Review Open Access

A review of genetic analysis and response to selection for resistance to Busseola fusca and Chilo partellus, stem borers in tropical maize germplasm: A Kenyan perspective

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Abstract

This paper provides a review on the context for the research in maize improvement for resistance to stem borers (*Chilo partellus* and *Busseola fusca*) in tropical environments. The following aspects are reviewed a) major productions constraints in East Africa, b) the stem borer problem in maize, c) genetic studies on maize resistance to stem borers. The explanations of key technical issues on progress and challenges in breeding for stem borer resistance in maize, inheritance of stem borer resistance and combining ability in maize, maize heterotic patterns, determination of heterotic orientations, application of the line x tester mating design, screening methods, selection indices, genotype x environment interactions, and response to selection for resistance to stem borers are addressed. Therefore, this paper forms a setting of reference for the study.

Keywords: *Chilo partellus*, *Busseola fusca*, genetic analysis, response to selection

Introduction

Maize is the dominant staple crop grown by a vast majority of rural households in Kenya. It is both a staple food and a cash crop for small-scale farmers (Government of Kenya, 2009). The production statistics of maize in Kenya is depicted (Table 1). Maize accounts for approximately 20% of the total agricultural production, and 25% of employment in the agricultural sector. It constitutes about 3% of Kenya's gross domestic product (GDP), 12% of the agricultural GDP and 21% of the total value of primary agricultural commodities (FAOSTAT, 2013). It contributes about 68% of daily per capita cereal consumption, 35% of total dietary energy consumption and 32% of total protein consumption, amounting to a *pro capita* consumption of 98 kg yr^{-1} (Government of Kenya, 2009). It is grown both for subsistence and as a commercial crop by smallholders (75%) and large-scale farmers (25%). This translates to between 2.7 and 3.1 million metric tons annually. In Kenya, the various maize agroecologies have different characteristics (Table 2). In Kenya, «when there is no maize, there is no food» because of the strong link between food security and the amount of annual maize produced. Unfortunately the yield is very low ranging between 1.6 and 2.0 t ha^{-1} (Tables 1 and 2). Thus the farmer's average maize yield is low when compared to world average of 4.3 t ha^{-1} (FAOSTAT, 2013). Yet an estimated five million farmers in Kenya grow maize at least once in a year on two out of every three farms (Government of Kenya, 2010). A yield potential of up to 6 t ha^{-1} is achievable with the use of improved maize hybrids, irrigation, and the use of fertilizers accompanied with good management depending on the agro-ecological zone (Table 2; Government of Kenya, 2010). However the yield is still compromised by stress factors. Surveys in major maize ecologies in Kenya indicate that most farmers consistently rank poor and erratic rains, low soil fertility, Striga, and stem borer infestation as their most important constraints (Mutunga et al, 2010).

Maize can be grown in almost every agro-ecological zone in Kenya (Figure 1; Hassan, 1998). Three of the agro-ecological zones namely; lowland tropics, dry mid-altitudes, and the dry transitional zones are characterized by low yields (< 1.5 t ha^{-1}). Although these zones cover 29% of Kenya's maize area, they only produce 11% of the maize. The highland tropics, moist transitional, and the mid altitude agro-ecological zones achieve high yields (> 2.5 t ha^{-1}) and produce 80% of Kenya's maize (Government of Kenya, 2010). However, even in this zone grain yield of maize has not reached its full potential due to many constraints.

Maize production constraints in Kenya

Despite the importance of maize and its widespread production and consumption, recent reports indicate dramatic reductions of expected maize yield in counties in the Eastern, Coast, and the Rift Valley regions of Kenya with respective decreases of 79%,

RECEIVED 12/08/2015

Table 1 - Maize area harvested, production, yield and amount of seed in Kenya between 2000 and 2011.

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Area Harvested (1,000 Ha)	1500	1640	1592	1671	1351	1771	1888	1615	1700	1884	2008	2131
Production (1,000 tons)	2160	2790	2409	2711	2607	2906	3247	2929	2367	2439	3464	3376
Yield (t ha^{-1})	1.44	1.701	1.513	1.622	1.929	1.641	1.72	1.813	1.392	1.294	1.725	1.584
Seed (1,000 tons)	49.20	47.77	50.13	40.54	53.13	53.00	48.46	51.00	51.00	57.00	63.96	63.96

Source: FAO Statistics Division 2013

32%, and 14% (Government of Kenya, 2010). These regions are considered the food and grain basket of Kenya. However, reduction in production of maize is about 250,000 metric tons (Government of Kenya, 2010). At the national level, this is likely to impact negatively on livelihood, market prices, and overall food security (Government of Kenya, 2009; Oscar, 2009).

The decline in grain yield can be attributed to various maize production constraints. These production constraints can be grouped into socioeconomic, technological, policy constraints, abiotic and biotic constraints (Oscar, 2009). Socioeconomic, technological and policy limitations facing farmers include use of poor quality seeds, population pressure, land constraints, limitations to market access, poor state of infrastructure, and high costs of farm inputs (De Groote et al, 2004; Government of Kenya, 2010). Abiotic factors affecting maize production include declining soil fertility, low soil pH with associated nutrient deficiencies and toxicities, and low and unreliable rainfall leading to recurrent droughts (Government of Kenya, 2010).

Biotic constraints that affect maize production are foliar diseases (maize lethal necrosis (MLN) disease, turcicum leaf blight, grey leaf spot, and maize streak virus), parasitic weeds (Striga and *Allectra vogelli* spps), and insect pests (stem borers, leaf hoppers, chafer grubs, cut worms, wireworms, maize weevils and the larger grain borer) (Ajala et al, 2010; Morais et al, 2012; Wangai et al, 2012). However the lepidopteran pests, mainly stem borers are one of the most devastating insect pests of maize in sub Saharan Africa (SSA) (Belay et al, 2010). The stem borers are major constraints to maize production because of their significant contribution to yield losses and grain quality degradation. The favourable climatic conditions are more conducive for the accelerated insect development with numerous overlapping generations leading to high infestation levels and losses. Stem borers, are most damaging in the larval stages when they tunnel inside the maize stem after hatching and therefore very difficult to control. Successful infestation of these borers into plants, and their feeding may cause death of growing points, reduction in number of harvestable ears or may cause structural damage that increases the likelihood of lodging (Morais et al, 2012). In some cases these pests also attack maize ears, cobs and the kernels, predisposing them to rots due to fungal attacks which produce mycotoxins.

Among these lepidopteran pests, primarily stem borers, the African stalk borer (*Busseola fusca* Fuller) and the spotted stem borer (*Chilo partellus* Swinhoe) are the most serious pests of maize in Kenya. Their biology of *Busseola fusca* and *Chilo partellus*, and distribution, and economic importance are discussed below.

Biology of *Busseola fusca*

The first concise information about the life cycle and the economics of Busseola fusca was carried out by Fuller in the 1900's (Kfir, 1997). *B. fusca* has two generations in one year; however it may have more than three generations in warm areas of sub Saharan Africa. Its importance increases at higher altitudes. *B. fusca* forms tunnels in stems of host plants towards the end of the rainy season, and the larvae may diapause in areas that experience winter or dry seasons. The eggs are white at first, but later turn darker with time. These eggs are globular and about 1 mm in diameter. They are laid in a long column stretching up the stem, under the leaf sheath. They hatch after about 10 days and the young larvae are deep purple or black in colour. In the early stages, the caterpillars feed on leaves in the whorl of the host plant, resulting in characteristic lines of holes and 'windows'. The larval period takes about 35 days or more. The fully grown caterpillar is about 40 mm long with a pinkish white colour and small black spots along the sides of the body. A mature caterpillar cuts a hole in the stem before pupating within the tunnel and eventually uses this hole to emerge. The pupa is about 25 mm long. The pupal stage lasts about 14 days. Before the crop ripens there are usually two generations whose eggs may be laid on the cob. The caterpillars feed on the cob and later move into the stem. Before pupating they may diapause for long which may last till the next rains. They prepare a pupal chamber in the stem and pupate. The adult is a pale brown nocturnal moth with a wing span of 35-40 mm (Kfir, 1997). The detailed description of the biology of *B. fusca* is given by Mally (1920).

Biology of *Chilo partellus*

The first concise information about the life cycle and the economics of *Chilo partellus* was carried out by Swinhoe in the 1900's (Kfir, 1997). The eggs are laid on the underside of the host plant near the midrib in 3-5 rows and in groups of 50-100. These eggs are flattened, ovoid, and about 8 mm long. Hatching takes place after 7-10 days. The young caterpillars form characteristic holes on leaves and «windowing» from their feeding. In early stages they may mine

Table 2 - Characteristics of maize growing regions in Kenya.

Characteristic	Highland	Mid-altitude Transitional	Mid-altitude moist	Mid-altitude dry	Lowland
Elevation (100 m)	>18	14-18	14-18	9-18	<9
Annual rainfall (100 mm)	<18	10-18	8-12	4-8	4-14
National maize area (1,000 ha)	307	461	118	118	33
National area (%)	30	46	10	10	4
National Production (%)	35	25	25	10	5
Potential yield (t ha^{-1})	6.7	5.2	3.7	2.7	3.3
Farmer yield (t ha^{-1})	2.0	0.7	1.1	0.5	1.0

Source: Government of Kenya.

in the leaves causing streaks. After a few days the young caterpillars bore down through the whorl into the stem of the host plant. In general, *C. partellus* young caterpillars resemble those of *B. fusca* larvae. They are creamy pink with groups of dark spots along the back. The head capsule is brown. When mature they are about 25 mm long. These caterpillars can be distinguished from *B. fusca* and *Sesamia calamistis* larvae by the presence of circular hooks on their prolegs. In *B. fusca* and *S. calamistis* these hooks are arranged in a crescent manner. The larval period takes about 28-35 days. Pupation takes place in 7 to 10 days in a small chamber in the stem of a host plant. The adult moths have a wing span of 20-30 mm. The males are smaller and darker than females. The forewings of males are pale brown while those of females are paler with the hind wings almost white (Kfir, 1997). The detailed description of the biology of *C. partellus* is given by Päts (1992).

Geographical distribution of *Busseola fusca* and *Chilo partellus* stem borers

The biology, habits, distribution and control measures for these injurious insect pests to maize have been described in various literature sources (see Figure 2; Belay et al, 2010; Chaudhary, 2013). *B. fusca* and *C. partellus* as pests of maize have generated a lot of interest for researchers since the last century. The geographical distribution of these two most damaging cereal stem borers of maize and sorghum are probably altitude-dependent (Kfir, 1997; De Groote et al, 2004). *Chilo partellus* reportedly occurs below 1500 m asl, whereas *B. fusca* is found at elevations greater than 600 m asl (Kfir et al, 2002). However, other studies have suggested that temperature, rainfall and humidity are key factors responsible for their distribution, with temperature being most important (Kfir et al, 2002; Ajala et al, 2010). Kfir et al (2002) indicated that *B. fusca* and *C. partellus* are found in warmer and cooler regions respectively.

The distribution and occurrence of *B. fusca* and *C. partellus* stem borers is diverse in Africa (Figure 2; Mailafiya et al, 2011). Several factors affect their population dynamics specifically; host availability, location and suitability, mate location, success of oviposition, larval survival and establishment, temperature and altitude (Mailafiya et al, 2011). The basic hosts are cultivated cereal crops mainly maize, sorghum, pearl millet, finger millet, and sugarcane. The non-cultivated hosts are the wild grasses namely; wild Sudan grass (*Sorghum verticilliflorum*), elephant grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), Johnson grass (*Sorghum halepense*), *Hyparrhenia rufa* and *Rottboellia exaltata* (Kfir, 1997; Mailafiya et al, 2011).

Economic importance of *Busseola fusca* and *Chilo partellus*

Currently, about 50% of the maize area in 25 key maize growing countries in the tropics and subtropics has approximately 60% area under infestation with lepidopteran pests (Ong'amo et al, 2012; FAOSTAT, 2013). These lepidopteran pests include maize stem borers which are most serious in Asia and Africa. In Africa, they are mainly *Busseola fusca* and *Chilo partellus*, the pink stem borer (*Sesamia calamistis* Hampson) and the sugar cane borer (*Eldana saccharina* Walker) (Mailafiya et al, 2011). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al, 2005). This amount represents an average of 13.54% of the farmers' total annual harvest of maize.

Management of the stem borers

A number of strategic approaches for the management of stem borers have potential to either mitigate the damaging effects of these borers; however, each option has its own limitations. For illustration, chemical control methods are most effective; though, they are expensive to most small scale farmers and pose risks to humans, livestock, and the environment. Biological control methods are efficient, cost-effective and environmentally safe; still, they may not be sufficient to manage the pest populations at below economic injury levels (Mailafiya et al, 2009).

Cultural control methods are easy to use and may not involve costs *per se*; however, they have a limited mode of application, may not be applicable to large scale farms, and they have challenges in the timing. The use of genetically engineered *Bacillus thuringiensis* (Bt) crops is a very effective method in the control of stem borers and other lepidopteran pests because the proteins are highly specific in their mode of action, and they control a narrow range of target pests (Yuan et al, 2009). Nevertheless, there are biosafety concerns ranging from ethical and moral, intellectual property restrictions and the payment of royalties,

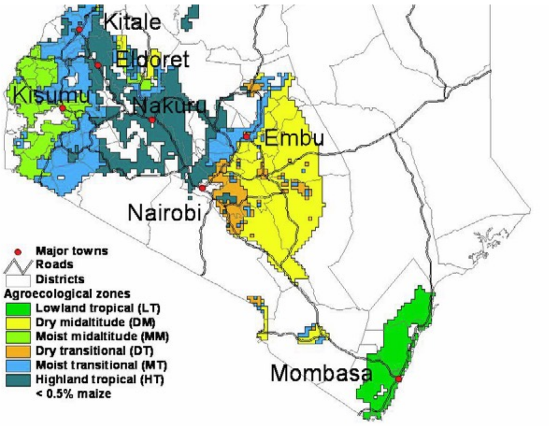

Figure 1 - Maize agro-ecological zones in Kenya. (source: Hassan, 1998).

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). Research on Bt-maize is in progress for implementation in Kenyan farming systems under collaborative projects between the Kenya Agricultural Research Institute (KARI) and its partners. However, even with the current biosafety law and the Agriculture, Fisheries, and Food Authority Act in place it may take longer before farmers realize the products (Mugo et al, 2005; Government of Kenya, 2013). Thus application of biotechnology that involves genetically engineered products is still an evolving option in sub-Saharan Africa.

Host plant resistance using conventional methods is an acceptable method for protecting plants against *B. fusca* and *C. partellus* because there are no biosafety concerns. However, its application is still limited due to the polygenic nature of the insect resistance trait, limited understanding of its inheritance and the high costs associated with plant breeding (Hallauer et al, 2010). Nonetheless, host plant resistance forms an important part of integrated pest management. It provides inherent control without environmental concerns. Host plant resistance is compatible with other pest management approaches (Morais et al, 2012). It is with this background that a large body of literature provides evidence that; farmers would probably continue to grow their accustomed varieties alongside the improved maize from conventional breeding. The genetics of *B. fusca* and *C. partellus* resistance is restricted to a few crosses of maize inbred lines. These challenges may be attributed to the lack of resistant varieties, limited genetic information on stem borer resistance, and limited information on response to selection for borer resistance. Therefore the need to study and improve the understanding of the genetics of *B. fusca* and *C. partellus* resistance in maize, and grain yield under artificial infestation. This will form the basis of a viable breeding strategy for deploying stem borer resistant maize hybrids.

Breeding for resistance to maize stem borers

Suitable maize germplasm should have resistance to both *B. fusca* and *C. partellus* species because the pests may occur together. Recent studies (Kfir, 1997) show that *C. partellus* is progressively displacing *B. fusca* from the high altitude areas in Kenya due to climate change. The problem is further exacerbated by farmers who exchange maize germplasm across agro-ecologies. Currently, there is lack of resistant varieties to both, limited genetic information on stem borer resistance, and limited information on response to selection for borer resistance. Therefore the need to identify resistance in tropical maize inbred lines to both *B. fusca* and *C. partellus* species key for the maize programme in Kenya. It is with this background that effective breeding methods for both pests could be designed by plant breeders using both improved and new sources of stem borer resistance.

Progress and challenges in breeding for resistance to maize stem borers

Various efforts have been undertaken by the International Maize and Wheat Improvement Center (CIMMYT) to include breeding for insect resistance in its breeding programs (CIMMYT, 2008; Tefera et al, 2010). The Insect Resistant Maize for Africa (IRMA) project was a collaborative initiative with the local partners to develop and deploy insect resistant maize for African farmers. Currently, the Water Efficient Maize for Africa (WEMA) project is testing the insect protected maize (Bt maize) at KARI, Kiboko with the long term view of deployment to Kenyan farms. Additionally, the international collaboration continues to enable exchange of germplasm with insect resistance to various countries in Africa, Asia, and others (CIMMYT, 2008). Maize varieties (open pollinated varieties and hybrids) have been identified and released by KARI; resistance levels are low to moderate (Ajala et al, 2010; Tefera et al, 2010) which has limited efficacy of the technology. Consequently, the identified sources of stem borer resistance have not been used extensively. Probably, this is due to linkage drag, pleiotropic effects, or low heritability which discourages breeders to emphasise insect resistance when there is huge pressure from donors to release new varieties in real time. Despite all these ominous efforts, there is a need to increase resistance levels through selection and other strategies, and to manipulate the basis of resistance through use of diverse resistance sources in cultivated germplasm. A lot of pre breeding work needs to be done so that commercial breeders and their NARS counterparts to readily find stem borer resistance in the right genetic background, and from the right heterotic group to make maize hybrids.

Figure 2 - Geographical distribution of *Busseola fusca* (left) and *Chilo partellus* (right) in Africa.
Source: http://www.infonet-biovision.org/default/ct/92/pests and http://www.infonet-biovision.org/default/ct/102/pests (accessed 27.11.2013).

Inheritance and combining ability for resistance to stem borers

Effective plant breeding programs for the development of stem borer resistant maize germplasm requires an elaborate understanding of the gene action involved in the inheritance of the traits. Breeders also want to know whether breeding for stem borer resistance can be achieved without affecting the grain yield potential of the hybrids. Gene action denotes how the expression of traits, separately or in combinations is affected through inheritance. The genetic components affecting quantitative or polygenic traits may be classified as additive, dominance, and epistasis variance (Falconer et al, 1996). Additive variance represents the proportion of a trait that can be transmitted from parents to the progeny, and it characterizes the degree of resemblance between offspring's and their parents (Falconer et al, 1996). It may be expressed by narrow sense heritability. Higher values of narrow sense heritability imply a higher probability of the transmission of the trait from the parent to the progeny. Non-additive gene action is not transmissible to the progeny, and represents all types of deviations that may not be explained by the additive model, and may include dominance and epistasis (Falconer et al, 1996). Given the foregoing, it is imperative to establish the mode of inheritance for stem borer resistance so that appropriate breeding strategies are devised.

Most studies on stem borer resistance in maize indicate both significant general and specific combining abilities, showing that additive and non-additive gene effects are important in governing the resistance (Udaykumar et al, 2013). Stalk resistance to stem borers is complicated because it is polygenic, and involves additive, dominance, and epistatic effects (Sandoya et al, 2010; Barros et al, 2011) which partly explains why breeding for resistance in maize has been really difficult. Furthermore, both additive and dominance effects influence the expression of resistance to *B. fusca* and *C. partellus* (Andre et al, 2003; Kamala et al, 2012) which compromises heritability especially when the non-additive portion is preponderant. However other previous genetic studies have indicated that at least 10 genes are involved in borer resistance, and that gene action is primarily additive (Singh et al, 2012) indicating that higher heritability could be found in some populations. Other studies indicate that in sweet corn resistance to ear damage caused by *Helicoverpa zea*, is controlled by epistatic and, additive dominance effects (Butrón et al, 2009; Singh et al, 2012). In addition, in different maize populations both GCA and SCA effects explain significant levels of variation for resistance to fall army worm, *Spodoptera frugiperda*, and the sugarcane borer, *Diatraea grandiosella* (Dyar) (Oloyede-Kamiyo et al, 2011). Given that stem borer resistance is a polygenic trait with low heritability (Falconer et al, 1996), recurrent selection approaches would be the most appropriate for the accumulation of favourable alleles for resistance.

Recurrent selection in maize

Recurrent selection is a method of that involves selection, recombination, and evaluation of the best test genotypes in successive cycles (Ana Paula et al, 2013) to accumulate high allele frequencies for traits of interest. Generally, the method improves the mean performance of the population, while at the same time maintains the genetic variation. The method is applied for the population improvement for polygenic traits hence it would be appropriate for improving stem borer resistance in maize.

Six different types of recurrent selection strategies have been identified namely; full sib, half sib, S1 progeny, S2 progeny, simple recurrent selection (SRS), and reciprocal recurrent selection (RRS) (Ana Paula et al, 2013). The traits under selection and the number of populations under consideration determine the method to be used in the selection. Both intra-population and inter population recurrent selection approaches are used, but more commonly the former is applied for improvement of a single population (Sandoya et al, 2010). It is predominantly applied for improvement of resistance to insect pests and germplasm adaptation. The effectiveness of recurrent selection approaches depend on trait heritability, selection intensity, and the level of genetic variation in the base population (Acquaah, 2009).

The application of the S1 progeny recurrent selection exposes lethal recessive alleles and reduces the genetic load in the target population and at the same time, it emphasizes additive gene effects which are more appropriate and effective in the improvement of most maize traits. Recent studies in quantitative genetics theory indicate that S1 progeny recurrent selection can be used in breeding for resistance to stem borers in maize populations (Sandoya et al, 2010). Through the S1 progeny recurrent selection, the expected genetic variation considering only the additive genetic effects is four times greater among half-sib families and two times among full-sib families (Sandoya et al, 2008; Hallauer et al, 2010). For these reasons, the S1 progeny recurrent selection was considered relevant for the current study. The strategy is most appropriate given the low heritability of the polygenic traits that constitute stem borer resistance (Hallauer et al, 2010). Given that there are limited studies on response of maize populations to selection for pest resistance, the present study serves as the reference for determining the value of S1 progeny recurrent selection for the improvement of *B. fusca* and *C. partellus* resistance in maize.

Line x tester mating design

Another strategy for breeding stem borer resistance would be exploitation of heterosis in hybrids. Therefore information regarding combining ability of insect resistant inbred lines would be required to expedite development of hybrids. The line x tester mating design developed by Kempthorne (1957) provides consistent information on the general and specific combining ability effects of parents and their hybrid combinations, respectively. The design has been applied in many previous quantitative genetic studies in maize (Sanghera et al, 2012). The design is mainly used to generate data on nature and magnitude of gene action, combining ability effects, heritability and nature and extent of heterosis for different traits. For example, Sprague et al (1942) on studies in maize yield observed that general combining ability is mainly due to the additive gene effects while specific combining ability is attributed to dominance or epistatic gene effects. The line x tester mating design has been used in determining the pattern of gene action for stem borer (*B. fusca* and *C. partellus*) resistance in maize (Sanghera et al, 2012). The application of line x tester mating design is generally in the early generations of breeding mostly S2 or S3 generations to reduce the genetic load. Populations and inbred lines or single cross hybrids have been used as testers (Aguiar et al, 2008). This mating design continues to be applied in determination of the maize heterotic orientations using different testers (Hallauer et al, 2010; Fato et al, 2012). The design was therefore applied in the current study to evaluate the experimental inbred lines and hybrids in the target environments in Kenya.

Heterotic orientations in maize

For efficient development of hybrids knowledge of heterotic groups and patterns is essential. A heterotic group is defined as a group of related or unrelated genotypes from the same or different populations that indicate similar combining ability and heterotic response when crossed with genotypes from other genetically diverse germplasm groups. Furthermore, a heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and therefore high hybrid performance in their crosses (Hallauer et al, 1988).

In maize hybrid breeding, the concept of heterotic groups and patterns is basic (Hallauer et al, 1988; Flint-Garcia et al, 2009). Genetic diversity of the maize germplasm is a key consideration in the design of hybrid-oriented breeding program, where preference is given to the choice of heterotic groups and patterns from divergent populations. The more genetically diverse the parent lines selected for crossing for the formation of hybrids, the higher the hybrid vigour or expression of heterosis (Aguiar et al, 2008). Variations in the gene and allelic frequencies in the inbred lines is the basis for the diverse heterotic orientations.

The basis for selection of the best parents into different heterotic groups varies. Some breeders use trait performance (Estakhr et al, 2012), pedigree information and testcross evaluation (Barata et al, 2006), adaptability and grain yield stability (Badu-Apraku et al, 2011). Also various mating designs (Carena et al, 2010), biometrical approaches (Mather et al, 1982) have been used to determine heterotic groups. Both morphological and genetic markers (Wang et al, 2011) have been widely used to determine genetic groups for maize germplasm. Generally, an array of approaches has been applied to simplify separation of parent lines into heterotic groups (Hartings et al, 2008) that are manageable.

The number of heterotic groups depends on the objective of the programme, but it is generally simplified into two groups, namely A and B. Derera (2005), for example, reported at least nine heterotic groups of maize used in breeding programmes in Eastern and Southern Africa. Similarly, in Kenya, there are nine major heterotic maize groups classified according to the maize growing agro ecological zones (Hassan, 1998). The mid altitude programme has six heterotic groups; Embu 11, Embu 12, Muguga A, Muguga

B, Kakamega pool A, and Kakamega pool B (KARI, 1992; KARI, 2000), while the high altitude programme has three heterotic groups; Ecuador 573 and Kitale Synthetic I and II (Hassan, 1998). At CIMMYT heterotic groups have been simplified into three groups, namely A, B, and AB, which can affect effective utilization of new inbred lines in the programmes. Good maize inbred lines may be discarded when effective testers with high discrimination capacity are not used in hybrid oriented programmes. Among other objectives the current study aims to determine whether CIMMYT single cross testers would be effective for discriminating new stem borer resistant inbred lines according to grain yield under stem borer infestation.

Determination of heterotic orientations

Germplasm variation is of primary importance for hybrid breeding and population improvement programs. Characterization of the maize germplasm and its assignment into different heterotic orientations is useful in providing information about the genotypes (Hallauer et al, 2010). Numerous methods have been applied in the allocation of maize lines into different heterotic orientations (Schnable et al, 2013). Heterotic orientations among inbred lines and the best hybrid combinations can be identified using information from several approaches namely: quantitative genetic analysis; testcrosses to testers; pedigree information; morphological traits; and molecular markers (Fato et al, 2012; Sanghera et al, 2012; Liberatore et al, 2013). Quantitative genetic analysis based methods depend on gene frequency variations among the parental genotypes used in the crossing. Variations in the genetic structure determine the relationships among heterotic orientations of germplasm. Inbred lines are assigned to different groups' relative heterosis to the mean of the testers or based on the SCA estimates. Based on the heterosis data, lines that display significant heterosis in their crosses are allocated to the opposite groups.

Clustering of lines into heterotic groups depends on the direction of the specific combining ability such that lines exhibiting positive SCA with tester are allocated to the opposite heterotic group, and vice versa, whereas lines displaying positive SCA to both testers are designated as both groups (Hallauer et al, 2010; Fato et al, 2012; Sanghera et al, 2012). In the literature, the SCA effects based classification is considered to be more reliable because they have better predictive value for F1 grain yield than heterosis based classification (Aguiar et al, 2008; Hallauer et al, 2010; Fato et al, 2012; Sanghera et al, 2012). The heterosis based grouping is subject to environmental effects which might mask expression of heterosis or that heterosis changes from one site to another due to genotype x environment interactions.

Applications of molecular markers are a more powerful tool to discriminate heterotic orientations and to allocate inbred lines into current heterotic groups and for diversity analysis (Aguiar et al, 2008) because the markers are not affected by genotype x environment interactions. It is reported in the literature that a combination of various approaches in the allocation of inbred lines into dissimilar heterotic orientations is more meaningful than a single method (Aguiar et al, 2008). In the current study, both SCA effects based classification and heterosis based grouping were applied in designation of genotypes to their different heterotic orientation.

Methods of screening maize germplasm for resistance to stem borers

Successful screening of maize materials for selection or evaluation requires normal vigorous plants. Plants exposed to different stress conditions (drought, salinity, heat, low soil fertility, etc) may obscure the expression of resistance or plants may be 'escapes' which contributes to low heritability or low repeatability for insect resistance in maize. Heterosis or different maturity groups may also determine screening methodology for comparison. The use of local resistant and susceptible checks may help in determining the threshold of comparison of maize test genotypes.

Screening methods and rating

Artificial infestation is the most effective manner for screening maize germplasm. However, the larval colonies used especially the insectary-reared stem borer larvae and egg masses, should be vigor-

Table 3 - Scale for scoring stem borer damage from seedling to whorl-stage in maize.

Numerical scores	Visual ratings of plant damage	Reaction to resistance
0	No damage	Probable escape
1	Few pin holes	Highly resistant
2	Few shot holes on a few leaves	Resistant
3	Several shot holes on leaves (<50%)	Resistant
4	Several shot holes on leaves (>50%) or small lesions (<2cm long)	Moderately resistant
5	Elongated lesions (>2cm long) on a few leaves	Moderately resistant
6	Elongated lesions on several leaves	Susceptible
7	Several leaves with long lesions with leaf tattering	Susceptible
8	Several leaves with long lesions with severe leaf tattering	Highly susceptible
9	Plant dying due to death of growing points (dead-hearts)	Extensively sensitive to damage

Source: Adapted and modified from CIMMYT (1989).

ous and survive to cause feeding damage to the test genotypes under field conditions. Infestation should be carried out mid-morning or in the late afternoon to limit desiccation of larvae. Consistency for the number of insect larvae per plant used for infestation is most critical in discriminating the test genotypes. Factors, such as plant vigour, plant age, temperature and relative humidity may influence the observations on the test genotypes (Ajala et al, 2010; Tefera et al, 2010). In maize screening for resistance to stem borers, the level of plant damage on leaves is used in the rating. Mostly, the visual rating scale system is used (Table 3).

There are two methods of infestation with stem borers namely; natural and artificial. Natural infestation is the use of hotspot areas where the pest incidence is very high and mostly coincides with the critical stage of crop growth. Uniformity in the distribution of the infestation is challenging due to lack of stable pest populations over seasons, and the possibility of test genotypes being «escapes» or be over infested. In contrast, artificial infestation is the most reliable and most effective method of screening maize germplasm. Through artificial infestation consistency is achieved since each test plant is infested with at least 20 first instar larvae or neonates or egg masses at the whorl stage 14 days after planting. Infestation may be carried out manually using camel hair brushes or through the use the bazooka applicator for large-scale testing (Tefera et al, 2010).

Leaf disk bioassays method

Breeding methods for resistance to borer damage requires reliable screening approaches. However, quick screening methods for maize genotypes for stem borer resistance are limited. Currently, screening involves splitting of stalks for measurement of cumulative tunneling, counting the number of exit holes and dead hearts, which are time consuming and labour intensive, therefore, the need to optimize a detached leaf bioassay screening method in the greenhouse and laboratory is essential. The use of isolated leaf bioassays for artificial screening of maize genotypes for stem borer resistance may be a practical alternative method than the splitting of stalks for measurements and counts. Natural infestation may not be reliable due to lack of uniformity and seasonal variations that occur (Tefera et al, 2010). The use of artificial infestation in a controlled environment allows multiple screenings within a short time. Leaf screening bioassays have been used as rapid methods for screening materials in a wide range of horticultural and agronomic crops against pests and diseases including Bt maize trials (Mugo et al, 2001; Murenga et al, 2011; González et al, 2013). However this has not been tested for its efficacy in discriminating genotypes for stem borer resistance in maize breeding. Therefore the current study, aimed at appraising this approach against traditional screening methods with a view to lower costs and increase speed, and heritability in breeding maize for stem borer resistance.

Selection indices

Selection indices are multivariate techniques that combine information of different traits of agronomic interest with the genetic properties of a population. In the application of selection indices, numerical values are weighted and serve as an additional hypothetical trait resulting from a combination of various traits of interest (Mutinda et al, 2013). Selection for resistance to stem borers, *B. fusca* and *C. partellus* based on a single parameter is difficult since a resistant genotype has a certain aspect of damage that may be susceptible to another form or when pressure is increased. Trait interactions associated with a reduction in the amount of grain yield include: leaf feeding damage, dead hearts tunneling and exit holes. Appropriate indices, are useful in assisting breeders for concurrent selection for resistance per se, in addition to grain yield performance. Various examples in the applications of selection indices with improvements in stem borer resistance and grain yield in maize have been reported in the literature (Ajala et al, 2010).

Genotype x Environment Interactions

Genotypes x environment interactions are of considerable influence to response to selection and efficiency of resistance breeding programmes (Butrón et al, 2004). There are two types of genotype x environment interactions namely; cross over and non-cross over interactions which affect genotype performance and crop improvement. The cross over type exemplifies the instability of genotype performance (Hallauer et al, 2010). The cross over type limits breeding progress due the alterations in constitution of selection at every environment and represents the genotypes' specific adaptation across environments. However, non crossover type represents stability of performance across the unfavourable environments, where cultivars are ranked consistently across environments resulting in analogous selection in all environments. The capacity of the new testcross hybrids to produce higher grain yield may be attributed to their ability to adapt to the biotic or abiotic stress conditions (Butrón et al, 2004; Carena et al, 2010). In the current study, experiments were set up in different mega environments because the genotype x environment interactions were an important consideration; because insect infestation also depends on whether favourable conditions prevail for insect feeding, fertility and development.

Conclusions from the literature review

From the review of literature, the two stem borers, *B. fusca* and *C. partellus* are identified as one of the most devastating insect pests limiting maize production in tropical environments. Suitable maize germplasm should have resistance to *B. fusca* and *C. partellus* borers where they occur. There is a need to breed and promote genotypes with *B. fusca* resistance, and to encourage wide adoption across maize agro-ecologies of the competitive hybrids with *B. fusca* resistance. Breeding for resistance to *B. fusca* and *C. partellus* requires a good understanding of heritability of resistance, gene action, and combining ability effects in relation to heterosis among the testcrosses. The S1 progeny recurrent selection was considered relevant for the current study and useful given the low heritability of the polygenic traits that constitute stem borer resistance, because a larger portion of additive genetic variance would contribute towards breeding progress. The line x tester mating scheme using single cross testers was preferred since in generating information on gene action, the products formed from the testcrosses would be three way crosses which will be deployed immediately into the national performance trials for further testing. The majority of the farmers in SSA use three way cross products since the cost of seed is less compared to that of single crosses. Screening for resistance to stem borers is an important component of breeding for resistance. Quick screening methods for borer resistance should be found because current approaches are time consuming and labour intensive. The use of detached leaf disk bioassay and whole plant assays methods for screening for *B. fusca* and *C. partellus* resistance maize in the laboratory and greenhouse trials would provide a rapid technique that would enable breeders to screen and make decisions faster towards breeding progress. Therefore the need to carry out an appraisal of the leaf disk bioassay and whole plant assays in both the greenhouse and laboratory.

Acknowledgements

Thanks to The Alliance for a Green Revolution in Africa (AGRA) for financial support during the research, CIMMYT, Kenya for hosting and for providing me with the germplasm, office of the Director General, Kenya Agricultural and Livestock Research Organization (KALRO) for granting me study leave, and for providing the research facilities at the KALRO Biotechnology Centre during the research period.

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