions maintained higher relative water content and lower cell membrane damage10 days after stress induction than the sensitive genotypes.

2.4.4.1.2. Photosynthesis

Ability of crop plants to adapt to a new environment is related to its acclamatisation ability of photosynthesis, which in turn affects biochemical and physiological processes and finally growth and yield of the plant (Chandra, 2003).

In a study on drought tolerance using four varieties of wheat, Siddique *et al*. (2001) concluded that higher photosynthesis of varieties is associated with higher leaf water potential, relative water content and low leaf temperature.

Photosynthesis is inhibited in the tissues subjected to drought stress owing to an imbalance between light capture and utilization (Foyer and Noctor, 2000). The gas exchange parameters of crop plants are impeded by drought resulting from fall in leaf expansion, impaired photosynthetic machinery, oxidation of chloroplast lipids, premature leaf senescence, change in structure of proteins and pigments (Menconi *et al*., 1995).

In soybean, drought stress led to a considerable decline in net photosynthesis (33.22 %) and other physiological parameters like stomatal conductance (25.54 %) and transpiration rate (37.84 %) compared to the plants under normal irrigation (Anjum *et al*., 2011).

2.4.4.1.3. Membrane stability

Cell membrane stability serves as an important physiological parameter to evaluate drought tolerance (Premachandra *et al*., 1991). Being the first target of many abiotic stresses, membrane stability is an essential component of drought tolerance in plants (Bajji *et al.*, 2002). In a drought related study in wheat, Dhanda *et al*. (2004) considered membrane stability of leaf as the prime trait in screening germplasm for drought tolerance.

Analysis of cell membrane stability as a measure of electrolyte leakage to assess degree of water stress tolerance by *in vitro* dessication of leaf tissue using a solution of Poly ethylene glycol (PEG) has been reported in *durum* wheat (Bajji *et al.,* 2002).

Cell membrane stability declined rapidly in Kentucky blue-grass on simultaneous exposure to drought and heat stress (Wang and Huang, 2004). In a study on drought tolerance in maize, potassium nutrition improved drought tolerence due to the improved membrane stability (Gnanasiri *et al*., 1991).

In a study to understand the response of drought stress, RWC and membrane stability was studied in 44 black pepper cultivars using saplings, only

6 varieties *viz*., Kalluvally 4, Karimunda, Padarpan, Panniyur-5, Poonjarmunda and Uthirankotta 2 survived after 4 days of induction of water stress (Thankamani and Ashokan, 2004).

2.4.4.1.4. Leaf temperature

Leaf temperature is one of the main factors controlling leaf water status under water scarcity conditions (Leopold *et al*.,1994). Water is one of the most essential component for plant life serving as a solvent to transport solutes between cells and organs. In a study by Surendra *et al*. (2013) a rise in 4°C was recorded in leaf temperature in drought stress imposed banana compared to unstressed ones.

2.4.4.1.5. Transpiration rate

Water stress is known to reduce transpiration rate in plants. Latha (1998) reported a reduction of transpiration rate in cashew seedlings to about 50 per cent when stress period was extended to 5 days from 2 days. In a field study in coconut genotypes, the drought resilient ones expressed a reduction of transpirational loss through greater stomatal sensitivity and induced stomatal closure (Apshara *et al*.,2013).

2.4.4.1.6. Chlorophyll content

Chlorophyll pigments are important to plants mainly for tapping light and production of electrons with reducing power. Low concentration of photosynthetic pigments can directly reduce photosynthetic potential of leaf. Kaiser *et al*. (1981) have reported that the loss of more chlorophyll is from mesophyll than from bundle sheath cells during stress.

The chlorophyll content decreased to a significantly low level on advanced stages of stress in sunflower (Kiani *et al*., 2008) and cotton (Massacci *et al.,* 2008). The ratio of chlorophyll ‘a’ and ’b’ were sensitive to soil drying conditions (Farooq *et al*., 2009). The decrease in total chlorophyll content observed may be resulted from a decrease in leaf water status in the soybean (Makbul *et al*., 2011).

2.4.4..1.7. Chlorophyll stability index

High stability index of chlorophyll bestows the plant to withstand stress condition through better availability of chlorophyll. In Cocoa, Ravindran and Menon (1981) used chlorophyll stability index for *in vitro* screening of drought tolerance *in vitro*.

2.4.4.1. 8. Stomatal conductance

Stomatal conductance is generally accepted to be the main determinant for the decreased photosynthesis under mild to moderate drought (Sharkey, 1990). It is clear that stomata closes progressively as drought progresses followed by a decrease in photosynthesis. A high degree of co-regulation of stomatal conductance and photosynthesis is usually found (Wong *et al*.,1979). Stomata closes as leaf to air vapour pressure deficit (VPD) increases (Raschke, 1979), irrespective of soil water availability. Stomatal conductance is controlling the regulation of the whole photosynthetic process by checking CO_2 availability in the mesophyll (Medrano *et al*., 2002).

2.4.4.2. Biochemical responses

A good comprehension of the biochemical and molecular responses to drought is a dependable tool to unravel plant resistance mechanisms to water stress conditions. Drought affects growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations, and photosynthetic activity (Benjamin and Nielsen, 2006). The degree of susceptibility of plants towards drought depends on degree of stress, different factors accompanying stress, plant species and their developmental stages (Demirevska *et al*., 2009).

2.4.4.2.1.Proline

Osmotic Adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine and other solutes in cytoplasm may also be achieved for maintenance of leaf turgor by improving water uptake from drying soil. The process of accumulation of such solutes in response to water stress is known as osmotic adjustment. Proline is the most widely studied osmoticum due to its considerable importance in the stress tolerance. Mobilization of proline was observed to enhance tolerance to water stress in wheat (Nayyar and Walia, 2003).

Accumulation of proline is the immediate response of plants to reduce injury of cells under water stress conditions. Proline content increased as the drought stress progressed and reached a peak after 10 days of stress, and then decreased under prolonged water stress in maize (Anjum *et al*., 2011b).

The proline accumulation under stress has been correlated with stress tolerance and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. Under moisture stress proline influences protein solvation and preserves the quaternary structure of complex proteins, maintaining membrane integrity and reducing oxidation of lipid membranes (Demiral and Turkan, 2004).

Endowed with various roles proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression and is found to be essential for plant recovery from stress (Szabados and Savoure, 2009). It can also contribute towards stabilizing sub-cellular structures removing free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration is usually higher in stress tolerant than in stress sensitive plants (Silvente *et al*., 2012).

Significant differences in proline content were observed among genotypes of Alfalfa under water stress (Irigoyen *et al*.,1992). Jaleel *et al*., 2007, reported a relationship between turgor pressure and proline accumulation which could be useful as a possible drought injury sensor, so that selection of new drought tolerant genotypes based on high proline accumulation can be used as a selection parameter for stress tolerance in *Catharanthus roseus*.

2.4.4.2.2. Reactive oxygen species (ROS)

The ROS generation is one of the earliest biochemical responses of eukaryotic cells to both biotic and abiotic stresses which leads to subsequent defense reaction in plants. The production of ROS, is an early event of plant defense response to water-stress. The ROS, which include oxygen ions, free radicals and peroxides form as a natural byproduct of the normal metabolism of oxygen and have important role in cell signaling.

The degree of oxidative stress in a cell is determined by the amounts of superoxide, H_2O_2 and hydroxyl radicals. Therefore, the balance of SOD, ascorbate peroxidase and catalase activities will be crucial for suppressing toxic reactive oxygen species levels in a cell (Apel and Hirt, 2004).

The interaction of membrane components with ROS causes membrane damage and loss of membrane integrity resulting in accelerated ion leakage and water loss in peppers (Kissinger *et al.,* 2006). Owing to drought induced over production of ROS, increase in Malon dialdehyde (MDA) content has been considered as an indicator of oxidative damage (Moller *et al*., 2007). In the leaves of pea plants, levels of lipid peroxidation increased two to four folds with an increase in the duration of drought stress, and this was highly correlated with protein peroxidation (Moran *et al*., 1994). Being highly reactive, ROS can seriously damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death.

2.4.4.2.3. Role of anti oxidant enzymes

A complex enzymatic and non-enzymatic antioxidant system have evolved in plants to minimize the effects of oxidative stress, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) (Apel and Hirt, 2004). The integrity of the photosynthetic membranes is maintained under oxidative stress by the co-operation of non-enzymatic antioxidants or enzymatic antioxidant to scavenge ROS.

Plants possess an internal protective enzyme catalyzed clean up system to avoid injuries of active oxygen, ensuring normal cellular function (Horvath *et al*., 2007). The involvement of antioxidative enzyme and ROS production, determines whether oxidative signaling and damage will occur (Moller *et al*., 2007). The susceptible carpet grass showed continuous increase in SOD activity, transient rise of catalase and a reduction of peroxidase activity leading to H_2O_2 accumulation resulting in injury at progressive moisture stress. But in drought tolerant tifdwarf grass transient rise in the activity of superoxide dismutase and prolonged increase in the activities of catalase and peroxidase were noticed (Shaoyun *et al*., 2003). SODs act as first line of defense against reactive oxygen species, dismutating superoxide to H_2O_2, ascorbate peroxidase, glutathione peroxidase and catalase subsequently detoxify H_2O_2.

A decrease in acid phosphate was reported with water stress during seed filling in beans (Pasin *et al*., 1991). Acid phosphatase activity decreased with stress intensity in black pepper. *Piper colubrinum* as well as all the *Piper nigrum* accessions had higher activity compared to other species. There are reports highlighting the importance of these antioxidant enzyme activities in relation to disease tolerance (Klessig *et al*., 2000; Mittler *et al*., 1999).

The estimation of antioxidant enzymes *viz*. catalase, peroxidase and super oxide dismutase as screening parameters for drought tolerance has been established in many crop plants (Dhindsa *et al*., 1981; Rahman *et al*., 1999; Chempakam *et al*., 1993; Krishnamurthy *et al*., 2000; Chowdhury and Choudhuri, 1985).

Increase in enzyme activities of peroxidase and SOD were observed in tolerant black pepper varieties by inducing soil moisture stress. In Panniyur-1 high levels of lipid peroxidation were observed, showing susceptibility of the variety.

The best biochemical characters were observed in Panniyur 5 during water stress (Thankamani *et al.,* 2004).

In coconut, the activities of SOD, catalase and peroxidase have been shown to increase during water stress and the tolerant varieties maintained higher enzyme activities than the susceptible ones (Chempakam *et al.,* 1993). Decreased activities of SOD and catalase resulted in increased levels of O_2^- , H_2O_2, OH^- and O_2 in the tissue. This in turn increased the level of lipid peroxidation.

2.4.3. Foliar nutrition and drought tolerance

Increasing evidence suggests that improvement of potassium (K)-nutritional status of plants can greatly lower the ROS production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport. Potassium deficiency causes severe reduction in photosynthetic CO_2 fixation and impairment in partitioning and utilization of photosynthates. Such disturbances result in excess of photosynthetically produced electrons and thus stimulation of ROS production by intensified transfer of electrons to O_2 (Cackmak, 2005).

Analysis of gas exchange characteristics between the control and *P. nigrum* applied with beneficial microorganism treatment revealed that the photosynthetic rate, stomatal conductance and transpiration rate in pepper applied with Fermented Plant Juice (FPJ) and (Fermented Fruit Juice (FFJ) were significantly ($p<0.05$) higher compared to that of Indigenous Micro Organisms (IMO), Latic Acid Bacteria Serum (LABS) and the control (Sulok *et al*., 2018).

Integrated fertilizer treatment was found to be significantly superior to organic and chemical fertilizers realizing higher yield producing various phenotypic alterations in physiological processes and plant characteristics such as large leaf area index, higher chlorophyll content, photosynthetic rate and low transpiration rate in black pepper compared to other treatments (Ann, 2012).

In legumes, damaging effects of drought can be diminished by ample K supply (Sangakkara *et al*., 2000).

Mengel and Kirkby (2001) observed that due to low K concentration, ROS production was induced during water deficit which caused disturbance in stomatal openingin maize .

2.4.4. Pink pigmented facultative methylotroph (PPFM)

Plant growth promoting Pink pigmented methylotrophic bacteria (PPFM) include both the rhizosphereic and phyllospheric bacteria that can enhance plant growth by a wide variety of mechanisms.

The potentiality of plant growth promoting methylobacteria in agriculture has steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Growth promoting substances are likely to be produced in large quantities by these microorganisms that influence indirectly on the overall morphology of the plants.

The application of PPFM as biofertilizer is also advocated for the protection of crops in heat and drought stress conditions. *Pseudomonas* foliar spray along with a biofertilizer enhanced the microbial population in soil, making nutrients more available to plants (Jayajyothi *et al*., 2014).

In a drought mitigation study on tomato the highest leaf water potential of -0.89 MPa was registered by PPFM (2%) which can protect the plant under drought. Foliar spray of 2 per cent PPFM recorded lowest leaf temperature (25.2°C) and highest stomatal conductance (0.40 $mmolm^{-2s-1}$) followed by brassinolide (25.50°C and 0.38 $mmol^{m-2s-1}$) respectively, (Sivakumar *et al*., 2018).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled “Characterization and identification of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality” was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara and ICAR-IISR, Calicut during the period 2016-2019. In this study study five experiments were conducted as detailed below.

Experiment 1: Morphological characterization and evaluation of black pepper accessions for yield, biotic and abiotic stresses

Experiment 2: Biochemical characterization of selected accessions

Experiment 3: Screening of selected accessions for foot rot disease tolerance

Experiment 4: Screening of selected accessions for drought tolerance

Experiment 5: Evaluation of response of selected accessions to foliar nutrition under moisture stress

3.1. EXPERIMENTAL MATERIAL

Fifty accessions of black pepper collected from various parts of Kerala in bearing stage and of uniform age maintained at ICAR- IISR, Calicut formed the base material for the study. The popular black pepper variety Panniyur 5 was used as check variety in the screening for foot rot and drought tolerance. The details of 50 accessions used in this study are presented in Table1.

Table 1. Passport details of accessions of black pepper used for the study

Sl No	Accession No	IC No	Place of collection	District	Latitude/longitude
1	7211	598866	Meenmutty	Wayanad	11.43.57/75.52.46
2	7215	598869	Mayyil-Velam	Kannur	11.99.79/75.44.92
3	7219	598872	Mayyil-Velam	Kannur	11.99.79/75.44.91
4	7220	598873	Mayyil-Velam	Kannur	11.99.79/75.44.94
5	7221	598874	Chapparakkunnu	Kannur	11.86.07/75.41.40
6	7222	598875	Chapparakkunnu	Kannur	11.86.07/75.41.40
7	7225	598877	Chapparakkunnu	Kannur	12.12.25/75.49.42
8	7229	598880	Vellora	Kannur	12.12.25/75.49.42
9	7230	598881	Vellora	Kannur	12.07.35/75.30.16

Table 1. (Contd)

10	7232	598883	Vellora	Kannur	12.07.35/75.30.11
11	7236	598887	Kakkara	Kannur	12.07.35/75.30.16
12	7237	598888	Kakkara	Kannur	12.07.35/75.30.16
13	7239	598889	Kayapoil	Kannur	11.86.07/75.41.40
14	7240	598890	Kayapoil	Kannur	12.92.25/79.13.90
15	7241	598891	Kayapoil	Kannur	11.99.36/75.45.09
16	7243	598893	Kakkara	Kannur	11.72.56/76.11.04
17	7249	598899	Ammankad	Kannur	12.51.03/75.20.14
18	7251	598901	Ammankad	Kannur	12.51.03/75.20.14
19	7252	598902	Oduvally	Kannur	12.51.03/75.20.13
20	7254	598903	Naduvil	Kannur	11.40.70/75.98.76
21	7255	598904	Naduvil	Kannur	11.40.70/75.98.76
22	7258	598905	Mandalam	Kannur	11.39.93/75.95.60
23	7259	598906	Mandalam	Kannur	11.39.93/75.95.55
24	7260	598907	Mandalam	Kannur	11.99.36/75.45.09
25	7262	598909	Mandalam	Kannur	11.86.07/75.41.40
26	7264	598911	Mandalam	Kannur	11.86.07/75.41.40
27	7276	598920	Kattippara	Calicut	12.92.25/79.13.90
28	7277	598921	Kattippara	Calicut	11.86.07/75.41.40
29	7283	598927	Kattippara	Calicut	12.07.35/75.30.16
30	7285	598929	Kodencherry	Calicut	11.86.07/75.41.40
31	7286	598930	Kodencherry	Calicut	12.92.25/79.13.90
32	7288	598932	Koodathai	Calicut	11.86.07/75.41.40
33	7289	598933	Koodathai	Calicut	11.99.36/75.45.09
34	7293	598936	Koodathai	Calicut	11.39.93/75.95.60
35	7295	598938	Kodencherry	Calicut	11.40.70/75.98.76
36	7296	598939	Kodencherry	Calicut	11.40.70/75.98.76
37	7297	598940	Norramthodu	Calicut	11.23.41/75.79.55
38	7298	598941	Norramthodu	Calicut	11.23.41/75.79.55
39	7302	598945	Pallickal	Malappuram	9.66.43/77.09.74
40	7305	598948	Pallickal	Malappuram	11.04.16/76.07.97
41	7306	598949	Pallickal	Malappuram	11.14.29/75.92.20
42	7309	598952	Anangapara-Pallickal	Malappuram	11.14.29/75.92.20
43	7325	598967	Vettom- Tirur	Malappuram	11.14.29/75.92.20
44	7339	598979	Nariampara-Kattappana	Idukki	9.84.75/76.98.09
45	7344	598984	Nariampara-Kattappana	Idukki	10.90.07/75.89.71
46	7352	598992	Kanjiyar-Udumbanchola	Idukki	9.66.43/77.09.74
47	7358	598997	Konginikadavu-Kattappana	Idukki	9.84.75/77.11.68
48	7385	599024	Santhigram-Irattayar	Idukki	9.84.75/76.98.09

Black pepper germplasm at IISR

Marking of healthy accessions

Multiplication of selected accessions by serpentine method

Saplings ready for evaluation

Plate 1. Black pepper germplasm at IISR and multiplication of selected accessions

Table 1. (Contd)

49	7386	599025	Santhigram-Irattayar	Idukki	9.84.75/76.98.09
50	7387	599026	Santhigram-Irattayar	Idukki	9.84.75/76.98.09

3.2. EXPERIMENTAL LOCATION

The standing vines in the field germplasm of ICAR-IISR at Calicut was used for morphological characterization. The planting material of selected accessions were multiplied by serpentine method at IISR Experimental Farm, Peruvannamuzhi and utilized for further evaluation. Biochemical evaluation was carried out at biochemistry lab of ICAR- IISR, Calicut using dried berries. Preparation of culture of *Phytophthora capsici* and screening for foot rot was conducted at lab and green house of crop protection division of ICAR- IISR at Calicut. The drought experimental trials were conducted in the field experimental shed of the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara. Field view of germplasm and multiplication of selected accessions are given as plate 1.

3.2.1. Brief note on agroclimate of experimental location

Calicut district is situated on the south west coast of India between north latitudes 11º 08' and 11º 50' and east longitudes 75º 30' and 76º 08'. The district has a humid climate with a hot season extending from March - May. The humidity ranges from 70 - 90 per cent. The district receives rains in two spells *viz*. the southwest and northeast monsoon. The southwest monsoon sets normally in the first week of June and extends up to September. The northeast monsoon extends from the second half of October to November. The major share of showers received during June to August is around 2000 mm. The average annual rainfall is around 3000 mm. During December to March, the rainfall received is very low and from October onwards the temperature gradually increases. The minimum temperature is generally around 23 ºC and the maximum temperature is up to 32.8 °C in May.

3.3. OUT LINE OF THE EXPERIMENT

All the five experiments were conducted in tandem succession. In the first phase, 50 bearing accessions in the germplasm were characterized using IPGRI descriptor, 1995.

In the second phase of the 50 accessions characterized 20 were selected based on superiority in yield and manifested field tolerance to foot rot and pollu beetle (*Longitarsus nigripennis*) infestation.

In the third phase the selected accessions were multiplied and subjected to artificial inoculation trial for tolerance to foot rot disease at IISR during the monsoon season of 2017. Later the same accessions were evaluated using dried berries for piperine, essential oil and oleoresin content in the laboratory at IISR during 2018 summer. The drought trials were conducted at Vellanikkara during the period from September to December 2018.

3.3.1.Experiment 1:Characterization of germplasm

3.3.1.1.Morphological characterization

Fifty black pepper accessions of uniform age maintained at IISR as field germplasm was utilized for characterization. The characterization was carried out using IPGRI descriptor, 1995 for black pepper with slight modifications.

3.3.1.1.2. Morphological characterization for qualitative traits

This included six vine characters, four leaf characters, four spike characters and one fruit character as given in Table 2 following IPGRI descriptor (1995) and the characters selected for observation are presented in Table 3.

Table 2. IPGRI descriptor (1995) used for recording qualitative characters in black pepper

Sl no	Character	Description
1.	Branching habit	Dimorphic Polymorphic
2.	Shoot tip colour	Greenish yellow Light purple Darkpurple

Table 2. (Contd)

		Light red
3.	Runner shoot production	Many Few
4.	Holding capacity	Strong Weak
5.	Adventitious root production	Many Few
6.	Lateral branch habit	Horizontal Hanging Erect
7.	Leaf lamina shape	Ovate Ovate-elliptic Ovate lanceolate Elliptic- lanceolate Cordate
8.	Leaf base shape	Round Cordate Acute Oblique
9.	Leaf margin	Even Wavy
10.	Type of venation	Acrodromous Campylodromous Eucamptodromous
11.	Spike orientation	Erect Prostrate
12.	Spike shape	Filiform Cylindrical Globular Conical
13.	Spike colour	Green Greenish yellow Light yellow Light purple
14.	Type of hermaphroditism	Staminate flowers only Pistillate flowers only Bisexual flowers only Predominantly male Predominantly female Predominently bisexual
15.	Fruit shape	Round Ovate Oblong

Table 3. Details of characters selected for morphological characterization of blackpepper

Sl. No.	Vine characters	Leaf characters	Spike characters	Fruit characters
1	Vine length	Leaf petiole length	Spike orientation	Fruit setting
2	Branching habit	Leaf length	Spike shape	No.of developed fruits/ spike
3	Shoot tip colour	Leaf width	Spike colour	Fruit shape
4	Runner shoot production	Leaf lamina shape	Spike length	100 fruit weight
5	Holding capacity	Leaf base shape	Type of hermaphroditism	100 fruit volume
6	Adventitious root production	Leaf margin type	Peduncle length	Green spike yield/ plant
7	Lateral branch production	Type of venation	Number of spikes/ lateral branch	Green berry yield/ plant
8	Lateral branch length			Driage
9	Number of nodes/ lateral branch			

3.1.1.3. Morphological characterization for quantitative traits

Quantitative evaluation was based on 15 quantitative characters as given below.

3.3.1.1.4. Vine characters

1. <u>Vine length (m)</u>

Measured as vine column height from ground level to the tip of orthotropic shoot.

2. <u>Lateral branch length (cm)</u>

Average length of 50 randomly selected lateral branches.

3. <u>Number of nodes per lateral branch</u>

Average number of nodes of 50 lateral branches selected at random.

3.3.1.1.5. Leaf characters

1. <u>Leaf petiole length (cm)</u>

Leaf petiole length was calculated as average length of 50 randomly selected mature leaves from lateral branches measured from base to the point of insertion with leaf blade.

2. Leaf length (cm)

Leaf length was calculated as the average length of 50 randomly selected mature leaves from branches measured from base of midrib to tip.

3. Leaf width (cm)

Leaf width was calculated as the average width of 50 randomly selected mature leaves from laterals.

3.3.1.1.6. Spike characters

1. Spike length (cm)

Spike length was calculated as the average length of of 50 randomly selected spikes measured from tip to base of spike at maturity recorded at harvesting using scale.

2. Peduncle length (cm)

Peduncle length was calculated as the average of 50 randomly selected spikes measured from tip to base of peduncle at maturity using scale.

3.3.1.1.7. Fruit characters

1. Fruit setting percentage

Fruit setting percentage was calculated as average of 50 spikes observed at harvest of mature spikes.

2. Number of well developed fruits/spike

Number of well developed fruits per spike was calculated as the average of 50 spikes observed at harvest of mature spikes.

3. Hundred fruit weight (g)

Hundred fruit weight was calculated as weight in grams of 100 fully developed fruits at maturity recorded soon after harvest.

4. Hundred fruit volume (cc)

Hundred fruit volume was calculated as the volume of 100 well developed mature berries measured soon after harvest following water displacement method by putting berries in a marked measuring cylinder containing known volume of water and noting the increase in volume.

5. Green spike yield/plant (g)

Green spike yield was calculated as weight in gram of mature whole spikes soon after harvest/ vine/year.

6.Green berry yield/plant (g)

For calculating green berry yield per plant, the berries were separated from spike after determining the spike yield and the total weight of green berries was determined in gram.

7. Driage (per cent)

Measured as dry weight of berries in gram / green weight of berries in gram multiplied by 100 recorded for each vine.

The mean values of the above 15 characters were analysed for correlation and cluster analysis.

Evaluation of susceptibility to biotic stress was scored on a five point score ranging from 1-9 as per IPGRI, 1995 descriptor as given in Table 4. The accessions with score of 1or 3 only were selected for further detailed evaluation.

Table 4. Score chart for biotic stress tolerance in black pepper

Sl No.	Score	Description
1	1	Very low or no sign of susceptibility
2	3	Low
3	5	Intermediate
4	7	High
5	9	Very high

Out of the 50 germplasm accessions characterized, twenty accessions were selected based on yield and field level tolerance to biotic and abiotic stresses (Table 5). These twenty accessions were further evaluated for biochemical constituents determining the quality of pepper, tolerance to foot rot disease and drought

Table 5. List of black pepper accessions selected for detailed evaluation

Sl. No	Accession No	IC. No
1	7211	598866
2	7215	598869
3	7219	598872
4	7221	598874
5	7222	598875
6	7229	598880
7	7232	598883
8	7240	598890
9	7241	598891
10	7243	598893
11	7249	598899
12	7252	598902
13	7254	598903
14	7255	598904
15	7259	598906
16	7276	598920
17	7285	598929
18	7286	598930
19	7289	598933
20	7293	598936

3.3.2. Biochemical characterization

The selected accessions were evaluated for commercially important biochemical constituents in dried berries. Content of piperine, volatile oil and oleoresin were analysed by following standard procedures (Plate 2).

Spikes were harvested from the vines of the selected accessions at maturity and quality dried black pepper was made following hot water treatment (dipping berries for one minute in boiling water before drying). Dried and cleaned

Pulverisation of dried berries for oleoresin extraction

Estimation of piperine

Estimation of volatile oil

Estimation of oleoresin

Plate 2. Biochemical evaluation of piperine, volatile oil and oleoresin

samples of 150g were taken from each accession for analysis of the above qualitative traits.

3.3.2.1. Determination of oleoresin content

The oleoresin content of the dried black pepper berries was estimated using extraction as per ASTA (1997). Dried pepper berries were ground to fine powder, then, 10 g of the powder was transferred into the extraction column after packing the bottom of column with cotton plug into which 50 ml of hundred percent acetone was added and kept for overnight standing. The extract was drained to a previously weighed and labeled empty beaker. An additional quantity of 30ml acetone was added to the column and allowed to stand for one hour. The final drain was collected into the beaker and was kept in water bath at 60-65°C to evaporate the solvent. The beaker containing oleoresin was kept in oven till constant weight was recorded. Oleoresin content was estimated as

$$\text{Percentage of oleoresin} = \frac{\text{weight of oleoresin in the sample} \times 100}{10}$$

3.3.2.2. Determination of essential oil content

The essential oil content of dried black pepper berries was estimated following hydro distillation (ASTA, 1997) using modified Clevenger apparatus. Twenty five gram of dried black pepper sample was coarsely crushed in granite mortar and transferred to 1000 ml round bottom flask and 500 ml distilled water was added. The Clevenger apparatus was connected to round bottom flask. After setting water supply and current, the mantle was switched on and run for 3 hours after boiling. The reading of oil collected was recorded after cooling, collected in appendorf tube and stored in refrigerated condition with a pinch of sodium sulphate. The percentage of essential oil was estimated as

$$\text{Percentage of essential oil} = \frac{\text{Quantity of oil collected}}{25} \times 100$$

3.3.2.3. Estimation of piperine

The percent piperine content of black pepper berries was determined by spectrophotometric procedure using materials as given in Table 6. Piperine is extracted into denatured alcohol and absorbance is measured between 342nm and 345 nm by following the method suggested by ASTA (1997).

A. Table 6. Materials used for estimation of Piperine in black pepper

Sl no.	Apparatus/materials	Specification
1	Spectrophotometer	Double beam, UV (deuterium) light source, capable of accurately measuring absorbance at 342-345 nm.
2	Cuvettes	1 cm. square silica
3	Ambe Erlenmeyer flask	125 mL capacity with S. T. 24/40 ground joint
4	Condenser	West type, with water cooled drip tip, 400 mm., S.T. 24/40 ground joint
5	Amber volumetric flasks	100 mL with S.T. stoppers
6	Pipettes	Volumetric type 1, 2, 3, 4, 5, and 10 ml
7	Funnel	65 to 75 mm. I.D.at top
8	Filter paper	Fluted, Whatman No. 2 or equivalent.
9	Reagents SDA No. 3A	Specially Denatured Alcohol formed by mixing 5ml of methyl alcohol and 100ml of 95per cent ethyl alcohol
10	Piperine	Sigma-Aldrich, AS

B. Preparation of standards

Accurately 0.10 g of piperine standard was taken into a 100 ml volumetric flask with 70 ml SDA No. 3 and shaken to dissolve. The volume was made up to 100 ml volume and mixed well. 10 ml of aliquot was pipetted into 100 ml volumetric flask and made up the volume with SDA No. 3A and shaken well. Again 1, 2, 3, 4, 5, and 6 ml aliquots from solution was pipetted and transferred into 100 ml volumetric flasks and made up the volume with SDA No. 3A. These solutions represent concentrations of 1, 2, 3, 4, 5, and 6 µg/mlof the standard solutions of piperine. Spectrophotometer was auto zeroed with SDA No. 3A in both cells. Absorbance readings of A1, A2, A3, A4, A5 and A6 of corresponding solutions were determined at absorbance maxima between 342-345 nm. Average absorbance of standards A1–A6 were calculated and normalized to 1 µg/ml for each standard.

C. Procedure for estimation of piperine in sample

Accurately 0.50 gram of sample was weighed and taken in a 125 ml Erlenmeyer flask. 70 ml SDA was added in the sample refluxed for one hour, cooled to room temperature and filtered quantitatively using whatman no 2 filter paper into a 200 ml volumetric flask. Extracted residue was transferred to the filter and washed thoroughly with SDA No. 3A and diluted to the mark with SDA No. 3A. Four ml of this solution was pipetted into a 100 ml volumetric flask and made up the volume with SDA No. 3A and shaken well. Using SDA No. 3A as the reference solution, absorbance reading of this solution was recorded within 15 minutes at absorbance maxima 342-345 nm.

Percentage of piperine is calculated as piperine per cent $= \frac{As \times F \times V \times 100}{Ws \times 10^6}$

Where,

A_s = Absorbance of sample

F = Factor =1/Absorbance of 1ppm standard piperine @343 nm

V = Volume of solution (200ml) and 1ml diluted to 25ml

Ws = Weight of sample (0.5g)

3.3.2.4 Clustering based on biochemical quality characters

The oleoresin, volatile oil and the piperine content of the samples from selected pepper accessions were estimated by standard procedures. All these three biochemical characters evaluated were considered for cluster analysis. The accessions were subjected to multivariate analysis of clustering using the free online soft ware R' SOFT.

3.3.3. Screening of accessions for tolerance to foot rot disease

The selected accessions were subjected to artificial inoculation (Plate 3 – 4.).Rooted cuttings were raised in standard polybags and grown till 3months maturity in nursery sheds . These rooted cuttings were artificially inoculated with *Phytophthora capsici* Leon. Five rooted cuttings of each accession were screened per replication and their average scores were taken (Eikemo *et al*., 2003).

3.3.3.1. Stem inoculation

The cuttings were inoculated by making a pin prick with sharp tip of a needle at the middle portion of third internode from the tip of the cutting. Inoculum disc of 3mm size was taken from the growing margin of 72 h old stem isolate culture of *Phytophthora capsici* and placed at the point of pin prick (Sarma *et al.,* 1982). The inoculated portion was covered with a wet cotton pad and tied with a polythene strip to keep the inoculum in place without drying (Plate 3.) . The inoculated cuttings were incubated for 72h in green house maintained at temperatures of 25-28 °C with relative humidity 75-90 per cent. After 72 h, the cotton pad was removed and lesion length was measured. Then the stem was split open longitudinally and depth of penetration was assessed using a visual score rating and used as index for rating the accessions on 0 - 4 scale. The scoring were as in Table 7.

Table 7. Score chart for stem inoculation in *Phytphora* foot rot screening of black pepper

Score	Stem lesion length	Score	Depth of penetration
0	No lesion	0	No penetration
1	1-5mm lesion length	1	Up to 25per cent penetration
2	6-20mm lesion length	2	Up to 25-50per cent penetration
3	21-30mm lesion length	3	50-75per cent penetration
4	>30mm lesion length	4	>75per cent penetration

3.3.3.2. Leaf inoculation

Along with the stem inoculation, the third or fourth leaf from the top was also inoculated but without making injury by placing mycelial plug of 3mm size

Preparation of culture of *Phytophthoracapsici*

Leaf inoculation technique

Stem inoculation technique

Humid chamber

Plate 3. Screening of accessions *to Phytophthora capsici* by artificial inoculation

taken from the edge of 72 h old actively growing leaf isolate of *Phytophthora capsici* on the lower surface of leaf lamina.The disc was kept intact after packing with cotton and tied with polythene strip and maintained wet by dripping distilled water. The inoculated plants were incubated for 72 h at a temperature of 24-25°C with relative humidity of 80- 90 per cent in a humid chamber. After 72 h, the cotton pad was removed and lesion length was measured using a marked scale. The leaf lesions were measured and scored on a 0-4 scale as in Table 8, (Plate 4).

Table 8. Score chart for leaf inoculation in *Phytphora* foot rot screening of black pepper

Score	Leaf lesion diameter (mm)
0	No lesion
1	1-5 mm lesion
2	6-20 mm lesion
3	21-30 mm lesion
4	=>30 mm lesion

The lesion diameters of both stem and leaf were statistically analyzed. From the lesion scorings, the disease severity index (DSI) was calculated for stem lesion, depth of penetration and leaf lesion using the formula of Kim *et al*., 2000.

$$DSI = \frac{\text{Sum of rating of each plant} \times 100}{\text{Maximum score} \times \text{No.of plants rated}}$$

Final rating of infection is made based on average disease severity index (DSI) of both stem and leaf for each accession is presented as below

Average DSI of stem and leaf (%)	Reaction
< 30	Resistant
31-40	Moderately resistant
>40	Susceptible

Development of stem lesions

Development of leaf lesions

Development of stem and leaf lesions for various accessions

Moderately tolerant accession

Plate 4. Development of lesions after artificial inoculation

3.3.4. Screening for drought tolerance

Selected accessions in the experiment 1 were artificially screened for tolerance to drought as per Krishnamurthy *et al*. (1998) and Krishnamurthy *et al*. (2000).

Six month old rooted cuttings were subjected to moisture stress under pot culture in controlled conditions in a protected shed. Physiological and biochemical changes were recorded during the induction of moisture stress. Panniyur 5 was used as local check. The rooted cuttings of the selected accessions were raised in cement pots of 12" diameter containing 10 kg potting mixture with 3 part soil:1part FYM:1part sand. Each pot was planted with 3 rooted cuttings. The plants were irrigated uniformly every day for one week until saturation. For induction of stress irrigation was withheld. Field capacity of the soil was observed after withholding irrigation for two days. The soil moisture regime was recorded gravimetrically by drawing soil sample from root zone by using an auger at different stages throughout the experiment (Plate 5).

3.3.4.1. Determination of soil moisture content:

Soil sample collected from 15-20cm depth from root zone of pepper plants in pots using soil auger at each stressed stage, were placed in petridish and weight was recorded after covering with glass top as W_1. The samples were placed in oven at 100 - 110°C for 24 hours, cooled to room temperature and weighed as W_2. The soil was discarded and weight of empty petri plate and lid was recorded as C_1.

The soil moisture content was calculated as per cent.

$$\text{soil moisture content} = \frac{[(W_1-W_2) - C_1] \times 100}{(W_2 - C_1)}$$

3.3.4.2. Stages of stress induced

The physiological and biochemical changes in response to induced water stress were studied at 3 stages *viz.* irrigation to field capacity, withdrawing irrigation up to 5 days after reaching field capacity and withdrawing irrigation for 10 days after reaching field capacity.

Experiment on screening for drought tolerance

Measurement of Physiological parameters with IRGA

Collection of leaf for analysis

Reading using visible spectrophotometer

Plate 5. Experiment on drought tolerance screening

3.3.4.3. Physiological parameters

Physiological parameters like photosynthesis, leaf temperature, transpiration, and stomatal conductance were directly noted as digitally recorded reading using IRGA by taking measurement using 3rd fully matured leaf from top of the plant. Other parameters like relative water content,chlorophyll content, chlorophyll stability index and membrane stability index were recorded by standard procedures at each stage.

3.3.4.3.1. Estimation of relative water content (RWC)

The concept of relative water content as a measure of water status of plant is an established method for water stress experiments. It can be used as an index to screen plants for drought tolerance. The RWC was estimated as percentage of turgid water content of leaf following slight modification of Barrs, (1968) . Ten leaf discs of uniform sizes were cut with single punch from third leaf from the top of plants from each accession at 10 am and recorded fresh weight(F_w). The leaf discs were then floated in distilled water for 3 hours for reaching full turgidity and, taken out,wiped with filter paper to remove sticking droplets and recorded turgid weight immediately (T_w). The discs were transferred to petriplates, covered and dried in hot air oven at 80°C for 24 hours and recorded dry weight (D_w). The relative water content was calculated using the formula

$$RWC = \frac{Fw - Dw \times 100}{T_w - D_w}$$

3.3.4.3.2. Estimation of chlorophyll content

Chlorophyll is the essential component for photosynthesis that occur in chloroplast as green pigments in all photosynthetic plant tissues. During water stress dehydration of plant tissues can result in increase in oxidative stress which causes deterioration in chloroplast structure and an associated loss in chlorophyll. This leads to a decrease in the chlorophyll content and photosynthetic activity. Chlorophyll content was estimated without homogenizing by using dimethyl sulfoxide (DMSO), following Hiscox and Israelstam (1979). Fresh leaf samples of

100mg collected from each accession cut to fine pieces were put in a test tube containing 7ml of DMSO and incubated overnight at 65°C. The extracted liquid was transferred to a graduated cylinder and made up to 10ml with DMSO. Absorbance at 645 and 663nm were recorded using a visible spectrophotometer keeping DMSO as blank. The total chlorophyll content was measured using Arnon's formula .

$$\text{Total chlorophyll/g tissue} = \frac{\text{OD at 652 nm x 1000 xV}}{34.5 \text{ x } 1000 \text{ x W}}$$

Where, OD = optical density, V= Final volume of supernatant (10ml) and W = weight of leaf sample taken in grams

3.3.4.3.3. Estimation of chlorophyll stability index (CSI)

Chlorophyll pigments are thermosensitive in nature and its degradation occur when it is subjected to high temperature or drought. Since chlorophyll stability index is a function of temperature, the property of chlorophyll pigment can be correlated with drought tolerance or susceptibility of crop plants. Based on CSI, plants are classified as tolerant, resistant and susceptible to drought as in Table 9.

Table 9. Categorization of drought tolerant black pepper plants based on chlorophyll stability index (CSI)

Sl no	CSI (%)	Drought tolerance capacity
1	>85	Highly drought tolerant
2	75-85	Moderately drought tolerant
3	65-75	Drought susceptible
4	< 65	Can not survive under drought

To estimate chlorophyll stability index , weighed 100mg of leaf sample cut into small pieces and transferred into two test tubes and added 20ml of distilled water (control) and hot water at 55°C (treatment) respectively. The treatment tube was kept for 30 minutes in a water bath. After the treatment time the leaf bits were taken out from each test tube and macerated with 10ml of 80 per cent acetone and absorbance measured at 652nm in a visible spectrophotometer. Total chlorophyll content was estimated using Arnon's formula

$$\text{Total chlorophyll/g tissue} = \frac{\text{OD at 652 nm} \times 1000 \times V}{34.5 \times 1000 \times W}$$

The chlorophyll stability index is the ratio of total chlorophyll content of the treated sample to the control sample expressed in percentage.

$$\text{Chlorophyll stability index (\%)} = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100$$

3.3.4.3.4. Estimation of membrane stability index (MSI)

Consequent to high temperature or stress, the permeability of plasma membrane is affected resulting in loss of compartmentation and leaking of cell causing collapse and death of cells. Determination of leaf membrane stability index helps to assess abiotic stress tolerance capacity of crop plants, high MSI indicates drought tolerance. At each drought stage, MSI was assessed for each accession following the method of Premachandra *et al.* (1991) as modified by Sairam (1994). Two sets of 100mg of leaf sample was taken in two test tubes containing 10ml of double distilled water from each accession. One test tube was kept at 40°C for thirty minutes in a water bath and the other at 100°C in a boiling water bath for fifteen minutes. Both samples were allowed to cool to room temperature. Electrical conductivity (EC) was measured as C_1 and C_2 respectively using a conductivity meter. The MSI was calculated using the formula

$MSI = [1- C_1/ C_2] \times 100.$

3.3.4.4. Biochemical parameters

Biochemical parameters like proline, superoxide dismutase, catalase and peroxidases were analysed following standard procedures at each stage. All enzyme extractions were done under ice cold conditions using a pre-chilled pestle and mortar. The procedure followed by Dhindsa *et al.* (1981) was followed for assaying SOD, catalase and peroxidase. For enzyme extraction youngest fully matured leaves were collected from plants and both surfaces were cleaned with cotton to remove dirt and soil adhering to leaf surface. Then 0.5g of the leaf material was used for enzyme extraction after weighing in an electronic balance. The sample was ground in 10ml of 0.1M phosphate buffer (pH 7.2). The homogenate was filtered through 4 layers of cheese cloth and centrifuged at 10000 rpm for 20 minutes. The supernatant was used to assay SOD, catalase and peroxidase activities.

3.3.4.4.1. Estimation of Proline

Proline is a basic amino acid that accumulates in plant system during stress. It acts as an osmolyte and increases turgor and helps in the tolerance of dehydration of protoplasm and acts as a storage compound for C and N during moisture stress. By selective extraction with aqueous sulphosalicylic acid proteins are precipitated as complex. The extracted proline is made to react with ninhydrin in acid medium to form chromophore and is read at 520nm wavelength in a visible spectrophotometer (Sadasivam and Manickam, 2016).

Fresh leaf sample of 500mg taken from 3rd fully mature leaf of each accession was macerated with10 ml of 3 per cent sulphosalicylic acid in a mortar using pestle. Then the contents were centrifuged at 3000 rpm for 10 minutes. Two milliliter of the supernatant solution is collected in a test tube and added with 2ml each of acid ninhydrin and glacial acetic acid.The contents of the test tube is boiled in a water bath for one hour and the reaction was terminated by placing under ice bath. Then 4ml toluene was added to the test tube and it was shaken for 30s using a vibrator. The test tube was warmed to room temperature and the upper pink layer is collected in cuvette and absorbance at 520 nm was read in a visible spectrophotometer. Proline standards were prepared by dissolving 0.1g L- Proline in a 100ml standard measuring flask and the volume was made up to prepare make 1000 ppm solution. A series of 1ppm, 2 ppm, 3 ppm, 4 ppm,5 ppm and 6 ppm standards were made and absorbance was read. Concentration of Proline in the test sample is calculated from the standard curve of Proline.

Proline content on fresh weight basis

$$(\mu moles/\ g\ tissue) = \frac{\mu g\ Proline/\ ml \times ml\ \ toluene \times 5}{115.5 \times 0.5}$$

3.3.4.4.2. Estimation of Superoxide Dismutase (SOD)

Environmental stress like water stress result in excess production of active oxygen species such as super oxide, hydrogen peroxide and hydroxyl radicals, the elimination of which is essential to protect DNA and tissues. Super oxide dismutase (SOD) a metal containing enzyme plays a vital role in scavenging superoxide (O_2^-) radical during moisture stress.

$$O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2.$$

Hydrogen peroxide is eliminated by peroxidases and catalases.

Super oxide dismutase activity was estimated by recording the decrease in optical density of formazone formed by superoxide radical and nitro-blue tetrazolium dye by the enzyme. (Dhindsa *et al* .,1981).

The 3ml reaction cocktail was prepared by mixing 50mM potassium phosphate buffer at a pH of 7.8, 13mM methionine, 2µM riboflavin, 0.1mM EDTA, 75µM Nitro Blue Tetrazolium (NBT) and 50µl of crude enzyme extract . This was prepared in duplicate. A blank without enzyme and NBT kept in darkness was used to calibrate spectrophotometer. Another control having NBT but no enzyme was used as reference. Reaction was started by adding 2µM riboflavin (0.1ml) and placing the tubes with blank and samples under two 15 w fluorescent lamps for 15 minutes. The absorbance was recorded at 560nm. One unit of enzyme activity was taken as that amount of enzyme which inhibited 50 per cent of the reaction between riboflavin and NBT in the presence of methionine. This will reduce the absorbance reading to 50 per cent in comparison with tubes lacking enzyme as shown below.

$$\text{Unit of enzyme} = \frac{\text{Blank- Sample}}{\text{Blank/2}}$$

3.3.4.4.3. Estimation of Peroxidase (POD)

Peroxidase catalyses the reduction of hydrogen peroxide with concurrent oxidation of substrate . Guaicol was used as substrate for assay of peroxidase.

$$\text{Guaicol} + H_2O_2 \longrightarrow \text{Oxidised guaicol} + 2H_2O$$

The rate of formation of guaicol dehydrogenation product is a measure of the POD activity and is assayed spectrometrically at 436 nm.

Pipetted out 3ml 0.1M phosphate buffer (pH 7), 0.05ml guaicol solution, 0.1ml enzyme extract and 0.03ml hydrogen peroxide solution in a cuvette and the spectrophotometer reading was taken immediately. Reading was noted by starting a stop-watch when absorbance increased by 0.05 and the time required in minutes (Δt) taken to increase the absorbance by 0.1. Since the extinction coefficient of guaicol dehydrogenation product at 436 nm under conditions specified is 6.39 per micromole, the enzyme activity units per litre of extract is calculated as

$$\text{Enzyme activity units/ litre} = \frac{3.18 \text{ x } 0.1 \text{ x}1000}{6.39 \text{ x } 1 \text{ x } \Delta t \text{ x } 0.1} = \frac{500}{\Delta t}$$

3.3.4.4.4. Estimation of Catalase

Enzyme catalase catalyses the reduction of hydrogen peroxide to water and molecular oxygen. It is localized in mitochondria and peroxisomes and is absent in chloroplast, the important site of H_2O_2 generation. So it is a less efficient system for scavenging reactive oxygen species.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase assay is based on the absorbance of H_2O_2 at 240nm in UV- range. A decrease in the absorbance was recorded over a time period as described by Anderson *et al*. (1995). Three millilitre of assay reaction mixture consisted of 1.5ml of 100mM phosphate buffer, 0.5 ml of 75 mM H_2O_2, 50µl of enzyme and distilled water and made up to 3ml. After adding H_2O_2, the reaction was started and decrease in absorbance at 240 nm was recorded for one minute.

Foliar nutrition experiment

Components of foliar nutrition

Application of foliar nurtition

Recording absorbance using UV spectrophotometer

Plate 6. Experiment on screening of black pepper accessions for response to foliar nutrition

Enzyme activity/g fresh weight was calculated as concentration of hydrogen peroxide reduced (initial reading –final reading) per minute.

3.5. Screening of accessions for response to foliar nutrition under moisture stress

The accessions used for screening for drought tolerance were also subjected to foliar nutrition for the evaluation of biochemical response to moisture stress (Plate 6). The rooted cuttings were raised in 12 inch pots and the plants at 6 months of uniform age were subjected to water stress by withdrawing irrigation for five and ten days after attaining field capacity. Foliar application of 1.5 per cent sulphate of potash, 0.1 per cent IISR power mix (A nutrient mix of Zn, B, Mg and K) developed for black pepper by ICAR- IISR, Calicut) and 0.5 per cent PPFM (Pink Pigmented Facultative Methylotrops) developed by TNAU, Coimbatore were applied on 5th and 10th day of stress using a hand sprayer. The foliar nutrition with both treatments were followed on the same plants as they are compatible. Foliar nutrition was done at 9 am and 3 pm respectively on treatment day using suphate of potash (1.5%) and IISR power mix (0.1%) in the morning and Pink Pigmented Facultative Methylotrops (PPFM) (0.5%) in the evening on all the plants. Biochemical parameters were recorded from these plants after 3 days of foliar application *ie*. 8th and 13th day after field capacity, following the standard procedures. Observations were also recorded from plants without foliar nutrition (control) on 8th and 13th day of progressive moisture stress.

Results and Discussion

4. RESULTS AND DISCUSSION

The present study entitled 'Identification and characterization of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality' was carried out at Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur and Indian Council of Agricultural Research-Indian Institute of Spices Research (ICAR-IISR), Calicut during the period from 2016 to 2019.

Fifty accessions of black pepper maintained in the germplasm of ICAR-IISR, Calicut collected from various places across Kerala were utilized for the characterization and initial screening. The result of the present investigation on the performance of these accessions are presented below.

4.1. Morphological characterization

Observations were recorded for both qualitative and quantitative characters. Morphological characterization has been proven to be a pragmatic tool for deriving economic breeding achievements from valuable germplasm collections and families of related accessions (Bekele *et al*., 2006).

Characterization of elite lines and varieties of high-value crops like spices is important for protection of bio-wealth in the present WTO era. Conventionally, characterization of biodiversity using morphological features, and visually scorable morphological markers that correspond to quantitative traits are used for morphological characterization. Genetic diversity analysis based on clustering of fifty one cultivars of black pepper was performed by Mathew *et al*. (2001). Intra cultivar or inter varietal variability has been observed for morphological and qualitative characters in black pepper (Ratnambal *et al*., 1985).

Black pepper accessions and varieties have been traditionally classified based on plant characters such as leaf length and breadth, shoot tip colour, leaf shape and size, features of leaf tip and base, berry size, spike length, spike composition (bisexual, female and male), fruit set, number of fruits spike,$^{-1}$ 1000

Dimorphic branching

Polymorphic branching

Plate 7. Variation in branching pattern of main stem

berry volume, 1000 berry weight, yield vine-[1] and dry recovery. Thus genetically divergent genotypes identified from the germplasm on morphological data basis can be used in future breeding programmes.

4.1.1. Qualitative evaluation

The observations on qualitative characters showed that considerable variability was there among the accessions for 10 out of 15 qualitative characters. The qualitative characters *viz*. leaf margin, type of venation, spike orientation, type of hermaphroditism and fruit shape were uniform in all accessions. Hence, these five characters were not included in further analysis.

Similarity of various characters among the accessions may be due to chance crossing of all accessions over generations due to their co-existence in the wild, sharing common support trees in the natural habitat and conservation of variability due to successful vegetative propagation as proposed by Ravindran,1991. Human selection for yield and number of berries per spike could be the reason for retention of predominantly hermaphrodite nature observed in all accessions. The oval fruit shape of accession 7240 resembles the fruit shape of *Piper attenuatum*, a wild species of black pepper.

4.1.1.1. Leaf and vine character

The leaf and vine characters of the accessions are given in the Table 10. The branching pattern of main stem of majority of accessions was dimorphic. However, few of them exhibited polymorphism as well (Plate7).

Sixtysix per cent of accessions had light purple coloured shoot tip (33nos.), followed by dark purple (32%) and light green (2%) (Table 12). The shoot tip colour in black pepper is proven to be due to the complementary factor interaction between two pairs of genes as reported by Ravinrdan *et al*. (1992d). Accordingly, the light purple coloured accessions may have a genotypic constitution of $A_1a_1A_2a_2$, deep purple accessions with $A_1A_1A_2a_2$ and light green with $A_1a_1a_2a_2$ or $A_1A_1a_2a_2$.

The accessions differed in runner shoot production from absent , few (<5 numbers per vine) to many (>10 numbers per vine) (Plate no 8). The holding capacity of vine to the standard ranged from weak to strong (Plate 9). Adventitious root production (Plate 10 .) varied from few to many. The accessions differed in lateral branch habit (Plate11.) as, horizontal, erect and hanging.

In majority of accessions leaf lamina shape was oval (42%) followed by ovate-elliptical (28%), ovate-lanceolate (14%), cordate (12%) and elliptic-lanceolate (4%) (Plate 12 .).

The genetics of leaf shape in black pepper was reported to be of three basic shapes *viz*. cordate, ovate and oblong-elliptical controlled by multiple alleles (Sasikumar et al, 1992). Slight variation in these basic classes were noted in the present experiment. The leaf base shapes (Plate no 13.) recorded include acute, round and cordate. The oblique shape reported in the descriptor was not observed in any of the accessions. Majority of accessions had horizontal branch habit (42%) followed by hanging type (38%). Erect type of lateral branch habit which ensures better light penetration was observed for only 20 per cent of accessions. The leaf margin was even in all the accessions. Similarly the venation was campylodromous type in all the 50 accessions studied.

Runner shoot production-many

Runner shoot production-few

Plate 8.R Variation in runner shoot production of accessions

Plate 9. Variation in holding capacity of accessions

Adventitious root production-few

Adventitious root production -many

Plate 10. Variation in adventitious root production of accessions

Horizontal

Erect

Hanging

Plate 11. Variation in lateral branch habit

Plate 12.   Variation of leaf lamina shape

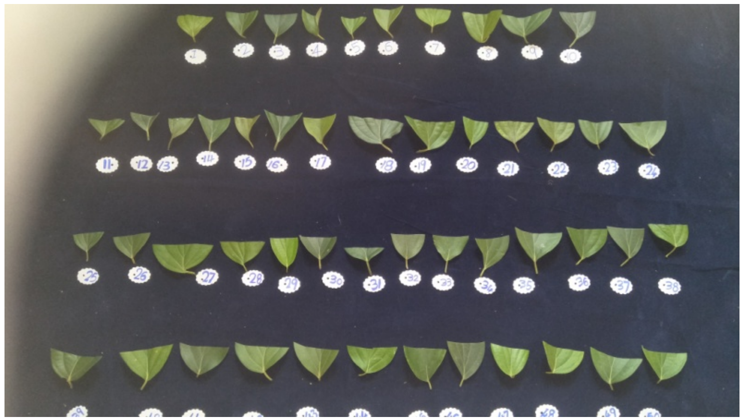


Plate 13. Variation of leaf base shape

Table 10. Evaluation of qualitative characters of vine and leaf of black pepper accessions

Accession	BH	STC	RSP	HC	ARP	LBH	LLS	LBS	LM	TV
7211	Dimorphic	Deep purple	Many	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous
7215	Dimorphic	Light purple	Few	Strong	Many	Erect	Ovate	Acute	Even	Campylodromous
7219	Dimorphic	Light purple	Few	Weak	Few	Horizontal	Ovate	Acute	Even	Campylodromous
7220	Dimorphic	Light purple	Few	Weak	Few	Horizontal	Ovate-lanceolate	Acute	Even	Campylodromous
7221	Polymorphic	Light purple	Few	Strong	Many	Horizontal	Ovate	Acute	Even	Campylodromous
7222	Polymorphic	Deep purple	Many	Weak	Few	Horizontal	Ovate	Acute	Even	Campylodromous
7225	Dimorphic	Light purple	Many	Weak	Few	Erect	Ovate-elliptic	Round	Even	Campylodromous
7229	Dimorphic	Light purple	Many	Strong	Many	Horizontal	Ovate-lanceolate	Acute	Even	Campylodromous
7230	Dimorphic	Light purple	Many	Weak	Few	Hanging	Ovate-elliptic	Round	Even	Campylodromous
7232	Dimorphic	Deep purple	Many	Strong	Many	Erect	Ovate	Acute	Even	Campylodromous
7236	Dimorphic	Light purple	Many	Weak	Few	Horizontal	Ovate	Acute	Even	Campylodromous
7237	polymorphic	Light purple	Many	Strong	Many	Hanging	Ovate	Acute	Even	Campylodromous
7239	polymorphic	Light purple	Many	Strong	Many	Erect	Ovate	Acute	Even	Campylodromous
7240	Dimorphic	Light purple	Few	Strong	Many	Erect	Ovate-elliptic	Acute	Even	Campylodromous
7241	Dimorphic	Light	Many	Weak	Few	Erect	Ovate-	Acute	Even	Campylodromous

Table 10. (Contd)

		purple					elliptic			
7243	Dimorphic	Light purple	Few	Strong	Many	Horizontal	Ovate-elliptic	Acute	Even	Campylodromous
7249	Polymorphic	Deep purple	Many	Strong	Many	Hanging	Elliptic-lanceolate	Acute	Even	Campylodromous
7251	Dimorphic	Deep purple	Few	Strong	Many	Erect	Cordate	Cordate	Even	Campylodromous
7252	polymorphic	Light purple	Few	Weak	Few	Horizontal	Ovate-elliptic	Acute	Even	Campylodromous
7254	Dimorphic	Light purple	Few	Strong	Many	Hanging	Ovate	Round	Even	Campylodromous
7255	Dimorphic	Light purple	Many	Strong	Many	Horizontal	Ovate	Round	Even	Campylodromous
7258	polymorphic	Light purple	Few	Weak	Few	Erect	Ovate-elliptic	Round	Even	Campylodromous
7259	polymorphic	Deep purple	Many	Strong	Many	Hanging	Ovate	Acute	Even	Campylodromous
7260	Dimorphic	Light purple	Few	Weak	Few	Horizontal	Cordate	Round	Even	Campylodromous
7262	Dimorphic	Light purple	Many	Strong	Many	Horizontal	Ovate-elliptic	Acute	Even	Campylodromous
7264	Dimorphic	Light purple	Few	Weak	Few	Erect	Ovate-elliptic	Acute	Even	Campylodromous
7276	Dimorphic	Light purple	Few	Weak	Few	Hanging	Ovate-elliptic	Acute	Even	Campylodromous
7277	Dimorphic	Light purple	Absent	Weak	Few	Horizontal	Elliptic-lanceolate	Round	Even	Campylodromous
7283	Dimorphic	Deep purple	Few	Weak	Few	Erect	Ovate	Acute	Even	Campylodromous
7285	Dimorphic	Light purple	Few	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous

Table 10. (Contd)

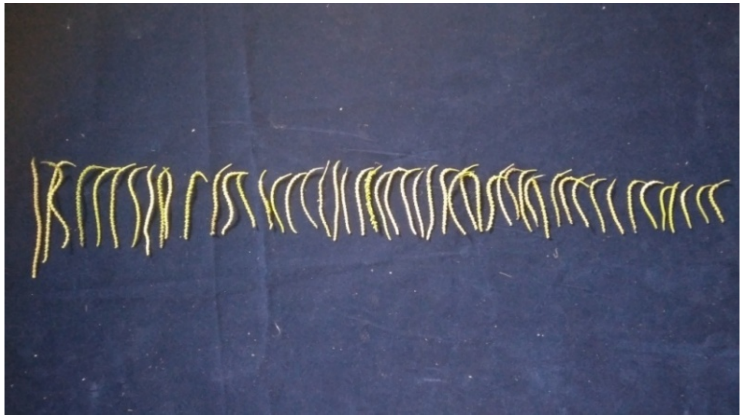
7286	Dimorphic	Deep purple	Few	Weak	Few	Horizontal	Ovate	Round	Even	Campylodromous
7288	Dimorphic	Deep purple	Absent	Weak	Few	Hanging	Ovate-elliptic	Round	Even	Campylodromous
7289	Dimorphic	Deep purple	Few	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous
7293	Dimorphic	Light purple	Few	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous
7295	Dimorphic	Light purple	Few	Weak	Few	Hanging	Ovate-elliptic	Acute	Even	Campylodromous
7296	Dimorphic	Light purple	Absent	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous
7297	Dimorphic	Light purple	Absent	Weak	Few	Hanging	Ovate -lanceolate	Acute	Even	Campylodromous
7298	Dimorphic	Light purple	Many	Strong	Many	Hanging	Ovate-Lanceolate	Acute	Even	Campylodromous
7302	Dimorphic	Deep purple	Few	Strong	Many	Erect	Cordate	Round	Even	Campylodromous
7305	Dimorphic	Deep purple	Many	Strong	Many	Erect	Ovate-lanceolate	Acute	Even	Campylodromous
7306	Dimorphic	Light purple	Few	Strong	Many	Hanging	Ovate	Acute	Even	Campylodromous
7309	Dimorphic	Light purple	Few	Weak	Few	Hanging	Ovate	Acute	Even	Campylodromous
7325	Dimorphic	Light purple	Absent	Weak	Few	Hanging	Ovate	Round	Even	Campylodromous
7339	Dimorphic	Light purple	Absent	Weak	Few	Hanging	Cordate	Round	Even	Campylodromous
7344	Dimorphic	Light purple	Absent	Weak	Few	Horizontal	Ovate-lanceolate	Acute	Even	Campylodromous
7352	Dimorphic	Deep purple	Few	Weak	Few	Hanging	Ovate-elliptic	Round	Even	Campylodromous

Table 10. (Contd)

7358	Dimorphic	Deep purple	Many	Weak	Few	Hanging	Ovate-lanceolate	Round	Even	Campylodromous
7385	Dimorphic	Light green	Absent	Strong	Many	Horizontal	Ovate-elliptic	Round	Even	Campylodromous
7386	Dimorphic	Deep purple	Absent	Weak	Few	Hanging	Cordate	Cordate	Even	Campylodromous
7387	polymorphic	Deep purple	Few	Weak	Few	Hanging	Cordate	Cordate	Even	Campylodromous

BH-Branching habit, STC- Shoot tip colour, RSP- Runner shoot production, HC-Holding capacity, ARP - Adventitious root production, LLS - Leaf lamina shape, LBS - Leaf base shape, LBH-Latera branch habit LM - Leaf margin, TV- Type of venation

Mature spikes



Immature spikes

Plate 14.Variation in spike characters of 50 accessions

4.1.1.2. Spike and fruit characters

The qualitative characters of spike and fruit of the different accessions like spike orientation, spike shape, type of hermaproditism and fruit shape showed perfect similarity (Table 11). There were three types of spike colour variation observed among the accessions *viz*. light yellow, greenish yellow and light green. The majority of accessions (60%) possessed light yellow, followed by greenish yellow (30%) and light green (10%) spike colour on complete emergence (Plate14.). The spike orientation and shapes were pendent and filiform, respectively, in all accessions. However, a rare type of spike orientation such as erect type was reported in related species such as *Piper longum* and *Piper chaba* and globose spike in *Piper sylvaticum* (Parthasarathy *et al*., 2006).Oval shaped fruit was observed only in the accession 7240 while the remaining accessions had spherical shaped fruits.

Table 11. Evaluation of qualitative characters of spike and fruit of black pepper accessions

Accession	Spike orientation	Spike shape	Spike colour	Type of hermaphroditism	Fruit shape
7211	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7215	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7219	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7220	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7221	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7222	Pendant	Filiform	Light green	Predominantly bisexual	Round
7225	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7229	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7230	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7232	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7236	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7237	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7239	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7240	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Oval
7241	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7243	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7249	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7251	Pendant	Filiform	Light yellow	Predominantly bisexual	Round

Table 11. (Contd)

7252	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7254	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7255	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7258	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7259	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7260	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7262	Pendant	Filiform	Light green	Predominantly bisexual	Round
7264	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7276	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7277	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7283	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7285	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7286	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7288	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7289	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7293	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7295	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7296	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7297	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7298	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7302	Pendant	Filiform	Greenish	Predominantly	Round

Table 11. (Contd)

			yellow	bisexual	
7305	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7306	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7309	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7325	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7339	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7344	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7352	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7358	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7385	Pendant	Filiform	Light yellow	Predominantly bisexual	Round
7386	Pendant	Filiform	Greenish yellow	Predominantly bisexual	Round
7387	Pendant	Filiform	Light yellow	Predominantly bisexual	Round

Based on morphological characterization of qualitative characters in black pepper accessions, the percentage of accessions falling into each category is presented in Table 12.

Table 12. Variation among qualitative characters of of black pepper accessions

Character	Description	Percentage of accessions
Branching habit	Dimorphic Polymorphic	(84) (16)
Shoot tip colour	Light purple Dark purple Light green	(66) (32) (2)
Runner shoot production	Many Few Absent	(26) (56) (18)
Holding capacity	Strong Weak	(38) (62)
Adventitious root production	Many Few	(40) (60)
Lateral branch habit	Horizontal Hanging Erect	(42) (38) (20)
Leaf lamina shape	Ovate Ovate-elliptic Ovate lanceolate Elliptic- lanceolate Cordate	(42) (28) (14) (4) (12)
Leaf base shape	Round Cordate Acute	(28) (4) (68)
Fruit shape	Round Ovate	(98) (2)

Branching pattern of main stem was dimorphic in majority of accessions. However, polymorphic branching with more than three branches were also observed in few accessions. Shoot tip colour in majority of accessions was light purple. However, dark purple and light green colour was also observed among few accessions. Runner shoot production of the accessions varied from absent, few (< 5 numbers per vine) and many (> 10 numbers per vine). Holding capacity of the vine indicated the strength of attachment of vine to the standard and it ranged from weak to strong across the accessions depending on the extent of cluster of sticky roots from nodes on the main stem . Adventitious roots produced ranged from few (< 5 numbers per node) to many (> 10 numbers per node) on the main stem.

Lateral branch habit referred to the orientation of lateral branches with respect to the horizontal axis. Majority of the accessions showed horizontal followed by hanging pattern. The erect branching, considered to be more efficient for tapping solar radiation was observed only in 20 per cent of the accessions. The oval leaf lamina shape was observed in majority of accessions. However, variations like ovate-elliptic, ovate - lanceolate and cordate were also observed. The elliptic-lanceolate leaf laminate shape was observed only for four per cent of the accessions. The major leaf base shapes observed were acute, round and cordate. The fruit shape was spherical for all accessions with the exception of ovate shape for the accession 7240. The oval fruit shape of accession 7240 resembles the fruit shape of *Piper attenuatum*, a wild species of black pepper.

4.1.2. Cluster analysis of accessions for qualitative characters.

Cluster analysis was done for qualitative characters of the 50 accessions based on binomial distance using online software R-soft and dendrogram was created.

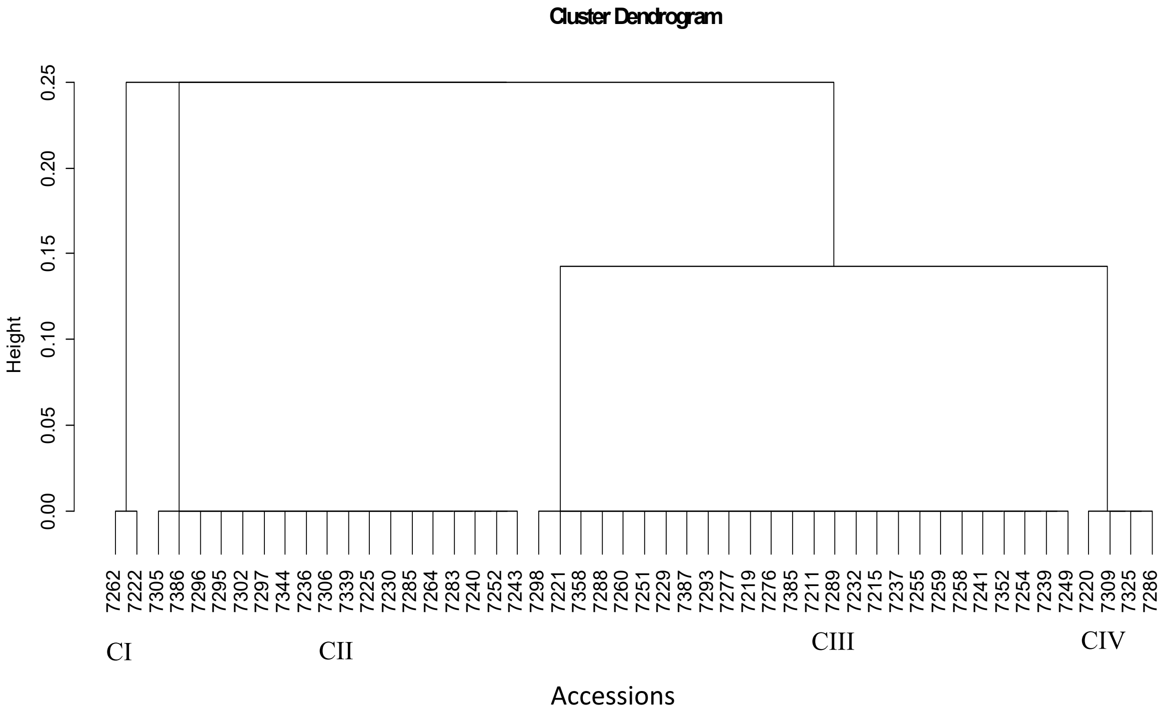


Fig.1. Dendrogram based on binomial distance for qualitative traits of fifty black pepper accessions

Table 13. Clustering/ grouping of black pepper accessions based on qualitative traits

Group	Accessions	No. of accessions	Characteristics
CI	7222 and 7262	2	Polymorphic branching with deep purple shoot tip colour, more runner shoots, bearing horizontal lateral branches and light yellow spikes.
CII	7225, 7230, 7236, 7240, 7243, 7252, 7264, 7283, 7285, 7295, 7296, 7297, 7302, 7305, 7306, 7339, 7344 and 7386	18	Dimorphic branching with deep purple shoot tip, few runner shoots, erect lateral branches, ovate - lanceolate leaves with acuminate leaf base and greenish yellow spike
CIII	7211, 7215, 7219, 7221, 7229, 7232, , 7237, 7239, 7241, 7249 ,7251, 7252, 7254, 7255, 7258, 7259, 7260 , 7276, 7277, 7288, 7289, 7293, 7298, 7358, 7385, and 7387	26	Dimorphic branching with few runner shoots, hanging lateral branches, oval leaf with acute leaf base , light yellow spikes and round berries
CIV	7220, 7286, 7309 and 7325	4	Dimorphic branching with few runner shoots, weak holding capacity, horizontal lateral branches and ovate - lanceolate leaf with acute leaf base and round fruits

Based on morphological similarity for qualitative characters the accessions are grouped. The dendrogram grouped the accessions in 4 clusters (Table 13).

The hierarchical cluster analysis showed that 26 out of 50 black pepper accessions are clustered in one group CIII, which indicates the absence of significant diversity for morphological characters among them. (Fig.1). The CI consisted of 2 accessions *viz.* 7222 and 7262 having deep purple shoot tip colour, polymorphic branch habit with more runner shoot production habit bearing horizontal lateral branches and with light yellow spikes.

The second cluster consisted of 18 accessions *viz*. 7225, 7230, 7236, 7240, 7243, 7252, 7264, 7283, 7285, 7295, 7296, 7297, 7302, 7305, 7306, 7339, 7344 and 7386 having dimorphic branching habit with deep purple shoot tip, erect lateral branch habit, greenish yellow spike colour and ovate-lanceolate leaves with acuminate shape of leaf base.

The third cluster is the major cluster consisting of 26 accessions *viz*. 7211, 7215, 7219, 7221, 7229, 7232, , 7237, 7239, 7241, 7249 ,7251, 7252, 7254, 7255, 7258, 7259, 7260 , 7276, 7277, 7288, 7289, 7293, 7298, 7358, 7385, and 7387. They are similar with respect to dimorphic branching pattern and other characters like few runner shoot production, hanging lateral branches, oval leaf shape and acute leaf base bearing light yellow spikes and round berries.

The fourth cluster comprised of 4 accessions *viz*. 7220, 7286., 7309, and 7325 They were having dimorphic branching, few runner shoot production, weak holding capacity, horizontal lateral branch habit and ovate-lanceolate leaf lamina shape with acute leaf base shape and round berries.

4.1.3. Morphological characterization for quantitative traits

4.1.3.1. Quantitative characters of leaf and stem

Quantitative characters of shoot, leaf and reproductive characters of 50 accessions were measured from each vine in the field germplasm as given in Tables 14 and 15.

Table 14. Quantitative characters of leaf and stem of black pepper accessions

Accession	Vine length (m)	Length of lateral (cm)	No.of nodes/lateral	Petiole length (cm)	Leaf length(cm)	Leaf width(cm)
7211	3.10	50.10	16.60	1.06	14.40	6.98
7215	3.00	57.20	42.80	1.32	12.18	5.40
7219	2.30	57.34	21.60	1.00	11.38	5.32
7220	2.10	36.74	20.00	1.66	12.16	7.46
7221	2.80	67.12	13.40	1.18	16.92	7.92
7222	1.70	66.54	27.40	1.02	11.94	6.32
7225	2.65	22.62	12.00	1.00	14.74	7.08
7229	3.50	38.48	22.00	1.56	17.66	9.24
7230	1.75	33.72	12.40	1.04	14.06	7.90
7232	3.95	70.64	28.20	1.76	15.38	8.84
7236	1.80	30.08	14.00	1.10	14.02	7.12
7237	2.70	55.88	22.40	1.76	12.58	7.16
7239	3.09	48.40	9.40	1.92	12.62	5.78
7240	2.73	36.34	8.40	1.54	14.04	8.18
7241	3.09	46.00	9.40	2.52	11.56	6.74
7243	3.30	55.12	21.80	1.32	11.54	6.44
7249	2.34	32.40	22.40	1.40	12.22	5.60
7251	3.64	34.26	16.20	3.49	16.26	11.60
7252	3.80	62.12	52.80	2.26	13.70	8.24
7254	3.27	47.94	40.40	1.30	11.20	6.40
7255	3.40	48.35	11.60	1.52	15.76	7.70
7258	2.10	50.12	24.50	1.24	13.06	6.70
7259	2.80	53.30	42.20	1.08	12.50	6.10
7260	1.85	28.92	11.2	1.72	14.88	8.62
7262	2.70	60.18	28.00	1.44	15.2	9.24

Table 14. (Contd)

7264	2.50	31.88	16.20	1.56	15.6	9.36
7276	2.14	44.56	30.80	1.02	15.94	8.46
7277	2.20	36.00	19.80	1.14	16.62	11.10
7283	2.84	58.94	38.00	2.10	14.56	6.62
7285	2.33	62.28	29.80	1.64	12.78	7.04
7286	2.75	65.20	35.20	1.58	13.08	7.18
7288	1.29	28.76	7.20	1.88	13.48	8.14
7289	2.30	42.48	10.80	3.08	13.34	7.60
7293	2.47	40.32	28.20	1.24	14.16	8.34
7295	1.82	30.98	11.80	1.26	16.56	8.28
7296	1.65	29.62	16.00	1.62	13.5	6.22
7297	1.73	29.36	8.40	1.46	15.44	9.66
7298	2.68	39.58	21.40	1.14	16.9	7.28
7302	2.80	66.06	23.20	2.32	16.34	9.28
7305	2.78	57.90	12.80	2.70	17.10	9.22
7306	3.10	39.60	26.40	1.66	14.10	8.90
7309	1.60	42.40	22.60	1.10	13.40	7.60
7325	1.80	30.82	22.60	1.36	11.40	6.42
7339	1.98	37.28	8.40	3.22	11.14	8.12
7344	1.80	19.10	12.80	1.22	14.6	9.10
7352	2.30	37.90	12.60	2.26	15.24	8.32
7358	1.80	31.08	14.60	3.18	15.02	9.48
7385	1.90	42.84	23.20	1.34	15.52	8.4
7386	1.80	25.98	11.60	1.56	13.34	8.08
7387	2.24	25.98	9.00	3.50	11.54	9.46
General Mean	2.48	43.64	20.49	1.79	14.05	7.83

Vine length was measured as the vine column length measured from ground level to the top tip of orthotropic shoot using measuring tape . The vine length ranged from 1.29 m to 3.95 m among the 50 accessions The lowest vine length was recorded for the accession 7288 and highest for the accession 7232. The overall mean of vine length was 2.48 m.

The length of lateral was measured as the average of 50 randomly selected laterals from the plant for every accession. Length of lateral also showed wide variation among the accessions ranging from 19.10cm to 70.64 cm with the overall mean of 43.64 cm. The accession 7232 recorded the longest lateral where as accession 7344 recorded shortest lateral.

Number of nodes per lateral showed wide variation among the accessions ranging from 7.20 to 52.80 with overall average of 20.33. The accession 7252 had the highest number of nodes / lateral and the accession 7288 had the minimum number of nodes/lateral.

The petiole length was measured as the average of 50 randomly selected mature leaves from lateral branches measured from base to the point of insertion with leaf blade. The overall mean of this character was 1.79 cm with the values ranging from 1.00 cm to 3.50 cm. The shortest petiole length was observed in two accessions *viz*.7219 and 7225 while the accession7387 registered the maximum value. The mean petiole length of leaves on lateral branches reported in cultivars ranged between 1.2 cm in Sreekara and Subhakara to 1.9 cm in Girimunda (Krishnamurthy *et al.,* 2010).

Leaf length was measured as the average of 50 randomly selected mature leaves from lateral branches measured from base of midrib to tip. Leaf characters serves as a major feature for cultivar identification in black pepper. The leaf size and shape of the emerging orthotropic shoots and runners differ from normal leaves on lateral fruiting branches. All the accessions showed wide variation ranging from 11.14 cm to 17.66 cm for this character with a mean of 14.05 cm. The maximum and minimum values for leaf length was recorded by the accessions 7229 and 7339 respectively. Similarity in spike length and leaf lamina length has been reported in black pepper cultivars (Ravindran, *et al*., 2000).

Leaf size in varieties / cultivars were earlier reported to vary from 8 to 20 cm or more in length and 4.00 to 12.00 cm or more in breadth. Leaf length and width were lowest in Sreekara (10.00 and 6.70 cm) and highest in HP 1411 (12.00 and 9.70 cm) (Purseglove *et al*., 1981).

In the present experiment leaf width was measured as the average of 50 randomly selected mature leaves from laterals. The variability of observed mean values ranged from 5.32 cm to 11.60 cm with the overall mean value of 7.83 cm. Maximum value for leaf width was observed in the the accession 7251 while the accession 7219 recorded the minimum.

4.1.3.2. Quantitative characters of spike and fruit of black pepper accessions

Eleven spike and fruit characters *viz.* spike length, peduncle length, number of spikes/lateral, fruit setting %, number of developed fruits/spike, 100 fruit weight, 100 fruit volume, green spike yield/ vine, green berry yield/ vine, dry berry yield/ vine and driage of accessions were recorded in 2017. The results are presented in Table 15.

Table 15. Quantitative characters of spike and fruit of black pepper accessions

Accession	Spike length (cm)	Peduncle length (cm)	No.of spikes /lateral	Fruit setting (%)	No. of fruits /spike	100 fruit weight (g)	100 fruit volume (ml)	Green spike yield/ vine (g)	Green berry yield/ vine (g)	Dry berry yield/ vine (g)	Driage (%)
7211	10.90	0.94	7.80	72.70	42.20	14.71	10.40	3350.00	2740.00	831.00	30.33
7215	8.08	1.16	16.00	87.06	44.00	13.98	14.00	3400.00	2500.00	926.00	37.04
7219	9.40	1.26	12.60	77.21	35.60	12.02	12.00	4050.00	3325.00	1128.00	33.92
7220	5.88	0.76	4.60	68.25	48.00	10.88	9.60	405.00	332.10	112.25	33.80
7221	8.66	1.24	6.00	80.71	45.60	12.75	15.00	9100.00	8200.00	2375.00	28.96
7222	7.76	1.26	12.2	86.10	59.00	13.43	10.30	9100.00	8200.00	2374.00	28.95
7225	11.22	1.10	9.20	66.48	52.71	9.65	9.00	365.00	277.40	87.94	31.70
7229	12.04	1.60	4.00	80.22	50.8	14.08	13.04	3000.00	2650.00	821.00	30.98
7230	10.12	1.14	18.8	68.00	53.13	10.25	9.70	381.00	308.61	100.29	32.49
7232	9.10	1.28	13.80	81.69	47.60	13.25	13.00	15650.00	14000.00	4518.00	32.27
7236	9.54	1.10	18.80	70.21	56.04	10.28	9.30	389.00	315.10	102.72	32.60
7237	7.98	0.90	26.40	10.25	61.23	10.10	9.80	430.00	344.00	95.98	27.90
7239	11.0	1.50	7.20	70.25	48.36	10.26	10.00	383.00	302.56	93.62	31.00

Table 15. (Contd)

	0										
7240	8.36	1.04	9.20	83.40	43.60	10.13	9.00	5035.00	3900.00	1300.00	33.33
7241	5.28	0.98	3.40	57.85	81.20	9.95	13.00	1100.00	825.00	225.00	27.27
7243	9.28	1.00	6.20	81.45	49.6	10.10	10.00	11450.00	8600.00	2641.00	30.71
7249	8.64	1.12	9.80	83.51	55.00	9.80	14.00	1200.00	1000.00	270.00	27.00
7251	7.18	1.26	5.00	68.00	54.13	9.85	9.10	371.00	290.50	90.06	31.00
7252	7.68	1.40	19.00	88.49	46.80	12.03	11.00	5000.00	4150.00	1380.00	33.25
7254	10.10	1.15	5.20	72.40	44.80	17.77	16.00	2560.00	2194.00	770.00	35.10
7255	8.96	1.32	40.40	83.00	59.40	12.70	15.00	6100.00	5000.00	1544.00	30.88
7258	7.02	1.04	14.20	68.65	57.16	9.85	9.00	375.00	288.75	82.08	28.42
7259	8.78	1.14	22.84	77.21	47.20	11.48	12.10	4250.00	3725.00	1138.00	30.55
7260	7.06	1.04	3.60	69.25	53.05	9.76	9.00	366.00	275.00	82.23	29.90
7262	8.54	1.12	11.40	71.00	58.12	10.05	9.20	386.00	310.70	87.00	28.00
7264	11.48	1.00	4.80	68.40	58.00	10.26	9.50	318.00	254.20	69.90	27.50
7276	11.58	1.06	8.80	54.45	43.00	10.76	10.00	550.00	450.00	158.00	35.11
7277	8.66	1.10	2.40	69.75	56.42	10.31	10.00	338.00	263.65	82.00	31.11
7283	9.60	1.12	5.20	69.20	56.11	10.25	9.40	425.00	340.00	107.10	31.50
7285	8.94	1.06	25.60	86.50	57.60	10.74	11.00	2560.00	2350.00	710.00	30.21
7286	8.20	1.10	14.80	87.29	52.80	14.39	13.00	2800.00	2500.00	621.00	24.84
7288	6.92	1.04	2.60	67.21	45.12	9.92	9.00	421.00	332.60	98.12	29.50
7289	8.24	1.12	6.40	66.60	37.20	12.68	12.00	885.00	700.00	243.00	34.71

Table 15. (Contd)

7293	9.00	1.58	10.00	80.26	45.20	13.47	13.00	1300.00	1250.00	411.00	32.90
7295	11.00	1.18	4.20	66.66	34.21	10.21	9.80	370.00	296.00	88.21	29.80
7296	8.14	1.22	4.00	69.25	42.01	9.58	9.10	386.00	308.80	89.55	29.00
7297	8.04	1.16	3.20	66.00	42.23	10.44	9.30	326.00	264.06	86.08	32.60
7298	7.76	1.38	5.80	69.21	37.00	10.78	9.80	372.00	302.00	83.96	27.80
7302	8.94	1.54	2.20	65.8	39.13	10.76	10.10	380.00	311.6	104.39	33.50
7305	9.04	1.08	2.80	68.40	36.41	10.52	9.30	385.00	311.85	92.00	29.50
7306	9.20	1.08	5.40	65.21	51.22	10.59	10.30	385.00	316.00	104.28	33.00
7309	8.30	1.08	10.6	71.00	54.22	10.45	9.50	387.00	317.34	104.09	32.80
7325	7.94	1.02	2.60	64.26	46.28	10.13	9.70	408.00	322.32	106.04	32.90
7339	6.62	1.04	2.40	59.65	42.40	10.43	10.20	420.00	340.00	109.48	32.20
7344	10.90	1.16	6.40	63.45	41.14	9.87	9.10	311.00	233.25	75.11	32.22
7352	5.20	1.00	6.80	65.43	47.53	10.68	10.40	383.00	310.00	93.00	30.00
7358	7.06	0.98	6.00	67.50	46.22	10.71	10.10	375.00	303.75	98.72	32.50
7385	10.80	1.16	4.80	69.45	49.05	10.43	10.10	376.00	302.65	96.85	32.00
7386	4.98	1.40	20.00	67.50	39.31	10.33	9.40	391.00	313.58	90.77	28.95
7387	6.22	1.16	3.40	62.15	49.03	9.95	9.10	431.00	336.18	96.82	28.80
General Mean	8.63	1.15	9.22	70.68	48.86	11.16	10.67	2077.58	1747.67	543.89	31.05

The spike length was recorded as the average of 50 mature spikes collected randomly at harvest and measured from tip to base using a scale. Similar to leaf length , the spike length is also a key character for the identification of a black pepper cultivar. The maximum spike length of 12.04 cm was recorded by the accession 7229. The spike and leaf characters of this accession resembles the cultivar Kalluvally suggesting that it may be derived from it. Minimum value of spike length of 4.98 cm was recorded by the accession 7386. The average spike length of the accessions was 8.63 cm. Length of spike is an important character contributing to yield in black pepper. Sujatha and Namboothiri (1995a.) reported a positive and significant association of spike length on yield.

Peduncle length of accessions ranged from 0.76 cm (7220) to 1.60 cm (7220) with a mean value of 1.15 cm among the accessions. The number of spikes per lateral showed wide variation ranging from 2.20 (7302) to 40.40 (7255) with a mean value of 9.22. Fruit setting percentage also showed wide variation ranging from 10.25 (7237) to 88.49 (7252) with a mean of 70.68 among the accessions. The number of developed fruits per spike ranged from 34.21 per cent (7295)) to 81.20 per cent (7241) with a mean of 48.86 per cent . Hundred fruit weight also showed significant variation among the accessions with a mean value of 11.16 g. The maximum value of 17.77 g was observed for the accession 7254 and the accession 7296 recorded the minimum value of 9.58 g for hundred fruit weight.

Hundred fruit volume which indicates the boldness of berries also showed wide variation among the accessions. Maximum value of 16.00 cc for this character was observed in the accession 7254 and the minimum value of 9.00 cc was observed in five accessions *viz.* 7225, 7240, 7258, 7260, and 7288 showing that these accessions have small berry size.

Green spike yield/plant ranged from 311g (7344) to 15650 g (7232) with a mean yield of 2077.58 g among the accessions. Berry yield per vine ranged from

233.25g (7344) to 14000 g (7232) with a mean value of 1747.67 g. Dry yield/ vine was estimated by following hot water treatment of berries. It ranged from 69.90 g (7264) to 4518 g (7232) with a mean value of 543.89 g among the accessions. The driage of accessions ranged from 24.84 per cent (7286)) 37.04 per cent to (7215) with a mean value of 31.05 per cent. The national average yield of pepper is 434 g per vine (Spices Board, 2017). However, in the present study 15 accessions showed dry yield of more than 500g per vine indicating superior performance of these accessions. This has to be evaluated further for establishing the superiority and stability over years.

4.1.4. Cluster analysis of the accessions for quantitative characters

Based on similarity in morphological characters, accessions were grouped. The hierarchical cluster analysis showed that 35 out of 50 black pepper accessions belong to one major group, which indicated the absence of significant morphological divergence among them (Fig 2). The dendrogram analysis indicated two major clusters comprising 50 accessions (Table 16).The first cluster (C_I) had 46 accessions grouped under the sub clusters with 5, 6 and 35 accessions each (SCA_1, SCA_2, SCA_3).The second cluster (C_{II}) had two sub clusters (SCB_1, SCB_2) grouped with one and three accessions each.

The first sub cluster (SCA_1) consists of 5 accessions *viz.* 7255, 7240, 7252, 7259 and 7219 with medium tall vine (2 - 3 m), medium long laterals (50 - 60 cm), medium no. of nodes (20 - 35), short petiole length , long leaf (> 14 cm), medium broad leaves (7 - 8 cm), short spikes (6-8cm), medium long peduncle (1- < 1.5 cm), medium dense berries (10 - 12 g), large berry size (> 13 cc), high yield (> 2000 g/ vine) and low driage (27 - 30 per cent).

Six accessions *viz.*7215, 7211, 7254, 7285, 7286, and 7229 with medium tall vine, medium long lateral, medium long petiole length, medium long leaf, narrow leaves (6-8 cm), short spikes (6-8 cm), medium long peduncle, medium number of nodes per lateral, high density berries (> 12 g), large berry size, medium yielders with high driage (> 33 per cent) were grouped in the second sub cluster, (SCA_2).

The third sub cluster (SCA_3)is the major cluster consisting of 35 accessions with characters *viz*. medium tall vine, long laterals, medium no of nodes/lateral, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spike intensity, low density berries, medium fruit size, low yield and high driage.

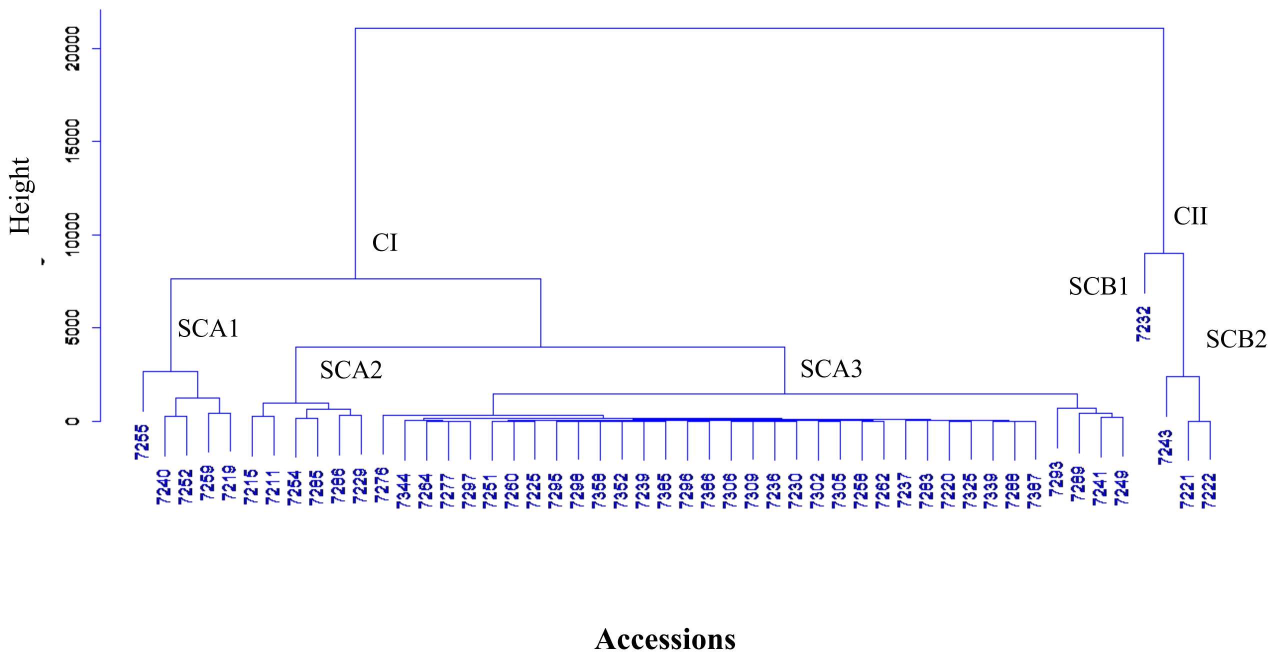


Fig 2. Dendrogram based on multivariate hierarchical cluster analysis for quantitative traits of black pepper accessions

The accession 7232 is the only accession grouped in the sub cluster SCB1 under the cluster CII. It differed from the rest of the accessions in all morphological and quantitative traits.The accession 7232 is high yielding, very tall, (> 3 m) with very long laterals, medium number of laterals , medium leaf petiole length , long leaf, medium wide leaf, medium long spike , medium long peduncle , low spiking intensity

, medium dense berries , medium sized berries , very high yield and medium driage. All these traits of 7232 have a close resemblance to the cultivar Kaniakkadan.

The sub cluster (SCB_2) under CII consists of 3 accessions *viz*. 7243, 7221 and 7222 with common characters *viz*. tall vine, medium long lateral, low number of nodes/ lateral, medium petiole length, medium long leaf, medium wide leaf, medium long spike, medium long peduncle, medium spike intensity, low density berries, high yield and low driage. The accession 7222 was with tall vine, medium long lateral, few number of nodes/lateral, medium petiole length, medium long leaf, medium wide leaf, medium long spike *etc*.

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Table 16. Quantitative cluster groups and characteristics of black pepper accessions

Group	Accessions	No. of accessions	Characteristics
SCA_1	7255,7240,7252,7259,7219	5	Medium tall vine, medium long laterals, medium no.of nodes, short petiole , long leaf, medium broad leaves, short spikes, medium long peduncle, medium dense berries, large berry , high yield and low driage.
SCA_2	7215,7211,7254,7285,7286,7229	6	Medium tall vine, medium long lateral, medium petiole length, medium long leaf, narrow leaves, short spikes, medium long peduncle, medium no.of nodes per node, high density berries, large berry size, medium yielders with high driage.
SCA_3	7276,7344,7264,7277, 7297,7251,7260,7225, 7295,7298,7358,7352, 7239,7385,7296,7386, 7306,7309,7236,7230, 7302,7305,7258,7262,	35	Medium tall vine ,long laterals, medium no of nodes/lateral, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spike intensity, low density berries, medium fruit size, low

Table 16. (Contd)

	7237,7283,7220,7325, 7339,7288,7387,7293, 7289,7241,7249		yield and high driage.
SCB_1	7232	1	Very tall vine, very long laterals, medium no. of laterals, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spiking intensity, medium dense berries, medium sized berries, very high yield and medium driage.
SCB_2	7243,7221,7222	3	Tall vine, medium long lateral, low no. of nodes/lateral, medium petiole length, medium long leaf, medium wide leaf, medium long spike, medium long peduncle, medium spike intensity, low density berries, high yield and low driage.

4.1.5. Ranking of black pepper accessions for yield and biotic stresses

In the first phase of the research, 50 bearing accessions in the germplasm were characterized using IPGRI descriptor, 1995. In the second phase, the accessions were scored based on yield and manifested field tolerance to biotic stresses as per IPGRI descriptor (Table 4).

4.1.5.1.Reaction to pollu beetle infestation

The accessions were observed for reaction to pollu beetle infestation (Table 17) . The scores obtained were ranging from 1-9 as per IPGRI descriptor . Fourteen accessions had a score of 1 indicating very low susceptibility, eight accessions scored 3 indicating low susceptibility and 14 accessions scored 5 showing moderate susceptibility to pollu beetle infestation. Fourteen accessions scored 7 and above indicating high to very high susceptibility to pollu beetle infestation.

4.1.5.2.Reaction to *Phytophthora* foot rot disease

Field reaction towards foot rot infestation of the accessions were scored adopting the score chart of IPGRI (Table17). Twenty two accessions recorded a score of 1 indicating field tolerance to phytophthora foot rot. Four accessions had the score of 5, fourteen accessions scored 7 and ten accessions had score of 9 indicating the susceptibility to phytophthora foot rot.

4.1.5.3. Green berry yield per vine

The green berry yield recorded in the accessions ranged from 90 g to 14000 g per vine (Table 17). Accession with berry yield of 450 g and above were only selected for further experiments. Four accessions selected had very high yield of more than 8000 g green berry per vine .They were 7232, 7221,7222 and 7243. Based on the stable performance, these accessions can be directly recommended for cultivation. Eleven accessions *viz.*7211, 7215, 7219, 7229, 7240, 7252, 7254, 7255, 7259, 7285, and 7286 were having an yield of more than 2000g green berry per vine. Considering their high yielding nature combining field tolerance to foot rot and pollu beetle, these accessions also can be recommended for multiplication and commercialization.

Table 17. Biotic susceptibility score and yield of black pepper accessions

Sl no	Accession	Score for foot rot infection	Score for pollu beetle infestation	Green berry yield/ vine (g)
1	7211	1	3	2740
2	7215	1	1	2500
3	7219	1	1	3320
4	7220	7	5	330
5	7221	1	3	8200
6	7222	1	1	8200
7	7225	7	5	280
8	7229	1	1	2650
9	7230	7	5	310
10	7232	1	1	14000

Table 17. (Contd)

11	7236	9	7	320
12	7237	7	5	340
13	7239	1	7	300
14	7240	1	1	3900
15	7241	1	1	820
16	7243	1	3	8600
17	7249	1	1	1000
18	7251	7	5	290
19	7252	1	3	4150
20	7254	1	1	2190
21	7255	1	3	5000
22	7258	7	7	280
23	7259	1	1	3720
24	7260	7	5	270
25	7262	5	5	310
16	7264	9	9	250
27	7276	1	1	450
28	7277	9	7	260
29	7283	7	5	340
30	7285	1	1	2350
31	7286	1	3	2500
32	7288	9	7	330
33	7289	1	3	700
34	7293	1	1	1350
35	7295	7	5	290
36	7296	7	7	300
37	7297	5	7	260
38	7298	5	7	300
39	7302	9	5	310
40	7305	7	5	310
41	7306	9	7	100
42	7309	7	9	100
43	7325	9	9	110
44	7339	5	7	110
45	7344	7	5	230
46	7352	1	1	90
47	7358	9	5	90
48	7385	9	3	90
49	7386	7	5	90
50	7387	9	7	90

4.1.5.4. Selection of accessions

Based on green berry yield, field tolerance to biotic stresses namely, foot rot and pollu beetle infection, 20 accessions were selected. Criteria fixed was field tolerance score of 1 for foot rot, 1and 3 for pollu beetle and green berry yield of more than 400g /vine. The details of the accessions advanced for further investigation are given in Table 18.

Table 18. List of 20 black pepper accessions selected for detailed evaluation

Sl.no.	Accession No.	IC No	Score for foot rot infection	Score for pollu beetle infestation
1	7211	598866	1	3
2	7215	598869	1	1
3	7219	598872	1	1
4	7221	598874	1	3
5	7222	598875	1	1
6	7229	598880	1	1
7	7232	598883	1	1
8	7240	598890	1	1
9	7241	598891	1	1
10	7243	598893	1	3
11	7249	598899	1	1
12	7252	598902	1	3
13	7254	598903	1	1
14	7255	598904	1	3
15	7259	598906	1	1
16	7276	598920	1	1
17	7285	598929	1	1
18	7286	598930	1	3
19	7289	598933	1	3
20	7293	598936	1	1

These twenty accessions were further evaluated for biochemical constituents determining the quality of pepper, tolerance to foot rot disease and tolerance to drought by biochemical and physiological analyses.

4.2. Biochemical characterization of selected accessions

The selected accessions were subjected to evaluation for commercially important biochemical constituents in dried berries. Content of piperine, volatile oil and oleoresin were analysed following standard procedures . The data were analysed statistically using online software RSOFT and the results are presented in Table 19.

Table 19. Quality parameters of 20 accessions of black pepper

Accession	Piperine (%)	Oil (%)	Oleoresin (%)
7211	5.68^{BC}	5.87^{A}	9.64^{C}
7215	5.49^{CD}	3.60^{IJ}	8.38^{E}
7219	4.33^{GHIJ}	3.87^{GHI}	10.79^{AB}
7221	4.28^{HIJ}	4.50^{CDE}	9.09^{CD}
7222	5.47^{CD}	4.67^{CD}	9.46^{C}
7229	3.61^{K}	3.47^{J}	7.10^{F}
7232	4.65^{FGH}	3.60^{IJ}	10.34^{B}
7240	4.28^{HIJ}	4.00^{FG}	7.43^{F}
7241	5.93^{B}	4.80^{C}	9.67^{C}
7243	4.35^{GHI}	4.27^{EF}	8.32^{E}
7249	5.68^{BC}	4.47^{DE}	9.58^{C}
7252	6.71^{A}	4.67^{CD}	10.28^{B}
7254	4.09^{IJ}	3.00^{K}	8.59^{DE}
7255	3.92^{JK}	5.47^{B}	7.52^{F}
7259	6.01^{B}	3.60^{IJ}	7.10^{F}
7276	3.99^{IJK}	3.93^{GH}	10.85^{AB}
7285	4.85^{EF}	4.00^{FG}	9.67^{C}
7286	4.73^{FG}	3.07^{K}	8.32^{E}
7289	5.18^{DE}	3.67^{HIJ}	11.18^{A}
7293	6.96^{A}	4.13^{FG}	9.04^{CD}
General Mean	5.00	4.13	9.12
SE	0.05	0.03	0.13
CV	4.52	4.29	4.02

The results of biochemical analysis (Table 19) has shown that there is significant variability among the accessions for all the characters of commercial value like piperine, oil and oleoresin content.

4.2.1.1. Piperine content of dry fruits

Maximum piperine content of 6.96 per cent was observed for the accession 7293 followed by 6.71 per cent for the accession 7252. The minimum piperine content was observed for the accession 7229 (3.61%). The mean value of piperine for all the accessions was 5.00 per cent. The accessions 7259, 7241, 7211 and 7249 were on par in high piperine content. Similarly accessions 7229, 7255, 7276, 7285, 7286 and 7232 were low in piperine content. In popular black pepper cultivars, piperine concentration varied from 2.8 per cent to 3.8 per cent (Zachariah *et al.*, 2005). Piperine content of 6.6 per cent has been recorded in Panniyur 2 (Zachariah , 2008).The piperine content of the accessions 7293 and 7252 recorded in the present experiment are greater than the maximum recorded value in the commercial variety Panniyur 2. Based on the quantitative estimation the accessions 7293, 7252, 7241, 7211 and 7249 were having piperine content of more than 5.5 per cent.

4.2.1.2. Volatile oil content of dry fruits

The volatile oil content of the accessions ranged widely among the accessions. Maximum oil content of 5.87 per cent was recorded for the accession 7211 followed by 5.47 per cent for the accession 7255. The mean value observed was 4.13 per cent .Few of the accessions shared similar oil content *viz.*7240 and 7285(4.00%), 7222 and 7252 (4.67%) 7215, 7232 and 7259 (3.60%). The minimum oil content of 3.00(%) was observed for the accession 7254. The accessions 7215, 7286, 7229, 7232 and 7289 were also poor in volatile oil content. The maximum value of oil content observed for the accession 7211 in the experiment (5.87%) was less than the maximum recorded value of 6.00 per cent, reported by Krishnamurthy *et al.*, (2010).

4.2.1.3. Oleoresin content of dry fruits

The oleoresin content of dry fruits ranged from 7.10 per cent (7229 and 7259) to 11.18 per cent (7289) among the accessions with a mean value of 9.12 per cent. There is significantly high oleoresin content for the accessions viz. 7289, 7276, 7219, 7232 and 7252 compared to rest of the accessions. Low oleoresin content was recorded by the accessions *viz.* 7229 (7.10%), 7259 (7.15%), 7240 (7.43%), 7255 (7.52%),7243 (8.32%) and 7286 (8.32%). However, all the accessions in the experiment expressed values lower than the maximum recorded value of oleoresin (15.45%) by the commercial variety PLD2.

4.2.2. Correlation of biochemical characters with yield

Correlation of yield with various biometrical characters *viz.,* piperine, volatile oil and oleoresin content were estimated (Table 20).

Table 20. Correlation between yield and biochemical characters of black pepper accessions

	Yield	Piperine	Oil	Oleoresin
Yield	1.00	-0.21	0.05	-0.03
Piperine		1.00	0.26	0.21
Oil			1.00	0.11
Oleoresin				1.00

Sainamlol *et al.*,(2008) had observed significant positive correlation between oil and oleoresin which was absent in the present study.

4.2.2.1. Cluster analysis of 20 selected accessions based on biochemical principles

Cluster analysis of 20 accessions was done based on various biochemical parameters. The dendrogram is depicted in Fig. 3

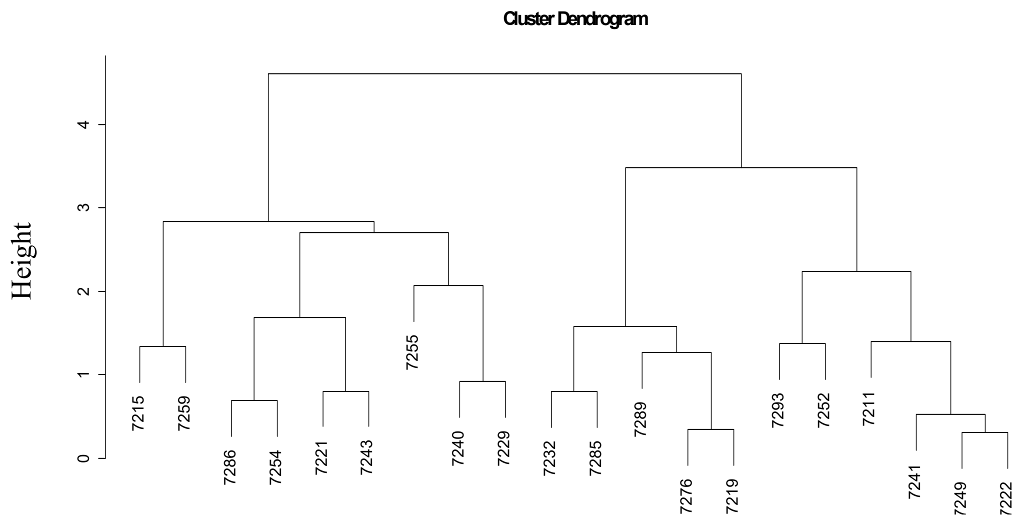

Fig.3. Dendrogram of 20 black pepper accessions for biochemical principles

The number of accessions falling under each cluster, based on piperine, oil and oleoresin content is presented in Table 21.

Table 21. Distribution pattern of black pepper accessions based on biochemical principles

Cluster	No. of accessions	Ac.no	Characteristics
1	2	7215 and 7259	High piperine Low oil Low oleoresin
2	4	7286,7254,7221and 7243	Medium piperine Medium oil Low oleoresin
3	3	7255,7240 and 7229	Low piperine Low oil Low oleoresin
4	5	7232,7285,7289,7276 and 7219	Medium piperine Medium oil Medium oleoresin
5	6	7293, 7252, 7211, 7241, 7249 and 7222	High piperine Medium oil Low oleoresin

The clustering showed that the accessions can be grouped into five major clusters (Fig.3.) on the basis of biochemical estimation of principles like piperine, oil and oleoresin content. Majority of the accessions belonged to the cluster 5 with high piperine, medium oil content and low oleoresin content. However , cluster 3 comprised of three accessions with inferior biochemical status as they are low in piperine, volatile oil and oleoresin. In a similar study on divergence of 42 black pepper germplasm accessions, oleoresin exhibited more variability than piperine (Pradeepkumar *et al*., 2003). Gopalam and Ravindran (1987) performed quality indexing of all important cultivars of black pepper by categorizing them into three classes *viz*. low, medium and high. The biometric observation and visual assessment of plants based on qualitative traits brought intra group, interclone and inter varietal variation of different characters among the tissue cultured and conventional clones of four pepper varieties (Sujatha, 2001). The Indian cultivars Kottanadan, Kumbakodi, Kuthiravally and Nilgiri recorded high piperine and oleoresin content, where as Balankotta, Kaniyakadan and Kumbakody were rich in essential oil (Krishnamoorthy and Parthasarathy, 2010). Sruthi *et al*. (2013) reported that there was profound variability in essential oil, oleoresin, piperine, total phenol, crude fibre, starch, total fat and bulk density in dried berries of the black pepper variety, Panniyur-1 collected from eleven locations

4.3. Screening for foot rot disease tolerance

The accessions were subjected to artificial inoculation and screening against *Phytophthora capsici* infection. The lesion diameters of both stem and leaf were statistically analyzed. From the lesion scorings, the disease severity index (DSI) was calculated for stem lesion, depth of penetration and leaf lesion using the formula adopted by Kim *et al*. (2000).

$$DSI = \frac{\text{Sum of rating of each plant}}{\text{Maximum score} \times \text{No.of plants rated}} \times 100$$

Final rating of infection is made based on average DSI of both stem and leaf for each accession. Scoring for *Phytophthora* infection is recorded as below, (Bhai *et al*, 2003).

Average DSI (stem and leaf)	**Reaction**
< 30%	Resistant
31- 40%	Moderately resistant
> 40%	Susceptible

Table 22. Reaction of black pepper accessions to *Phytophthora capsici* inoculation

Accession	DPDSI (%)	SLLDSI (%)	LLLDSI (%)	Overall Mean Disease Severity Index (OMDSI)(%)	Rating of accession
7211	46.67^{CDE}	56.67^{CD}	56.67^{CD}	53.34	S
7215	56.67^{CD}	53.33^{CD}	81.67^{BCDEF}	63.89	S
7219	30.00^{EFG}	43.33^{CDEF}	88.33^{ABCDE}	53.88	S
7221	38.33^{DEFG}	43.33^{CDEF}	75.00^{DEFG}	52.22	S
7222	58.33^{CD}	35.00^{EF}	96.67^{AB}	62.22	S
7229	43.33^{DEF}	40.00^{DEF}	86.67^{ABCDE}	56.65	S
7232	46.67^{CDE}	41.67^{CDEF}	81.67^{BCDEF}	61.67	S
7240	25.00^{FG}	50.00^{CDE}	80.00^{CDEF}	51.67	S
7241	63.33^{BC}	55.00^{CD}	73.33^{EFG}	63.88	S
7243	78.33^{AB}	78.33^{AB}	98.33^{A}	84.99	S
7249	43.33^{DEF}	31.67^{F}	61.67^{G}	45.56	S
7252	28.33^{EFG}	55.00^{CD}	70.00^{FG}	51.11	S
7254	95.00^{A}	91.67^{A}	88.33^{ABCDE}	91.66	S

Table 22 (Contd)

7255	30.00^{EFG}	35.00^{EF}	80.00^{CDEF}	48.33	S
7259	23.33^{G}	33.33^{EF}	61.67^{G}	39.44	MR
7276	20.00^{G}	31.67^{F}	81.67^{BCDEF}	44.44	S
7285	56.67^{CD}	45.00^{CDEF}	80.00^{CDEF}	60.55	S
7286	55.00^{CD}	41.67^{CDEF}	90.00^{ABCD}	62.22	S
7289	81.67^{A}	73.33^{B}	100^{A}	85.00	S
7293	43.33^{DEF}	58.33^{C}	95.00^{ABC}	65.55	S
P5	86.67^{A}	76.67^{AB}	100.00^{A}	87.78	S
General Mean	50.00	50.95	84.13		
SE	108.33	81.75	72.62		
CV(%)	20.82	17.74	10.13		

DPDSI-Depth of penetration disease severity index, SLLDSI-Stem lesion length disease severity index and LLLDSI- Leaf lesion length disease severity index.

Varying degrees of resistance was noticed in screening and the reactions significantly differed among the accessions (Table 22 and figure 4). Among the 20 accessions subjected for screening, none of them showed complete resistance to *P. capsici*. Only one accession *viz.*7259 has shown moderate resistance with an overall mean DSI of 39.44 for characters like stem lesion length, depth of penetration and leaf lesion. It is a promising accession worth further field level evaluation.

As depicted in Table 22, the lowest DSI value (20.00) was observed for depth of penetration followed by stem lesion length (31.67) and leaf lesion diameter (56.67 %). In the screening maximum DSI (100 %) was observed in 7289 and P 5 for leaf lesion length diameter after 72 h of inoculation . The accession 7254 recorded maximum value for stem lesion length (91.67 %). and depth of penetration (95.00%). The overall mean DSI was highest (91.67%) for the accession 7254 and the lowest (39.44 %) for accession 7259.

The accessions 7254, P5, 7289, 7243, 7211, 7222, 7293, 7286, 7219 and 7229 were on par in resistance response and were significantly different from other accessions. In a screening study of pepper germplasm, Bhai *et al*. (2007) reported that

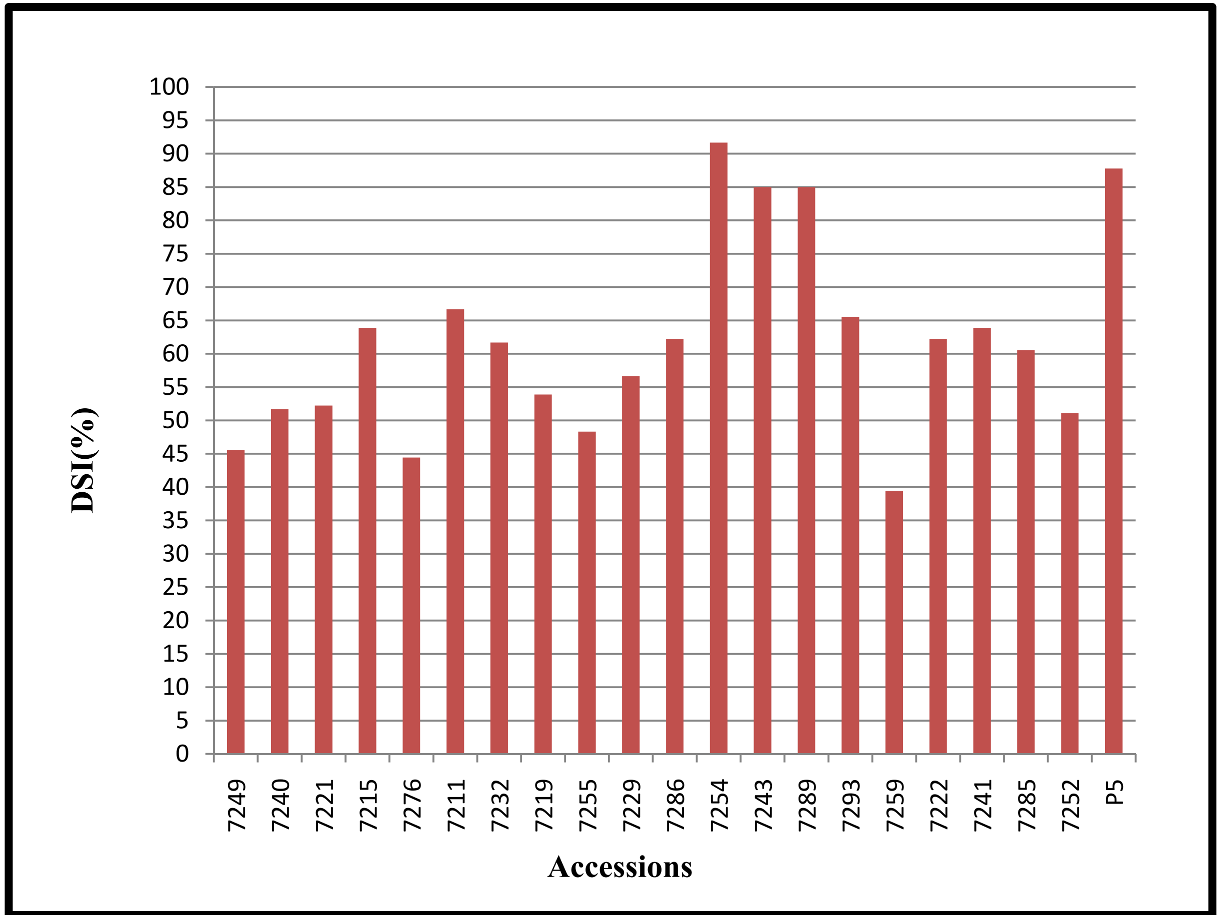

Fig 4.Variation in foot rot susceptibility of black pepper accession

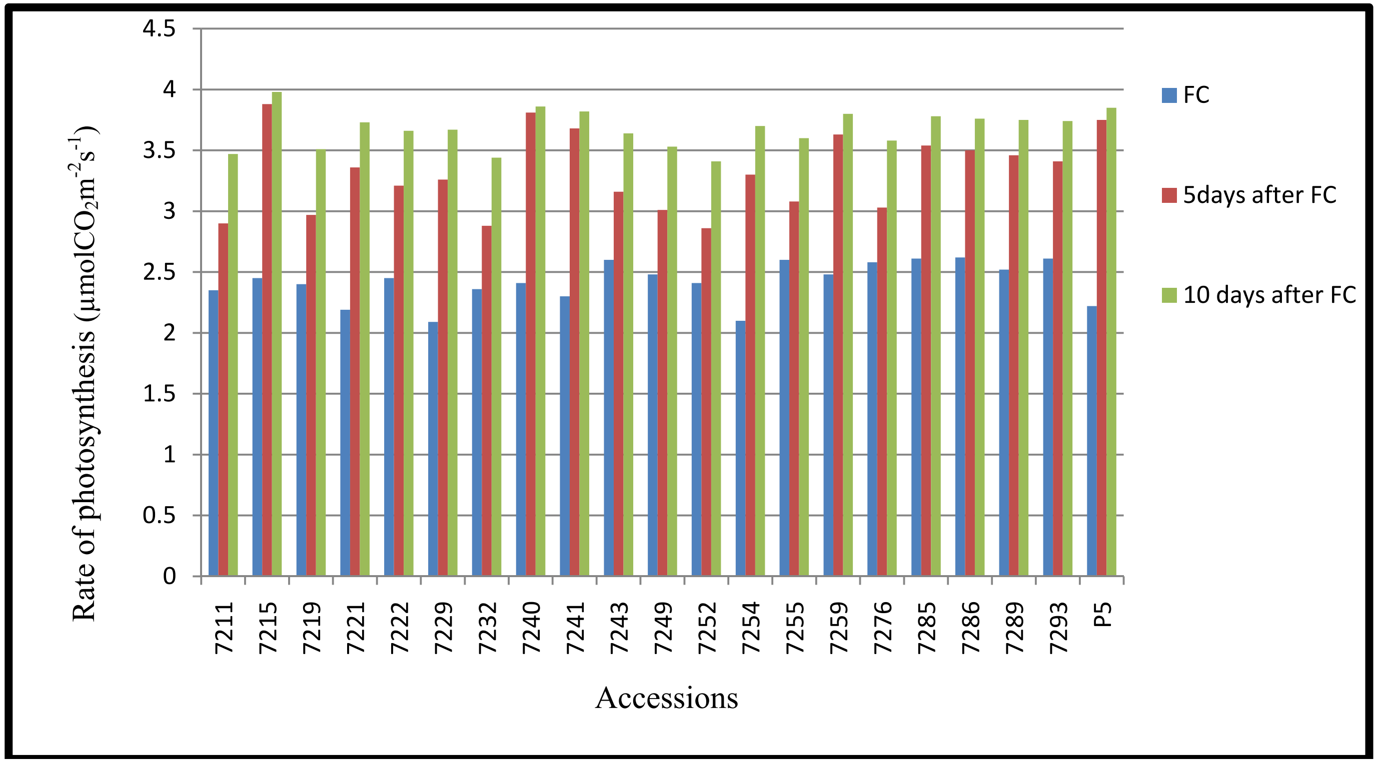

Fig.5. Variation in rate of photosynthesis of black pepper accessions under progressive moisture stress

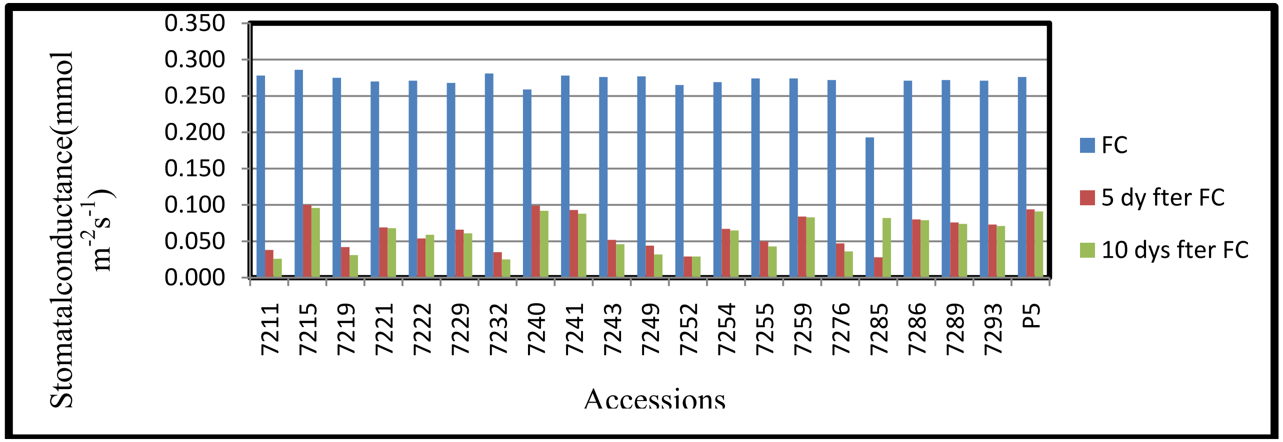

Fig.6.Variation in rate of stomatal conductance of black pepper accessionsunder progressive moisture stress

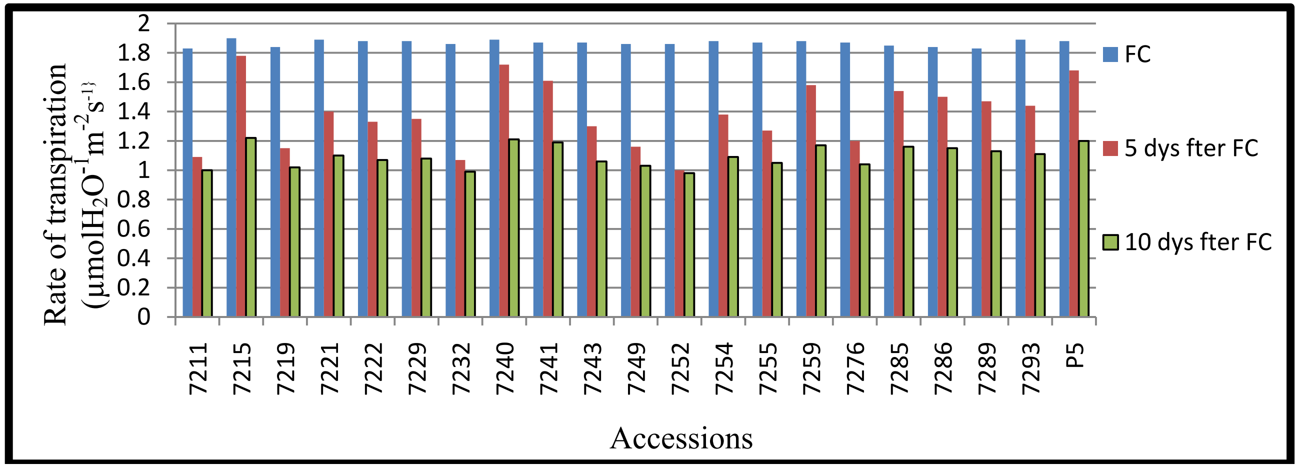

Fig.7.Variation in rate of transpiration of black pepper accessionsunder progressive moisture stress

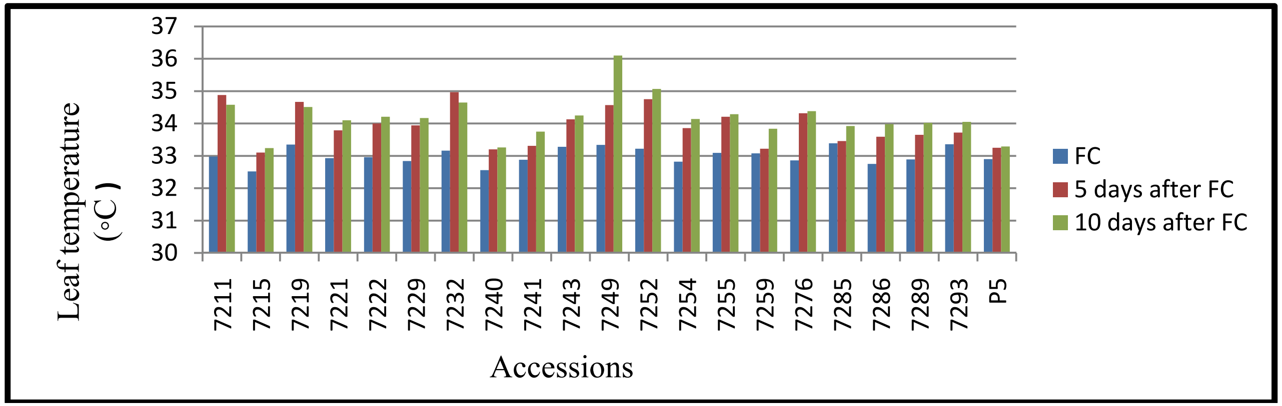

Fig.8.Variation in leaf temperature of black pepper accessionsunder progressive moisture stress

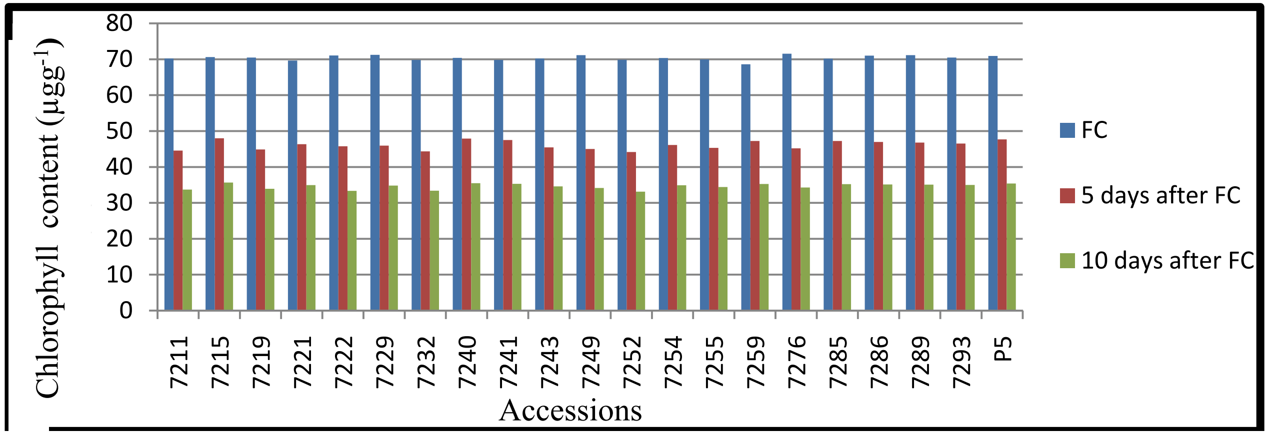

Fig.9.Variation in chlorophyll content of black pepper accessionsunder progressive moisture stress

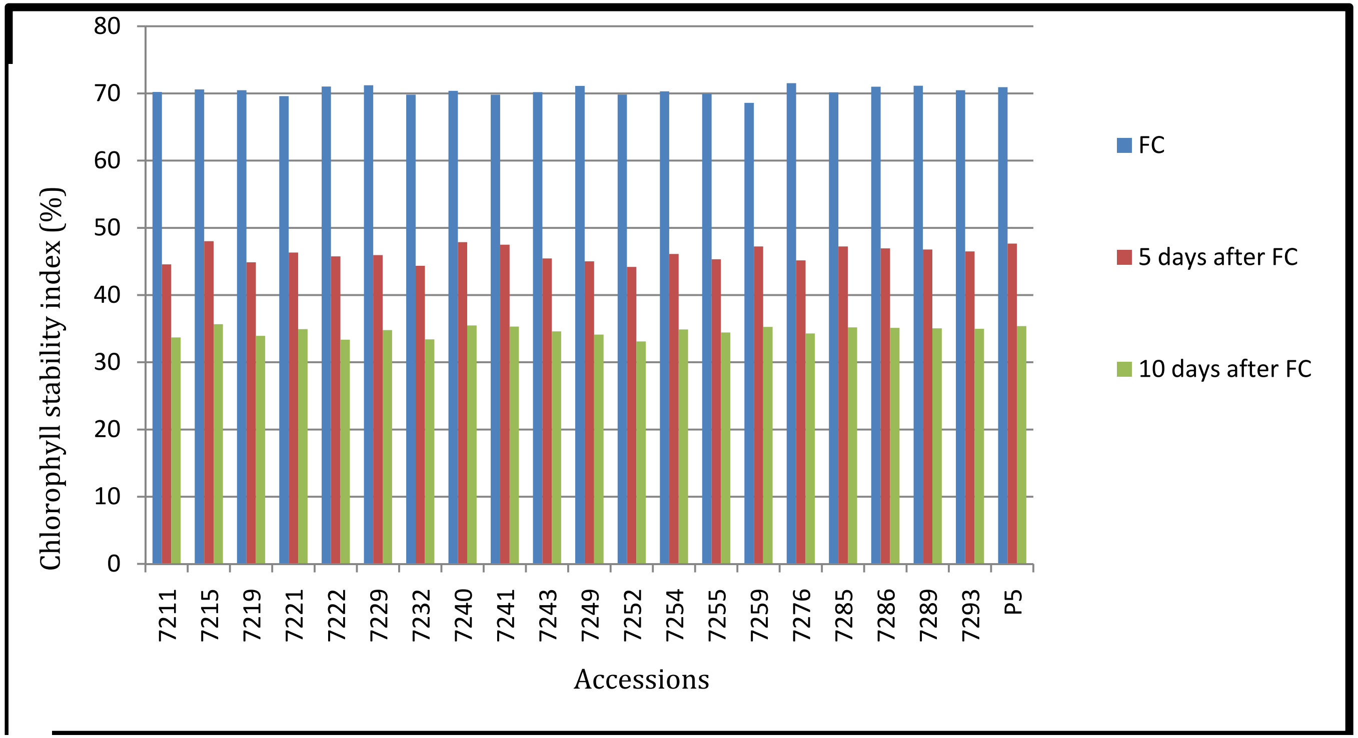

Fig. 10. Variation in chlorophyll stability index of black pepper accessionsunder progressive moisture stress

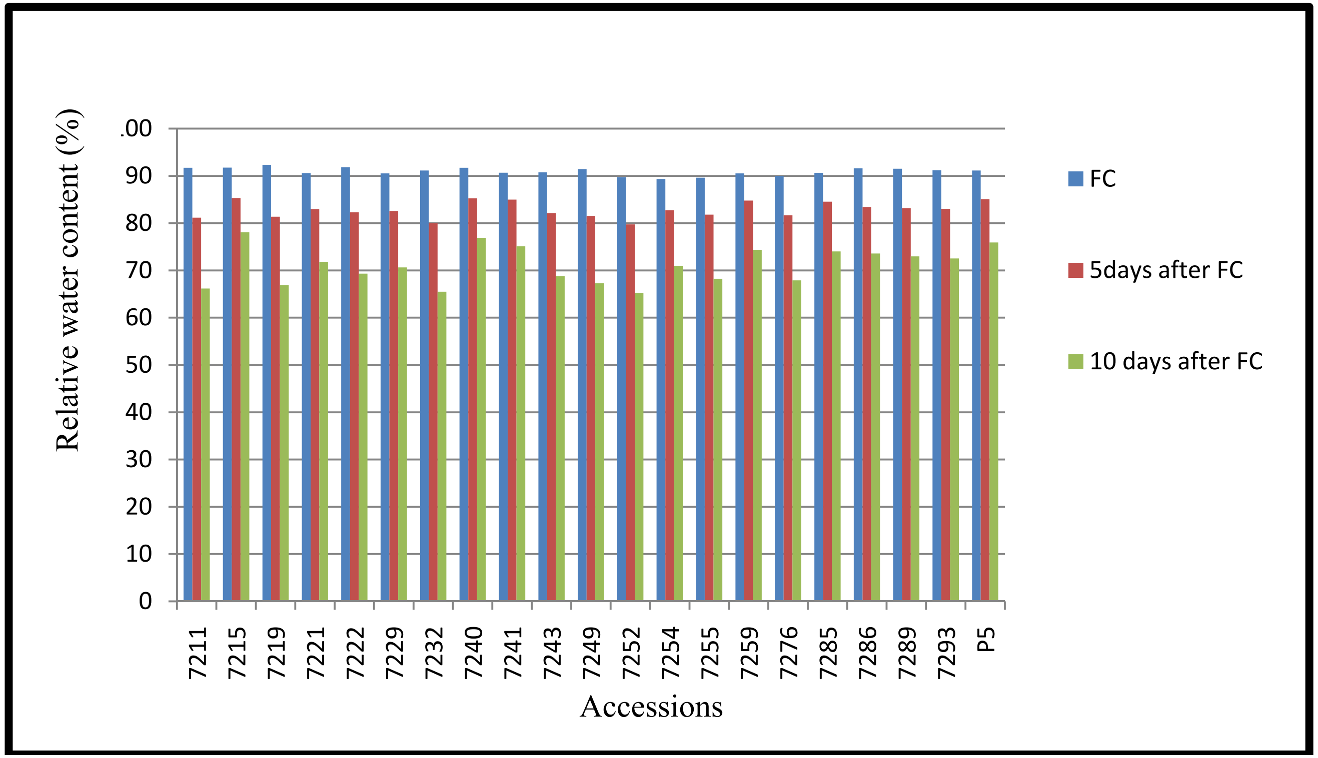

Fig.11. Variation in Relative water content of black pepper accessionsunder progressive moisture stress

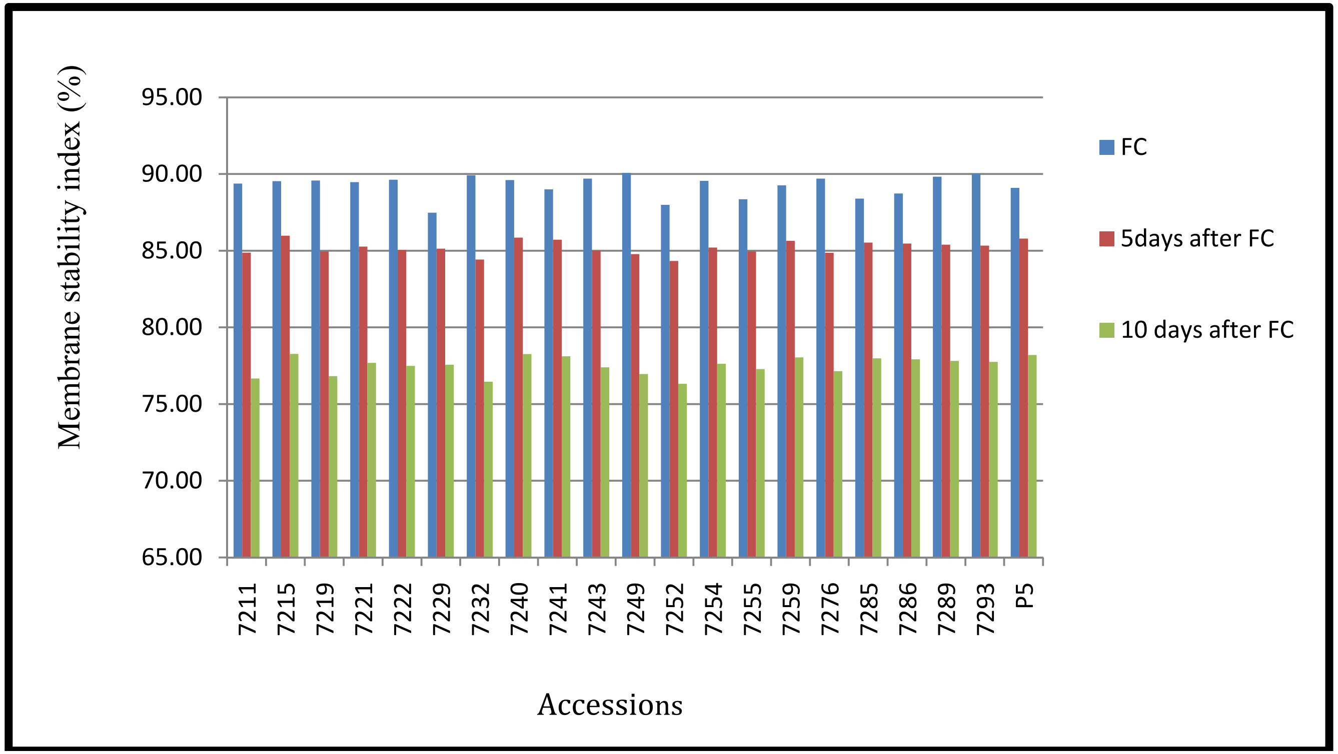

Fig.12.Variation in membrane stability index of black pepper accessionsunder progressive moisture stress

the hybrid *viz*. Acc.1375 and two wild accessions *viz*. Acc. 3160 and 3260 and four Kottanadan selections (2466, 2471, 2515 and 2433) were promising with a disease index of 1 with no external lesion after stem inoculation.

Screening of forty one black pepper cultivars and 73 wild *Piper* species gainst *Phytophthora palmivora* adopting root dip inoculation technique showed that cultivars *viz*. Narayakodi, Kalluvalli, Uthiaramkotta and Balankotta exhibited low percentage of infection (Sarma *et al.* 1982). None of the wild species showed any level of resistance in this study. Divya and Sharda, (2014) reported Panniyur 5, Aribally and Karimunda as resistant among the 9 varieties/ cultivars tested by root inoculation using soil drenching with zoospore suspension, whereas Panniyur 1, Panniyur 3, Panniyur 4, Panniyur 6 and Panniyur 7 were highly susceptible.

In another study Mammootty *et al*. (2008) found that the black pepper genotypes Karimunda I, Karimunda II, Panniyur 1, Panniyur 2, Panniyur 3, Panniyur 4, Panniyur 5, Panniyur 6 and Panniyur 7 showed varying levels of infection and mortality of the genotypes by inoculation of zoospore and culture discs of *Phytophthora capsici* on leaves, stems and roots.

In the present study also Panniyur 5, the check variety showed high susceptibility (Overall mean DSI - 87.78%) to foot rot fungus through the stem and leaf inoculation trials (Table 22.).

Based on the study, all the 20 accessions evaluated and the check variety were found to be susceptible to Phytophthora foot rot. Only one accession 7259 was showing moderate reistance. Hence this accession can be used in further breeding programme for development of foot rot resistant line / variety.

4.3.1 Correlation of *Phytophthora* resistance to yield and biochemical characters

Table 23. Correlation of *Phytophthora* resistance to yield and biochemical characters

	Yield	Piperine	Oil	Oleoresin	Foot rot resistance
Yield	1.00	-0.21	0.05	-0.03	-0.03
Piperine		1.00	0.26	0.21	-0.11
Oil			1.00	0.11	-0.23
Oleoresin				1.00	0.22
Foot rot resistance					1.00

Among the three biochemical parameters, oil content was having a very low and positive association with yield while oleoresin and piperine had a negative and non significant association with yield. Foot rot resistance had a negative and non significant association with piperine, oil content and yield. Overall, it is observed that there was no significant correlation between foot rot resistance, yield and biochemical characters (Table 23).

4.3.2 Ranking of accessions

Considering yield, foot rot tolerance and quality parameters, twenty potential accessions were ranked as suggested by Arunachalam and Bandyapadhyay (1984). The accessions were ranked based on mean values for yield, piperine (per cent), essential oil (per cent), oleoresin (per cent) and inverse of disease severity index (for depth of penetration, stem lesion length and leaf lesion length), (Table 24).

The accessions *viz*. 7211, 7221, 7222, 7232, 7249, 7252 and 7259 were found to be most promising with respect to these characters.

Table 24. Ranking of accessions on the basis of foot rot resistance, yield and quality

Accession	Rank for foot rot tolerance			Rank for quality parameters			Rank for yield	Total rank	Final rank
	DSI(Depth of penetration)	DSI(Stem lesion length)	DSI(Leaf lesion length)	Piperine(%)	Essential oil(%)	Oleoresin(%)	Green berry yield/vine (g.)		
7211	4	6	1	3	1	4	9	28	2
7215	5	6	7	4	10	7	11	50	10
7219	3	7	5	10	9	3	8	45	8
7221	4	5	5	11	4	5	3	37	5
7222	5	2	9	4	5	4	3	32	3
7229	5	3	8	15	11	8	10	60	13
7232	4	4	7	8	10	3	1	37	5
7240	2	4	6	11	7	8	6	44	7
7241	6	6	4	2	3	4	15	40	7
7243	7	8	10	9	6	7	2	49	9
7249	5	1	2	3	6	4	14	35	4
7252	3	6	3	1	5	4	5	27	1
7254	8	10	8	12	13	6	11	68	14
7255	3	2	5	14	2	8	4	38	6
7259	1	2	2	2	10	8	7	32	3
7276	1	1	7	13	8	2	17	50	10
7285	5	5	6	6	7	4	12	44	7
7286	5	5	9	7	12	7	11	56	11
7289	8	8	10	5	9	1	16	57	12
7293	5	7	10	1	7	5	13	48	8

4.4. Screening of black pepper accessions for drought tolerance

The 20 selected accessions were screened for physiological and biochemical parameters under progressive moisture stress.

4.4.1. Physiological parameters under progressive moisture stress

Plants exposed to environmental stress often exhibit a variety of symptoms or indications. Stress indicators are indicative of visible growth or morphological modifications or invisible physiological and biochemical changes that relate to resistance and repair mechanisms. Plant species and cultivars differ in their response to the environmental stress. Multiple morphological, physiological and biochemical changes may occur simultaneously in response to an environmental stress. Drought and increasing atmospheric temperature are the important limiting factors faced by pepper farmers. Stress tolerance against drought is assuming more importance in the scenario of climate change. The cultivation of black pepper is challenging if the atmospheric temperature increases beyond 40 degree centigrade.

Hence, the present study was undertaken to assess the tolerance to water stress in black pepper accessions. These included physiological parameters like photosynthesis, stomatal conductance, transpiration, leaf temperature, chlorophyll content, chlorophyll stability, relative water content and membrane stability. In the present experiment, the response of various accessions were evaluated at progressive moisture stress *viz.,* field capacity, after five days of stress and after ten days of stress, induced.

Table 25. Physiological response of black pepper accessions at field capacity

Accession	Photosynthesi ($\mu molCO_2m^{-2}s^{-1}$)	Stomatal conductance ($mmolm^{-2s-1}$)	Transpiration ($\mu molH_2O^{-1}m^{-2}s^{-1}$)	Leaf temperature (°C)	Chlorophyll Content (μgg^{-1})	Chlorophyll stability (%)	Relative water content (%)	Membrane stability index (%)
7211	3.82	0.278	1.83	32.98	2.80	70.21^{BCD}	91.72	89.38
7215	3.80	0.286	1.90	32.52	2.84	70.59^{ABCD}	91.73	89.54
7219	3.80	0.275	1.84	33.35	2.82	70.47^{ABCD}	92.33	89.58
7221	3.76	0.270	1.89	32.93	2.86	69.61^{DE}	90.59	89.47
7222	3.77	0.271	1.88	32.96	2.79	71.04^{AB}	91.86	89.63
7229	3.75	0.268	1.88	32.84	2.78	71.23^{AB}	90.54	87.48
7232	3.74	0.281	1.86	33.16	2.80	69.82^{D}	91.12	89.92
7240	3.75	0.259	1.89	32.56	2.81	70.38^{BCD}	91.71	89.61
7241	3.82	0.278	1.87	32.88	2.83	69.82^{D}	90.65	89.00
7243	3.83	0.276	1.87	33.28	2.79	70.19^{BCD}	90.75	89.70
7249	3.80	0.277	1.86	33.34	2.78	71.13^{AB}	91.45	90.08
7252	3.79	0.265	1.86	33.22	2.81	69.85^{D}	89.76	87.99
7254	3.76	0.269	1.88	32.82	2.8	70.32^{BCD}	89.34	89.56
7255	3.81	0.274	1.87	33.09	2.80	69.93^{CD}	89.60	88.36
7259	3.81	0.274	1.88	33.08	2.82	68.60^{E}	90.52	89.26
7276	3.81	0.272	1.87	32.86	2.76	71.53^{A}	89.98	89.70
7285	3.81	0.193	1.85	33.39	2.83	70.18^{BCD}	90.63	88.4

Table 25. (Contd)

7286	3.80	0.271	1.84	32.75	2.82	71.00^{AB}	91.61	88.73
7289	3.82	0.272	1.83	32.89	2.82	71.14^{AB}	91.52	89.83
7293	3.77	0.271	1.89	33.36	2.83	70.47^{ABCD}	91.19	90.03
P5	3.77	0.276	1.88	32.90	2.82	70.93^{ABC}	91.14	89.1
General Mean	3.79	0.269	1.87	33.01	2.81	70.4	90.94	89.25
SE	0.03	0.036	0.02	0.42	0.03	0.52	1.26	1.033
CV(%)	1.16	16.70	1.81	1.57	1.32	0.91	1.71	1.42

4.4.1.1. Physiological response of black pepper accessions at field capacity

The observations on the physiological parameters *viz*. photosynthesis, stomatal conductance, transpiration, leaf temperature, chlorophyll content, and membrane stability index showed no significant difference among the 21 black pepper accessions (Table 25). This is in agreement with earlier report by Krishnamurthy *et al.* (2000) in which *Piper* species showed on par performance of major physiological parameters at non stressed condition.

The mean value of rate of photosynthesis recorded at field capacity was 3.79 μmol $CO_2m^{-2}s^{-1}$. For stomatal conductance , mean value registered by the accessions was 0.269 mmol $m^{-2}s^{-1.}$ All the accessions showed high mean values for all the parameters like transpiration, chlorophyll content, chlorophyll stability index, relative water content and membrane stability index and low mean values for leaf temperature at field capacity.

However, there was significant variation shown by the accessions for chlorophyll stability index at field capacity. The maximum value was recorded by the accession 7276 (71.53%) followed by 7229 (71.23%). The lowest chlorophyll stability index of 68.60 per cent was shown by the accession 7259. This shows that genetic variability is present among the accessions for this character which is expressed even without moisture stress.

High values of chlorophyll stability index indicated that the performance of accessions *viz.*7276 (71.53%), 7229 (71.23%) and 7289 (71.14%) was on par with the check P 5 for chlorophyll stability which was visible in stress free condition as well.

Table 26. Physiological response of black pepper accessions after five days of moisture stress induction

Accession	Photosynthesis ($\mu mol\ CO_2 m^{-2} s^{-1}$)	Stomatal conductance ($mmol m^{-2} s^{-1}$)	Transpiration ($\mu mol\ H_2O^{-1} m^{-2} s^{-1}$)	Leaf temperature (°C)	Chlorophyll content ($\mu g\ g^{-1}$)	Chlorophyll stability (%)	Relative water content (%)	Membrane stability index (%)
7211	1.43^{IJK}	0.038^{MN}	1.09^{NO}	34.88	1.18^{PQ}	44.58^{M}	81.16^{H}	84.87^{IJ}
7215	3.00^{A}	0.100^{A}	1.78^{A}	33.10	1.95^{A}	48.01^{A}	85.33^{A}	85.98^{A}
7219	1.57^{HIJK}	0.042^{LM}	1.15^{MN}	34.67	1.24^{OP}	44.88^{L}	81.35^{GH}	84.96^{HIJ}
7221	2.26^{BCDEF}	0.069^{FG}	1.40^{GHIJ}	33.79	1.58^{HI}	46.33^{GH}	82.99^{BCD}	85.27^{EFGHI}
7222	1.97^{DEFGH}	0.054^{I}	1.33^{JK}	34.00	1.45^{JK}	45.77^{J}	82.30^{CDEF}	85.05^{GHIJ}
7229	2.04^{CDEFGH}	0.066^{HI}	1.35^{IJK}	33.94	1.51^{IJ}	45.94^{IJ}	82.57^{BCDE}	85.14^{FGHIJ}
7232	1.27^{JK}	0.035^{N}	1.07^{NO}	34.97	1.14^{Q}	44.35^{MN}	80.01^{I}	84.43^{KL}
7240	2.98^{A}	0.099^{AB}	1.72^{AB}	33.20	1.91^{AB}	47.88^{AB}	85.24^{A}	85.86^{AB}
7241	2.92^{A}	0.093^{BC}	1.61^{BCD}	33.31	1.82^{CD}	47.49^{CD}	84.97^{A}	85.72^{ABCD}
7243	1.88^{EFGHI}	0.052^{IJ}	1.30^{JKL}	34.13	1.42^{KL}	45.46^{K}	82.14^{DEFG}	85.00^{GHIJ}
7249	1.67^{HIJ}	0.044^{KL}	1.16^{MN}	34.57	1.28^{NO}	45.02^{L}	81.52^{FGH}	84.78^{JK}
7252	1.16^{K}	0.029^{O}	1.00^{O}	34.75	1.06^{R}	44.18^{N}	79.75^{I}	84.33^{L}
7254	2.21^{BCDEFG}	0.067^{GH}	1.38^{HIJ}	33.86	1.54^{HI}	46.12^{HI}	82.75^{BCD}	85.21^{FGHI}
7255	1.83^{FGHI}	0.050^{JK}	1.27^{KL}	34.21	1.36^{LM}	45.32^{K}	81.80^{EFGH}	84.96^{HIJ}

Table 26. (Contd)

7255	1.83 ^{FGHI}	0.050 ^{JK}	1.27 ^{KL}	34.21	1.36 ^{LM}	45.32 ^{K}	81.80 ^{EFGH}	84.96 ^{HIJ}
7259	2.67 ^{AB}	0.084 ^{CD}	1.58 ^{CDE}	33.22	1.77 ^{DE}	47.24 ^{D}	84.78 ^{A}	85.65 ^{ABCDE}
7276	1.76 ^{GHI}	0.047 ^{JKL}	1.20 ^{LM}	34.32	1.33 ^{MN}	45.17 ^{KL}	81.68 ^{EFGH}	84.86 ^{IJ}
7285	2.53 ^{AB}	0.028 ^{DE}	1.54 ^{DEF}	33.46	1.73 ^{EF}	47.24 ^{D}	84.55 ^{A}	85.53 ^{BCDEF}
7286	2.45 ^{BC}	0.080 ^{E}	1.50 ^{EFG}	33.59	1.69 ^{FG}	46.95 ^{E}	83.44 ^{B}	85.47 ^{BCDEF}
7289	2.40 ^{BCD}	0.076 ^{F}	1.47 ^{FGH}	33.65	1.66 ^{FG}	46.77 ^{EF}	83.18 ^{BC}	85.40 ^{CDEFG}
7293	2.33 ^{BCDE}	0.073 ^{F}	1.44 ^{FGHI}	33.72	1.62 ^{GH}	46.50 ^{FG}	83.01 ^{BCD}	85.33 ^{DEFGH}
P5	2.94 ^{A}	0.094 ^{B}	1.68 ^{ABC}	33.25	1.86 ^{BC}	47.66 ^{BC}	85.09 ^{A}	85.79 ^{ABC}
General Mean	2.16	0.063	1.38	33.90	1.53	46.14	82.84	85.22
SE	0.235	0.09	0.05	0.53	0.039	0.147	0.457	0.207
CV(%)	13.36	6.35	4.72	1.91	3.16	0.39	0.68	0.30

4.4.1.2. Physiological response of black pepper accessions after five days of progressive moisture stress

The result of the data on physiological parameters observed after five days of induced moisture stress is presented in Table 26. There is a significant difference observed among the accessions for various physiological parameters owing to progressive moisture stress for five days.

4.4.1.2.1. Photosynthesis

Under most of the water stressed conditions, reduced CO_2 diffusion to the site of carboxylation is the main reason for decreased photosynthetic rate (Erismann *et al.*, 2008). Tolerant hybrids showed higher rate of photosynthesis in Cocoa compared to the susceptible ones (Juby, 2019). In the present study high value of rate of photosynthesis was observed for the accessions 7215, 7240, P5, 7241, 7259 and 7285 indicating that these accessions are capable of retaining comparatively high rate of photosynthesis to cope up with moisture stress.

Relatively low rate of photosynthesis was observed for accessions *viz.* 7252, 7232, 7211, 7219 and 7249 after five days of moisture stress. This may be an indication that they are more prone to moisture stress. In maize, compared to well watered plants drought stress led to decline in net photosynthesis by 33.22 percent as reported by Anjum *et al.* (2011). However in the present experiment, the rate of photosynthesis declined by about 43.01 per cent in mean value after five days of moisture stress. This indicates that black pepper is more affected by moisture stress than maize.

4.4.1.2.2. Stomatal conductance

Stomatal closure is a well-known first responsive reaction of plants to drought stress. It is more closely related to soil moisture status than leaf water status and is mostly controlled by chemical signals such as Abscisic acd (ABA) produced in dehydrating roots (Brodribb and Mc Adam, 2011).

The mean value of stomatal conductance registered a decrease of 76.57 per cent after five days of moisture stress compared to that at field capacity. The maximum value of 0.100 $mmolm^{-2}s^{-1}$ was noted for the accession 7215 and minimum of 0.028 $mmolm^{-2}s^{-1}$ for the accession 7285. The higher value observed indicates more vulnerability of the accessions to stress where as the lower values of stomatal conductance shows the ability to reduce water loss by transpiration despite curtailment of photosynthesis. In the present experiment the accessions 7285 and 7252 registered the lower values for stomatal conductance indicating their tolerance to moisture stress.

4.4.1.2.3. Rate of transpiration

Water stress is reported to cause decline of transpiration rate in many crops. In cashew seedlings stressed for two days, transpiration rate declined from 4.75 $\mu molH_2O^{-1}m^{-2}s^{-1}$ to 2.1175 $\mu molH_2O^{-1}m^{-2}s^{-1}$ when stress was given for five days (Latha,1998)**.** Balasimha *et al.* (1988) reported that drought tolerance in cocoa is mainly attributed to effective stomatal regulation resulting in decreased transpiration. In the present experiment, the mean value of rate of transpiration also showed a decline (26.20 %) after five days compared to field capacity. The maximum value of 1.78 $\mu molH_2O^{-1}m^{-2}s^{-1}$ was recorded by the accession 7215 and minimum of 1.00 $\mu molH_2O^{-1}m^{-2}s^{-1}$ by the accession 7252. Lowest value observed for the accession 7252 indicates its tolerance to moisture stress .

4.4.1.2.4. Leaf temperature

The leaf temperature of all the accessions did not show significant variation after five days of moisture stress . This corresponds to the earlier observation made by Juby (2019) in cocoa where there was no change in leaf temperature under moisture stress .

4.4.1.2.5. Chlorophyll content

The chlorophyll content estimated after five days of moisture stress showed marked decrease in mean value by 45.55 per cent compared to the non stressed condition. The response of the accessions were significantly different for this parameter. The maximum value of 1.95µg g^{-1} was shown by the accession 7215 and minimum value of 1.06 µg g^{-1} by the accession 7252. The check variety, P5 had recorded a moderate photosynthesis of 1.86.µg g^{-1} after five days of moisture stress.

Low values of chlorophyll content were considered as indication of susceptibility of the accessions to moisture stress in cocoa hybrid seedlings maintained at 40 per cent field capacity (Juby, 2019). The accessions 7252, 7232 and 7211 in the present experiment can be considered as susceptible to moisture stress as they have recorded very low chlorophyll content when subjected to moisture stress.

4.4.1.2.6. Chlorophyll stability index

Chlorophyll stability index of the accessions showed a decline by 34.46 per cent in the mean value after five days of stress compared to the field capacity. This is in agreement with the finding of Juby (2019) in cocoa where the observed value of susceptible ones was below 60 per cent and tolerant ones above 60 per cent where as control had 80 per cent at 40 per cent moisture content of field capacity. In the present experiment, maximum value (48.01%) for this parameter was shown by the accession 7215 and minimum (44.18%) by the accession 7252, showing superior and inferior performance by the respective accessions for moisture stress tolerance after five days of progressive moisture stress.

4.4.1.2.7. Relative water content

The mean value of relative water content (RWC) also registered a decrease by 8.91 per cent after five days of stress. The maximum value of 85.33 per cent was shown by the accession 7215 and minimum (79.75%) by the accession 7252. The higher value of relative water content is considered as a criterion for drought tolerance in hybrid cocoa (Juby, 2019). This shows that the accessions 7215, 7240, P5, 7241, and 7259 were on par for RWC showing better tolerance to moisture stress compared to others. Two accessions *viz.*7252 and 7232 were on par for their RWC indicating their susceptibility to moisture stress.

4.4.1.2.8. Membrane stability index

As a major impact of environmental stress, the cellular membrane dysfunctions by showing increased permeability and leakage of ions out which can readily be measured by the efflux of electrolytes. Membrane stability is associated with water and high temperature stress tolerance in various crop plants (Sairam *et al.*, 1998). In cocoa, the drought tolerant hybrids showed high membrane stability under stress compared to drought susceptible ones (Juby, 2019).

In the present experiment, the membrane stability values showed a decline in mean value by 4.52 per cent after five days of stress compared to field capacity. The maximum value of 85.98 per cent was observed for the accession 7215 and minimum of 84.33 per cent observed for 7252. The high value is indicative of better tolerance of the accession to moisture stress. The accessions 7215, 7240, P5, 7241, and 7259 were on par for this parameter showing better tolerance to moisture stress compared to others. The accessions 7252 and 7232 were found to be susceptible to moisture stress as they recorded comparatively lower MSI values.

In general, a decrease in percent mean values of physiological parameters like photosynthesis (43.01%), stomatal conductance (76.57%), rate of transpiration

(26.20%), chlorophyll content (45.55%), chlorophyll stability (34.46%), membrane stability (4.52%) and relative water content (8.91%) were observed after five days of stress. The high values observed for photosynthesis, chlorophyll content, chlorophyll stability, relative water content and membrane stability suggests the greater stress tolerance capacity of the accessions 7215, 7240, P 5 and 7241 compared to other accessions after five days of moisture stress. However, these accessions showed high values for stomatal conductance and rate of transpiration. The low values of physiological parameters like photosynthesis, chlorophyll content, chlorophyll stability, relative water content and membrane stability along with high value of leaf temperature observed indicated greater susceptibility of the accessions 7252, 7232, 7211, 7219 and 7249.

Table 27. Physiological response of black pepper accessions after10 days of moisture stress induction

Accession	Photosynthesis(μ molCO$_2$m^{-2}s^{-1})	Stomatal conductance (mmolm^{-2s-1})	Transpiration(μmolH$_2$O^{-1}m^{-2}s^{-1})	Leaf temperature (°C)	Chlorophyll content(μg g^{-1})	Chlorophyll stability (%)	Relative water content (%)	Membrane stability index (%)
7211	0.60^{QR}	0.026	1.00^{PQ}	34.58^{B}	1.03^{PQ}	33.70^{HIJ}	66.18^{PQ}	76.67^{P}
7215	1.40^{A}	0.096	1.22^{A}	33.24^{D}	1.38^{A}	35.66^{A}	78.08^{A}	78.27^{A}
7219	0.64^{PQ}	0.031	1.02^{OP}	34.51^{B}	1.04^{OP}	33.94^{GHIJ}	66.90^{OP}	76.83^{O}
7221	1.01^{IJ}	0.068	1.10^{HI}	34.10^{BCD}	1.17^{H}	34.94^{ABCDEF}	71.83^{I}	77.69^{H}
7222	0.90^{LM}	0.059	1.07^{JK}	34.21^{BCD}	1.12^{JK}	33.36^{IJ}	69.30^{K}	77.49^{JK}
7229	0.94^{KL}	0.061	1.08^{JK}	34.17^{BCD}	1.13^{IJ}	34.80^{BCDEF}	70.63^{J}	77.56^{IJ}
7232	0.55^{RS}	0.025	0.99^{QR}	34.65^{B}	1.01^{Q}	33.41^{IJ}	65.51^{QR}	76.46^{Q}
7240	1.36^{AB}	0.092	1.21^{AB}	33.26^{CD}	1.35^{B}	35.49^{AB}	76.90^{B}	78.26^{A}
7241	1.29^{CD}	0.088	1.19^{CD}	33.75^{BCD}	1.31^{C}	35.31^{ABC}	75.09^{D}	78.12^{BC}
7243	0.86^{MN}	0.046	1.06^{KL}	34.25^{BCD}	1.11^{KL}	34.60^{CDEFG}	68.82^{KL}	77.40^{KL}
7249	0.67^{P}	0.032	1.03^{NO}	36.10^{A}	1.06^{NO}	34.13^{FGHI}	67.30^{NO}	76.96^{N}
7252	0.52^{S}	0.029	0.98^{R}	35.07^{B}	1.02^{PQ}	33.10^{J}	65.27^{R}	76.32^{R}
7254	0.96^{JK}	0.065	1.09^{IJ}	34.14^{BCD}	1.15^{I}	34.89^{ABCDEF}	70.97^{J}	77.63^{HI}
7255	0.83^{N}	0.043	1.05^{LM}	34.29^{BCD}	1.09^{LM}	34.42^{DEFGH}	68.22^{LM}	77.28^{L}
7259	1.25^{DE}	0.083	1.17^{DE}	33.84^{BCD}	1.29^{D}	35.26^{ABCD}	74.36^{DE}	78.05^{CD}

Table 27. (Contd)

7276	0.76^{O}	0.036	1.04^{MN}	34.38^{BC}	1.07^{MN}	34.28^{EFGH}	67.90^{MN}	77.15^{M}
7285	1.21^{EF}	0.082	1.16^{EF}	33.92^{BCD}	1.27^{E}	35.20^{ABCD}	74.03^{EF}	77.98^{DE}
7286	1.16^{FG}	0.079	1.15^{F}	33.98^{BCD}	1.24^{F}	35.13^{ABCD}	73.58^{FG}	77.92^{EF}
7289	1.11^{GH}	0.074	1.13^{G}	34.02^{BCD}	1.21^{G}	35.06^{ABCDE}	72.96^{GH}	77.82^{FG}
7293	1.05^{HI}	0.071	1.11^{GH}	34.05^{BCD}	1.19^{GH}	34.98^{ABCDE}	72.53^{HI}	77.75^{GH}
P5	1.32^{BC}	0.091	1.20^{BC}	33.29^{CD}	1.33^{BC}	35.39^{ABC}	75.90^{C}	78.20^{AB}
General Mean	0.97	0.054	1.10	34.16	1.17	34.62	71.06	77.51
SE	0.029	0.061	0.008	0.532	0.011	0.420	0.373	0.061
CV(%)	3.61	7.63	0.92	1.91	1.19	1.49	0.64	0.10

4.1.3. Physiological response of black pepper accessions after ten days of progressive moisture stress

The same accessions were further subjected for longer period of stress of ten days to understand the response of various physiological parameters at longer periods of moisture stress and the results are given in Table 27. Significant differences were observed for most of the physiological characters after ten days of moisture stress. There was a decrease in the rate of most of the physiological parameters.

4.4.1.3.1. Photosynthesis

Under most of the water stressed conditions reduced CO_2 diffusion to the site of carboxylation is the main reason for decreased photosynthetic rate (Erismann *et al.*, 2008). Tolerant hybrids showed higher rate of photosynthesis in cocoa compared to susceptible ones (Juby, 2019). The mean value for photosynthesis registered 55.09 per cent reduction after ten days of stress compared to that after five days of moisture stress. The maximum rate of photosynthesis was observed for the accession 7215 and the minimum for the accession 7252 indicating the better tolerance of the former and higher susceptibility of the later.

4.4.1.3.2. Stomatal conductance

Stomatal closure is a well-known first responsive reaction of plants to drought stress. It is more closely related to soil moisture status than leaf water status and is mostly controlled by chemical signals such as Abscisic acid (ABA) produced in dehydrating roots (Brodribb and Mc Adam, 2011).

The mean value of stomatal conductance (0.054 $mmolm^{-2}s^{-1}$) registered a decrease of 14.29 per cent after ten days of moisture stress compared to that after five days of stress. The maximum value of 0.096 $mmolm^{-2}s^{-1}$ was noted for the accession 7215 and minimum of 0.025 $mmolm^{-2}s^{-1}$ for the accession 7232. The higher value for this parameter observed indicates more vulnerability of the accessions to stress where as the lowering of stomatal conductance shows the ability to reduce water loss by transpiration inspite of curtailment of photosynthesis.

4.4.1.3.3. Transpiration

Water stress is reported to cause decline of transpiration rate in many crops. In cashew seedlings stressed for two days, transpiration rate declined from 4.75 $\mu molH_2O^{-1}m^{-2}s^{-1}$ to 2.1175 $\mu molH_2O^{-1}m^{-2}s^{-1}$ when stress was given for five days (Latha, 1998). Balasimha *et al.* (1988) reported that drought tolerance in Cocoa is mainly attributable to effective stomatal regulation resulting in decreased transpiration.

Transpiration rate recorded after ten days of stress showed a reduction in mean value by 20.29 per cent compared to that after five days of moisture stress and the accessions were significantly different. from each other The rate of transpiration was maximum in the accession 7215 (1.22 $\mu molH_2O^{-1}m^{-2}s^{-1}$ compared to the minimum (0.98 $\mu molH_2O^{-1}m^{-2}s^{-1}$) for the accession 7252.

4.4.1.3.4. Leaf temperature

Leaf temperature registered slight increase after 10 days of moisture stress in all the accessions. There was an increase in mean leaf temperature by 0.26°C (0.77 %) compared to the mean value of 33.9 °C after five days of moisture stress and the accessions differed significantly. Relatively low leaf temperature shown by the accessions *viz.* 7215 (33.24°C), 7240 (33.26° C), P5 (33.29 °C), 7241 (33.75 °C) and 7259 (33.84 °C) indicated their tolerance to the moisture stress. High values of leaf temperature was shown by the accessions *viz.* 7249 (36.10 °C), 7252 (35.07 °C), 7232 (34.65 °C), 7211 (34.58 °C) and 7219 (34.51°C). Hence, these accessions appear to be more susceptible to moisture stress. In cocoa, hybrid seedlings with leaf temperature above 31.6°C are reported as tolerant to moisture stress at 40 per cent field capacity (Juby, 2019).

4.4.1.3.5. Chlorophyll content

The chlorophyll content of accessions also reduced on progress of moisture stress to ten days and the accessions differed significantly. The mean value of chlorophyll content declined by 23.53 per cent compared to the mean value after five days of stress. Moisture stress has direct influence on decline of efficacy of photosynthesis. Maximum value was shown by the accession 7215 (1.38 µg g^{-1}) and minimum (1.01 µg g^{-1}) by the accession 7232. The higher value of chlorophyll content after ten days of moisture stress indicated that the accessions 7215, 7240 and P5 can be more tolerant to moisture stress than other accessions. The accessions 7252, 7232 and 7211 can be more susceptible to moisture stress as they recorded low values of chlorophyll content.

4.4.1.3.6. Chlorophyll stability

Chlorophyll stability of the accessions also showed a decline by 24.97 per cent in the mean value after ten days of stress compared to that after five days. This is in agreement with the finding by Juby (2019) in cocoa where the observed value of susceptible ones was below 60 per cent and tolerant ones above 60 per cent where as control had 80 per cent chlorophyll stability at 40 per cent moisture content of field capacity. In the present experiment, minimum value (33.10 %) for this parameter was shown by the accession 7252 and maximum (35.66 %) by the accession 7215, showing inferior and superior performance by the respective accessions for moisture stress tolerance. The accessions 7252, 7232, 7211 and 7219 were on par with low values for chlorophyll stability indicating more vulnerability to moisture stress.

4.4.1.3.7. Relative water content

The mean value of relative water content (RWC) also registered a decrease by 14.22 per cent after ten days of stress. The maximum value of 78.08 per cent was shown by the accession 7215 and minimum (65.27%) by the accession 7252. The higher value of relative water content is considered as a criterion for drought tolerance in hybrid Cocoa (Juby, 2019).

This shows that the accession 7215 exhibited its superiority for moisture stress tolerance after ten days of progressive moisture stress . The accessions 7215, 7240, P5, 7241, and 7259 were on par for this parameter showing better tolerance to moisture stress compared to others. The accessions with low values *viz*.7252 and 7232 can be more vulnerable to moisture stress. In black pepper accessions which maintain relative water content greater than 70 per cent and membrane stability above 80 per cent after 12 days of moisture stress (complete with holding of of water) are considered to be relatively tolerant (Krishnamurthy *et al*., 2006). Based on this criteria, none of the accessions in this experiment can be considered as tolerant to moisture stress.

4.4.1.3.8. Membrane stability index

As a major impact of environmental stress, the cellular membrane dysfunctions by showing increased permeability and leakage of ions which can readily be measured by the efflux of electrolytes. Membrane stability is associated with water and high temperature stress tolerance in various crop plants (Sairam *et al*., 1998). In cocoa, the drought tolerant hybrids showed high membrane stability under stress compared to drought susceptible ones (Juby, 2019).

In the present experiment, the membrane stability values showed a decline in mean value by 9.05 per cent after ten days of stress compared to that of five days of stress. The maximum value of 78.27 (%) was observed for the accession 7215 and minimum of 76.32 (%) observed for 7252. The high value is indicative of better tolerance of the accession to moisture stress. The accessions 7215, 7240, 7241, and P5, were on par for this parameter showing better tolerance to moisture stress compared to others. The accessions with low values *viz*. 7252 and 7232 may be showing susceptibility to moisture stress.

After ten days of progressive moisture stress, the accessions differed significantly for all physiological parameters. Most of the physiological parameters that showed sharp decline in mean value compared to values after five days of stress.

This included photosynthesis (55.09%), transpiration (20.29%), chlorophyll content (23.53%), chlorophyll stability (24.97%), stomatal conductance (14.28%), membrane stability (9.05%) and relative water content (14.22 %). Leaf temperature only registered an increase in mean value (0.77%). The accessions 7215, 7240, 7241, 7259 and P5(check) with high values of the physiological parameters favouring stress tolerance *viz*. photo synthesis, chlorophyll content, chlorophyll stability, membrane stability, relative water content and inferior values for leaf temperature showed that the accessions may have better stress tolerance. The low values exhibited by the accessions *viz*. 7252, 7232, and 7211 for these parameters and high value of leaf temperature can be considered as an indication of susceptibility to moisture stress.

The rate of photosynthesis decreased in all the accessions on progress of moisture stress. Maximumum value was observed at field capacity. The rate of stomatal conductance registered a progressive decrease in value across the accessions on progress of moisture stress. The value was maximum at field capacity. A similar trend was was observed for stomatal conductance, rate of transpiration, chlorophyll content, chlorophyll stability and membrane stability. However, leaf temperature registered a gradual increase across the accessions on advancement of moisture stress. (Fig.6-12.).

4.4.2. Biochemical response of black pepper accessions under progressive moisture stress.

Moisture stress induces changes in the biochemical structure in plant cell. The changes in different biochemical components at different levels of moisture stress is compared among the black pepper accessions to study the changes happening at different levels of moisture stress.

Use of antioxidant enzymes *viz.* catalase, peroxidase and superoxide dismutase as screening parameters for drought tolerance has been established in many crop plants (Dhindsa *et al*., 1981; Chowdhury and Choudhuri, 1985., Chempakam *et* *al.*, 1993., Rahman *et al.*, 1999., Krishnamurthy *et al.*, 2000). The integrity of the photosynthetic membranes is maintained under oxidative stress by the cooperation of non-enzymatic antioxidants or enzymatic antioxidant to scavenge reactive oxygen species (ROS) developed inside the plant tissues developed through various metabolism and stress.

Biochemical analysis was carried out for all the 21 accessions including the check variety Panniyur 5 by collecting sample at three different stages of stress *viz*. field capacity, after five days of stress induction and after ten days of stress induction. The results on biochemical responses of 21 black pepper accessions at field capacity is detailed in Table 28.

4.4.2.1. Biochemical response of black pepper accessions under field capacity.

At field capacity all the accessions are on par with respect to all biochemical parameters (Table 28). A mean value of 2.42.µg g^{-1} fresh weight of proline was recorded by the accessions .Mean superoxide dismutase activity of the accession was 13.29 a.u. g^{-1} fresh weight . The catalase content of the accessions had a mean of 6.09 a.u. g^{-1} fresh weight. The mean peroxidase content of the accessions at field capacity was 12.53 a.u. g^{-1} fresh weight.

Krishnamurthy *et al.*, 2000 reported that there was no significant difference in catalase activity among *Piper colubrinum, Piper chaba* and *Piper hymenophyllum.* at field capacity. The SOD activity at control (field capacity) was high in *Piper colubrinum* compared to other species and was on par with *Piper nigrum* accessions .All the accessions were on par for the biochemical parameters at field capacity as they were devoid of stress.

Table 28. Biochemical response of black pepper accessions at field capacity

Accession	Proline ($\mu g\ g^{-1}$ fresh weight)	Superoxide dismutase (a.u. g^{-1} fresh weight)	Catalase (a.u. g^{-1} fresh weight)	Peroxidase (a.u. g^{-1} fresh weight)
7211	2.35	13.25	5.97	12.68
7215	2.45	13.13	6.27	12.47
7219	2.40	13.26	6.02	12.55
7221	2.19	13.44	6.20	12.63
7222	2.45	13.34	5.88	12.87
7229	2.09	13.43	5.86	12.71
7232	2.36	13.23	5.97	12.39
7240	2.41	13.16	6.38	12.42
7241	2.30	13.35	6.04	12.21
7243	2.60	13.46	5.93	12.73
7249	2.48	13.32	5.98	12.80
7252	2.41	13.12	6.04	12.45
7254	2.10	13.28	6.23	12.51
7255	2.60	13.47	6.31	12.69
7259	2.48	13.26	6.04	12.48
7276	2.58	13.20	6.27	12.64
7285	2.61	13.20	6.06	12.58
7286	2.62	13.12	6.09	12.19
7289	2.52	13.23	6.13	12.38

Table 28. (Contd)

7293	2.61	13.58	5.98	12.67
P5	2.22	13.22	6.15	12.05
General Mean	2.42	13.29	6.09	12.53
SE	0.185	0.158	0.254	0.285
CV (%)	9.38	1.46	5.10	2.79

4.4.2.2. Biochemical response of black pepper accessions under five days of moisture stress induction

The result on the evaluation of black pepper accessions after five days of moisture stress induction is presented in Table 29.

Table 29. Biochemical response of accessions after five days of moisture stress induction

Accession	Proline ($\mu g\ g^{-1}$ fresh weight)	Superoxide dismutase (a.u. g^{-1} fresh weight)	Catalase (a.u. g^{-1} fresh weight)	Peroxidase (a.u. g^{-1} fresh weight)
7211	2.90^{MN}	14.15	3.18^{L}	28.61^{P}
7215	3.88^{A}	15.76	4.87^{A}	36.52^{A}
7219	2.97^{LMN}	14.34	3.41^{KL}	29.53^{OP}
7221	3.36^{FGH}	15.16	4.27^{DEF}	33.80^{GHI}
7222	3.21^{IJ}	14.81	4.02^{FGH}	32.64^{JK}
7229	3.26^{HIJ}	14.94	4.13^{EFG}	32.96^{IJK}
7232	2.88^{N}	14.00	2.77^{M}	27.24^{Q}
7240	3.81^{AB}	15.74	4.80^{AB}	36.30^{AB}
7241	3.68^{C}	15.56	4.71^{AB}	35.78^{ABCD}
7243	3.16^{JK}	14.74	3.93^{GHI}	32.03^{KL}

Table 29. (Contd)

7249	3.01 LM	14.49	3.58 JK	30.30 NO
7252	2.86 N	13.85	2.45 N	26.36 Q
7254	3.30 GHI	15.04	4.18 DEFG	33.41 HIJ
7255	3.08 KL	14.68	3.84 HIJ	31.63 LM
7259	3.63 CD	12.18	4.65 ABC	35.50 BCD
7276	3.03 KL	14.59	3.69 IJK	30.96 MN
7285	3.54 DE	15.45	4.60 ABC	35.17 CDE
7286	3.50 E	15.41	4.55 BC	34.86 DEF
7289	3.46 EF	15.35	4.43 CD	34.47 EFG
7293	3.41 EFG	15.33	4.39 CDE	34.10 FGH
P5	3.75 BC	15.67	4.76 AB	36.13 ABC
General Mean	3.32	14.82	4.06	32.78
SE	0.064	1.030	0.139	0.483
CV (%)	2.37	8.51	4.21	1.80

The result indicated that the accessions differed significantly for most of the biochemical characters on progress of moisture stress for five days.

4.4.2.2.1.Proline

Proline gets accumulated in plant in response to the stress and is able to protect cells from damage by functioning both as an osmotic agent and a scavenger of free radicals. The proline content of the accessions ranged from 2.86 µg gfreshweight^{-}1(7252) to 3.88 µg g^{-1} fresh weight (7215) after five days of moisture stress induction. It registered an increase of 37.19 per cent in the accessions following increase in moisture stress after five days compared to that at field capacity. The higher values of proline content is considered as a criterion for drought tolerance screening in hybrid cocoa seedlings (Juby, 2019). The higher value of proline observed may indicate superior tolerance of the accession 7215 and 7240 to moisture stress after five days of with holding water.

4.4.2.2.2. Superoxide dismutase (SOD)

Superoxide dismutase is acting as the most important anti oxidant first line defense system operating in plants that provide resistance against the oxidative damage due to oxide radicals (Larson, 1988) . After five days of stress, the accessions did not show significant difference in the value of superoxide dismutase activity. However, the mean value of superoxide dismutase (14.82 a.u.g^{-1} fresh weight) activity showed an increase of 11.51 per cent compared to that at field capacity. In a similar experiment, conducted at ICAR-IISR, Calicut, superoxide dismutase activity did not change much during control as well as early stress in all *Piper* species (Krishnamuthy *et al.*, 2000).

4.4.2.2.3.Catalase

Catalase present inside peroxisomes, mitochondria and chloroplast of cells plays an important role in plant defense system against drought. It acts as a scavenger of reactive H_2O_2 and dismutes to harmless H_2O and oxygen. The catalase content of the accessions differed significantly after five days of moisture stress. The catalase activity ranged from 2.45 a.u. g^{-1} fresh weight (7252) to 4.87 a.u. g^{-1} fresh weight (7215) with mean value of 4.06 a.u. g^{-1} fresh weight registering a decrease in mean value of 33.33 per cent after five days of stress compared to that at field capacity. The accession 7215 appears to be tolerant to early moisture while the accession 7252 behaved as susceptible with respect to catalase activity. The higher value of catalase is indicative of better tolerance to moisture stress of the accession compared to others. The report by Krishnamurthy *et al.,* (2000) on significant difference of catalase observed among *Piper nigrum* accessions and decline in mean value in four wild species of black pepper after five days under water stress compared to control corresponds to this observation.

4.4.2.2.4. Peroxidase

The peroxidase content also showed significant variation among the accessions after five days of stress. The values of peroxidase ranged from 26.36 a.u. g^{-1} fresh weight (7252) to 36.52 a.u. g^{-1} fresh weight (7215) with the mean value of 32.78 a.u.g^{-1} fresh weight showing an increase of 161.61 per cent compared to that at field capacity. The maximum value was shown by the accession 7215 and minimum by 7252. Peroxidase is a haemoprotein catalizing the oxidation of a number of substances by hydrogen peroxide with concurrent oxidation of a substrate. Most species show increase or maintenance of peroxidase in early phase of drought and then a decrease with further increase in magnitude of stress (Jingxian and Kirkham, 1994). The report by Krishnamurthy *et al.,* (2000) on significant difference of peroxidase among four *Piper nigrum* accessions and increase in mean value of four wild species of black pepper were observed after five days under water stress compared to control is supportive of this observation. Prolonged increase in activity of peroxidase was noticed in drought tolerant tif dwarf grass (Shaoyun *et al.*, 2003). So, the high value of peroxidase shown by the accession 7215 in the present experiment indicates higher activity of the enzyme present and may contribute to better tolerance to moisture stress .

4.4.2.3. Biochemical response of black pepper accessions under ten days of moisture stress induction

The results on biochemical parameters under further advancement of moisture stress with the accessions are presented in Table 30.

Table 30. Biochemical response of accessions after 10 days of moisture stress induction

Accession	Proline (µg g^{-1} fresh weight)	Superoxide dismutase (a.u. g^{-1} fresh weight)	Catalase (a.u. g^{-1} fresh weight)	Peroxidase (a.u. g^{-1} fresh weight)
7211	3.47^{LMN}	13.78^{NO}	2.59^{O}	13.68^{LM}
7215	3.98^{A}	15.07^{A}	4.09^{A}	15.42^{A}
7219	3.51^{KLMN}	13.89^{N}	2.81^{N}	13.78^{KLM}
7221	3.73^{DEFG}	14.49^{GHI}	3.52^{G}	14.72^{DEF}
7222	3.66^{FGHI}	14.31^{JK}	3.31^{IJ}	14.44^{FG}
7229	3.67^{FGHI}	14.39^{IJ}	3.38^{HI}	14.61^{EF}
7232	3.44^{N}	13.71^{O}	2.30^{P}	13.49^{MN}
7240	3.86^{B}	15.00^{AB}	3.98^{AB}	15.30^{A}
7241	3.82^{BCD}	14.89^{BCD}	3.89^{BC}	15.21^{AB}
7243	3.64^{GHIJ}	14.25^{KL}	3.21^{JK}	14.25^{GHI}

Table 30. (Contd)

7249	3.53^{JKLM}	14.06^{M}	2.94^{M}	13.94^{JKL}
7252	3.41^{N}	13.73^{O}	2.09^{Q}	13.30^{N}
7254	3.70^{EFGH}	14.44^{HIJ}	3.46^{GH}	14.43^{FGH}
7255	3.60^{HIJK}	14.19^{KLM}	3.13^{KL}	14.13^{HIJ}
7259	3.80^{BCDE}	14.84^{CD}	3.84^{CD}	15.17^{ABC}
7276	3.58^{IJKL}	14.13^{LM}	3.02^{LM}	14.03^{IJK}
7285	3.78^{BCDE}	14.77^{DE}	3.75^{DE}	15.33^{A}
7286	3.76^{BCDEF}	14.69^{EF}	3.71^{EF}	14.93^{BCD}
7289	3.75^{CDEF}	14.62^{FG}	3.66^{EF}	14.89^{CDE}
7293	3.74^{CDEFG}	14.58^{FGH}	3.59^{FG}	14.81^{DE}
P5	3.85^{BC}	14.96^{ABC}	3.95^{BC}	15.27^{A}
General Mean	3.68	14.42	3.34	14.53
SE	0.054	0.068	0.065	0.152
CV (%)	1.79	0.57	2.37	1.28

All the biochemical parameters showed significant differences among the accessions on progress of moisture stress for ten days.

4.4.2.3.1. Proline.

The proline content registered an increase of 10.84 per cent in mean value compared to that of five days of moisture stress. The proline content of the accessions ranged from 3.41µg g^{-1} fresh weight (7252) to 3.98µg g^{-1} fresh weight (7215). Proline gets accumulated in plant in response to the stress and is able to protect cells from damage by functioning both as an osmotic agent and a radical scavenger. The higher values of proline content is considered as a criterion for drought tolerance screening

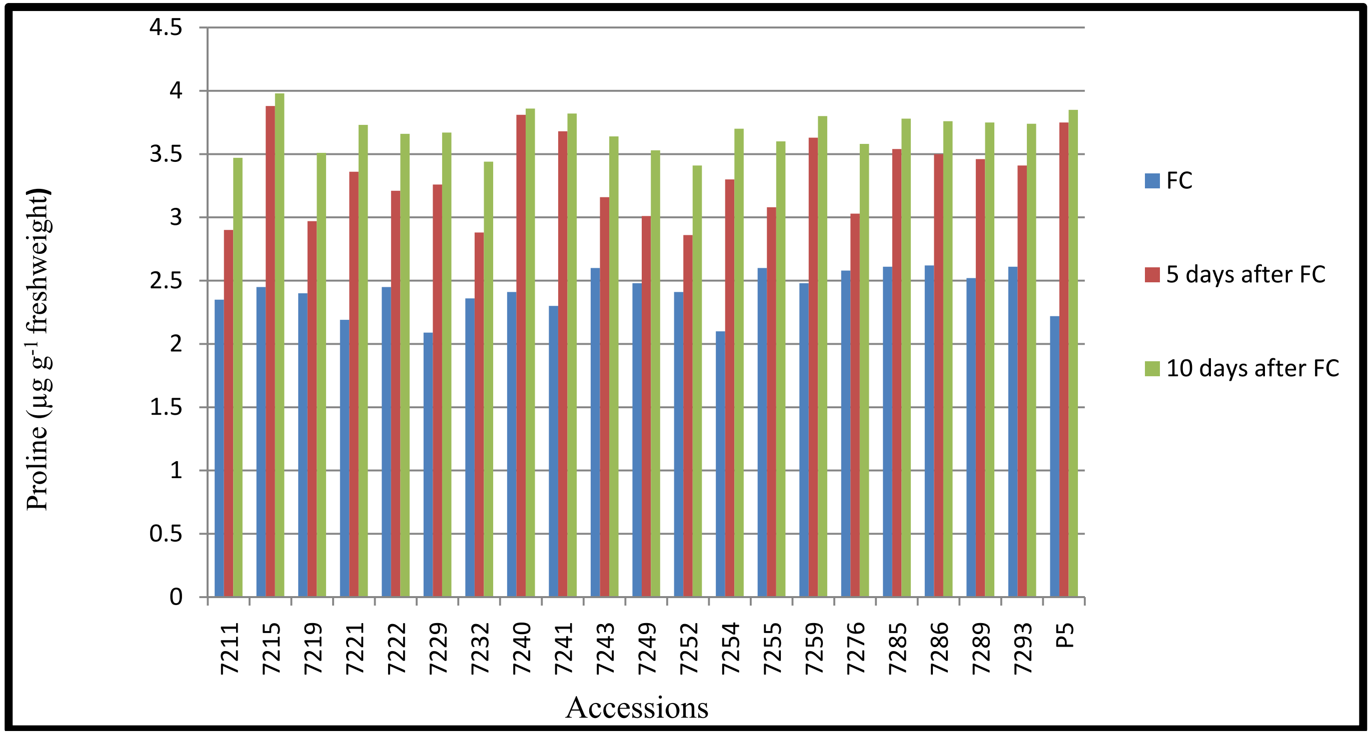

Fig.13. Variation in prolinecontent of black pepper accessionsunder progressive moisture stress

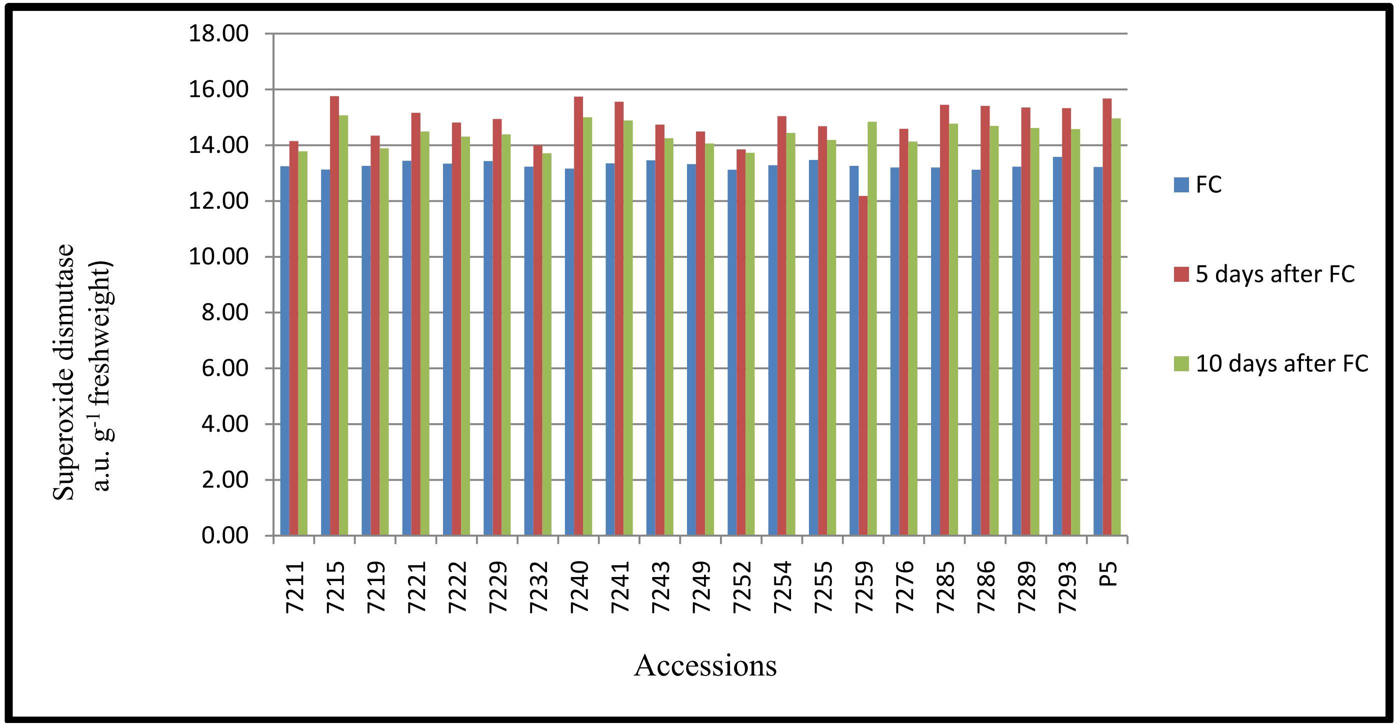

Fig.14. Variation in SOD activityof black pepper accessions under progressive moisture stress

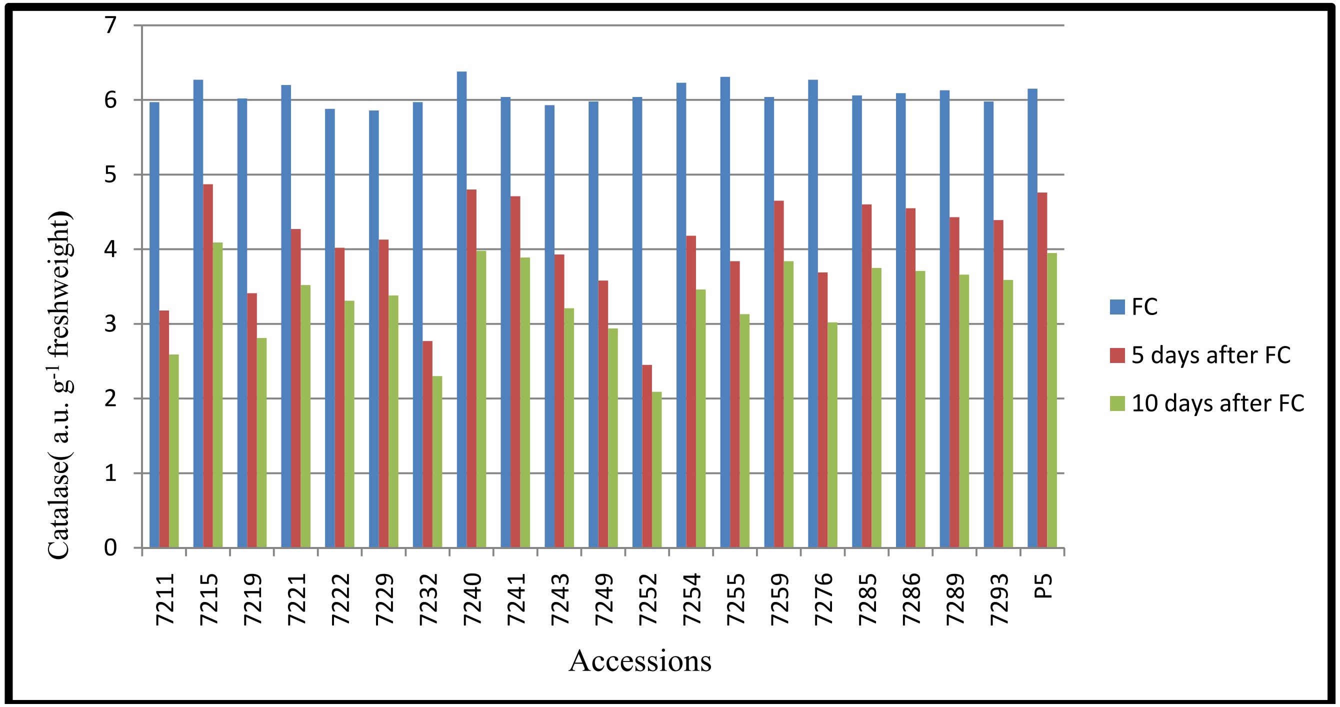

Fig.15.Variation in catalase activity of black pepper accessionsunder progressive moisture stress

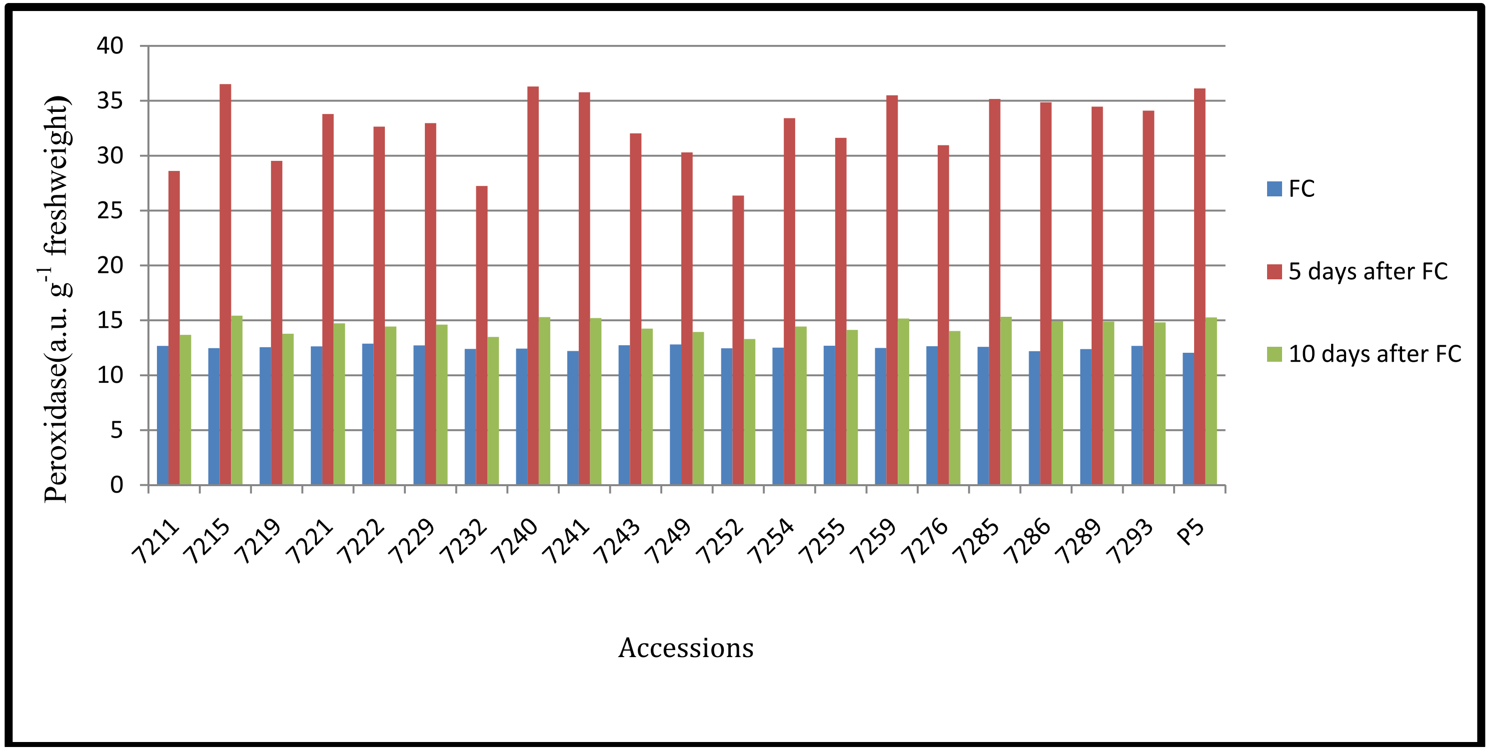

Fig.16.Variation in peroxidase activity of black pepper accessionsunder progressive moisture stress

hybrid Cocoa seedlings (Juby, 2019). The accession 7215 showed superior proline content compared to all other accessions and hence can be tolerant to moisture stress.

4.4.2.3.2. Superoxide dismutase

Unlike five days of moisture stress, the accessions showed significant difference in values of SOD. The mean value registered a decline by 2.70 per cent compared to that of five days of moisture stress. In a trial using the black pepper variety Panniyur1, the SOD activity was reduced by 6 per cent compared to that of five days of moisture stress (Krishnamurthy *et al.*2006). Hence, the accession, Panniyur 5 in the present study was able to tolerate stress better than Panniyur 1 as indicated by lower reduction (4.53) per cent of SOD recorded by it. The accessions varied in the SOD activity with a range of 13.71 a.u. g^{-1} fresh weight (7232) to 15.07 a.u. g^{-1} fresh weight (7215) with a mean value of 14.42 a.u. g^{-1} fresh weight . The high value of SOD observed in the accession 7215 may indicate its genetic potential to tolerate moisture stress while the accession 7232 may be susceptible.

4.4.2.3.3. Catalase

The catalase activity of the accessions differed significantly after ten days of moisture stress. It ranged from 2.09 a.u. g^{-1} fresh weight (7252) to 4.09 a.u. g^{-1} fresh weight (7215) with mean value of 3.34 a.u. g^{-1} fresh weight exhibiting a marked decrease of 17.73 per cent compared to that of five days of stress. In a similar experiment, the mean catalase activity showed 21.43 per cent decline after ten days of stress compared to that of five days of stress in Panniyur 1 variety of black pepper (Krishnamurthy *et al*. 2006). Hence, the accession Panniyur 5 in the present study was able to tolerate stress better than Panniyur 1. The superior value of catalase in the accession 7215 may be an indication of its tolerance. to moisture stress.

4.4.2.3.4. Peroxidase

The peroxidase content also showed significant variation among the accessions after ten days of stress. The peroxidase activity ranged from 13.30 a.u. g^{-1} fresh weight in the accession 7252 to 15.42 a.u. g^{-1} fresh weight in 7215 with the mean value of 14.53 a.u. g^{-1} fresh weight. There was a sharp decline in mean peroxidase activity by 55.67 per cent compared to the value after five days of stress. A decline of 38.46 per cent was reported for peroxidase activity in a similar study for variety Panniyur1 compared to the value after five days of with holding water by Krishnamurthy *et al.*, 2006. Hence, the accessions in the present study were more susceptible to moisture stress with respect to peroxidase activity . Higher value of peroxidase activity shown by the accession 7215 in the present experiment can be an indication of better tolerance to moisture stress .

Biochemical parameter, proline registered progressive increase on advancement of moisture stress in all the accessions. The SOD activity increased during initial stress and then showed a declining trend across the accessions.The catalase activity registered progressive decline among the accessions on advancement of moisture stress. The peroxdase activity registered a sharp rise in the early stress followed by high decline in value across the accessions (Fig.13-16.).

4.4. 3. Visual observation of black pepper accessions at permanent wilting point.

All the accessions were retained until they reached permanent wilting point (PWP) to observe the number of days taken to reach PWP by each accession. The results are presented in Table 31.

Table 31. Visual scoring of black pepper accessions at permanent wilting point

Accession	Days taken for permanent wilting	Symptoms of moisture stress			
		No.of fallen leaves	No.of leaves retained	No of flaccid leaves	No.of necrotic leaves
7211	16	5	9	9	7
7215	20	1	14	14	5
7219	16	5	8	8	6
7221	17	3	9	9	5
7222	16	4	10	10	6
7229	17	3	9	9	5
7232	16	5	7	7	5
7240	20	2	13	13	5
7241	19	2	13	13	6
7243	16	4	9	9	4
7249	16	5	8	8	6
7252	16	6	7	7	6
7254	17	3	10	10	6
7255	16	4	8	8	3
7259	19	3	12	12	6
7276	16	4	7	7	5
7285	19	3	12	12	7
7286	18	3	11	11	7
7289	18	3	11	11	6
7293	18	3	10	10	6
P5	19	2	12	12	5

Visual observations such as number of days taken for PWP, number of leaves fallen, number of retained leaves , number of flaccid leaves and number of necrotic leaves present on the plants at PWP per pot was noted and compared. The result shows that the accessions showed notable variation in visible symptoms of moisture stress tolerance. The accessions with superior tolerance had lesser number of fallen leaves and / more net number of leaves retained at PWP. Accessions 7215 and 7240 took 20 days to reach PWP. Four accessions viz. P5, 7245, 7259, and 7285 wilted in 19 days showing tolerance to moisture stress. Three accessions reached PWP in 17 days of moisture stress. Eleven accessions which reached PWP in 16 days were least tolerant to moisture stress, with lowest number of leaves retained.

Table 32. Ranking of accessions on the basis of physiological and biochemical parameters

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
7211	21	2	2	13	2	16	12	4	10	14	17	12	125	20	16
7215	1	9	10	1	1	1	1	1	1	1	1	1	29	1	20
7219	14	2	2	13	2	15	11	4	10	13	16	12	114	18	16
7221	9	7	6	8	1	9	5	2	5	7	8	5	72	10	17
7222	11	5	4	10	4	12	6	2	6	9	11	7	87	13	16
7229	10	6	5	9	2	11	6	2	6	8	10	6	81	12	17
7232	15	1	1	14	4	16	13	4	11	15	18	13	130	21	16
7240	1	8	9	2	1	2	1	1	2	1	1	1	30	2	20
7241	3	8	8	3	1	4	2	2	3	3	2	1	40	4	19
7243	11	5	4	11	2	12	7	2	7	10	12	8	91	14	16
7249	14	3	3	12	2	14	10	5	9	12	15	11	110	17	16
7252	15	1	1	14	4	16	14	4	12	15	11	14	121	19	16
7254	10	6	5	9	1	10	5	2	6	7	9	7	77	11	17
7255	12	4	3	12	2	13	8	2	8	10	13	9	96	15	16
7259	4	8	8	4	1	4	2	2	3	3	3	2	44	5	19
7276	13	3	3	12	3	14	9	3	9	11	14	10	104	16	16
7285	5	8	7	5	1	5	3	2	3	4	5	1	49	6	19
7286	6	8	7	6	1	6	4	2	3	5	5	3	56	7	18
7289	7	8	6	7	1	7	4	2	4	6	6	4	62	8	18
7293	8	7	6	7	1	8	4	2	4	6	7	5	65	9	18
P5	2	8	9	2	1	3	1	1	2	2	2	1	34	3	19

1-Accession,2-photosynthesis,3-stomatal conductance,4-transpiration,5-chlorophyll content,6-chlorophyll stability,7-relative wter content,8-membrane stability index,9- leaftemperature,10-proline,11-SOD,12-catalase,13-peroxidase,14-total score,15-rank and 16-days taken for PWP.

4.4.4 Ranking of accessions on the basis of physiological and biochemical parameters for drought tolerance

Accessions were ranked based on physiological and biochemical response at 10 days of moisture stress and is presented in Table 32. The ranking of the accessions were in agreement with the visual scoring. The result shows that the accessions 7215, 7240, P5 and 7241 were having high values for most of the positively influencing physiological and biochemical parameters of drought tolerance The accessions *viz.* 7252, 7232, 7211 and 7219 exhibited low values for the various physiological parameters associated with efficient tolerance of water stress and were reaching PWP early. These results confirms the effect of different physiological and biochemical parameters in imparting tolerance to moisture stress.

4.5. Response of black pepper accessions to foliar nutrition under moisture stress

Foliar nutrition has been advocated as a measure for mitigating losses due to moisture stress on crop growth and to reap moderate growth and yield in many crops. Foliar nutrition of key nutrients like potassium, boron, zinc *etc* can alleviate their deficiencies and increase drought tolerance maintaining physiological and biochemical processes. Pink pigmented facultative methylotrophs (PPFM) are bacteria isolated from plant tissues capable of using methanol as carbon source and are distinguished by pink colour. The application of PPFM as biofertilizer is also advocated for the protection of crops towards heat and drought. *Pseudomonas* foliar spray along with a biofertilizer enhanced the microbial population in soil, making nutrients more available to rice plants (Jayajyothi *et al*., 2014).

In a drought mitigation study on tomato, the highest leaf water potential of -0.89 MPa was registered by PPFM (2 %) which can protect the plant under drought. Foliar spray of 2 per cent PPFM recorded lowest leaf temperature (25.2 °C) and highest stomatal conductance (0.40 mmol m^{-2} s^{-1}) followed by brassinolide (25.50 °C and 0.38 mmol m^{-2} s^{-1}) respectively (Sivakumar *et al*., 2018).

Foliar spray of potassium and aminoacids significantly improved morphological and biochemical characters of wheat when grown under drought as well as normal irrigated conditions conditions (Ahamad *et al*., 2019).

Analysis of gas exchange characteristics between the control and *P. nigrum* applied with beneficial microorganism treatment revealed that the photosynthetic rate, stomatal conductance and transpiration rate in pepper applied with Fermented Plant Juice (FPJ) and Fermented Fruit Juice (FFJ) were significantly ($p<0.05$) higher compared to that of Indigenous Micro Organisms (IMO), Lactic Acid Bacteria Serum (LABS) and the control (Sulok *et al*., 2018).

Integrated fertilizer treatment was found to be significantly superior to organic and chemical fertilizers realizing higher yield producing various phenotypic alterations in physiological processes and plant characteristics such as large leaf area index, higher chlorophyll content, photosynthetic rate and low transpiration rate in black pepper trial (Ann, 2012).

4.5.1. Biochemical response of black pepper accessions under foliar nutrition on 5th day of moisture stress following field capacity

Foliar nutrition was done on fifth day of moisture stress as described in materials and methods and observations were recorded following standard procedures on third day of application. The results are presented in Table 33.

Table 33. Biochemical response of black pepper accessions under foliar nutrition and control on 8th day of moisture stress induction (MSI)

Accession	Without foliar nutrition				With foliar nutrition			
	Proline (µgg^{-1} fresh weight)	Superoxide dismutase (a.u.g^{-1} fresh weight)	Catalase (a.u.g^{-1} fresh weight)	Peroxidase (a.u.g^{-1} fresh weight)	Proline (µg g^{-1} fresh weight)	Superoxide dismutase (a.u.g^{-1} fresh weight)	Catalase (a.u.g^{-1} fresh weight)	Peroxidase (a.u.g^{-1} fresh weight)
7211	3.01^{E}	14.10^{BCD}	2.87^{F}	14.38^{G}	2.83	14.08^{I}	2.76^{K}	15.18^{O}
7215	3.89^{A}	15.51^{A}	4.34^{A}	25.27^{A}	3.85	15.53^{A}	4.61^{A}	26.46^{A}
7219	3.05^{E}	14.25^{BCD}	3.10^{EF}	16.45^{F}	2.91	14.06^{IJ}	3.06^{J}	17.29^{M}
7221	3.49^{C}	14.81^{BC}	3.78^{C}	20.63^{C}	3.30	14.58^{E}	3.72^{F}	22.68^{GH}
7222	3.40^{D}	14.62^{BC}	3.55^{D}	20.15^{D}	3.10	14.34^{FG}	3.45^{GH}	21.17^{J}
7229	3.43^{D}	14.75^{BC}	3.65^{CD}	20.52^{CD}	3.18	14.40^{FG}	3.56^{FG}	21.71^{IJ}
7232	2.95^{E}	13.93^{D}	2.54^{FG}	15.76^{FG}	2.85	14.15^{HI}	2.26^{L}	17.22^{M}
7240	3.82^{A}	15.37^{AB}	4.20^{AB}	25.12^{A}	3.79	15.52^{A}	4.62^{A}	26.45^{A}
7241	3.71^{AB}	15.13^{B}	4.11^{B}	23.06^{B}	3.63	15.27^{B}	4.53^{AB}	25.48^{B}
7243	3.37^{D}	14.51^{BCD}	3.47^{DE}	19.52^{D}	3.04	14.29^{GH}	3.43^{GH}	20.51^{K}
7249	3.08^{E}	14.39^{BCD}	3.22^{E}	16.92^{F}	3.01	14.10^{I}	3.20^{IJ}	18.80^{L}
7252	2.90^{EF}	13.70^{D}	2.35^{G}	14.28^{G}	2.84	13.93^{J}	2.18^{L}	16.02^{N}
7254	3.48^{C}	14.79^{BC}	3.76^{CD}	20.35^{CD}	3.19	14.47^{EF}	3.65^{F}	22.30^{HI}
7255	3.32^{DE}	14.49^{BCD}	3.40^{DE}	19.18^{DE}	3.10	14.27^{GH}	3.36^{HI}	19.96^{K}
7259	3.65^{B}	14.98^{BC}	4.08^{B}	22.93^{B}	3.57	15.21^{BC}	4.47^{AB}	25.09^{BC}
7276	3.25^{DE}	14.45^{BCD}	3.26^{E}	18.75^{E}	3.21	14.19^{HI}	3.29^{HI}	19.28^{L}
7285	3.62^{B}	15.09^{B}	3.98B^{C}	22.52^{B}	3.49	15.17^{BC}	4.36^{BC}	24.64^{CD}
7286	3.60^{B}	14.89^{BC}	3.96B^{C}	22.06^{BC}	3.44	15.13^{BC}	4.19^{CD}	24.17^{DE}
7289	3.55^{BC}	14.85^{BC}	3.90^{C}	21.82^{C}	3.39	15.08^{C}	4.10^{DE}	23.65^{EF}
7293	3.54^{BC}	14.79^{BC}	3.81^{C}	21.58^{C}	3.30	14.92^{D}	3.97^{E}	23.07^{FG}

Table 33. (Contd)

P5	3.77 AB	15.28 AB	4.16 B	24.98 A	3.71	15.53 A	4.60 A	26.16 A
General Mean	3.42	14.69 BC	3.59	20.29	3.12	14.68	3.69	21.78
SE	0.059	0.96	0.12	0.39	0.297	0.073	0.090	0.317
CV (%)	2.05	7.56	3.92	2.10	11.66	0.61	2.99	1.78

The result indicated that all accessions differed significantly in their response to foliar nutrition for various biochemical parameters.

4.5.1.1. Proline

In the case of proline, the accessions registered a decrease in mean value by 8.77 per cent following foliar nutrition compared to mean proline content (3.34 µg g^{-1} fresh weight) without foliar nutrition. However, the accessions were not significantly different. The lowering in proline following foliar nutrition indicates the positive effect of foliar nutrition on reducing impact of stress irrespective of genotypic differences at early stress.

4.5.1.2. Superoxide dismutase

The accessions registered a marginal decrease in mean SOD values by 0.06 per cent after foliar nutrition compared to that without foliar nutrition. The accessions were significantly different indicating genetic variability for this character. The accessions 7215, 7240 and P 5 were showing similar trend of high SOD content as observed for without foliar nutrition .

4.5.1.3. Catalase

The mean value of catalase increased by 2.71 per cent after foliar nutrition compared to that without foliar nutrition. The accessions were significantly different for the catalase activity with and without foliar nutrition. The accessions *viz.*7215 ,7241, P 5 and 7259 were having higher catalase activity under both the conditions .

4.5.1.4. Peroxidase

The mean value of peroxidase increased by 7.34 per cent after foliar nutrition compared to that without foliar nutrition. The accessions were significantly different for the peroxidase activity under both situations. The accessions *viz.* 7215, 7240, and P 5 had high peroxidase activity.

Ahamed *et al.* 2019, in a study observed that wheat plants under drought stress conditions attained maximum biochemical traits when potassium was applied as foliar nutrition . The present study indicated the positive effect of foliar nutrition in tolerating moisture stress. Also the results indicated that the differences among the accessions was mainly attributed to the genetic constitution of the plants.

4.5.2. Biochemical response of black pepper accessions under foliar nutrition on 10th day of moisture stress following field capacity.

Foliar nutrition was given on tenth day of moisture stress as detailed in materials and methods.Observations were recorded following standard procedures on third day after foliar application. The results are presented in Table 34.

Table 34. Biochemical response of black pepper accessions under foliar nutrition on 13th day of moisture stress induction (MSI)

Accession	Without foliar nutrition				With foliar nutrition			
	Proline (µg g^{-1} fresh weight)	Superoxide dismutase(a.u. g^{-1} fresh weight)	Catalase (a.u. g^{-1} fresh weight)	Peroxidase (a.u. g^{-1} fresh weight)	Proline(µ g g^{-1} fresh weight)	Superoxide dismutase(a.u. g^{-1} fresh weight)	Catalase(a.u. g^{-1} fresh weight)	Peroxidase(a.u. g^{-1} fresh weight)
7211	3.48^{DE}	13.10^{H}	2.24^{GH}	9.01^{F}	3.50^{LMN}	13.55^{JKL}	3.47^{LMN}	10.57^{LM}
7215	3.93^{A}	14.08^{A}	3.75^{A}	11.25^{A}	3.95^{A}	14.40^{AB}	4.09^{A}	11.91^{A}
7219	2.88^{H}	13.08^{H}	2.42^{G}	9.04^{F}	3.53^{KLMN}	13.61^{JK}	3.51^{KLMN}	10.73^{KL}
7221	3.48^{DE}	13.51^{D}	3.24^{D}	9.48^{D}	3.75^{CDEFGHI}	14.04^{EFGH}	3.52^{G}	11.47^{DEFG}

Table 34. (Contd)

7222	3.29^{F}	13.36^{EF}	2.96^{E}	9.16^{E}	3.68^{FGHIJK}	13.91^{GHI}	3.31^{IJ}	11.26^{GHI}
7229	3.35^{EF}	13.41^{E}	3.01^{E}	9.21^{DE}	3.71^{EFGHIJ}	13.97^{FGH}	3.38^{HI}	11.35^{FGH}
7232	2.89^{H}	13.16^{H}	2.09^{H}	9.03^{F}	3.46^{MN}	13.46^{KL}	3.44^{MN}	10.47^{M}
7240	3.90^{A}	14.02^{A}	3.64^{B}	11.10^{A}	3.93^{AB}	14.37^{ABC}	3.98^{AB}	11.84^{AB}
7241	3.85^{AB}	13.91^{B}	3.57^{AB}	10.28^{C}	3.88^{ABCD}	14.29^{BCD}	3.89^{BC}	11.76^{ABC}
7243	3.19^{F}	13.32^{F}	2.91^{E}	9.14^{E}	3.66^{GHIJKL}	13.86^{HI}	3.21^{JK}	11.12^{HIJ}
7249	3.06^{H}	13.12^{G}	2.61^{FG}	9.08^{EF}	3.57^{JKLMN}	13.72^{IJ}	$3.53^{jkl.M}$	10.93^{JK}
7252	2.87^{H}	12.96^{H}	2.00^{HI}	9.00^{FG}	3.44^{N}	13.38^{L}	3.41^{N}	10.36^{M}
7254	3.36^{E}	13.46^{DE}	3.11^{DE}	9.25^{DE}	3.73^{DEFGHI}	14.00^{EFGH}	3.46^{GH}	11.39^{EFG}
7255	3.12^{G}	13.34^{F}	2.80^{EF}	9.12^{E}	3.63^{HIJKL}	13.64^{JK}	3.13^{KL}	11.07^{IJ}
7259	3.82^{B}	13.94^{AB}	3.49^{ABC}	10.14^{CD}	3.85^{ABCDE}	14.22^{BCDE}	3.84^{CD}	11.72^{ABCD}
7276	3.06^{G}	13.21^{G}	2.72^{F}	9.13^{E}	3.60^{IJKLM}	13.75^{IJ}	3.08^{LM}	10.99^{JK}
7285	3.79^{B}	13.87^{B}	3.42^{C}	10.10^{CD}	3.83^{ABCDEF}	14.19^{BCDEF}	3.75^{DE}	11.68^{ABCD}
7286	3.80^{B}	13.73^{C}	3.39^{C}	9.11^{CD}	3.82^{ABCDEF}	14.15^{CDEF}	3.71^{EF}	11.65^{BCD}
7289	3.61^{C}	13.63^{C}	3.36^{C}	9.62^{CD}	$3.79^{ABCDEFG}$	14.12^{DEFG}	3.66^{EF}	11.61^{BCDE}
7293	3.57^{C}	13.59^{D}	3.26^{D}	9.50^{D}	$3.77^{BCDEFGH}$	14.08^{DEFGH}	3.59^{FG}	11.54^{CDEF}
P5	3.86^{A}	13.98^{AB}	3.42^{C}	10.97^{AB}	3.90^{ABC}	14.56^{A}	3.95^{BC}	11.80^{AB}
General Mean	3.26	13.51	3.02	9.60	3.71	13.97	3.34	11.30
SE	0.071	0.11	0.051	0.132	0.078	0.108	0.065	0.128
CV (%)	2.16	0.87	2.28	1.28	2.59	0.94	2.37	1.39

4.5.2 1. Proline

The mean value of proline registered an increase of 13.80 per cent compared to that without foliar application and the accessions showed significant variation in the proline content. The accessions 7215 and 7240 were on par with higher proline content after foliar application.

4.5.2.2. Superoxide Dismutase

Accessions were significantly different for SOD both under control and foliar nutrition .There was an increase of 3.40 per cent in the mean value of SOD after foliar nutrition.

4.5.2 3.Catalase

The accessions recorded an increase in the activity of catalase following foliar nutrition than without foliar nutrition. The mean value of catalase increased by10.59 per cent after foliar nutrition. The accessions also showed significant difference under both conditions

4.5.2.4. Peroxidase

The accessions recorded an increase of 17.70 per cent in mean peroxidase activity after foliar nutrition. The accessions were significantly different for the peroxidase activity with and without foliar nutrition.

A similar trend was observed on tenth day of foliar nutrition.The accessions recorded a positive effect by recording increased values of all biochemical parameters after foliar application. Also it was observed that the tolerance to moisture stress under both the conditions are determined by the genotypes. The foliar nutrition was more effective on accessions having natural tolerance . The accessions 7215, 7240 , P5 and 7241 were tolerating moisture stress under both the conditions . As the experiment was carried out with potted plants of six month age and black pepper is grown by trailing on trees, the effectiveness of foliar nutrition on a commercial scale has to be validated further.

Comparison of visual observation at permanent wilting point (PWP) showed that the foliar nutrition did not extend the PWP of the genotypes . The accessions attained PWP regardless of foliar nutrient application.

4.5.3. Response of black pepper to foliar nutrition

Response of black pepper to foliar nutrition is presented in Table 35 and 36. The results indicated that there was no significant difference in the biochemical parameters of black pepper with and without foliar nutrition upon moisture stress induced on eighth and thirteenth day after field capacity.

Table 35. Response of black pepper to foliar nutrition on 8th day of MSI

Category	Mean of parameters			
	Proline content ($\mu g\ g^{-1}$ fresh weight)	SOD activity (a.u. g^{-1} fresh weight)	Catalase activity (a.u. g^{-1} fresh weight)	Peroxidase activity (a.u. g^{-1} fresh weight)
Foliar nutrition	3.29	14.69	3.67	21.75
Control	3.42	14.69	3.59	20.29
t -value	1.32^{NS}	0.05^{NS}	0.38^{NS}	1.48^{NS}

Table 36. Response of black pepper to foliar nutrition on 13th day of MSI

Category	Mean of parameters			
	Proline content ($\mu g\ g^{-1}$ fresh weight)	SOD activity (a.u.g^{-1} fresh weight)	Catalase activity (a.u.g^{-1} fresh weight)	Peroxidase activity (a.u.g^{-1} fresh weight)
Foliar nutrition	3.73	13.93	3.54	11.27
Control	3.71	13.96	3.57	11.30
t -value	0.50^{NS}	0.30^{NS}	0.36^{NS}	0.15^{NS}

Summary

. SUMMARY

The study entitled 'Characterization and identification of black pepper accessions *(Piper nigrum* L.) for stress tolerance and quality ' was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, College of Forestry, Vellanikkara (Kerala Agricultural University) and Indian Institute of Spices Research (ICAR-IISR), Calicut during 2016-2019. The objective of the study was to characterize black pepper accessions collected across Kerala and maintained at ICAR-IISR and identify superior ones for stress tolerance and quality. Fifty accessions of *Piper nigrum* maintained at IISR, Calicut constituted the basic experimental material. The salient findings are summerised below.

- Characterizeation of the accessions as per the descriptor for black pepper developed by IPGRI, 1995 showed that majority of accessions had dimorphic branching habit, light purple shoot tip colour, many runner shoots, weak holding capacity, few adventitious roots, horizontal lateral branch habit, ovate leaf lamina, round leaf base shape and round fruit shape .
- Clustering based on qualitative characters grouped the black pepper accessions numbering fifty into 4 clusters
- Multivariate hierarchial cluster analysis based on quantitative traits of vine , leaf, and spike characters grouped the accessions into two major clusters. The first cluster had three sub- clusters with 5, 6,and 35 accessions.The remaining four genotypes were grouped under the second cluster.
- The accessions were scored for high yield and low biotic susceptibility for foot rot infection and pollu beetle infestation as per IPGRI descriptor .
- Twenty accessions were selected based on superior yield and field tolerance to pollu beetle infestation and foot rot disease incidence.
- Biochemical characterization of selected accessions for piperine, essential oil and oleoresin content identified two accessions with high piperine content *viz.* 7293 with 6.96 per cent followed by 7252 with 6.71 per cent . Both are greater

than the highest reported value of 6.6 per cent in the commercial variety Panniyur 2.

- Essential oil content of the accessions varied from 3.00 (7254) - 5.87 (7211) per cent with a mean value of 4.13 per cent .
- Oleoresin content of accessions ranged from 7.10 (7229) - 11.18 (7289) per cent with a mean value of 9.12 per cent .
- Correlation analysis between resistance to *Phytophthora* , yield and biochemical parameters indicated absence of significant association between them
- Screening of selected accessions for foot rot disease tolerance showed that none of the accessions were tolerant to foot rot. However, the accession 7259 was moderately resistant to foot rot.
- Ranking of accessions based on yield, foot rot tolerance and quality parameters showed that the accessions 7252, 7211, 7259 7222, 7249,7232 and 7211 are promising.
- Screening of selected accessions for drought tolerance showed that at field capacity all the accessions were on par in response with respect to all biochemical parameters.
- A decrease in per cent mean values of physiological parameters like photosynthesis (59.60 %), stomatal conductance (76.57 %), rate of transpiration (26.20 %), chlorophyll content (45.55 %), chlorophyll stability (34.46 %), membrane stability (4.51 %) and relative water content (8.90 %) were observed after five days of stress induction.
- The accessions *viz.* 7215, 7240, P 5 and 7241 had high value of photosynthesis, chlorophyll content, chlorophyll stability, relative water content and membrane stability after five days of moisture stress
- Leaf temperature registered an increase in mean value (0.77 %) compared to that after five days stress induction

- After 10 days of progressive moisture stress induction, most of the physiological parameters showed decline in mean value
- The accessions 7215, 7240, P5, 7241 and 7259 had high values of physiological parameters favouring moisture stress tolerance *viz*. photo synthesis, chlorophyll content, chlorophyll stability, membrane stability, relative water content and inferior values for leaf temperature
- All accessions were on par in biochemical parameters at field capacity.
- After 5 days of stress induction, all the accessions differed significantly for most of the biochemical parameters except SOD activity. All accessions registered an increase in mean value of proline (37.19%), SOD (11.51%) and peroxidase (161%). Catalase activity showed a decline (33.33%) in per cent mean value.
- For biochemical parameters associated with moisture stress tolerance 7215, 7240, P 5 and 7241 were the accessions recording high values .
- After 10 days of moisture stress induction, most of the biochemical parameter's registered decrease in the mean value *viz*. SOD (2.70 %), catalase (17.73 %) and peroxidase (55.67 %).
- Proline content registerd an increase by 10.84 per cent in mean value compared to that after five days of stress induction
- The high value of proline, SOD, catalase and peroxidase for the accessions 7215, 7240, P5 and 7241 suggested superior tolerance to stress.
- The visual scoring observed by retaining the accessions until permanent wilting point (PWP) showed that accessions 7215, 7240, P 5 and 7241 were having high value for most of the positively influencing physiological and biochemical parameters of drought tolerance and survived longer The accessions 7252, 7232, 7211 and 7219 with low values for physiological and biochemical parameters favouring susceptibility to moisture stress reached PWP early.

- Comparison of responses of accessions to foliar nutrition on various biochemical parmeters showed that there is a positive effect of foliar nutrition on reducing the impact of stress
- Foliar nutrition was more effective on accessions having natural tolerance.
- The accessions 7215, 7240, P5 and 7211 were tolerating moisture stress better under both conditions.
- Comparison of visual observations at permanent wilting point showed that the foliar nutrition did not extend the PWP of the genotypes.

References

REFERENCES

Ahamad, A., Aslam, Z., Ilyas, M. Z., Ameer, H., Mahamood, A. and Rahman, M. 2019. *J. Environ. Agric. Sci.* 18: 10-18.

Anderson, M. D., Prasasd.,T. K. and Stewart, C. R. 1995. Changes in isozyme profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant physiol*.109:1247-1257.

Anjum, S.A., Wang, L. C. Farook, M., Hussain , M., Xue, L. L. and Zou, C.H. 2011. Brassinolide application improves the drought tolerace in maize through modulation of enzymatic oxidants and leaf gas exchange. *J. Agron. Crop Sci.*197:177-185.

Anjum, S. A., Xie, X., Wang,L. C., Saleem. M. F., Man, C. and Lei, W. 2011a. Morphological, physiological and biochemical responses of plants to drought stress. *African J of Agric Res*.6 (9): 2026-2032.

Anjum, S. A.,Wang, L. C., Farooq, M., Hussain, M., Xue, L. L., and Zou,C. M. 2011b. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic anti oxidants and leaf gas exchange. *J. Agron. Crop Sci*. 197:177-185.

Ann,Y. P.2012. Impact of different fertilization methods on the soil, yield and growth performance of black pepper (*Piper nigrum* L.). *Malasian J.of Soil Sci*.16:71-87.

Apel, K. and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Ann. Rev. Plant Biol.* 55: 373-399.

Apshara, S. E., Rajesh, M. K., and Balasimha, D. 2013. Assessement of morphological, physiological and molecular characteristics of cocoa ccessions from Central and South America in relation to drought tolerance. *J. Plant.Crops*.41(3); 389-39.

Archana, C. P. and Rajan, P. P. 2013. In vitro screening of black pepper (*Piper nigrum* L.) cultivars collected from Malabar region of Kerala, India, against *Phytophthora capsici* Leonian emend, Alizedeh and Tsao. *Int. J. of Applied Res. and Studies*. 2(5): 2028-2030.

Arnon, D. 1949. Measurement of chlorophyll content and chlorophyll stability. *Plant physiology* 24: 1- 15.

Arunachalam,V. and Bandyopadhayay,A. 1984. A method to make decisions jointly on a number of dependent characters. *Indian J Genet*. 44 (3): 419 - 424.

Ashraf, M. and Foolad, M. R. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59: 206-216.

ASTA [American Spice Trade Association] 1997. Official Analytical Methods (4thEd.). American Spice Trade Association, New York, p.175.

Athar, H. R., and Ashraf, M. 2005. Photosynthesis under drought stress. In: Pessarakli, M. (ed.), *Handbook of Photosynthesis.* (11th Ed.). CRC Press, Taylor and Fransis group, New York, pp. 793-804.

Bajji, M. Kinet, J. M., and Lutts, S. 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul*.36: 61-70.

Balasimha, D., Rajagopal, V., Daniel, E.V., Nair, R.V. and Bhagavan, S. 1988. Comparative drought tolerance of cacao accessions', *Plantation Crops Res. Inst. Reg. Sta*., 574 (3), p. 243.

Bekele, F. L., Bekele, I., Butler, D. R., and Bidaisee, G. G. 2006. Patterns of morphological variation in a sample of cocao (*Theobroma cacao* L.) germplasm from the International Cocoa Gene Bank,Trinidad.*Genet. Resour. Crop Evol.*33(5):933-948.

Benjamin, J. G. and Nielsen, D. C. 2006. Water deficit effects on root distribution of soyabean, field pea and chickpea. *Field Crops Res.* 97: 248-253.

Bhai, R.S. Joseph, T., Potty, S.N., Geetha, L. and Soloman, J. J. 2003. Mosaic disease of vanilla (*Vanilla planifolia* Andrews) the first report from India: *J.Spices and Aromatic Crops.*12:80-82.

Bhai, R. S., Ananadaraj, M., Sarma, Y. R., Veena, S. S., and Saji, K.V. 2007. Screening of black pepper (*Piper nigrum* L.) germplasm for resistance to foot rot disease caused by *Phytophthora capsici* Leoni. *J. Spices and Aromat. Crops.* 16(2) : 115-117.

Brodribb, T. J. and Adam, M. 2011. Passive origins of stomatal control in vascular plants. *Science* , 331: 582-587.

Cakmak, I. 2005. The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J *Plant. Nutr. Soil. Sci.* 168:521– 530.

Chandra, S. 2003. Energy exchange characteristics as as an indicator of biomass production potentialin tree species.*Belg. J. Bot.*136(1):45-51.

Chempakam, B., Kasturi-Bai, K. V., and Rajagopal, V. 1993. Lipid peroxidation in relation to drought tolerance in coconut (*Cocos nucifera* L.). *Plant Physiol. Biochem.* 20: 5-10.

Chowdhury, R.S., and Choudhuri, M.A., 1985, Hydrogen peroxide metabolism as an index of water stress tolerance in jute. *Physiologia Plantarum*. 65: 503-507

Demiral, T., and Turkan, I. 2004. Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? *J. Plant Physiol.*161: 1089-1110.

Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M., and Feller, U. 2009. Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. *Acta Physiol. Plant* 31: 1129-1138.

Dewaard, P. W. F. and Zeven, A. C. 1969. Pepper, *Piper nigrum*, L. *The Outlines of Perennial Crop Breeding in Tropics*. Wageningen. pp. 409- 426.

Dhanda, S. S., Sethi, G. S.and Behl. R. K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop Sci*. 190:6-12.

Dhindsa, R. S., Plumb-Dhindsa, P. and Thorpe, T. A. 1981. Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*. 32: 93-101.

Divya, C. R. and Sharada, M. S. 2014. Screening of *piper nigrum* l. Varieties/cultivars against quick wilt caused by *Phytophthora capsici* L. under green house condition. *Int. J.of Recent Scientific Res.*5 (11), 2028-2030.

Eikemo, H., Stensvand, D. J. and Tronsmo, A. M. 2003. Resistance to crown rot (*Phytophthora cactorum*) in raspberry cultivars and in offspring from crosses between cultivars differing in susceptibility to the disease *Ann. Appl. Biol*. 142:83–89.

Erismann, N.M., Machado, E.C. and Tucci, M.L.S. 2008. Photosynthetic limitation by CO_2 diffusion in drought stressed orange leaves on three root stocks. *Photosynth. Res*. 96 :163-172.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. A. 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*. 29:185-212.

Foyer, C. H. and Noctor, G. 2000.Oxygen processing in photosynthesis: regulation and signaliing. *New Phytol*.146:359-388.

George. K. J., Malik, N., Kumar, V. I. P., and Krishnamurthy, K. S. 2017. Gene expression analysis in drought tolerant and susceptible black pepper (*Piper nigrum* L.) in response to water deficit stress. *Acta Physiologicae Plantarum* 39:1-9.

Gnanasri, S. P., Saneoka, H., and Ogata, S. 1991. Cell membrane stability and leaf water relations as affected by potassium nutrition of water stressed maize. *J. Exp .Bot*. 42: 739-745.

Gopalam A. and Ravindran, P. N. 1987. Indexing of quality parameters in black pepper cultivars. *Indian Spices*. 22/23:8-11.

Hiscox, J. D. and Israelstam .G. F. 1979. A method for the extraction ofchlorophyll with out masceration. *Can.J.of Bot*. 57(12):1332-1334.

Horwath, E., Pal, M., Szaai, G., Paldi, E., and Janda, T. 2007. Exogenous4-hydroxy benzoic acid and salicylic acid modulate the effect of short term drought and freezing stress on wheat plants. *BiolPlan*.51: 480-487.

Ibrahim, K. K., Pillai,V. S., and Sasikumar, S. 1985. Variability, heritability and genetic advance for certain quantitative characters in black pepper. *Agric. Res. J. Kerala* 23:45-48.

IPC [International Pepper Community]. 2017. *Pepper Statistical Year Book*, International Pepper Community, Jakarta, Indonesia. [www.ipcnet.org]

IPGRI [International Plant Genetic Resources Institute]. 1995. Descriptor for black pepper. Rome, Italy. 180 p.

Irigoyen, J. J., D. W. Emerich, and M. Sanchez-Diaz. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in alfalfa (*Medicago sativa*) plants. *Physiol. Plant*. 84: 55-60.

Jaleel, C. A., Gopi, B. Sankar, P. Manivannan, A. Kishorekumar, R. Sridharan and Panneerselvam, R. 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. *South Afr. J. Bot.* 73: 190 -195

Jeyajothi, R., Subbalakshmi, L. and Nalliah, D. 2014. Effect of PPFM application on microbial population in transplanted Rice. Trends in Biosciences. 7: 3573–3574.

Jingxian, Z and Kirkham, M. B. 1994. Drought - stress - induced changes in activities of super oxide dismutase,catalase and peroxidase in wheat species. *Plant and Cell Physiol* 35(5): 785- 791.

Joy, N., Abraham, Z., and Soniya, E.V.2007. A preliminary assessement of genetic relationships among agronomically important cultivars of black pepper**.** *BMC Genetics* 8(42): 1-7.

Juby, B. 2019. Breeding for drought tolerance in Cocoa (*Theobroma cacao* L.), PhD thesis submitted to Kerala Agricultural University, Thrissur, 149p.

Kaini, S. P., Maury, P., Sarrafi, A., and Grieu, P. 2008. QTL analysis of chlorophyll fluorescence parameters in Sunflower (*Helianthus annus* L.) under well-watered and well- stressed conditions. *Plant Sci*.175:565-573.

Kaiser, W. M., Kaiser, G., Schoner, S. and Neimanis, S. 1981. 1981. Photosynthesis under osmotic stress-differential recovery of photosynthetic activities of stroma enzymes,intact chloroplasts and leaf slices after exposure to high solute concentration. *Planta.*153:430-435.

Kim, K.Y., Park, S. K., Shin, Y. A., and Lee, E. J. 2000. Survey on the actual condition of cultural methods and continuous cultivation injury of red pepper in field. Research Reports of the Rural Development Administration, Horticulture: 32 1-10.

Kissinger, M., Alkalai, T.S. and Shalom, Y. 2006. Characterization of physiological and biochemical factors associated with post harvest water loss in ripe pepper fruit during storage. Journal of The American Society for for Horticultural Science, 130:735-741.

Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne D. 2000. Nitric acid and salicylic acid signaling in Plant defense. Proceedings of the National Academy of Sciences, USA 97: 8849-8855.

Krishnamurthy, K.S., Ankegowda, S.J. and Johnson G. K. 1998. Impact of water stress on some physiological parameters in black pepper. In: Water and nutrient management for sustainable production and quality of spices (eds) A.K. Sadanandan, K.S. Krishnamurthy, K. Kandiannan and V.S. Korikanthimath., Indian Society for Spices, Calicut. pp 153-157.

Krishnamurthy, K. S., Ankegowda, S. J., and Saji, K. V. 2000. Water stress effects of membrane damage and activities of catalase, peroxidase and super oxide dismutase in black pepper (*Piper nigrum* L.). *J.of Plant Biol.* 27 (1): 39-42.

Krishnamurthy, K. S, Ankegowda, S. J. and Saji, K. V. 2006. Response of *Piper* species to water stress. *Indian J. Hort.* 63(4): 433-438.

Krishnamoorthy, B. and Parthasarathy, V. A. 2010. Improvement of Black Pepper. In : *CAB Reviews in Agriculture, Veterinary Sciences, Nutrition and Natural Resources* 5(3):1-12.

Larson, R. A. 1988. The antioxidants of higher plants. *Phytochemistry*. 27:969-978.

Latha, A. 1998.Varietal reaction to moisture stress in Cashew (*Anacardium occidentale* L.). Ph.D Thesis, Kerala Agricultural University,Vellanikkara, Thrissur, pp.177.

Leopold, A. C., Sun, W. Q. and Lugo, L. B.1994. The glassy state in seeds: analysis and function. *Seed Sci Res*.4:267-274.

Makbul, S. Guler, N. S., Durmus, N., and and Goven, S. 2011. Changes in anatomical and physiological parameters of soybean under drought stress. *Turk. J. Bot*.35:369-377.

Mammootty, K. P., Abraham, K. and Vijayaraghavan, R. 2008. Screening black pepper (*Piper nigrum* L.) varieties / cultivars against Phytophthora disease in nursery. *J. of Tropical Agri*. 46(1-2):70-72.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*, 2, Academic Press, London, U. K., 889p.

Massacci, A., Nabiev, S. M., Pietrosanti, l., Nemalov, S. K., Chernikova,T.N, Thor, K., and Leipner, J. 2008. Respose of photosynthetic apparatus of Cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas – exchange analysis and chlorophyll fluoresecence imaging. *Plant Physiol. Biochem*. 46:189-195.

Mathai, C. K., Kumaran, P. M., and Chandy, K. C.1981. Evaluation of commercially important wild black pepper types. *Qual. Plantfood. Hum. Nutrn*.30:194-202.

Mathew, P. G., Mathew, P. M., and Kumar,V. 2001. Graph clustering of *Piper nigrum* L.(black pepper) *Euphytica*.118 (3):257-264.

Medrano, H., Escalona, J. M., Bota, J., Gulias, J. 2002. Regulation of photosynthesis of C3 plants in response to progressive drought; stomatal conductance as reference parameter. *Annals of Bot.* 89 (7) 895-905.

Menconi, M., Sgherri, C. L. M., Pinzino, C., and Navari-Izzo, F.1995.Activated oxygen production and de toxificationin wheat plants subjected to a water deficient programme. *J.Exp.Bot*.46 (9):69-76.

Mengel K. and E. A. Kirkby 2001. Principles of Plant Nutrition. 5th ed., Kluwer Academic Publishers, Netherlands, 243p.

Menon, A.N, Padmakumari, K. P. 2005. Essential oil composition of four major cultivars of black pepper (*Piper nigrum* L.). *J. Essent. Oil Res.* 17: 206–208.

Mittler, R., Herr, E. H., Orvar, B. L., Van Camp, W., and Willekens H. 1999. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hypersensitive to pathogen infection. Proceedings of the National Academy of Sciences, USA, 96: 14165-14170

Moller, I. M., Jensen, P. E., Hansson, A. 2007. Oxidative modifications to cellular components in pants. *Annu. Rev. Plant Biol.* 58:459-481.

Moran, J. F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S. Klucas, R. V., Apparicio-Tejo, P. 1994. Drought induced oxidative stress in pea plants. *Planta,* 194:346-352.

Nair, R. R. and Ravindran, P.N. 1992. Induced polyploidy in black pepper. *J. of Spices and Aromatic Plants:*1, 151-153.

Nair, R. R. , Sasikumar, B. and Ravindran, P. N. 1993. Polyploidy in a cultivar of black pepper (*Piper nigrum* L.) and its open pollinated progenies. *Cytologia*, 58:27-31.

Nayyar, H. and Walia, D. P. 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. *Bio. Plant*. 46: 275-279.

Nayyar, H. and Gupta, D. 2006.Differential sensitivity of C3 and C4 plants to water deficit stress; Association with oxidative stress and anti oxidants. *Environ. Exp.Bot*.58:111: 373-382.

Nguyen, V. L. 2015. Spread of *Phytophthora capsici* in black pepper (*Piper nigrum*) in Vietnam. *Engineering*, **7**, 506-513.

Parthasarathy, U., Saji, K. V. Jayaraj, K and Parthasarathy,V. A. 2006. Biodiversity of Piper in South India- Application of GIS and cluster analysis. *Curr. Sci* 9(5) 652-658.

Pasin, N. H., Santos-Filho, B. G. D., Santos, B. S. B. D., and Mello, V. D. C. 1991, Performance of bean seeds derived from plants subjected to water stress at two stages. Pesquisa Agropecuaria Brasileira 26 (2): 183-192

Pradeepkumar, T., Sajith Babu, D., Aipe, K. C., and Mathew, S. 2003. Clonal variability in black pepper hybrid Panniyur 1. *J. of Spices and Aromat. Crops* 12 (2): 154-157.

Preethy, T. T., Aswathy,T. S., Sathyan, T., Dhanya, M. K. and Murugan, M. 2018. Performance, diversity analysis and character association of black pepper

(*Piper nigrum* L.) accessions in the high altitude of Idukki district, Kerala. *J.o f Spices and Aromatic Crops* l. 27 (1): 22-31 .

Premachandra, G.S., Saneoka, H., Kanaya, M. and Ogata, S. 1991. Cell membrane stability and leaf surface wax content as affected by increasing water deficit in maize. *J.Exp.Bot.*42: 167-171.

Purseglove, J. W., Brown, E. G., Green, C. L. and Robbins, S. R. J. 1981. *Spices* Vol.1.Longman, London, 439p.

Rahman, S. M. L., Nawata, E., and Sakuratani,T. 1999. Effects of water stress on superoxide dismutase and water content of tomato cultivars at different plant ages. *Hortic Sci.*34: 490-494.

Rajagopal, V. and Balasimha, D. 1994. Drought tolerance in plantation crops. *Adv. Hortic.* 10: 1185-1200.

Raju, K., Ravindran, P. N., and Nair. M. K. 1983. Quality evaluation of black pepper cultivars. *Indian Spices*. 20: 3-5.

Raschke, K. 1979. The stomatal turgor mechanism and its response to Co_2 and Abscisic acid: Observations and hypothesis.In:Regulation of cell membrane activities. Marre, E and Cifferi,O. (eds), Elsevier, North Holland.173-183

Ratnambal, M. J., Ravindran, P. N., and Nair, M. K.1985. Variability in Karimunda. *J. Plantat. Crops*. 13:154-158.

Ravindran, P.N.1991. Studies on black pepper and some of its wild relatives. Ph.D.thesis, Univ.Calicut. 130

Ravindran, P. N. and Menon, M. A. 1981. Chlorophyll stability index as an aid in breeding heat tolerant Cocoa (*Theobroma cacao* L.) *Planter*.57: 581-583.

Ravindran, P. N. Sasikumar, B., and Nirmalbabu, K. 1992d. Genetics of shoot tip colour in black pepper. *J. Plantation Crops*. 20:76 -78.

Ravindran, P. N. and Sasikumar, B. 1993. Variabilitv in open pollinated seedlings of black pepper *J. Spices Aromatic Crops* 2: 60-65.

Ravindran, P. N. and Babu, N. K. 1994. Genetic resources of black pepper. In: Chadha, K. L. Rethinam ,P. (Eds.), *Advances in Horticulture* Vol. 9. *Plantation Crops and Spices Part* I., New Delhi, Malhotra Publishing House, pp.99-120.

Ravindran, P. N and .Johny, A. K. 2000. High yielding varieties in spices. *Indian Spices* 37(1):17-19.

Ravindran, P. N., Babu, K. N., Sasikumar, B., and Krishnamurthy, K.S. 2000. Botany and crop improvement of black pepper.In:Ravindran,P.N.(ed.), *Black Pepper*: *Piper nigrum*. Harwood Academic Publishers, The Netherlands, pp.23-142.

Ristaino, J. B. and Gumpertz, M. L. 2000. New frontiers in the study of dispersal and spatial analysis of epidemics caused by species of the genus *Phytophthora*. *Annual Review of Phytopathol*, 38: 541-576.

Ritchie, S.W., Nguyan, H. T., and Holaday, A. S.1990. Leaf water content and gas exchange parameters of two genotypes differing in drought resistance. *Crop Sci.* 30 (1): 105- 111.

Sadasivam, S. and Manickam, A. 2016 . *Biochemical Methods*. (Third Ed.).New Age International (P) Ltd, Publishers, New Delhi. 254 p.

Sairam, P. K., Desmukh, P.S. and Saxena, D. C. 1998. Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biol.Plant.* 41:387-394.

Sangakkara, U. R., M. Frehner and J. Nosberger 2000. Effect of soil moisture and potassium fertilizer on shoot water potential, photosynthesis and partitioning of carbon in mung bean and cowpea. *J. Agron. Crop Sci*. 185: 201–207.

Sarma, Y. R. and Nambiar, K. K. N .1979. Screening of pepper (*Piper nigrum* L.) against *Phytophthora palmivora.* In: Venkataram C. S . (Ed.) Proc. PLACROSYM-II 1979. pp. 403- 406.

Sarma, Y. R, Nambiar, K. K. N., and Nair, M. K. 1982. Screening of black pepper (*Piper nigrum* L) and *Piper* species against *Phytophthora palmivora.* In: Nambiar K. K. N. (Ed.), Proc. Workshop on *Phytophthora* Diseases of Tropical Cultivated Plants, 242 - 247). Central Plantation Crops Research Institute, Kasaragod.

Saikumar, B. George, J. K. and Ravindran, P. N. 1992. Breeding behaviour of black pepper (*Piper nigrum* L.), *Indian J.Genet*. 52: 17-21.

Sasikumar, B., Saji, K. V., Lijini, K. R.,. Kandiannan, K., Prasath, D., and Anandaraj, M. 2013. Agali black pepper-A unique accession with high dry recovery and an ideal commodity for gi registration. 2013. *Focus on Pepper*, 5(1):55-60.

Sasikumar, B., George, J. K. Saji, K.V., Gowda, A.S. J. and Zachariah, T. J. 2014. Two unique black pepper accessions with very long spikes from the centre of origin. *Plant Genetic Resources: Characterization and Utilization* 13(2); 183-185.

Sasikumar, B., Chempakam, B. George, G. K. Remashree, A.B., Devasahayam, S., Dhamayanthi, K. P. M., Ravindran, P. N., and Peter, K.V. 1999. Characterization of two interspecific hybrids of Piper. *J. of Hort. Sci. and Biotech.* 74 (1):125-131.

Scheiermeier, Q. 2008. A long dry summer. *Nature*, 452: 270-273.

Shaoyun, L., Si Ping, C., SiMan, C., Xiao, L. and ZhenFei, G. 2003. Responses of proline content and activity of antioxidant enzymes in warm season turfgrasses to soil drought stress. *Acta Horticulturae Sinica.* 30 (3): 303-306.

Sharkey,T.D. 1990. Water stress effects on photosynthesis. *Photosynthetica* 24:651-655.

Siddique, K. H. M., Regan, K. L., Tennant, D., and Thomson, B. D. 2001.Water use and water use efficiency of cool season grain legumes in low rainfall-Mediterranean-type environments. *Eur.J.Agron*.15 (4): 267-280.

Silvente, S., Sobolev, A. P., Lara, M. 2012. Metabolite adjustments in drought tolerant and sensitive soybean genotypes in response to water stress. *PLoS One*. 7(6): 38554.

Sivakumar, R., Chandrasekaran, P., Srividhya, S., and Vijayakumar, M. 2018. Impact of pink-pigmented facultative methylotroph on physiological, growth analytical traits and yield of tomato (*Solanum lycopersicum*) under drought condition. *Int. J. Microbiol. Res*. 10(5):1205-1208.

Spices Board, 2017. *Annual Report 2017-18*. Spices Board, Cochin.63p.

Sreedevi, M., Syamkumar, S., and Sasikumar, B. 2004. Molecular and morphological characterization of new promising black pepper *(Piper nigrum* L.) lines. *J. of Spices and Aromatic Crops*. 14 : 1-9.

Sruthi, D., Zachariah, T. J. N. K., Leela, N. K., and Jayarajan, K. 2013. Correlation between chemical profiles of black pepper (*Piper nigrum* L.) var. Panniyur-1 collected from different locations. *J.of Medicinal Plants Res.* 7(31): 2349-2357.

Sujatha, R. 2001. Characterisation of field established tissue culture derived Black pepper (*Piper nigrum* L.) plants using morphological, cytological and molecular markers. Ph.D thesis submitted to Kerala Agricultural University, Thrissur. 250p.

Sujatha, R. and Namboodiri, K. M. N. 1995a. Influence of plant characters on yield in black pepper (*Piper nigrum* L.). *J. Trop. Agric.* 33:11-15.

Sulok, K. M. T., Zainudin, S. R., Chen, Y. S., Zehnder, J. A. M. and Ambi, W. 2018. Photosynthetic response of pepper to application of beneficial microorganisms on sandy textured soil. *Trans. Malaysian Soc. Plant Physiol. 25*: 69-74.

Surendar, K., Devi, D., Ravi, I., Jayakumar, P., and Velayudham, K. 2013. Studies on the impact of water deficit on morphological, physiological and yield of banana (*Musa* spp.) cultivars and hybrids. *Int.J.Agr.Sci*.3(4): 473-482.

Szabados, L. and Savoure, A. 2009. Proline: a multifunctional amino acid. *Trends Plant Sci.* 15: 89-97.

Thankamani, C. K. and Ashokan, P. K. 2004. Screening of black pepper *(Piper nigrum*L.) cultivars for water stress. *Agric. Sci. Digest* 24(3): 162.

Truong, N.V., Liew, E. C., and Burgess, L.W.2010. Characterisation of *Phytophthora capsici* isolates from black pepper in Vietnam. Fungal Biol. 114(2-3):160-70.

Vasantha, S. and Ramadasan, A. 1990. Drought tolerance in black pepper (*Piper nigrum* L.) cultivars: an annual evaluation of physiological parameters. *Indian J. Plant Physiol.* 33(4): 363-366.

Vasantha, S. 1996. Physiological and biochemical studies on drought tolerance in black pepper (*Piper nigrum* L.) cultivars. Ph.D. thesis, National Research Centre for Spices, University of Calicut, 79p.

Waraich, E. A., Ahmad, R, Saifullal, Asharaf, M.Y and Ehsanullah. 2011. Role of mineral nutrition in alleviation of drought stress in plants *Australian J. Crop Sci.* 5(6): 764-777.

Wang, W. X., Vinocur, B., Shoseyov, O., and Altman, A. 2001. Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. *Acta Hortic*. 560: 285-292.

Wang, Z. and Huang, B. 2004. Physiological recovery of Kentucky blue grass from simultaneous drought and heat stress. *CropSci*. 44:1729-1736.

Wong, S.C., Cowan, I. R., and Farquhar, G. D. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282: 424-426.

Wu, B. D., Fan, R. Hu,. LS., Wu, H.S.and Hao, C.Y. 2016. Genetic diversity in the germplasm of black pepper determined by EST-SSR markers. *Genet. Mol. Res.*15(1):1-9.

Zachariah, T.J., Mathew, P.A. and Gobinath, P. 2005. Chemical quality of berries from black pepper varieties grafted on *Piper colubrinum*. *Jounal of Medicinal and Aromatic Plant sciences* 27 (1):39 - 42.

Zachariah ,T. J. and Parthasarathy V. A. 2008. *Black Pepper: The Book of Chemistry of Spices.* Indian Institute of Spices Research Calicut, Kerala, India.p.445.

Abstract

Characterization and identification of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality

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ABSTRACT OF THE THESIS

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ABSTRACT

Black pepper *(Piper nigrum* L.), often described as the ‘King of spices’ is the most important spice crop, grown for its berries in the world. Indian pepper is preferred across the globe due to its intrinsic qualities. Foot rot is a devastating disease of black pepper. In the changing climate, drought can be a major threat in black pepper production. Hence, the present study was taken up at College of Horticulture, Vellanikkara and ICAR-IISR, Kozhikode to characterise and to identify superior accessions of black pepper for yield, quality and tolerance to biotic and abiotic stresses. Fifty accessions of black pepper in the bearing stage maintained in the National Active Germplasm Site of ICAR-IISR, Kozhikode formed the base material for the study.

The accessions were characterised for fifty qualitative and fifty quantitative characters following the descriptor developed by IPGRI (1995). Wide variability was observed among the accessions for ten qualitative characters. Quantitative characters of shoot, leaf, spike and fruit also showed wide variability.

Field tolerance to foot rot disease and pollu beetle infestation was observed among the accessions. Twenty accessions were selected from the base collection based on superiority of yield (> 450g green berries/vine) , field tolerance to foot rot disease infection (biotic susceptibility score 1) and pollu beetle infestation (biotic susceptibility score 1-3). They were further evaluated for biochemical principles of quality, tolerance to foot rot disease under artificial inoculation and tolerance to drought by physiological and biochemical analyses.

Piperine, essential oil and oleoresin ranged from 3.61 - 6.96 per cent, 3.00 - 5.87 per cent and 7.10 - 11.18 per cent, respectively, across the accessions. The accessions with high value of piperine, essential oil and oleoresin were identified as 7293, 7211 and 7289 respectively. The two accessions identified *viz*. 7293 and 7252

contained more piperine than the highest of Panniyur 2 (6.6 per cent) reported among the released varieties .

Artificial inoculation of selected accessions using *Phytophthora capsici* culture for screening for foot rot disease resistance based on over all disease severity index of both stem and leaf lesions showed that accession 7259 was moderately resistant.

The selected accessions did not exhibit significant variation for various physiological and biochemical parameters at field capacity. However higher value of photosynthesis, chlorophyll content, chlorophyll stability index, relative water content and membrane stability index and low leaf temperature were observed for accessions *viz*. 7215, 7240, P 5 and 7241 after five days and ten days of moisture stress induction following field capacity compared to other accessions. Higher values of proline, SOD, catalase and peroxidase were also observed for these accessions.

The visual scoring showed that accessions with higher values for most of physiological and biochemical parameters of drought tolerance *viz*. 7215, 7240, P5, and 7241 had lesser number of fallen leaves and more number of leaves retained at permanent wilting point (PWP). The accessions 7215 and 7240 took twenty days to reach PWP compared to eleven accessions which took only 16 days to reach PWP.

Foliar nutrition with sulphate of potash, IISR - Power mix and Pink Pigmented Facultative Methylotrophs (PPFM) had positive effect on drought tolerance for the accessions (7215, 7240, P5 and 7241) having natural tolerance. The identified accessions with high yield , quality and tolerance to biotic or abiotic stress can be used for further breeding programme.