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Relationship of Coffee Wilt Disease (*Gibberella xylarioides* Heim and Saccas) Resistance and Morphological Traits in Selected Arabica Coffee (*Coffea arabica* L.) Genotypes

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Abstract

Understanding the association between coffee wilt disease (CWD) resistance and morphological traits are important in designing appropriate resistant breeding approaches. The study was conducted to estimate the relationship among characters and to determine path coefficient analysis (direct and indirect effects) of the characters on resistance in 17 selected genotypes (experiment 1); and eight parents with 28 F1 crosses (experiment 2) using randomized complete block design (RCBD) with three replications in artificial seedling inoculation test at the greenhouse, Jimma Agricultural Research Center (JARC). The analyses of variance showed that highly significant differences ($p < 0.01$) among the genotypes for wilted seedling percentage, incubation period, number of defoliated leaves, and all seedling growth characters (height, stem diameter, average inter-node length, petiole length, leaf area, number of nodes and leaves) for both experiments. Wilted seedling percentage revealed a highly significant negative genotypic correlation with the incubation period, leaf area, and stem diameter in both experiments; while it showed a positive association with the number of defoliated leaves. The incubation period, leaf area, and stem diameter observed high negative direct effects on CWD resistance. Additionally, seedling height also showed a direct negative effect, but low in magnitude. The indirect effect of average internodes length found mainly through leaf area, and seedling height through incubation period and stem diameter. We concluded that emphasis should be given to the incubation period and stem diameter along with the number of defoliated leaves for the indirect selection of resistance Arabica coffee genotypes. These traits could be used as a selection criterion to improve resistance in Arabica coffee; however, it is not the only factors that contributes to selection. Therefore, further study is important on the association of CWD resistance to these morphological and vascular discoloration traits at natural field conditions (multi-locations).

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Keywords

Arabica coffee, coffee wilt disease, correlation, path coefficient analysis, resistance.

Introduction

Coffee is the most exported and traded agricultural commodity in the world. The production and

consumption have risen over the past 50 years. Some coffee producing countries have seen considerable benefits through higher yield and growing volumes of sales. However, many, especially small producers who

produce the majority of the world's coffee are facing growing challenges from climate change and more difficult natural growing conditions (FAO, 2015). Additionally, the production is low due to the widespread of coffee diseases such as, coffee berry disease (CBD), coffee leaf rust (CLR), coffee wilt disease along with other limiting factors (Eshetu *et al.*, 2000; Girma *et al.*, 2009a).

Coffee wilt disease (CWD) is a fungal disease that causes a vascular disease in coffee; caused by *Gibberella xylarioides* or *Fusarium xylarioides* (Heim and Saccas, 1950; Geiser, *et al.*, 2005). The fungus invades the coffee tree and colonizes the xylem system. Successive surveys by different scholars on the occurrence and prevalence of the pathogen in major coffee-growing regions ascertained the existence of the disease with varying intensity (Van der Graaff and Pieters, 1978; Merdassa, 1986; Girma, 1997; Eshetu *et al.*, 2000; Sihen *et al.*, 2012). The prevalence and importance of CWD has been markedly increasing throughout coffee producing areas of the country (Girma *et al.*, 2001; CABI, 2003; Girma, 2004).

A host-pathogen association involves a three-dimensional interaction between host varieties, pathogen strains and environmental variables that can affect disease expression. To be able to limit the effect of these factors on host-pathogen interactions, standard artificial screening protocols that discriminate between resistant and susceptible genotypes have developed (Flood, 2006). Different inoculation procedures, such as stem nicking, root dipping and syringe injection have to screen and identify resistant genotypes by different countries (Pieters and Van der Graaff, 1980; Girma and Mengistu, 2000; Musoli, *et al.*, 2001; Musoli, 2005). Stem nicking method of young coffee seedlings with inoculum suspension 2×10^6 spore per milliliter of *Gibberella xylarioides* isolate at cotyledon stage (2 to 2.5 months old) using a scalpel has adopted as the preferred standard practice on Arabica coffee. Thus, standardizing the inoculation protocols (methodologies), identifying proper growth stages of the seedlings (host) that show differential reactions, selection of aggressive strain/isolate and conditions that favor infection and wilt disease development are paramount important in designing an effective screening and breeding program for CWD management (Girma *et al.*, 2009b).

Correlation is a measure of the degree of association between traits and the association may be based on genetics or non-genetic between two or more traits (Hallauer and Miranda, 1988). If a genetic association

exists, selection for one trait will cause the changes on other traits. This response to change by genetic association is called correlated response; it may be caused by pleiotropism or linkage disequilibrium. Pleiotropism is the multiple effect of a single gene (i.e., a single gene simultaneously affects several physiological pathways). In a random mating population, the role of linkage disequilibrium in correlated response is only important if the traits of interest are closely linked (Acquaah, 2012).

Several studies have suggested that morphological traits (height, basal diameter of the axis, and the number of branches) of Japanese pines are associated with resistance to pine wilt nematodes (PWN) (Toda *et al.*, 1986; Toda and Fujimoto, 1987; Kuroda, 2004). These morphological traits showed different levels of relevance among individuals. Yamanobe (2009) supposed that a thicker basal diameter well survived. Pine trees can survive as long as there is a partial passage for xylem and phloem transport, even if almost no transport occurs (Kuroda, 1999). Trees with a wider diameter at the base may have a greater potential to retain functional passages than thinner trees. With respect to the number of branches, subjects with more branches would survive better as long as there are more branches below the inoculation position. For upland cottons, significant negative correlations observed between foliar damage or vascular discoloration with number of nodes and plant height. These significant correlations indicated that the reduction in plant growth related to symptoms (Ulloa *et al.*, 2006).

Agrios (2005) stated that inoculation of *Fusarium wilt* resulted in clogging of xylem vessels by mycelia, spores and tyloses. Crushing of vessels by proliferating adjacent parenchyma cells also observed, which hamper the translocation of water on the infected plants. The leaves of infected plants transpire more water than the roots and stem can transport, resulting in wilting symptoms. That is why growth and transpiration reduced in *Fusarium wilt* infected plants. Glasshouse experiments conducted to assess the effects of wilt fungus on growth and transpiration of chickpea. Results showed inoculation of *Fusarium oxysporum* reduced plant growth, transpiration and caused severe wilting (Siddiqui and Singh, 2004). Walyaro and Van der Vossen (1979) also studied on 16 Arabica coffee varieties and they reported phenotypic correlation is generally much lower than the genotypic ones, indicating that the inherent association between characters strongly influenced by environmental cause. The girth at the base of the main stem is

genotypic-ally correlated with height. According to El-bramawy *et al.*, (2009), the regression analysis of branch number and seed color in sesame showed significantly correlated with *Fusarium wilt* and charcoal rot diseases infection percentages. Therefore, these traits might use for direct selection of sesame accessions that are resistant to fusarium wilt and charcoal rot disease. The associations between characters are important selection parameters for plant breeder to select the required traits (Panwar *et al.*, 2015). So far, information has been lacking in correlation of CWD resistance with other characters. Therefore, the study conducted to determine the association and path coefficient analysis (direct and indirect) of resistance with other wilt and seedling growth characters.

Materials and Methods

Coffee genotypes and experimental design

The study was conducted on two interrelated experiments at JARC (in greenhouse), Southwest Ethiopia. The first experiment consisted of 17 Arabica coffee genotypes with different CWD resistance reactions based on artificial inoculation test or naturally infested soils (Table 1). The genotypes obtained from Jimma and Gera Agricultural Research Centers.

Then, eight promising coffee parents (from experiment one), namely 75227 (P1), 971 (P2), 74110 (P3), 8136 (P4), 79233 (P5), Arbagugu (P6), 974 (P7), 370 (P8) and one susceptible check (Geisha) were selected based on the first experiment, yield and some other agronomic traits.

These parental lines were generated from different CWD reaction groups; parental lines 971 (P2) and 974 (P7) resistant; 79233 (P5), 370 (P8), 8136 (P4), 74144A (P6) moderately resistant; 75227 (P1) and 74110 (P3) susceptible parents (Table 1). Then, eight parents were crossed in 8 x 8 half diallel mating design using Griffing (1956) method 2 and model I at Gera, southwest of Ethiopia in 2014. The resulting 28 F1 crosses along with eight parents and one susceptible check were studied in experiment two from 2015 to 2016. The above symbols and designation of the parental lines are the same throughout this study. Both experiments were laid out in randomized complete block design (RCBD) with three replications at greenhouse using heat sterilized and moistened sandy soil on disinfected plastic pots (each has 5652 cm³ capacity). Five seedlings from 20 inoculated seedlings in each pot sampled and the growth

traits recorded for each genotype. The methods and procedures used for coffee seedling raising, inoculum preparation, seedlings inoculation, management, disease assessment and types of data collected were the same for both experiments based on Pieters and Van der Graaff (1980); (Girma and Mengistu, 2000). Selfing and crossing techniques were made based on Carvalho (1988) procedures.

Data recorded on a plot (pot) basis for wilted seedling Percentage and incubation period. In addition, numbers of yellow and defoliated leaves per seedling recorded per seedling and analyzed for average of five sampled seedlings at four months old seedling. On the other hand, seedling growth characters: stem height, stem diameter, average inter node length on stem based on Walyero (1983), number of stem nodes per seedling, number of leaves per seedling, leaf petiole length and leaf area were measured and analyzed for average of five sampled seedlings at four months old seedling stage.

Data analysis

Data was analyzed using SAS program version 9.2 (SAS, 2008). Fisher's least significant difference (LSD) mean separation test was performed to identify and comparison of genotypes means that were significantly different from each other. Wilted or dead seedling percentage calculated from cumulative number of wilted over total number of seedlings (wilted plus healthy) after six months of inoculation as follow;

$$\text{Wilted seedling Percentage} = \frac{\text{cumulative number of dead seedlings}}{\text{total number of seedlings (dead plus healthy)}} * 100$$

Estimates of correlation and path coefficient analysis

The associations between wilted seedling percentage and incubation period, number of yellow and defoliated leaves per plant and seedling growth characters were calculated as Singh and Chaudhary (1985):

$$r_p = \frac{pcovx.y}{\sqrt{\delta^2_{px} \cdot \delta^2_{py}}}$$

$$r_g = \frac{gcovxy}{\sqrt{\delta^2_{gx} \cdot \delta^2_{gy}}}$$

Where; r_p and r_g are phenotypic and genotypic correlation coefficients, respectively, $pcovx.y$ and

σ_{xy} are phenotypic and genotypic covariance between variables x and y , respectively, σ^2_{px} and σ^2_{gx} are phenotypic and genotypic variances for variable x , and σ^2_{py} and σ^2_{gy} are phenotypic and genotypic variances for the variable y , respectively. The significance of phenotypic and genotypic correlation coefficient was tested at 5 % and 1 % significance level by comparing the computed 'r' value to the tabular 'r' value at $n-2$ degree of freedom. Path coefficient analysis and residual effect were estimated for wilted seedling percentage and morphological traits following the method described by Dewey and Lu (1959).

$$r_{ij} = P_{ij} + \sum r_{ik}P_{kj}$$

$$\text{Residual effect (R)} = \frac{\sqrt{1 - R^2}}{\sqrt{1 - R^2}} \text{Where: } -R^2 = \sum p_{ij}.r_{ij}$$

Where, r_{ij} =Mutual association between the independent character (i) and dependent Character (j) as measured by genotypic correlation coefficient; P_{ij} =Component of direct effects of the independent character (i) on dependent character (j) as measured by genotypic path coefficient; $\sum r_{ik}P_{kj}$ =Summation of components of indirect effect of a given independent character (i) on the given dependent character (j) via all other independent character (k) and R = residual effect

Results and Discussion

Analysis of variance

In both experiments, analysis of variance (ANOVA) mean squares for four CWD parameters and seven seedling morphological characters are presented in Table 1. ANOVA mean squares showed that highly significant differences ($p < 0.01$) between 17 genotypes for all characters in experiment one. Genotypes also showed variable reaction to wilted seedling percentage in area under disease progress curve (AUDPC).

Moreover, ANOVA mean squares showed significant different ($p < 0.01$ or $p < 0.05$) between genotypes for wilted seedling percentage, incubation period, number of defoliated leaves, seedling height, seedling stem diameter (girth), average inter-node length, number of nodes, petiole length, leaf area and number of leaves when compared 28 F1 crosses with eight parents in experiment two. However, number of yellow leaves exhibited non-significant differences.

Correlation among characters

Phenotypic and genotypic correlation estimates between characters for experiment one is shown in Table 2. The genotypic correlation coefficient was higher than their corresponding phenotypic correlation in all characters, which indicated that the association was largely due to genetic factors. Wilted seedling percentage showed negative and significant phenotypic and genotypic association with incubation period ($r_p = -0.88$, $r_g = -0.93$), seedling height ($r_p = -0.73$, $r_g = -0.76$), average inter node length ($r_p = -0.50$, $r_g = -0.52$), stem diameter ($r_p = -0.072$, $r_g = -0.78$) and leaf area ($r_p = -0.54$, $r_g = -0.60$) in experiment one (Table 2). However, it showed positive and significant association with number of yellow leaves. In contrary, incubation period manifested significant, but reverses relationship with the above-mentioned characters. Both correlations were also detected positive and significant between seedling height with seedling diameter, average inter node length, petiole length and leaf area; while, negative and significant ($p < 0.01$) association with number of yellow leaves. Moreover, seedling diameter had positive and significant correlation with average inter node length, petiole length and leaf area; whereas, seedling diameter and leaf area showed negative and significant association with number of yellow leaves for both types of correlations. But, number of nodes and leaves had positive and significant relationship with number of defoliated leaves per seedling.

This significant correlation of traits may be caused by pleiotropism (multiple effect of a single gene) or linkage disequilibrium (different traits genes linked or closely connected; and during segregation and recombination they pass together to the next generation). In order to select CWD resistance (primary trait of interest) and other CWD and growth characters (secondary traits) simultaneously, it is clear that the effectiveness of indirect selection depends on the magnitude of genetic correlation and the heritability of the secondary traits being selected. Therefore, such a situation would occur when the secondary trait is less sensitive to environmental change (or can be measured under controlled conditions) and it is easier to measure. Generally, low wilted seedling percentage (CWD resistance) had a desirable association with incubation period, seedling height, stem diameter, leaf area and average inter node length; but it showed negative correlation with number of yellow leaves, defoliated leaves, number of leaves and number of nodes (undesirable association). Therefore, such

interrelationships of characters with wilted seedling percentage and between each other aid to identify CWD resistant and suggest to plan efficient selection.

The estimates of phenotypic and genotypic correlation between and among characters for experiment two are shown in Table 3. There were found highly significant ($p < 0.01$) and negative correlation between wilted seedling percentage and incubation period ($r_p = -0.86$, $r_g = -0.91$) and following components: leaf area ($r_p = -0.59$, $r_g = -0.64$) and stem diameter ($r_p = -0.34$, $r_g = -0.41$) in experiment two. While, it was positive and highly significant correlation with number of defoliated leaves ($r_p = +0.86$, $r_g = +1.00$). Therefore, these correlations indicated that wilted seedling percentage showed strong negative correlation with incubation period; while strong positive associations with number of defoliated leaves. Longer incubation period of CWD was positive and highly significance ($p < 0.01$) phenotypic and genotypic association with leaf area, but negative and highly significant correlation with number of defoliated leaves. Furthermore, incubation period showed positive and non-significant association with the rest quantitative characters, except seedling diameter and number of leaves for genotypic correlation (significant association). Therefore, low wilted seedling parentage (CWD resistance) could be improved by considering direct selection of longer incubation period, wide stem diameter and leaf area with minimum number of defoliated leaves and number of nodes. Selections for characters based on its positive and significant association are very useful for simultaneous improvement of the associated characters. On the other hand, characters manifesting negative association, simultaneous improvement of characters could be quite difficult and independent selection may have to be carried out to improve the characters (Sylva and Carvalho, 1997).

The current wilted seedling percentage or CWD resistance with other characters associations result supported by Toda *et al.*, (1986); Toda and Fujimoto (1987); Kuroda (2004); they studied in Japanese pines resistant to Pine wilt nematodes and suggested that height, basal diameter of the axis and the number of branches increment are associated with PWN resistance. According to Yamanobe (2009) on pine tree also a thicker pine basal diameter and more branches increase survival to PWN, considered as resistance factors.

Furthermore, a thicker basal diameter predicted to survive better and also trees can survive as long as there is a partial passage for xylem and phloem transport. Likewise, Siddiqui and Singh (2004) conducted an experiment to assess the effects of wilt fungus on the growth and transpiration of chickpea at glasshouse. Results showed that inoculation of *Fussarium oxysporum* reduced plant growth, transpiration and caused severe wilting. The existing quantitative characters correlation results are in agreement with Olika *et al.*, (2011) and Yonas *et al.*, (2014). Different scholars also confirmed the positive association between seedlings vigorous, such as height and stem diameter at the nursery with a better growth and yield performance in the field condition. Walyaro (1983) reported a positive correlation between three-year average yield and early record of seedling leaf area, seedling girth, height, number of leaves, number of laterals and longest lateral branch length. He also noted a positive relationship between height at the nursery and field. Likewise, Mesfin (1982) reported a desirable association between 21 months old seedling growth and three years old F1 plants girth, number of primary nodes and height. According to Fernie (1970), noted positive and highly significant correlation between stem girth and six years mean yield in F1 hybrids. Walyaro and Van der Vossen (1980) also reported highly significant genotypic correlation between three-year yield with height and girth in Arabica coffee. In most studies stem girth had a good association with yield. Therefore, the current good seedling vigorous and CWD resistant genotypes might also have good growth and yield production in the field.

Path coefficient analysis

Path coefficient analysis is an extension of correlation analysis and it has been used to evaluate selection criteria in several plants. This technique is useful in determining the direct influence of one variable on another (Lenka and Misra, 1973). The genotypic and phenotypic correlation coefficients were further divided into direct and indirect effects using path coefficient analysis.

Such analysis leads to the identification of important component traits useful in indirect selection of complex traits like CWD resistance. In this study, CWD resistance was considered as a complex dependable trait (resultant) while the rest of the variables that were negatively correlated with it were used as causal (independent) traits.

Table.1 Description of Arabica coffee genotypes used for the study

| No. | Coffee Genotypes | Origin | Released or collection year | Some characters description |
|-----|------------------|--------------------------|-----------------------------|--|
| 1 | 75227 | Gera, Jimmy | 1980/81 | Open growth habit, good yielder, CBD resistant, susceptible to CWD (DemelashandKifle., 2015) |
| 2 | 971 | Gelana Abaya, Borena | 2010 | Resistant to CWD (Chalaet <i>al.</i> , 2012) |
| 3 | 74110 | Metu, Illubabor | 1978/79 | Resistant to CBD, susceptible to CWD, good yielder, compact growth habit (DemelashandKifle., 2015) |
| 4 | 8136 | Gera, Jimmy | 2006 | High yielding potential, resistant to CBD & CLR, moderately resistant to CWD (Girma, 2004) |
| 5 | 79233 | International collection | 1979 | CWD resistant under natural infested soil (personal observation) |
| 6 | Arbagugu | Arsi, Oromia | 1978/79 | Moderately resistant to CWD under natural infested soil, susceptible to CBD (personal observation) |
| 7 | 974 | Gelana Abaya, Borena | 2010 | Resistant to CWD (Chalaet <i>al.</i> , 2012) |
| 8 | 370 | Seka-Chekorsa, Jimmy | – | Resistant to CWD, susceptible to CBD (Demelash, 2013) |
| 9 | Catimor J-19 | International collection | 1998 | Resistant to CWD (Girma, 2004) |
| 10 | Catimor J-21 | International collection | 1998 | Resistant to CWD (Girma, 2004) |
| 11 | 7440 | Washi, Kaffa | 1979/80 | Moderately resistant to CWD (Girma, 2004) |
| 12 | 279/71 | Sokoru, Jimmy | 2013 | CWD resistant, CBD susceptible (Demelash, 2013) |
| 13 | B-64/04 | Balle, Oromia | 2004 | CWD resistant (Kifleet <i>al.</i> , 2015) |
| 14 | B-70/04 | Balle, Oromia | 2004 | CWD resistant (Kifleet <i>al.</i> , 2015) |
| 15 | 74144B | Arsi, Oromia | 1978/79 | Moderately susceptible to CBD (personal observation) |
| 16 | Geisha* | International collection | 2002 | Highly susceptible to CWD (Demelash, 2013) |
| 17 | SN-5* | Kaffa, SNNPR | | Susceptible to CWD (Girma, 2004) |

*= susceptible check, Source: JARC / Coffee Breeding and Genetics division database for genotypes origin and some characters description; breeding method is pure line selection

Table.2 Analysis of variance mean squares and probability levels for CWD and growth characters

| Characters | Characters mean squares and p' | | | | | |
|--|--------------------------------|--------------------------|-------------------|------------------------------------|--------------------------------|---------------------------|
| | Experiment 1 | | | Experiment 2(F1 crosses & parents) | | |
| | Block (df=2) | Genotypes (df=16) | Error (df=32) | Block (df=2) | Genotypes (df=35) | Error (df=70) |
| Disease parameters | | | | | | |
| Wilted coffee seedlings Percentage | 1000.98** (666.29**) | 2161.31** (1179.73**) | 103.11 (58.35) | 1065.19** (801.08**) | 1743.23** (823.29**) | 204.48 (118.02) |
| Incubation period | 456.37 ^{ns} | 1290.27** | 157.77 | 397.34** | 610.80** | 52.69 |
| Number of Defoliated leaves per seedling | 0.01 ^{ns} | 0.24** | 0.03 | 8.22** | 1.73** | 0.66 |
| Number of yellow leaves per seedling | 0.04 ns | 0.26** | 0.05 | 1.58** | 0.14ns | 0.10 |
| Morphological characters | | | | | | |
| Seedling height (cm) | 0.89 ^{ns} | 6.31** | 0.40 | 9.97** | 2.62** | 0.44 |
| Seedling stem diameter (mm) | 0.024* | 0.039** | 0.005 | 0.01 ns | 0.022* | 0.013 |
| Number of nodes | 0.04 ^{ns} | 0.27** | 0.01 | 0.03* | 0.17** | 0.009 |
| Average internode length (cm) | 0.42* | 1.83** | 0.10 | 0.18 ns | 1.16** | 0.16 |
| Number of leaves | 0.54* | 0.77** | 0.13 | 2.85** | 0.57** | 0.18 |
| Leaf area (cm ²) | 1.06 ^{ns} | 9.49** | 0.97 | 24.11** | 18.02** | 1.80 |
| Petiole length (cm) | 0.002* | 0.005** | 0.0005 | 0.0065** | 0.0032** | 0.0004 |
| AUDPC | 5110325.2 | 16205358.7** | 784550.8 | – | – | – |

Data in bracket is arcsine transformed value of wilted seedlings percentage

df= degree of freedom of block, genotypes and error, ** = highly significant at P <0.01, * = significant at p<0.05 and ns =non-significant

Table.3 Phenotypic (r_p) (above diagonal) and genotypic (r_g) (below diagonal) correlation of 11 characters for experiment 1

| | WS (%) | IP (days) | SH (cm) | SSD (mm) | AINL (cm) | NN (no.) | PL (cm) | LA (cm ²) | NL (no.) | NDL (no.) | NYL (no.) |
|-----------------------|-----------|--------------|------------|-------------|--------------|-------------|------------|--------------------------|-------------|--------------|--------------|
| WS (%) | | -0.88** | -0.73** | -0.72** | -0.50* | 0.23 | -0.17 | -0.54* | 0.17 | 0.40 | 0.62** |
| IP (days) | -0.93** | | 0.75** | 0.57* | 0.55* | -0.26 | 0.11 | 0.53* | -0.18 | -0.43 | -0.76** |
| SH (cm) | -0.76** | 0.81** | | 0.81** | 0.75** | -0.27 | 0.59* | 0.73** | -0.25 | -0.19 | -0.68** |
| SSD (mm) | -0.78** | 0.64** | 0.82** | | 0.51* | 0.05 | 0.48* | 0.66** | -0.02 | 0.03 | -0.50* |
| AINL (cm) | -0.52* | 0.63** | 0.80** | 0.53* | | -0.66** | 0.54* | 0.81** | -0.71** | -0.48 | -0.63** |
| NN (no.) | 0.24 | -0.30 | -0.30 | 0.05 | -0.68** | | -0.15 | -0.38 | 0.93** | 0.62** | 0.31 |
| PL (cm) | -0.21 | 0.12 | 0.61** | 0.48* | 0.58* | -0.18 | | 0.71** | -0.21 | 0.24 | -0.20 |
| LA (cm ²) | -0.60* | 0.60* | 0.74** | 0.69** | 0.87** | -0.42 | 0.74** | | -0.47 | -0.32 | -0.53* |
| NL (no.) | 0.23 | -0.27 | -0.35 | -0.09 | -0.76** | 1.00** | -0.28 | -0.62** | | 0.63** | 0.33 |
| NDL (no.) | 0.41 | -0.46 | -0.20 | 0.07 | -0.50* | 0.66** | 0.26 | -0.36 | 0.75** | | 0.34 |
| NYL (no.) | 0.68** | -0.85** | -0.74** | -0.54* | -0.70** | 0.40 | -0.23 | -0.56* | 0.48 | 0.41 | |

cm= cent meter, cm²= cent meter square, mm= mill meter

AINL= average inter node length, IP = incubation period, LA =leaf area,NDL = number of defoliated leaves, NL= number of leaves, NN = number of nodes NYL =number of yellow leaves, PL= petiole length, SH = seedling height, SSD = seedling stem diameter, WS = wilted seedling percentage

Table.4 Phenotypic (rp) (above diagonal) and genotypic (rg) (below diagonal) correlation among characters for experiment two

| | WS (%) | IP (days) | SH (cm) | SSD (mm) | AINL (cm) | NN (no.) | PL (cm) | LA (cm ²) | NL (no.) | NDL (no.) | NYL (no.) |
|-----------------------|--------|-----------|---------|----------|-----------|----------|---------|-----------------------|----------|-----------|-----------|
| WS (%) | | - | -0.18 | -0.34* | -0.22 | 0.08 | -0.22 | -0.59* | -0.10 | 0.86** | -0.17 |
| IP (days) | 0.91** | | 0.17 | 0.29 | 0.19 | 0.02 | 0.16 | 0.68** | 0.28 | - | 0.15 |
| SH (cm) | -0.17 | 0.20 | | 0.44** | 0.89** | -0.41* | 0.58** | 0.60** | -0.08 | -0.33* | -0.05 |
| SG (mm) | 0.41** | 0.44** | 0.52** | | 0.36* | 0.10 | 0.26 | 0.51** | 0.30 | -0.28 | 0.23 |
| AINL (cm) | -0.25 | 0.23 | 0.97** | 0.57** | | - | 0.55** | 0.65** | -0.24 | -0.34* | -0.03 |
| NN (no.) | 0.08 | 0.01 | - | 0.18 | - | | -0.13 | -0.18 | 0.68** | 0.23 | 0.02 |
| PL (cm) | -0.23 | 0.18 | 0.44** | 0.27 | 0.64** | -0.15 | | 0.46** | 0.25 | -0.33* | 0.39* |
| LA (cm ²) | 0.64** | 0.75** | 0.66** | 0.69** | 0.72** | -0.17 | 0.48** | | 0.20 | - | 0.16 |
| NL (no.) | -0.09 | 0.34* | -0.24 | 0.25 | -0.29 | 0.83** | 0.23 | 0.19 | | 0.002 | 0.28 |
| NDL (no.) | 1.00** | - | -0.31 | -0.02 | - | 0.27 | -0.35* | - | 0.13 | | -0.06 |
| NYL (no.) | -0.37* | 0.96** | -0.03 | 0.55** | -0.02 | 0.004 | 0.76** | 0.58** | 0.57** | 0.01 | |

Note: Values without asterisk (*) are non-significant; cm= centimeter, cm² = centimeter square, mm=millimeter
 AINL= average inter node length, IP = incubation period, LA= leaf area, NL= number of leaves, NDL= number of defoliated leaves per seedling, NN= number of nodes, PL= petiole length, SH= seedling height, SSD= seedling stem diameter, WS%= Wilted coffee seedling percentage

Table.5 Direct (diagonal) and indirect effects of path coefficient analysis for wilted seedling percentage by different traits for experiment 1

| | IP | SH | SSD | AINL | NN | PL | LA | NL | NDL | NYL | Total genotypic correlation with WS% |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------------------------------------|
| IP | -0.71 | -0.78 | 0.07 | 0.74 | -0.01 | 0.07 | -0.53 | -0.15 | 0.15 | 0.22 | -0.93 |
| SH | -0.58 | -0.96 | 0.09 | 0.94 | -0.01 | 0.34 | -0.66 | -0.19 | 0.06 | 0.19 | -0.76 |
| SSD | -0.45 | -0.79 | 0.11 | 0.63 | 0.00 | 0.27 | -0.61 | -0.05 | -0.02 | 0.14 | -0.78 |
| AINL | -0.45 | -0.77 | 0.06 | 1.18 | -0.02 | 0.32 | -0.77 | -0.41 | 0.16 | 0.18 | -0.51 |
| NN | 0.21 | 0.29 | 0.01 | -0.80 | 0.03 | -0.10 | 0.37 | 0.54 | -0.21 | -0.10 | 0.24 |
| PL | -0.09 | -0.59 | 0.05 | 0.68 | -0.01 | 0.56 | -0.66 | -0.15 | -0.08 | 0.06 | -0.21 |
| LA | -0.43 | -0.71 | 0.08 | 1.03 | -0.01 | 0.41 | -0.89 | -0.33 | 0.12 | 0.15 | -0.60 |
| NL | 0.19 | 0.34 | -0.01 | -0.90 | 0.03 | -0.16 | 0.55 | 0.54 | -0.24 | -0.12 | 0.22 |
| NDL | 0.33 | 0.19 | 0.01 | -0.59 | 0.02 | 0.15 | 0.32 | 0.41 | -0.32 | -0.11 | 0.41 |
| NYL | 0.60 | 0.71 | -0.06 | -0.83 | 0.01 | -0.13 | 0.50 | 0.26 | -0.13 | -0.26 | 0.67 |

Residual effect = 0.246374

AINL= average inter node length, IP = incubation period, LA= leaf area, NDL= number of defoliated leaves per seedling, NL= number of leaves, NN= number of nodes, NYL= number of yellow leaves, PL= petiole length, SH= seedling height, SSD= seedling stem diameter, WS%= Wilted coffee seedling percentage

Table.6 Direct (diagonal) and indirect effects of path coefficient analysis for wilted seedling percentage by different traits for experiment 2

| | IP | SH | SSD | AINL | NN | PL | LA | NL | NDL | NYL | Total genotypic correlation with WS% |
|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------------------------------|
| IP | -0.69 | 0.09 | -0.15 | 0.07 | 0.00 | -0.10 | -0.17 | 0.32 | -0.27 | -0.01 | -0.90 |
| SH | -0.14 | 0.43 | -0.18 | 0.30 | 0.20 | -0.32 | -0.15 | -0.23 | -0.09 | 0.00 | -0.17 |
| SSD | -0.30 | 0.22 | -0.34 | 0.18 | -0.08 | -0.14 | -0.15 | 0.24 | -0.01 | -0.02 | -0.41 |
| AINL | -0.16 | 0.42 | -0.19 | 0.31 | 0.27 | -0.34 | -0.16 | -0.27 | -0.12 | 0.00 | -0.24 |
| NN | -0.01 | -0.19 | -0.06 | -0.19 | -0.45 | 0.08 | 0.04 | 0.78 | 0.08 | 0.00 | 0.08 |
| PL | -0.12 | 0.26 | -0.09 | 0.20 | 0.07 | -0.53 | -0.11 | 0.22 | -0.10 | -0.02 | -0.23 |
| LA | -0.52 | 0.28 | -0.23 | 0.22 | 0.08 | -0.25 | -0.22 | 0.18 | -0.16 | -0.01 | -0.64 |
| NL | -0.23 | -0.10 | -0.09 | -0.09 | -0.37 | -0.12 | -0.04 | 0.94 | 0.04 | -0.02 | -0.09 |
| NDL | 0.66 | -0.13 | 0.01 | -0.13 | -0.12 | 0.19 | 0.13 | 0.12 | 0.28 | 0.00 | 1.00 |
| NYL | -0.21 | -0.01 | -0.19 | -0.01 | 0.00 | -0.40 | -0.06 | 0.54 | 0.00 | -0.03 | -0.37 |

Residual effect = 0.214709

AINL= average inter node length, IP = incubation period, LA= leaf area, NDL= number of defoliated leaves per seedling, NL= number of leaves, NN= number of nodes, NYL= number of yellow leaves, PL= petiole length, SH= seedling height, SSD= seedling stem diameter, WS%= Wilted coffee seedling percentage

The path analysis was done among 10genotypic correlated traits with wilted seedling percentage. Direct and indirect effects of these traits determined on resistance and their contribution ratios are summarized in table 4 and 5. Incubation period (-0.71), seedling height (-0.96) and leaf area (-0.89) in experiment one, and Incubation period (-0.69), petiole length (-0.53), number of nodes (-0.45), and seedling stem diameter (-0.34) in experiment two showed the highest negative direct effect on resistance genotypes selection. Average internode length and number of leaves showed positive direct effect on resistance at both experiments; which is insignificant traits as a selection criterion.

Alternatively, seedling height and number of defoliated leaves showed negative direct effect in experiment one, but positive direct effect in experiment two. However, petiole length exhibited positive and negative direct effect, but closer in magnitude in experiment one and two, respectively. Generally, petiole length, number of leaves, average internodes length, seedling height number of yellow and defoliated leaves were not the stronger traits used as a selection criterion for CWD resistance variety development.

These traits varied in the two experiments and it may be due to the presence of genotypic variabilities in these two experiments. Overall, incubation period and leaf areas were the best selection criterion with considering other traits in resistance genotypes selection. The indirect effect of average internodes length found mainly through

leaf area, and seedling height through incubation period and stem diameter.

In this study, the genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients for all traits. These results indicated that the traits are predominantly governed by genetics factor rather than environmental factors.

In both experiments, CWD resistant genotypes were significantly associated with extended incubation period, wide stem diameter, extensive leaf area and minimum number of defoliated leaves than the susceptible genotypes.

As a result, CWD resistance could be improved by indirect selection of these correlated characters. Traits such as, Incubation period and leaf area observed negative direct effects on CWD resistance. Therefore, incubation period and leaf areas traits were the best selection criterion with considering other traits in resistance genotypes selection. The indirect effect of average internodes length found mainly through leaf area, and seedling height through incubation period and stem diameter.

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Conflict of Interest

The author has declared that there is no conflict of interest.

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