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Effect of *Phytophthora cinnamomi* inoculation on chlorophyll concentration and chlorophyll fluorescence parameters of four avocado (*Persea americana* Mill.) rootstocks seedlings grown under water logging conditions.

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Abstract: **Avocado plant`s fruits have high amounts of fats, proteins and fibre. Phytophthora cinnamomi fungal causes avocado root rot that is devastating to growth. The pathogen combined with flooding leads to asphyxiation, root decay and eventually death. Several studies have evaluated the tolerance of different avocado rootstocks to Phytophthora cinnamomi, there are few studies in Kenya that characterized the pathogen and evaluated the effect of the pathogen on the physiology of avocado rootstocks under flooding. The objectives of this study therefore were to determine physiological responses of avocado rootstocks to the fungus under water logging conditions. Rootstocks; Puebla, Fuerte, Booth7 and Pinkerton were obtained from orchards in Maseno, Nyando, Kisumu East and Budalangi of Western Kenya. The fungus was isolated from the soil by planting on selective medium and by baiting methods. Avocado rootstocks were planted in 10 litre plastic pots containing sand - soil mixture of ratio 1:2 for 3 months, inoculated under flooded and non-flooded conditions. Experiment was laid in a CRD within greenhouse. Data on chlorophyll concentration and chlorophyll fluorescence were collected then subjected to analysis of variance. Means separation and comparison was done using LSD at 0.05. Flooding and inoculation significantly ($p \leq 0.05$) reduced chlorophyll a, b, total chlorophyll concentration and, reduced Fv/Fm, ΦPSII and ETR of rootstocks. This was an indication that photosynthetic apparatus were affected. This contributed to the reduction in overall avocado growth. Interactions between treatments and avocado rootstocks were significantly different for the parameters. Puebla rootstocks responded better to flooding and P. cinnamomi inoculation, and were therefore recommended for growing in flood affected regions of Kenya where P. cinnamomi infestation is also a common problem.**

Keywords: **Avocado rootstocks, Flooding, Phytophthora cinnamomi, Physiological response, Chlorophyll fluorescence.**

1. INTRODUCTION

In many countries seed propagated avocado rootstock are still chosen according to availability and nursery performance rather than as to orchard performance (Allen *et al*., 1980). After a new rootstock clonal propagation method was developed in California, USA, it was spread to Israel where green ovacado cutting mist spray was developed. Unfortunately the methods never became commercial (Baum and Pinkas, 1988). Avocado is highly susceptible to *P.*

cinnamomi and is a commercially valuable fruit tree cultivated in tropical climates throughout the world, producing a green-skinned, pear-shaped fruit that ripens after harvesting (Menge *et al.*, 2012). They occupy small areas, but give good yields which fetch high prices when compared to other field crops. Earlier before it became commercial, it was planted in home gardens in its countries of origin that include Mexico, Colombia and Ecuador (Pandey *et al.*, 2010). According to Menge *et al.* (2012) among other factors of avocado industry, rootstocks are more important. More so, avocado tree development, health and productivity in fruits are dependent on rootstock type (Christie, 2012). In Kenya, there is slow rate of increase on avocado rootstock research and that a lot of damages are being caused by the pathogen *Phytophthora cinnamomi* (Gimeno *et al*., 2012). Also, no clear method of elimination has been discovered to be functional in Kenya (Colmer and Voesenek, 2009).

The production of avocado in many cases is negatively affected by factors such as reduction in soil fertility, poor agronomic practices and lack of healthy seeds (Arpaia *et al*., 1993). Root rot disease of avocado is ranked the first devastating disease of avocado that is caused by *Phytophthora cinnamomi* (Reeksting *et al*., 2014). This disease has led to reduction in avocado production in most avocado producing countries (Randy *et al.*, 2001). Limited root rot resistance varieties, poor avocado tree crop management practices, poor market information, pests (thrips, scales, fruit fly and systates weevil) and diseases (root rot, anthracnose and *Cercospora* leaf spot), and limited utilisation of avocado (MoA, 2005) also attribute to these low yields

There are several varieties of avocado. These include; Bacon, Duke, Anaheim, Ganter, Jim, Lula, Lyon, and nabal, Puebla, Pinkerton, Gueen, Reed, Sama, Tambarina and Winter Mexican (Menge *et al.*, 2012). There are two major varieties grown in Kenya, namely Fuerte and Puebla (Njuguna, 2005). Fuerte's is green even when ripe and mostly oval shaped. Its inside is creamy to pale green and is sweet to taste. It grows to a large or medium size. (Njuguna, 2005). Puebla is round in shape and turns to purple when ripe. It`s flesh is creamy in color and the variety is available in Kenya throughout the year (ICRAF, 2007).

In regions of Lake Victoria Basin of Kenya, avocado production has expanded to swampy areas that have soils that drain slowly (MoA, 2005). This combined with poor irrigation design and management has increased the potential for flood-induced root asphyxiation leading to root decay due to *P. cinnamomi* (EPPO, 2004). The root rot caused by *P. cinnamomi* Rands, a soil borne organism that is initiated to spread by flooding as water spreads motile zoospores that are chemotactically attracted to, infect, and kill root tips of avocado (Randy *et al*., 2001). This makes it most severe in poorly drained and flooded soils with asphyxia conditions. In avocado trees, root asphyxiation has resulted in a delay of vegetative spring shoot growth, a reduction in fruit yield (40%), reduced biomass accumulation, loss of foliage and tree death that is associated with reduction in plant chlorophyll, chlorosis and reduction in photosynthetic apparatus performance (Gil *et al*., 2007). It is not known the extent of avocado losses due to *P. cinnamomi* in Kenya, but, annual losses in USA have been estimated at 30million. There are limited studies that have determined the effect of flooding and *Phytophthora cinnamomi* inoculation on chlorophyll concentration and chlorophyll fluorescence.

Propagules of the *Phytophthora cinnamomi* spread by soil movement, including wind-blows or debris, or by water flow and runoff in drainage/irrigation ditches. There is therefore need to determine *Phytophythora* (PRR) resistant avocado rootstocks. For instance, Menge *et al*. (2012) worked with three tolerant varieties `Steddom` (PP24), Zentmyer` (PP4) and Uzi (PP14) and found Steddom and Zentmyer to be resistant to *P. cinnamomi* in America. However, there is need to check the responses of varieties commonly grown in Kenya. Approximately a third of farmers in Kenya grow avocado (FAOSTAT, 2004) and 40 - 50% of the population consumes avocados regularly. Avocado is mainly grown in Western, Nyanza and Rift valley regions, but unfortunately some areas of Nyanza e.g. Nyando (Okeyo *et al*., 2008) where avocado is grown are water logged and infected with *Phytophthora cinnamomi*. Much more badly, the cure of avocado root rot has not yet been identified in most farming regions in Kenya and even other countries (FAOSTAT, 2004).

Local and seasonal variations in environmental conditions influence the virulence of *P. cinnamomi*. For instance, if environmental conditions for *P. cinnamomi* are not optimal, despite its presence, it may not be active (Van Rooyen, 2011). Soil moisture levels and temperatures significantly influence the activity of *P. cinnamomi*. Temperatures between 15 °C and 36 °C accompanied by high soil moisture levels due to rains favours the production of free swimming spores. This increases their potential for infection due to the spread of spores downhill (Colmer and Voesenek, 2009). Plants with damaged roots will naturally succumb more rapidly, especially with greater sun exposure. Mountain Ash (*Eucalyptus*

regnans) is one of important timber trees, that is susceptible to *P. cinnamomi*. However, the combination of cold winters, dry summers and high organic matter in the soil act to safeguard plants against *P. cinnamomi* (Dinis *et al.*, 2011)

Chlamydospores germinate to produce sporangia that release motile spores (zoospores). Zoospores are able to locate and attach to root tips after which they give rise to fine filamentous threads (hyphae) that may invade the roots of the host (Christie, 2012). However, there is variation in the response of hosts to the pathogen. Some hosts may show no obvious symptoms due to the ability to curtail spread of the pathogen within their tissue (Corcobado *et al*., 2013). Such plants are said to have low susceptibility. In highly susceptible host species, P. *cinnamomi* hyphae spread throughout the root system until they girdle the major roots and stems. It impedes the ability of plant's vascular tissues to absorb nutrients and water. This leads to symptoms in some larger plants that are alike to those of drought stress (Gil *et al*., 2007**).**This includes outermost parts of vascular tissue becoming yellow and dying first ('dieback'). The expression of dieback has been mostly limited to Ash species [e.g. Silvertop Ash (*Eucalyptus sieberi*)] and stringy bark species of low timber productivity coastal forest in east and south Gippsland (Pegg *et al.,* 2002**).** Warm wet summers followed by warm dry autumns that favour *P. cinnamomi* development. Water-logged conditions in plants, has led to epidemic outbreaks of *P. cinnamomi* in this forests, as well it becomes worse as these situations tends to be cyclic (Kong *et.al*, 2003).

Early symptoms of infection include wilting, yellowing and retention of dried foliage and darkening of root colour. Infection often leads to death of the plant, especially in dry summer conditions when plants may be water stressed (Whiley *et al.*, 2002). Root infectedrhododendrons and azaleas and tree saplings develop above ground leaf chlorosis, necrosis, wilt, leaf curl, and death. Stem necrosis may not occur for many weeks after the development of wilting symptoms. Below-ground symptoms are most severe in poorly drained soils and include necrosis of young feeder roots and the lower vascular tissues around the crown and just below the soil line (Gimeno *et al.*, 2012). Cankers may become visible at the base of 1-2 year old plants. The roots of older plants may recover from disease and may not develop a canker of the base of the stem. Older plants may remain symptomless, or display only mild `dieback` despite severe root rot (Christie, 2012).

The host range is very wide; *P. cinnamomi* is the most widely distributed *Phytophthora* species, with nearly 1000 host species (Fleischmann *et al.*, 2002). The principal food crop hosts of *P. cinnamomi* are avocados (*Persea americana*), with which the European Union is exclusively concerned (Bergh and Ellstrand, 1986) and pineapples (*Ananas comosus* on which it causes root and heart rot. *P. cinnamomi* also attacks *Castanea*, *Cinnamomum*, *Coniferales*, *Ericaceae* that include *Rhododendron* spp., *Eucalyptus*, *Fagus*, *Juglans*, *Quercus* and many ornamental trees and shrubs (Colmer and Voesenek, 2009). Temperate fruit trees are not important hosts in practice, but in the EPPO region, avocados are a significant host, in the limited areas where they are grown (Dias and Marenco, 2006).

Conditions of high soil moisture are known to favor *Phytophthora* root rot (Corcobado *et al.,* 2013**)**. Earlier studies by Colmer and Voesenek, (2009) showed that a serious decline of avocado in South California, had been associated with excess water and variously termed to be water injury melarnorhiza, asphyliation or apoplexy (Zentmeyer, 1983). This was actually found to be a root disease caused by *P. cinnamomi*. Zentmeyer (1983) found that 2-3 years old trees were not sensitive to flooding as plants grown in soils in absence of *P. cinnamomi* could be subjected to up to 9 days of continuous flooding with no apparent ill effects. However, if the soil was infested by the pathogen then flooding of about two days could result into severe root rot.

Historically the occurrence of *Phytophthora* root rot in flooded soils has been attributed to requirements of the pathogen for high soil moisture (Gil *et al.*, 2007). Evidence exists which indicates that soil-water status can exert a determining influence on several epidemiologically important stages in the life of *P. cinnamomi* (Pezeshki, 2001). Some species require a drained soil with matric potentials less than -20 millibars for optimum formation while others require flooded soils of matric potential of 0 millibars (Gil *et al.*, 2007). If the specific water requirements are not satisfied, sporangium formation is significantly reduced (Robin *et al.*, 2001). Once sporangia have formed, their ability to germinate indirectly by release of zoospores also has exacting water requirements. In experiment with *P. megasperma* and *P. cryptogeal* optimum release of zoospores occurred in fully water saturated soils and even a slight drainage of soil to matric potential of -5 or -10 millibars caused significant reduction in the number of zoospores released **(**Smith *et al.*, 2011**)**. Therefore, the ability of zoospore to swim through soil and infect plant roots is dependent upon the availability of large completely water-filled pores in soils that have maximum saturation of water (Kong *et al.*, 2003**).**

Mittler (2006**)** found that host factors associated with oxygen deficiency can also play a significant role in disease development**.** Oxygen quickly becomes deficient in flooded soils when pores are filled with water rather than air, although the degree of anaerobiosis which develops is mediated by drainage properties of the soil, distribution and continuity of soil pores and respiration of roots and microorganisms (Pezeshki, 2001**)**. Such stress predisposes plants to infections of *Phytophthora cinnamomi*. Although oxygen deficiency could increase disease severity, scientists considered it to be only a secondary contributing factor which prevented regeneration of roots decayed by *Phytophthora cinnamomi* and thus made plants less able to tolerate the effects of chronic root rot (Müller *et al*., 2001). Certainly, the sensitivity of plant roots to periodic flooding could vary greatly depending upon species and season and this could be a determining factor in predisposition (Van Rooyen, 2011). Clearly, the conditions which exist in the flooded soils do not only favour pathogen activity but in some cases can predispose roots to severe infection (Hardham, 2005).

Waterlogged or flooded soils in lake Victoria regions of Kenya may result from high rainfall, river overflow, elevated water tables, inadequate drainage and improper irrigation management as it occurs in other areas (Colmer and Voesenek, 2009; Pandey *et al*., 2010). Avocado growth is sensitive to flooding and *Phytophthora* root rot (PRR) and therefore tolerant rootstocks need to be investigated and identified for recommendation for growth in these regions of Kenya.

The world's production of avocado in 2004 was 3.2 million tonnes (ICRAF, 2007) that included a major contribution from North and Central America. Kenya was ranked 6th in export value of the avocado in the world (FAOSTAT, 2004). About 85% avocado produce in Kenya was by smallholder farmers. Avocado crop is therefore an important crop to rural communities and economies (Cooper *et al*., 2003). Despite the favourable climate in the Lake Victoria Basin, production of avocado in this region is still low and this is due to the constraints such as diseases, salinity and water logging (Schaffer *et al*., 1992).

Reeksting *et al*. (2014) found out that in America avocado`s total chlorophyll decreases under *Phytopthora cinnamomi* inoculation. This decrease in chlorophyll due to *Phytopthora cinnamomi* was related to chlorophyll photo-bleaching. These decreases in chlorophyll reflect a reduction in the chlorophyll antenna size of the photosystems from photo-inhibition by reducing energy delivery to the reaction centres (Kate and Giles, 2000). In most plants, chlorophyll antenna size is an adaptive strategy to reduce light absorption and avoid damage of the photo systems due to *Phytopthora cinnamomi* (Mmayi 2015).This was expected to occur in avocado varieties under study. In most of the varieties at the beginning of exposure to *Phytopthora cinnamomi* inoculums, chlorophyll will reduce but after 48h of treatment the chlorophyll antenna becomes similar to the controls (Reeksting *et al.*, 2014).These varieties have favourable root growth to support faster acclimatization of photosynthetic apparatus to *Phytopthora cinnamomi* stress by increasing water absorption and nutrient uptake (Steward *et al*., 2012). This has also been reported in evergreen species under *Phytopthora cinnamomi* stress (Sayed, 2003).

Light energy absorbed by chlorophyll molecules in a leaf can be used to drive photosynthesis (photochemistry), excessive energy will be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence (Liu *et al*., 2012). Any increase in the efficiency of one of the three will result in a decrease in the yield of the other two (Maxwell and Johnson, 2000). It is well known that photoinhibition is one of the primary physiological consequences of water-logging and that alteration of PSII activity under water stress are related to photoinhibition rather than to a direct damage to PSII (Efeoglu, 2009).

Measuring chlorophyll fluorescence has become a very useful technique in obtaining rapid qualitative and quantitative information on photosynthesis (Rohácek and Bartak, 1999), and it can provide information on the relationship between structure and function of photosystem II (PSII) reaction centre (Stewards *et al*., 2012). Chlorophyll fluorescence analysis is a useful, non-invasive, powerful, and reliable technique to assess the changes in function of PSII under different environments (Liu *et al*., 2012; Mauchamp and Methy, 2004). It can check the composition and organization of photosystems (Jiang *et al*., 2008), the excitation energy transfer, the photochemistry, and the effects of various stresses on plants (Liu *et al*., 2012). Chlorophyll fluorescence provides useful information about leaf photosynthetic performance of many plants under drought stress (Liu *et al*., 2012). Furthermore, chlorophyll fluorescence can tell the extent to which PSII is using the energy absorbed by chlorophyll (Zhou *et al*., 2011), and the extent to which it is being damaged by excessive light (Maxwell and Johnson, 2000). Waterlogged avocado plants that were infected were expected to show a reduction of the photochemical chlorophyll fluorescence quenching, PSII quantum yield and electron transport rate and more heat dissipation (Dias and Bruggemann, 2010).

Under high irradiance, however, the PSII reaction centres will absorb excessive light energy which eventually results in the impairment or inactivation of the chlorophyll-containing reaction centres of the chloroplasts (Bertaminia *et al*., 2006). When studying maize genotypes, Liu *et al*. (2012) analyzed chlorophyll fluorescence that showed that photosystem (PSII) was rather tolerant to the water stress imposed. According to them, water stress caused a slight decrease in the efficiency of excitation capture by open PSII reaction centre. Declining values of Fv/Fm are an indicator of stress (Liu *et al*., 2012). Dark adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and have previously been used as sensitive indicators of plant photosynthetic performance, with optimal values for healthy plants generally being 0.83 (Burke, 2007; Liu *et al*., 2012). Values are lower when the plants are exposed to stress, indicating the phenomenon of photo-inhibition or the degree of damage to PSII complex (Kate and Giles, 2000).

Fv/Fm was used to screen maize (Liu *et al*., 2012) and was found to be correlated with decreased CO_2 assimilation and electron transport (Sayed, 2003). Decline in Fv/Fm in avocado genotypes when water-logged, suggested that photo-inhibition is accompanied by an over-reduction of PSII (Reeksting *et al*., 2014). This study just like that of Colom and Vazzana (2003) hen studying maize showed that with increasing irradiance, there was a steady decline in qP,ϕPSII, open PSII energy capture efficiency (Fv/Fm) and a clear increase in non-photochemical quenching (NPQ). It is however not clear how these parameters are affected by water logging and inoculation by *P. cinnamon* on avocado. The qP is an indication of the proportion of open PSII reaction centres, and translates light quantum energy into chemical energy process, which reflects the photosynthetic efficiency and the light use situation of plant (Fracheboud *et al*., 1999). NPQ can represent the energy which cannot be utilized to transport photosynthetic electrons but be dissipated harmlessly as heat energy from PSII antennae (Kate and Giles, 2000) and (Fracheboud *et al*., 1999). In avocado rootstocks, a decrease of the qP was observed in response to the *P. cinnamomi* treatment, indicating that a larger percentage of the PSII reaction centres would close at any time (Reekstings *et al*., 2014), which also indicated that the balance between excitation rate and electron transfer rate have changed (Efeoğlu, 2009). It is therefore interesting to establish the trend of these effects in avocado rootstocks under water logging. Fv/Fm is of great value in assessing the relative contributions of PSII photochemical capacity and thermal decay processes to the overall efficiency of photochemistry at PSII in avocado plants (Liu *et al*., 2012).

ϕPSII is the effective quantum yield of photochemical energy conversion in PSII (Ronácek and Bartak, 1999). ϕPSII increase is related to significant reductions of Fv/Fm (Colom, 2003). Such reductions occur with increase in thermal energy of dissipation indicated by NPQ. Increase in non-photochemical fluorescence quenching, as one means of estimating the level of energy dissipation, is expected to have increase incident photon flux densities at waterlogging and *P. cinnamomi* in avocado leaves. The decrease of qP and ϕPSII is also expected under this stress (Shangguan *et al*., 2000). Xanthophyll cycle relying on photo-protection is believed to be the main mechanism for plants to deal with excessive light energy (Liu *et al*., 2012), and it plays an indirect role in thermal dissipation by mediating a critical conformational change within the PSII antenna (Ort, 2001). With the increase of NPQ of xanthophyll cycle, excessive energy was dissipated as thermal energy to protect the maize leaf from light-induced damage in draught. The variation trend of NPQ increased along with the increasing irradiation. Ort (2001) indicated that the NPQ got involved in the competition between the thermal dissipation of chlorophyll *a* and fluorescence emission as well as photosynthesis.

Flooding inhibits root growth, shoots and knew leave development, reduce net photosynthetic rate, photosynthetic electron transport rate, photosystem II photochemical efficiency and cause reactive oxygen species metabolism disorder (Reeksting *et al*., 2014). Some plants sense oxygen levels and bring morphological, physiological and biochemical changes that improve flood tolerance and also reduce in carbohydrate consumption. Zebin *et al*., 2014 while researching on restoration of *Distylium chinense*, ashrub after a dam construction, found it to maintain stable P_n. A decrease in Fv/Fm, qP and ETR accompanied by an increase in qN have found to reflect increased photo-protection through the xanthophyll cycle rather than photo-damage (Zebin *et al*., 2014). It is therefore necessary to determine chlorophyll fluorescence parameters, in order to determine if avocado rootstocks have a stronger adaptability to soil flooding, which may be a factor to enable them survive in flooded areas.

Tedious efforts have been made to increase avocado fruit production by extirpating *P. cinnamomi* infected avocado rootstocks or treating with fungicides but still no remarkable success (Griesbach, 2005). Infection of avocado by *P. cinnamomi* poses a big problem as it has caused a lot of losses in avocado producing regions in Kenya and mostly where flooding is common. Knowledge pertaining to the physiological tolerance of avocado rootstocks such as Puebla, Fuerte, Pinkerton and Booth7 to *P. cinnamomi* and flooding is limited. Little is understood on the effect of *Phytopthora*

cinnamomi inoculation on chlorophyll content and chlorophyll fluorescence of avocado rootstocks under flooding conditions in Kenya. There is also limited scientific initiative that has documented on the morphology and physiology of avocado rootstocks in Kenya under flooding and *P. cinnamomi* inoculation. As Kenya's population continue to rise, there is pressure on land for diversified food production and increased yield, hence the need to evaluate the response of avocado rootstocks to soil flooding and *P. cinnamomi* inoculation.

Most parts of Kenya are arid and semi-arid thus, avocado growth becomes difficult due to need for irrigation. To increase food security and raw avocado to industries, further expansion of avocado production in Kenya that may call for growing it in perennially flooded areas and wetlands that include lake basin water logged areas of Nyanza that are infested with *P. cinnamomi*. These efforts to expansion cannot succeed because of water logging and *Phytophthora* infestation in these areas, and therefore need to have rootstocks that are resistant to these abiotic and biotic stresses (Pegg *et al*., 2002). With the current shrinking of arable land, avocado plants are being grown in potentially flooded areas such as the Lake Victoria region. There is need for research work to select rootstock varieties that can survive well in waterlogged areas and those that are prone to diseases caused by pathogens such as *P. cinnamomi*.

The general objective of the study was to evaluate selected avocado rootstocks commonly found growing in the Lake Victoria Basin of Kenya for response to water logging and *Phytophthora cinnamomi* inoculation under greenhouse conditions. A specific objective of the study was to determine the effect of *Phytophthora cinnamomi* inoculation on chlorophyll concentration and chlorophyll fluorescence parameters of avocado rootstocks seedlings grown under water logging conditions.

2. MATERIALS AND METHODS

Study Area and Experimental Materials;

Avocado fruit varieties were collected from the following parts of Kenya; Maseno division, Nyando division, Kisumu East division (Kisumu county) and Busia-Budalangi division (Busia county), and were taken to Museums of Kenya Herbarium for confirmation and identification.

Avocado fruits were kept to ripen then seeds extracted and planted at Maseno Botanical Garden in polythene bags. A total of four rootstocks commonly grown in these regions were planted, namely; Fuerte, Booth 7, Puebla and Pinkerton. The varieties were identified with the following characteristics according to Reeksting *et al*. (2014): Fuerte fruits were pear with flat area on bottom corner, green colour ripe fruit, thin skin, smooth fruit surface and fruits mature early. Booth7 were spheroid oborte, fruit apex rounded, small in size, bright green fruit that are slightly pebbed, Glossy, thick and woody skin. Puebla fruits were small in size, onyx black skin that is thin and smooth, very creamy and succulent flesh. Pinkerton had fruits that are pear shaped with well-developed long neck, course dark green ripe fruits and medium thick skin that are mid in maturing.

Mwai (2001) classified Maseno to be located at Latitude extent 0^0 1`N – $0^0$ 12`S and Longitude extent 34^0 25`E – $34^0$47`E, Maseno is at approximate 1500m above sea level, soils in Maseno are acrisol deep reddish brown clay and well drained and that Maseno receives rains averaging to 1750 mm per annum with a mean temperature of 28.7^0C. The seedlings were then transferred to a greenhouse in KALRO (Kibos) research centre after three months. Greenhouse growth conditions were 25°C-40°C/20°C-30°C (day/night) temperature, 14/10-h (light/dark) photoperiod, and 64-77% relative humidity. Flooding and inoculation treatments were then induced after three weeks of acclimation. According to Japheth *et al.* (2014), Kibos is located at 34^0 48`E, $0^0$ 04`N and1144m above sea level with clay loamy soil and long term mean annual rainfall of 1440mm. It`s temperatures range from 15.3 ^{0}C to 30 ^{0}C.

Flooding and Inoculation Treatments

Flooding treatments were introduced when seedlings were three months old and this was after two weeks of transplanting of avocado rootstock seedlings (plate 2). Inoculations were done at 7 days intervals thereafter. The avocado rootstocks seedlings planted in 10 litre pots were immersed in 20 litre plastic pots containing distilled water almost half full. Two control treatments involved avocado seedlings grown in 10 litre plastic pots, and daily provided with water and another one where the avocado seedlings were planted into 10 litre plastic pots and immersed into 20 litres plastic pots containing water half full but were not inoculated.

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The treatments were as indicated below and were replicated three times.

T_1; - Un-flooded-un-inoculated (Control), T2;-Unflooded-inoculated, T3;-Flooded uninoculated and T_4; - Flooded-inoculated. The pots containing the avocado rootstock seedlings were laid out in a greenhouse in a completely randomized design. The inoculums were soaked with dilute vegetable juice broth as described by Wilcox (1989). The test fungus was serially diluted to a concentration of approximately $1x10^7$cfu/ml. It was added to potted plants at a rate of $30cm^3$ per $1000cm^3$ of potting medium.

Plate 2 Arrangement of avocado rootstocks flooded and inoculated with *P. cinnamomi* in a greenhouse at kibos KARI.

Determination of chlorophyll concentration;

Chlorophyll concentration was determined according to Netondo (1999). The chlorophyll was extracted by the 80% acetone. Absorbance of the chlorophyll determined by a spectrophotometer (Nova spec II, Pharmacia Biotech, Cambridge, England) at 645 and 663nm to determine the chlorophyll *a* and *b* content. Chlorophyll concentration in the leaf was calculated as follows: -

Chl a = 12.7(D663) - 2.67(D645) × V/1000 × W [mg *Chl a* g^{-1}leaf tissue];

Chl b = 22.9(D645) -4.68(D663) × V/1000 × W [mg *Chl b* g^{-1}leaf tissue];

Total chlorophyll concentration was calculated as chla +*chlb*.

Where:

Chl a is chlorophyll a concentrations; *chl b* is chlorophyll b concentrations; D= absorbance measured at wavelengths 645nm and 663nm; V= volume in ml of acetone extract used and W= fresh weight (g) of leaf from which the extract was made.

Determination of chlorophyll fluorescence parameters;

Chlorophyll fluorescence measurements were determined using a portable fluorescence monitoring system (Hansatech model FMS 2; Hansatech Instruments, England) on the first fully opened and exposed leaf at an interval of two weeks. Leaves were dark-adapted for 15 minutes, using the dark adaptation clips and then illuminated for six seconds to induce fluorescence. The leaves were continuously illuminated with a white actinic light (200 µmol m^{-2} s^{-1}). The initial fluorescence (Fo) and the maximum fluorescence (Fm) was measured, and the variable fluorescence (Fv = Fm - Fo) and the Fv/Fo ratio calculated. The potential minimum efficiency of PSII (Fv/Fm) of dark-adapted leaves was calculated as Fv/Fm = (Fm-Fo)/Fm. The parameters of fast chlorophyll fluorescence, maximum fluorescence yield from PSII following a saturating pulse of photons in a light-adapted plant (Fm'), steady state yield of PSII fluorescence in the light (Fs), and electron transport rate through PSII (ETR) was determined during the day between 11:00 am and 1:00 pm according to Maricle *et al*. (2007).

Statistical data analysis

The data collected from this study were subjected to analysis of variance (ANOVA) using SAS statistical computer package (Steel *et al*., 2006). Fisher's LSD test at 5% level was used to separate the treatment means.

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3. RESULTS

Chlorophyll concentration

Chlorophyll *a;* Chlorophyll *a* was significantly ($p \leq 0.05$) decreased by flooding and *phytophthora cinnamon* inoculation. Fuerte and Booth 7 rootstocks were much affected (Figure 1). There were significant interactions in Chlorophyll *a* concentrations between treatments and rootstocks. Chlorophyll a content values were as follows; control (26.75), Flooded –uninoculated (17.24), Flooded –inoculated (10.05), and Inoculated (20.42). Mean of variety Pinkerton (21.31) and Puebla (22.15) were significantly different when each was compared to each of the following varieties; Fuerte (15.33) and Booth 7 (15.56).

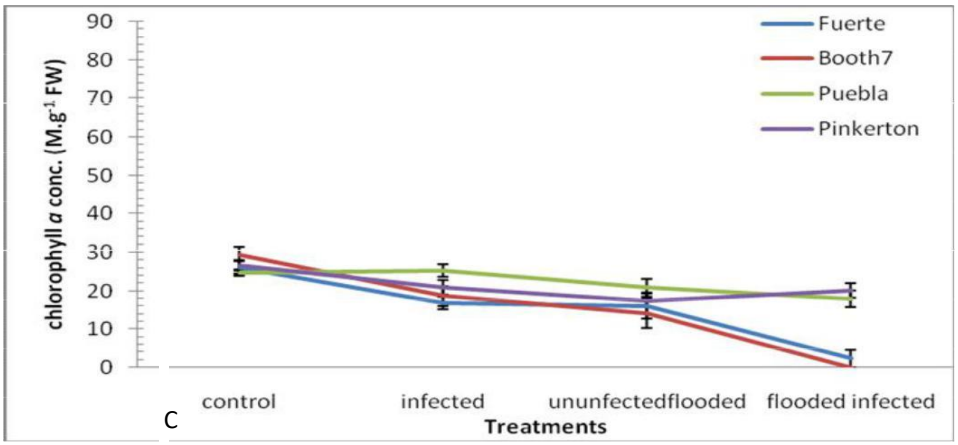

Fig. 1. Chlorophyll *a* conc. (m.g^{-1} FW) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

Chlorophyll *b;* There were significant ($p \leq 0.05$) decreases in chlorophyll *b* concentration in Fuerte and Booth7 rootstocks under Flooded and inoculated treatments (Figure 2). Significant differences in chlorophyll *b* concentration occurred among treatments and rootstocks. There were significant interactions between treatments and rootstocks. Mean of Pinkerton rootstock (22.97) and Puebla (22.040) were significantly different when compared to other avocado rootstocks, i.e Fuerte (15.840) and Booth 7 (16.112).

Total chlorophyll (mg.g^{-1}); Total chlorophyll concentration significantly ($p \leq 0.05$) decreased in Fuerte and Booth 7 under flooded–uninoculated and flooded-inoculated conditions (Figure 3). Pinkerton Rootstocks were less affected under Flooded and inoculation treatment. There were significant differences in total chlorophyll content among treatments and rootstocks. The interactions between treatments and rootstocks were also significant.

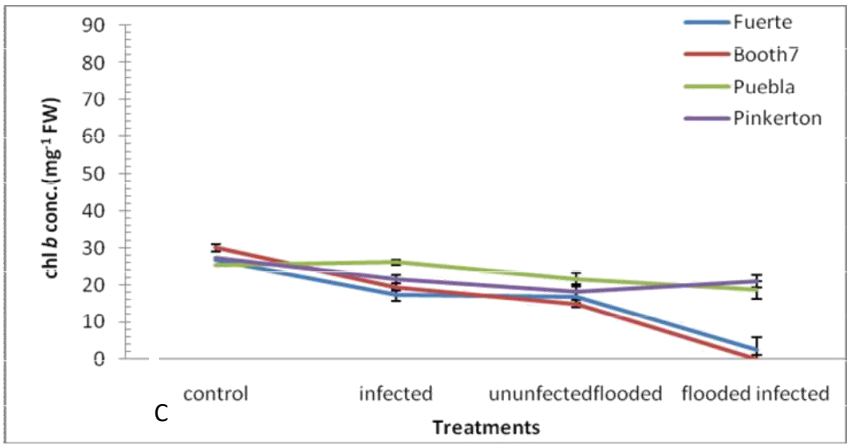

Fig. 2. Chlorophyll *b* conc. (m.g^{-1} FW) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

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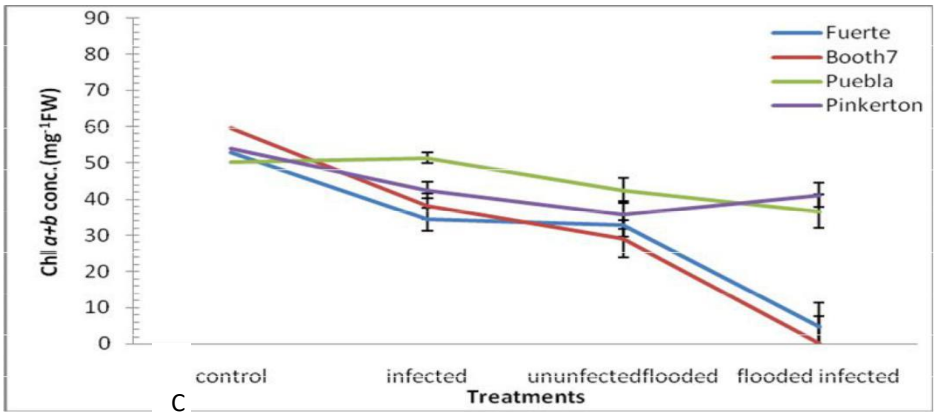

C

Fig. 3. Chlorophyll *a+b* conc. (m.g^{-1} FW) of avocado rootstocks: control (Un-flooded-uninoculated), unflooded-inoculated, flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

Chlorophyll Fluorescence

Maximum quantum yield (FV/FM); Pinkerton and Puebla rootstocks were less affected by flooding and inoculation treatments (Figure 4). There were significant ($p \leq 0.05$) decreases in FV/FM among Fuerte and Booth 7 rootstocks under Flooded-uninoculated and flooded-inoculated treatments. There was a significant interaction between treatments and rootstocks. Mean of variety Puebla (0.63) was significantly different when compared to each of the following rootstocks; Pinkerton (0.42), Fuerte (0.44) and Booth 7 (0.32).

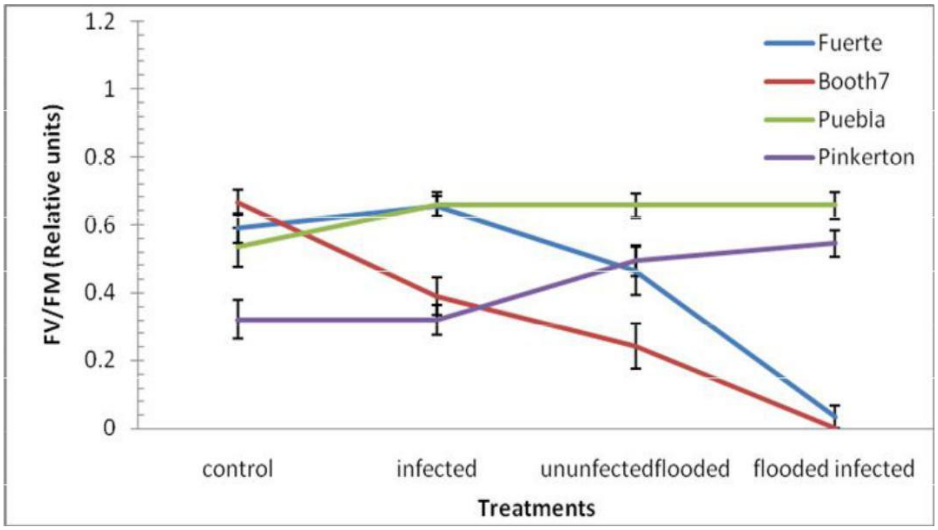

Fig. 4. FV/FM (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

Effective quantum yield (ϕPSII); Fuerte and Booth7 rootstocks were much affected by the flooded-uninoculated and flooded -inoculated treatments (Figure 5). There were significant ($p \leq 0.05$) decreases in ϕPSII among treatments and rootstocks. The interactions between treatments and rootstocks were also significant. Mean of rootstock Puebla (0.60) was significantly different when compared to each of the following; Pinkerton (0.45), Fuerte (0.42) and Booth 7 (0.30).

Non-photochemical quenching (NPQ); Non-photochemical quenching decreased significantly ($p \leq 0.05$) in Fuerte and Booth 7 avocado rootstocks under flooded-uninoculated and flooded- inoculated conditions (Figure 6). On the other hand Puebla and Pinkerton rootstocks experienced some increases in NPQ under similar conditions. There were no significant differences in NPQ between Fuerte and Pinkerton rootstocks under unflooded inoculated and uninoculated flooded. There were no significant interactions between treatments and rootstocks.

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Electron transport rate (ETR); ETR values were significantly ($p \leq 0.05$) reduced under uninoculated-flooded and flooded- inoculated conditions in both Booth7 and Fuerte rootstocks (Figure 7). Electron transport rate showed significant decreases ($p \leq 0.05$) among treatments and rootstocks. There was a significant interaction between treatments and rootstocks.

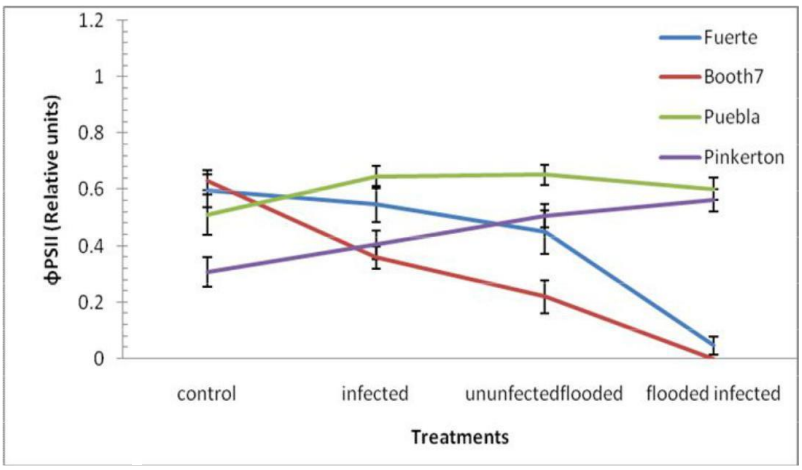

Fig. 5. ϕPSII (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

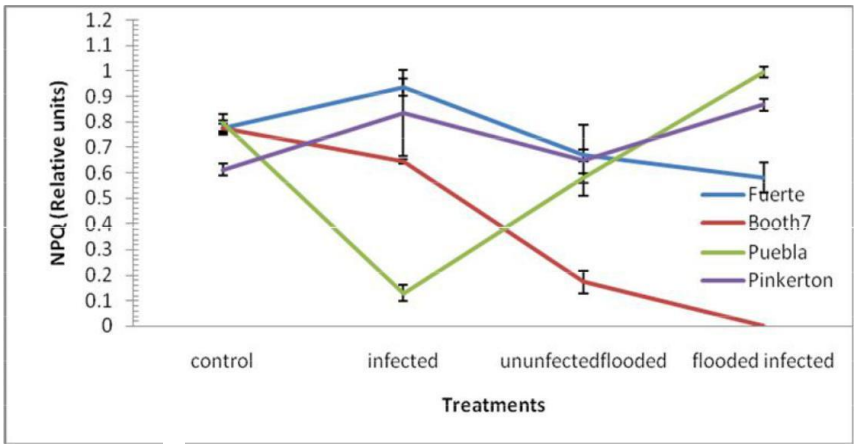

Fig. 6. NPQ (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

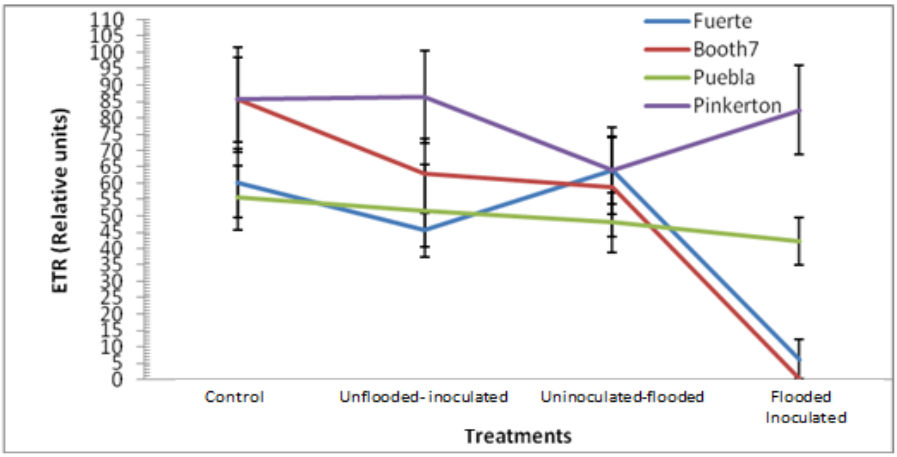

Fig. 7. ETR (Relative Units) of avocado rootstocks: control (Un-flooded-un-inoculated), unflooded-inoculated, flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates±S.E.

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4. DISCUSSION

Influence of *P. cinnamomi* and flooding stress on chlorophyll content; Water-logging and *P. cinnamomi* inoculation induced a decrease in chlorophyll a concentration in avocado rootstocks. This has similarly been reported earlier in other plant species under environmental stresses, such as sorghum (Peixoto *et al*., 2002), beech (Ridolfi and Garrec, 2000) and barely (Abdalla, 2008). It should, however be noted that a decrease in chlorophyll concentration of avocado rootstocks in response to *P. cinnamomi* inoculation and flooding treatments was probably not the primary factor to limit CO_2 assimilation (Jiang *et al*., 2008). A study by Reeksting *et al*. (2014) support this postulate since chlorophyll concentration values were lower in Waterlogging and *P. cinnamomi* inoculation than in control leaves. Reeksting *et al*. (2014) found that a combination of such factors reduced photosynthetic pigment concentration, impaired PSII photochemistry and the distribution of enzymatic machinery accounted for the treatment induced decrease in CO_2 assimilation in avocado rootstocks.

Flooding and *P. cinnamomi* inoculation might have caused a decrease in chlorophyll *a* synthesis in avocado rootstock leaves when compared to the control by inhibiting the activity of - aminolevulinic acid (-ALA) dehydratase (Pereira *et al*., 2006; Mmayi (2015). Mihailovic *et al*. (2008) found that in stressed sensitive maize inbred line, chlorophyll reduction coincided with 5-ALA synthesis inhibition, chlorophyllase activation and leaf deprivation of Fe and Mg. Therefore decrease in chlorophyll *a* with water-logging and *P. cinnamomi* inoculation for the rootstocks in this study may be attributed to the inhibition of the activity of - aminolevulinic acid (-ALA) dehydratase (Mmayi, 2015)

There was a reduction in chlorophyll *b* concentration under initiation of both water-logging and *Phytopthora cinnamomi* inoculation treatments majorly in Puebla and Pinkerton rootstocks. This may have been due to decreased uptake of Magnessium ions by roots under water-logging and *Phytopthora cinnamomi* inoculation conditions, resulting in a correspondingly decreased PAR utility efficiency which affected the photosynthetic capacity of the rootstocks (Steward *et al*., 2001). The low levels in chlorophyll *a* and *b* decreased total chlorophyll content in avocado rootstocks. Chlorophyll a and b concentration in Fuerte and Booth 7 rootstocks were significantly decreased under flooded and inoculation treatments at the same time. This suggests that there was chlorophyll photo bleaching within PSI and PSII (Reeksting *et al*., 2014) at high rates in these rootstocks, resulting in a smaller fraction of absorbed light energy for electron transport.

Generally plants under water logging and *Phytopthora cinnamomi* inoculation had low chl *a*, chl *b*, and total chlorophyll concentration. *Phytopthora cinnamomi* and water-logging treatments significantly affected the concentration of chl *a*. water logging and *Phytopthora cinnamomi* treatments did not significantly affect the concentration of photosynthetic pigments; chl *b*, chl *a* and chl *a*+*b*.

Influence of *P. cinnamomi* and flooding stress on chlorophyll fluorescence

Phytopthora cinnamomi and flooding treatments affected the photochemical efficiency of Fv/Fm, ΦPSII, NPQ and ETR of the avocado rootstocks investigated differently. In Puebla leaves, *Phtopthora cinnamomi* inoculation, flooding, and inoculation during flooding caused a significant decrease of the photochemical efficiency of PSII (Chen *et al*., 2005b). Photochemical parameters of PSII have the potential to estimate photosynthetic performance of stressed plants (Maxwell and Johnson, 2000). The Fv/Fm ratio measured in the four rootstocks of avocado after exposure to different treatments showed significant ($p \leq 0.05$) differences. Mean values for maximum quantum yield were high at the control treatment compared to uninoculated-flooded and flooded-inoculated treatments for Fuerte and Booth7 avocado rootstocks. This shows that photosynthetic apparatus of these rootstocks were highly affected compared to Puebla and Pinkerton and that inoculation alone had less effect on the rootstocks similar to findings of Reeksting *et al*. (2014). The Fv/Fm values found in this study did not show a consistent reduction with treatments. According to Kate and Giles (2000), Fv/Fm ratios for normal plants have an optimal value of 0.83. Therefore low Fv/Fm ratios of avocado rootstocks in this study showed that they exhibited normal photosynthesis despite being grown under uninoculated-flooded and inoculated conditions. Fuerte and Booth 7 rootstocks showed abnormal growth with very low values of Fv/Fm that ranged from 2.9 and 0 under flooded and *P. cinnamomi* inoculation conditions. Fv/Fm of plants exposed to a combination of the two stresses (flooding and *P. cinnamomi* inoculation) dropped to level significantly lower than both non-flooded, inoculated plants and control plants. These trends were similar to those reported by Reeksting *et al*. (2014). Fv/Fm ratio is a useful indicator of early responses to flooding or disease infection in plants (Xiao-Bin *et al*., 2007; Maxwell and Johnson, 2000; Marenco and Dias, 2006).

Effective quantum yield (ΦPSII) and ETR had low values at the control treatments compared to uninoculated-flooded and flooded-inoculated treatments of Puebla and Pinkerton rootstocks. The ΦPSII in Fuerte and Booth 7 rootstocks were lower even under the control treatment compared with the other rootstocks. Fuerte and Booth 7 rootstocks may have been intrinsically less efficient at managing their energy for photochemical processes than the other avocado rootstocks (Giannakoula *et al*., 2008). This rootstock specific behaviour indicates that they might be having lower productivity as compared to the other rootstocks. High values of ΦPSII in Puebla rootstocks showed that the photochemical activity was the main way to dissipate safely the excess energy of excitation. This was an indication that ETR was never saturated showing that other sinks, different from the assimilatory process, were likely to accept electrons (Erwin *et al*. 2014). In this way the excess energy of excitation is dissipated by photochemical activity avoiding the over reduction of PSII reaction centres (Ambrosio *et al*., 2003). Reeksting *et al*. (2014) observed Mehler reaction in mesophyll chloroplasts of C3 species and proposed a role in the production of extra ATP for the pseudocyclic photophosphorylation. Differences in ϕPSII were noticeable at even the onset of visible symptoms between flooded and non-inoculated treatments of both Booth 7 and Puebla rootstocks with flooded and inoculated avocado rootstocks exhibiting lower values, thus highlighting the importance of the combination of the stresses in avocado rootstocks. The reduction in ϕPSII indicates a decrease in the proportions of radiations absorbed by chlorophyll associated with PSII, which is used in photochemistry (Maxwell and Johnson, 2000; Reeksting *et al*., 2014). Decrease in ϕPSII and ETR of avocado rootstocks under this study were not accompanied by an increase in NPQ, possibly suggesting a reduced ability of these plants to dissipate excess energy resulting from a decline in photochemistry.

Thermal energy dissipation measured as NPQ in the four avocado rootstock rootstocks did not have a clear pattern with different treatments although treatment means were significantly different. In Fuerte, Pinkerton and Puebla cases NPQ was high in flooded-inoculated plants compared to control (Fig. 6). Less energy was dissipated in treatments of inoculated and un-inoculated flooded, that show the two treatments each to have less effects to growth of avocado plants as compared to inoculated-flooded plants (Lu *et al*., 2003). In this case other metabolic pathways such as the water cycle (Mehler reaction) and photorespiration in flooded-inoculated avocado rootstocks may have been up regulated to cope with the increased excess of excitation (Reeksting *et al*., 2014) in Fuerte rootstock that had a high NPQ.

It is accepted that PSII is the most vulnerable part of the photosynthetic apparatus to stress-induced damage (Marjorie *et al*., 2010). Inoculated-flooded avocado rootstocks therefore might have used a smaller fraction of the absorbed light in electron transport compared with control leaves which had more excess excitation energy. The main role of NPQ is to indicate dissipation of the excess energy of excitation. The low non-photochemical quenching (Chen and Cheng, 2003) in control plants, indicated that there was less thermal energy dissipation. A higher mean value in flooded and *P. cinnamomi* inoculated avocado rootstocks contributed to excess of thermal energy of dissipation (NPQ). This explains the fact that apart from photochemistry, fluorescence strategy was adopted to dissipate excess energy to some extent. Fuerte avocado rootstocks appeared to have been strongly affected by *P. cinnamomi* and flooding stress since it exhibited high fluorescence and was found to have dissipated more energy.

Generally avocado rootstocks under inoculated and flooded treatments had low values of Fv/Fm, ΦPSII and ETR showing that photosynthetic apparatus were affected by *P. cinnamomi* and flooding treatments. Flooding and *P. cinnamomi* inoculation interfered with chlorophyll fluorescence parameters of the rootstocks. Puebla and Pinkerton rootstocks had high Fv/Fm and ETR values. Booth7 rootstocks behaved differently compared to the rest of avocado rootstocks.

5. CONCLUSIONS

Puebla had high concentration of chlorophyll *a, b* and total chlorophyll in the leaves. Generally avocado rootstocks under flooding and *P. cinnamomi* had low chlorophyll a concentration. Avocado rootstocks that were grown under flooded-inoculated treatments had low chlorophyll concentration.

Avocado rootstocks under *P. cinnamomi* and flooding treatments had low values of Fv/Fm, ΦPSII and ETR as an indication that their photosynthetic apparatus were negatively affected. The *P. cinnamomi* did interfere with chlorophyll fluorescence parameters in the avocado rootstocks. Some rootstock differences were evident in Fuerte, Puebla and Booth 7 in Fv/Fm, ΦPSII and ETR values. Pinkerton behaved somehow differently compared to the avocado rootstocks.

Puebla rootstocks responded better to *P. cinnamomi* inoculation and flooding stress as compared to the other avocado rootstocks. They may be recommended for growing in *P. cinnamomi* infested and waterlogged regions. Additional research is needed to determine the relationships among A, gs, and Ci for more flooded and non-flooded avocado rootstocks inoculated with *P. cinnamomi.*

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