Crop Protection 51 (2013) 24–31

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

An evaluation of gains in breeding for resistance to the cocoa swollen shoot virus disease in Ghana

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ARTICLE INFO

Article history:
Received 21 November 2012
Received in revised form
24 March 2013
Accepted 22 April 2013

Keywords:
Cocoa
Cocoa swollen shoot virus
CSSV
Mealybug
West Africa

ABSTRACT

The present study examines the gains in resistance to cocoa swollen shoot virus (CSSV) infection from investments in breeding over the past seven decades. The general susceptibility to CSSV infection of the West African Amelonado that dominated plantings prior to the start of formal research in 1938 necessitated the introduction of germplasm of Upper Amazon origin to better contain the disease spread. Included in this study are findings of two recent experiments. In the first, the genetic basis for resistance in the clone mvT85, developed from gamma radiation of clone T85/799 and putatively resistant to CSSV disease was investigated. In the second experiment, the comparative levels of resistance in sets of old, current and new cocoa varieties were tested following inoculations with the severe CSSV strain 1A. Absence of nucleotide differences at 29 single nucleotide polymorphism (SNP) loci between mvT85 and T85/799, and lack of segregation for resistance in the full-sib and backcross populations derived from mvT85 indicated that mvT85 did not carry novel genes for improving cocoa for CSSV disease resistance. Moreover, there were no differences in resistance to CSSV disease between mvT85 and T85/799. These observations conflict with the previous report that mvT85 is immune to CSSV disease, and distinct from T85/799. Between variety groups, disease severity scores based on three successive leaf flushes after inoculation were not effective in discriminating among them. Disease severity assessed eight months after inoculation was the most important criterion for separating varieties for resistance to CSSV disease. As expected, the older varieties were the most sensitive to infection. No differences were found between current varieties derived exclusively from Upper Amazon clones and new varieties. Contrary to the generally held opinion of a higher level of resistance in existing inter Upper Amazon cultivars, varieties derived from crosses using Catongo, RB 49 and C-SUL 7 (all of Lower Amazon origin) as males with specific Upper Amazon varieties were among the most resistant. A re-appraisal of variety recommendations for areas of mass infection and for less affected areas is advocated.

1. Introduction

Cocoa (*Theobroma cacao* L.) was domesticated in Southern Mexico and the North Central American region (Cuatrecasas, 1964; Hunter, 1990; Motamayor et al., 2002). The centre of genetic diversity is hypothesized to be located in the Upper Amazonian region (Cheesman, 1944). Two morphogeographic groups are recognized within *T. cacao* namely Criollo and Forastero (Cheesman, 1944). The Criollo group, characterized by white or rosy beans that produce the finest chocolate, represents the first domesticated cocoa and was originally cultivated in Central America (Soria, 1970). The Forastero group is recognized to have higher vigour and is further subdivided into Lower and Upper Amazon Forastero with the Upper Amazon group more genetically diverse and exhibiting superior agronomic performance (Cheesman, 1944). Lower Amazonian cocoa now constitute the most prevalent cultivated type worldwide, grown notably in West Africa and Brazil. A third group, Trinitario, is described either as an intermediate type between Criollo and Forastero (Lockwood and Gyamfi, 1979) or a group of hybrids that display characteristics that include the total range of variation (Cheesman, 1944). More recently though, Motamayor et al. (2008) proposed 10 genetic clusters, as opposed to the two genetic groups traditionally recognized within *T. cacao*. These include the Marañon, Guiana, Contanama, Curaray, Nanay, Iquitos, Nacional, Purus, Criollo and Amelonado.

In West Africa where approximately 70% of the world's cocoa output is obtained, the cocoa swollen shoot virus (CSSV) disease is one of the most important limitations to cocoa production. The

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http://dx.doi.org/10.1016/j.cropro.2013.04.007

disease is transmitted by at least 20 mealybug species (Strickland, 1951; Leston, 1970). Differences in the preferences of mealybug vectors for feeding sites on the cocoa host plant, mobility, mutualistic associations with ant species and overhead shade intensity have acted together to perpetuate the disease. Several strains of CSSV have been characterized based on symptom severity and their effects on host tree vigour and yield loss (Crowdy and Posnette, 1947; Thresh et al., 1988). Following infection, the characteristic symptoms of leaf chlorosis and stem swellings (Thresh and Tinsley, 1959) are often followed by dieback of branches and death of plants (Posnette, 1951; Crowdy and Posnette, 1947).

Originated from the Lower Amazon basin of Brazil, the genetically uniform and less vigorous Amelonado variety that formed the basis of the successful West African cocoa industry was found to be highly susceptible to the CSSV disease (Crowdy and Posnette, 1947). Though the variety initially established well under the heavy shade of the then available primary forests, the gradual loss of the complex agroforest associated with the cocoa ecosystem later constrained the use of Amelonado and encouraged introduction of more vigorous Upper Amazon germplasm (Posnette, 1951). Incidentally, these varieties also possessed higher tolerance to CSSV disease, and experienced slower deterioration and lower loss of yield following infection (Brunt, 1975). The anticipated reduction in the rate of spread of the disease several years after these Upper Amazon varieties were recommended for large-scale plantings was, however, not realized (Thresh and Owusu, 1986; Ollennu et al., 1989; Domfeh et al., 2011).

Various control measures have been tested and recommended since Posnette (1940) showed that the swollen shoot and dieback of cocoa trees reported in Ghana in 1936 were due to infection by a virus. Whiles it is generally agreed that removing visibly infected trees and trees in contact with these is the most practical means of control, and have been practiced since 1944 (Thresh and Owusu, 1986), the disease is more prevalent now than ever before (Domfeh et al., 2011). Discontinuities in the eradication program (Ollennu et al., 1989), farmers opposition to removal of infected trees (Beeton, 1948), and the latent and missed infections particularly on farms planted with tolerant varieties (Thresh and Owusu, 1986) have accounted for inefficiencies in the cutting-out program. Progenies of some Upper Amazon clones were considered to combine low preference by mealybugs, resistance to CSSV infection, and longer latent periods than progenies of Amelonado and Trinitario clones (Posnette and Todd, 1951; Bigger, 1975; Firempong, 1984). The rate of spread of CSSV disease was therefore expected to decline with large-scale plantings of varieties derived from these clones. In large-scale field observations, however, evidence for the positive impact of these varieties in reducing rate of spread of the disease is lacking (Ollennu et al., 1989). Moreover, Legg et al. (1984) found the overall incidence of latent and missed infections in fields planted to resistant Upper Amazon varieties to be higher than those planted with the susceptible local Amelonado.

In attempts to develop varieties with higher levels of resistance to CSSV disease, Adu-Ampomah et al. (1996) used gama rays to induce mutants in the Upper Amazon clone T85/799. Mutant clones (hereafter referred to as mvT85) that remained symptomless after inoculation with high concentration of the severe strain 1A of CSSV were obtained. The genetic basis of this resistance however, was not determined. The objective of this study is to determine the gains in resistance to CSSV disease obtained from various breeding efforts at developing resistant varieties. To meet this objective, the genetic basis of resistance in the mutant clone mvT85 was determined. Secondly, the study examined the relative resistance to CSSV infection of recommended varieties that dominated plantings in Ghana and much of West Africa across different timescales.

2. Materials and methods

Two sets of experiments were conducted. The clones used to generate the progenies in the recent study are described by source or genotype in Table 1. At the outset, all clones used in the experiments were genotyped at 29 highly single nucleotide polymorphism (SNP) loci to ensure that selected clones were true-to-type. These 29 SNP markers are a subset of 70 used by Kun et al. (2013) to study genetic diversity among farmers' cocoa varieties in Honduras and Nicaragua. In that study, this subset of SNP markers provided more than 99.9% confidence in identifying an individual cocoa tree.

The first set of experiments had the objective of determining the genetic basis of the resistance to CSSV disease in mvT85. In the second experiment, the relative resistance to CSSV disease among thirty-five varieties that represents the main varieties planted over different time periods in Ghana and much of West Africa was assessed.

2.1. Determination of the genetic basis of CSSV resistance in the clones mvT85 and mvP30

To confirm the level of resistance of mvT85 previously reported as immune to CSSV disease, 20 plants each of clones mvT85 and T85/799 (the progenitor of mvT85) were tested for their resistance to CSSV infection. At the start of the present study, all plants of mvT85 available were previously inoculated with the virus (Adu-Ampomah et al., 1996). To obtain plants of mvT85 that are initially free of the virus so as to inoculate and undertake the test for resistance, we used somatic embryogenesis of floral explants of mvT85 following the procedure of Li et al. (1998). Scion-wood of these plants were grafted to generate the twenty individuals used

Table 1
Description of clones used by source or parentage[a].

Clone	Parentage or source
A1/154	A clone of Upper Amazon origin, developed by CRIG for resistance to *Phytophthora* spp. Exact pedigree unknown
A1/213	A clone of Upper Amazon origin, developed by CRIG for resistance to *Phytophthora* spp. Exact pedigree unknown
ACU 85	Local Trinitario
AMAZ 3-2	A clone selection made around river Iquitos, Upper Amazon basin
Catongo	A clone selection from Bahia, Brazil
C-SUL 7	A clone selection in the vicinity of Cruzeiro do Sul, Brazil
Mocorongo	Clone of Lower Amazon (Brazil) origin, but unknown pedigree.
mvT85	Clone derived from mutants following irradiation of the clone T85/799 and described as resistant to CSSV (Adu-Ampomah et al., 1996)
mvP30	Clone derived from mutants following irradiation of the clone P 30 and described as resistant to CSSV (Adu-Ampomah et al., 1996)
P 30	Local Amelonado selection
P 4	Local Amelonado selection
PA 121	A clone selections made by Pound at Parinari
PA 150	A clone selections made by Pound at Parinari
PA 7	A clone selections made by Pound at Parinari
Pound 10	Material selected in the headwaters of the Amazon by Pound
Pound 7	Material selected in the headwaters of the Amazon by Pound
RB 49	A clone selection made in the vicinity of River Branco, Brazil
S84	Local Amelonado selection
T16/613	Clone selection derived from open pollinated tree of IMC 24
T60/887	Clone selection derived from PA 7 × NA 32
T63/967	Clone selection derived from PA 35 × NA 32
T63/971	Clone selection derived from PA 35 × NA 32
T65/238	Clone selection derived from PA 7 × IMC 35
T65/326	Clone selection derived from PA 7 × IMC 35
T79/501	Clone selection derived from NA 32 × PA 7
T85/799	Clone selection derived from IMC 60 × NA 34

[a] Full descriptions given in Lockwood and Gyamfi (1979).