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Maize resistance to stem borers and storage pests: The need for new genetic and functional genomics approaches in future research

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ABSTRACT

Insect pests are primary constraints in maize (*Zea mays*) production in many places in sub-Saharan Africa. Stem borers and storage pests are responsible for severe yield losses and health hazards due to mycotoxin contamination. Integrated pest management (IPM) strategies have moved from control methods and transgenic resistance to recognizing the necessity of host plant resistance (HPR) especially in the context of an ever changing climate and its forecasted negative consequences. For that, a wealth of scientific knowledge has been generated over the years although the goals are far to be reached. Here, we first review current literature on maize resistance mechanisms as regards to insect herbivory. We show that there are numerous insect species that feed on maize before narrowing down to stem borers and storage pests. We also look at the basis of maize resistance in terms of its biochemical components and analyse the progress of genetic studies in terms of QTL mapping and trait genes identification. Finally, we highlight the usefulness of new genetic and functional genomic approaches in underpinning the genetic basis of maize resistance to insect pests in general and particularly stem borers and storage pests.

Key words: Biochemical resistance, functional genomics, genetics, insects, maize

RÉSUMÉ

Les insectes ravageurs constituent une contrainte majeure pour la production de maïs (*Zea mais*) et les insectes foreurs de tiges et les ravageurs de stocks de gain sont responsables de graves pertes de rendement et risques pour la santé dues à la subséquente contamination mycotoxines. Les stratégies de gestion intégrée des insectes ravageurs ont évolué pour passer des méthodes de contrôle et de résistance transgénique à la reconnaissance de la nécessité de renforcer la résistance hôte dans le maïs pour mieux lutter contre les pertes de rendement et les défis résultant de insectes herbivores dans un contexte changement climatique avec son corollaire de conséquences négatives. Pour cela, une riche information scientifiques a été générée au fil des ans, cependant il semble que les objectifs sont loin d'être atteints. Ici, nous examinons d'abord la littérature actuelle sur les mécanismes de résistance du maïs en ce qui concerne insectes herbivores. Nous montrons le caractère pléthorique des espèces d'insectes qui attaquent le maïs en nous appesantissant sur les insectes foreurs et les ravageurs de stocks de grains en Afrique sub-saharienne dans un contexte d'échec général des méthodes de contrôle proposées. Nous examinons également la base résistance biochimique du maïs à ces insectes analysons l'avancement des études génétiques en termes d'indentification de QTL et de gènes responsables de traits de résistance. Enfin, nous mettons en évidence l'utilité des nouvelles approches de génétiques et de génomiques fonctionnelles dans l'étude de la base génétique de la résistance du maïs aux insectes en général et les insectes foreurs et les ravageurs de stocks de grains en particulier.

Mots clés: résistance biochimique, génomique fonctionnelle, génétique, insectes, maïs

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INTRODUCTION

Maize is one of the most important crops in the world. It feeds more than 4.5 billion people in 100 countries. It is also a multi-purpose crop as it is also used for biofuel production and livestock feed (Shiferaw *et al.,* 2011; Gafishi Kanyamasoro *et al.,* 2012; Cairns *et al.,* 2013). However, its yield is limited by several stresses among which insect herbivory plays a central role. Insect-related yield losses begin in the field with a range of pests with high economic and social impact (Meihls *et al.,* 2012) resulting in very huge losses and health hazards. Stem borers and storage pests are primary maize pests found in many climatic zones including Sub Saharan Africa (SSA). In Uganda, stem borers, *Chilo partellus* and the storage pest, *Sitophilus zeamais* are major problems. They are responsible for losses estimated at 90% and 80% for stem borers and the maize weevil, respectively (Sylvain *et al.,* 2015). Their attack on maize ears and grain lead to contamination with mycotoxins that are harmful to human beings and livestock. The control methods proposed for these pests have challenges (for example use of insecticides is associated with human and environmental health risks) (Wanja *et al.,* 2015). Additionally, environmental factors are key in influencing plant defensive mechanisms (Stam *et al.,* 2014). Climate change is predicted to impact on plant-insect interaction possibly leading to less fitness of plants with aggravated yield losses (DeLucia *et al.,* 2012). In fact, forecasted CO_2 and temperature rises are predicted to negatively affect plant-insect interactions (DeLucia *et al.,* 2012) through modification of the environment.. Host plant-resistance (HPR) built on a thorough understanding of the genetic basis of plant defensive mechanism is the best option to manage insect herbivory on maize in the context of global climate changes.

In this paper, we present a brief summary of the current knowledge on insect species that challenge maize production particularly stem borers and storage pests and review biochemical and genetic studies so far undertaken with regard to maize resistance to insect pests. Lastly, we present prospects for the future of genomics and functional genomics research in this interest areas and shed light on the next generation sequencing technologies (NGS) that are being made available.

Maize pests and their management. Insect species that feed on cultivated maize are estimated to be more than ninety. They attack the crop as individual species or as combination of species. They attack every part of the plant, from the roots to the tassel (Meihls *et al.,* 2012). They are thus classified into four groups: leaf feeders, stem borers, phloem feeders, and root feeders (Meihls *et al.,* 2012). The leaf feeders are responsible for damage of foliar and reproductive tissues. Among them are the fall armyworm (*Spodoptera frugiperda*), the beet armyworm (*Spodoptera exigua*), the corn earworm (*Helicoverpa zea*), and the grasshoppers (*Melanoplus* spp). These feed on the whorls of young maize plants, producing small holes and irregular notches on the leaf margins. Stem borers damage maize by boring tunnels within the stems of the plant. They include the southwestern corn borer (*Diatraea grandiosella*), sugarcane borer (*Diatraea saccharalis*), Asian corn borer (*Ostrinia furnacalis*), European corn borer (*Ostrinia nubilalis*) and the Mediterranean corn borer (*Sesamia nonagrioides*). Phloem feeders on the other hand obtain nutrients from phloem sap of all aboveground plant tissues. They are piercing-sucking insects and include the corn leaf aphid (*Rhopalosiphum maidis*), greenbug (*Schizaphis graminum*) and the bird cherry-oat aphid (*Rhopalosiphum padi*). A complex of Diabrotica species, among others, the western (*Diabrotica virgifera virgifera)*, northern (*Diabrotica barberi*), and Mexican (*Diabrotica virgifera zeae*) corn rootworms attack maize roots (Meihls *et al.,* 2012). All those insects are maize field pests and are responsible for severe yield losses through damaging photosynthetic tissues, impeding water and nutrient movement, stem logging and breaking, leaf curling and wilting, affecting pollination, etc (Meihls *et al.,* 2012).

Found in many environments, corn borers are primary maize pests (Meihls *et al.,* 2012). They feed on the pith of the stem which results in yield losses as stem damage interferes with assimilate movement to developing kernels (Samayoa *et al.,* 2015). Corn borers also attack ears, resulting in secondary fungal infection, leading to contamination of grain with mycotoxins like aflatoxin, which is harmful to human and animal health (Visconti *et al.,* 1999). The maize weevil (MW) (*Sitophilus zeamais*), on the other hand, is among the key storage pests in maize and affects mostly susceptible genotypes leading to great losses in both quality and quantity (Gafishi Kanyamasoro *et al.,* 2012). Classified as primary category of grain insects, both adults and larvae are internal feeders and can cause up to 80% losses on untreated maize grains (Dhliwayo and Pixley, 2003). In SSA, the most challenging stem borers are *Chilo partellus* and *Busseola fusca* and in East Africa, they are the most abundant field insect species. Of the two, *C. partellus* is the most competitive species able to displace any indigenous one within only two years (Sylvain *et al.,* 2015). They account for losses ranging from 20 to 90%. In Uganda, the indigenous stemborer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae), and the invasive *Chilo partellus* (Swinhoe) (Crambidae), which was introduced into Africa sometime before the 1930s, are the most important biotic constraints to cereal production (Matama-Kauma *et al.,* 2008).

A number of strategies have been used for managing maize stem borers to either prevent or mitigate their damaging effects (Sylvain *et al.,* 2015). Chemical control methods, although deemed to be the most effective, are expensive to most small-scale farmers and risky to humans, livestock, and the environment (Tefera *et al.,* 2011). In terms of efficiency, biological control rank second. Biological control is also cost-effective and environmentally safe. However, the approach is limited by its inability to sufficiently maintain pest populations below economic injury levels (Mailafiya *et al.,* 2009). Control of stem borers has also relied on cultural methods which are easy to use and much less expensive. The limitation of these methods however, is their inapplicability to large scale farms in addition to being difficult to time their application (Munyiri *et al.,* 2015). A very effective approach in the control of stem borers and other lepidopteran pests is the use of Bt crops. These are crops genetically engineered to carry *Bacillus thuringiensis* genes. These genes produce proteins that are toxic to lepidopteran pests. They are highly specific in their mode of action leading to a narrow range of target pests (Yuan *et al.,* 2009). However, their acceptability is limited because of biosafety concerns like ethical and moral issues as well as intellectual property restrictions and the payment of royalties, environmental health considerations on biodiversity, food safety and human health, labeling and trade issues, traceability, and the need for monitoring of Bt-derived products (Tabashnik *et al.,* 2009). In storage, pesticides have proven effective but still present the same concerns (Mwololo *et al.,* 2012). Pests may develop insecticide-resistance (Derera *et al.,* 2014) and give rise to secondary insect species. All those challenges associated with pest control methods led to the reduction of their usage and success in SSA with the risk of encountering huge losses since in developing countries storage facilities are not efficient to prevent pest attacks. Therefore, the necessity of building host plant resistance (HPR) becomes obvious. To achieve this goal, several biochemical and genetic studies have been carried out with promising results.

Maize-pests'interaction: physical and biochemical resistance basis. Painter (1951) has explained the basis of plant resistance to insects as a three-fold mechanism: antibiosis, antixenosis (non-preference) and tolerance. Tolerance is characterized by a complex set of genetic traits that enable a plant to withstand or recover from insect damage. Antixenosis occurs when plant morphological or chemical factors adversely affect insect behavior, leading to delayed acceptance and possible outright rejection of a plant as a host. By contrast, the antibiosis category of plant resistance occurs when a resistant plant adversely affects the life-history traits (survival, development, fecundity) of an insect attempting to use that plant as a host (Meihls *et al.,* 2012; Smith and

Clement, 2012). In maize grain resistance to MW, antibiosis is considered as the main resistance factor. It is also called biochemical resistance and based on phenolic compounds mainly, which act in two ways: through mechanical resistance and antibiosis in the pericarp and aleurone layer, respectively (García-Lara *et al.,* 2004). This resistance is positively correlated with the high phenolic content of the maize grain (Serratos *et al.,* 1987). A study conducted by Classen *et al.* (1990) suggested that the phenolic content of a maize grain might allow it to show storage pest resistance by an antibiosis mechanism. Furthermore, those phenolic compounds may have the ability to sustain human health if they carry an antioxidant activity (García-Lara and Bergvinson, 2014; Kardas and Durucasu, 2014). On the other hand, several studies have investigated and shown the presence of phenolic compounds in immature and mature leaves and their related role in the mechanism of resistance to field insect pests. Bergvinson *et al.* (1997) pointed out the increase of Di-Ferulic Acids (DFAs) in leaves and other tissues over cycles of selection to European Corn Borer (ECB) (*Ostrinia nubilalis*) resistance in the Iowa Stiff Stalk Synthetic BS9. For ECB resistance, increased levels of Ferulic Acid (FA) monomer have been quantified in epidermal cell walls of leaves in resistant inbred lines (Bergvinson *et al.,* 1994a) while the content of FA dimers in leaves was negatively correlated across genotypes with leaf damage (Bergvinson *et al.,* 1994b). Cross-linking of hemicellulose by DFAs was suggested to be a mechanism of resistance through early cell wall fortification hence increasing the hardness of the leaf tissue (Ramputh, 2002). In addition, both DFA and p-Coumaric Acid (p-CA) were negatively correlated with damage parameters in the node and pith (Bergvinson *et al.,* 1997). In a recent study, Barros-Rios *et al.* (2015) showed the first direct negative relationship between diferulates concentration in pith stem tissues and corn borers larvae weight and stem tunneling.

In general, the production of defensive proteins and secondary metabolites have been linked to the natural variation in maize insect resistance (Betsiashvili *et al.,* 2015). In fact, herbivores or their egg deposition on plant, affects their phenotype through changes in the production of primary and secondary metabolites, morphological traits, and architecture (Stam *et al.,* 2014). For example, protection against lepidopteran herbivory is deemed to be provided by protease inhibitors (Tamayo *et al.,* 2000), the cysteine proteases (Pechan *et al.,* 2000), and ribosome-inactivating proteins (Chuang *et al.,* 2014). In maize defensive metabolites include chlorogenic acid (Cortés-Cruz *et al.,* 2003), maysin (Rector *et al.,* 2003), and benzoxazinoids (Frey *et al.,* 2009). The later ones are known to provide resistance to a large number of herbivores and pathogens (Niemeyer, 2009) and have been studied most extensively. For instance, the core biosynthetic pathway for 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one acid glucoside (DIMBOA-Glc) has eight encoding genes (Bx1–Bx8) that are tightly linked at the top of maize chromosome 4 (Betsiashvili *et al.,* 2015). Igl1 and Bx9 are two additional genes which encode the same enzymatic functions as Bx1 and Bx8, respectively (Gierl and Frey, 2001; Ahmad *et al.,* 2011), and are located on chromosome 1. DIMBOA-Glc is activated by glucosidases to form DIMBOA when insects attack the plant (Betsiashvili *et al.,* 2015). Later on the decay of the DIMBOA forms active-insect-deterrent metabolites (Gierl and Frey, 2001). When the attack is caused by chewing herbivores, DIMBOA-Glc is converted to the more toxic 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one acid glucoside (HDMBOA-Glc) (Glauser *et al.,* 2011) whereby the Bx10 methyl transferases of maize is considered as the most likely causative enzyme (Meihls *et al.,* 2012).

Additionally, Gill *et al.* (2010) reviewed the role of oxidative enzymes in plant insect herbivory resistance. Those enzymes are the peroxidases, the lipoxygenases and the polyphenol oxidases. They are induced upon biotic stress occurrence in plant and are believed to be a mechanism of host plant defense against insect herbivory (Gill *et al.,* 2010). Furthermore, in mature maize grain, numerous isozymes of peroxidase are present; their genetic loci have been previously documented (Brewbaker *et al.,* 1985). Besides that, in the UniProt1 database (Magrane and Consortium, 2011), more than 400 *Zea mays* proteins are tagged as PODs. More specifically cell wall associated class III PODs are involved in the loosening and stiffening of cell walls during plant development. López-Castillo *et al.* (2015) identified and characterized a class III POD B6T173 (ZmPrx35), which accounts for about 80% of the POD activity in maize (*Z. mays* p84C3) kernels.

QTL mapping for maize resistance to pests: what have we learnt? The majority of maize chromosomes contain insect-herbivore resistance QTL, although there seems to be a concentration in some, like at the top of chromosome 1, the bottom of chromosome 2, and on chromosome 7 and only 10% of maize bin are not known to be involved in some sort of insect resistance (Meihls *et al.,* 2012). Furthermore, stem-boring QTL are co-localized with both chemical defense (51 bins) and plant traits (60 bins). More interestingly, QTL for benzoxazinoid content, stem-borer resistance and DIMBOA-glucoside biosynthetic loci co-localize in bin 4.01 suggest a cause-and-effect relationship (Meihls *et al.,* 2012) as benzoxazinoids provide defense against a wide variety of pathogens and herbivores (Niemeyer, 1988). Another interesting fact is that maize lepidopteran and *Sitophilus zeamais* resistance and defense chemicals (DIMBOA, DIMBOAGlc, maysin, chlorigenic acid, lignin components, hydroxycinammic acid etc.) share several maize bins (Meihls *et al.,* 2012) which suggests that the genes under those chromosome regions may be responsible for maize resistance to both stem borers and storages pests through the production of defense metabolites. Besides, it is noteworthy to consider that those genes encode for metabolites of different defense pathways that could be interacting to assure defense mechanisms against pest attacks. García-Lara *et al.* (2009 and 2010), and Castro-Alvarez- *et al.* (2015) have detected QTLs for MW resistance in an F_2 and F_6 (RIL) populations respectively using SSR and restriction fragment length polymorphism (RFLP) markers. Garcia-Lara *et al.* (2009) identified 21 chromosomal regions as putative QTLs responsible for mechanical resistance of a maize F_2 population. Repeating the QTL analysis for eleven grain phenolic acids identified as biochemical basis of resistance, Garcia-Lara *et al.* (2010) defined 17 QTL regions out of which nine regions were common between QTL associated with MW susceptibility and cell wall bound compounds, suggesting a strong genetic association with MW resistance in tropical maize. Alvarez-castro *et al.* (2015) performed the QTL analysis for mechanical resistance using only SSR on a RIL population and revealed 15 QTL regions out of which three were co-localized in chromosomes 4.08, 10.04, and 10.07, where no resistance-associated genes had been reported previously. Comparing their results with those found by Garcia-Lara *et al.* (2009 and 2010), Castro-Alvarez *et al.* (2015) suggested that further studies should be conducted using denser molecular markers set such as single nucleotide polymorphism (SNPs) to corroborate the importance of these QTLs and form the basis of marker based selection in high-throughput genotyping schemes.

Presently, almost all the genetic studies on maize resistance to pests are done through QTL mapping which does not give precise information on the underlying genes and their variability determining susceptibility index among maize inbred lines (Meihls *et al.,* 2012). Narrowing the QTL regions combined with candidate genes approaches will help to tackle this important question and further allow the investigation of DNA sequence variation modulating resistance. This could be as well done by first identifying consensus QTL for both stem borers and storage pest to analyze the commonality of the involved pathways regarding their synergistic action and interactions. QTL mapping has proven to be a powerful method for the identification of regions of the genome that co-segregate with a given trait in either F_2 populations or Recombinant Inbred Line (RIL) families (Corte and Farlow, 2013). However, this technique uses a limited resolution (10–20 cM) which necessitates further fine mapping to isolate the possible candidate gene (s) (Xue *et al.,* 2013).

Yet, higher resolution is needed to detect genes involved in traits like plant defense mechanism (Samayoa *et al.,* 2015). In fact, bi-parental QTL mapping has an inherent low allele frequency with a subsequent low recombination rate which leads to lack of resolution (Korte and Farlow, 2013) leading to very scarce reports of QTL usefulness in marker assisted selection (Bernardo, 2008). Therefore, the best way of mapping QTLs or genes would be by increasing the resolution to the nucleotide level and such a target is no longer out of reach since the recent development of next generation sequencing technologies.

WHAT IS THE WAY FORWARD?

Narrowing QTL stretches to a single nucleotide.

Single nucleotide polymorphism (SNPs) are DNA markers classified as high-throughput sequence-based makers (Wang *et al.,* 1998), universal and as well as the most abundant forms of genetic variation among individuals of the same species (Ghosh *et al.,* 2002). Although less polymorphic than SSR markers because of their biallelic nature, they easily compensate this drawback by being abundant, ubiquitous, and amenable to high- and ultra-high throughput automation (Mammadov *et al.,* 2012). Therefore, they can provide the highest map resolution compared to other marker systems (Jones *et al.,* 2007). Genome-wide association study (GWAS) is a complementary tool for QTL analysis (Tang *et al.,* 2015) and allows relating traits of interest to a single SNP. Furthermore, GWAS overcomes QTL mapping major limitations pertaining to low recombination rate and low allele frequency that arise from bi-parental populations (Corte and Farlow, 2013). GWAS is based on linkage disequilibrium (LD) analysis in a diverse and natural population and constitutes a relatively new approach, which offers higher resolution mapping (Samayoa *et al.,* 2015). Under optimal conditions, GWAS can identify causal genes underlying quantitative trait variation exploiting advances in genotyping and sequencing technologies, which made it successful in detecting genes associated with different traits (Samayoa *et al.,* 2015). GWAS have been run for SNPs-trait association for diseases in humans (Duerr *et al.,* 2006, Sladek *et al.,* 2007; Weiss *et al.,* 2009), animals (Barendse *et al.,* 2007; Kijas *et al.,* 2009; Bolormaa *et al,* 2011; Fan *et al.,* 2011) and different quantitative traits in plants (Tang *et al.,* 2015; Samayoa *et al.,* 2015). The fact that GWAS is based on LD, a large number of markers covering the whole genome are required (Peiffer *et al.,* 2013). LD is found to be low in in diverse maize samples, therefore, many more markers are needed than in autogamous species (with higher LD) to adequately explore the genetic architecture of complex traits (Romay *et al.,* 2013). However, the low LD has the benefit of offering a better mapping resolution to delineate potential causal genes within small LD blocks (Samayoa *et al.,* 2015). Furthermore, tropical maize germplasms have more genetic variability and show rapider LD decay than in temperate lines, which is an advantage for GWAS (Lu *et al*., 2011). The advances in molecular genetics have led to the facilitation of SNPs discovery passing from high-throughput to ultra-high-throughput with the event of Next-Generation-Sequencing (NGS) through whole-genome sequencing or resequencing for various species (Deschamps *et al.,* 2012). NGS have driven the costs for DNA sequence as low as allowing for Genotyping-By-Sequencing (GBS) for high diversity, large genome species and mostly for crops with a reference genome (Elshire *et al.,* 2011). GBS uses specific restriction enzymes that reduce genome complexity like ApeKI in maize, which is a methylation sensitive restriction enzyme, hence, avoiding sequencing repetitive genome regions (Elshire *et al.,* 2011). These methods allow the construction of highly dense genetic maps with a great usefulness in genetic and plant breeding (Poland *et al.,* 2012). GBS is an ultra-high throughput tool that allows the calling of thousands and millions of SNP (Poland *et al.,* 2012), hence very useful for GWAS in plants especially in maize. As GWAS relates trait to SNP (Corte and Farlow, 2013), it becomes therefore, possible to associate the phenotypic variation to a single gene or a limited number around the SNP. Further studies can be undertaken to determine the metabolic pathways involved in the expression of the trait and other bioinformatics studies can be run on the candidate genes, like in Samayoa *et al.* (2015) or in Tang *et al.* (2015).

The candidate gene approach: straight forward for trait causative genes identification. The selection of putative genes is based on either positional or functional gene choice dependent on the state of the genetic knowledge of those genes in terms of involvement in the biosynthetic and/or physiological pathway. A positional gene choice pertains to basing the choice on linkage data of the locus being characterized where all closely linked genes may be candidate genes. This is accomplished by evaluating the closeness of linkage with the trait loci, or performing a comparative map analysis (synteny), or instead looking at the conservation of gene sequences, order and distribution between species. On the other hand, a functional gene choice consists in the selection of genes based on their involvement in the biosynthesis of the products responsible for the expression of the phenotypic trait (Pflieger *et al.,* 2001). In plant genetics, the selection of the most likely candidate genes can be performed using two strategies. Genetic map positions of putative functional CGs and target loci involved in the studied trait can be compared. Simultaneously, correlation analyses between phenotypic variation and molecular polymorphisms within the CG can also be conducted in a set of genealogically unrelated individuals. These two strategies are not exclusive but complementary (Pflieger *et al.,* 2001). Several studies have been achieved so far in maize and some other species trying to identify candidate genes for several traits of agronomic importance (Wong *et al.,* 2004 through Gande *et al.,* 2015). More recently, genome-wide-association study (GWAS) results are combined with trait candidate genes and pathway determination (Samayoa *et al.,* 2015; Tang *et al.,* 2015).

Inferring information from related species to further unravel maize resistance to pests' mechanisms. Another way of understanding the role of herbivore and pathogen-imposed selection on plants is by shedding light on the evolution of defense-related traits, including a diverse array of morphological structures, physiological responses, secondary metabolites, RNAs and proteins (Tiffin and Moeller, 2006). This approach could provide information that could help to guide the development of durable resistant crop varieties and more sustainable pest management strategies (Tiffin and Moeller, 2006). Grass species sequenced to date share an ancient Whole-Genome duplication (WGD) dated around 70 million years ago (MYA) (Yu *et al.,* 2005). Furthermore, of all plant families, the grasses are represented by the most published sequenced genomes with brachypodium, maize, rice, and sorghum, representing three subfamily-level grass lineages (The International Brachypodium Initiative, 2010). This means maize share numerous orthologues with the other three grasses although the second WGD undergone by maize around 5–12 MYA (Swigoȍová *et al.,* 2004) resulted in the existence of two homologous locations within the maize genome co-orthologous to any single location in the genomes of rice, sorghum, and brachypodium (Schnable *et al.,* 2012). Additionally, syntenic analysis of the grasses has also detected evidence of more ancient WGD events shared by most, if not all, monocot species (Tang *et al.,* 2010). It is also estimated that monocot and dicot diverged between 120 and 200 MYA (Wolfe *et al.,* 1989) therefore, maize orthologous mining can be extended to both the other monocot and dicot species. This highlights the possibility of using those evolutionary relationships to infer the functions of genes in one specie from related species and furthermore determine the evolution forces that drove their genomic expansion.

The necessity of considering multi-herbivory attacks in maize defense mechanisms. Numerous studies have been carried out to assess the effect of pairwise interactions between one insect and one plant species at a time (Barah and Bones, 2015). However, in a natural environment, plants do not deal with only one insect; rather they are exposed to combinations of herbivores simultaneously (Heidel-Fischer *et al.,* 2014). Furthermore, plant defense is under influence of complex web of interactions with co-existing biotic and abiotic factors in their respective environment (Rasmussen *et al.,* 2013). Such interaction effects are known to be always severe to plants (Heidel-Fischer *et al.,* 2014). Barah and Bones (2015) observed that very few studies have been carried out to study the responses of plants to a combination of different herbivores. Some of the studies reported the uniqueness of plant responses to a combination of attacks which is not directly inferable from that of an individual attack (Pieterse and Dicke, 2007; Dicke *et al.,* 2009; Utsumi *et al.,* 2010). In fact, Gols (2014) considers that the cumulative effect of a putative defense response may be under-or over-estimated when other interactions are excluded. It is believed that attack by different herbivores comprises dynamic and differential impacts on different levels of the biological organization: trophic levels, phenotypic traits, molecular level etc. (Stam *et al.,* 2014). Therefore, maize being prone to numerous pests (Meihls *et al.,* 2012), it is more useful to conduct studies that combine several attackers through a multidisciplinary approach (Stam *et al.,* 2014).

Additionally, plants show different gene expression responses under herbivore attacks depending on the mode of herbivore feeding, the amount of tissue damage, the specific temporal and spatial patterns at the feeding site, as well as the host plant species (Kessler and Baldwin, 2002). Therefore, studies on local stem borers need to be undertaken to understand local genotypes responses to those pests under local environment. As resistance traits are costly and frequently up-regulated after attack which negatively affects growth, reproduction or storage, this increases assimilation to meet their metabolic demands (Schwachtje and Baldwin, 2008). In maize, this suggests that field attacks by insects such as stem borers may negatively impact on grain filling which may lead to a lesser resistance to storage pests. However, this possibility still remains unclear and need to be investigated.

Benefits of new approaches in studying mechanisms of maize resistance to pests. Breeding for maize resistance to insects is the most suitable approach for integrated pest management. For that, new approaches need to be explored for a better understanding of maize resistance mechanisms. Maize being prone to both field and storage pest, it is not very efficient to develop resistance strategies for one particular pest. Rather combining several pest-resistance genes in one plant would be more useful and safer regarding the importance of the pest on maize both in field and under storage. A major step toward the development of multi-pest resistant maize is by the identification of common resistance mechanisms for pre- and post-harvest pests in maize.

It is important to determine the commonality of the plethora of QTLs reported for maize resistance to pests, particularly stem borers and storage pests. The identification consensus QTL regions (meta-QTL, MQTLs) that determine the genetic basis of resistance to all those pests requires conducting a comparative study of all the QTL results through meta-analysis (Arcade *et al.,* 2004). QTL Meta-analysis is an analytical method that could integrate independent QTL results to identify co-localized QTL (Arcade *et al.,* 2004). Unfortunately, the maize genome database (MaizeGDB) has no curation for QTLs related to pests. Such data curation would ease the meta-analysis of those QTLs and, if meta-QTLs are revealed, it would be very interesting to use them in different breeding strategies for multi-pest resistance in maize.

GWAS is an up-to-date method associating trait variation to a single SNP. It allows relating phenotypic variability to a single or some genes unlike QTL mapping (Korte and Farlow, 2013) which is very useful for gene introgression and pyramiding for crop improvement. This is a promising approach in maize as linkage disequilibrium (LD) is of rapid decay in diverse maize samples. High LD has the benefit of offering a better mapping resolution to delineate potential causal genes within small LD blocks (Samayoa *et al.,* 2015). Currently, obtaining the necessary number of marker for high LD species is quite easy since the event of the NGS technologies especially GBS (Elshire *et al.,* 2011).

Furthermore, tropical maize germplasms have more genetic variability and show faster LD decay than in temperate lines, which is an advantage for GWAS (Lu *et al.,* 2011). The GWAS results can be used to first confirm the new QTL identified by Casto-Alvarez *et al.* (2015) and secondly compare them with QTLs for stem borers resistance. In fact, after identification of consensus QTLs, they can be compared with GWAS results to assess whether SNPs is significantly associated to a trait co-localized with a meta-QTLs. This can be done by BioMercator 4.2, which allows the use of reference genomes information to locate genes or markers on a genetic map (Sosnoswki and Joets, 2012). Such an achievement will be very useful to breeding programs that aim at developing single or multi-pest resistance through introgression, gene pyramiding or more advanced techniques such as genetic engineering.

In determining the genes and pathways involved in maize resistance to local insects, associating GWAS with candidate genes is a promising approach. It can be achieved through a functional and/or positional candidate genes selection (Pflieger *et al.,* 2001). Relating the candidate genes designation to pathway analysis allows revealing the specific biological pathways that are involved under given specific host-insect interactions. Such genetic studies based on candidate genes selection and pathway analysis need to be confirmed through other approaches such as fine mapping and transgenic methods which are long and costly (Pflieger *et al.,* 2001). Fortunately, a first validation step is achievable through differential gene expression analysis to assess whether those genes have different transcriptomic levels between resistant and susceptible genotypes and to identify inducible and consecutive defense-related genes in maize against the concerned insects. Inferring information from known function orthologues from related species through comparative analysis, which is a dynamic and systematic approach for integrating relevant information across species and domains of biology, could also help to achieve that confirmation (Chen *et al.,* 2012). Additionally, this will permit revealing the evolutionary forces that guided the expansion of those genes (Tiffin, 2004) and a comparative analysis to integrate genomic information across several crops (Chen *et al.,* 2012). This will strengthen the understanding of the molecular and metabolic basis of maize stem borers and storage pest resistance.

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STATEMENT OF NO CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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