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Mode of Inheritance and Combining ability studies on Epicuticular wax Production in Resistance to Black pod disease in Cacao (*Theobroma cacao* L.)

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ABSTRACT

Black pod caused by Phytophthora species is a devastating disease of cacao (*Theobroma cacao* L.) in production regions worldwide. Breeding for cacao genotypes resistant to black pod disease is crucial for sustainable cocoa production and profitability. Although breeding programmes in the past have made considerable efforts in improving resistance of cocoa genotypes with diverse genetic background, the disease continues to cause unacceptable yield losses in cacao production. To understand the mode of inheritance of epicuticular wax on the surface of cocoa pod known to play a major role in resistance to black pod disease, a 6 × 6 full diallel and a M x N (6 × 4) mating designs were used and data was analysed using Griffing's method I model I approach and the North Carolina design –II approach, respectively. The results of the two mating designs were consistent and showed that epicuticular wax on leaf and pod surfaces of cacao were under the control of additive genetic effects. The non-additive effects were generally much smaller compared to the additive genetic effects. The significant association between GCA estimates of parents and their means indicates that parental values could be used as indicators of progeny performance. The best general combiners for epicuticular wax load on leaf and pod surfaces were Pa7/808, T60/887 and Pa 150. The larger additive components and heritability observed in this study, indicates that pedigree selection could be used to develop cacao cultivars with high amount of epicuticular wax on pod surface to enhance resistance to black pod disease.

1. Introduction

The significance of black pod disease of cacao (*Theobroma cacao* L.) caused by *Phytophthora palmivora* and *Phytophthora megakarya* in the humid tropical regions of the world such as South America, West Africa and Southeast Asia is well documented (Opoku et al., 2000; Adomako, 2007; Dakwa, 1987; McMahon and Purwantara, 2004; Rosmana et al., 2006). The disease is more prevalent in humid environments and its severity and incidence are highly correlated with the amount of rainfall received (Efombagn et al., 2004; Lockwood, 1971; Dakwa, 1974). From 1985, black pod turned into a major cocoa disease in Ghana, which was attributed to the emergence of *P. megakarya* as a pathogen of cacao (Dakwa, 1987). *P. megakarya* has become a major threat for cocoa production in affected areas (Opoku et al., 2000) and has spread to major cocoa production regions in Ghana (Akrofi et al., 2015). Depending on the cocoa variety grown (Bowers et al., 2001; Nyassé et al., 2007), conditions of high rainfall and when no control is done, *P. megakarya* causes yield losses as high as 80% in Cameroon (Deberdt et al., 2008) and 60–100% in Ghana (Dakwa, 1987). In 2012, Ghana lost over 25% (212,500 M T) of its annual output of 850,000 M T of cacao beans to black pod disease, representing a revenue loss of about GH7.5 million (COCOBOD, 2014).

Several control measures have been employed against black pod disease. The most effective measure is the use of fungicides, however considering the high cost of fungicides and the fact that cacao is mostly grown by resource poor farmers in marginal areas, the most economic, practical and appropriate means to control black pod disease is resistance breeding (Adomako, 2007; Nyadanu et al., 2009; Nyassé et al., 1995; 2002; Tahi et al., 2000, 2006a, 2006b). Host plant resistance to black pod disease in cacao has been described as polygenic and additively inherited (Tan and Tan, 1990; Adomako, 2006; Nyadanu et al., 2012a; Iwaro et al., 1999; Blaha and Latodé, 1997). Heritability of resistance to black pod disease is reported to be low and much influenced by environment (Nyadanu et al., 2012a; Nyassé et al., 2002, Tahi2006a, 2006b). Host factors of resistance to a disease are of significant importance as they could be utilized for strengthening resistance (Wink,

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1988). This is of more relevance for a disease like black pod where immunity is not known and breeders struggle to enhance resistance beyond a certain level. The poor understanding of host factors of resistance to black pod disease and their genetics was considered by Bartley (1986) and Kennedy et al. (1987) as the reason for lack of progress in breeding for black pod disease resistance in cacao.

Morpho-physiological, histological and biochemical factors of resistance could be combined to provide a solid base for selection of durable resistance to black pod disease (Nyadanu, 2011; Omokolo et al., 2002). Selection for cultivars that possess morphological traits that reduces the severity of black pod disease would have tremendous effect on cacao productivity in the humid tropics. The presence of epicuticular wax on the leaf and pod surfaces of cacao has been considered to be involved in host resistance to Phytophthora species (Iwaro et al., 1997; Sena Gomes and Machado, 1994; Sena-Gomes et al., 1995; Nyadanu et al., 2012b). Smith et al. (2006) also reported substantial evidence of epicuticular wax playing a major role in blister rust resistance in *Pinus strobus*. Wax occlusions were regarded as the likely reason for fewer infections on the resistant genotype. The accumulation of moisture on the pod and leaf surfaces is a condition required for the establishment and germination of the spores (Stover, 1980; Wadia and Butler,1994). Waxy pod and leaf surfaces accumulate lesser moisture than non-waxy pod and leaf surfaces in cacao (Nyadanu et al., 2012b). The hydrophobic waxes on leaf surfaces form a water repellant surface and thereby facilitate evaporation of films of water on pod and leaf surfaces, in which pathogens may multiply and enter the leaf or pod interior using their own mobility (Cooper et al., 2001). The chemical makeup of epicuticular wax is also known to affect resistance to fungus and insects (Kolattukudy, 1985; Jenks and Ashworth, 1999). Therefore, pod and leaf waxiness could be regarded as an evasion resistance mechanism which protect plants against infection by pathogens (Ortiz et al., 1995).

Differences in total wax amount within populations of plant species like *Oryza sativa* L. (rice), *Musa spp*. (banana and plantain) and *Leymus angustus* (Trin) Pilger Dewey (Altai wilddrye) showed clear polygenic inheritance (Haque et al., 1992; Ortiz et al., 1995; Jefferson et al., 1988). These species also responded to genetic selection for increased total wax quantity. However, the mode of inheritance, heritability and combining ability of amount of epicuticular wax on leaf and pod of cacao has not been reported. These genetic parameters are important in order to develop an efficient breeding strategy and more effectively guide the selection of improved cacao genotypes with increased amount of wax and resistance to black pod disease. Knowledge of the breeding value (general and specific combining ability) of the parental forms used in crossbreeding programmes as well as the fundamental knowledge on the genetic determinants of the intensity of quantitative traits at the population level (Masny et al., 2016) accelerates and increases the likelihood of achieving the intended purpose.

The objective of this study was to determine the inheritance, combining ability and heritability of amount of epicuticular wax on leaf and pod of cacao.

2. Materials and methods

The experimental designs of the field trials used for the study were a 6 × 6 complete diallel and 6 × 4 factorial mating designs (without the selfs). The parents involved were selected on the basis of their yield records and their variable levels of resistance to black pod and cocoa swollen shoot virus diseases. These were (PA7/808, NA33, T60/887, T63/971, IMC 76, PA 150, SCA 9, IMC 67, IMC 53 and SCA 6). PA7/808, NA33, T60/887, T63/971, IMC 76 and PA 150 were used as male parents and SCA 9, IMC 67, IMC 53 and SCA 6 as female parents in a 6 × 4 mating design. PA7/808, NA33, T60/887, T63/971, IMC 76 and PA 150 were used as parents in a 6 × 6 full diallel mating design. T85/799 and T79/501 were used as parents of the control progeny, T85/799 x T79/501. The cocoa trees were planted in a Randomized Complete Block Design (RCBD), with 10 trees per plot (family) and four blocks. The cocoa trees were spaced at 2.5 m x 2.5 m on 3.5 acres land called N 19 plot. The crop was grown under shade trees with uniform and optimal cover (approximately 50% of the solar radiation passed through the canopy). No fertilizers were applied. Regular pruning was performed on the exceeding plagiotropic and orthotropic branches, along with the removal of parasitic epiphytes and chupons. The trial was established at the Cocoa Research Institute of Ghana (CRIG) station (Ghana–Akim Tafo) in year 2000.

2.1. Assessment of epicuticular wax load on pod and leaf surfaces

Matured pods and leaves of uniform ages were used. This was determined by tagging flower buds and leaf flushes of the progenies. The harvested pods and leaves were placed in airtight plastic bag and transported to the laboratory. The pods were covered with cotton wool to avoid bruises on their surfaces.

Surface wax was extracted by dipping the distal end of pods up to the equator into chloroform for 30S. The extract was transferred into a clean, dry and pre-weighed flask (W1) (Galeano et al., 1986). The chloroform was evaporated using Rotary evaporator (Ratavapor BÜCHI, EL 131, made in Switzerland) set at 75 °C. The flask with the sample was placed in desiccators in a cool dry place for 24 h and allowed to equilibrate overnight at room temperature. The flask with the sample was weighed (W2) on an electronic balance. The extracted part of the pod (which turns brown) was cut into four pieces and after removing the inner tissue, their outlines were traced on brown paper. This was used to determine the pod area (A)/cm^2 using a leaf area meter. Three pods were assessed per genotype per replication. Wax was extracted from both abaxial and adaxial surfaces of leaf by washing each surface at a time with chloroform. The area of the leaf was also determined using leaf area meter. Five leaves were assessed per genotype per replication.

The weight of the wax load on pod/leaf surface was calculated using the formula:

$$Wax\ load\ \mu g/cm2 = \frac{W2-W1}{A} \times 1000000$$

Where W2 = Weight of sample + flask, W1 = Weight of empty flask, A = Surface area of pod or leaf.

2.2. Statistical analysis

Analysis of variance was carried out to determine the significance of genotypic differences for the 6 × 6 full diallel experiment and the significance of male parent and female parent effects and interaction effects for the 6 × 4 mating experiment using Diallel SAS05.

Genetic analysis of data from the 6 × 6 diallel experiment proceeded using Griffing's (1956) Method I Model I approach (Singh and Chaudhary, 1996; Kearsey and Pooni, 1996), while that for the 6 × 4 factorial experiment was analyzed using the North Carolina Design II approach (Hallauer and Miranda, 1981; Dablokar, 1999).

General combining ability (GCA) and specific combining ability (SCA) effects were calculated using Griffing's approach for the diallel experiment (Griffing, 1956).

The additive (σ^2A), non-additive (σ^2 NA), and environmental (σ^2SE) variance were computed using mean squares for GCA (MSg), SCA (MSs), and error (MS*E*) extracted from the analysis of variance table and outlined as follows:

$\sigma^2A = (MSG - MSs)/(P + 2)$; $\sigma^2NA = MSs - MSE$; $\sigma^2E = MSE$

P: number of parents.

Broad-sense heritability (h^2b) and narrow-sense heritability (h^2n) estimates were computed as below.

$h^2b = (\sigma^2A + \sigma^2NA) / (\sigma^2A + \sigma^2NA + \sigma^2E)$