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Chapter 11

Phenotyping Tools to Understand Effects of Climate Change

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1. Plantation Crops in India

The major plantation crops in India are coconut, arecanut, cocoa, rubber, tea, coffee, cashew, oil palm and rubber. The organized development of some of the plantation crops like tea, coffee and rubber started at colonial era. At the same time, coconut and arecanut were already established before colonial era. According to the statistics from Food and Agriculture Organization (FAO) in 2014, India stands one among the top countries with highest production of coconut (11,078,873Mt), coffee (318,200Mt), tea (1,208,780 Mt), cashew (753,000Mt) and rubber (900,000 Mt). Number of industries are in India based on plantation crops. For an instance, India is one of the major producer of coconut. Coconut mainly consumed by the domestic market and it's also used for the edible oil and coir production. Coconut plantations in India provides livelihood for 10 million people including farmers and small and large scale industrialist. Coconut is grown in over 200 districts of different states in India, the major producing area is 20 districts, which add almost 70 per cent of national production (Naresh Kumar and Aggarwal, 2013).At the same time, India is one of the leading exporter of coffee, tea, cashew and rubber. Plantation crops have a major role in Indian economy because of the large and small scale farmers and industries in the growing areas based on the production of planation crops.

Global agriculture production facing threat due to the climate variability. Global climate change models predict daily mean temperature to increase by about 1.0 to 3.7°C by end of the 21st century with increase in the variability in rain fall

and increased frequency of extreme weather events like heat waves, cold waves, drought and floods (IPCC, 2013).Coastal and hilly area considered as the major vulnerable areas to climate change, where the majority of the plantation crops are grown in India. The change in climate can impact on plantation crops more than the seasonal crop because of the perennial nature and it can adversely affect the economy of particular region. Additionally, farmer are investing more precious resources like land, inputs and time on plantation crops than other annual crops (Hebbar *et al.*, 2013; Naresh Kumar and Aggarwal, 2013).Coconut is one of the major plantation crops in India and it is projected that productivity in high yield areas in India mainly Andhra Pradesh, Odissa, south and central parts of West Bengal, Gujarat, plains of Karnataka, and eastern and southeastern parts of Tamil Nadu is going to decrease under future climate change conditions due to drought and high temperature stress (Naresh Kumar and Aggarwal, 2013). At the same time, it is predicted that coffeeand rubber plantationsfacing ill effects of climate variability and going to receive the negative impact of drought due to the decrease in rain fall and high temperature in future (Majumder *et al*, 2014; Ovalle-Rivera *et al.*, 2015). The tea production in north east part of India is predicted to decrease in year 2050 due to increase in average temperature (Dutta, 2014). Selection of genotypes which are appropriate for the future climatic condition are really important for plantation crop because of the perennial nature. It is really challenging to manage an established plantation which are not suitable for the changed climatic condition and a financial burden for the farmers, so planting scientifically proven tolerant genotypes can help the farmers to save time and money. In this chapter we are explaining different tools with physiological, morphological and biochemical background for screening high temperature and drought tolerance in plantation crops. We acknowledge that threshold of physiological and biochemical changes due to the individual effect of each stress factors or combination of both can vary in different crops. Hence, the screening tools are explained in the generalized fashion to fit for individual effect of each stress factors or combination of both and diverse response of different crops.

2. Screening Tools for Temperature and Moisture Deficit Tolerance

2.1. Vegetative Stage

2.1.1. Photosynthesis and Leaf Night Respiration

The physiological response among crops arediverse under drought and high temperature stress conditions. Mainly gas exchange fluctuates under stress conditions and in turn results in changes in photosynthesis (Prasad *et al.*, 2006). Increased demand for photosynthates for the maintenance due to the damage caused by stress can decrease the growth respiration and increase the maintenance respiration. Hence, respiration effect the plant growth, development and yield. Photosynthesis and night respiration are not directly correlated, but both are important for plant growth and yield. Photosynthesis and night respiration are two important traits for screening the plants under stress conditions (Prasad *et al.*, 2011; Djanaguriraman *et al.*, 2013). Portable photosynthesis systems (Infra-red gas

analyzers; IRGA; *e.g.* Li-6400 xt, LiCor. Lincoln, Nebraska, USA) are widely used to measure the photosynthesis and respiration.

Under drought condition photosynthesis decreases due to stomatal and non stomatal limitation (Zhou *et al.*, 2007). Stomatal limitation involves the partial or complete closing of stomata and that decreases the flow of CO_2 to the mesophyll cells. The initial decrease in the photosynthesis in drought stress is mainly due to the closure of stomata which is associated with content of abscisic acid (ABA). The plant hormone ABA, transfer signal of stress to the all parts of plants and that leads to the stomatal closure and related changes (Stoddard *et al.*, 2006). Non stomatal limitations will appear in later stages as the severity of drought increases causing changes in the biochemical processes essential for the photosynthesis. The main biochemical changes includes regeneration of ribulosebisphosphate (RuBP) and ribulose1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein content (Bota *et al.*, 2004), decreased Rubisco activity (Parry *et al.*, 2002), impairment of ATP synthesis, and photophosphorylation or decreased inorganic phosphorus (Prasad *et al.*, 2008). There are some contrasting results showing the occurrence of non stomal limitation in initial stage leading to a temporary increase in intercellular CO_2 causing stomatal closure (Briggs *et al.*, 1986). The high temperature is also an important environmental stress that negatively affect the photosynthesis. Each crop have different threshold for the optimum temperature and temperature increase beyond that level can decrease photosynthesis. Under high temperature conditions, photosynthesis is related to the Rubisco and its dependence to subtract CO_2 and oxygen and due to the decreased solubility of oxygen to a lesser extent than CO_2 inducing photorespiration and decreased photosynthesis. Rubisco and Rubisco activase are inhibited under high temperature condition and that lead to the decreases in photosynthesis (Lea and Leegood, 1999; Crafts-Brandner and Salvucci, 2000; Prasad *et al.*, 2004).

Plant respiration is equally important as photosynthesis while considering plant growth and developments under high temperature and drought stress conditions. A combination of day time photosynthesic rate and leaf night respiration rate can give an output of carbon gain and carbon loss under stress conditions (Sunoj *et al.*, 2016). The photosynthesis occurs only in day time and mainly occurs in leaf, but the respiration is a continuous process (day and night) and occurs in all the organs. About 30 to 70 per cent of carbon gained by photosynthesis is lost through respiratory metabolism (Mohammed and Tarpley, 2011). Glucose, fructose and maltose are the reducing sugars and sucrose is the major transported non-reducing sugar in plants and respiration oxidizes these carbon rich carbohydrates, to generate energy for the growth and maintenance. In addition, leaf night respirationcomplete dependence on degradation of starch and sucrose is documented (Atkin *et al.*, 2000). Respiration is a group of two portions that includesmaintenance respiration and growth respiration. Maintenance respiration is the most responsive to the changes in growing conditions. The main functions of respiration is turn over protein and lipids and maintenance of ion transport across the membranes. Under stress conditions, maintenance respiration increases to manage these important functions. The high leaf night respiration due to the damage caused by stress uses

carbohydrates for the maintenance and that decreases allocation of carbohydrates towards biomass accumulation (Halford *et al.*, 2010). Increased leaf night respiration under high temperature conditions reported in several crops (Loka and Oosterhuis 2010; Djanaguriraman *et al.*, 2013).Contrasting results are observed in leaf night respiration under drought condition. Researchers reported no change, decrease or increase in leaf night respiration in different crops at different temperatures and it proves that changes in night respiration depends on the leaf temperature (Atkin and Macherel, 2009; Gauthier *et al.*, 2014).

2.1.2. Chlorophyll Fluorescence

Chlorophyll florescence is used to evaluate the plant health status and photochemical efficiency of photosystem II (PS II; Fv/Fm) is routinely used as an indicator of the degree of stress (Laxman *et al.*, 2013; Djanaguriraman *et al.*, 2013). PS II plays an important role in photosynthesis under stress conditions.Chlorophyll fluorescence is widely used for quantifying the impact of drought and temperature stress. High temperature and drought can damage the thylakoid membrane and that causes malfunction of PSII light harvesting complex (Djanaguriraman *et al.*, 2011). PSII is more sensitive to high temperature condition as compared to drought. There are several types of chlorophyll fluorescence techniques used in broader fields related to biophysics, biochemistry and physiology.The most commonly used technique in the field of abiotic stress research are pulse amplitude modulation (PAM) fluorometryand fast chlorophyll fluorescence technique (Chlorophyll Fluorometers; *e.g.* OS 30+, OptiScience, Hudson, USA).

Photosynthetic antenna molecules present in the thylakoid membranes absorb light energy through light harvesting pigments chlorophyll and carotenoids (Horton and Ruban, 2004). Light energy absorbed by the chlorophyll can be used for three processes such as to drive photosynthesis (photochemistry), dissipated as heat or emitted as red fluorescence. These three process occur in competitions and decrease in one process can increase other two. Therefore measuring chlorophyll fluorescence can provide details on photochemical efficiency and heat dissipation. Using above fluorescence techniques can provide the informations like minimal fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv = Fm-F0), photochemical efficiency of PSII (Fv/Fm; which represents maximum quantum yield of PSII), (F0/Fm; which represents damage to thylakoid membranes), OJIP fluorescence curve, yield, photochemical quenching (PQ; quenching in the form of photochemistry), non-photochemical quenching (NPQ; quenching in the form of heat dissipation and fluorescence) and linear electron transport in PSII (ETR).The important informations used in abiotic stress screening are Fv/Fmratiothat indicates stress and F0/Fm ratio indicates damage to thylakoid membranes due to stress (Prasad *et al.*, 2008). The ratio of Fv/Fm in a healthy plant ranging from 0.78- 0.84 is considered as the maximum quantum yield of PSII photochemistry. The plants give values less than this range considered as under stress at high temperature and drought conditions. Plants severally affected with stress can give values below the mentioned range because of the damage to PSII (Djanaguriraman *et al.*, 2013; Laxman *et al.*, 2013).

2.1.3. Chlorophyll Index

Foliar chlorophyll content is a good indicator of plant stress and plant health because of its effects on photosynthesis and growth (Datt, 1999). Environmental (drought and high temperatures) and nutrient (particularly N) stresses commonly cause loss of leaf chlorophyll content leading to poor photosynthesis, growth, biomass, and economic yield. Drought and high temperature can decrease leaf chlorophyll index and intensity of decrease varies with different crops (Asharef and Harris, 2013). Degradation of chlorophyll can affect the photosynthesis, because of importance of chlorophyll as a light harvesting pigment. Slower degradation of chlorophyll content identified in some crops are termed as stay green types and the stay green character and chlorophyll retention in leaves considered as the tolerance of particular genotype to high temperature and drought (Fokar *et al.*, 1998; Araus *et al.*, 2012).Stay green genotypes are mostly expressed under drought condition and the genotypes with this particular trait can maintain the photosynthes is longer than the senescent type. Yield benefits are high in stay green genotypes as compared with the senescent type (Borrell *et al.*, 2001; Jordan *et al.*, 2003; Hebbar *et al.*, 2013). Stay green like trait is not identified in any of the plantation crops and investigations are required in this particular aspect while screening genotypes for drought and high temperature stress. Decrease in chlorophyll index under high temperature condition is mainly due to the reduced chlorophyll biosynthesis or increased chlorophyll degradation or effect of both. Numerous enzymes involved in the chlorophyll biosynthesis get deactivated under high temperature and that leads to the cessation of chlorophyll biosynthesis. For an instance, the activity of 5-aminolevulinate dehydratase (ALAD), the first enzyme of pyrrole biosynthetic pathway, decreased under high-temperature conditions (Mohanty *et al.*, 2006; Asharef and Harris, 2013). Under drought condition decrease in chlorophyll index is reported in several crops. However, studies on chlorophyllase and peroxidase shown that the decrease in chlorophyll is due to the elevated break down of chlorophyll as compared to the slow synthesis (Harpaz-Saad *et al.*, 2007; Kaewsuksaeng, 2011). The chlorophyll content can be quantified by destructive and non-destructive methods. Destructive methods includes the wet lab analysis using spectrophotometers (Lichtenthaler and Buschmann, 2001). Using chlorophyll meters (eg. SPAD 502, Konica Minolta Inc., Japan) is time saving, nondestructive and reliable for large number of plant in breeding population in a limited time frame. In coconut a portable non-destructive meter (atLeaf chlorophyll meter) was validated using experimental data from plant type (tall vs. dwarf and green foliage vs. colored foliage), cropping systems, fertigation method, soil type to establish the relationship between at Leaf chlorophyll meter measurements and chlorophyll and N concentration were quantified. The atLeaf chlorophyll meter provided good estimates for chlorophyll concentrations in coconut, with coefficients of determination greater than 0.81 (Hebbar *et al.*, 2016).

2.1.4. Leaf Water Relation

Water is a major component for majority of the crops and it is no exception for plantation crops.The water availability for the plantation crop getting decreased due to the irregular pattern and intensity of precipitation and increased demand for fresh water for domestic consumption. Changing climate will make situation more

serious. In future, availability and quality of water going to be a serious issue around the globe (IPCC 2008). Under a situation like this creates the need for utilizing water resource in efficient ways. Hence, screening for diverse genotypes in plantation crops or suitable irrigation practices has to be implemented. While screening genotypes for traits related to efficient use of available water under drought and high temperature condition, traits like water use efficiency (WUE) become more important. WUE is the amount of water used to produce the dry matter in plants through evapotranspiration and it involves a series of above and below ground morphological, physiological and biochemical modification in plants. Gravimetric method is commonly used for calculating the water use efficiency in different crops and it is laborious and time consuming in most of the plantation crops because of the canopy spread and size. Intrinsic water use efficiency can be calculated using the gas exchange measurements. Instantaneous water use efficacy (P_N/E) is the ratio of photosynthesis rate (P_N) and transpiration (E). On the other hand, intrinsic water use efficiency (P_N/gs) also be calculated from the ratio of photosynthesis rate (P_N) and stomatal conductance (*gs*). The intrinsic and instantaneous water use efficiency provides information regarding the carbon fixed by photosynthesis in relation to the water lost by stomatal conductance and transpiration or capacity of plants to preserve water and maximizing carbon fixing (Rymbai *et al.*, 2014).All over again, calculating water use efficiency from gas exchange measurements are time consuming for large breeding populations.

Hence, scientists have depended on the surrogate methods of assaying the WUE. Measurement of carbon-isotope discrimination (CID) is an indicator of instantaneous water use efficiency (P_N/E) and it can be used as screening tool for the high temperature and drought (Richards *et al.*, 2010; Djanaguriraman *et al.*, 2011).It is already been used as a tool for screening large population in breeding programs to identify the genetic variation in transpiration efficacy and use for further breed to improve water use efficiency and yield (Condone *et al.*, 2004). The main advantage of this technique is collection of samples at the end of growing season and it can provide the details of instantaneous water use efficacy of entire growth season. There are two isotopic form of carbon (^{12}C and ^{13}C) in atmosphere. ^{12}C is more preferred by plants for photosynthesis as compared to ^{13}C. Carbon isotope discrimination ($\Delta^{13}C$) is ratio of $^{13}C/^{12}C$ in plant material relative to the value of the same ratio in the air which used by plants for photosynthesis. The ratio of $^{13}C/^{12}C$ in plants tissue is less than the ratio of $^{13}C/^{12}C$ in the atmosphere, which indicates the discrimination against heavy isotope of carbon (^{13}C) present in the atmospheric CO_2 during photosynthesis. The differences in the discrimination is due to the stomatal limitation and enzymatic activities (Condone *et al.*, 2004). Rubisco prefers $^{12}CO_2$ as compared with $^{13}CO_2$ because of the isotope effect.This isotopic discrimination is remain in the plant dry matter, which can be used to calculate discrimination. Increased photosynthesis and decreased stomatal conductance causes decrease in intercellular CO_2 resulting in decrease in discrimination against $^{13}CO_2$ (Farquhar *et al.*, 1982) or it can be explained as the increase in intercellular CO_2 implies greater carbon isotope discrimination for the heavier ^{13}C isotope.The CID is not a good tool for the studies where the ambient $^{13}C/^{12}C$ ratio is unknown (eg. Growth chambers and greenhouses without CO_2 controls).

Relative water content and leaf water potential are other methods to calculate the leaf water status in plant and it is related to several leaf physiological traits such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration. Leaf water content expresses the relative amount of water present on the plant tissues. Alternatively, water potential measures the energetic status of water inside the leaf cells (Yamasaki and Dillenburg, 1999).

2.1.5. Osmotic Adjustments

Osmotic adjustments are really important physiological mechanism to combat with stress conditions, mainly under water deficit condition (Tangpremsri *et al.*, 1995; Morgan, 2000). Plants can resist dehydration due to stress by reducing the cellular osmotic potential by accumulating certain organic compounds of low molecular mass, generally referred to as compatible osmolytes (O'Neill, 1983; Wahid *et al.*, 2007). Different crops responds in different ways to accumulate variety of osmolytes such as sugars and sugar alcohols (polyols), amino acids, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi, 2004). Plants can enhance the stress tolerance by the accumulation of such solutes and changing osmotic potential in leaves. Examples for some of the osmolytes which identified in wide range of crops are sugars, glycinebetaine (amphoteric quaternary amine), proline (immino acid) and 4-aminobutyric acid (GABA; non-protein amino acid). The accumulation of cellular solutes have a key role in turgor maintenance and cell volume during stress conditions and consequently withstand the water loss and it can help to increase the biomass and yield under water stress condition (Blum, 2005). At the same time, including osmotic potential as a tool for drought stress is questioned by Serraj and Sinclair (2002), pointing towards the survival and yield of crops. In annual crops, occurrence of osmotic adjustments happens at the late stage and it helps the plant to survive, but not helps to maintain the yield. On the other hand, plantation crops are perennial in nature and with a greater recovery rate from the stress condition can make the osmotic adjustments important trait for screening stress tolerance. Leaf osmotic potential measurements can provide information regarding the capacity of particular genotypes which can withstand under stress conditions. Osmometer (*e.g.*, Wescor VAPRO Model 5600, Wescor, Inc., Logan, USA) is widely used to measure the osmotic potential (Laxman *et al.*, 2013).

2.1.6. Cell Membrane Thermo-stability

Cellular membrane modification is a result of various environmental stresses includes high temperature and drought, which result in the partial or total dysfunction of cellular membrane. Persistent functioning of cellular membrane under stress condition is essential for the proper photosynthesis and respiration (Prasad *et al.*, 2011; Djanaguriraman *et al.*, 2013). Membrane damage is associated with increased fluidity of membrane lipids, lipid peroxidation, and protein degradation in various metabolic processes under high temperature condition (Savchenko *et al.*, 2002). At the same time, exact cell membrane structural and function modifications caused by stress is not well understood.The reactive oxygen species (ROS; singlet oxygen [$^1O_2$], superoxide radicals [O^{2-}], hydrogen peroxide [H_2O_2] and hydroxyl radical [OH^-]) induced peroxidation of membrane lipid (in terms of malondialdehyde

[MDA] content) is considered as the manifestation of stress induced damage at the cellular level. The damage due to the lipid peroxidation of membrane can cause increased membrane fluidity (Jain *et al.*, 2001). Cell membrane thermo stability is considered as the surrogate method to assume the proper functioning of enzymatic (superoxide dismutase [SOD], peroxidase [POX] and catalase [CAT]) and non-enzymatic(proline, phenols and flavonoids) ROS quenching activity under high temperature and drought conditions (Sunoj *et al.*, 2014). The cellular dysfunction under high temperature and drought stress conditions will result in increased leaf permeability and leakage of ions, which canbe calculated by measuring the changes in electric conductivitydue to the leaked ionsfrom the leaves (Tripathy *et al.*, 2000;Bajji *et al.*, 2001; Lu etal., 2003). Measuring the changes in the lipid composition of membrane lipids are another method to assess the drought and high temperature stress (Repellin *et al.*, 1997; Narayanan *et al.*, 2015).

2.1.7. High Throughput Screening for High Temperature and Drought Stress

2.1.7.1. Infra-Red Thermography for Monitoring Canopy Temperature

Canopy temperature is an important pointer towards the plant water status and it can be used as a screening tool for genotypic selection for the drought and high temperature tolerance. The widely used methods for the infra-Red thermography for monitoring canopy temperatureare infra-Red thermometers, field mounted Infra -Red sensors (eg. Smart crop sensors; Smart field, Inc., USA) and infra-Red cameras (hand held, field mounted or Unmanned Arial Vehicle [UAV] mounted). Basic principle behind the infrared thermal imaging is all the objects above 0 degrees Kelvin (-273 °C), emit infrared energy. The Infra-Red energy emitted from the measured object is converted into an electrical signal by the imaging sensor in the camera and displayed on a monitor as a color or monochrome thermal images and in thermometers it is shown as digits.

Canopy temperature is influenced with different microclimatic and environmental factors such as solar radiation, cloud, wind, atmospheric temperature, relative humidity and soil moisture. At the same time, canopy temperature is determined by several physiological, biochemical and morphological traits as well, such as epicuticular wax, leaf angle, transpiration, stomatal conductance, root growth (root biomass and length), capability of plants to move water through the vascular system, and source and sink metabolism (Mason and Singh, 2014). Under high temperature and drought conditions, which is linked with vapor pressure deficit that increases the loss of water from leaves through transpiration. Increased transpiration helps to keep the cooler canopy temperature and it is possible when plants are grown under fully irrigated conditions or when plant have a better root growth. Growth and development of root systems are really important for absorbing water in these conditions to keep the canopy cooler for longer duration of time (Prasad *et al.*, 2008). Improved root system under water limited condition (moderate drought) and high temperature can help tolerant genotypes to absorb water from the deeper soil. Under these stressed conditions, measuring canopy temperature and stomatal conductance can be considered as the surrogate measurement of root growth. Lower canopy temperature and increased stomatal conductance indicates

the increased root growth and that can help plant absorb water from deep soil to keep the canopy temperature cooler, which help plants to minimize damage due to drought stress.

2.1.7.2. Spectral Reflectance Signature

The fraction of solar radiation enter to the atmosphere of earth is transmitted, absorbed or reflected. Different vegetation or any other materials (eg. soil, turbid water and clear water) have capacity to absorb and reflect at different wave length and that makes unique spectral reflectance signature for that particular vegetation or material. Theoretically, spectral reflectance signature can be used to identify the vegetation or material, if the sensing system has sufficient spectral resolution to distinguish particular spectrum of the targeted vegetation or material. This is the basic principle for multispectral remote sensing.High temperature and drought stress changes canopy spectral reflectance signature of different vegetation and these change were recorded using spectroradiometer (Dobrowski *et al.*, 2005; Genc *et al.*, 2013). The data of canopy spectral reflectance can be used to calculate the different vegetative indices (Vina *et al.*, 2011) such as Simple Ratio (SR), normalized difference vegetation index (NDVI), enhanced vegetation index (EVI), green atmospherically resistant vegetation index (GARI), wide dynamic range vegetation index (WDRVI), green and red-edge chlorophyll indicesand MERIS terrestrial chlorophyll index, MTCI. Among the vegetative indices, NDVI is commonly used by researchers to calculate the effect of high temperature and drought stress conditions on plants.

2.1.7.3. Normalized difference Vegetation Index (NDVI)

Normalized difference vegetation index (NDVI) is most popular mathematical algorithms used to calculate vegetation indices (VIs). The NDVI is used to calculate the crop nutrient deficiency, vegetative greenness, indicator of biotic and abiotic stress and canopy photosynthetic rate (Raun *et al.*, 2001; Rodriguez *et al.*, 2004). It is also useful for precision agriculture, weed detection for herbicide spraying, and rate and timing of nitrogenous fertilizer application (Pietragalla and Vega, 2011). The popularity and advantage of NDVI is availability of data from different sources such as hand held sensors (eg. Green seeker), data from spectroradiometer, camera (handheld, UAV mounted and field mounted; *e.g.* Canon Power shot SX280HS) and satellite. In abiotic stress research, NDVI is a good method to calculate intensity of drought and high temperature stress (Peter *et al.*, 2002; Hazratkulova *et al.*, 2012). NDVI is calculated by measuring light reflectance in the red (600–700 nm) and near infra-Red (700-900 nm) regions of spectrum. The healthy plant will absorb most of the red light because of the high photosyntheticactivity (chlorophyll absorbs red and blue light for the photosynthesis) and reflects most of the NIR light. The formula used to calculate the NDVI is given below.

$$NDVI = (R_{NIR} - R_{Red})/(R_{NIR} + R_{Red})$$

Where, R_{NIR} is the reflectance of NIR radiation and R_{Red} is the reflectance of visible red radiation in the spectrum.

2.2. Reproductive Stage

2.2.1. Pollen Viability, Germination and Stigma Receptivity

The reproductive success is controlled by the particular reproductive stages such as developmental stage of micro (pollen development) and megasporogensis (stigma development), anthesis, pollen tube growth, fertilization, and early embryo development. Incongruous circumstances (biotic or abiotic) at the time of any above these stages can directly affect the yield. These stages are highly susceptible to drought and high temperature stresses. Changes in theses stages can decrease fertilization or increase early embryo abortion, leading to lower crop yield (Boyer and Westgate, 2004; Prasad *et al.*, 2006). Keeping this in mind, pollen viability, pollen germination and stigma receptivity can be used as a tool for assessing the genotypes to make selections for the tolerant genotype under stresses at reproductive stage. Even though, complete physiological and biochemical mechanisms which interferes pollen viability, germination and stigma receptivity is not clear and these three processes are important for the reproductive success of any crop. When discussing the issues of pollen and pollination, there involves three major steps, 1) pollen adhesion, 2) pollen hydration and 3) pollen polarization and germination. The complete mechanism for each step is not clear. The nature of contact at pollen adhesion, transport of water, nutrients and other small molecules to the pollen grain from stigma at pollen hydration, perceiving signals and transduction to select a single point for pollen tube emergence at pollen polarization and germination and pollen tube invasion to stigma remains unclear (Edlund *et al.*, 2004). There are some hypotheses projected as accountable for the dysfunction of pollen under drought and high temperature stress, some of them are 1) premature dislocation of microspores (Saini *et al.*, 1984); 2) abnormal vacuolization leads to dysfunction of tapetal cells (Lalonde *et al.*, 1997); 3) lack of endothecial development and damage of tapetal cells at early stages (Ahmed *et al.*, 1992) 4) changed carbohydrate metabolism and accumulation (Saini, 1997; Jain *et al.*, 2007); 5) loss of gametophyte viability due to oxygen starvation in the developing microspores. At the same time some researches are showing the important role of hormones such as abscisic acid (ABA) and cytokinin. The plant hormone ABA is important under stress condition to prevent the water loss, improving the root hydraulic conductivity and maintain root growth, which delays the dehydration. ABA plays an important role in the signal transduction of stress and that leads to sterility or abortion. The cytokinin under stress condition disrupt the cellular and nuclear integrity of cells in the periphery of the endosperm (Jones and Setter, 2000; Prasad *et al.*, 2008). At high temperature, probabilities of changes in pollen function and anatomy is high. Pollen shape, pollen viability, pollen germination, pollen tube growth, thickness of tapetum and exain layer altered under high temperature stress. Further, pollen tapetal cells were vacuolated and showed autolysis, generative cell acquired a fusiform shape and the chromatin showed condensation (Prasad *et al.*, 2000; Prasad *et al.*, 2006; Djanaguriraman *et al.*, 2013; Djanaguriraman *et al.*, 2013a).

The stigma is the first reproductive structure that comes in contact with pollen grain on the way to fertilization. Stigma receptivity is defined as the ability of stigma

to facilitate pollen germination and that is also an important part for reproductive success in various crops. The effective pollination period is closely linked with the duration (longevity) of stigmatic receptivity and it can vary among different crops (Sanzol and Herrero, 2001). High temperature and drought stress can play a major role in longevity of stigma receptivity. High temperature reduces stigma receptivity by lack of support to pollen for adhesion, elongation and penetration (Hedhly *et al.*, 2003).

2.2.2. Crop Yield and Yield Indices

High or sustainable yield are the ultimate aim for the success of the screening of genotypes for stress tolerance. In plantations crops, final output from the screening criterion will take more time as compare to other seasonal crops and that adds more importance while executing above mentioned screening tools. Final yield and related traits can also be considered for the screening while conducting long term experiments and it is necessary for the successful conclusion of other screening methods. In other crops like vegetables, legumes and cereals, fruit (expressed as grain, seed or nut) set per cent, size and number are considered as important traits for the selection of tolerant genotypes and high temperature and drought stress reported to decrease the yield in vegetables, legumes and cereals (Sato *et al.*, 2000; Prasad *et al.*, 2008). These yield related traits are generally get affected when the stress occurs at the time of reproductive developments (micro and megasporogensis) (Prasad *et al.*, 2006; Valliyodan and Nguyen, 2006).

The relative yield performance of genotypes in drought stressed and more favorable environments seems to be a common starting point in the identification of traits related to drought tolerance and the selection of genotypes for use in breeding for dry environments (Clarke *et al.*, 1992 ; Hebbar et al., 2016)). To differentiate drought resistance genotypes, several selection indices have been suggested on the basis of a mathematical relationship between favorable and stress conditions (Clarke *et al.*, 1982; Huang, 2000). Tolerance (TOL) (McCaig and Clarke, 1982; Clarke *et al.*, 1992), mean productivity (MP) (McCaig and Clarke, 1982), stress susceptibility index (SSI) (Fischer and Maurer, 1978), geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez, 1992) have all been employed under various conditions.

The drought intensity index (*DII*) was calculated using the following formula of Ramirez-Vallejo and Kelly, 1998.

$$DII = (1 - Xs \div Xi)$$

Where, *Xs* is the mean experiment yield of all genotypes grown under stress, and *Xi* is the meanexperiment yield of all genotypes grown under non-stress conditions. Values exceeding 0.7 would indicate severe drought.

Ramirez-Vallejo and Kelly (1998) also concluded that the most effective approach to breed beans for resistance to drought would be based first on selection for high geometric mean seed yields followed by selection for low Fischer Maurer drought susceptibility index values. The Fischer and Maurer drought susceptibility index (*DSI*) is calculated as follows:

$DSI = (1 - Ys \div Yi) \div DII$ (Fischer and Maurer, 1978)

Schneider *et al.* (1997) showed the geometric mean (*GM*) of seed yield to be the best predictor of genotype performance in stress and non-stress environments. They recommended a breeding strategy that involved genotypic selection based first on *GM*, followed by selection based on seed yield in the stress environment. The *GM* is calculated as follows:

$$GM = \sqrt{Ys \times Yi}$$

Where, *Ys* is the mean seed yield of a line under drought stress and *Yi* is the mean seed yield of the line grown under non-stress. The square root of the product (*Ys*×*Yi*) from two treatments is used to calculate the *GM* for an individual genotype.

Drought resistance can only be estimated by comparing the performance of breeding lines under stress and non-stress (irrigated) conditions. Using data from the two water treatments (rainfed and irrigated) at Arsikere district of Karnataka state, India, drought intensity index, different susceptibility indices and means to assist in selection of drought resistant genotypes of coconut was calculated (Hebbar, unpublished data). Arasikere was under severe drought for the years 2011, 2012 and 2013. Yield data was collected from rainfed and irrigated coconut orchards of experimental farm at Arasikere for different genotypes as shown in the Table 11.1.

Table 11.1: The Relative Yield Performance of Coconut Genotypes in Rainfed and Irrigated Conditions of Arasikere, Karnataka and the Calculation of Tolerant Indices

Cultivar	*Water Treatments*		*Per cent Reduction*	*DSI*	*GM*
	Irrigated	*Rainfed*			
WCT	52	4	92	1.14	14
LCT	63	19	70	0.86	35
ADOT	54	5	91	1.12	16
Sanramon	74	2	97	1.20	12
WCTXGBGD	180	25	86	1.06	67
BS1	44	21	52	0.65	30
PHOT	73	0	100	1.23	0
WCTXCOD	85	5	94	1.16	21
CODXWCT	72	3	96	1.18	15
TPT	62	25	60	0.74	39
Zangiber	130	36	72	0.89	68
Java	47	20	57	0.71	31
Mean	78	14	81	1.00	29

In this experiment, *GM* was high for cv.Zangiber and WCT x GBGD which also had high yield under stress and non-stress conditions.Drought susceptibility index (*DSI*) was low for BS1 and Java, but they were low in nut production. Caution in using this index is advised as certain genotypes with the lowest *DSI* rankings had

the lowest overall yield potential (White and Singh, 1991). Small yield differences between the stress and non-stress treatments produce low *DSI* values even though the potential yield of the line is low. Therefore in coconut *GM* is the best indicator of drought tolerance and can be used in breeding programs across different environments.

2. Conclusions

Maintaining growth and yield under high temperature and drought is the major challenges to plantation crops. Climate variability is going to enhance in future and it can impact more on the plantation crops because the major growing areas in India are vulnerable to climate change. Plantation crops are experiencing the ill effects of adverse climate than annual crops. Selection of tolerant genotypes to high temperature and drought using scientific tools are really important to maintain the growth and yield in future climatic conditions. Farmers are investing their valuable time, land and inputs for plantation crops and outputs from that is crucial for the financial stability of famers and related industries. Integration of physiological, biochemical, molecular, breeding and modeling tools can play a major role in the selection of tolerant genotypes. Additionally, better understanding of physiological, biochemical, molecular and genetic basis of the mechanisms promoting the tolerance to high temperature, drought and combined effect of both will improve the capacity to enhance the yield or maintenance of yield under hostile environmental conditions.

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