A COMPARATIVE ANALYSIS OF CONVENTIONAL AND MARKER ASSISTED SELECTION METHODS IN SCREENING FOR RESISTANCE TO MAIZE (*Zea mays L.*) STREAK VIRUS DISEASE

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GENERAL ABSTRACT

Maize (*Zea mays* L.) streak virus disease (MSD) is the most important virus disease in Africa but farmers are unaware of its status. A project was initiated to assess the current status of MSD and to breed for its resistance. Four populations comprised of two BC1F1 and two F2 progenies developed by backcrossing and selfing the F1 progenies of two crosses between a donor line (CML 202) and two susceptible lines (CML 321 and CML 384) were developed. Conventional and molecular marker assisted selection (MAS) methods were used to screen for resistance to MSD in each of the four populations. To facilitate unbiased comparison, separate screening nurseries were established for MAS and conventional screening. The objectives of the study were five-fold; 1) to assess the status of MSD in Uganda and understand farmers' preferences and varietal selection criteria for maize using a participatory rural appraisal (PRA), 2) to screen for MSD resistance in early generations of segregating maize populations using conventional method, 3) to screen for resistance to MSD using SSR marker assisted selection , 4) to compare the effectiveness of marker assisted selection and conventional methods for selection for resistance to MSD, and 5) to compare costs associated with MAS and conventional selection methods.

Results of PRA showed that unreliable rainfall and insect pests were the dominant constraints to maize productivity in Uganda. Diseases were ranked fifth among the production constraints. Maize streak virus disease was considered the most important disease constraint. Farmers showed common preference for high yielding and early maturing cultivars. However, farmers had other special preferences which were diverse and included large, white and high test density kernels for marketing, and sweet taste, particularly for home consumption. Farmers' research priorities included tolerance to drought, resistance to insect pests and diseases, sweetness, prolificacy, resistance to lodging, and drooping leaves because theyt cover the soil fast and prevent weed growth.

Conventional screening for resistance to MSD showed that backcross and selfing populations segregated in 1:1 and 3:1 Mendelian ratios confirming the presence of one major gene with simple inheritance. Severity and incidence of disease were positively correlated suggesting a non-preference by the insects. In the selfing populations, the presence of complete resistance against MSD was suggested because frequency distribution patterns were highly skewed in favour of resistance. There was a decrease in disease severities with selection from BC1F1 to BC2F1 and from F2 to F3 generations indicating that high response to selection was achieved. On the other hand, one marker, *umc*1917, consistently polymorphic and co-dominant was selected and used in MAS protocol. Results showed that the observed outcomes fitted the expected ratio of 1:2:1 for a F2 population and 1:1 for a BC1F1 population (X^2 not significant). Evaluation of F3 and BC2F1 progeny selected using markers showed low disease severity suggesting that marker assisted selection was effective. However, the study showed that the presence of the QTL was not consistent with symptom expression in the field.

Evaluation of lines in three-way crosses identified ten potential lines that were high yielding, highly resistant to MSD and stable across three locations. Both MAS and conventional selection were equally effective in identifying high yielding lines although resistance was higher under MAS.

Costs of MAS and conventional method varied depending on the units for comparison. The total costs of conventional method were higher than that of MAS in both first and second selection cycles. Comparing costs per row for conventional and costs per plant or data point for MAS showed that conventional selection was 2.4 times more expensive than costs per sample for MAS. However, costs per plant for MAS were 6.6 times higher than for conventional selection.

DECLARATION

The thesis studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged in the text.

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DEDICATION

To my Lord and Saviour, Jesus Christ, to the Glory of God the Father. You are Ebenezer; the Lord who brought me thus far! To my family: my husband, Mr. John Bosco Wabwire, to my baby, Santanita Wabwire, and to my mother, Santa Acayo. Your love for me is the reason I have persevered and studied this far.

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General Introduction

Maize (*Zea mays*. L) is a staple food crop in Uganda and chiefly produced by resource-poor, small-scale farmers (Pingali, 2001). It also plays a major role as a food security crop in both rural and urban areas. Production per unit area in Uganda has not increased much over the last decade and yet its demand is steadily rising (FAOSTAT, 2005). Total production registered in the past was a result of increase in area under production but not increase in yield.

Varietal adoption has been slow because breeders have not often been well informed of the special needs and preferences of farmers (Banziger and De Meyer, 2002; Toomy, 1999). There was a need to incorporate farmers' needs and preferences in the selection. Hence a PRA was included as part of the initial study to understand and integrate farmers' special needs in the selection process.

Both socio-economic and biophysical factors lie behind the persistent gap between potential yield and yield on farmer's fields. In subtropical regions, the dominant biotic and abiotic constraints to bridging the gap between potential and actual yield are declining soil fertility, gray leaf spot (*Cercospora zeamaydis*), maize streak virus, weevils, borers and drought (Pingali, 2001). Maize streak virus is considered the most extensively distributed biotic constraint in Africa (DeVries and Toenniessen, 2001).

Maize streak virus (MSV), belonging to genus Mastrevirus and Geminiviridae family, is an indigenous African virus transmitted by a range of leafhoppers in the genus *Cicadulina* (Storey, 1925). It causes maize streak virus disease (MSD), the most damaging virus disease of the crop in Africa (Thottappilly, 1992). Maize streak virus has become a major disease of maize in Africa and it is most prevalent in tropical lowlands (≤ 800m altitude) and part of the tropical mid altitude (800-1500m altitude) maize growing areas (DeVries and Toenniessen, 2001; Okori *et al.*, 1999). The pathogen causes serious yield losses, but its occurrence is sporadic and unpredictable (Mesfin and Bosque-Perez, 1998; Diallo, 1999). Yield losses due to the disease are not easy to quantify and may range from 0-100% (Ampong-Nyarko *et al.*, 1998; Diallo, 1999; ISAAA, 2001) depending on the year and stage of growth of maize plant when it is attacked. For example, plants attacked at early stages of growth (up to seven-leaf stage) sustained losses of 80% or more, while those attacked

shortly thereafter (at the nine-leaf stage), incurred only 20% yield loss (Ampong-Nyako *et al.*, 1998).

Practices such as timely planting and treatment of seed with systemic insecticides can help control yield losses, but a more pragmatic and effective solution for resource poor or subsistence farmers would be use of high yielding maize that carries genetic resistance to MSD. This could be done through breeding for durable maize host resistance.

While significant progress has been made on raising the yield potential of tropical maize, most of those genetic materials do not possess the tolerance and resistance needed to overcome the biological stress encountered by maize farmers in a particular ecological and / or geographical region (Pingali, 2001). Fortunately, resistance sources to all the major diseases have been identified and used to improve a number of agronomically acceptable cultivars (Pratt *et al.*, 1997; CIMMYT, 2002).

Currently, the National Maize programme (NMP) in Uganda focuses on extraction of inbred lines from adapted locally developed or introduced lines for the production of 3-way hybrids and synthetics. With support from the International Maize and Wheat Improvement Centre (CIMMYT) and the Rockefeller Foundation, some inbred lines have been derived from popular cultivars Longe 1 and LP 16. In addition, the NMP has studied and characterised many CIMMYT inbred lines that have good combining ability and are resistant to northern leaf blight (NLB) and gray leaf spot (GLS) but susceptible to MSD. What remains then is to improve these inbred lines for resistance to MSD.

Rationale and significance

The National Agricultural Research Organisation/NMP of Uganda, like many breeding programmes, relies on conventional breeding methods for the improvement of maize cultivars. While this has produced important genetic gains in maize e.g. release of agronomically acceptable and disease resistant cultivars such as Longe 1 and 4, the breeding progress has been slow. Breeding of new cultivars using conventional methods are time consuming. In Uganda, it takes a minimum of seven years to produce a cultivar. The successes of such breeding programmes depend on environmental conditions that determine the presence of diseases. Consequently, where the desired diseases fail to

develop, the researcher would have to defer selection or wait for another season to acquire needed data.

Maize streak virus disease development under natural conditions depends on year and season (Mesfin and Bosque-Perez, 1998). In Uganda, screening for resistance to MSD has been done with the use of spreader rows. This technique has been effectively used in screening for MSD resistance in Zimbabwe (Caulfield, 1997) and Mozambique (Denic, 1997) and has produced successful results. However, the main challenge faced when using spreader row techniques is that disease symptoms usually appear after four weeks (Caulfield, 1997). Whenever such delays occurred, disease assessment would be done late: at flowering stage. This would coincide with the critical stage for NLB and GLS infection making accurate severity rating difficult. An artificial inoculation method is used to achieve relatively uniform infection at early stage. This procedure involves rearing leafhoppers in cages on pearl millet (*Pennisetum americanum* (L.) K. Schum). The leafhoppers then acquire the virus by making them feed on infected maize plants before transferring them on seedlings in the screening nurseries (Bosque-Perez and Alam, 1992). This is an expensive procedure in terms of time and labour. DNA markers, described as alleles of loci at which there is sequence variation or polymorphism in DNA that is neutral in terms of phenotype (Jones *et al.* 1997) can be employed in breeding in a procedure known as marker assisted selection (MAS). Marker assisted selection technique is most likely to confer an advantage over conventional breeding techniques when phenotypic selection is difficult, time-consuming or expensive (Dreher *et al.*, 2000).

To date, economic constraints due to the prohibitive costs of the laboratory work involved in molecular screening are a major obstacle to the widespread incorporation of MAS in breeding programmes (Moreau *et al.*, 1998; Dekkers and Hospital, 2002; Dreher *et al.*, 2000; Koebner and Summers, 2003). The cost of MAS can be high especially when beginning from quantitative trait loci (QTL) mapping (Wilcox *et al.*, 2002). However, a critical practical evaluation of different breeding schemes for an inbred line conversion to quality protein maize has shown that cost of MAS varied depending on circumstances (Dreher *et al.*, 2000). For example, when comparing the cost of actual application of MAS and conventional selection, MAS was reported to be cheaper than conventional selection (Ragot and Hoisington, 1993; Gu *et al.*, 1995; Yu *et al.*, 2000). These authors considered different parameters in estimating the cost of MAS. Ultimately, cost effectiveness of MAS over

conventional selection would depend on the interests of individual programmes, but the practicality of it can not disregard their economic implications. For Ugandan situation, the use of MAS for selection for resistance to MSD could be advantageous due to the problems associated with natural virus infection in the breeding nurseries. The use of MAS would then improve on the efficiency of selection for resistance to MSD.

Considerable research has been undertaken to investigate suitable molecular markers linked to resistance loci for MSD (Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a, b). The loci for MSD have been mapped consistently to the same chromosome location in multiple field tests in Zimbabwe (Kyetere *et al.*, 1999; Pernet *et al.*, 1999a and b), Reunion (Welz *et al.*, 1998; Pernet *et al.*, 1999a and b) and Uganda (Kyetere *et al.*, 1999). Following the mapping of MSD QTL, molecular markers associated with MSD resistance QTL have been identified by CIMMYT and are accelerating the development of resistant cultivars by many breeding programmes. Application of MAS in breeding process has been carried out mostly based on available information on map position of traits with agronomical importance and on the linked molecular markers. This study was one of those that relied on such available information. Simple sequence repeat (SSR) marker, known to amplify within the same location where a major MSD QTL was detected (chromosome 1, bin 1.04) was used (Kyetere *et al.*, 1998; Pernet *et al.*, 1999a, b; Welz *et al.*, 1998). Simple sequence repeats are PCR based multi-allelic markers which are rapidly becoming the molecular markers of choice for linkage map development in plants (Chin *et al.*, 1996; Cregan and Quigley, 1996; Taramino and Tingley, 1996) and for MAS.

There are other different molecular markers available for MAS procedure. However, not all the markers available can be used efficiently. Mohan *et al.* (1997) suggested two criteria for selection of markers for MAS: 1) the markers should be efficient in screening of large populations, and 2) markers should show a high degree of reproducibility across laboratories. Molecular markers should all be cost-effective (Thomas, 2003). Restriction fragment length polymorphisms (RFLPs) are reliable and yield co-dominant data, but are also time-consuming and expensive, requiring relatively large amount of highly purified DNA and they do not lend themselves to automation (Gupta *et al.*, 1999). Random amplified polymorphic DNA (RAPD) markers are unreliable with poor replication success among laboratories (Penner *et al.*, 1993; Hallden *et al.*, 1996). Sequence characterised amplified regions (SCAR) markers are more reliable than RAPD markers, but are often developed

from RAPD markers (Paran and Michelmore, 1993), which might limit their utility. Simple sequence repeats (SSR) markers, however, combine reliability and genomic abundance with high levels of polymorphism and co-dominance (Chin *et al.*, 1996; Cregan and Quigley, 1996; Taramino and Tingley, 1996; Mohan *et al.*, 1997). Simple sequence repeats have become the marker of choice in the public sector. Recently, there is increasing use of single nucleotide polymorphism markers (SNPs) in maize (http://www.maizegdb.org/)). However, this is mostly in the private sector as start-up costs are very expensive. Simple sequence repeats, on the other hand, do not require sophisticated DNA extraction methods (Tang *et al.*, 2003) making them suitable for Ugandan situation where old-fashion DNA extraction techniques (e.g. manual leaf grinding in liquid nitrogen) and there is no proper bio-safety measures/ equipments to handle radioactive compounds required for some of the techniques. The main drawback of SSRs is the initial identification of primer sites to amplify SSR loci, a procedure which is time- and resource demanding. In the present case, a large number of SSR markers are already available. Thus, MAS using SSR markers are most likely to become a valuable tool in plant breeding.

Objectives of the study

The overall objective was to determine an efficient and effective method for breeding for resistance to MSD resistance in early generations of selection in Uganda.

The specific objectives were to:

1. assess the status of MSD in Uganda and understand farmers' preferences and varietal selection criteria for maize,
2. screen for MSD resistance in early generations of segregating maize populations using conventional method and SSR marker assisted selection,
3. compare lines from marker assisted selection and conventional methods for resistance to MSD and yield in testcrosses, and
4. compare cost associated with marker assisted selection and conventional selection methods.

The hypotheses tested in the study were:

1. farmers' preferences for maize cultivars correspond to the traits breeder select for, and

2. marker assisted selection and conventional selection methods are equally efficient and effective in breeding for resistance to MSD in maize.

Regionally adapted MSD resistant donor line (CML202) was crossed with susceptible lines, CML321 and CML384, which have important desirable traits to generate the source populations for selection. The choice of F2 and backcross (BC) populations was based on the fact that breeders usually use them for developing inbred lines (Jenkins, 1978; Bauman, 1981).

The study was conducted in Uganda from 2003 to 2006. Field research was based at Namulonge Agricultural and Animal production Research Institute (NAARI). The Laboratory work was done at Makerere University, Faculty of Agriculture Biotechnology Laboratory. The final evaluation of the materials was done at three locations, namely, NAARI, Iganga and Masaka districts.

The thesis structure was as follows:

Thesis structure

General Introduction	
Chapter One	Literature Review
Chapter Two	Participatory Rural Appraisal
Chapter Three	Conventional selection for MSD resistance
Chapter Four	Marker Assisted Selection (MAS) for MSD resistance
Chapter Five	Comparison of Conventional and MAS selection methods
Chapter Six	Comparison of costs of MAS and conventional methods
Chapter Seven	Research overview

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CHAPTER ONE

Literature Review

1.1 Introduction

A review of literature has been compiled to cover different areas of advances in maize research. This review tackled areas on importance and utilisation of maize around the world. It also covered production and major factors hampering production. Critical analysis of gains realised from conventional and marker assisted selection (MAS) methods has been made. Efficiency of MAS, challenges and limitations of using MAS are highlighted as well. Farmers' preferences were also reviewed.

1.2 Importance of Maize

Maize (*Zea mays* L.) is a major cereal crop and was considered to be the third most important cereal crop in the world after wheat and rice up to the end of the 1980's (Rouanet, 1987). Recently, worldwide, maize have been ranked second in production among the major cereal grains (Duvick and Farnham, 1997) but due to a shift in cereal demand, maize is expected to be the leading cereal surpassing both wheat and rice (Pingali, 2001). The trend could be different now, with maize ranking as the leading cereals but there is no data to support.

Maize is grown for both human and animal consumption. In the developed countries, maize is used primarily as animal feed and secondarily for production of food and industrial products such as starch, sweeteners, and alcohol (Duvick, 1997). In developing countries, maize is often grown as a food crop for home consumption as well as for the market. Increasingly it is also used for animal feed. For several countries in Africa and Latin America, maize is the food of first choice.

To date, the industrialized world still uses more maize than the developing world, but the trend indicates that within a couple of decades (by 2020) developing countries will demand more maize than the industrialized world as a consequence of both population growth and increasing urbanization (Duvick, 1997; Pingali, 2001). In the last decade, it was estimated

that by 2020 maize demands in industrialised and developing countries would be 216 MMT and 412 MMT, respectively (Rosegrant *et al.*, 1995). World demand is predicted to rise to about 138 percent of 1995 demand (Rosegrant *et al.*, 1995, USDA, 1996). According to IFPRI (2000) higher demands are expected. In the developing world alone, maize requirement is predicted to increase from 282 million tons as it was in 1995 to 504 million tons in 2020 (IFPRI, 2000). While globally, the area planted to maize is expected to increase during the next 25 years, it will be at a slower rate than in the past quarter of last century.

1.3 World production

It is estimated that 140 million hectares of maize is grown globally and approximately 96 million of that total production area is in developing countries (Pingali and Pandey, 2001). Despite that, only 46% of the world maize is produced in developing countries. Low average yield in the developing world is considered one of the causes of the wide gap between the global share of area and share of production. Food and Agricultural Organisation (FAOSTAT, 2004, 2005) reported worldwide average maize productivity at 4 t ha^{-1}, but yield in Africa averages only 1.7 t ha^{-1}. Due to significant social and cropping systems, the USA yield alone is 92% above the global average followed by China with 19% above average). Wide disparities in climatic conditions (tropical versus temperate) and in farming technologies account for the yield differential between the developed and the developing world (Pingali, 2001). For developing countries, Latin America and sub-Saharan Africa produce the most tropical maize, while temperate maize production is mainly from China and Argentina.

Maize is also increasingly becoming an important non-traditional agricultural export crop. A 3% increase in maize demand per year is reported for East Africa due to expanding population (CIMMYT, 2002). Although its palatability is often cited as a reason for maize's continued popularity among rural populations of eastern and southern Africa, higher productivity and lower labour demands can probably be assumed to be at least as important (DeVries and Toenniessen, 2001). Sorghum and millet yields in eastern and southern Africa since 1980 have averaged 0.765 and 0.729 t ha^{-1}, respectively, compared with 1.19 t ha^{-1} yields of maize over the same period. While a portion of these differences can be attributed to the different environments in which the crops are grown, even when grown under identical conditions in semi-arid southern Africa, maize was shown to yield higher than sorghum.

1.4 Maize production and utilisation in Uganda

In Uganda, maize is a staple food crop chiefly produced by resource-poor small-scale farmers (Bigirwa *et al.*, 2001; CIMMYT, 2002; FEWS Uganda, 2002; Doss *et al.*, 2003). It also plays a major role as a food security crop in both rural and urban areas (Doss *et al.*, 2003).

Production per unit area in Uganda has not increased much over the last six years and yet its demand is steadily rising. Total production increase registered in the past was a result of increase in area under production but not yield (Table 1.1) (FAOSTA, 2005). Production trends over five years from 2000 to 2004 have been fluctuating within a limited range (Table 1.1).

Table 1.1 Maize production trend in Uganda

Year	Area harvested (1000/ha)	Yield (tonnes/ha)	Production (1000 tonnes)
2000	629	1.74	1096
2001	652	1.8	1174
2002	676	1.8	1217
2003	710	1.7	1207
2004	750	1.8	1350

Source: FAOSTAT (FAO 2005)

Per capita consumption of maize in Africa is highest in eastern and southern Africa. In Uganda, the average per capita consumption of maize was estimated at 39 kg per year with an annual growth rate of 3.6%. This figure indicated that production of maize fell short of demand (Aquino *et al.*, 2001). To cope with an ever-increasing population and export demand, current production needs to be increased. According to DeVries and Toenniessen (2001), this can be done through additional resource inputs and protection of potential yield level from various biotic and abiotic stresses.

1.5 Maize production constraints

Despite maize increasingly having become such an important cereal and staple food crop, the average yield in most developing countries particularly in Africa is still the lowest in the world. World-wide average maize yield is about 4 metric tons per hectare while in Africa it is estimated at 1.7 t ha^{-1} only (FAO, 2005). Unless this situation is reversed, food dependency will increase in much of the continent. Both socio-economic and biophysical factors are the main contributor to the persistent low yields on farmer's fields. DeVries and Toenniessen (2001) summarised these factors in five categories which include: a) the range and intensity of biophysical constraints; b) large agro-ecological variation; c) the under developed state of seed sectors in most developing countries; d) the absence of policies which encourage crop improvement; and e) very low and declining soil fertility in much of Africa.

1.6 Biotic and abiotic constraints

Maize is attacked by an array of biotic and abiotic factors that curtail productivity. These abiotic constraints include drought, declining soil fertility, high acidic soils, soil erosion, late and early maturing germplasm, high temperatures and lack of improved germplasm for the tropical highlands (Pingali, 2001). Biotic factors are primarily related to tropical insects and diseases, and weeds. According to Pingali (2001), the dominant diseases that significantly hinder bridging of the yield gap between potential and actual yields in Sub-Saharan Africa are downy mildew (*Peronosclerospora sorghi)*, turcicum leaf blight (*Exserohilum turcicum)*, maize streak virus (MSD), gray leaf spot (*Cercospora zeaemaydis*) and various species of stalk borers (*Chilo patelus, Sesamia calamistis, S. cretica, Busseola fusca* and *Eldana saccharina*). These constraints are not reviewed in the current study because the focus of the study is on MSD.

1.7 Farmers' preferences

In developing new cultivars and extending them to farmers, the formal breeding sector has often encountered two setbacks (De Groote *et al.*, 2002). First, most new cultivars have been unacceptable to farmers (Witcombe *et al.*, 2003). Secondly, breeders have necessarily discarded many crosses because of traits considered undesirable yet these traits may be of interest to farmers (De Groote *et al.*, 2002).This is because the breeders are not often well

informed of the needs and preferences of farmers (Toomy, 1999; Banziger and De Meyer, 2002).

Breeders and farmers' mode of varietal selection vary considerably. Breeders' selections are based on data generated from highly controlled experiments. Farmers, however, select cultivars based on small experiments and observations in the field and visual evidence, using intuitive multifactor analysis. Some of the major selection criteria used by farmers were reported as grain colour, yield, plant height, and maturity (Sthapit *et al.*, 1996).

Farmers' preferences and selection criteria are not always the same in different agro-ecologies. Kernel colour, taste and ear aspect were found to be among the most important characteristics in Kenya (De Groote and Siambi, 2002). In Uganda, cob size, kernel size and colour were also reported among the top priorities of farmers' needs (Gibson *et al.*, 2005). These preferences showed that they were not interested in yield *per se*. In fact, they were willing to trade yield for other traits like storability and weevil resistance (Williams *et al.*, 2006).

Maize farmers in developing countries often save seed from their own production to plant in the following season (Morris *et al.*, 1999; Gibson *et al.*, 2005). The choice of seeds saved by farmers appeared to be influence by market demands. Farmers have indicated that white large kernels were easy to market (Gibson *et al.*, 2005) as opposed to yellow kernels. Involving farmers more closely from the beginning in varietal development process is likely to increase the varietal adoption (De Groote *et al.*, 2002).

Farmers' requirements have to be identified first so that they can be given more appropriate genetic materials for their utilisation (Witcombe *et al.*, 1996). Unfortunately, formal research systems in developing countries are highly centralised and do not target the problems of resource-poor farmers (Sthapit *et al.*, 1996). As a result, appropriate cultivars are not reaching farmers; instead inappropriate cultivars are being recommended (Joshi and Witcombe, 1996). This was because research in the recent past had paid little attention to farmers' preferences but this changing with the realisation that if farmers' preferences are not included, varieties released would not fit farmers' expectation, hence would not contribute to improvement of their livelihoods. Furthermore, recent approaches by CIMMYT using Mother-Baby Trials have shown that inclusion of farmers in selection and variety

selection and development enhances identification of appropriate varieties. As such, the inclusion of PRA at the onset of this study was to involve farmers in identifying their preferences and direct selection to meet those preferences.

1.8 Maize streak virus disease

Maize streak virus disease was reported first from East Africa and has now extended to many other African countries. MSD is a major disease of maize in Africa and most prevalent in tropical lowlands (< 1000masl) and part of tropical mid altitude (1000– 1800masl) maize growing areas (Okori *et al.*, 1999; DeVries and Toenniessen, 2001; Pingali, 2001). According to DeVries and Toenniessen (2001), it is estimated that 60% of the total production area is affected by MSD, ranking it the most widespread biotic constraint in Africa.

1.8.1 Economic importance of MSD

Maize streak virus is transmitted by leafhoppers and causes serious yield losses, but its occurrence is sporadic and unpredictable (Diallo, 1999). A severe outbreak in Kenya in 1988, for example, destroyed more than half the crop over large areas (Pingali, 2001). However, yield losses due to the disease are not easy to quantify and in susceptible cultivars yield reduction often exceeds 70% (Guthrie, 1978; Bosque-Perez *et al.*, 1998). Symptom severity depends on genotype susceptibility and plant age at the time of infection (van Rensburg and Kuhn, 1977; Bosque-Perez *et al.*, 1998). When plants were infected at a younger age greater reductions in yield resulted than from plants infected at an older age (Guthrie, 1978; van Rensburg, 1981; Mzira, 1984; Bosque-Perez *et al.*, 1998). A similar observation was made by Ampong-Nyako *et al.* (1998). Pingali (2001) concluded that MSD is one of the dominant constraints to bridging the gap between potential and actual yield in mid altitude/ subtropical regions.

1.8.2 MSD causal agent

Twenty-two species of Cicadulina leafhoppers have been reported; 18 of these occur in Africa (Webb, 1987). The species known to transmit MSD include *C.mbila, C.similis, C.storey, C. arachidis, C. latens, C. bipunctata, C.ghauri, C. parazaea.* The distribution of Cicadulina leafhoppers varies considerably across Africa. *Cicadulina. mbila* and *C. storeyi*

are widely distributed across Africa. *Cicadulina mbila* is most important vector species (Nielson, 1968; Okoth and Dabrowski, 1987).

1.8.3 Transmission of MSD geminivirus

Cereal geminiviruses are transmitted only by leafhoppers (Storey, 1928; Harrison, 1985; Jonker and Flett, 1997). Grass geminiviruses are transmitted by a wide range of genera and species in the *Cicadellidae*. Maize streak virus is one of the seven viruses that attack maize crops in Africa although worldwide there are 32 viruses reported in maize. Maize streak virus is believed to have evolved with native grasses and is indigenous to Africa and the adjacent Islands (Storey, 1936; Bock, 1974; Rose, 1978; Thottappilly *et al.*, 1993). *Cicadulina* species vary in their ability to transmit MSD. Within a population the occurrence of virus transmitters and non-transmitters has been reported (Storey, 1931, 1932). However, the transmission is inherited, dominant and sex linked (Storey, 1931, 1932; ISAAA, 2001) with males being heterozygous (ISAAA, 2001). It is a single stranded DNA virus requiring leafhoppers, *Cicadulina spp*, for transmission and neither the virion nor DNA of the virus is mechanically transmitted (ISAAA, 2001). The viral sequence or genome, however, can be delivered into the plant by agro inoculation techniques via *Agrobacterium tumiefaciens*. Such transmission mechanism can be useful in breeding because it can substitute the tedious rearing of leafhoppers and their inoculation.

1.8.4 Maize streak virus disease epidemiology

Maize streak virus epidemics are more severe in the tropical regions (Rose, 1978; Autrey and Ricaud, 1983). It is reported that the virus spread by leafhoppers is facilitated by continuous cropping systems and the presence of wild grasses as reservoirs of both virus and vectors (Autrey and Ricaud, 1983). Maize streak virus epidemics are also reported to be facilitated by availability of a wide host range for the vector, the ability of the vector to transmit MSD persistently, and the insect's ability to migrate for long distances (Rose, 1978). Maize streak virus disease occurrence has been observed to be sporadic and unpredictable (Pixley *et al.*, 1997; Bosque-Perez, 2000) and it also depends on the environmental factors. This could be explained by the different forms of *C. mbila* (the predominant vector) produced in different climatic conditions and environments (Rose, 1972). It has been shown that in a warm and wet season, as is common in sub Saharan Africa, the fecund, long-bodied form of *C. mbila* is produced (Rybicki, 1988). Such forms fly less than 10m and are associated with

localised or pockets of disease development. A stronger-flying, short-bodied form of *C.mbila* is produced when the crops mature or when drought sets in (Rose, 1978). This is followed by extensive migration into young crop fields (late planted crops) or into irrigated crops causing extensive yield losses.

Unpredictability of MSD occurrence and existence of the migratory form of *C. mbila* indicate difficulty in control of the vectors/disease. Only few options are available for controlling MSD and they may not be effective in most cases. For example, various cultural practices have been suggested for control. These include 'barriers' of bare ground between early and late planted maize fields to reduce leafhopper movement and subsequent spread of MSD (Gorter, 1953). Avoiding maize plantings downwind from older cereal crops and the use of crop rotations was reported to reduce infection by minimising invasion by viruliferous leafhoppers (Rose, 1978). These cultural methods may be effective in reducing infection, but where land fragmentation exists, like in the maize growing regions of Uganda, the method would not be applicable. While insecticides have been recommended for control of leafhopper vectors (Rose, 1978; Rothwell, 1979; Mzira, 1984; Barrow, 1992), the inability of resource-poor farmers to use chemicals renders the method unsuitable for such farmers. The difficulty of achieving effective control of MSD through cultural and chemical methods implies that the use of plant resistance would probably be the most effective and economically feasible option for the control of MSD.

1.8.5 Maize streak virus disease symptoms

Maize streak disease symptom development starts as minute pale circular spots on the lowest exposed portion of the leaf. The symptoms then develop only on newly formed leaves after the infection has occurred, leaving healthy leaves below the point of infection (Storey, 1936). The older the plant is when it is infected, the more disease free lower leaves there are on the plant. The spots develop into streaks up to several millimetres in length along the leaf veins, particularly along secondary or tertiary veins. The streaks often fuse laterally to give narrow, broken, chlorotic stripes, which may extend over the entire length of fully affected leaves (Storey, 1936). In highly susceptible genotypes, chlorotic streaks may coalesce to form large chlorotic and later necrotic leaf areas, whereas partially or highly resistant genotypes produce few or no streaks (Welz *et al.*, 1998).

In general, diseased plants are characterized by broken to almost continuous, longitudinal chlorotic streaks along the leaf surface (Storey, 1925; Pinner *et al.*, 1988; Thottapilly *et al.*, 1993). Severely diseased plants are stunted, yield poorly, misshapen or give no yield at all (Storey, 1925; Fajemisin *et al.*, 1976; Rose, 1978; Rossel and Thottappilly, 1985; Pinner *et al.*, 1988; Thottappilly, *et al.*, 1993) and may appear very pale green or white from a distance (Storey, 1925; Pinner *et al.*, 1988; Thottappilly *et al.*, 1993). Those that are affected at later stages may suffer insignificant yield reduction (Ampong-Nyako *et al.*, 1998).

The described mode of symptom development has direct implication on disease rating and efficiency of selection during the breeding process. When the disease appears late, plants would be rated low and if disease infection occurs early, severity rating would be high. Data from a season with low disease infection would not be useful to discriminate between the resistant and susceptible plants. In conventional breeding, therefore, the use of spreader rows (Caulfield, 1997; Denic, 1997) or artificial inoculation (Bosque-Perez and Alam, 1992) would be required to facilitate effective screening for resistance. Marker assisted selection, on the other hand, would not require the presence of disease for selection and hence would be a suitable tool for MSD resistance breeding.

1.8.6 Sources of resistance to MSV

Sources of resistance to MSD have been known and used in many breeding programmes. A few sources of resistance have been identified. Resistance in maize was first reported in South Africa as early as 1931 and later, tolerance was found in other materials which were deployed in breeding (Soto *et al.*, 1982). Storey and Howland (1967) identified resistance to MSD in East Africa. Resistance was documented on CIMMYT inbred line CML202 (Welz *et al.*, 1998), although its source of resistance remains unknown (Pernet *et al.*, 1999a). From Réunion Island two other sources of resistance have been identified, namely, D211 and L61, which were completely resistant to MSD (Pernet *et al.*, 1999a). Research undertaken by International Institute for Tropical Agriculture (IITA) also reported IB32 as a streak-resistant line developed from the maize population TZ-Y and "La Revolution" developed in Reunion Island (Soto *et al.*, 1982). Other sources of resistance include CIRAD's C390, IITA's Tzi3, CIMMYT's OSU23I, PANNAR's AO76, and KARI's Embu 11 (ISAAA, 2001). CIRAD's C390 is considered immune to MSD. CML202 though known for resistance to MSD, is rated 2 on a scale of 0 – 5, where 0 = immune and 5 susceptible. Other studies have identified materials with higher resistance to MSD than CML202. However, the use of

CML202 in this study was based on the fact that the inbred line has been well studied; its highly adapted, and its resistance quantitative trait loci (QTLs) have been consistently mapped to the same location on the short arm of chromosome one at bin 1.04 of the maize genome.

1.8.7 Mode of inheritance

Studies of genetic control of resistance to MSD at IITA showed that resistance was controlled by two or three gene pairs (Kim *et al.*, 1989). Analysis of the genetic control of resistance in MSD-tolerant line Tzi 4 was conducted by Kyetere *et al.* (1999). They reported a single major gene (designated *MSD*1) controlling the tolerance. In CML 202, a major QTL, allelic or identical to that observed in Tzi was identified plus other genes of minor effects (Welz *et al.*, 1998). Studies undertaken in Zimbabwe confirmed the presence of a major QTL common to all resistance sources and other minor QTLs scattered within the genomes of the different sources of resistance (Pernet *et al.*, 1999a, b), although the minor QTLs appeared to be specific for one source of resistance or the other.

The observation made by Kyetere *et al.* (1999) in backcross progenies fitted 1:1 segregation ratio which indicated monogenic inheritance. Resistance in IITA material was reported to be inherited quantitatively, mainly additively but involving two or three major genes (IITA, 1981; Kim *et al.*, 1989). Rodier *et al.* (1995) reported two systems of inheritance; a major system supposedly monogenic and a minor system, which they considered polygenic. In CML 202, a major QTL showed partially dominant gene action with a bimodal frequency showing a clear 3:1 segregation (Welz *et al.*, 1998). This indicated a monogenic inheritance (Welz *et al.*, 1998). Pixley *et al.* (1997) reported that results from most field trials showed simple mode of inheritance. However, partial resistance was observed in Tzi 4 and was confirmed to be monogenic inheritance (Pernet *et al.*, 1999b). They also concluded that two resistance mechanisms existed.

The variation observed in mode of inheritance of MSD resistance from different sources points out the importance of using the right sources of resistance combined with the appropriate selection methods. Evidently most studies have suggested a simple mode of inheritance for MSD, and, in a source of resistance like CML202, the major QTL was shown to be monogenic (Welz *et al.*, 1998). Thus when using backcrosses the segregation ratio

should fit a 1:1 Mendelian phenotypic ratio (Kyetere *et al.*, 1999). The expectations for selfing would be 3:1 Mendelian phenotypic ratio (Welz *et al.*, 1998). Selection with marker associated backcrossing and selfing should produce 1:1 and 1:2:1 genotypic ratios if there are no distorted segregations (Lu *et al.*, 2002). Generally, the expected frequency for each marker type is 0.5 for BC and F2 populations and the frequencies are 0.25 for the parental marker types and 0.5 for the heterozygote (Moreau *et al.*, 1998).

1.9 Selection gains of conventional selection in breeding

Conventional breeding has been notably successful in producing important genetic gains in maize breeding. Through conventional selection resistant cultivars have been developed and have continued to play a major role in reducing the threat posed by MSD. For example, many high-yielding maize cultivars have been developed and released to farmers in sub-Saharan Africa (Timothy *et al.*, 1988). Efforts to improve resistance to MSD in maize germplasm have produced significant selection gains. At IITA, resistance to MSD in open-pollinated cultivars and hybrids has been developed (Soto *et al.*, 1982; Asanzi *et al.*, 1994; Bosque-Perez *et al.*, 1998). The resistance manifests itself as reduced symptom severity combined with low virus incidence in the field. Successful conversion of susceptible, but high yielding cultivars and landraces in various countries in Africa, into MSD-resistant ones at IITA have been made (Efron *et al.*, 1989). The Pannar seed company of South Africa has also developed and released MSD-resistant hybrids in several African countries (Barrow, 1992, 1993).

The pedigree method is often used for developing inbred lines in maize. Lines expressing complete resistance to MSD were developed from five cycles of inbreeding and selection (Rodier *et al.*, 1995). Selections in maize with inbreeding from S_0 to S_3 generations have resulted in open pollinated cultivars (OPVs) that were 16, 8, 2, and 1% improved for MSD and GLS resistance, ear height, and days to anthesis, respectively (Pixley *et al.*, 2006). The results demonstrated improvement of a maize population for MSD resistance and other traits by selection during inbreeding (from S_0 to S_3), without negative impact on gains for grain yield achieved by evaluation and selection among the progenitor FS families (Pixley *et al.*, 2006). Presello *et al.* (2005) assessed the effectiveness of pedigree selection for improving resistance to Gibberella ear rot in four maize populations. Their results showed significant

responses to selection which were more evident in later than in earlier generations of inbreeding.

Population improvement selection methods have also been successful in improving MSD resistance. Tang and Bjarnason (1993) reported that both modified full-sib recurrent selection and backcrossing were highly effective in improving MSD resistance without sacrificing yield. They suggested that simple backcross and backcrossing with selfing were equally efficient in converting susceptible cultivars to MSD resistance. In selection for machine harvestable yield, Landi and Frascaroli (1993) showed positive responses to selection in all populations and they attributed this to the changes in the frequencies of alleles with important additive effects. This demonstrated the usefulness of conventional selection in concentrating favourable alleles. Half-sib and full-sib reciprocal recurrent selection (RRS) have successfully been used to improve maize populations for yield and other traits (Peiris and Hallauer, 2005). The authors reported no significant difference between half-sib and full-sib RRS methods. Choice of method, therefore, should depend on the breeding objective, availability of resistance within the germplasm and resources. Monneveux *et al.* (2006) concluded that recurrent selection under drought was effective as a means of improving tropical maize source populations for performance under water deficits.

Conventional selection methods have been used successfully to achieve significant genetic gains as demonstrated by various studies. The limitations faced with the use of conventional methods usually arise from difficulties in working with some traits. Most quantitatively inherited traits have low heritability and are subject to genotype by environment interactions. The process of selection may also take long and can be laborious. Dreher *et al.* (2000) mentioned that phenotypic selection can be difficult to use to efficiently select the best allelic combinations for linked target regions. This is because of the difficulty of breaking linkages between two linked loci in repulsive phase. For certain traits phenotypic screening is difficult, time-consuming, and/or expensive, and for these applications MAS methods may offer advantages (Dreher *et al.*, 2000). Marker assisted selection was shown to produce more predictable results between season and years than conventional methods (Stuber and Edwards, 1989; Stuber, 1992). Thus, MAS may be a reliable method of screening for resistance to MSD.

1.10 Molecular maker assisted selection

1.10.1 QTL for MSD resistance and its stability

Pernet *et al.* (1999) investigated QTLs responsible for resistance to MSD and showed that MSD was quantitatively inherited. They detected at least five significant QTLs on chromosomes 1, 2, 3, and 10 in cultivar D211. These QTLs explained between 48% and 62 % of the total phenotypic variation observed. In a different study with cultivar CIRAD390 as a source of resistance, they identified eight QTLs (Pernet *et al.*, 1999b). Still, MSD resistance was reported to be controlled by a few genes with two QTLs on chromosomes 1 and 10 (bin 1.05 and 10.05) stable across dates and environments in two populations. Their chromosomal locations were consistently mapped in the different populations (Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a; Pernet *et al.*, 1999b). According to Pernet *et al.* (1999), two systems exist: one major gene conferring stronger resistance and the other, polygenic, conferring partial resistance. In terms of gene action, the major QTL in bin 1.05 appeared to be partially dominant at the early stage of disease and additive for all the other scoring dates.

Stability of QTLs across populations has been shown to be variable. Pilet *et al.* (2001) mapped QTLs for blackleg in two populations and observed that some of them were consistent across the two populations and the QTLs expressed dominance or over-dominance effects. Reyna and Sneller (2001) also concluded that it may be difficult to realise the value assigned to QTL alleles derived from diverse parents with variable relative genetic values when the alleles are introgressed into populations with different genetic backgrounds, or when tested in different environments. This, however, may not be the case for MSD QTLs. The major QTL for maize streak virus has been mapped consistently to the same position on the first chromosome in different populations (Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a, b). Henry *et al.* (2001) also demonstrated that QTLs were stable across different populations. Yousef and Juvik (2002) have shown that QTL identified in one mapping population has a positive effect across different genetic backgrounds and across different environments. Their outcomes appear to negate the view that QTLs mapped in one population may not be effective in another genetic background (Pilet *et al.*, 2001; Reyna and Sneller, 2001).

Unfortunately, practically it appears that most of the work done on MAS is still limited and concentrated only on the same populations used in mapping the QTL (Wilcox *et al.*, 2002). There is limited literature on actual application of markers in MAS; most of the reports are on simulation studies. It is apparent, therefore, that more work should be done on mapping QTLs in many populations to determine their stability across populations.

The feasibility of using MAS in breeding programmes is determined by the reproducibility of marker-QTL associations across generations, populations and environments (Dudley, 1993). However, markers are also subject to environmental influence. The study by Stromberg *et al.* (1994) showed significant marker by location interaction effects in F2 maize populations. Such differences in marker by location effects were also observed by Stuber (1992) and Pernet *et al.* (1999). These interactions appeared to be associated with markers linked to minor QTLs controlling a trait, because no such interactions have been observed for markers linked to major QTLs or genes (Huang *et al.*, 1997; Chen *et al.*, 2000).

There are also situations that may reduce the efficiency of MAS; when the environment or the genetic background, or both, affect the final contribution of the QTL. For example, when genotype–environment (G x E) and epistatic interactions are involved in the phenotypic value (Gupta and Lewontin, 1982; Gurganus *et al.*, 1998). Epistasis occurs when the combined effect of two or more genes on a phenotype cannot have been predicted as the sum of their separate effects (Fisher, 1918; Frankel and Schork, 1996). In populations segregating for an entire genome the presence of epistasis between QTL assayed has been found at a frequency close to that expected by chance alone (Stuber *et al.*, 1992; Xiao *et al.*, 1995), yet when, recombinant inbred lines (RILs), di-haploids (DHs) and isogenic lines are used, epistasis is detected more frequently (Doebley *et al.*, 1995; Lark *et al.*, 1995; Eshed and Zamir, 1996; Lukens and Doebley, 1999; Simko *et al.*, 1999; Maheswaran *et al.*, 2000; Subudhi *et al.*, 2000). The presence of epistasis would imply that the QTL detected are not completely independent of QTLs located elsewhere in the genome. As such, MAS would not be effective if the epistatic QTLs are not both selected for in the segregating population. This would result in differences in genetic and phenotypic variation of the traits of interest making MSD a difficult trait to select for (Pernet *et al.*, 1999a). In the case of this study, only the major QTL is being introgressed using MAS and yet there were other QTLs within the genome contributing to the resistance. If they exhibited epistatic interaction then the

phenotypic value from the selection would not meet the expected value. The remedy to that would be to use markers for most of the QTLs identified to achieve the desired phenotype.

1.10.2 Application of markers in selection methods

Marker assisted selection is a breeding strategy applying indirect selection. Instead of selecting for the gene itself, the molecular markers closely linked to the genes are used to monitor the incorporation of the desirable alleles from the donor source (Dudley, 1993). The strategy also finds application for quantitative traits. By the use of markers linked to specific QTLs, it is possible to introgress specific regions of the genome that confer desirable quantitative characteristics to an elite cultivar (Smith *et al.*, 1987; Hillel *et al.*, 1990, 1993; Hospital *et al.*, 1992). Marker screening within the early generations of a breeding programme means that MAS can help to accelerate the backcrossing and develop improved lines or populations. It may especially have advantages in some cases where phenotypic selection is difficult (Chen *et al.*, 2000).

Marker assisted selection with backcrossing has been suggested as a breeding strategy for the introgression of a limited number of QTLs into elite germplasm (Dudley, 1993; Stuber, 1994; Bernacchi *et al.*, 1998). For example, Yousef and Juvik (2000) introgressed beneficial QTLs using MAS to improve seedling emergence in a BC2F1 generation. Their results showed improvement of seedling emergence of up to 40.8 %. In rice, the use of MAS in improving bacterial blight resistance produced lines with resistance comparable to the donor parent (Chen *et al.*, 2000). Bouchez *et al.* (2002) also showed that MAS was useful in improving earliness in their selections. The authors observed that the magnitude and sign of QTL effects agreed to their expectations.

The use of marker assisted selection for introgression of major QTLs for disease resistance and other agronomic traits is increasing in crop improvement. Once a target trait has been genetically dissected, and assuming the genes that code for the trait have been identified and tagged, breeders can use molecular markers to accelerate germplasm improvement. This is achieved by (1) tracing favourable alleles in the genomic background of genotypes to be improved, and (2) identifying in large segregating populations individual plants that carry the favourable alleles (Mohan *et al.*, 1997; Ribaut and Hoisington, 1998; Crouch, 2000).

Two factors are considered critical for MAS procedure (Chen *et al.*, 2000): The first factor is the selection for recombinants between the adjacent gene loci and the flanking markers linked to the target gene locus. The selection can be for simultaneous recombination on both sides of the flanking markers. This has the advantage of shortening the time required, but it is also an expensive option. It is also complicated by the fact that double cross-overs are not very common. The other option is tandem selection in which selection is made for recombination on only one side in the first generation and selection for the recombination on the other side in the second generation. The latter approach is much less expensive than the former but takes longer.

Secondly, background selection of the recurrent parent genotypes was also viewed as a vital consideration for improving the efficiency of MAS (Chen *et al.*, 2000). Genetic markers could be used in two ways in introgression programmes: 1) using markers for the QTL or gene to be introgressed and 2) using markers to select for (or against) a particular background genotype (Visscher, 1996). On average 0.875 proportions of loci for individuals are expected to be homozygous for the recurrent parent genotypes in the BC3F1, but this depends on the number of loci involved. By carrying out background selection for the parental genotypes using markers that cover the entire genome, the recovery of the parent genotype would be achieved within a short duration. Application of an additional one round of background selection in BC1F1 to the MAS scheme may greatly increase the efficiency of MAS (Chen *et al.*, 2000).

The observations made by Chen *et al.* (2000) indicate that MAS can have an advantage over conventional selection by shortening the time required to introgress a QTL into elite material through backcrossing. If double crossovers were easily achievable, MAS with backcrossing plus one round of background selection would require not more than two generations to fully recover the parent genotypes fixed for the QTL being introgressed. Successful marker assisted selection for QTL of importance with additional rounds on background selection at BC2 and BC3 recovered almost all the recipient genotypes within the first two backcross generations (Bouchez *et al.*, 2002). This would make MAS an ideal tool in crop improvement because breeders are looking for procedures that can shorten time and save resources (Thomas, 2003).

1.11 Comparison of selection using conventional and marker assisted selection

There is limited base of practical experimental data on comparison of MAS and conventional or phenotypic selection (PS) methods. A few researchers have put forward their findings regarding the subjects and so far not all their outcomes are distinctively in favour of MAS. In most attempted MAS schemes either with single or multiple genes, results showed that MAS was either equally effective (Stromberg *et al.*, 1994; Groh *et al.*, 1998; Wilcox *et al.*, 2002) or more effective compared to conventional selection (Huang *et al.*, 1997; Chen *et al.*, 2000; Yousef and Juvik, 2001). In comparing phenotypic and marker assisted selection on quantitative trait in sweet corn, Yousef and Juvik (2001) found out that MAS had a significantly higher gain than the phenotypic selection across different populations. Yousef and Juvik (2002) also observed that marker associated backcrossing enhanced seedling emergence of sweet corn by 28 – 40 % over the unmodified hybrid. Yousef and Juvik (2001) showed that the use of MAS is most appropriate when traits are difficult and costly to measure.

Stromberg *et al.* (1994) compared yield of test crosses from MAS and PS and their results showed that both MAS and PS were equally efficient in improving yield. Marker assisted backcrossing with 15 marker loci, representing between 30 -40% of the genome was used to select for grain yield in elite single crosses in comparison with phenotypic selection (which would be expected to involve the whole genome) (Stuber *et al.*, 1999). Their results indicated that marker-facilitated selection was as effective as phenotypic selection. A comparative analysis of a population at BC2F3 using MAS and phenotypic selection also showed no significant difference between the two approaches. Both MAS and PS produced lines with improved resistance to southern corn blight over susceptible parents (Wilcox *et al.*, 2002). In another study (Yousef and Juvik, 2001), the average MAS and PS gains across composite populations for selected traits were 10.9% and 6.1%, respectively. Higher genetic gains were realised from MAS. The population size used was smaller and yet there was improved selection gain. This signified that MAS had a potential of reducing costs by reducing the population size unlike in PS where large numbers are required to achieve a similar gain in selection.

The efficiency of MAS is enhanced, especially when coupled with the genetic background selection. With only three backcrosses, Chen *et al.* (2000) were able to improve resistance

to bacterial blight in inbred lines without compromising on the yield and yield components. Their results showed that with heavy disease infestation, the improved lines were not only resistant, but also had higher yield than the parents.

Unlike in phenotypic selection where the goal is to select a line from the upper 1 % of the genotypic distribution by selecting the upper 10 % of the phenotypic distribution, the results from Knapp's (1998) study showed that 94 to 96 % of the selected progeny were usually inferior when heritability estimates ranged from 0.1 – 0.5. When heritability estimates of the selected traits are low or moderate and worse still when small samples of progeny are screened, the probability of selecting superior genotypes is very low (Johnson, 1989). Visscher *et al.* (1996) observed that response to selection using markers did not vary with heritability. This was because the proportion of the genetic variance explained by markers was constant. Under such circumstance, the top ranking genotypes usually correlated strongly with the top ranking phenotypes and could be selected with any selection intensity. The use of markers in breeding is regarded as a technology that has the potential to substantially decrease resources needed to accomplish a selection goal for a low to moderate heritability trait when selection goal and selection intensity are high (Stuber *et al.*, 1999). The advantages associated with marker assisted selection method makes it a cost effective procedure since it tends to reduce the need for resources for progeny testing and avoids disproportionate allocation of resources to inferior progeny (Knapp, 1998; Dreher *et al.*, 2000).

Marker assisted selection has been viewed as a strategy for increasing selection gains (Lande and Thompson, 1990; Lande, 1992; Dudley, 1993; Knapp, 1994). However, Knapp (1998) argued that QTL and MAS index (weighted sum of phenotypic and marker scores) parameter estimation errors, genetic drift and disequilibrium between selected and unselected QTL can reduce the gains from MAS and may lead to lower selection gains for MAS than for phenotypic selection, particularly in recurrent selection. This is because the accuracy of QTL and MAS index parameter estimates is shown to be low when heritability is low and samples are small (Gimelfarb and Lande, 1995).

Selection gains over cycles of selection also appear to decrease from one cycle to the next using MAS. Edwards and Page (1994) reported that rapid responses from MAS observed in the early generation of selection are not consistent over further cycles of selection. A similar

observation was also made by Yousef and Juvik (2001) whose results showed increment of favourable allelic frequencies in the first cycle of selection. In their second cycle, they observed a decreased increase in favourable allelic frequencies, suggesting that MAS would be of more advantage than phenotypic selection over the first two to three cycles of selection, thereafter conventional methods might replace MAS to achieve further responses. This implies that MAS may not be economical on its own to produce cultivars, but can be used to complement conventional selection methods to improve the rate of return in breeding (Lande and Thompson, 1990).

Marker assisted selection can be a very expensive procedure especially when the QTL is not yet mapped (Wilcox *et al.*, 2002; Dreher *et al.*, 2000). For example, Wilcox *et al.* (2002) estimated the cost of QTL mapping only of US$ 37000. The author estimated the actual application of MAS for selection up to BC2F2 generation without mapping at only $ 70 compared to $ 11 000 for conventional selection. This suggests that the application of mapped QTL in MAS could substantially reduce costs associated with conventional breeding. The same authors also pointed to the problem of phenotypic screening involving insect rearing and artificial infestation followed by disease severity. Insect infestation and individual plant leaf damage ratings require large time commitment from the scientist and highly qualified technicians, which are the most expensive labour fraction (Wilcox *et al.*, 2002). This is a valuable piece of information for breeders and especially for developing countries where MAS practicability would probably rely on the already mapped QTL, hence substantial reduction in the cost for MAS.

Marker assisted selection has advantage over conventional methods by the reduction of years required to achieve results (Yousef and Juvik, 2001), selection in the absence of stress factor and reliable way of improving the target trait (Chen *et al.*, 2000; Wilcox *et al.*, 2002). For conventional selection to be effective field conditions should be favourable, that is, when plants are not subjected to biotic or abiotic stresses (Dreher *et al.*, 2000) or when the disease pressure permits screening. This would practically make conventional selection easy, fast, and inexpensive. The reality, however, is different because field conditions are not always favourable and for insect transmitted disease it is even more unreliable to achieve uniform and high level of disease development early in the season. A technology that would permit the screening for disease resistance even in the absence of the disease

would be more likely to cut down on time and save resources. Molecular marker selections then derive their advantages over conventional selection because of this mode of selection.

The impact of marker-based QTL analysis on the development of new lines or cultivars with enhanced quantitative traits has been less than expected (Tanksley and Nelson, 1996). Three reasons were stated: "1) the discovery of QTL and cultivar development have been two separate processes, 2) most breeding-related QTL studies have been targeted toward the manipulation of quantitative traits in elite germplasm (Tanksley and Nelson, 1996), and 3) for traits such as grain yield, QTL expression usually is dependent upon the genetic background in which it is found; therefore QTL evaluation must be done independently each time a new population or cross is used (Stuber *et al.*, 1999). For less complex traits, such as disease and insect resistance, this is usually not the case, however.

Although MAS combines different advantages of increasing reliability and increasing efficiency (Peleman and van der Voort, 2003) not many breeding programme are currently using MAS. Economic constraints of the prohibitive costs of the laboratory work involved in molecular screening are a major obstacle to the widespread incorporation of MAS in breeding programmes (Moreau *et al.*, 1998; Dekkers and Hospital, 2002, Dreher *et al.*, 2000, Koebner and Summers, 2003). It is necessary, therefore, that before deciding to follow DNA marker-assisted approaches, practical concerns and cost-benefit analysis need to be addressed. Many breeding programmes have not conducted their own cost-benefit analysis. Available data on cost-benefit analysis does not offer a clear distinction between MAS and conventional approaches. Perhaps these and other factors such as the absence of laboratory facilities for molecular analysis and technical competence are some of the factors behind lack of adoption of MAS technology. The best option would be the integration of the two approaches (MAS and conventional methods) to obtain the maximum improvement in economic value of domesticated populations (Lande and Thompson, 1990).

1.12 Choice of source population for maize inbred line development

There are different choices of source population to effect selection or for inbred line developments. Previously, open-pollinated cultivars that were popularly grown were used as source population of inbred line, segregating population of elite line crosses are the commonly used source populations (Hallauer, 1989). Single cross and one backcross as

source populations were rated the highest among the sources of germplasm for selection (Bauman, 1981). Pedigree selections particularly F2 and backcross, coupled with topcrossing have been the most commonly used methods of developing inbred lines (Hallauer, 1989).

It is important, however, to consider selection intensity used when choosing between F2 and backcross as source population. Lamkey *et al.* (1993) reported that for high selection intensity, the F2 was a better source population, and for low selection intensity, the BC to the better parent was the best source population. Backcross to the better parent is critical for yield traits but may not be so for disease resistance like maize streak virus disease. This is because no significant epistatic interactions have been reported for MSD (Pernet *at al.*, 1999), that render BC superior to F2 (Lamkey *at al.*, 1993). In the current study, backcrossing was done to the worse parent that was to be improved. Results of Lamkey *et al.* (1993) study also showed that F2 had superior genetic gains than BC. He also reported that F2 had more superior usefulness under high selection intensity than BC. However, the superiority of both F2 and BC is reported to depend on the number of loci with favourable alleles (Dudley, 1984). He reported that if the two parents had equal or different number of favourable alleles then selfing and backcrossing, respectively were advisable. In the current study both F2 and BC source populations were used to effect selection because of two reasons: 1) due to greater genetic variance in F2 than in BC, the probability of selecting a new line with more favourable alleles than either of the parents was high (Dudley, 1984). Hence lines more resistant to MSD could be obtained, through selfing, for hybrid production, and 2) through backcrossing the useful traits expressed by the susceptible parents would be reconstituted.

1.13 Challenges and limitation

There are a few reports on mapping QTL for MSD resistance and suggestions for using MAS to introgress the QTL. The limitation has been the inadequate work and limited publications or reports that critically evaluate the use of identified QTLs to introgress important traits into elite material (Reyna and Sneller, 2001; Yousef and Juvik, 2001). Most studies are theoretical computer simulations (Lande and Thompson, 1990; Zhang and Smith, 1992; Edwards and Page, 1994; Gimelfarb and Lande, 1994; Knapp, 1998) and their results suggest that MAS can be effective in improving quantitative traits in breeding of both plants and animals.

Currently, little information is publicly available concerning the costs of phenotypic and genotypic selection procedures. The few papers that have addressed this issue (Ragot and Hoisington, 1993; Moreau *et al.*, 2000; Yu *et al.*, 2000; Dreher *et al.*, 2000; Morris *et al.*, 2003) have considered different parameters. This is an indication that there are no standards for cost comparison involved in MAS yet, just like it is in any breeding scheme. MAS for different traits may involve different numbers of QTLs mapped for the traits and different numbers of markers. This would make cost comparisons between MAS and phenotypic selection difficult to achieve or generalise (Yousef and Juvik, 2001). The cost of MAS also depends on the time when the MAS is applied and the number of cycles of MAS (Chen *et al.*, 2000), making an accurate comparison between the cost of MAS and phenotypic selection very difficult and may not be applicable to every breeding programme. Overall, cost comparison depends on selected traits, number of evaluated traits, population size, labour costs, number of environments and replication, selection intensity, number of polymorphic markers detected and types of DNA markers used in the breeding programme (Yousef and Juvik, 2001).

In conclusion, MAS can only increase the rate of genetic gain in the long term, when there is a continuous advantage of new identified QTL (Montaldo and Mezo-Herrera, 1998). With more cycles of selection the extra genetic gain due to the MAS decreases very quickly with increase in the number of generations of selection for the same QTL. Nonetheless, the rate of identification of new QTL is difficult to predict. As suggested by Montaldo and Meza-Herrera (1998), the application of MAS can be effective on characters that are controlled by a few pairs of alleles as is the case with MSD or disease resistance with simple inheritance.

1.14 References

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CHAPTER TWO

Farmers' perceived maize production constraints and MSD status and varietal selection preferences in two districts of Uganda

2.1 Abstract

In spite of diligent efforts by national and international scientists, plant breeding for many important crops in Africa has been plagued by low productivity and low adoption rates among the majority of small scale farmers. A participatory rural appraisal (PRA) was conducted in Sironko and Iganga districts in Uganda to determine maize production constraints, assess the status of maize streak virus disease and to determine farmers' preferences and varietal selection criteria. Semi-structured interviews and survey questionnaires were used for focus group discussions and individual interviews of selected farmers, respectively. Transect walks followed focus group discussions. Results showed that unreliable rainfall and insect pests were the dominant constraints to maize productivity in Uganda. Diseases were ranked fifth among the production constraints. Among the diseases, MSD was considered the most important disease constraint in maize productivity. Farmers showed common preference for high yielding and early maturing cultivars. However, farmers had other special preferences which were diverse and included large, white and high test density kernels for marketing, and sweet taste, particularly for home consumption. Research priorities as perceived by the farmers included tolerance to drought, resistance to insect pests and diseases, sweetness for green maize, prolificacy, resistance to lodging, and drooping leaves.

2.2 Introduction

Maize (*Zea mays* L.) production in East Africa is dominated by resource-poor farmers. These subsistence and small-scale commercial farmers grow the crop under highly variable and stress-prone environments usually hampered by resource constraints (Banziger and de Meyer, 2002). Unfortunately, the current formal plant breeding programmes have not been successful in improving maize productivity under these conditions. Productivity of maize in developing countries and especially in sub-Saharan Africa has remained low due to, among other things, low adoption rate of new or superior improved cultivars being released by breeding institutions, biotic and abiotic production constraints.

Maize productivity in Uganda is curtailed by an array of biotic factors. The most important biotic factors reported were diseases, namely, gray leaf spot (*Cercospora zeae maydis),* turcicum leaf blight (*Exserohilum turcicum)* and maize streak virus (MSD). These diseases

were also reported among the dominant constraints to maize productivity in Sub-Saharan Africa (Pingali, 2001). Maize streak virus (genus Mastrevirus; family Geminiviridae) is an indigenous African virus transmitted by a range of leafhoppers in the genus Cicadulina (Storey, 1925). Maize streak virus is a major disease of maize in Africa and most prevalent in tropical lowlands and part of tropical mid altitude maize growing areas (Thottappilly, 1992; Okori *et al.*, 1999; DeVries and Toenniessen, 2001; Pingali, 2001). Maize streak virus occurrence is sporadic (Pingali, 2001) and can cause yield losses of up to 100% even in high potential agricultural zones (ISAAA, 2001). It is estimated that 60% of the total production area is affected by MSD, ranking it the most widespread biotic constraint in Africa (DeVries and Toenniessen, 2001). Breeding for disease resistance is still regarded as an important means of bridging the yield gap between potential and actual yield. A survey done in 2001 by the National Maize Programme ranked MSD as the third most important disease constraint in Uganda. Since MSD is sporadic and damage varies in years and seasons, its current status is unpredictable. This study was aimed at assessing MSD status and establishing the major maize production constraints in the maize growing regions of Uganda.

Farmers also have taste and other preferences that form key factors when selecting cultivars for production. Farmers have special preferences for maize cultivars such as taste, cooking qualities and high biomass for stock feed. In other words, farmers are heterogeneous in that their needs, priorities, and preferences are diverse. Failure to consider these differences would result in rejection of an otherwise promising technology (Pingali, 2001). Engaging farmers in the varietal development process would help breeders understand their needs and preferences which in turn would help in selecting appropriate genetic materials for them (Witcombe *et al.*, 1996). Unfortunately, formal research systems in developing countries are highly centralised and do not target the problems of resource-poor farmers (Sthapit *et al.*, 1996). While enormous resources have been directed towards breeding maize in most of Africa over the past three decades, only an estimated 37% of the farmers regularly plant improved cultivars (Morris, 1998, cited by DeVries and Toenniessen, 2001). It has been reported that, in most cases, breeders do not have a clear understanding of the farmers' requirements; hence breeding programmes might not have sufficiently considered the needs and preferences of farmers (Toomey, 1999; Banziger and Cooper, 2001; Banziger and de Meyer, 2002). Consequently, cultivars released might not meet the specific and local conditions of the farmers. In developing countries, most cultivars grown by

farmers are old and only a few of the recently released cultivars are grown by farmers (Joshi and Witcombe, 1996; Witcombe *et al.*, 1996). Studies in India have shown that the average age of cultivars grown by farmers is more than 12 years for rice (*Oryzae sativa* L.), 15 years for groundnuts (*Arachis hypogea* L.), 16 for sorghum (*Sorghum bicolor* (L.) Moench), and 17 for maize, but in Africa the cultivars grown could be older.

Therefore, in a bid to improve maize inbred lines for maize streak virus resistance, which subsequently will be used to develop new cultivars, it was imperative that farmer's needs and preferences were understood for integration into the breeding programmes and hence, the objectives of the study.

2.3 Problem Statement

Maize productivity in farmers' fields in Uganda is still low compared to the potential achievable in research stations. There are factors that are contributing to such low productivity of which farmers may not be aware. Maize streak virus could be one of the major production constraints and yet farmers may not be aware of its current status. The continuous low adoption rate of new or superior improved cultivars being released by breeding institutions could be attributed to insufficient consideration of farmers' needs and preferences by breeding programmes (Banziger and de Meyer, 2002). Consequently, very few cultivars released meet farmers needs.

2.4 Research Goal and Objectives

The overall goal of the study was to establish the importance of MSD relative to other production constraints to maize production and to identify farmers' needs and preferences for objective formulation and cultivar development to suit farmers' needs using PRA.

The specific objectives of the study were to:

1. identify farmer's maize production constraints,
2. assess the current status of maize streak virus disease, and
3. understand farmer's preferences and selection criteria for future varietal development.

The hypotheses being tested were:

1. Maize streak virus disease is an important constraint of maize productivity, and
2. Farmer's preferences correspond to the qualities breeders select for.

2.5 Research Methodology

A participatory rural appraisal (PRA) approach which entails interaction between breeders and farmers and leads to mutual exchange of knowledge and experience was conducted in two districts of Uganda in 2005.

2.5.1 Site selection

The PRA was conducted in Sironko and Iganga districts in the eastern region of Uganda. The two districts were among the leading in maize production in Uganda. The areas chosen represented different agro-ecologies. This was important since the maize steak virus disease epidemic is influenced by environmental factors (Mesfin and Bosque-Perez, 1998) and hence there was a need to assess the differences of MSD severity in those areas. Another reason for the choice of the two districts was that they lie at different altitudes, which has influence on the choice of maize cultivars to be grown. Iganga lies at 1200m (mid-altitude), while Sironko lies at 1650m (high-altitude). In the recent years, MSD epidemics have been noted to cover all areas ranging from lowlands to highly productive highlands of 2300m altitude (Diallo, 1999).The cultivars that are adapted to the highland may not necessarily be suitable for the mid-altitude conditions.

2.5.2 Selection of farmers

Thirty farmers were selected from each of the two districts in the following pattern: In Iganga district, the study was conducted in Bukanga sub-county, a maize growing area. Two parishes were selected and 15 farmers were selected from each of the two parishes. The choice of farmers from each parish was done by the area extension worker. Both male and female farmers, from all socio-economic classes were represented adequately in the study.

From Sironko district, Bulegeni sub-county was selected for this study. Two parishes were chosen but the choice was based on altitude to include parishes from the high and the low altitudes. Fifteen farmers were selected from each of the two parishes. There were both

male and female farmers with females comprising fifty percent of the total number of farmers selected.

In addition to the 30 farmers per district, two groups of 15 farmers each were formed for focus group discussions from each district. These groups were selected with recommendations from the area extension workers. They were grouped on the basis of their income, maize production capacity and social status based on information from the area extension worker.

2.5.3 Data collection tools

Different PRA techniques were used to obtain information about the farming problems, varietal selection criteria, research priorities and opportunities. A combination of three data collection techniques was employed. These included (1) semi-structured interviews for focus group discussion (FGD), (2) transect walks for field observation with the groups and (3) survey questionnaires for individual interviews.

Semi-structured interviews were conducted with 15 maize growers per parish in two parishes per district. Fifteen farmers were selected for FGD per parish. The discussions were followed by transect walks through 20 maize fields during which maize streak virus disease and other biotic and abiotic constraints were identified and scored with the farmers' groups and the area extension worker. Group discussions were held with a selected sample of 15 farmers to confirm results from questionnaires for individual farmer interviews including a key informant.

Rank matrices were drawn to rank the constraints. Individual farmers ranked biotic and abiotic constraints independently. The constraint with the highest score was considered the most important. Transect walks in the fields of farmers were conducted and observations made. Different traits and plant characters that were considered by farmers in cultivar selection were recorded. In addition to providing information on cultivar preferences, farmers identified other special requirements, which they considered important for home consumption and marketing.

2.5.4 Data Analyses

Data from questionnaires for individual interviews were coded and analysed using SPSS computer package (version 10.0). Average scores and average ranks were calculated for data obtained from both group discussions.

2.6 Results

2.6.1 Maize production constraints

The use of unimproved seeds for production was noted as the commonest practice among farmers. The results (Table 2.1) revealed that, in general, most farmers (35%) used home saved seeds followed by those who obtained seeds through home associations (21.7%) or purchased seeds from agro-input shops (21.7%). Home associations were farmers' groups who came together to produce seeds for contract buyers or seed companies. However, clear differences could be seen between the two districts in terms of seed sources. While Iganga farmers formed farmer groups to access seeds for its members, there were no such farmer groups in Sironko district. The use of own saved seeds was high in the two districts but a relatively higher number of farmers from Sironko bought seeds from agro-input shops or from the markets.

Table 2.1 Sources of seed for farmers in Sironko and Iganga Districts

Seed source	District		Total Frequency	Percentage
	Sironko	Iganga		
Market	4	1	5	8.3
Own home saved	9	12	21	35.0
Purchase from in put shops	11	2	13	21.7
From associations		13	13	21.7
2 and 3		2	2	3.3
unknown	6		6	10
Total	24	30	54	100

There were a number of factors that hampered maize production in the eastern region of Uganda. Seven production constraints were listed as the most important in maize production although their importance was variable. Unreliable rainfall was ranked as the most important problem (43.3%) farmers were facing in maize production in the two districts (Table 2.2).

Insect pests (18.3 %), mainly stalk borers, were ranked as the second most important constraint, and followed by declining soil fertility (13.3%). Diseases were fifth in the ranking.

Table 2.2. Maize production constraints and their order of importance

Maize production constraints	Frequency	Percentage	Ranking
Unreliable rainfall	26	43.3	1
Insect pests	11	18.3	2
Declining soil fertility	8	13.3	3
Land shortage	6	10.0	4
Disease	5	8.3	5
Lack of access to improved seeds	3	5.0	6
High input costs	1	1.7	7
Total	60	100	

Farmers did not have alternative approaches for controlling insect pests. Eighty three percent of the farmers mentioned they would pluck and burn the affected plants and the others (16.7%) said they had no solution to control insect pests devastating their crops.

2.6.2 Importance of maize streak virus disease

There were differences between the two districts in terms of disease prevalence (Table 2.3). Farmers described diseased devastating their crops and were able to identify them from photographs. Almost half (48.3%) of the farmers interviewed in Sironko district reported that head smut (*Sphacelotheca reiliana*) was the most important disease reducing maize yield in their fields. The second most important disease affecting maize production was MSD (41.7%). The other diseases were not considered to be important.

The order of importance was different for Iganga district. In Iganga district, MSD and northern leaf blight were ranked similarly, that is, 33.3% and 30%, respectively. In Iganga, ear rots were also considered as very important diseases causing yield losses from the field and post harvest.

Table 2.3. Importance of diseases of maize and their ranking in Sironko and Iganga

Disease	Sironko		Iganga	
	Frequency	Percentage	Frequency	Percentage
Head smut (*Sphacelotheca reiliana*)	29	48.3	5	5.0
Maize streak virus disease (MSD)	25	41.7	20	33.3
Gray leaf spot (*Cercospora zeae-maydis*)	1	1.7	1	1.7
Northern leaf blight (*Excerohilium turcicum*)	1	1.7	18	30.0
Rust (*Puccinia sorghi*)	1	1.7	1	1.7
Ear rot (*Stenocarpella maydis*)	3	5.0	15	25.0

Table (2.4) shows farmers' observations of maize cultivars which were severely attacked by MSD. It was apparent that local cultivars were the most affected in both first and second seasons (35.0%), while improved cultivars succumbed to attack differently in either the first or second season.

.Table 2.4. Cultivars attacked by MSD in relation to season's variations in Sironko and Iganga districts

Cultivar	Season	Frequency	Percentage
Local cultivar	All seasons	21	35
Longe 1(improved)	First	18	30.0
Longe 5 (improved)	Second	11	18.3
Longe 4 (improved)	Second	5	8.3
Uganda hybrid	Second	1	1.7
all cultivars	All seasons	1	1.7
Not sure	Not sure	1	1.7

Results showed that farmers' adopted various strategies for MSD control. The majority of the farmers (55%) mentioned that they would remove the growing tip of affected (Table 2.5). About 23.3 % had no idea on how to control MSD. Early planting (6.7%) and changing the planting season (6.7%) were approaches considered by a few farmers as control measures for MSD. There were only a few farmers (1.7 %) who sought advice from the extension workers.

Table 2.5 Farmers' approach for MSD control in Sironko and Iganga districts

Strategy	Frequency	Percentage
Remove the growing part of the affected plant	33	55.0
Nothing	14	23.3
Change the planting season	4	6.7
Seek advice from the extension worker	1	1.7
Early planting	4	6.7

Twenty two percent of the farmers interviewed perceived yield losses from MSD at above 10% of the total yield, while the rest of the farmers interviewed reported less than 10% loss (Table 2.6).

Table 2.6. Perceived yield losses of maize due to MSD in Sironko and Iganga districts

Percentage loss	Frequency	Percent
Less than 5%	11	18.3
5-6%	13	21.7
7-10%	10	16.7
Above 10%	22	36.7
NO response	4	6.7
Total	60	100

2.6.3 Farmers' varietal preference and selection criteria

There were varied sources of information on new technologies which farmers could utilise. The study revealed that information from farmer to farmer was a major factor (21.7%) in new technology awareness and perhaps adoption. Extension service providers and radio broadcasts constituted 16.7% each. Some farmers also reported that they received information on new technologies through farmer associations (Table 2.7).

Table 2.7. Farmers' sources of information on new technologies

Source	Frequency	Percent
Extension workers	10	16.7
Radio and NARO[1]	5	8.3
Fellow farmers	13	21.7
Radio and fellow farmers	8	13.3
Radio	10	16.7
Associations	8	13.3
Never had anything	3	5.0
ARDC[2]	1	1.7
No where	2	3.8
Total	60	100

[1]National Agricultural Research Organisation; [2]Agricultural Research Development Centre

Farmer preferences tended to vary across districts with differences in preferences of qualities for market and home consumption (Table 2.8a and b). Farmers from Sironko district preferred maize that was high yielding, with large grains and heavy grain weight for

the market whereas farmers from Iganga district mostly opted for any cultivar as long as there was a ready market for them (Table 2.8a). Qualities most preferred for home consumption among Sironko farmers included sweet taste, high yield, large grains and early maturity. In Iganga, on the other hand, farmers mentioned that they preferred cultivars which give high energy when eaten. High energy was assumed to refer to high starch content.

Table 2.8a. Preferences for maize for marketing

Preferences	District		Total
	Sironko	Iganga	
One which can be planted twice a year		2	2
One with available market		20	20
One which matures quickly		2	2
All the above		3	3
One which meets the basic needs		3	3
High yielding, big grains, high test density	21		21
No preference	9		9
Total	30	30	60

Table 2.8b. Preferences for maize for home consumption

Preferences	District		Total
	Respondent (%)		
	Sironko	Iganga	
High carbohydrate content	2	15	28.3
Easy to cook and easily stored		13	21.7
1 and 2 above	2	2	6.7
High yielding, resistant to lodging, resistant to rotting,	9		15
Tastes sweet, high yielding, large grains and quick maturing	17		28.3
Total	30	30	60

Figure (1) shows farmers' response on how often maize cultivars were to be changed/ developed by breeders. It was evident that farmers would appreciate replacement of the existing cultivars at various intervals. Over 50% of the farmers interviewed said maize cultivars need to be changed after one to two years (Table 2.9). The reasons given for the change were diverse but two reasons appeared to weigh more, namely, i) enough time was given to each cultivar in order to assess its performance and, ii) high yields were obtained if

the cultivars were changed regularly. Another benefit of changing cultivars was that cultivars with insect pest and disease resistance could be assessed in a period of three years.

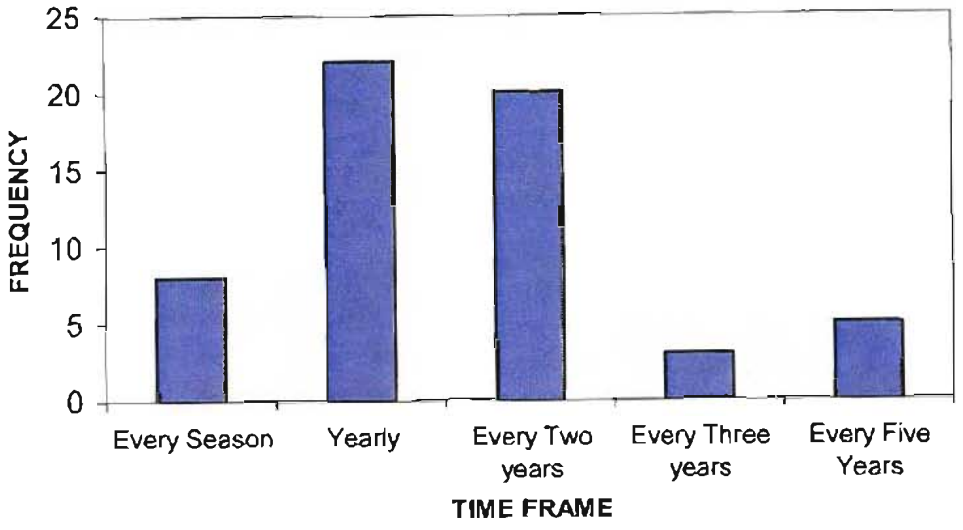

Figure 2.1 Farmers' views on how often maize cultivars should be changed

Table 2.9. Reasons for changing maize varieties by farmers' over given time frame

Reasons for changing maize cultivars	Frequency	Percentage
Enough time is given to each cultivar to assess its performance	20	33.3
High yields are got if the cultivars were changed seasonally	16	26.7
Yields get reduced after two years	6	10.0
Each year needs to be planted with new seed if high yields were to be realised	3	5.0
Crops with pest and disease resistance can be assessed in a period of three years	13	21.7

2.6.4 Farmers' involvement in cultivar development

Most farmers (71.7%) were willing to participate in the process of varietal development only towards the end of the process when the cultivars were ready for on-farm evaluations (Table 2.10). Only 5 % of the respondents would want to be involved from the onset, during breeding objective formulation. However, a few farmers thought that breeding could best be done by scientists at research institutions. About 23.3% of the respondents were not willing to participate in varietal development. Fifty seven percent of the farmers thought that it was

resource (time and money) consuming and 35.7% thought that it required highly trained persons.

Table 2.10. Stages of involvement of farmers in participatory plant breeding

Stages of involvement	Frequency	Percent
Objective formulation	3	5.0
On-farm trials and evaluations	43	71.7
No involvement	14	23.3
Total	60	100

2.7 Discussion

Results from Participatory Rural Appraisal revealed that farmers still used home saved seeds. The cultivars they grew were not improved for stress tolerance and therefore they succumbed to disease and insect pest damages. Gibson *et al.* (2005) also observed that most farmers selected seeds for planting from the best plants in their fields. Hence production of unimproved cultivars that were susceptible to both biotic and abiotic stress continued.

Some farmers obtained their seeds from home associations, which are farmers' groups. Such community based groups played a key role in helping farmers get access to improved seeds from NARO or through the National Agricultural Advisory Services (NAADS) programmes that target such farmer groups. For example in Iganga, a group known as Bakusekamajja, which began seed production in the mid-1990s, is involved in planting, harvesting, and marketing seed (Bakaira *et al.*, 2004). However, such groups are lacking in Sironko district where relatively more farmers used home saved seed.

A number of factors were listed as constraints to maize production across the two districts. These included unreliable rainfall, insect pests, declining soil fertility, disease and lack of access to improved seeds. While insect pests (mainly stalk borers) appeared the major biotic constraints, control methods being employed by the farmers were notably limited and consisted of rouging only. Many did not practise any control method, either because they lacked knowledge on control technologies, or they could not afford to use chemical control. The use of insect resistant maize cultivars would improve on farmers' yields. Breeding for

insect resistant cultivars is, therefore, suggested as the only economically affordable means of control to insect pests damages.

The order of importance of maize disease constraints had changed since a previous survey conducted in 2001 (Bigirwa *et al.*, 2001). Then, gray leaf spot was the most important disease curtailing maize production, followed by northern leaf blight. Maize streak virus was ranked fourth among the diseases. In this study differences were observed in disease ranking across the two districts. In Sironko, smut disease was ranked the number one disease constraint. This could probably be attributed to two factors. The first one was the under developed state of seed sectors in most developing countries (DeVries and Toenniessen, 2001). As such most farmers have continued to use own saved seeds or local cultivars. The second factor would be lack of resistance to head smut in improved maize cultivars being grown. Head smut has not been a disease of importance in Uganda and there has not been any active breeding programme in Uganda specifically addressing resistance to head smut. The environmental conditions in the breeding area may not have favoured the selection for resistance against smut. Hence most of the materials released might not have the genetic resistance against the disease.

Maize streak virus disease was also acknowledged as an important constraint in bridging the yield gap across the two districts. Overall MSD appeared to be the most important disease constraints to maize production. This is in agreement with other literature (Okori *et al.*, 1999; Bigirwa *et al.*, 2001; DeVries and Toenniessen, 2001; Pingali, 2001; CIMMYT, 2002). One of the reasons for the devastating effect of MSD was probably because the farmers still used unimproved cultivars that do not have resistance to the disease. It should be realised that MSD resistance is controlled by partially dominant genes that account for about 45-67% of the total variation in disease response (Pernet *et al.*, 1999a), which implies that there will always be some symptoms even when improved materials that carry the resistance genes are used. Farmers who grew improved seeds still mentioned that those cultivars suffered MSD damage showing there were different tolerance levels. For example Longe 1, an open-pollinated Uganda cultivar, is known to be resistant to MSD. Variations in yield losses due to MSD suggested that infection occurred late and/or at various infection levels. MSD infection can be uneven in a field leaving pockets of uninfected plants. It is reported that farmers usually bought seeds and grew them for many cropping cycles (Gibson *et al.*, 2005). As a result of out crossing in the field, most of their cultivars are not

genetically homogenous. By selecting seeds from uninfected maize plants, farmers tend to accumulate susceptible materials hence increased MSD level of damage. Thus farmers would be recommended to renew their cultivars at least every three years.

Ear rot was another disease that farmers perceived to be an important constraint to maize production. Like head smut, ear rot was not important until recently. The disease appeared to be more important in Iganga compared to Sironko. The two districts differ in altitudes and rainfall distributions. Sironko receives higher rainfall than Iganga, hence providing favourable condition for ear rot. Ear rot was not considered as one of the dominant constraints to bridging yield gap in sub-Saharan Africa (Pingali, 2001). Nevertheless, it is becoming an important production constraint factor in Uganda (Dr. J. Imanywoha, personal communication) that requires the attention of breeders. Most released cultivars grown by farmers were not improved for ear rot resistance and an epidemic could cause a great loss. The National Maize Programme has begun improving the exiting cultivars for resistance to ear rot.

Control measures for MSD employed by farmers consisted of removal of growing tip of young affected plants, which was similar to rouging. The removal of the growing tip kills the plants before producing any ear. The practice could be done to avoid replanting seeds from disease plant since they used own saved seed from their previous crops. A few farmers practised early planting to help crops escape MSD but most farmers do not have the machinery to prepare land early before the rains. Land preparation usually delayed planting, subjecting the crops to coincide with high leafhopper population build-up for infection during the season. Early planting has been criticised because it is not an effective control measure for MSD where continuous cropping was practiced (ISAAA, 2001). Some farmers change of cultivars in each season and planted some cultivars in one or the other season which was only effective where MSD occurrence was sporadic but did not work where there continuous occurrence of MSD. The effectiveness of these control methods depended on a number of factors including, among others, season, crop growth stage at which the infection occurred and the genetic make up of the plant cultivar in question. The most commonly used control by farmers According to Pingali (2001), practices such as timely planting and treatment of seed with systemic insecticides could help control yield losses. A more effective and practical solution for subsistence farmers is high yielding maize cultivars that are resistant to MSD (DeVries and Toenniessen, 2001; ISAAA, 2001). Breeding for resistance to MSD is

easy because symptoms are very clear and easy to rate especially when the disease infection occurred early.

Relatively more farmers indicated that their main source of information on new technologies was other farmers. This could be improved upon if farmers were organised into groups. The National Agricultural Advisory Services has been formed by the Ministry of Agriculture, Uganda to offer technical services to farmers based on their needs. Such services could be easily accessed by farmer groups who indicated the type of support they needed. Organised farmer groups could receive training on disease control or access improved cultivars or even collaborate with the National Maize Programme (NMP) in varietal trials and hence improve dissemination of improved technologies. The Bakusekamajja women's group in Iganga is a classical example (Bakaira *et al.*, 2004).

The results also showed that relatively a small percentage (16.7%) of farmers sought advice from extension workers. This is probably because extension workers are few and therefore are unable to reach all farmers. In addition, farmers tend to view technology transfer facilitated by extension workers are expensive in terms of agro-input. They are most time reluctant to seek advice from extension worker but would prefer going to another farmer like modal farmers in their areas.

Variations in farmers' preferences when choosing cultivars for market and home consumption were noted. However, results showed that farmers pointed out yield and early maturity as common selection criteria and priority requirements in any improved cultivar. Improvement of the two traits is therefore fundamental for the adoption of new cultivars. Yield and early maturity have been central in every breeding programme thus providing a basic need. With unreliable rainfall cited as the most important constraint, the development of early maturing cultivars would improve on the varietal adoption. High yield and maturity duration are negatively correlated and to combine the two traits remains a daunting task for the breeder. Farmers' preferences for market cultivars were also apparently different from preference for home consumption. In this regard different breeding objectives should be formulated with input from the farmers to address these special preferences. Breeding for speciality cultivars designed for marketing purposes is suggested.

Besides yield and earliness, farmers had some preferences that were identified by the survey. They included sweet taste, large kernels and high test density. These kernel qualities or preferences were also reported from a survey in Uganda and Tanzania (Gibson *et al.*, 2005). Breeders have not been focussing on such qualities. Sweet taste, large kernels and high test density, therefore, form farmers' special preferences. There is need to integrate such special farmer preferences into selection criteria. It is easy to breed for early maturity *per se* because it is highly heritable, but not so for yield, which has low heritability (Hallauer and Miranda, 1988). Selection for large and high density kernels would also be easy as these can be selected for by visual assessment and by determining the weight of a 100 kernels, respectively. Sweet taste for green maize can be selected for using palatability tests. The challenge is sweet taste would the time of testing is critical, in that if done late most sucrose would have been converted to starch hence less sweet taste. Yet in Africa, green maize is normally consumed when maize is approaching physiological maturity when most conversion of sucrose to starch has taken place.

From the results presented here, these preferences varied across the regions. Involvement of farmers in varietal selection would help bridge the information gap between farmers and breeders. It has been reported that farmers grow the same cultivars for long periods of time. This could probably be because they have failed to find new cultivars with special qualities they desire. In this study, farmers showed their willingness to change cultivars regularly suggesting that appropriate cultivars would be adopted. Breeding for wide adaptation, as has been the case over the years, can no longer work because of differences in preferences. Therefore breeding for specific ecologies is being suggested.

Fortunately farmers were interested in participating in the breeding process although at the later stages of varietal testing. Farmers expressed concerns that being involved from the start of the varietal development process was resource (time and money) consuming. They were, however, willing to participate in varietal selection at the later stage of varietal development, a concept known as participatory varietal selection (PVS). Participatory varietal selection and/or participatory plant breeding are becoming a necessity because farmers' needs and preferences are quickly changing with changes in agricultural environments and market demand. While meeting these needs requires continuous engagement of the farmers in the breeding process, the reality of this remains a daunting task to breeders. On the other hand, when a cultivar fails to meet farmers' needs during

PVS, farmers would not adopt it and the effort and resources spent would have been wasted. For example, when working together with farmers to produce improved cultivars of maize for the low-resource farmers in India, farmers were involved towards the end (PVS). The results were not very successful. When they got involved in plant selection from the segregation stage (PPB), the method produced success. The cultivars were adopted by the farmers themselves (Witcombe *et al.*, 2003) because they were able to identify and select for the special qualities at early stage when genetic variation is large. Other advantages reported were that the approach was cheaper and benefits to farmers were realised earlier.

2.8 Conclusion

The survey revealed two dominant factors as maize production constraints, namely unreliable rainfall and insect pests (stalk borers). Other factors were; declining soil fertility, land shortage and diseases. Among the diseases MSD was the most important in Iganga and was the second most important production constraint in Sironko. Overall, MSD was the most important disease in bridging the yield gap between research stations and farmer's fields. Results also showed that farmers still have a basic preference for high yielding and early maturing cultivars. Farmer's also had special preferences which included large, white and high test density kernels for marketing, and sweet tastes, particularly for home consumption. Priorities crop improvement as perceived by the farmers included resistance to drought, insect pests and diseases, sweetness for green maize, prolificacy, resistance to lodging, and drooping leaves. Drooping is preferred because they form canopy and prevent weeds from getting sunlight, hence control them. Farmers had high interest in participatory varietal selection but not the whole process of participatory plant breeding, which they perceived to be time consuming.

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CHAPTER THREE

Selection for resistance to maize streak virus disease using conventional breeding methods

3.1 Abstract

Maize streak virus disease, a geminivirus, is exclusively transmitted by *Cicadulina* leafhoppers and continues to devastate farmers' fields in Uganda. There are a few options for controlling the disease but plant genetic resistance is the only economically viable one. A breeding programme was therefore initiated with the aim of screening for resistance to MSD using conventional method and to determine mode of inheritance for MSD resistance. Two crosses were made between CML202 (resistant parent), and CML321 and CML384 (susceptible parents). From the two cross, four populations were generated; two by backcrossing to the susceptible parents and the other two, by selfing progenies. First and second cycles of conventional selection for MSD resistance were applied at BC1F1 and BC2F1 for backcross populations and at F2 and F3 in the selfed progenies. Results showed that backcross and selfed populations segregated in 1:1 and 3:1 Mendelian ratios, respectively, confirming simple inheritance and the presence of one major gene with dominant gene action for resistance to MSD. Severity and incidence of disease were positively correlated suggesting a non-preference form of resistance by the insects. A bimodal frequency distribution for disease severities at BC1F1and BC2F1 generations of backcrossing indicated that resistance was controlled by both a major and minor genes. In the selfed populations, the presence of complete resistance against MSD was observed. The frequency distribution patterns at both F2 and F3 generations were highly skewed in favour of resistance. There was a decrease in disease severities with selection from BC1F1 to BC2F1 and from F2 to F3 generations indicating that high response to selection was achieved. As expected, selfed populations were more superior source populations to effect selection than backcrosses.

3.2 Introduction

Surveys conducted in Uganda have shown a wide distribution of maize streak virus (MSD) among other persistent foliar diseases (Okori, 1999; Bigirwa *et al.*, 2000; Gibson *et al.*, 2005). Maize streak virus is still one of the dominant constraints to bridging the gap between potential and actual yield in mid altitude or subtropical regions (Pingali, 2001). Sporadic epidemics of MSD continue to occur in much of Africa and the resulting losses can be devastating (Bosque-Perez, 2000). Yield losses due to the disease are not easy to quantify but range from 0-100% (Bosque-Perez, 2000), depending on the year and stage of growth of maize plant when it is attacked. Reduction in yield due to MSD is reported to be a function of the age of plants at time of infection and younger plants sustain greater yield reductions

than older ones (Guthrie, 1978; van Rensburg, 1981; Mzira, 1984; Ampong-Nyarko *et al.*, 1998; Bosque-Perez *et al.*, 1998). Yield loss is a function of age (Ampong-Nyako *et al.*, 1998) and of the relative level of susceptibility or resistance of the maize cultivar (Vogel *et al.*, 1991, 1993; Barrow, 1992; Bosque-Perez *et al.*, 1998), indicating that breeding for resistance would be effective against the disease.

There are few options for controlling maize streak virus available for farmers to choose from. While insecticides have been used to control leafhopper vectors (Rose, 1978; Rothwell, 1979; Barrow, 1992), they are too expensive for the resource-poor farmers who are hardest hit by MSD. There are also other issues regarding the use of chemicals (ISAAA, 2001). For example, human and environment hazards, short-lived effects of chemicals and development of resistance to commercially available products by insects. According to Boulton (2003), pesticide application against the insect vectors is time-consuming, costly, and environmentally undesirable.

Early sowing can also help the crop reach a safe age (beyond seven leaf stage) before populations of the leafhopper build up. However, this strategy is considered to be ineffective in areas with multiple sowing dates, especially those with two growing seasons (ISAAA, 2001). Instead such changes in cultural practices promote population build up of leafhoppers, hence providing a ready source of infection for the following season. A more pragmatic and effective solution for subsistence farmers would be to breed for high yielding maize cultivars that carry genetic resistance to MSD (DeVries and Toenniessen, 2001; Pingali, 2001).

The occurrence of MSD epidemics in Tanzania in 1979 (Buddenhagen and Bosque-Pérez, 1999) and in Kenya in 1988 (Pingali, 1999) has reawakened scientists in the region to come up with an appropriate solution for resource poor farmers if they are to overcome effects of MSD epidemics. Genetic resistance is generally viewed the most economical solution to MSD problem (Buddenhagen, 1983; Buddenhagen and Bosque-Pérez, 1999; DeVries and Toenniessen, 2001; ISAAA, 2002). Most breeding programmes have released MSD resistant maize cultivars. In Uganda, for example, the national maize program bred cultivars that are tolerant to MSD including Longe1.

There are resistance sources to maize streak virus disease which have been used to improve a number of agronomically acceptable cultivars (Barrow, 1993; Pratt *et al.*, 1997;

CIMMYT, 2002). It is the availability of a resistance source, the need for improved plant materials and the demand for more food to feed the world that forms the underlying principle for improving resistance to agronomically acceptable inbred lines.

Improving maize cultivars for disease resistance using conventional approaches can be quite challenging, especially when the success of selection depends on environmental conditions that determine the presence of diseases. The other drawback of the commonly employed conventional breeding is that it takes a minimum of 7 years to produce a new cultivar (Dreher *et al.*, 2000). However, conventional breeding for MSD has been notably successful in producing important genetic gains in maize. A diversity of conventional breeding methods have been utilised including intra- and inter-population improvement schemes, pedigree selection, and use of selection indices. The most commonly used conventional breeding method to improve agronomically acceptable cultivars or cultivars for disease resistance is backcrossing. The method is used especially when dealing with major genes. Resistant inbred lines have also been developed through generations of selfing. Hence, to realise the high yield potential of the mid-altitude maize growing areas of Uganda (1000 – 1600m above sea level), maize cultivars and hybrids must possess resistance to the specific foliar diseases which occur in that zone.

Resistance to MSD is controlled by a major gene (Rodier *et al.*, 1995; Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a, b) and other minor genes (Rodier *et al.*, 1995, Welz *et al.*, 1998; Pernet *et al.*, 1999a, b). The major gene appeared to be simply inherited (Rodier *et al.*, 1995; Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a, b), and the minor genes showed quantitative mode of inheritance (Kim *et al.*, 1989; Rodier *et al.*, 1995).

Conventional selection using backcrossing is done to recover the recurrent parents. For traits controlled by major genes backcrosses with selection can be done without interval of selfing between backcrosses as the phenotypes will be different. In the case of a trait controlled by a recessive gene, backcrossing should be followed by selfing for the trait to be selected for phenotypically. Selfing and selection method is done to generate new recombination and selection of new lines different from the parents. The success of screening for resistance to maize streak virus disease under natural conditions depends on year and season (Mesfin and Bosque-Perez, 1998). In Uganda, screening for resistance to MSD has been done with the use of spreader rows. The technique involves planting rows of

susceptible materials at ±10m intervals between experimental materials. This technique has been used in screening for MSD resistance in Zimbabwe (Caulfield, 1997) and Mozambique (Denic, 1997) and has produced successful results. Being vector transmitted the distribution is normally not uniform in the field. Such variations may allow for some plants to escape the disease. Artificial inoculation method is used to achieve relatively more uniform infection at early stage than the spreader rows. This procedure involves rearing leafhoppers in cages (Bosque-Perez and Alam, 1992), which is expensive procedure in terms of time and labour.

3.3 Objective of the study

The specific objectives of the study were:

- to screen for MSD resistance using conventional method in backcross and selfed populations at early segregating generations
- determine the inheritance of resistance in backcross and selfed populations

The hypothesis tested was that:

- resistance to MSD can be improved through selection in early segregating generations, and is simply inherited.

3.4 Materials and Methods

3.4.1 Location of the study

The experiment was conducted at Namulonge Agricultural and Animal Research Institute (NAARI), which is located 23 kilometres north of Kampala (0° 31' N, 32° 35' E), with a mean altitude of 1,150m above sea level. The long term mean annual rainfall is 1,270mm, and the distribution is bimodal, with peaks in April to May, and October to December respectively. The relative humidity averages 76% to 88%. The area consists of highly weathered ferralitic soils mainly of the clay-loam type.

3.4.2 Parent lines and source of resistance

Three maize inbred lines obtained from CIMMYT-Zimbabwe were used in this study and they included CML 384, CML 321and CML 202 (Table 3.1). CML384 and CML 321 were released by CIMMYT maize program (Mexico) and are adapted to subtropical environments.

They are late maturing, with white flint kernels and are highly resistant to NLB (*Exerohilum turcicum* Pass), but susceptible to MSD (CIMMYT, 2002). CML202 was released by CIMMYT Maize Programme in Zimbabwe and is adapted to mid-altitude environments. It is characterised by late maturity, white semi-dent kernels and is resistant to MSD, NLB and rust (Table 3.1).

Table 3.1. Pedigree information of inbred lines used in the study

Line	Germplasm source	Pedigree	Adaptation	Maturity	Stress tolerance and resistance
CML202		ZSR923S4BULK-5-1-B	Africa MA/ST*	Late	MSD, E. turcicum, P. sorghi
CML321	P502	P502C0F1-1-3-1-B*4	Subtropical	Late	E turcicum
CML384	P502	P502C1#-771-2-2-1-3-B	Subtropical	Late	GLS, E turcicum

Source: CIMMYT (2001). *Mid-altitude/ Sub-tropical

3.4.3 Development of segregating populations for MSD screening

For the backcross selection method, two crosses were made between CML321 x CML202 and CML384 x CML202 to generate F1 progenies. The F1 generations were backcrossed to the recurrent parents CML321 and CML384 to give two BC1F1 populations. Their F1s were also self-pollinated to generate two F2 populations.

The populations were denoted as follows:

- BC1F1a derived by backcrossing F1 between CML321 and CML202 to recurrent parent, CML321.
- BC1F1b derived by backcrossing F1 between CML384 and CML202 to recurrent parent CML384.
- F2a derived by self-pollinating F1 of CML321 and CML202.
- F2b derived by self-pollinating F1 of CML321 and CML202.

BC1F1 and F2 generations were screened for resistance and the selected plants were again backcrossed to the recurrent parents (CML321 and CML384) and self-pollinated to produce BC2F1 and F3 generations as follows:

- BC1F1a backcrossed to CML321 to generate BC2F1a
- BC1F1b backcrossed to CML384 to generate BC2F1b.
- F2a self-pollinated to generate F3a.

- F2b self-pollinated to generate F3b.

3.4.4 Establishment of screening nurseries

Each of the segregating populations was planted for screening for MSD resistance in 2004 at Namulonge Agricultural and Animal production Research Institute (NAARI). Population size of 500 plants for each population was established. Screening nurseries were laid out in three blocks for each population. The blocks were made up of 42 rows of 5m long. Field lay out design involved planting four rows of susceptible checks (spreader rows) at the beginning of each block, then after every five rows of segregating populations, and at the end of the block. In backcross nurseries, three rows of the recurrent parents were planted following the first four rows of susceptible checks on either side of the blocks.

The progenies of selected plants from BC1F1 and F2 generations, that is, BC2F1 and F3 generations were planted in an ear-to-row method in which progenies of each plant were planted in one row only. The same pattern of spreader rows layout was used. Agronomic practices included fertilizer application at planting with diammonium phosphate (DAP), weeding and top dressing with urea during the vegetative stage. Termite control measures involved spraying with pesticides at regular intervals to reduce crop loss.

3.4.5 Enhancement of *Cicadulina* leafhopper population for MSD screening

The experiment required artificial inoculation of the seedlings with *Cicadulina* leafhoppers. While the breeding programmes at the station have relied on natural *Cicadulina* population for screening, the population build up depended largely on environmental factors. In order to augment the leafhopper population, the infester (spreader) row technique (Caulfield, 1997; Denic, 1997) was adopted. Infester row technique is where susceptible materials are planted to increase the vector population to a high level in order to increase disease pressure. This technique involved planting a susceptible hybrid from private seed dealers (thus pedigree or brand name is not mentioned) three times at two weeks intervals beginning four weeks before planting the test materials. The susceptible hybrid was planted between every five rows of the test materials. Planting at different dates was intended to produce a continuous supply of a preferred growth stage when the plants are at a height of 25 – 40 cm (NARO Information Sheet) so that there would be increased leafhopper population before planting the test materials. The test materials were planted at once after the second planting of spreader rows. The pattern of planting also ensured relatively late

planting of the test materials and coincided with a build up of leafhopper population and hence increased disease incidence.

3.4.6 Data collection

Disease development was monitored during the growth of the crops and data was taken. At flowering, individual plants scoring above 3 for MSD were not selected for selfing or backcrossing in the F2 and BC1F1, respectively. Maize streak virus severity was rated using a scale of 0 – 5 (Bosque-Perez and Alam, 1992) as follows:

Scale	Description
0	no visible disease symptoms
1	very few streaks on some leaves
2	light streak symptoms on most leaves
3	moderate streak symptoms on most leaves
4	abundant symptoms on all leaves (>60%) leaf area affected
5	severe symptoms on all leaves (>80%) of leaves affected with no yield

Maize streak virus disease incidence and severity were scored twice; at four weeks after emergence and at flowering (critical stages for MSD effect on yield). Disease incidence was scored by recording the number of plants in each population showing MSD symptoms and then expressing that as a percentage of the total plant population. Disease severity was scored on the whole plant as a proportion of total leaf area diseased. Other diseases (GLS, NLB and ear rot) were also observed though their incidence and severity were too low for statistical considerations (data not presented).

3.4.7 Selection method

Single plant selection method was applied at the F2 and BC1F1 selection stages during which each individual plant is genetically unique. Selection of plants for advancement to the next generation was done in two stages: First, the plants were selected basing on MSD severity scores. Plants with 0 severity scores were not selected because they could not be separated from escapes. Only plants that showed symptoms with severity scores of 1 and 2 indicating high resistance were selected. Ten percent (50/500) of the plants with score of 1 to 2 were selected, equivalent to selection intensity of 1.74 (Falconer, 1981). To achieve that

intensity of selection, the number of plants selected was reduced based on agronomic characteristics.

3.4.8 Data analysis

Data from F2 and BC1F1 populations were subjected to chi-square goodness of fit test to determine whether the phenotypic observation was consistent with expected segregation ratios of 1:1 and 3:1 for backcross and selfed populations. The expected fitted frequencies were computed basing on the expected Mendelian ratios as follows:

For a 1:1 ratio in a backcross population, the expected outcome would be:

= 1/ (1+1) x the total observations, given total observation is 500,
= ½ x 500
= 250 for each observation.

For a 3:1 ratio in selfed population, the expected outcome would be given as:

= 3/ (3+1) x the total observations, given total observation is 500,
= ¾ x 500
= 375 for one observation;

For the other observation,

= 1/ (3+1) x 500
= ¼ x 500
= 125

Pearson chi-square test was also performed to determine the frequency of plants in each severity class using GenStat statistical software (GenStat, 2005). Overall mean severity for all plant and severity for plants exhibiting symptoms were calculated using the formula described by Rodier *et al.* (1995). Standard deviations were computed for overall mean severity, percentage incidences and mean rating for plants with symptoms.

3.5 Results

3.5.1 Selection in BC1F1 and F2 segregating populations

Results of chi-square goodness of fit test analysis of BC1F1 and F2 populations showed that plants segregated in a ratio of 1:1 and 3: 1, respectively. The X^2cal values were not significant for all the four populations (Table 3.2). It is important to note that we considered all plants with MSD rating from 0 to 2 as resistant and those that were rated 4 and 5 were considered susceptible.

Table 3.2. Chi-squared goodness of fit test results for backcross and selfed populations

	Backcross populations			
	BC1F1a		***BC1F1b***	
	Resistant	Susceptible	Resistant	Susceptible
Ratio	1	1	1	1
Observed Outcome	265	235	229	271
Expected Outcome	250	250	250	250
X^2cal	0.18		0.06	
X^2tab (α = 0.05, df = 1)	3.84			
	F2 Selfed populations			
	F2a		***F2b***	
	Resistant	Susceptible	Resistant	Susceptible
Ratio	3	1	3	1
Observed Outcome	401	99	397	103
Expected Outcome	375	125	375	125
X^2cal	0.01		0.02	
X^2tab (α = 0.05, df = 1)	3.84			

There was significant variation (X^2cal = 126.27, df = 15, P <0.001) in incidence and severity of MSD between populations. While most plants showed no symptoms in each population, the number varied across populations (Table 3.3). The percentages of plants in populations BC1F1a, BC1F1b, F2a and F2b that had disease severity of 0 were 31.2%, 29.4%, 55.6% and 54.8%, respectively. Less than 10% of plants within each population had severity rating of 5.

The disease evaluation showed little variation in overall mean severity across populations and mean severity ranged from 1.0 to 1.5 across the populations (Table 3.3). There was no significant correlation between overall mean severity and percentages of symptom-free plants (r = -0.3643, p < 0.081) and also between overall mean severity and percentage disease incidence (r = 0.3644, P < 0.079).

Table 3.3. Variation in mean severity, incidences and frequency distributions across populations

Population	Sample size	Overall mean severity		Percentage of symptom-free plants	Percentage incidence		Mean severity for plant with symptoms		Frequency distribution pattern for severity
		Mean	SD*		Mean	SD*	Mean	SD*	
BC1F1a	500	1.4	1.6	31.2	57.8	33.5	2.6	1.4	Bimodal
BC1F1b	500	1.3	1.5	29.4	52.4	30.1	2.8	1.2	Bimodal
F2a	500	1.0	1.4	55.6	44.4	22.2	2.3	1.2	Skewed left
F2b	500	1.5	1.4	54.8	45.2	24.8	2.3	1.1	Skewed left

* Standard deviations

Strong positive correlation (r = 0.995, $P < 0.001$) was observed between the mean rating of plants with symptoms and percentage incidence. A strong but negative correlation existed between mean severity for plants exhibiting symptoms and percentage of symptom-free plants. Intra-population severity rating showed bimodal frequency distribution patterns for the backcross populations (Fig3.1 and b) and skewed in favour of resistance for selfed populations (Fig 3.1c and d). This is also showed in table 3.4.

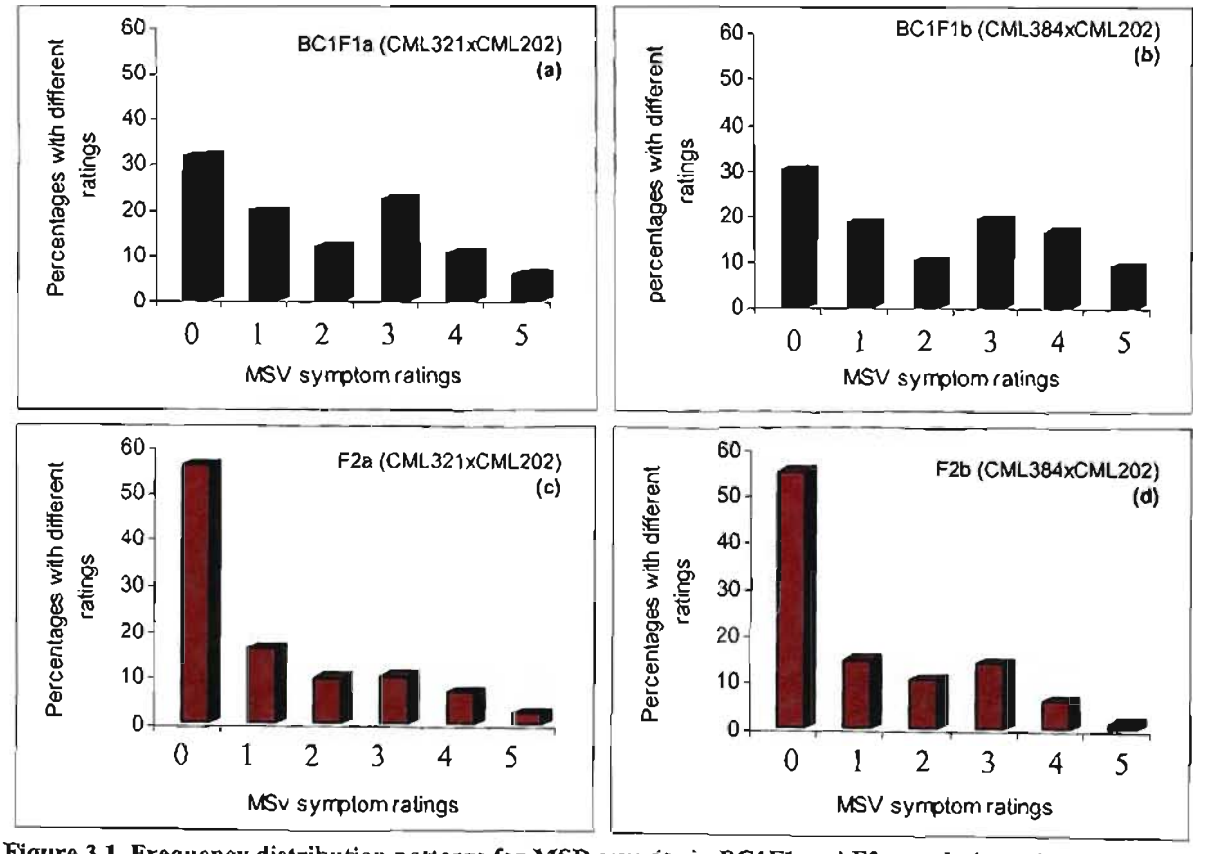

Figure 3.1. Frequency distribution patterns for MSD severity in BC1F1 and F2 populations: (a) and (b) are BC1F1 populations; (c) and (d) are F2 populations.

Table 3.4. Pearson chi-square contingency table showing observed and fitted frequency of plants for different disease severity ratings in four populations

Population	MSD_Sev	Observed	Fitted	Residual
BC1F1a	0	156.00	150.25	-4.05
	1	97.00	75.75	2.36
	2	58.00	25.00	0.36
	3	109.00	100.25	-3.52
	4	51.00	65.75	8.17
	5	29.00	35.00	-0.37
BC1F1b	0	147.00	150.25	-1.27
	1	89.00	75.25	0.45
	2	48.00	25.25	-2.37
	3	94.00	100.25	6.02
	4	79.00	65.75	-4.02
	5	43.00	35.25	1.48
F2a	0	278.00	250.25	2.87
	1	79.00	85.75	-0.92
	2	48.00	44.00	0.73
	3	50.00	67.25	-2.61
	4	34.00	42.75	-1.62
	5	11.00	10.00	0.37
F2b	0	274.00	250.25	2.45
	1	72.00	85.75	-1.88
	2	51.00	44.00	1.28
	3	68.00	67.25	0.11
	4	29.00	42.75	-2.54
	5	6.00	10.00	-1.48

Pearson chi-square value is 126.3 with 15 d.f. Probability level (under null hypothesis) $p < 0.001$

3.5.2 Selection in BC2F1 and F3 segregating populations

Disease severity on selected lines at F3 and BC2F1 generations varied within each population and between populations. Severity ratings observed across the populations ranged from 1 to 4. Differences between backcross populations were not significant (X^2cal = 3.86, df = 3, (α = 0.05) (Table 3.5a) although there were variations in the number of lines with similar MSD severity scores within backcross populations. However, significant variations were observed between populations BC1F1a and F3a (X^2cal = 13.54) (Table 3.5b), and between populations BC1F1a and F3b (X^2cal = 17.45) (Table 3.5c). No significant differences were observed in severity between populations BC2F1b and F3a (X^2cal = 3.86) (Table 3.5d), between BC2F1b and F3b (X^2cal = 5.97) (Table 3.5e), and between F3a and F3b (X^2cal = 1.55) (Table 3.5f). Number of lines with the same severity scores varied

between the populations. Differences were noted when lines from backcrosses selection were compared with lines from selfing methods.

Table 3.5(a). Observed and fitted frequency of plant for different disease severity ratings in BC2F1a and BC2F1b populations

Population	MSD_Sev	Observed	Fitted	Residual
BC2F1a (CML321xCML202)	1	5.00	8.50	-1.86
	2	21.00	19.00	0.82
	3	19.00	17.00	0.84
	4	5.00	5.50	-0.32
BC2F1b (CML384xCML202)	1	12.00	8.50	1.86
	2	17.00	19.00	-0.82
	3	15.00	17.00	-0.84
	4	6.00	5.50	0.32

Pearson chi-square value is 3.86 with 3 d.f., Probability level (under null hypothesis) p = 0.276

Table 3.5(b). Observed and fitted frequency of plant for different disease severity ratings in BC2F1a and F3a populations

Population	MSD_Sev	Observed	Fitted	Residual
BC2F1a (CML321xCML202)	1	5.00	13.00	-3.65
	2	21.00	16.50	1.91
	3	19.00	16.00	1.29
	4	5.00	4.50	0.35
F3a (CML384xCML202)	1	21.00	13.00	3.65
	2	12.00	16.50	-1.91
	3	13.00	16.00	-1.29
	4	4.00	4.50	-0.35

Pearson chi-square value is 13.54 with 3 d.f., Probability level (under null hypothesis) p = 0.004

Table 3.5(c). Observed and fitted frequency of plant for different disease severity ratings in BC2F1a and F3b populations

Population	MSD_Sev	Observed	Fitted	Residual
BC2F1a (CML321xCML202)	1	5.00	14.00	-4.01
	2	21.00	17.50	1.47
	3	19.00	13.50	2.48
	4	5.00	5.00	0.00
F3b (CML384xCML202)	1	23.00	14.00	4.01
	2	14.00	17.50	-1.47
	3	8.00	13.50	-2.48
	4	5.00	5.00	0.00

Pearson chi-square value is 17.45 with 3 d.f., Probability level (under null hypothesis) p < 0.001

Table 3.5(d). Observed and fitted frequency of plant for different disease severity ratings in BC2F1b and F3a populations

Population	MSD_Sev	observed	Fitted	Residual
BC2F1b (CML384xCML202)	1	12.00	16.50	-1.91
	2	17.00	14.50	1.10
	3	15.00	14.00	0.45
	4	6.00	5.00	0.67
F3a (CML321xCML202)	1	21.00	16.50	1.91
	2	12.00	14.50	-1.10
	3	13.00	14.00	-0.45
	4	4.00	5.00	-0.67

Pearson chi-square value is 3.86 with 3 d.f., Probability level (under null hypothesis) p = 0.277

Table 3.5(e). Observed and fitted frequency of plant for different disease severity ratings in BC2F1b and F3b populations

Population	MSD_Sev	Observed	Fitted	Residual
BC2F1b (CML384xCML202)	1	12.00	17.50	-2.30
	2	17.00	15.50	0.65
	3	15.00	11.50	1.66
	4	6.00	5.50	0.32
F3b (CML384xCML202)	1	23.00	17.50	2.30
	2	14.00	15.50	-0.65
	3	8.00	11.50	-1.66
	4	5.00	5.50	-0.32

Pearson chi-square value is 5.97 with 3 d.f., Probability level (under null hypothesis) p = 0.113

Table 3.5(f). Observed and fitted frequency of plant for different disease severity ratings in F3a and F3b populations

Population	MSD_Sev	Observed	Fitted	Residual
F3a (CML321xCML202)	1	21.00	22.00	-0.40
	2	12.00	13.00	-0.46
	3	13.00	10.50	1.23
	4	4.00	4.50	-0.35
F3b (CML384xCML202)	1	23.00	22.00	0.40
	2	14.00	13.00	0.46
	3	8.00	10.50	-1.23
	4	5.00	4.50	0.35

Pearson chi-square value is 1.55 with 3 d.f., Probability level (under null hypothesis) p = 0.672

Calculations of mean severity at F3 and BC2F1 generation of selection showed a variation in mean severity ranging from 1.9 to 2.5 (Table 3.6). Backcross populations had consistently higher mean severity ratings than selfed populations. Unlike in BC1F1 generation, backcross populations at BC2F1 generation showed a unimodal pattern of frequency distributions (Fig. 3.2a and b). On the other hand, the two selfed populations maintained a skewed left pattern of frequency distribution for mean severity (Fig. 3.2c and d).

Table 3.6. Mean severity ratings and frequency distribution for MSD

Population	Sample size	Mean symptom rating		Frequency distribution pattern for severity
		Mean	SD	
BC2F1a	50	2.5	0.8	bimodal
BC2F1b	50	2.3	1.0	Unimodal
F3a	50	2.0	1.0	Skewed
F3b	50	1.9	1.0	Skewed

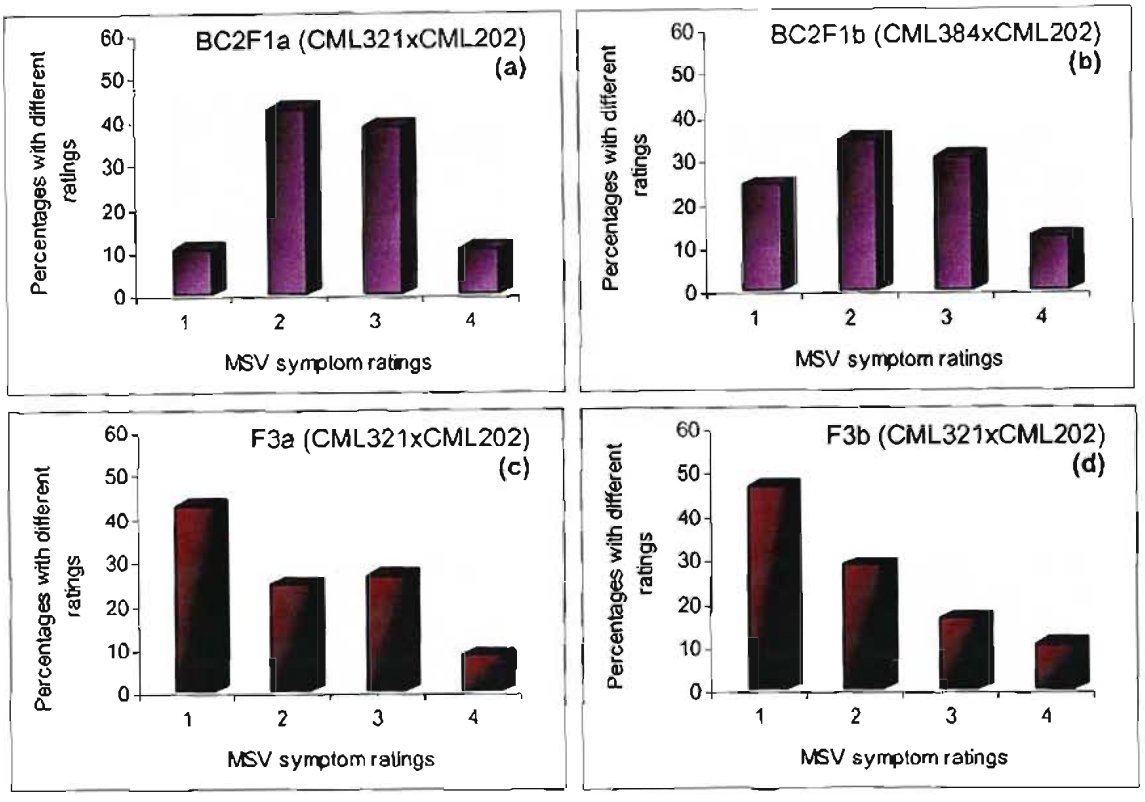

Figure 3.2. Frequency distribution for severity in BC2F1 and F3 populations: (a) and (b) are BC2F1 populations; (c) and (d) are F3 populations.

Disease incidences, like severity, also varied between families within each population. Incidences in populations BC2F1a and BC2F1b varied across the lines and ranged from 22% to 100% and from 36% to 100%, respectively (Table 3.6). Incidences also varied across lines between populations F3a and F3b (Table 3.6). The values of incidences ranged from 7% to 100% in F3a population, and from 13% to 100% in F3b population. Correlation of incidence and severity variations within each population showed a strong positive relationship (r_1 to $r_4 > 0.8$, $P < 0.001$) between occurrence of the disease and the extent of its damage within every family of each population (Table 3.6). The higher the disease incidence observed, the higher the severity recorded, and vice versa.

Table 3.6. Disease severities and percentage incidences across populations

Lines Within population	BC2F1a (CML321xCML202)		BC2F1b (CML321xCML202)		F3a (CML321xCML202)		F3b (CML321xCML202)	
	Severity	Percentage incidence	Severity	Percentage incidence	Severity	Percentage incidence	Severity	Percentage incidence
1	1	22	1	36	1	33	1	42
2	1	40	1	38	1	38	1	21
3	1	41	1	42	1	24	1	23
4	1	38	1	42	1	29	1	23
5	1	38	1	44	1	8	1	23
6	1	43	1	45	1	7	1	10
7	1	45	1	46	1	31	1	27
8	1	47	1	46	1	9	1	25
9	1	28	1	50	1	13	1	35
10	1	60	1	50	1	21	1	13
11	1	29	1	57	1	36	1	33
12	1	55	1	63	1	20	1	21
13	1	69	2	40	1	43	1	17
14	1	50	2	42	1	33	1	33
15	1	40	2	46	1	40	1	29
16	1	40	2	50	1	44	1	17
17	1	44	2	50	1	14	1	33
18	1	64	2	50	1	50	1	33
19	1	40	2	50	1	43	1	27
20	1	30	2	50	1	38	1	23
21	1	31	2	50	1	36	1	38
22	2	38	2	53	2	31	1	14
23	2	47	2	54	2	44	1	27
24	2	58	2	54	2	21	2	31
25	2	53	2	54	2	38	2	44
26	2	57	2	54	2	46	2	36
27	2	57	2	55	2	47	2	38
28	2	53	2	55	2	35	2	29
29	2	67	2	56	2	36	2	46
30	2	56	3	56	2	56	2	36
31	2	71	3	57	2	71	2	56
32	2	50	3	57	2	62	2	36
33	2	60	3	57	2	100	2	27
34	3	54	3	62	3	38	2	50
35	3	59	3	62	3	40	2	27
36	3	53	3	63	3	43	2	44
37	3	63	3	67	3	50	2	30
38	3	92	3	67	3	56	3	60
39	3	67	3	70	3	71	3	46
40	3	69	3	71	3	56	3	50
41	3	69	3	75	3	94	3	50
42	3	87	3	75	3	42	3	50
43	3	67	3	77	3	69	3	69
44	3	67	3	82	3	64	3	70
45	3	47	4	63	3	73	3	60
46	3	88	4	73	3	100	4	56
47	4	79	4	77	4	92	4	100
48	4	100	4	89	4	100	4	100
49	4	88	4	100	4	81	4	100
50	4	86	4	100	4	100	4	100
Correlations:	$r_1 = 0.8374$, $P < 0.0001$		$r_2 = 0.8135$, $P < 0.0001$		$r_3 = 0.8481$, $P < 0.0001$		$r_4 = 0.9615$, $P < 0.0001$	

3.5.3 Response to selection

Mean severities varied between backcross and selfing populations during the first and second cycles of selections. In the first cycle of selection, backcross populations had higher mean severities than selfing populations (Table 3.7). In the second cycle of selection, backcrosses still had higher severities than selfing populations. There was a reduction in disease severity levels in the second cycle of selection for each of the populations (Table 3.7). BC1F1 generations scored higher severities than BC2F1 generations. F2 generations also had higher severities than F3 generations. These showed that there was a response to selection in each of the populations. During the first cycle of selection there was low incidence but disease severities ranged from 0 – 5. In the second cycle of selection, there was high incidence and severity ranged from 1-4.

Table 3.7. Mean severities at two selection cycles of backcross and selfing populations

First cycle of selection		Second cycle of selection	
Populations	Mean severity	Populations	Mean severity
BC1F1a	2.6	BC2F1a	2.4
BC1F1b	2.8	BC2F1b	2.3
F2a	2.3	F3a	2.0
F2b	2.3	F3b	1.9
		Standard Deviation = 0.21	

3.6 Discussion

Until recently, selection for resistance to diseases in maize using conventional methods has been the only practical tool used by breeders to develop cultivars resistant to MSD. A diversity of conventional breeding methods have been utilised including intra- and inter-population improvement schemes, inbreeding and hybridisation, backcross selection, and use of selection indices. This is because most of the traits of major importance are under polygenic control. However, when improving a line or a cultivar for resistance to diseases controlled by a single gene or a few genes with dominant gene action, the method commonly used has been backcrossing (Frisch and Melchinger, 2005). In this study both backcrossing and selfing selection methods were utilised to select for resistance to MSD.

The aim was to improve resistance to MSD in the recurrent parents through backcrossing and also generate new inbred lines with resistance to MSD through selfing.

A phenotypic segregation ratio of 1:1 observed for resistance to susceptible in backcross populations indicated that MSD resistance was simply inherited. This is consistent with previous findings of Kyetere *et al.* (1999) and Pernet *et al.* (1999) who revealed that MSD was controlled by one major gene and it appeared to be dominant, in favour of resistance. In selfing, a 3:1 ratio was observed confirming simple inheritance of MSD genes. This was consistent with the bimodal frequency distribution observed in the mapping population showing a clear 3:1 segregation. The results suggested the presence of one major gene. However, variations observed for differences in number of plants within each population with the same disease severity suggested that plants were still segregating for MSD resistance. The plants were still at F3:4 generation and most of the loci had not been fixed yet.

The pattern of frequency distribution (unimodal and bimodal) for mean disease severity suggested the presence of two control systems: i) a system of gene action involving major gene(s) controlling high resistance, and ii) a system of gene action involving loci with minor genes controlling partial resistance. The frequency distribution patterns in backcross populations showed bimodal distribution pattern. This implied that MSD resistance was controlled by a major gene, conferring complete resistance and several minor genes conferring partial resistance. In the second generation of backcross, there was a unimodal pattern of frequency distribution indicating the presence of several genes conferring partial resistance. Perhaps the plants with no symptoms carried complete resistance but they were not selected. On the other hand, frequency distribution patterns in populations derived by selfing were consistently skewed in favour of resistance. The observation suggested the presence of complete resistance. The second generation of selfing still showed frequency distribution skewed towards resistance. This is consistent with the observations made earlier on MSD resistance control (Rodier *et al.*, 1995; Pernet *et al.*, 1999a, b).

Incidence varied in the same direction as disease severity (intensity) across the population. Correlation coefficient values were very high; above 80% for all populations. All populations that had low incidence also experienced low disease severity; those with MSD rating of 5 had incidence of 100% as compared to those with disease rating of 1 having incidence as low as 7%. This observation appeared to suggest preferences by leafhoppers to feed on

some plants. Confirmatory study should be done to prove this theory of antixenosis-type of resistance.

Breeding for resistance using pedigree methods have shown improvement in the levels of resistance from BC1F1 to BC2F1 and also from F2 to F3 generations. In all cases, higher population means for MSD severity were recorded in the first generation of selection than in the second generation of selections. The decrease in the mean severities from one generation to the next generation indicated that conventional methods produced genetic gains; therefore, conventional methods were effective in selecting superior genotypes. The response to selection could have been higher if the plants with no symptom were selected. However, high response to selection was observed in selfed populations than in backcross population confirming the superiority of selfed populations over backcross population as source populations for selection. The superiority of selfed over backcross population is consistent with the observation made in previous studies (Dudley, 1984; Lamkey, *et al.*, 1993).

The study confirmed that maize streak virus disease is controlled by a few genes with resistance showing dominance. The disease is also shown to have high heritability. Once disease occurs early, the symptoms are highly visible and therefore easy to score making phenotypic screening easy. These observations agreed with early reports (Welz *et al.*, 1998; Kyetere *et al.*, 1999, Pernet *et al.*, 1999a, b).

3.7 Conclusion

Breeding for resistance to MSD using conventional methods was highly effective. Backcross and selfing populations segregated in 1:1 and 3:1 Mendelian ratios confirming the presence of one major gene with dominant gene action. Severity and incidence of disease were positively correlated suggesting a form of resistance by non-preference by the insect. A bimodal frequency distribution for disease severities at BC1F1 generation of backcrossing indicated that resistance was controlled by both a major and minor genes. However, the second cycle of backcrossing generated a unimodal frequency distribution pattern suggesting the presence of a few genes with additive gene action. On the other hand, in selfing populations, the presence of complete resistance against MSD was observed because frequency distribution patterns in both F2 and F3 generations were highly skewed

in favour of resistance. There was a decrease in disease severities with selection from BC1F1 to BC2F1 and from F2 to F3 generations suggesting response to selection. As expected selfed populations were more superior source populations than backcrosses. Improvement of existing lines for MSD resistance using backcrossing and selecting for new lines by selfing were achieved.

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CHAPTER FOUR

Application of SSR molecular markers in MSD resistance screening

4.1 Abstract

Thirty three pairs of simple sequence repeats (SSR) markers were screened for polymorphism in inbred lines (parents) and their F1 progenies and one marker, *umc* 1917, known to amplify within bin 1.04, a location for a major quantitative trait loci (QTL) for maize streak virus (MSD) resistance, was found consistently polymorphic and co-dominant and therefore used for marker assisted selection for resistance to MSD. The objectives of the study were to screen for resistance to MSD and determine the segregation of the marker. Four segregating populations developed from two crosses between CML202 (donor parent) and CML321 and CML384 (susceptible parents), comprised of two backcrosses and two selfed progenies from each of the two crosses. Two cycles of marker assisted selection were applied: at BC1F1 and F2, and at BC2F1 and F4 generations. Selection was done in two stages: 1) plants with the QTL (homozygous dominant or heterozygous) were selected, and 2) agronomic data was used to reduce the number of selected plants to 10% of population screened. The observed outcomes were tested for goodness of fit to a 1:1 and 1:2:1 ratios for backcrosses and selfed progenies, respectively. Results showed that X^2 values for all the four populations were not significant indicating that the observed outcomes fitted the expected ratio of 1:2:1 for a F2 population and 1:1 for a BC1F1 population. Field evaluation for MSD showed phenotypic segregations of 3:1 ratio, confirming the presence of one dominant gene for MSD with simple inheritance. This was also in agreement with the presence of a major QTL with dominant gene action in favour of resistance. Evaluation of F3 and BC2F1 progeny selected using the marker showed low disease severity scores ranging from 1.5 to 2.1 across the four populations suggesting that marker assisted selection was effective in selecting for resistance to MSD. However, the study showed that the presence of the marker was not consistent with symptom expression in the field. The variation in severity levels were probably attributed to the fact that marker selection focuses on the major QTL conferring resistance to MSD which explains only 45% of phenotypic variations on its own. Some few plants without the marker showed resistance to MSD suggesting possible occurrence of recombination due to crossing over between the marker and the QTL. There is a need to further identify other major genes or QTLs to improve on the effectiveness of MAS for resistance to MSD in African maize.

4.2 Introduction

Previous studies have identified a major QTL responsible for MSD resistance in the short arm of chromosome 1. This QTL known as MSD-1 (Kyetere *et al.*, 1999) accounts for 43 to 67 % of the total phenotypic variation of resistance (Pernet et al., 1999b), which makes it an attractive tool for marker assisted selection (MAS). Marker assisted selection for simply

inherited traits is gaining increasing importance in breeding programmes allowing an acceleration of the breeding process. Traits related to resistance to pathogens and to the quality of some crop products are offering some important examples of a possible routine application of MAS (Francia *et al.*, 2005). There are extensive examples of where MAS has been used for manipulating genes controlling qualitatively inherited disease resistance (Huang *et al.*, 1997; Lee and Penner, 1997; Geffroy *et al.*, 1998; Kawchuk *et al.*, 1998; Kelly and Miklas, 1998; Lawson *et al.*, 1998; Myburg *et al.*, 1998; Hernandez *et al.*, 1999; Hausner *et al.*, 1999).

Quantitative trait loci (QTL) for disease resistance have been identified for many diseases affecting crop plants. In the case of MSD, considerable research has been undertaken to investigate suitable molecular markers linked to resistant loci to MSD (Welz *et al.*, 1998; Kyetere *et al.*, 1999; Pernet *et al.*, 1999a and b). Markers have also been reported for gray leaf spot (GLS) (Bubeck *et al.*, 1993; Saghai-Maroof *et al.*, 1996; Clements *et al.*, 2000; Lehmensiek *et al.*, 2001) and Northern (*turcicum*) leaf blight (NLB) (Freymark *et al.*, 1993; Dingerdissen *et al.*, 1996; Welz *et al.*, 1998; Schechert *et al.*, 1999). Only the loci for MSD have been mapped consistently to the same chromosome location in multiple field tests in Zimbabwe (Kyetere *et al.*, 1999; Pernet *et al.*, 1999a and b), Reunion (Welz *et al.*, 1998; Pernet *et al.*, 1999a, b) and Uganda (Kyetere *et al.*, 1999).

Following the mapping of MSD QTL, molecular markers associated with MSD resistance QTL have been identified by CIMMYT. However, there have been few reports published to date regarding the actual use of MAS for improving crops for quantitatively inherited disease resistance (Concibido *et al.*, 1996; Toojinda *et al.*, 1998). The potential of MAS as a tool for improvement of quantitatively inherited traits has been explored mostly in theoretical studies (Lande and Thompson 1990; Dudley, 1993; Hospital and Charcosset, 1997; Knapp 1998; Xie and Xu, 1988; Frisch *et al.*, 1999; Stuber *et al.*, 1999; Reyes-Valdes, 2000; Wang *et al.*, 2003).

A few practical applications of MAS showed variable outcomes. Marker assisted selection has been applied in maize breeding programmes for early generation selection for yield. Results showed that it was an effective selection method (Stromberg *et al.*, 1994). Wilcox *et al.* (2002) employed MAS in breeding for resistance to Southern corn borer and their results indicated that MAS was effective in improving resistance in the materials. Selection with

markers was also shown to be useful in backcross programmes for simultaneously introgressing an allele and selecting for desired genomic background (Visscher, 1996). Marker-facilitated selection was effectively used in improvement of resistance to bacterial blight diseases in rice (Chen *et al.*, 2000) and beans (Yu *et al.*, 2000). The usefulness of MAS has also been exploited in enhancing agronomically important traits in sweet corn (Yousef and Juvik, 2001, 2002). These are few of the cases where MAS methods were put into practical use. Despite the effectiveness of MAS demonstrated theoretically and in some few practical cases, many breeding programmes are not utilising it. Cost of MAS appears prohibitive and limited critical analysis of cost effectiveness has been done (Dreher *et al.*, 2000). In Africa, a few breeding programmes may have the facilities and the competence but the majority of the programmes may not have established molecular laboratory to handle the task.

A breeding programme was initiated in Uganda to apply MAS in practical breeding to test the effectiveness of marker-assisted selection in breeding for resistance to MSD. Marker-assisted backcrossing was considered for introgression of MSD resistance QTL into lines susceptible to MSD, but which have important agronomic attributes which were to be reconstituted. Marker-assisted selection through selfing was included for generation of new inbred lines resistant to MSD.

4.3 Objectives of the study

The objectives of the study were:

1. to select for MSD resistance using SSR marker assisted selection, and
2. determine segregation pattern of MSD resistance in backcross and selfing populations.

The hypotheses tested were:

1. Marker assisted selection was effective in selecting for resistance to MSD, and
2. marker segregation ratios in backcrosses and selfing populations were 1:1 and 1:2:1, respectively.

4.4 Materials and Methods

4.4.1 Plant materials

The study was conducted on four maize populations during a maize breeding programme. Two (2) maize inbred lines CML 321 and CML 384, susceptible to maize streak virus disease (MSD) were selected (upon consultation with NARO maize breeder) as parents for improvement for MSD resistance. CML 202 was selected for study as the donor line. The choice of CML 202 was considered in the study, because it has been employed on several occasions in MSD resistance breeding in the Ugandan National Maize Programme, and found to be adaptable to the tropical condition in Uganda.

Two crosses were made between maize inbred lines CML321 and CML202, and between CML384 and CML202 at CIMMYT-Zimbabwe in 2003. The F1 generations were backcrossed to the recurrent parents (CML321 and CML384) and also self-pollinated to generate both BC1F1 and F2 populations, respectively.

Four populations were generated and denoted as follows:

- BC1F1 (A) derived by backcrossing F1 between CML321 and CML202 to recurrent parent, CML321 = (CML321xCML202)/CML321.
- BC1F1 (B) derived by backcrossing F1 between CML384 and CML202 to recurrent parent CML384, = (CML384xCML202)/CML384
- F2 (A) derived by self-pollinating F1 of CML321 x CML202.
- F2 (B) derived by self-pollinating F1 of CML384 x CML202.

BC1F1 and F2 generations were screened for resistance and the selected plants were again backcrossed to the recurrent parents (CML321 and CML384) and self-pollinated to produce BC2F1 and F3 generations as follows:

- BC1F1 (A) was backcrossed to CML321 to generate BC2F1 (A).
- BC1F1 (B) was backcrossed to CML384 to generate BC2F1 (B).
- F2 (A) was self-pollinated to generate F3 (A).
- F2 (B) was self-pollinated to generate F3 (B).

The recurrent parents were used as males, assuming there was no maternal effect for MSD resistance trait. This was also to ensure that adequate seed was produced in a resistant

plant. The difference in disease resistance between CML321 (recurrent parent and CML202 (donor parent) can be seen below (Plate 4.1).

CML 321 (Susceptible parent) CML 202 (Donor parent)

Plate 4.1. Maize streak virus symptoms on susceptible inbred line CML321 and resistant inbred line CML202 (symptom-free).

4.4.2 DNA Extraction and quantification

To screen for polymorphism leaf material was sampled from parent inbred lines and their F1s. Representative leaf samples were picked from 10 plants for each genotype. The plants were sampled at four weeks from planting. Leaf samples were picked, placed in an ice box and then transported to Makerere University Biotechnology Laboratory for analysis. Lyophilisation was not done, but the leaves were stored at -180°C. Genomic DNA was extracted using cetyltri-methylammonium bromide (CTAB) protocol adopted from CIMMYT laboratory protocol (CIMMYT, 2003), but with the following modifications: i) leaf tissues were ground to powder in liquid nitrogen and 1 ml of pre-warmed (65°C) X2 CTAB buffer [100mM Tris pH 8, 30mM EDTA, 1.4M NaCl, 2% (w/v) and CTAB, 2% (w/v); ii) samples were incubated at 37°C for 60 minutes and frequently mixed by inversion . There was no addition of Protease K and DTT in the mixture.

DNA was quantified using a spectrophotometer. OD_{260}/ OD_{280} ratios of the samples ranged from 1.8 to 2.0 with an average value of 1.9. This ratio was within the acceptable range of 1.65 to 2.0. The DNA was then diluted to 50ng/µl and stored at -20°C.

4.4.3 PCR Analysis

Polymerase Chain Reaction (PCR) analyses were carried out in a DNA Thermal Cycler (Bio Rad). While trying to optimise the PCR reaction conditions, different standard PCR conditions with one annealing temperature were tried but no product was detected. A touchdown PCR procedure described by Mellersch and Sampson (1993) was successful and therefore adopted. The annealing temperatures ranged from 64°C to 54°C. The procedure consisted of an initial denaturation of DNA at 94°C for 2 minutes, followed by 10 cycles of 94°C for 1.30 minutes, 64°C for 1 minute and 72°C for 1.30 minutes, with a decrease in annealing temperature by 1°C in each cycle until it reached 54°C, then followed by 25 cycles at annealing temperature of 54°C. In the last cycle, after extension time at 72°C, the reaction was maintained at 4°C. A 15µl volume reaction mix consisted of 50 ng of each primer, 1 unit of Taq DNA polymerase, 250 ng of template genomic DNA, 1 X *Taq* buffer ($MgCl_2$-free), 2.5 $MgCl_2$, 1.2 mM dNTPs and de-ionised water.

4.4.4 Gel analysis and scoring for marker presence

PCR products were separated in 4% Metaphor agarose gels (3 Metaphor: 1 Seakam agarose) and 1XTBE buffer. Samples were run into gels at 120 Volts for 3 hrs, removed and stained in 1µg/ml ethidium bromide (100 µl of 10 mg/ml ethidium bromide in 1000 ml de-ionised water (dH_2O) for 20 minutes. The gel was removed and washed in dH_2O for 20 minutes and then visualized under UV light.

4.4.5 Marker selection

Applying the above procedure in selecting the primers (markers) for polymorphism, 32 pairs of SSR markers acquired from CIMMYT-Mexico laboratory, known to amplify bin 1.04-1.05 on chromosome 1, a region containing the QTL responsible for MSD resistance, were analysed to test their discriminating powers using the inbred line parents and their F1 progenies. SSR markers were chosen if they showed different band sizes of the susceptible

and resistant parents (polymorphism) and consistently amplified the parents and their F1s. Basing on those criteria *umc1917* marker was selected for MAS.

4.4.6 Marker Assisted Selection for resistance to MSD in segregating populations of maize

Each of the four segregating populations was planted for MSD resistance screening in 2004 at Namulonge Agricultural and Animal production Research Institute (NAARI). Population size of 200 plants for each population was used for the analysis.

Plant genotyping was done using *umc* 1917 marker at F2 and F3 generations and BC1F1 and BC2F1 generations. In all these, plant leaf sampling, DNA extraction, PCR analysis and gel electrophoresis were done as described above. Data was scored by observing gels under UV light and counting and recording the number of samples showing single bands (homozygous dominant), double bands (heterozygous) and single bands (homozygous recessive) for the QTL. For each population the total number of homozygous dominant, heterozygous and homozygous recessive individuals was computed. Selection for advancement was done in two stages: 1) all plants homozygous dominant or heterozygous for the QTL were selected, 2) to achieve a selection intensity of 10 % for each of the population, the number of plants selected was reduced based on agronomic characteristics.

4.4.7 Field Data Collection

To determine whether there was association between molecular and phenotypic data, disease severity level was also scored on each of the populations for MAS. Maize streak virus disease incidence was taken by counting all the plants infested in a population then expressed as a percentage of plants infested over the total plant population. Maize streak virus disease severity was scored at flowering only (critical stages for MSD effect on yield). Disease severity was scored on the whole plant as a proportion of total leaf area diseased using a scale of 0 – 5 (Bosque-Perez and Alam, 1992):

Scale	Description
0	no visible disease symptoms
1	very few streaks on some leaves
2	light streak symptoms on most leaves
3	moderate streak symptoms on most leaves

4 abundant symptoms on all leaves (>60%) leaf area affected

5 severe symptoms on all leaves (>80%) of leaves affected with no yield whatsoever

Other diseases observed during the plant growth were gray leaf spot, northern leaf blight and ear rot disease. Data was also taken on the following traits: anthesis and silking interval, plant height, ear placement (number of leaves above ground at which the ear is placed) and number of ears per plant (prolificacy). At dry harvest maturity, the plants were scored for stem and root lodging in addition to the grain attributes. Yield evaluation was not considered at this stage. Data for all these parameters except MSD are not presented.

4.4.8 Data analysis

Molecular data from F2 and BC1F1 generations was subjected to Chi-Square Goodness of Fit to test whether the data fitted the genetic ratio of 1:1 for BC1F1 and 1:2:1 for F2 populations.

For a 1:1 ratio in a backcross population, the expected outcome would be

= 1/ (1+1) x the total observations, given total observation is 153,

= ½ x 153

= 76.5 for each observation

For a 1:2:1 ratio in selfed population, the expected outcome would be given as:

= 1/ (1+2+1) x the total observations, given total observation is 193,

= ¼ x 193

= 48.25 for one observation

For the other observation,

= 2/ (1+2+1) x 193

= 2/4 x 193

= 96.5

The analysis of a chi-square test of independence was done to test whether the presence of QTL for MSD resistance in a plant would correspond to the resistance level expressed in the field, that is, if there was any direct association between presence of QTL and low disease severity. Data from F3 and BC2F1 generations were used to select lines for advancement into the next generation.

4.4.9 Line selection and advancement

Selection for advancement was done in two stages, namely, i) molecular data was used to select all those individual plants carrying single bands (homozygous dominant) from the donor parent and those with double bands (heterozygous for the trait) from both parents (Foreground selection), and ii) the selected plants were screened for good agronomic traits, hence reducing the number of plants selected further to only 10% of the planted population. Field severity rating for MSD was not used in selection and advancement of plants.

Ten percent of the F2 and BC1F1 population were selected and advanced to F3 and BC2F1 through self pollination and backcrossing to recurrent parents, respectively. The selected F3 and BC2F1 plants were planted ear-to-row in which progenies of each plant (from one ear) were planted in one row only to facilitate ear-to-row selection for agronomic traits. In ear-to-row selection, first selections of the best rows were done followed by selection of the best individuals from the best rows. Maize streak virus resistance was again scored at flowering.

4.5 Results

4.5.1 Marker Assisted Selection

One marker, namely *umc* 1917, was consistently polymorphic and able to distinguish between the susceptible and resistant parents. The marker was also co-dominant in the F1 generation (Plate 4.2).

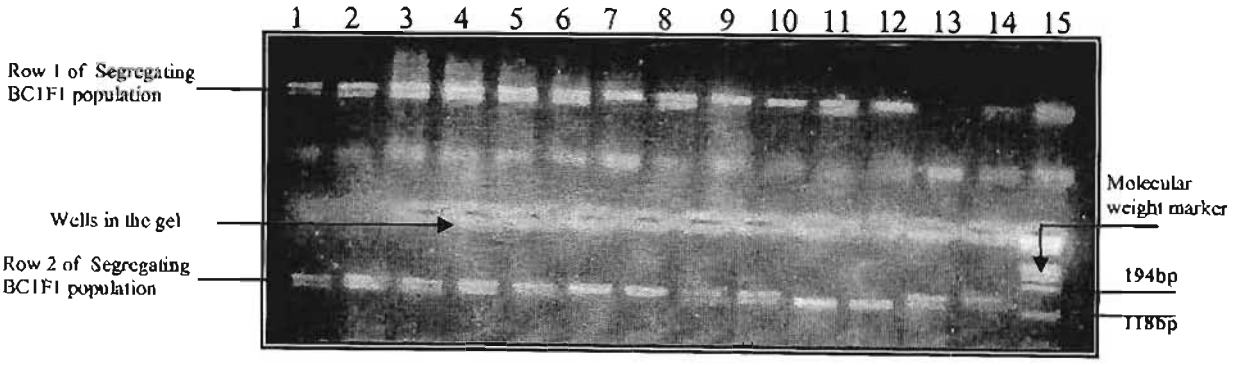

Plate 4.2. BC1F1 population segregating for *umc* 1917 marker in a co-dominant manner: Two rows of 15 wells were loaded in the gel; wells 1 to 14 were plant samples; well 15 was molecular weight marker (PhiX174 HaeIII ladder). The gel picture was a section cut from a bigger gel which left out the molecular weight marker (PhiX174 HaeIII ladder).

From the database (Maize Mapping Project, 2002), the IBM2 neighbour map distance is 374.8 cM on chromosome 1, bin 1.04. The repeat sequences of the primer are as shown below (Table 4.1).

Table 4.1. Structure of SSR primer, umc1917, used in MAS procedures

Name	Repeat Sequence	Region amplified
umc 1917 (F)	ACTTCCACTTCACCAGCCTTTTC	1.04
umc 1917 (R)	GGAAAGAAGAGCCGCTTGGT	

Analysis of plant tissues from the BC1F1 (population A) using Chi-Square Goodness of Fit Testing a ratio of 1:1 showed that there was no significant (X^2cal =1.104) difference between observed and expected outcomes indicating that the outcome fit the ratio being tested (Table 4.2).

Table 4.2. PCR analysis data showing observed and expected outcome in BC1F1 (population A) (CML202/CML321//CML321)

	No. single bands (DP)	No. Double bands (H)	Total
Observed Value	70.0	83.0	153
Expected Outcome	76.5	76.5	153
	X^2 cal= 1.104; X^2tab = 3.84, α =0.05, df = 1		

DP = bands from donor parent; H = heterozygous

Application of markers in population B also showed that there was no significant (X^2cal = 0.405) difference between the observed and the expected outcome of the analysis (Table 4.3). The marker segregated at a ratio of 1:1 as expected.

Table 4.3. PCR analysis data showing observed and expected outcome in BC1F1 (population B) (CML202/CML384//CML384).

	No. single bands (DP)	No. Double bands (H)	Total
Observed Value	57.0	64.0	121
Expected Outcome	60.5	60.5	121
	X^2 cal= 0.405; X^2tab = 3.84, α =0.05, df = 1		

DP = bands from donor parent; H = heterozygous

There was no significant difference between observed and expected marker segregation ratios in F2 (population C). The expected segregation ratio in an F2 population segregating for two alleles was 1:2:1. Results of Chi-Square Goodness of fit test analysis fitted the ratio of 1:2:1. The X^2cal (4.285) was not significant (Table 4.4). The segregation obtained was also consistent with a phenotypic ratio of 3:1 segregation expected for a single major dominant gene.

Table 4.4. PCR analysis data showing observed and expected outcome in F2 (population C) (CML202/CML321)

	No of single bands (DP)	No of double bands (H)	No of single bands (SP)	Total
Observed Value	38.00	110.00	45.00	193
Expected Outcome	48.25	96.5	48.25	193
X^2 cal = 4.285; X^2 tab = 5.99, α = 0.05, df = 2				

DP = bands from donor parent; H = heterozygous; SP = bands from susceptible parent

Similarly, the observed marker segregation in the F2 (Population D) followed the same pattern as in population C. The X^2(cal) was not significant (Table 4.5) indicating that there was no significant difference between observed and expected marker segregation ratios in F2 (population D).

Table 4.5. PCR analysis data showing observed and expected outcome in F2 (population D) (CML202/CML384)

	No of single bands (DP)	No of double bands (H)	No of single bands (SP)	Total
Observed Value	36	64	40	140
Expected Outcome	35	70	35	140
X^2 cal = 1.257; X^2 tab = 5.99, α = 0.05, df = 2				

DP = bands from donor parent; H = heterozygous; SP = bands from susceptible parent

Marker analysis at F3 and BC2F1 generations showed variations between selection techniques. Selfing populations C and D had more lines which were homozygous dominant for the marker than the backcross populations (Table 4.6). The results also showed that

after two cycles of selection, relatively more lines in F3 populations than lines in BC2F1 were still heterozygous for the marker but very few were homozygous recessive.

Table 4.6. Variation of QTL rate of fixation at F3 and BC2F1 generations

Population	Number of lines		
	homozygous dominant	heterozygous	homozygous recessive
BC2F1 (Population A)	4	10	10
BC2F1 (Population B)	3	13	8
F3 (Population C)	7	15	2
F3 (Population D)	8	14	2

4.5.2 Field evaluation

MSD development occurred late during the season when the plants were about to flower and hence low incidence and severity was observed. Disease incidences on the populations A and B were 70.5% and 65.5%, respectively (Table 4.7). Disease incidences on populations C and D were 60% and 52.5%, respectively (Table 4.8).

Table 4.7. Variation of MSD severity levels between populations A and B

Pop.	MSD_sev	Observed	Fitted	Residual
Pop. A	0	59.00	93.79	4.88
	1	35.00	43.28	0.79
	2	23.00	48.53	3.55
	3	45.00	39.78	-4.03
	4	29.00	19.62	-1.57
	5	9.00	5.57	-0.71
Pop. B	0	69.00	85.21	4.88
	1	44.00	55.72	-0.79
	2	21.00	62.47	-3.54
	3	37.00	51.22	-4.03
	4	18.00	12.38	1.57
	5	11.00	2.38	1.57

Pearson chi-square value is 36.73 with 4 df. Probability level (under null hypothesis) $p < 0.001$

Table 4.8. Variation of MSD severity levels between populations C and D

Pop.	MSD_sev	Observed	Fitted	Residual
Pop. C	0	80.00	91.53	-2.88
	1	33.00	43.62	-1.57
	2	26.00	21.79	1.21
	3	23.00	19.28	4.03
	4	13.00	10.78	-0.59
	5	3.00	8.78	-0.79
Pop. D	0	95.00	79.78	4.98
	1	30.00	33.72	-0.79
	2	27.00	35.45	-3.54
	3	29.00	36.27	-4.03
	4	19.00	14.78	11.47
	5	9.00	4.78	1.47

Pearson chi-square value is 29.63 with 4 df. Probability level (under null hypothesis) $p < 0.001$

4.5.3 Association between phenotypic and molecular data

Results of Chi-Square Test of Independence between molecular and the morphological data indicated that there was no significant difference ($X^2 = 0.0243$; $X^2tab = 3.84$, $\alpha = 0.05$, df = 1) between presence of the QTL (molecular data) and MSD severity scores (field data). The presence of QTL for MSD resistance was not associated with symptom expression. Analysis for the rest of the populations B, C, and D also showed that MSD severities in the field were independent of the marker analysis results. Chi-Square values (X^2cal) were 0.0221, 0.3121 and 0.0072, respectively, (given $X^2tab = 3.84$ at $\alpha = 0.05$ and df = 1; $X^2tab = 5.99$ at $\alpha = 0.05$ and df = 2). Frequency distribution for the populations at BC1F1 generations of the two populations A and B showed bimodal pattern (Fig 4.1a and b). The frequency distribution of F2 population C and D were skewed in favour of resistance (Fig 4.1c and d), which was consistent with 1:2:1 segregation ratio.

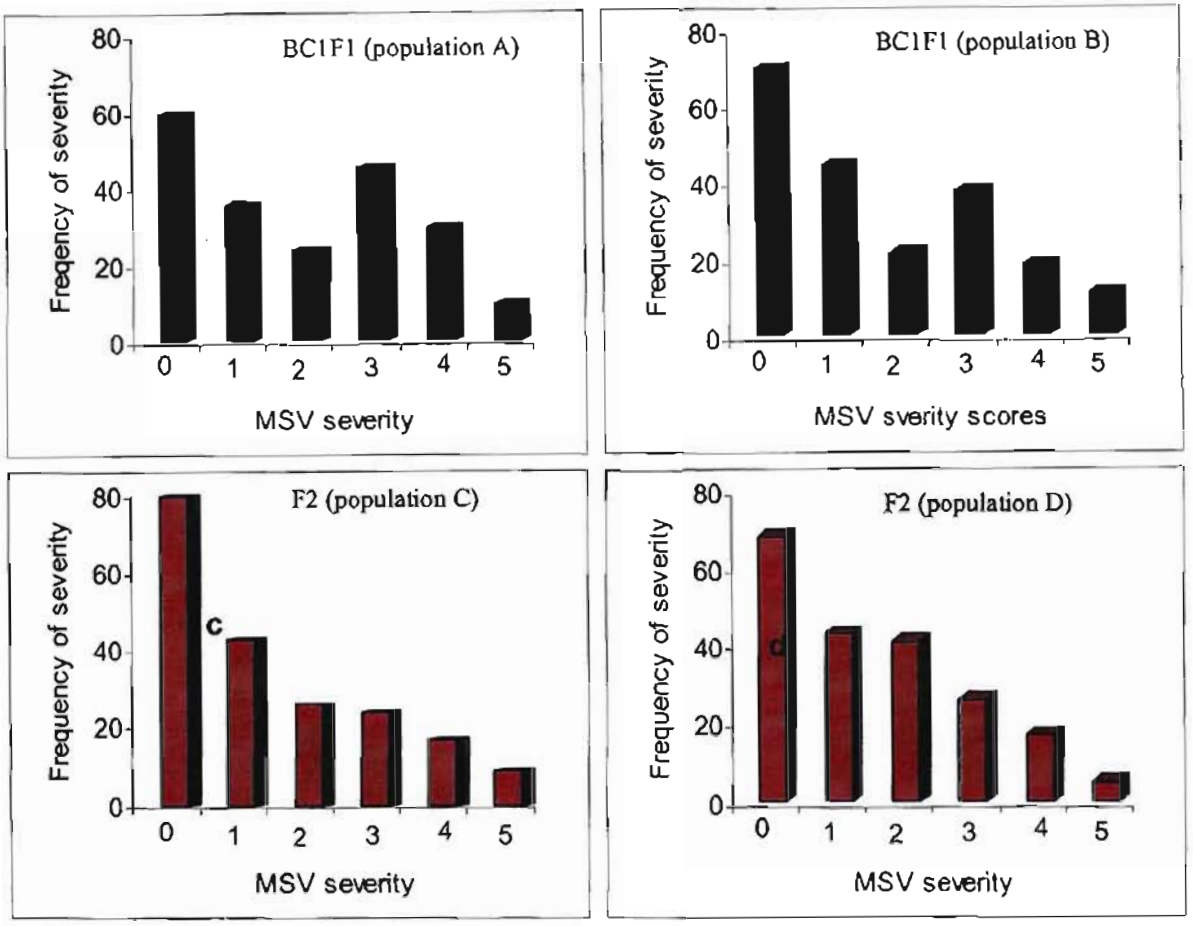

Figure 4.1. Frequency distributions for MSD severity in BC1F1 (a and b), and in F2 populations (c and d) under MAS method

Field evaluation of the populations at BC2F1 and F3 segregation showed that all the lines selected had low disease severity (Table 4.8). The mean severity scores were relatively lower for population C and D (F3 generation), which were 1.65 and 1.5, than population A and B (BC2F1 generation).

Table 2 MSD mean severity scored at F3 and BC2F1 generations

Selected Lines	MSD Mean severity scores			
	BC2F1 Population A	BC2F1 Population B	F3 Population C	F3 Population D
1	1	1	1	2
2	2	2	1	2
3	2	2	1	2
4	2	2	1	2
5	3	1	1	1
6	2	1	1	2
7	2	1	1	1
8	2	3	2	1
9	1	3	2	1
10	3	3	2	1
11	3	2	1	1
12	3	2	2	3
13	3	2	2	3
14	2	2	2	1
15	3	1	1	1
16	2	2	1	1
17	1	2	2	1
18	2	2	2	1
19	2	3	3	1
20	1	2	1	2
21	1	4	2	2
22	1	3	2	1
23	2	3	3	2
24	1	2	2	1
Means	**1.96**	**2.13**	**1.63**	**1.50**

*0 = no symptoms; 5 = > 80% of the plant leaves disease infected

4.6 Discussion

Application of marker assisted selection in the breeding process has been carried out mostly based on available information on map position of traits with agronomical importance and on the linked molecular markers. Our programme was one of those that relied on available information. Markers known to amplify within the same chromosomal location where a major MSD QTL was detected (chromosome 1, bin 1.04) (Welz *et al.*, 1998; Kyetere *et al.*, 1998; Pernet *et al.*, 1999a,b) were screened for polymorphism and subsequently one of the them was selected and its effectiveness in MAS selected was tested.

As expected, the marker segregation showed a ratio of 1:1 for backcross populations. This observation indicated that MSD resistance was under dominant gene action. This is consistent with previous finding of Kyetere *et al.* (1999), Pernet *et al.* (1999) who revealed that MSD was controlled by one major gene and it appeared to be partially dominant, in favour of resistance. In selfing populations, a 1:2:1 ratio of marker segregation was observed. This was also consistent with the frequency distribution observed in the same F2 populations showing a clear 3:1 phenotypic segregation. The results confirmed the presence of one major gene, which has been observed in CML202 inbred line although two or more major genes controlling resistance to MSD have been reported in IITA germplasm (Kim *et al.*, 1989).

Marker segregations did not depart from the expectations based upon simple Mendelian inheritance. SSR markers have been reported to have low incidence of segregation in a non-Mendelian fashion (Smith *et al.*, 1997). Studies have shown that effectiveness of MAS depended on the genetic distance between the QTL and the linked molecular marker(s), the number of markers used per QTL for MAS, and the relative size of the QTL phenotypic effect (Lande and Thompson, 1990; Hospital *et al.*, 1992; Dudley, 1993). The marker used in current studies is known to amplify within bin 1.04, a region where the major QTL for MSD resistance was mapped to. Inconsistent introgression due to recombination is therefore unlikely unless the marker was in fact outside this small mapped interval (Robert *et al.*, 2001).

Frequency distribution patterns for BC1F1 and F2 generations showed bimodal and skewed distributions in favour of resistance, respectively. These all confirmed that MSD resistance was controlled by a major gene or QTL with simple Mendelian inheritance. However, plants that were homozygous dominant for the marker did not show consistent disease severity levels in the field. The variations in severity levels were probably due to two factors, i) the major QTL conferring resistance to MSD on chromosome 1 explains only 44% of phenotypic variations and in combination with the minor QTLs they explain 43-67% of phenotypic variation (Pernet *et al.*, 1999b), and ii) MAS targeted only one QTL and yet the high resistance observed in the donor parent CML202 was as a result of all the QTL identified (Welz *et al.*, 1998, Pernet *et al.*, 1999b).

Disease severity at F2 and BC1F1 generations ranged from 0 to 5. The average disease severity for F2 and BC1F1 before MAS was 3. However, after one cycle of selection with markers, severities at F3 and BC2F1 generations ranged from 1 to 3 and the susceptible checks (in this case were the recurrent parents) had mean severity of 4.5. The mean severities for the four populations were low, ranging from 1.5 to 2.1, suggesting that marker assisted selection was effective. These results showed response to selection by MAS indicating that MAS was effective in selecting for resistance to MSD. Other studies have showed that MAS was an effective tool in selection for disease resistance (Groh *et al.,* 1998; Yu *et al.*, 2000; Wilcox *et al.,* 2002). Marker assisted selection for MSD resistance is currently based on one major QTL, which does not explain 100% phenotypic variations. There is a possibility that the other minor QTLs scattered across the genome may not all be present in the observed individuals. The trait has also shown additive gene action (Welz *et al.*, 1998; Pernet *et al.*, 1999b) and this usually is associated with genotype by environment interaction resulting in variation of phenotypes. Presence of additive gene action might explain the response to selection observed in all the populations.

This brings us to a similar conclusion to Yousef and Juvik (2001) that MAS can be a tool of choice in introgression of MSD resistance QTL in a breeding programme, particularly because it does not rely on presence of disease for selection in which case MSD is sporadic. The application of a molecular marker in selection for MSD resistance proved to be effective. The success of this study attests to the fact that QTL identified in one mapping population has a positive effect across different genetic backgrounds and across different environments (Yousef and Juvik, 2002) as opposed to the view that QTL mapped in one population may not be effective in another genetic background (Pilet *et al.*, 2001; Reyna and Sneller, 2001).

Some few plants showed resistance to MSD even though they were not showing the presence the marker. This might imply that recombination might have taken place hence separating the marker from the QTL. In that case there would be some plants with the marker but without the QTL and some plants with the QTL and without the marker. Such plants without the QTL would be susceptible to MSD. Future studies should be directed towards identification of other markers for the other QTLs for their introgression together with the major QTL.

4.7 Conclusion

Marker assisted selection for resistance to MSD was conducted in early generations of four populations. Results showed that the two selfing populations segregated in a ratio of 1:2:1 and, and the two backcross populations in a ratio of 1:1. This confirmed the presence of a major QTL with partial dominant gene action in favour of resistance. Marker selected lines showed disease severity scores ranging from 1.5 to 2.1 across the F3 and BC2F1 generations indicating marker assisted selection was effective in selecting for resistance to MSD. However, the presence of the QTL was not consistent with symptom expression in the field. The variation in severity levels were probably attributed to the fact that marker selection focused on the major QTL conferring resistance to MSD which explains only 45% of phenotypic variations on its own. Some few plants without the marker showed resistance to MSD suggesting possible recombination between the marker and the QTL.

4.8 References

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CHAPTER FIVE

Comparison of marker assisted selection and conventional selection methods for resistance to maize streak virus and yield performance in testcrosses

5.1 Abstract

There is no information regarding the comparative use of marker-assisted selection (MAS) and conventional selection for maize streak virus (MSD) resistance for maize breeding programme in Uganda. A few studies, mainly theoretical, have indicated that MAS is likely to confer advantages over conventional selection. The objectives of this study were; (i) to compare the effectiveness of MAS and conventional selection methods for resistance to maize streak virus (MSD), and (ii) to compare yields of lines selected for resistance to MSD through MAS and conventional breeding in Uganda from 2003 to 2006. Four populations comprising of two backcrosses and two selfed progenies derived from two crosses, CML 202 x CML 321 and CML 202 x CML384, were advanced to BC3F1 and F4 generations using MAS and conventional methods, separately. Inbred line CML202 was the donor parent for MSD resistance. Three lines with high resistance to MSD were drawn from each of the four populations derived from conventional methods and also from the four corresponding populations derived from MAS. The selected lines were evaluated under artificial inoculation with viruliferous leafhoppers with 100% transmission rate. The same lines were also crossed to two elite standard single cross testers and the testcrosses were evaluated for yield across three locations. Results showed that the MSD incidence was significantly higher in populations under conventional selection (79.3 %) than in those under MAS (64.8 %). Mean area under disease progress curves (AUDPC) for MSD in populations under conventional selection were higher (ranging from 1.6 to 2.4) than AUDPC from corresponding populations under marker assisted selection (ranged from 1.4 to 1.8). However both selection methods produced lines with higher resistance than those for susceptible parents. A significant positive correlation was observed between incidence and severity on all the populations, which suggested a non-preference form of resistance by the insect. Evaluation of test-cross progenies identified ten potential lines that can be advanced and eventually used for hybrid production for farmers' utilization. Ten crosses had mean yield above the overall mean for all the crosses and their yields ranged from 6.54 to 9.24 t ha^{-1} and were superior to standard check hybrids. Five of these crosses were of lines from MAS and the other five were crosses of lines from conventional selection, indicating that both methods were effective in identifying high yielding inbred lines. These ten lines x tester crosses also showed good levels of resistance to MSD, with AUDPC ranging from 1.2 to 2.4, and were highly stable across environments for both yield and resistance to MSD.

5.2 Introduction

The use of marker assisted selection (MAS) for introgression of major quantitative trait loci (QTLs) for disease resistance and other agronomic traits are increasingly being used in crop improvement. In the last decade, MAS has been employed in the disease resistance screening mainly in the improvement of elite lines through backcrossing (Frisch *et al.*, 1999;

Chen *et al.*, 2000; Frisch and Melchinger, 2001; Reyna and Sneller, 2001; Bouchez *et al.*, 2002; Reyes-Valdes, 2002; Frisch and Melchinger, 2005). Thomas (2003) argued that molecular marker assisted selection methods are only useful if they can accelerate the breeding process, or improve the cost-effectiveness of breeding programmes, besides improving genetic gain of the traits in question.

By the use of markers linked to specific QTLs it is possible to introgress specific regions of the genome that confer desirable quantitative characteristics to an elite germplasm. Marker screening within the early generations of a breeding programme means that MAS can help to accelerate the development of improved lines or populations by reducing the number of years required to achieve results (Yousef and Juvik, 2001). It may especially have advantages in some cases where phenotypic selection is difficult (Dreher *et al.*, 2000). Marker assisted selection can facilitate selection in the absence of stress factors, and offering a reliable way of improving the target trait (Chen *et al.*, 2000; Wilcox *et al.*, 2002).

Conventional selection methods, on the other hand have been the methods of selection over the years. The methods have produced tangible results and genetic gains. For example, in the case of MSD, many high-yielding maize cultivars have been developed and released to farmers in sub-Saharan Africa (Timothy *et al.*, 1988). In Uganda, resistant cultivars released were Longe 1 and Longe 4. Under induced artificial epidemics in the field high yielding and MSD resistant cultivars selected phenotypically have been released in several African countries by Pannar Seed Company (Barrow, 1992; 1993). CIMMYT has produced inbred lines resistant to MSD (Pixley *et al.*, 1997) including CML202, the donor line used in the current study. It is important, however, to acknowledge the difficulties experienced when screening for resistance to MSD using conventional methods. The success of conventional screening for MSD in a breeding programme depends on the environmental factors and the occurrence of MSD is sporadic and unpredictable (Pixley *et al.*, 1997; Bosque-Perez, 2000). To enhance natural virus infection, additional resources are required for rearing leafhoppers and inoculate each plant in the nurseries or establishing spreader rows. These add to screening costs. Besides, the disease may not always be uniformly distributed and, for natural virus enhancement through spreader rows, disease may not always develop early in the season to allow for effective screening. In such a case, MAS may be an ideal selection tool, because it facilitates selection in the absence of stress factors (Chen *et al.*, 2000; Wilcox *et al.*, 2002) and, therefore, offers a reliable way of selection for MSD resistance.

There is no published information on comparison of MAS and conventional selection for MSD resistance using both backcrossing and pedigree selection methods. Where comparisons of MAS and conventional selection have been made, the methods compared MAS with backcrossing only. In most cases, the comparison has been based on theory rather than practice. In Uganda, this is the first study done on MSD.

5.3 Hypothesis

The hypothesis being tested in this study was that marker assisted selection for MSD resistance is as effective as conventional selection methods.

5.4 Objectives of the study

The objectives of the study were twofold:

1. to compare the resistance of lines selected through conventional and marker assisted selection methods, and
2. to compare yield of selected lines in three-way cross hybrids.

5.5 Materials and methods

5.5. 1 Line selection

To compare the relative efficiency of MAS and conventional breeding methods, lines at BC3F1 and F4 generations were drawn from eight populations basing on resistance to MSD and their agronomic qualities. Three lines were selected from each of the four breeding populations in MAS methods and three other lines from the corresponding populations under conventional breeding approach. The highly resistant lines were selected based on their resistance scores at BC2F1 and F3 generations. The resistant parent and the susceptible parents were also included as checks. A total of 27 lines were planted for artificial inoculation. For the purposes of ease of discussion the eight populations and lines were designated as shown (Table 5.1).

Table 5.1 Structure of populations and lines used in the study

Population generation	Breeding method	Designation	Lines
BC3F1 of (CML 202 x CML 321)	MAS with backcrossing	Population 1	1, 2, 3
BC3F1 of (CML 202 x CML 384)	MAS with backcrossing	Population 2	4, 5, 6
F4 of (CML 202 x CML 321)	MAS with selfing	Population 3	7, 8, 9
F4 of (CML 202 x CML 384)	MAS with selfing	Population 4	10, 11, 12
BC3F1 of (CML 202 x CML 321)	Conventional backcrossing	Population 5	13, 14, 15
BC3F1 of (CML 202 x CML 384)	Conventional backcrossing	Population 6	16, 17, 18
F4 of (CML 202 x CML 321)	Conventional selfing	Population 7	19, 20, 21
F4 of (CML 202 x CML 384)	Conventional selfing	Population 9	22, 23, 24
Resistant parent (CML202) and Susceptible parents (CML321 and CML384) were included as checks		Population 9	CML202, CML321, CML384

5.5. 2 Artificial inoculation of lines with *viruliferous* leafhoppers

Leaf hoppers were reared on pearl millet in cages as described by Bosque-Perez and Alam (1992). Transmission rate was determined by inoculating twenty seedlings with one leafhopper each. There was 100 % transmission rate. Each line was planted in two-litre buckets with three replications. Each bucket (plot) had five plants in them. Infected maize plants were introduced in each of the cages for virus acquisition by the leafhoppers. The acquisition access period was extended up to 48 hours to improve transmission rate (Okoth *et al.*, 1987). After the 48 hours had elapsed, the infected maize plants were removed and buckets of seedlings were placed in the cages. The seedlings were left in the cages for 72 hours which was to accommodate an average latent period of 16 - 20 hours before transmission can occur. This was because the frequency of transmission by and persistence of MSD in individual insects have been shown to increase with virus concentration in the plant, length of the acquisition access period, and duration of inoculation (Okoth *et al.*, 1987). The length of the latency period is also varied with temperature (Okoth and Dabrowski, 1987; Storey, 1928), and the medium period reported is 16–20 hours (Okoth Okoth and Dabrowski, 1987).

5.5. 3 Evaluation of line x tester crosses

To evaluate the performance of lines from different selection methods, the same lines described above were used. Two standard single cross testers, CML442/ CML312 (tester A) and CML444/ CML395 (tester B), acquired from CIMMYT- Zimbabwe were crossed to each of the lines. However, due to drought during the season not all the crosses generated enough seeds for evaluation. The details of tester are as shown below (Table 5.2). CML312/CML442 is an intermediate maturity tester, while CML395/CML444 is a late maturity tester.

Table 5.2. Pedigree information for testers

Tester	Pedigree	Heterotic Group	Adaptation
CML312/CML442 (Tester A)	S89500 F2-2-2-1-1-B*5 x [M37W/ZM607#bF37 sr-2-3sr-6-2-X]-8-2-X-1-BBB	A	Subtropical/ Africa MA*/ST**
CML395/CML444 (Tester B)	90323(B)-1-B-1-B*4 x P43C9-1-1-1-1-1-BBBB	B	Africa MA*/ST**

Source: CIMMYT (2001); *MA = midaltitude; **ST = subtropical

Twenty four crosses yielded enough seeds and were therefore evaluated in three different locations in Uganda, namely, Namulonge (1150masl), Masaka (1250masl) and Iganga (1081masl). Six standard cultivars were included and they comprised of: one susceptible check (from a private seed stockist), two open-pollinated cultivars as resistant checks (Longe 1 and Longe 4) and four locally popular hybrids (Longe 6H, Longe 7H and SC 407). A susceptible check hybrid was planted both as an entry in the experiment and also as border plots. The experiments were set up in randomised complete block design (RCBD) with two replications. Plants were spaced at 0.75m and 0.50m between and within rows, respectively. Standard cultural practices such as weeding and fertilizer were applied as follows: weeding was done twice, and fertilisers were applied at a rate of 30kg/ha and 45kg/ha for phosphate and Nitrogen fertilisers.

5.5. 4 Data collection

Data was collected from one week after inoculation at weekly interval for five weeks on lines inoculated artificially. Both incidence and severity were recorded. Severity was scored using a scale of 0 – 5 (Bosque-Perez and Alam, 1992), as detailed below:

Scale	Description
0	no visible disease symptoms
1	very few streaks on some leaves
2	light streak symptoms on most leaves
3	moderate streak symptoms on most leaves
4	abundant symptoms on all leaves (>60%) leaf area affected
5	severe symptoms on all leaves (>80%) of leaves affected with no yield

On the line x tester evaluation, diseases were monitored and data was collected on MSD at weekly intervals for six week. Gray leaf spot damage was negligible at all sites with only a few lines showing traces of symptoms. Northern leaf blight disease data was collected at one site only (Iganga), and therefore not presented. At harvest data was recorded on plant stand (number of plants/ plot), number of ears/plant, field or cob weight (kg/ plot), grain texture and ear rot (percentage of diseased ears/ plot). Moisture content (%) and grain yield (t ha^{-1}) were determined immediately after harvest.

5.5. 5 Data analysis

Data from artificially inoculated lines collected at several dates was used to calculate area under disease progress curves (AUDPC) basing on formula given by Campbell and Madden (1990). Area under disease progress curve values were first standardised by dividing them by the total time of the epidemics (Fry, 1977), then subjected to analysis of variance using GenStat computer software (GenStat, 2005). Significant means were separated by Fischer least significant difference (LSD) at 5 % level of significance. In this analysis, the three parental lines were grouped as one population giving a total of nine populations in the analysis such that eight degrees of freedom for populations was obtained in the ANOVA.

Data from evaluation of testcrosses were also subjected to analysis of variance using GenStat. Standardised AUDPC values were computed for MSD severity over 6 weeks as described above. Since MSD AUDPC had many zero values for some genotypes, the analysis had very high coefficient of variation (72.9 %). AUDPC data was therefore transformed using inverse logit given by formula:

Inverse logit = c/ (1 + exp (-x)); (GenStat, 2005)

Where x is the data value, c is a constant at a specified value of 10. The final results presented are in untransformed format.

Yield data and AUDPC were subjected to additive main effects and multiplicative interactions (AMMI) analysis to determine genotype x environment interaction. Spearman's rank correlation analysis was also applied on yield data to determine the performance of crosses. Average ranks and rank standard deviations were also calculated from Spearman's ranking to determine stability of the crosses Correlation analysis was performed between yield data and MSD AUDPC values. It is important to note that line x tester analysis to establish combining ability (breeding values) of the lines was not performed because not all the crosses produced enough seeds for yield evaluation.

5.6 Results

5.6.1 Comparison of lines from MAS and conventional selection methods for resistance to MSD

Analysis of variance showed that there were significant variations in percentage incidence of MSD between populations (Table 5.3). Significant variations were also observed among lines across populations and among lines within each population (Table 5.3).

Table 5.3. ANOVA summary for final MSD incidence on 27 maize lines

Source of variation	D.F	S.S	M.S	Probability
Replication	2	494.4	247.2	
(Lines + Checks)	26	42314.1	1627.5	<.001
(Population + Checks)	8	17756.4	2219.5	<.001
Population/Lines	18	24557.7	1364.3	0.004
Total	80	70621.8		

Disease incidence on all lines evaluated ranged from 29.2 % to 100 % (Table 5.5). Three out of 24 lines evaluated had disease incidence levels lower than that of CML 202 (resistant check). Percentage incidences varied on lines within populations. In BC3F1 (of CML321xCML202) population 1 from MAS, the best line had the least incidence (29.2%) and worse line had the highest incidence of 83.3%. In the corresponding BC3F1 of CML321xCML202 (population 5) from conventional selection, the best line had incidence of 68.8% and worse line had incidence of 95.2%. Lines from F4 of CML384xCML202 with MAS and conventional method showed incidences ranging from 35.4 to 97.9% and from 97.9 to 100%, respectively.

Significant variations were also observed for AUDPC values for severity (Table 5.4). Significant variations were observed within and across populations. AUDPC for disease severity among lines ranged from 1.2 to 2.7 (Table 5.5). AUDPC on checks lied at the two extremes; the lowest AUDPC value of 0.7 for severity was recorded on CML 202 (resistant check) and the highest AUDPC value of 3.0 was on CML 321 and CML 384 (Susceptible checks). For populations from MAS, AUDPC variation on lines within BC3F1 of CML321xCML202 (population 1) was from 1.2 in the best line to 2.4 in the worse line. In F4 of CML384xCML202 (population4), the lines had AUDPC ranging from 1.3 in the best line to 2.7 in the worse line. BC3F1 of CML283xCML202 (population 2) showed the least variation in AUDPC among the populations from MAS. Lines from F4 of CML321xCML384 (Population 7) from conventional method showed the highest variations in AUDPC values. The best lines from population 7 had AUDPC value of 1.4 and the worse lines had AUDPC value of 2.0.

Table 5.4 ANOVA of AUDPC for MSD severity on 27 maize inbred lines

Source of variation	D.F	S.S	M.S	Probability
Rep	2	485.20	242.60	
Lines + checks	26	27.39	1.05	0.001
(Populations + Checks)	8	8.20	1.03	0.018
Population/ Lines	18	19.19	1.07	0.003
Total	80	49.01		

Highest incidences were recorded in F4 of CML384xCML202 (Population 9) followed by BC3F1 of CML321xCML202 (population 5) (Table 5.6). The least disease incidences were 53.2% and 54.3%, recorded on BC3F1 of CML384xCML202 (population 2) and on F4 of CML321xCML202 (population 7), respectively. The average incidence was higher in populations under conventional selection (79.3%) than in those under marker assisted methods (64.8%). Higher variations were observed in populations selected through MAS than in populations from conventional methods. Standard deviation for lines within populations from MAS ranged from 17.5 to 32.7; while in populations from conventional methods, the standard deviations ranged from 1.2 to 13.9 (Table 5.6).

Average AUDPC for populations from marker assisted selection ranged from 1.4 to 1.8. Marker assisted selection with backcrossing and selfing showed similar mean AUDPC of 1.6 and 1.7, respectively. While a range of 1.6 to 2.4 was observed for AUDPC on populations under conventional selection (Table 5.6). AUDPC means for populations from marker

assisted selection were lower than those from corresponding populations under conventional selection methods. There were relatively lesser variations for AUDPC on lines within populations from conventional selection methods than variations on lines from populations from MAS. The standard deviations for AUDPC on lines within populations from MAS were varied ranging from 0.2 to 0.8. While the standard deviations within populations from conventional methods ranged from 0.1 to 0.3. None of the populations had AUDPC values lower than that of resistant check and all the populations had AUDPC values lower than the values shown by susceptible checks. Correlation coefficient between incidence and severity was 0.732 ($P < 0.001$).

Table 5.5 Means of MSD incidences and standardised AUDPC values for MSD severity on 27 maize lines

Lines within population	Populations	Breeding method	MSD Incidence (%)	Standardised AUDPC For MSD severity
1	BC3F1 of CML321xCML202 (Population 1)	MAS with backcrossing	83.3	2.4
2			70.8	1.5
3			29.2	1.2
4	BC3F1 of CML384xCML202 (Population 2)	MAS with backcrossing	65.9	1.4
5			60.4	1.6
6			33.3	1.2
7	F4 of CML321xCML202 Population 3	MAS with selfing	52.1	1.3
8			85.4	1.9
9			91.7	2.3
10	F4 of CML384xCML202 Population 4	MAS with selfing	97.9	2.7
11			35.4	1.3
12			50.0	1.5
13	BC3F1 of CML321xCML202 (Population 1)	Conventional backcrossing	95.2	2.3
14			68.8	2.4
15			89.6	2.3
16	BC3F1 of CML384xCML202 (Population 2)	Conventional backcrossing	75.0	2.0
17			75.0	1.9
18			89.6	2.2
19	F4 of CML321xCML202 Population 3	Conventional selfing	54.2	1.4
20			52.1	1.4
21			56.8	2.0
22	F4 of CML384xCML202 Population 4	Conventional selfing	100.0	2.2
23			97.9	2.3
24			97.9	2.6
CML 202	Resistant check	Inbred line	37.5	0.7
CML 321	Susceptible check	Inbred line	100	3.2
CML 384	Susceptible check	Inbred line	100	3.0
Mean			72.00	1.93
SED			4.57	0.51
$LSD_{0.05}$			9.17	1.03
C.V (%)			32.10	32.60

Table 5.6 Pooled means of MSD incidence and AUDPC for lines within populations

Pooled means of lines within population				
Populations*	MSD Severity AUDPC	SD for severity	MSD incidence	SD for incidence
Population **1** (BC3F1 of CML321xCML202)	1.7	0.6	61.1	28.3
Population **2** (BC3F1 of CML384xCML202)	1.4	0.2	53.2	17.5
Population **3** (F4 of CML321xCML202)	1.8	0.5	76.4	21.3
Population **4** (F4 of CML384xCML202)	1.8	0.8	61.1	32.7
Population **5** (BC3F1 of CML321xCML202)	2.3	0.1	84.5	13.9
Population **6** (BC3F1 of CML384xCML202)	2.4	0.2	79.6	8.4
Population **7** (F4 of CML321xCML202)	1.6	0.3	54.3	2.4
Population **9** (F4 of CML384xCML202)	2.0	0.2	98.9	1.2
CML 202 (resistant check)	0.7		37.5	
CML 321 (susceptible check)	3.2		100	
CML 384 (susceptible check)	3.0		100	
Means	1.9		72.0	
SED	0.3		10.9	
LSD0.05	0.6		21.9	
C.V (%)	32.6		32.1	

*Populations 1-4 were from MAS; Populations 5-8 were from conventional methods.

Time-course analysis for incidence and severity

Analysis of MSD incidence against time showed that there was significant variation for disease incidence between populations ($P<0.001$) (Table 5.7). Time-course of disease incidences and disease symptom development showed differences between populations from marker assisted selection and conventional selection methods. Disease assessment at one week after inoculation showed no symptoms. From the fourth week after inoculation, disease incidence remained constant (Fig 5.1a and b). MSD symptom severity also varied significantly among the different populations (Table 5.8). There was a general increase in severity over time (Fig 5.1c and d), although some lines showed relatively smaller marginal increase in severity over time. Changes in both disease incidence and severity were consistent over time hence neither incidence x time nor severity x time interactions were observed ($P > 0.05$).

Table 5.7 ANOVA for maize streak virus disease incidence over time

Source of variation	D.F	S.S	M.S	Probability
Rep stratum	2	1977.5	988.8	
Lines	26	169256.4	6509.9	<.001
Time	3	40899.1	13633.0	<.001
Time x Lines	78	18014.5	231.0	0.180
Total	323	370248.6		

Table 5.8 ANOVA for maize streak virus disease severity over time

Source of variation	D.F	S.S	M.S.	Probability
Replication	2	4.77	2.38	
Lines	26	86.44	3.32	0.001
Time	3	180.10	60.03	<.001
Time x Lines	78	15.34	0.20	0.118
Total	323	375.21		

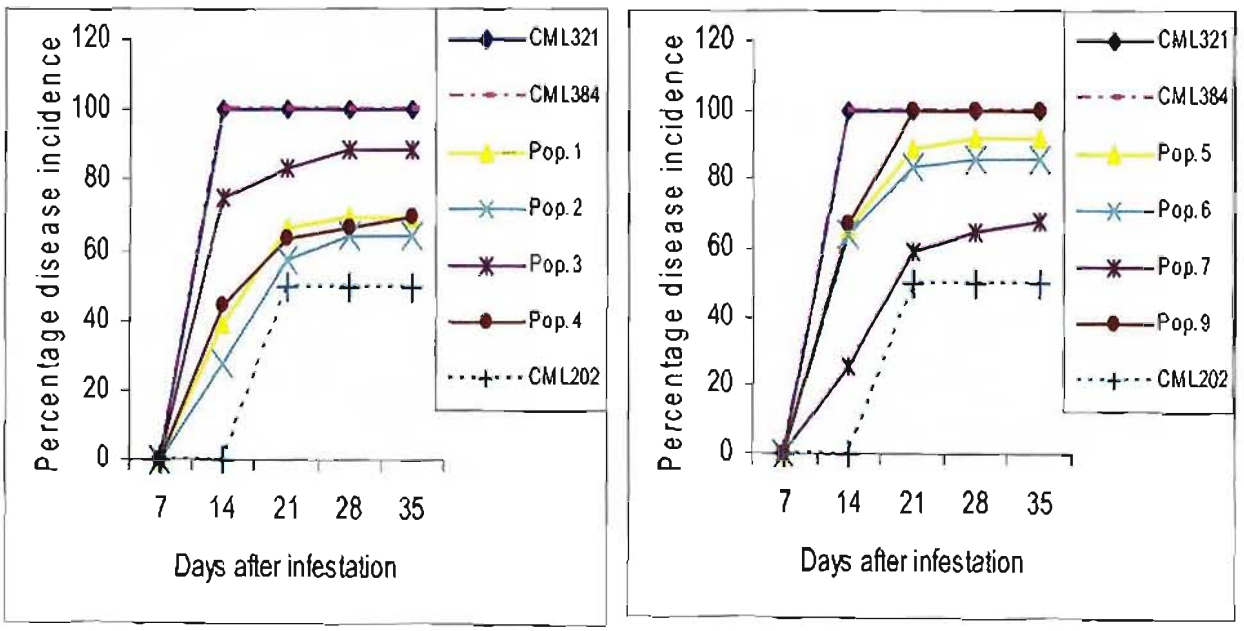

Figure 5.1 (a) Percentage incidence on populations 1, 2, 3 and 4 from MAS; (b) on populations 5, 6, 7 and 8 from conventional selection methods.

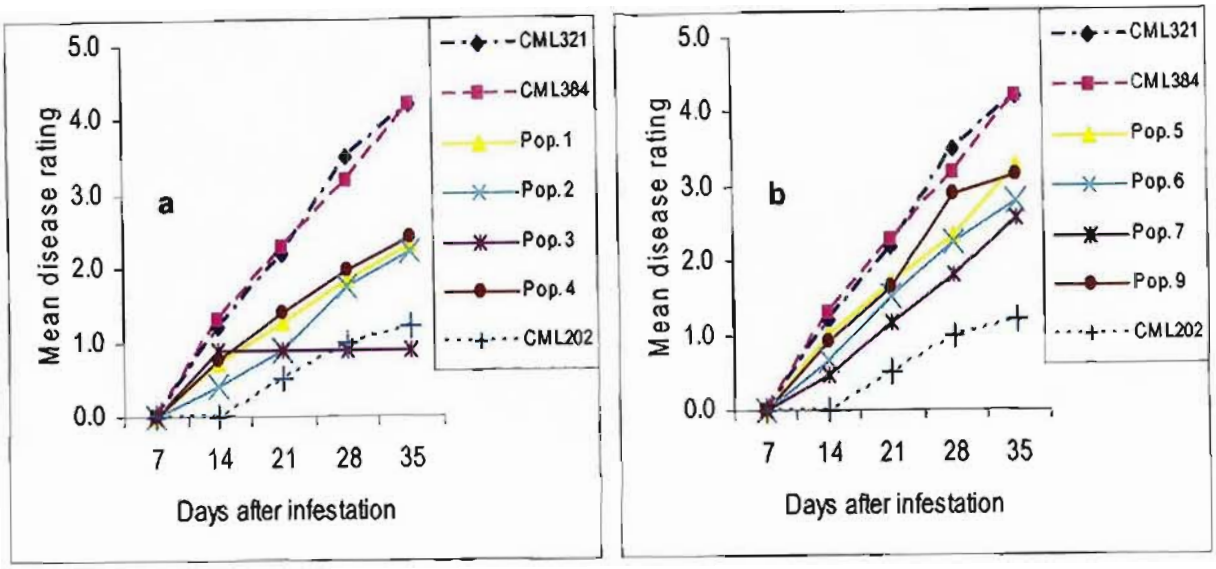

Figure 5.2 (a) Time-course of MSD severity on populations 1, 2, 3 and 4 from MAS; (b) on populations 5, 6, 7 and 8 from conventional selection methods

5.6.2 Evaluation of lines x tester crosses for resistance to MSD and yield

Analysis of variance for AUDPC for crosses showed that there were significant differences between crosses (P = 0.021) and also between locations (P < 0,001) (Table 5.9). AMMI analysis for AUDPC data also showed that crosses and location main effects were significantly different (Appendix 5.1). There was no significant crosses x location interaction at 5 % level of significance even though IPCA 1 scores were significant (P = 0.00368) (Appendix 5.1).

Table 5.9. Summary of ANOVA of AUDPC for 30 crosses across 3 locations

Source of variation	d.f.	s.s.	m.s.	
Locations	2	86.27	43.13	< 0.001
Crosses	29	46.47	1.60	0.021
Locations x Crosses	58	72.45	1.25	0.083
Total	179	286.26		

Area under disease progress curve values for MSD were generally low and ranged from 0.6 to 2.1 on the crosses (Table 5.10). All crosses showed lower AUDPC than the susceptible check (3.2). Except Longe 4 (a resistant check) which had AUDPC value of 0.7, thirteen crosses had higher resistance to MSD than the other standards used. Eight out of the best 13 crosses were from marker assisted selection. Susceptible check had AUDPC of 3.2.

Table 5.10. Means of yields (t ha^{-1}) and AUDPC of MSD severity of 30 crosses across 3 locations

Lines	Population	Breeding method	Mean Yield (t ha^{-1})	Mean AUDPC)
Tester A				
22 x tester A	F4 of CML384xCML202	Conventional	9.24	1.8
17 x tester A	BC3F1 of CML384xCML202	Conventional	8.54	1.0
9 x tester A	F4 of CML321xCML202	MAS	8.15	0.9
21 x tester A	F4 of CML321xCML202	Conventional	7.56	1.1
6 x tester A	BC3F1 of CML384xCML202	MAS	6.69	1.0
3 x tester A	BC3F1 of CML321xCML202	MAS	6.54	0.7
15 x tester A	BC3F1 of CML321xCML202	Conventional	6.41	2.1
8 x tester A	F4 of CML321xCML202	MAS	6.40	1.9
23 x tester A	F4 of CML384xCML202	Conventional	6.26	0.8
20 x tester A	F4 of CML321xCML202	Conventional	6.20	1.0
14 x tester A	BC3F1 of CML321xCML202	Conventional	6.11	0.6
5 x tester A	BC3F1 of CML384xCML202	MAS	6.05	1.2
18 x tester A	BC3F1 of CML384xCML202	Conventional	5.67	1.1
2 x tester A	BC3F1 of CML321xCML202	MAS	5.53	1.6
13 x tester A	BC3F1 of CML321xCML202	Conventional	5.07	1.6
Tester B				
12 x tester B	F4 of CML384xCML202	MAS	8.91	2.1
17 x tester B	BC3F1 of CML384xCML202	Conventional	8.01	2.0
1 x tester B	BC3F1 of CML321xCML202	MAS	7.46	0.8
14 x tester B	BC3F1 of CML321xCML202	Conventional	6.88	0.8
13 x tester B	BC3F1 of CML321xCML202	Conventional	6.48	1.1
6 x tester B	BC3F1 of CML384xCML202	MAS	6.15	1.8
8 x tester B	F4 of CML321xCML202	MAS	5.37	1.8
3 x tester B	BC3F1 of CML321xCML202	MAS	5.35	1.7
4 x tester B	BC3F1 of CML384xCML202	MAS	3.92	0.8
Longe 7H	Check		7.56	1.2
SC407	Check		7.14	1.2
Susceptible check	Check		6.93	3.2
Longe 4	Resistant check		5.87	0.7
Longe 6H	Check		5.00	2.2
Longe 1	Resistant check		4.75	1.3
Mean			6.51	1.4
SED			1.11	0.5
$LSD_{0.05}$			2.21	1.1
C.V (%)			29.60	19.8

Analysis of variance for yield results showed that there were significant differences among the crosses ($P < 0.001$) and among locations ($P < 0.001$) (Table 5.11). AMMI analysis of yields showed no significant ($P = 0.497$) crosses x location interactions (Appendix 5.2). Since there were no significant interactions between crosses and locations, only the pooled mean yields for crosses across locations are presented (Table 10). Mean yields over 3 locations ranged from 3.92 t ha^{-1} to 9.24 t ha^{-1}. Five crosses performed better than the best

standard (Longe 7H). The best standard yielded 7.56 t ha^{-1} and the best five crosses had yields which ranged from 8.01 t ha^{-1} to 9.24 t ha^{-1}. Ten crosses had mean yields above the overall mean for all the crosses evaluated. Five of those crosses were from lines under MAS and the other five were from lines under conventional methods.

Genotype ranking was consistent across locations and genotype x environment interactions was shown to be non-significant through both analysis of variance and AMMI model. However, Spearman's rank correlation coefficient for yield across locations was significant (r = 0.687, P = 0.001) (Table 5.11).

Table 5.11 Average ranking and standard deviation for crosses with respect to yield

Lines x tester Crosses	Generation/ Pedigree	Breeding methods	Average ranking	Standard deviation
22 x tester A	F4 of CML384xCML202	Conventional	28	1.5
15 x tester A	BC3F1 of CML321xCML202	Conventional	27	1.7
12 x tester B	F4 of CML384xCML202	MAS	24	6.5
21 x tester A	F4 of CML321xCML202	Conventional	24	5.3
17 x tester A	BC3F1 of CML384xCML202	Conventional	23	8.4
1 x tester B	BC3F1 of CML321xCML202	MAS	22	7.0
9 x tester A	F4 of CML321xCML202	MAS	22	11.6
17 x tester B	BC3F1 of CML384xCML202	Conventional	19	6.8
6 x tester A	BC3F1 of CML384xCML202	MAS	18	11.3
23 x tester A	F4 of CML384xCML202	Conventional	15	5.2
3 x tester A	BC3F1 of CML321xCML202	MAS	14	10.1
6 x tester B	BC3F1 of CML384xCML202	MAS	14	7.8
8 x tester A	F4 of CML321xCML202	MAS	14	7.6
14 x tester B	BC3F1 of CML321xCML202	Conventional	14	9.5
5 x tester A	BC3F1 of CML384xCML202	MAS	13	4.9
13 x tester B	BC3F1 of CML321xCML202	Conventional	13	8.1
18 x tester A	BC3F1 of CML384xCML202	Conventional	13	7.5
14 x tester A	BC3F1 of CML321xCML202	Conventional	12	7.6
20 x tester A	F4 of CML321xCML202	Conventional	12	6.7
13 x tester A	BC3F1 of CML321xCML202	Conventional	10	8.1
2 x tester A	BC3F1 of CML321xCML202	MAS	9	5.5
8 x tester B	F4 of CML321xCML202	MAS	9	3.8
3 x tester B	BC3F1 of CML321xCML202	MAS	8	7.4
4 x tester B	BC3F1 of CML384xCML202	MAS	1	0.6
Longe 1	Check		4	1.0
Longe 6H	Check		9	6.1
Longe 4	Check		12	6.2
Susceptible check	Check		19	3.6
SC407	Check		19	10.6
Longe 7H	Check		23	4.5
Spearman's Rank Correlation coefficient			0.687; P < 0.001	

Average rank for grain yield across all locations were calculated and results showed that the high yielding crosses had higher average ranks than crosses with low yields, and vice versa. Rank standard deviation varied ranging from 0.6 in the worse cross (line 4 x tester B) to 11.6 in line 9 x tester A. the variation in rank standard deviation showed that the stability of the crossed were variable.

5.7 Discussion

Marker assisted selection for simply inherited traits is gaining increasing importance in breeding programmes, allowing an acceleration of the breeding process. Traits related to resistance to pathogens and to quality of some crop products are offering some important examples of a possible application of MAS (Francia *et al.*, 2005).

When comparing resistance to MSD based on field observation of disease incidence and severity, it is important to subject all the maize lines to the same disease pressure and at the same time. Any differences observed in disease incidence and symptom severity are most likely due to the genetic potential of the plants. In this study, all the plants were artificially inoculated with viruliferous leafhoppers with 100 % transmission rate. Chances of having escapes were minimised and hence the low incidences of disease observed were attributed to resistance. This view is also supported by the highly significant correlation between disease incidence and severity.

Among all populations, disease symptoms progressed at slower rate and the final percentage infection was also lower than that of the susceptible checks. A corresponding lower percentage incidence was also observed on the populations than on the susceptible checks. This confirmed a definite level of resistance was present in the populations. Differences between lines within each population were observed in both disease incidences and severities. However, the variations between lines within population were lesser in populations from conventional selection than in populations from MAS. Lines within F4 population of CML384xCML202 (Population 4) from MAS had AUDPC values ranging from 1.3 to 2.7. Lines from the corresponding population under conventional methods (Population 9) had AUDPC values which ranged from 2.2 to 2.6. The standard deviations were 0.8 and

0.2 for MAS and conventional methods. The variations observed between lines within MAS populations could be attributed to the fact that MAS selected for lines with fixed QTL for resistance and those that were still heterozygous for the QTL. In general, MSD Incidence and severity were lower in lines drawn from marker assisted selection than those from conventional selection methods. The results showed that MAS was more efficient in selecting for resistance to MSD than conventional methods. These findings were in agreement with reports that MAS was more efficient than conventional selection in MSD (Huang *et al.*, 1997; Chen *et al.*, 2000; Yousef and Juvik, 2001). However, low resistance in populations from conventional selection methods might have resulted because of the elimination of zero scores because of possible confounding with escapes.

A high positive correlation coefficient ($r = 0.732$; $P < 0.001$) between MSD incidence and severity was observed suggesting a form of resistance probably by non-preference. It has been reported that low incidence is associated with resistance due to non-preference (Kairo *et al.*, 1995; Mesfin and Bosque-Perez, 1998). However, in the current study no confirmation was done to determine whether low incidence observed on lines with high resistance was, in fact, due the non-preference.

Resistance to MSD also varied between conventional backcross and selfing populations. Relatively higher levels of resistance were achieved through selfing, which was expected, due to segregation and recombination. These results suggest that different selection approaches determined the levels of resistance achieved. According to Caulfield (1997), sensitivity to viruses increased with inbreeding and infected materials produced no ear. Thus elimination of susceptible materials can be achieved faster through selfing. Selections through selfing have produced lines with a resistance level higher than those of the donor parents (Pixley *et al.*, 1997). The study indicated that higher selection gain is realised when lines are derived from a segregating F2 than BC source populations in conventional methods.

In contrast, no significant difference was observed between MAS with backcross and MAS with selfing. Results showed that the efficiency of MAS was the same in MAS with backcross and with selfing. All lines from the MAS method were selected for the presence of major QTL which is partially dominant and accounts for 45% of the phenotypic variations

(Welz *et al.*, 1998; Pernet *et al.*, 1999a). It is apparent, therefore, that there is no superiority of F2 or BC source population when using MAS in selection.

Analysis of variance for resistance to MSD on lines x tester crosses showed consistency across locations, but locations differed significantly from each other. This indicated that the crosses have stable resistance. Stability of resistance across environments compares with results of a study by Flett *et al.* (1997). Their field evaluation of hybrids over six seasons showed that the relative resistance of the hybrids was stable. These results contradict results obtained by Dintinger *et al.* (1997) in which they observed significant genotype x environment interaction for resistance to MSD.

Results also showed that neither incidence nor disease severity affected yield of testcrosses. Correlation coefficient between yield and disease incidence was -0.0895 (P = 0.7462) and between yield and disease severity was 0.0868 (P = 0.08132), indicating there was no effect on yield due to the presence of MSD. This was because the lines were fairly resistant and because of the partial dominant nature of resistance, the crosses were also resistant to MSD. As such, disease incidences on crosses were low across locations. A corresponding low disease severity was also observed on the lines compared to the susceptible check. Differences in ecological zones may exist but incidence of MSD has been found to correlate with severity.

Yields differed significantly between crosses but no significant cross x location interactions were observed suggesting that hybrids were generally stable across environments. The environment was largely significant with average yield of 7.21 t ha^{-1}, 5.37 t ha^{-1} and 6.94 t ha^{-1} for Namulonge, Masaka and Iganga, respectively. The differences in yields across locations were perhaps due to differences in the levels of disease pressure and altitude. Namulonge, Iganga and Masaka also differ in soil fertility, rainfall amount and distribution. Masaka site is characterized by acidic soils.

Although line x tester analysis to determine the general and specific combining abilities of the lines were not done due to failure of some crosses due to drought, performance of lines with respect to testers are indications of their breeding values. As such, with respect to tester A (Heterotic group A), line 22 showed the highest breeding value. This was followed by lines 17 and 9. Line 22 was from F4 of CML384xCML202 from conventional selection.

Line 17 was from BC3F1 of the same cross as line 22 (i.e. CML384xCML202). Line 9 was from F4 of CML321xCML202 from MAS method.

With respect to tester B (Heterotic group B), line 12 showed the highest breeding value, followed by lines 17 and 1. Line 12 was from F4 of CML384xCML202 from MAS. Line 17 was from BC3F1 of the same cross as line 12 (CML384xCML202) but from conventional selection. Line 1 was from BC3F1 of CML321xCML202 from MAS method. Higher yields were observed for crosses with tester A than with tester B. This is because tester A (CML395/CML444) is a higher potential tester since it is later maturing than tester B. In addition, all lines tested were from B-heterotic orientation hence they were complementary to tester, which is from A-heterotic group. However, some lines, although from the same heterotic group, produced high in the cross. This is because the classification is very broad such that some lines can still show heterosis within the same grouping. For example, lines 12 and 17 performed well with tester B although they are from the same heterotic groups. Yields for crosses with tester A ranged from 5.07 to 9.24 t ha^{-1} and from crosses with tester B, yields ranged from 3.92 to 8.91 t ha^{-1}.

Most lines from conventional selection combined high yield and high stability as indicated by high ranking values and low standard deviations across environments. However, it was apparent that lines from MAS showed variable performance as indicated by high values of standard deviation, suggesting that unlike in conventional selection where selected plants are results of genotypic and environmental effective, MAS methods are independent of environmental effects. The performance of lines from MAS in the field would depend on the environments because the traits are not independent of environmental influence as markers. Perhaps these explain the relatively higher rank standard deviation values for crosses of lines from MAS than from conventional methods. This suggests that MAS selection can be improved if integrated with field evaluations.

Evaluation of test-cross progenies identified potential lines that can eventually be used for hybrid production for farmers' utilization. Ten crosses had mean yields above the overall mean for all the crosses evaluated and their yields ranged from 6.54 to 9.24 t ha^{-1}. Five of these crosses were with lines from MAS and the other five were crosses of lines from conventional selection. These ten lines that performed well in the test cross also showed good levels of resistance to MSD, with AUDPC ranging from 1.2 to 2.4. These results

indicate potential three-way cross hybrids that could eventually be released for use by farmers in MSD prone-environments of Uganda. The results also showed that early testing can be used to identify beneficial lines for potential hybrid production. The ten outstanding inbred lines will be advanced by selfing for possible use in hybrids production in Uganda.

5.8 Conclusion

Molecular marker selection with backcrossing and selfing was used to obtain resistant maize lines. Marker assisted selection was more efficient than conventional selection because average incidence of MSD was higher in populations under conventional selection (79.3 %) than in those under marker assisted methods (64.8 %). Area under disease progress curve for populations under marker assisted selection were also lower than those from corresponding populations under conventional selection methods confirming superiority of MAS. However both selection methods were effective in selecting lines with higher resistance than the susceptible checks. A positive correlation between incidence and severity of disease in all the populations suggested presence of non-preference mechanism for the insect. Evaluation of lines in three-way crosses identified ten potential lines that can eventually be used for hybrid production for farmers' utilization. The hybrids also showed high stability across three locations. Ten crosses; five from MAS and five from conventional selection methods, had mean yields above the overall mean for all the genotypes and were highly superior to standard hybrids evaluated. Both MAS and conventional selection were equally effective in identifying high yielding lines although resistance was higher under MAS. As expected, higher selection progress was realised when selection for resistance to MSD was effected in F2 than BC populations using conventional breeding, but with MAS both source populations achieved similar selection progress.

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5.9 Appendices

Appendix 5.1 Summary of ANOVA for AMMI model for AUDPC in 3 locations

Source of variation	D.F	S.S	M.S	F-Probability
Treatments	89	205.19	2.31	0.00001
Genotypes	29	46.47	1.60	0.02568
Environments	2	86.27	43.13	0.00000
Interactions	58	72.45	1.25	0.09743
IPCA 1	30	58.52	1.95	0.00368
Total	179	286.26	1.60	

Appendix 5.2 Summary of ANOVA for AMMI model for yields (t ha^{-1}) in 3 locations

Source of variation	D.F	S.S	M.S	F-Probability
Treatments	89	646271687	7261480	0.00103
Genotypes	29	311901646	10755229	0.00008
Environments	2	118541942	59270971	0.00000
Interactions	58	215828099	3721174	0.49782
IPCA	30	173816312	5793877	0.05927
Total	179	976625381	5456008	

CHAPTER SIX

Analysis of cost of marker assisted selection and conventional selection at two early generations of selection

6.1 Abstract

Comparison of marker assisted selection (MAS) and conventional selection has been done with a lot of precautions. So far there has not been any generalisation on cost of MAS, and no recommendation of best choice between MAS and conventional selection methods has been put forward. This study was designed with the objective of comparing the costs of MAS and conventional selections at two cycles of selections for resistance to maize streak virus. Selections were applied on four segregating populations which comprised of BC1F1 and F2 from CML202 with CML321, and BC1F1 and F2 from CML202 with CML384. A second cycle of selection was done at BC2F1 and F3 of the respective populations. Costs for field operations and laboratory procedures during the selections were computed from payment vouchers, receipts and invoices. Results showed that costs of MAS and conventional methods varied, depending on the units for comparison. Considering the total costs of selection for MSD using MAS and conventional methods, costs of conventional methods were higher in both first (US$ 2322.58) and second (US$ 1955.60) selection cycles than the total costs of MAS in first (US$ 1951.27) and second (US$ 1113.01) cycles. The costs of field activities associated with MAS were about 30.4% of the field evaluation cost for conventional methods. Comparing costs per row for conventional method and costs per plant or data point for MAS showed that conventional method was 2.4 times more expensive than MAS. However, by standardising costs per plant to facilitate appropriate economic comparison, MAS was shown to be 6.6 times more expensive than conventional selection. The largest proportion (60%) of costs of MAS was from laboratory consumables. Costs of MAS can be reduced by using relative smaller population size for screening.

6.2 Introduction

With the development of highly variable molecular markers and decrease of genotyping cost, genetic markers have received increasing attention (Wang and Hill, 1999). Breeders are searching for a tool that can either increase the rate of advance, or offer an improvement in the cost-effectiveness of breeding (Thomas 2003). For MAS to be more effective, it is necessary to decrease further the cost associated with molecular marker assays (Xie and Xu, 1998). Continuous changes in marker technology should be able to lower the costs of marker selection substantially.

There is limited work done concerning comparison of cost of MAS and conventional selection methods. The few papers that have addressed these aspects have considered

different parameters and breeding methods in their analyses (Ragot and Hoisington, 1993; Gu *et al.*, 1995; Moreau *et al.*, 2000; Yu *et al.*, 2000; Dreher *et al.*, 2000; Morris *et al.*, 2003). For example, Dreher *et at.* (2003) and Morris *et al.* (2003) compared costs of markers versus conventional selection in quality protein maize (QPM), which approach consists of chemical analysis for tryptophan and lysine. Yu *et al.* (2000) compared cost of selection for bacterial blight resistance in greenhouse experiment with MAS. The variable methodologies applied in the recent studies showed the absence of general standards for comparing costs associated with MAS and conventional selection methods. For example, comparisons have been computed based either on one cycle of selection (Gu *et al.*, 1995; Moreau *et al.*, 2000; Yu *et al.*, 2000) or on multistage selection (Dreher *et al.*, 2000; Morris *et al.*, 2003). Comparisons have also been done involving different numbers and types of markers, different population size and at different stages of selection.

The costs of MAS have been shown to be much lower than conventional depending on the choice of markers. The estimated cost for using sequence characterised amplified regions (SCAR) and random amplified polymorphic DNA (RAPD) markers to analyse 100 bean lines would be about 3.13 and 3.48 (Canadian dollars) per data point, respectively, after the markers were developed (Yu *et al.*, 2000). In contrast, conventional greenhouse screening was estimated to cost approximately, 5.88 Canadian dollars per data point. The cost of using PCR-based markers for selection was estimated to be about 1.14 US dollars per data point (Ragot and Hoisington, 1993). A much lower cost of MAS at less than $0.40 per sample was estimated (Gu *et al.*, 1995). With respect to their particular conditions, the authors concluded that MAS was more cost-effective than conventional methods. Dreher *et al.* (2000), however, concluded the cost of application of MAS were variable depending on the circumstances. Dekkers and Hospital (2002) have recently reviewed some of the potential limitations of MAS application and concluded that the use of MAS will be determined by the economic benefit relative to conventional selection.

Phenotypic selection methods, on the other hand, can be fast and inexpensive especially, when selection is done under optimum environmental conditions (Dreher *et al.*, 2000). The relative cost of conventional selection depends on a number of factors including nature of trait (qualitative or quantitative), type of gene action involved, the ease of measuring target trait (Morris *et al.*, 2003). However, Dreher *et al.* (2000) also acknowledged that for some traits conventional selection could be difficult, time-consuming and may be even expensive.

In such cases MAS would have an advantage over conventional screening by either saving on time or reducing cost or improving genetic gains.

This study was designed to compare the costs associated with conducting a breeding programme to screen for resistance to maize streak virus (MSD) using MAS and conventional selection method in early generations of segregating populations. The costs of initial development of SSR marker linked to the QTL for MSD resistance were not included. This study relied on the available information in the maize data-base on available SSR markers and their linkage to MSD resistance QTL. The costs of equipment for PCR were not considered since the study was conducted at Makerere University with an established biotechnology laboratory.

Conventional screening for MSD is normally best done under artificial inoculation of plants with viruliferous leafhoppers. At Namulonge leafhopper rearing unit was not in place until this year 2006. To increase leafhopper population during field screening an alternative way has to been the use of spreader rows. With artificial inoculation or the use of spreader rows, MSD infections are shown to be low at the beginning of the season (Caulfield, 1997). Moreover, such techniques are laborious and increase cost of field screening. Accurate selection is also complicated by the fact that symptoms development depend on time of infection (Bosque-Perez et al., 1998). Marker assisted selection does not rely on symptoms or field condition. The overall purpose of the study was to determine if cost associated with MAS can warrant its use for screening for resistance to MSD.

6.3 Objective of the study

The objective of the study was to compare the cost of marker assisted selection for MSD resistance with conventional selection methods.

6.4 Hypothesis tested

The hypothesis tested was that marker assisted selection was more expensive than the conventional selection methods.

6.5 Material and method

A maize breeding programme was initiated with the aim of selecting for resistance to MSD using MAS with backcrossing and selfing and conventional backcrossing and selfing. Two crosses were made between maize inbred lines CML321 and CML202 (CML321/ CML202) and, CML384 and CML202 (CML384/CML202). Their F1 generations were backcrossed to the recurrent parents (CML321 and CML384) and also self-pollinated to generate both BC1F1 and F2 for each of the 2 crosses, respectively. For conventional method each of the four populations was planted in screening nurseries for conventional screening. Each population was planted in three blocks, made up of 42 rows each with 15 plants per row. Two bordering blocks planted with a susceptible hybrid to enhance natural virus infection for screening was added, giving a total of 14 blocks for conventional selection in the first generation of selection. In the second generation of selection (BC2F1 and F3) each population was planted in two blocks and a susceptible hybrid also in two blocks bordering the nursery. A total of ten blocks consisting of 42 rows each were established.

Separate nurseries were established for each of the four populations for MAS methods. Each population was planted in only one block consisting of 42 rows. Lines selected from BC1F1 and F2 generations were backcrossed to recurrent parents and self pollinated to advance then to BC2F1 and F3 generations. These were also established for screening. Each population was planted in one block of 42 rows with 15 plants per row. A selection intensity of 10 percent was applied for both MAS and conventional methods to advance the populations to BC2F1 and F3 of the respective populations.

Field costs for conventional methods were computed from a total of 588 breeding rows in the first selection cycle and from 420 rows in the second cycle of selection. On the other hand, field cost for MAS was computed from 168 breeding rows in each selection cycle. Costs of laboratory procedures were calculated from 800 plant samples (200 plants per population) in the first MAS selection and 400 plant samples (100 plants per population) in the second round of MAS.

Costs for field operations performed during two cycles of selection were computed from payment vouchers. The prices of laboratory consumables and chemical inputs used in the field operations for both MAS and conventional breeding were obtained from receipts and/or

supplier quotations (proforma invoices). Capital costs were not included here since all the research was done in an existing fully functional biotechnology laboratory. Overhead cost (bench fee) was used based on Makerere University rate of 7%. Reagents like *Taq* polymerase and the primers were imported and their costs of shipment were also included in the estimates. The cost of MAS per sample was determined as the total sum of unit costs of reagents and disposable equipment used for each sample in PCR amplification. Technician labour cost was determined from their monthly salary rate.

The following assumptions and considerations were made during cost computation for both MAS and conventional methods:

1. Suitable markers were already available.
2. The analysis was done in an established laboratory so costs of equipment were not included.
3. Scientists were paid their monthly salaries that were the same for government institutions.
4. Students were paid monthly stipend and not additional payment for time spent in the field or laboratory.
5. Overhead costs were 7% of the total expenditure based on Makerere University rate where the money for research came through.
6. An exchange rate of 1800 Uganda shillings to 1 US dollar was used.

6.6 Results

Results showed that cost of conventional screening for MSD during the first and second cycles of selection were different. In the first selection cycle the cost of field screening was US$ 2322.58 and in the second cycle of selection the cost was estimated at US$ 1955.60 (Table 6.1). Costs per row of conventional selection were about US$ 3.95 and US$ 4.76 for first and second cycles of selection, respectively.

Table 6.1 Field costs for conventional selection methods for two generations of selection

Item	First generation		Second generation		Mean per row
	Cost for 588 rows	Cost/ row*	Cost for 0.2ha	Cost/row*	
Chemicals:					
Fertilizer	62.52	0.11	41.68	0.11	0.11
Pesticides	75.24	0.13	50.16	0.13	0.13
Other chemicals	28.38	0.05	18.92	0.05	0.05
Travel	475.00	0.81	475.00	1.13	0.97
Supplies:					
Pollination bags	120.00	0.20	80.00	0.20	0.20
harvesting bags	17.70	0.03	11.80	0.03	0.03
Seed packets	49.98	0.09	33.32	0.09	0.09
Other supplies	52.50	0.09	35.00	0.09	0.09
Labour					
Technical Assistance	333.30	0.57	333.30	0.79	0.68
Field supervisor	166.70	0.28	166.70	0.40	0.34
Tractor operations	166.70	0.28	166.70	0.40	0.34
Field activities labour:					
Spreader rows labour	61.32	0.10	40.88	0.10	0.10
Planting	120.00	0.20	80.00	0.20	0.20
Weeding	101.82	0.17	67.88	0.17	0.17
Pollination	196.98	0.34	131.32	0.34	0.34
Termite control	25.02	0.04	16.68	0.04	0.04
Harvesting	79.98	0.14	53.32	0.14	0.14
Seed processing	37.50	0.06	25.00	0.06	0.06
Sub-total	**2170.64**	**3.69**	**1827.66**	**4.46**	**4.08**
Overhead	**151.94**	**0.27**	**127.94**	**0.30**	**0.28**
Grand Total	**2322.58**	**3.96**	**1955.60**	**4.76**	**4.36**

* 5m long rows with plants at spacing of 0.5m within rows

Costs incurred in the first and second cycles of Laboratory procedures without field cost were US$ 1493.71 and US$ 655.45 (Table 6.2). Cost of molecular analysis using one SSR marker without cost of field activities was at US$ 1.86 per plant sample. Among laboratory requirements for molecular analysis, reagents costs contributed to the largest proportion (60%) of the total laboratory procedure costs (Table 6.2). The cost of field activities of MAS was 30.4% of the field evaluation cost for conventional method. Total field costs associated with MAS procedures for first and second cycles of selection were the same, US$ 457.56 each (Table 6.3).

Table 6.2 Unit cost of SSR molecular marker analysis for MSD resistance selection for two cycles of selection

Components	First cycle	Second cycle	Percentage two cycles	Cost per data point
Reagents	**899.19**	**385.37**	**60**	**1.04**
Liquid Nitrogen	43.75	18.75	3	0.05
Taq polymerase	280.00	120.00	19	0.33
dNTPs	37.80	16.20	3	0.04
Primer (S)	4.20	1.80	0	0.00
Metaphor Agarose	462.88	198.38	31	0.54
Seakam Agarose	53.76	23.04	4	0.06
DNA Ladder	16.80	7.20	1	0.02
Supplies	**296.80**	**127.20**	**20**	**0.34**
Pipette tips	179.90	77.10	12	0.21
Eppendorf tubes	77.00	33.00	5	0.09
PCR tubes	39.90	17.10	3	0.05
Technician	200.00	100.00	14	0.25
Sub-total	**1395.99**	**612.57**	**93**	**1.64**
Overhead	97.72	42.88	7	0.11
Grand Total	**1493.71**	**655.45**	**100**	**1.75**

Table 6.3 Field cost associated with MAS method

Item	First generation Cost for 0.08 ha	Cost/ row	Second generation Cost for 0.08 ha	Cost/ row
Chemicals:	**46.10**	**0.27**	**46.10**	**0.27**
Fertilizer	17.35	0.10	17.35	0.10
Pesticides	20.85	0.12	20.85	0.12
Other chemicals	7.90	0.05	7.90	0.05
Travel	**158.50**	**0.59**	**158.50**	**0.59**
Supplies:	**106.30**	**0.63**	**106.30**	**0.63**
Pollination bags	33.35	0.20	33.35	0.20
harvesting bags	3.36	0.02	3.36	0.02
Seed packets	13.90	0.08	13.90	0.08
Other supplies	9.59	0.06	9.59	0.06
Labour:	**47.28**	**0.28**	**47.28**	**0.28**
Technical Assistance	27.78	0.17	27.78	0.17
Field supervisor	12.50	0.07	12.50	0.07
Tractor operations	7.00	0.04	7.00	0.04
Field activities labour:	**115.55**	**0.69**	**115.55**	**0.69**
Planting	33.60	0.20	33.60	0.20
Weeding	28.30	0.17	28.30	0.17
Pollination	25.20	0.15	25.20	0.15
Termite control	6.95	0.04	6.95	0.04
Harvesting	11.10	0.07	11.10	0.07
Seed processing	10.40	0.06	10.40	0.06
Sub-total	**427.63**	**2.19**	**427.63**	**2.19**
Overhead	29.93	0.18	29.93	0.18
Grand Total	**457.56**	**2.37**	**457.56**	**2.37**

Comparison based on selection cycles showed that total costs of MAS were less than the costs for conventional selection. MAS incurred costs of US$ 1951.27 and US$ 1113.01 in the first and second selection cycles (Table 6. 4), while conventional selection incurred costs of US$ 2322.58 and 1955.60 in the respective selection cycles (Table 6.1). Marker assisted selection costs reduced to about 57% of costs of conventional selection in the second generation of selection.

Table 6.4 Proportion of costs from laboratory and field activities associated with MAS

Procedure	Generation 1	Generation 2	Percentage	Average cost per sample
Laboratory procedure	1493.71	655.45	70	1.75
Field production cost	475.56	475.56	30	0.18
Total	**1951.27**	**1113.01**	**100**	**1.93**

Field cost per plant for MAS was US$ 0.18 (Table 6.5). Total costs of MAS including laboratory procedures and field cost of using one SSR marker was US$ 1.93 per plant (Table 6.4 and 6.5). The largest proportion of the costs came from laboratory procedures.

Comparison of conventional and MAS procedures showed that costs associated with the two procedures were different. The results showed that the cost of field screening one plant for resistance to maize streak virus US$ 0.29, while the cost of growing one plant in the field and analysing it in the laboratory using one SSR marker was US$ 1.93 (Table 6.5). Costs of conventional selection basing on one plant were lower than costs of marker assisted selection.

Table 6.5. Costs per plant associated with MAS and conventional breeding for resistance to MSD in Uganda

	Conventional					Marker assisted selection				
	First selection cycle		Second selection cycle			First selection cycle		Second selection cycle		
Item	Total cost (588 rows)	Cost/plant	Total cost (420 rows)	Cost/plant	Average cost/plant	Field cost per plant	Lab cost per plant	Field cost per plant	Lab cost per plant	Average cost/plant
Total	2322.58	0.26	1955.60	0.31	0.29	0.18	1.86	0.18	1.64	1.93
Total cost per plant per selection cycle						2.04		1.82		

* A row is 5m long; Number of plants per row was 15.

6.7 Discussion

The efficiency of any breeding method is usually measured in terms of genetic gain over time (Fehr, 1987) and relative cost (Ragot and Hoisington, 1993). However, the choice between MAS and conventional methods will involve a trade off between money and time (Morris *et al.*, 2003). This study has shown costs associated with conventional screening and MAS for MSD resistance at two early generations of selections.

Average costs of the conventional method were different for the two cycles of selections. Conventional screening cost increased from US$ 3.96 per row in the first cycle of selection to US$ 4.76 per row in the second selection cycle. This was because in the second cycle of selection, fewer plants were used and yet the costs of transport, technical assistance and others did not change. The cost per row would be smaller if screening involved larger population size where overhead costs are divided by a large area size.

Marker associated costs per cycle were not very different; US$ 2.04 and 1.82 per sample for first and second cycles of selection, respectively. This could be reduced farther if smaller sample size was considered. It is possible to use small sample at one generation of selection. Laboratory reagents costs contributed to 60% of the total costs of laboratory procedures, suggesting that costs could be reduced by bulk purchase of reagents, taking advantage of economies of scale.

Comparison of conventional and MAS methods is a big challenge, especially given that in conventional selection breeders consider cost per rows while in MAS cost are calculated per sample or data point. The difference in methodology or units used does not allow for a good economic comparison between the two methods. However, by standardising the units in both conventional and MAS in terms of costs per plant, clear differences in cost estimates were observed. Marker selection for MSD resistance was showed to be more expensive than conventional selection methods. The cost of conventional selection was US$ 0.29 per plant, while the cost of MAS was US$ 1.93. This showed that MAS was 6.6 times more expensive than conventional method. However, using costs per plant may only be appropriate for economic comparison but in practice some plants established may not be

sampled for analysis or evaluated in the field thereby the cost per plant may be either over or under valued.

Overall MAS for MSD screening has been shown to be more expensive than conventional screening method. To cut down on cost, MAS for disease resistance can be applied at one stage to select plants fixed for the traits and continue with conventional screening for other agronomic characters. This approach involves selecting plants at an early generation with a fixed, favourable genetic background at specific loci, conducting a single large-scale marker-assisted selection, while maintaining as much as possible of the allelic segregation in the rest of the genome (Ribaut and Betran, 1999). This would require a large population for selection. The results of this study implicitly showed that costs depended on the population sizes. Therefore, any change in population size would change the cost of MAS in the same direction. Therefore by using a combination of MAS and conventional approach, in which a small population is used in MAS to help identify the plants fixed for the trait, and at the same time apply phenotypic selection, high selection gains are likely to be realised and, faster.

Other costs could not be computed with precision. For example, labour for technician, temporary labour and field supervision and tractor operations were hired and at a rate different from that of the National Agricultural Research organisation. Since research was done by a student, computing cost for scientist labour as reflected by Dreher *et al.* (2000) was not done. This may not have affected the cost as the scientist labour from MAS and conventional selection would be the same, therefore, the cost of MAS and conventional methods would vary proportionately.

Although costs associated with MAS can be high, conventional genetic improvement programmes can also be expensive (De Koning, cited by FAO, 2004). Various stages in the MAS development and application process were regarded as being costly. Labour and DNA extraction were viewed as representing the major costs (Williams cited by FAO, 2004). However, Collard (cited by FAO, 2004) considered equipment, consumables and infrastructure to be among the most costly items in a MAS programme. Depending on the programmes and institutions, cost of MAS can be higher or lower than cost of conventional selection methods. In this study, laboratory consumables represented the major costs of MAS (60 %). Labour cost, particularly, depends on countries and in Africa labour may still be relatively cheap.

6.8 Conclusion

Costs of MAS and conventional method varied depending on the units for comparison. Considering the total costs of selection for MSD using MAS and conventional methods, costs of conventional methods were higher in both first (US$ 2322.58) and second (US$ 1955.60) selection cycles than the total costs of MAS in first (US$ 1951.27) and second (US$ 1113.01) cycles. Comparing costs per row for conventional and costs per plant or data point for MAS showed that conventional selection was 2.4 times more expensive than costs per sample for MAS. However, by standardising costs per plant to facilitate appropriate economic comparison, MAS was shown to be 6.6 times more expensive than conventional selection. The largest proportion of costs of MAS was from laboratory consumables. Costs of MAS can be reduced by using relative smaller population size for screening.

6.9 References

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Research Overview

Introduction

The study was conducted with the aim of determining an efficient method for breeding for resistance to maize streak virus (MSD) in early generations of selection in Uganda. In the overview, the discussion will fall under two sections as follows:

1. Summary of research findings,
2. Implication of the findings on maize breeding in Uganda, and
3. Recommendations.

Summary of research findings

The research produced some significant outcomes that give us insight into genetic control of MSD resistance, the efficiency of conventional and marker assisted selection methods and the costs associated with the two selection methods. The findings can be summarised as follows:

- Unreliable rainfall and insect pests are the dominant maize production constraints.
- Maize streak virus disease has become the most importance disease constraining maize productivity in Uganda.
- Head smut and ear rots are also emerging as important diseases in Uganda.
- Farmers preferred sweet taste, large kernels and high test density that breeders may not normally consider in varietal selection.
- Farmers are willing to be involved in participatory varietal selection as opposed to the whole process of participatory plant breeding.
- Resistance to maize streak virus is controlled by two systems: one major gene with a major effect, the major gene is under simple Mendelian inheritance. The second system involves minor genes with minor effects and appeared to be quantitatively inherited.
- A positive correlation between severity and incidence indicated a possible non-preference form of resistance was exhibited by the maize plants.
- Higher levels of resistance were observed on lines selected through selfing and partial resistance was observed on lines in backcross populations.

- Choice of source population was critical for conventional selection and not MAS.
- Both MAS and conventional selection methods showed selection gains for resistance to MSD.
- Marker assisted selection and conventional methods were equally effective and efficient in selecting for resistance to maize streak virus.
- No differences in yield were observed due to MAS and conventional methods.
- Testcross performance identified high yielding and stable lines.
- Costs of MAS and conventional selection methods varied depending on the units used in the comparison.

Implications of research findings

Unreliable rainfall and insect pest damage mainly from stalk borers are the most important factors lying behind low yield in farmers' fields. Farmers have no appropriate control technologies for those constraints which mean that yields will continue to be low. In the year of serious drought farmers are likely to loss all their crops because of growing non-drought tolerant cultivars. The low yields may still be reduced if insect pests are not controlled. Unfortunately farmers are growing cultivars susceptible to insects.

Maize streak virus disease has become the most important disease constraint in maize productivity in Eastern Uganda, the major maize growing region of the country. To help farmers in reducing crop losses due to MSD epidemics, all cultivars being released must carry genetic resistance needed to overcome the disease. MSD resistant cultivars are the only economical control method for the resource poor farmers of Uganda. The current status of MSD in the maize producing regions of Uganda also calls for sensitisation of farmers on the available control methods particularly on resistant cultivars which can avoid yield losses due to the disease. The change in the rank of important disease constraints of maize, from gray leaf spot which ranked the most important disease in 2001 (Bigirwa *et al.*, 2001) to MSD are indications that environmental conditions, on which MSD epidemic depend are also changing. Farming practices are also changing with most farmers in Iganga and Sironko growing maize twice a year. This system tends to provide continuous source of inoculum for MSD making cultural control methods ineffective. Breeding for resistance is therefore justified and would be easy to manage and sustain as it is packaged with the seed.

Maize head smut disease and ear rot disease, which previously were not considered important, are also becoming limiting factors of maize production. The outbreaks of these diseases also show that climatic factors are changing in favour of these diseases. It also points to the fact that farmers are growing susceptible cultivars. There has not been any active programme for improving resistance to head smut and ear rot caused by *Sphacelotheca reliana* and *Stenocarpella maydis,* respectively. The National Maize Programme has just begun improving their elite lines for ear rot disease, but not head smut. The continuous change in importance of production constraints puts breeders in a situation where they have to continue to select for resistance to most of the diseases, such that when an epidemic occurs, the appropriate resistant cultivars are provided and farmers do not have to wait for years before resistant cultivars are released.

Farmers' have some special preferences that breeders normally do not select for in their programmes. These preferences are part of the reasons farmers do not easily adopt improved cultivars even if they have important traits like resistance to diseases. These preferences are very diverse posing a big challenge to the breeders. However, by involving farmers in the process of varietal development, breeders can appreciate and comprehend those preferences and include them in their selection. Since farmers have indicated their willingness to participate in varietal selection, this can be a means of ensuring adoption of the cultivars selected by farmers themselves.

A positive correlation observed between severity and incidence of disease indicated a form of non-preference resistance may occur. Other studies have shown that leafhoppers have preferences for plants they attack, an antixenosis-type of resistance (Kairo *et al.*, 1995; Mesfin and Bosque-Perez, 1998). This has not been confirmed in the current study. If, however, non-preference resistance exists for insects, then selection for it would reduce disease spread in the field by preventing the vector from carrying disease from one plant to the other.

In conventional selection the expectation is that F2 results in higher levels of resistance because of the new recombination whereas the BC are limited by the recurrent parents used and more so if backcrossing is done to worse parent. However, when using molecular markers for selection, there are no differences between F2 and BC because in both cases the methods would target the marker(s). This implies that marker assisted selection is very

efficient in selecting superior genotypes regardless of the source population used. This points to the fact that marker assisted selection is more efficient than conventional selection (Yousef and Juvik, 2001, 2002

Marker assisted selection for MSD was slightly less expensive than the conventional selection procedures. These results imply that the use of MAS can be employed if there are no established leafhopper-rearing units or where the environmental conditions do not favour disease development. Where the cost of labour is very high conventional screening, which involves rearing leafhoppers, inoculation of each plant and disease scoring may be more expensive than molecular analysis of few plant samples. In such cases integrated approach using MAS and conventional selection methods will likely to result in more progress. The study has shown the potential of using a small population under MAS and at the same selecting using phenotypic markers. Marker assisted selection on small population should be enhanced by the use of flanking markers.

There was lack of association between molecular data indicating the presence of MSD QTL and the symptom severity of the corresponding plants in the field. This may imply that selection focusing on the major MSD QTL was not adequate and loss of the other minor QTLs detected could go unnoticed. If there was epistasis between the major QTL and other QTLs in the genome, then selecting one without the other could lead to such difference in phenotypes. In addition, symptoms in the field are results of total genetic and environmental variances, which influence the symptom expression even though all plants carried the QTL. Where some few plants showed resistance to MSD without the marker implied that the marker could be separated from the QTL through recombination due to crossing over. In such cases selecting for the presence of marker would select for susceptible. The Recombination is rarer with flanking markers than a single marker. In this study MAS produced higher levels of resistance than conventional selection implying that the marker, umc1917, is a good marker.

Ten high yielding and highly resistant to MSD were identified as potential lines that can eventually be used for hybrid production for farmers' utilization. The lines also showed high stability across three locations. Yield was not selected for in the early generations of selection and accounted for the similarity in yield of lines from MAS and conventional selection methods. The ten lines had yields above the overall mean for all the genotypes

and were highly superior to standard hybrids evaluated. Breeding can be aimed at fixing those lines for their use later in hybrid production.

Recommendations

The problems of unreliable rainfall and insect pest damages call for the use of drought tolerant and insect resistant cultivars. Other methods of control such as irrigation to mitigate the problem of unreliable rainfall or use of chemicals to control insect damage are not economically feasible for the resource-poor farmers. Breeding for drought tolerance and resistance to insects are therefore recommended.

Maize streak virus appeared as the most important disease constraint and yet farmers have no appropriate control. It is recommended that farmers should use MSD resistant cultivars to reduce on losses of their crop. This availability of resistant hybrids should be promoted of and is supported by an effective small-scale seed production system to improve maize production as the technology will compel farmers to buy new seed every.

The study has identified high yielding and MSD resistant lines that can be deployed in hybrid production. These should be advanced through selfing to fix all the loci and then be tested for specific combining ability for their use later in hybrid development.

There were consistent significant positive correlations between MSD incidence and severity which indicated a non-preference form of resistance by the insect. A study should be done to confirm the observation.

In conclusion, the current study showed that both MAS and conventional methods were effective in generating MSD resistant and high yielding lines. However, the level of resistance was slightly higher under MAS than under conventional selection. In terms of cost, MAS was 2.4 times cheaper than conventional selection when costs per sample or data point for MAS were compared with costs per row for conventional method. On the other hand, when the same units were used for comparison, which is cost per plant for both methods; conventional method was shown to be 6.6 times cheaper than MAS. The advantages of MAS in this study should be taken with caution due to many other costs not included in the calculations. Seemingly, a high selection progress would be realised if

breeding is effected on F2 segregating population than BC to recurrent parent under conventional. Thus the choice of source population would be critical for conventional breeding, but not for MAS.

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