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Cocoa plant diseases and importance of *Bacilus subtilis* to induce resistance on crops in agriculture-A review

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ABSTRACT

Fungal and viral infections represent a major cause of cocoa disease in agriculture. Plant protection through biological control is an alternative strategy in agriculture to control pests. Recognition of certain non-pathogenic rhizobacteria by plants can trigger a systemic resistance reaction that renders the host less susceptible to subsequent infection by a virulent agent. The impact of cocoa diseases as black pod, Cocoa Shollen Shoot Virus (CSSV), witches' broom, and frosty pod rot, has increased dramatically in the world.This paper review emphasizes cocoa disease and utilisation of *Bacillus subtilis* as biocontrol agent against fungal and virus from cocoa disease in Côte d'Ivoire.

Key words: Agriculture, *Bacillus subtilis*, Black pod, Cocoa disease, Côte d'Ivoire, CSSV, Systemic resistance.

Cocoa is a very important ingredient in several kinds of foods, such as cakes, biscuits, child-foods, ice-creams and sweet. Cocoa beans are seed from the fruit of *Theobroma cacao* tree. Cocoa beans is an important source of cocoa powder and of food worldwide and constitutes an inexpensive fat source from Africa, Central and South America. The economy of some countries rests largely on this commodity. Cote d'Ivoire is the world's leading exporter of cocoa beans. In Cote d'Ivoire, cocoa beans represents 10% of the GDP and 40% of the export returns (Martens, 2011). Unfortunately the crop of cocoa in Cote d'Ivoire is easily exposed to fungi and virus diseases.

Nowadays, growers still rely heavily on chemical pesticides to prevent, or control these diseases. However, the high effectiveness and ease of utilization of these chemicals can result in environmental contamination and the presence of pesticide residues on food. Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides. One of the biggest ecological challenges being faced by the microbiologists and plant pathologists is the development of environmental friendly alternatives to the currently used chemical pesticides for combating a variety of crop diseases (Ongena and Jacques, 2008). Indeed, these biopesticides present many advantages in term of sustainability, mode of action and toxicity compared to chemical pesticides. One of the best solutions to overcome disease problem in long-term period is to induce systemic plant resistance (IRS) by using *Bacillus subtilis* (Al-Mughrabi, 2008) which provides an efficient disease control and increases crop yields.

In plants, resistance to pathogen infection is accomplished through protective physical barriers and a diverse array of antimicrobial chemicals and proteins. Many of these antimicrobial compounds are part of an active defense response, and their rapid induction is contingent on the plant's ability to recognize and respond to an invading pathogen (Kumar and Kumar, 2012). Here, we focus in detail on the versatile utilization of *Bacillus subtilis* as biopesticide in agriculture. More precisely, a special emphasis is given to the main specific mechanisms involved in biocontrol of plant diseases by this bacterial genus : production of inhibitory chemicals and induction of so-called systemic resistance in host plants. Beside this, strategies for enhancing the efficacy of *Bacillus*-based biopesticides are also discussed (Roongsawang *et al*. 2011).

Cocoa deseases : Cocoa is affected by a range of diseases, caused by fungi, viruses, and nematodes, with a negative effect on the global production. Fungal and viral diseases are the most destructible in west Africa mainly in Cote d'Ivoire. Globally, the three most frequent fungal diseases that affect the cocoa tree are Black Pod, Frosty Pod Rot, Witches' Broom. But there are other cocao diseases that are infrequent. Worldwide it is estimated that approximately 30 to 40% of all potential cocoa production is lost to diseases and pests. In localities with exceptional disease and/or pest infestation, losses can exceed 80%. In dollar terms, annual losses to the tune of approximately $2 billion occur. While

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these losses have an impact throughout the supply chain, it is the cocoa farmer that feels the most immediate and direct impact on family income. Depending on tree variety and region, cocoa farmers can face a variety of fungal diseases and insect pests (ICCO, 2015).

Fungal disease

***Phytophthora* pod rot :** *Phytophthora* spp. are destructive plant pathogens. Most of the species illustrated in this guide are commonly associated with root rot and/or dieback on ornamentals. Pod Rot, also know as Black Pod, is caused by the fungus *Phytophthora* spp. (End *et al*. 2014). Black pod causes 20% to 25% loss of world production, and making it the biggest constraint to production. Among cacao diseases by fungi, black pod caused by *Phytophthora* spp. can be considered the most important, because it occurs in all cocoa-producing countries (Nyasse *et al*. 2007 ; ICCO, 2015). *P. palmivora* is the most widely distributed in the world. Its occurrence has increased considerably in recent years due to increased rainfall (Susilene *et al*. 2011) and cause 20-30% global yield loss and 10% tree deaths annually**.** The situation has changed considerably over the past few decades, and it is now known that each continent has a complex of species which can induce black pod symptoms in cacao. *P. palmivora* is cosmopolitan and occurs in in all countries producing cocoa. *P. megakarya* is endemic in Cameroun, Nigeria, Gabon and Ghana. Both the species produce the similar symptoms (Opoku *et al*. 2007).

P. megakarya is most aggressive in Africa and can cause between 60 and 100 % crop loss. In Cote d'Ivoire, *Phytophthora* spp is the most widespread and the most important cocoa disease and the main pathogen is *P. Palmivora* which caused almost 41 % crop loss. The equivalent of Brazil is *P. capsici*, Other *Phytophthora* species have been reported causing black pod disease without causing significant damage are *P. botryosa* **(**Chee and Wastie, 1970), *P. heveae* (Lozano and Romero, 1984), *P. katsurae* and *P. megasperma* (Liyanage and Wheeler, 1989). Fig 1 shows the most notable symptoms of black pod disease. The lesions start as small yellow spots on the pod that darken to brown or black color and grow fast covering the whole pod and internal tissues. Infected pods become reservoirs of inoculum for extended time periods. *P. palmivora* spores on mummified pods remain active for at least 3 years (ICCO, 2015).

Fig 1: Cocoa pods affected by black pod disease caused by *Phytophthora* sp.

Dieback due to *Lasiodipolodia theobromae* : *Lasiodiplodia theobromae* is most common in the tropics and subtropics. It also occurs as an endophyte (Rubini *et al*. 2005; Mohali *et al*. 2005 ; Twumasi *et al*. 2014). L. *theobromae* (syn. *Botryodiplodia theobromae*), was most commonly associated with dieback and was consistently isolated from various tissues (twigs, bark, vascular tissue and fruits) of affected cocoa plants. Irrespective of age, affected cocoa trees manifest typical dieback symptoms. Leaves on the outer twigs yellow ûrst and the damage may then extend along the whole branch, reaching the main trunk, eventually resulting in tree death. The twigs and branches of diseased trees show internal discolouration with brown streaks in the vascular tissue. Although sudden widespread wilting and death may occur, affected trees more typically decline over several months. Although tree mortality occurs throughout the year, symptoms are more severe during the dry season, especially on trees with nil or slight shade. This disease can affect 100 % of cocoa trees at farms (Mohali *et al*. 2005).

Frosty Pod Rot and witches' broom: Frosty Pod Rot disease is caused by the fungus, *Moniliophthora roreri*. It is one of the bigest constraints to cocoa cultivation in Latin America countries (Andrade *et al*. 2009). The disease is confined to Central and South America. It first appeared in Columbia in 1917, and has spread to Ecuador, western Venezuela, Panama, Costa Rica, Peru, Nicaragua, Honduras, Guatemala, Belize and Mexico (Evans *et al*. 2013).

The fungus produces spores that are spread naturally by wind, water and movement of the infected pods (Evans, 2012). The disease infected young pods show light yellow swellings and distortion. Older pods ripen prematurely. Internally, the beans appear reddish brown and necrotic. In advance stages, the pod typically shows chocolate-coloured lesions and the white/creamy fungus on the pod surface. Frosty Pod Rot disease has been reported to be twice as destructive as Black Pod rot disease. Average pod losses is over 30%, but can exceed 90% under favourable conditions.

Witches' broom : The causal agent of witches' broom disease of cacao is *Moniliophthora perniciosa*. In South and Central America, this disease causes a lot of damage to the cacao crops. It was recorded for the first time in 1929 in the region of Tumaco. Between 1960 and 1977, the disease spread to almost all areas of Colombia (Barros, 1978). The infection caused by *M. perniciosa* is due to infectious propagules, germinating on young cacao tissues such as apical and axillary meristems, young leaves, developing flowers, and fruits (Meinhardt *et al*. 2008), and subsequently penetrating plant tissues directly or entering through stomata

(Frias et Purdy, 1991). The disease occurs in two phases such as the biotrophic phase and the necrotrophic phase. Germinated basidiospores form a swollen (5–20µm) monokaryotic hyphae that grows intercellularly as a biotroph and causes symptoms that include shoot swelling, petiole swelling, and loss of apical dominance (Meinhardt *et al*. 2008). The biotrophic phase usually lasts for about 60 days in artificially inoculated plants (Silva *et al*. 2002 ; Scarpari *et al*. 2005). In the necrotrophic phase of infection, from the apex downwards, the green broom becomes brown and dies within a week (Meinhardt *et al*. 2008). This phase is associated with the appearance of intra-cellular hyphae, which is dikaryotic and exhibits clamp connections. When the dried broom is exposed to alternate periods of dry and wet weather, basidiocarps emerge to complete the life cycle (Pires *et al*. 2009).

Virus disease : Cocoa swollen shoot virus (CSSV): The pathogen responsible for the disease is Cacao swollen shoot virus (CSSV) belonging to the genus *Badnavirus* and family *Caulimoviridae* (Dzahini-Obiatey and Adu 2010). Swollen shoot disease has always been described as a disease endemic to West Africa, because it has never been reported in South America, the cocoa tree's area of origin. A viral disease causing similar leaf symptoms has been reported in Trinidad, but it is not associated with swellings and the disease is no longer being reported. The existence of swollen shoot disease in Malaysia and Sri Lanka has not been clearly established (Vos *et al*. 2003). Among all the regional diseases of cocoa, CSSV is probably of greatest importance, while Thresh (1991) reported that the disease is the most intractable and destructive to strike at the cocoa industry in West Africa. Countries mainly affected are Nigeria, Togo, Ghana, and Cote d'Ivoire (Kouakou *et al*. 2012). The economic importance is evidenced by the serious decline in cocoa production experienced in these countries. Loss production vary from 40 to 100%. Many distinct variants can be recognized usually named after the locality of their origin. The disease was first discovered in the Eastern Region of Ghana in 1936 and is now found in all cocoa growing areas of the country (Steven 1936 ; Ollenu *et al*. 1989).

Six CSSV isolates from Ghana and Togo have been entirely sequenced thus far and are genetically structured in three groups: A, B and C (Muller, 2008). In Cote d'Ivoire, swollen shoot disease was identified for the first time in 1943 (Alibert, 1946). Losses attributable to swollen shoot disease were considerable in eastern Cote d'Ivoire, where two forms of the disease were described : the Kongodia form, similar to the New Juaben form in Ghana, which is very virulent, leading to the rapid death of plants within 3 to 5 years, and the benign Sankadiokro form, which has not been found to have an impact on yields. Unlike in Ghana, where swollen shoot disease has prevailed. The sustainability of cocoa production in Cote d'Ivoire, the world's leading producer, has been under threat since the discovery of cocoa swollen shoot disease outbreaks in the Centre West of the country, notably at Bouafle and Sinfra (Kebe *et al*. 2006).

According to Kouakou *et al*. (2012), the CSSV isolates in Cote d'Ivoire belonged to four of the six groups detected (B, D, E, and F) along with the B-C intermediate subgroups. The distribution of the different groups of CSSV isolates in the regions affected by swollen shoot disease in Cote d'Ivoire showed the presence of group B isolates in all the outbreaks studied, except at Adzope (Fig 2). From east to west of the country, it was seen that all the isolates collected in the different plots of the Moyen Comoe region (Abengourou and Agnibilékrou) contained isolates belonging to group B only. In the center-west region, we found isolates from groups B and D at the same time in the Bouafle, Sinfra, and Issia outbreaks near the Daloa region. The isolates belonging to the B-C intermediate subgroups were mainly detected in outbreaks of Bouaflé and Sinfra, where the disease was discovered in 2003 (Kouakou *et al*. 2012).

CSSV severely reduces the yields of an affected cocoa tree and kills the tree in under 5 years. However, between these two extreme phases (reduced yields and tree death), the leaves gradually turn yellow and fall, then the branches dry out from their tips (die-back). The typical symptoms of the disease are a red vein banding of young leaves, mosaic on older leaves, and, above all, swelling of the orthotropic shoots. Natural transmission of the virus is via mealybugs of the family *Pseudococcidae* in a semipersistent manner (Dufour *et al*. 1993). The only effective way of controlling swollen shoot disease is to remove infected cocoa trees and their nearest neighbors, then replant with healthy hybrids selected for their partial resistance to the virus (Kouakou *et al*. 2012).

Cocoa swollen shoot virus can infect cocoa plants at any stage of development. The disease causes a wide range

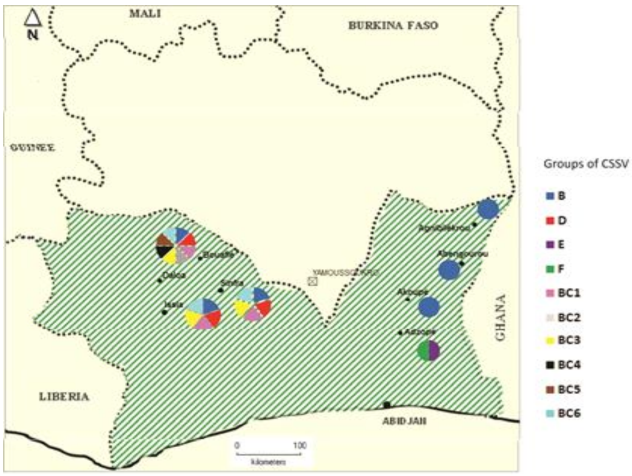

Fig 2: Spatial distribution of cacao swollen shoot virus groups of isolates in the different regions of Cote d'Ivoire (Kouakou *et al.*, 2012).

of symptoms depending on the strain of the virus, the stage of infection and the susceptibility of the cocoa variety. Amelonado cocoa varieties are particularly susceptible to infection and diseased plants show the following characteristic symptoms : reddening of primary veins or 'banding' in young leaves, yellow banding along the main veins of leaves, vein clearing in leaves, sometimes producing a 'fernlike'pattern, chlorosis or flecking and mottling of mature leaves, stem and root swellings (some mild strains of the virus do not cause swellings in infected plants), abnormally shaped pods, usually smaller and spherical (Dzahini-Obiatey *et al*. 2010).

Bacillus subtilis

Lipopeptides produced by *Bacillus subtilis*: *Bacillus* sp. is an important organism for the molecular genetic study of peptide synthesis. Lipopeptides represent a unique class of bioactive microbial secondary metabolites, and many of them show attractive therapeutic and biotechnological properties (Maget-Dana and Peypoux, 1994). *B. subtilis* are known to produce a wide range of secondary metabolites including cyclic lipopeptides (c-LPs), some of the most powerful ones with regard to their antifungal and biosurfactant activity (Ongena and Jacques, 2008; Jacques, 2011). Secondary metabolites produced by *B. subtilis* consist mainly of three families of non-ribosomally synthesizedc-LPs. These are the iturins, the fengycins, and the surfactins. These c-LPs contain a peptide ring with seven (iturins and surfactins) or 10 (fengycins) amino acids linked to a b-hydroxy (fengycins and surfactins) or b-amino (iturins) fatty acid. Each lipopeptide family is further subdivided into groups based on its amino acid composition. For example, the fengycin family comprises fengycinA and fengycin B, which differ in a single amino acid in the sixth position (D-alanine and D-valine, respectively). Within each group, there are homologues differing in the length, branching, and saturation of their acyl chain (Ongena and Jacques, 2008). Members of the iturin familyrange from C14 to C17, fengycins from C14 to C19 and surfactins from C12 to C17. Both iturins and fengycins are mainly known for their anti-fungal properties, while surfactins are mostly anti-viral and anti-bacterial.When different families are co-produced, their interaction can become synergistic and enhances each of their respective activities (Ongena *et al*. 2007; Romero *et al*. 2007).

Importantce in agriculture : Bacterial products represent the majority of the microorganism-based biopesticides but fungal biocontrol agents have also been developed as efficient products (Shoresh *et al*. 2010). The *Bacillus* genus encompasses a large genetic biodiversity. They are present in an extremely large palette of environments ranging from sea water to soil, and are even found in extreme environments like hot springs. Firstly, *Bacilli*, such as *B. subtilis*, are well-studied organisms which facilitates their rational use. Secondly, the US Food and Drug Administration (USFDA) has granted the "generally regarded as safe" (GRAS) status to *B. subtilis* which is thus recognized non-pathogenic (Cawoy *et al*. 2011). Thirdly, *B. subtilis* has the capacity to produce spores (Piggot and Hilbert, 2004) which can withstand high temperatures, unfavorable pH, lack of nutrients or water, etc. They are produced by the bacteria when environmental conditions are unfavorable which helps these microorganisms to survive in the phytosphere. The phenomenon can also be exploited in industrial production as sporulation can be induced at the end of cultures (Monteiro *et al*. 2005). Beside its spore forming ability, *B. subtilis* possess several characteristics that enhance its survival in the rhizosphere and thus its effectiveness as a biopesticide (Rosas-Garcia, 2009). *B. subtilis* is intensively studied for their importance in biotechnological industry and agriculture, as phytopathogenic antagonists or plant growth promoters (Sicuia *et al*. 2015). Another reason for the high interest is the diversity of their modes of action. They can display almost all the mechanisms of biocontrol and bio-stimulation/ fertilization.

Potential biocontrol agent for management of plant diseases : One of the mechanisms of such a protection includes production of bioactive secondary metabolites which can be directly involved in antibiosis. This secondary metabolites consist mainly of three families of non-ribosomally synthesized c-LPs. These are the iturins, the fengycins and the surfactins. Surfactins are potent antibacterian, antiviral and biosurfactants. Iturins and fengycins are potent antifugal angent (Ongena, 2014). Malfanova *et al*. (2011) have described the isolation and partial characterization of the plant-beneficial endophytic bacterium *B. subtilis* HC8. This strain shows strong in vitro antifungal activity against various fungal phytopathogens. When applied to seeds, *B. subtilis* HC8 is able to significantly decrease symptoms of tomato foot and root rot which is caused by *F. solani, Forl* and *P. ultimum*. It has been reported that *B. subtilis* forms adhering biofilms on inert surfaces under the control of a variety of transcription factors (Stanley *et al*. 2003). *B. subtilis* biofilms are dependent on the secretion of surfactin. There is a growing recognition that biosurfactant production not only affects biofilm architecture but can influence the attachment of bacteria to surfaces (Davey *et al*. 2003).

Plant growth promotion: The term "Plant Growth Promoting Rhizobacteria" – PGPR, was coined by Kloepper and Schroth in 1978. They described the PGPR as naturally occurring soil bacteria that have the ability to colonize the roots and stimulate plant growth. Various species of *B. subtilis* have been reported as PGPR. According to Gupta *et al*. (2016), *Bacillus* spiece isolate B18 were found effective as PGPR. The PGPR improve plant physiological state by phytohormones production or by releasing beneficial organic compounds (Kumar and Kumar, 2012). Polyamines, for example, have important physiological role, being involved in cell division and differentiation, protein synthesis, and membrane stability, moreover they have protective role against various abiotic stresses (Xie *et al*. 2014). PGPR fix atmospheric nitrogen, produce phytohormones, siderophore, solubilize phosphate, potassium and zinc, alleviate the various stress by secreting ACC (1-aminocyclopropane-1-carboxylate) deaminase enzyme. Several other workers have also found the biocontrol activities of *B. subtilis* against many common phytopathogens (Toure *et al*. 2004 ; Chung *et al*. 2008 ; Gajbhiye *et al*. 2010). Seed treatment with PGPR has been used to enhance growth of several crops and to suppress the growth of plant pathogens and deleterious rhizosphere microorganisms (Cleyet-Marcel *et al*. 2001, Sudhapriyadharsini *et al*. 2016). *B. subtilis* as PGPR can produce Indole acetic acid (IAA). The main effect of IAA is to promote growth of roots and stems, through stretching of the newly formed cells in the meristem (Chagas *et al*. 2015). IAA produced by *B. subtilis* can stimulate cell elongation by modifying certain conditions like, increase in osmotic contents of the cell, increase in permeability of water into cell. It promotes embial activity, inhibit or delay abscissionof leaves, induce flowering and fruiting (Zhao, 2010).

Soil fertility management by biofertilizers using phosphate solubilizing bacteria (PSB) is one of the basic components of sustainable agriculture. Phosphorus is one the most essential elements for plant growth, second only to nitrogen in requirement for plants (Abd-Alla, 1994). Approximately 95-99% of soil phosphorous is present in the form of insoluble phosphates and cannot be utilized by the plants (Assileva *et al*. 2001). Biofertilizers are one of the best modern tools for agriculture. *Bacillus* strain from Ashvgandha soil were able to solubilize phosphorus (Mohinder *et al*. 2011). *B. subtilis* isolated from the soil by Persello-Cartieaux *et al.* (2003) had effective properties of phosphate solubilizers which could be used as a biological fertilizer in agriculture.

Induced systemic resistance: Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance (ISR) in the treated plant. ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens. Such plant microbe interactions lack any visible hypersensitive response with colonization of the roots by PGPR (Ongena, 2014). The PGPR strains were shown previously to elicit ISR on several crops against fungal, bacterial, and viral pathogens under greenhouse and field conditions (Bhattacharyya and Jha, 2012). Rhizobacterial strains that triggered ISR by VOC signaling included *B. subtilis* GB03 (Ryu *et al.*, 2004).

Fengycins have antagonistic activity against a broad range of fungal targets and have also been shown to elicit induced systemic resistance (ISR) in plant hosts. Surfactins are also elicitors of ISR in plants (Ongena and Jacques, 2008 ; Raaijmakers *et al*. 2010).

Zehnder *et al*. (2000) assessed *B. subtilis* IN937b for ISR activity against CMV on tomato plants under ûeld conditions for two consecutive cropping seasons. *B. subtilis* IN937b were applied as seed treatments at the time of transplanting to the pots prior to their transplantation in the ûeld, while CMV inoculation was done on plants 1 week before transplantation to the ûeld. Treatment with *B. subtilis* IN937b strains resulted in signiûcant reduction of disease compared to the nonbacterized control. According to Mohiddin and Khan (2018) *B. subtilis* was effective decreased wilting 51-55% (seed treatment) and 47-51% (soil application).

The induction of the host plant defence system is a promising strategy to reduce pesticide use in conventional agricultural (Harm *et al*. 2011). Pathogen-derived metabolites (elicitors) are recognised by putative plant cell receptors and activate a complex network of signal transduction pathways and a variety of biochemical and molecular defence mechanisms. The signalling pathways mediated by microbial elicitors involve secondary signals such as salicylic acid (SA), jasmonic acid (JA) and ethylene. These bioactive molecules can either act independently or in combination to orchestrate local and systemic induction of defence responses (Yang *et al*. 2011). According to studies in *Arabidopsis thaliana*, defense against biotrophic pathogens usually involves dependent signaling of SA, while the induced defense against herbivorous insects and necrotrophic pathogens depend of JA (Gutjahr and Paszkowski, 2009).

CONCLUSION

Cocoa is very important for humanity but pests and diseases are major constraints for its his production in world mainly in Cote d'Ivoire and yield losses are huge. Agriculture across the world is dependent on pesticides. However, *B. subtilis* by its ability to promote plant growth and its antimicrobial properties would be an excellent biological control agent against phitopathogenics agents. In the future, given the negative impact of pesticides and chemical fertilizers, the development of organic strains capable of stimulating growth and the natural defense mechanisms of plants would be a very holds much promise.

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