Published March, 2009

RESEARCH

# A Comparative Analysis of Conventional and Marker-Assisted Selection Methods in Breeding Maize Streak Virus Resistance in Maize

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**ABSTRACT**

Reliable information regarding comparative advantage of marker-assisted selection (MAS) over conventional selection (CS) in breeding for maize streak virus (MSV) resistance in maize (*Zea mays* L.) is scarcely available. A comparative study was, therefore, conducted to determine the efficiency of both methods in breeding for MSV resistance in Uganda. Backcross and selfed-progenies were derived from inbred lines CML202 (resistant), CML321, and CML384 (susceptible) using MAS and CS. The experimental lines and their testcross progenies were evaluated for MSV resistance and yield across three locations. Although both breeding approaches were effective in generating MSV-resistant lines, disease incidence was higher in populations under CS (79%) than MAS (62%). A similar trend was observed for area under disease progress curve. However, an equal number of lines generated by MAS and CS displayed high yield potential and MVS resistance in testcrosses. Because all required DNA analysis was performed in an existing laboratory and on a well-characterized quantitative trait locus, costs of capital, equipment maintenance, and marker development were excluded in costing the MAS procedure. Considering total running costs, MAS was cheaper than CS by 26%, which was realized by using fewer plants. Therefore, when laboratory facilities are already established MAS would be recommended in breeding for MSV resistance.

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**Abbreviations:** asl, above sea level; AUDPC, area under disease progress curve; CS, conventional selection; MAS, marker-assisted selection; MSV, maize streak virus; PCR, polymerase chain reaction; QTL, quantitative trait locus; SSR, simple sequence repeat.

The use of marker-assisted selection (MAS) for introgression of major quantitative trait loci (QTLs) for disease resistance is increasingly being used in crop improvement. In the last decade, MAS has been used in improvement of elite lines for disease resistance (Reyes-Valdés, 2000; Bouchez et al., 2002; Frisch and Melchinger, 2005). Besides improving genetic gain, MAS is also useful to accelerate breeding process and reduce costs of a breeding program through reduced number of years and breeding population size (Yousef and Juvik, 2001; Thomas, 2003). Marker-assisted selection might have advantages in cases where conventional selection (CS) is difficult (Dreher et al., 2000) and in the absence of stress factors (Chen et al., 2000; Willcox et al., 2002). Marker-assisted backcrossing is also a valuable tool for improving simply inherited traits such as maize streak virus (MSV) resistance in existing varieties (Frisch et al., 1999; Frisch and Melchinger, 2001; Xu and Crouch, 2008).

Conventional selection has been the method of selection over the years, and has produced tangible results and genetic gains. For example, in the case of MSV, many high-yielding maize (*Zea mays*

Published in Crop Sci. 49:509–520 (2009).
doi: 10.2135/cropsci2008.03.0162

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L.) cultivars have been developed and released to farmers in sub-Saharan Africa (Timothy et al., 1988; Barrow, 1992, 1993; Pixley et al., 1997), including Longe1 and Longe4 in Uganda. However, the success of CS in breeding MSV resistance depends on environmental factors because occurrence of MSV is sporadic and unpredictable (Bosque-Perez, 2000). To enhance natural virus infection, additional and expensive resources are required for rearing leafhoppers (*Cicadulina mbila* Naudé) that act as vector for MSV, and inoculating each plant or establishing spreader rows in the nurseries. Besides, the disease might not be uniformly distributed; for natural virus enhancement through spreader rows, disease might not develop early in the season to allow effective screening. In such a case, MAS would be ideal and reliable to facilitate selection.

There is limited work done concerning comparison of costs of MAS and CS methods in breeding for MSV resistance in sub-Saharan Africa. The few papers that have addressed these aspects in breeding other traits have considered different parameters and breeding methods in their analyses. For example, Dreher et al. (2003) and Morris et al. (2003) compared costs of MAS vs. CS in breeding quality protein maize, which consists of chemical analysis for tryptophan and lysine. Yu et al. (2000) compared costs of CS for bacterial blight resistance in the 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 CS 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 sizes, and at different stages of selection.

The costs of MAS have been shown to be much lower than those of CS depending on the choice of markers. The estimated costs for using sequence characterized amplified regions and random amplified polymorphic DNA markers to analyze 100 bean (*Phaseolus vulgaris* L.) lines were estimated at Can$3.13 and Can$3.48 per data point, respectively, after the markers were developed (Yu et al., 2000). In contrast, conventional greenhouse screening was estimated at Can$5.88 per data point. The cost of using polymerase chain reaction (PCR)–based markers for selection was estimated at US$1.14 per data point (Ragot and Hoisington, 1993), while a much lower cost of MAS at <$0.40 sample$^{-1}$ was estimated (Gu et al., 1995). With respect to their particular conditions, they concluded that MAS was more cost-effective than CS. Dreher et al. (2000), however, concluded that the costs of MAS were variable depending on the circumstances. Dekkers and Hospital (2002) have recently reviewed some of the potential limitations of MAS applications and concluded that the use of MAS will be determined by the economic benefit relative to CS.

Conventional selection for MSV, the most important disease of maize in Africa, is normally best done under artificial inoculation of plants with viruliferous leafhoppers (*C. mbila*). At Namulonge Research Station in Uganda, a leafhopper rearing unit was not in place until 2006. An alternative way to increase leafhopper population during field screening is the use of spreader rows. With artificial inoculation or spreader rows, MSV infections were low at the beginning of the season (Caulfield, 1997). Moreover, such techniques are laborious and increase costs of field screening. Accurate selection is also complicated because development of symptoms depended on time of infection (Bosque-Pérez et al., 1998). Marker-assisted selection does not rely on symptoms or field conditions.

There is no published information on comparison of MAS and CS for MSV resistance using both backcrossing and pedigree selection methods. Where comparisons of MAS and CS have been made for other traits, the methods compared MAS with conventional backcrossing only. In most cases, the comparison has been based on theory rather than practice. In Uganda, this is the first practical study done on MSV. The objective of the study was to determine if costs associated with MAS can warrant its use for screening for resistance to MSV and to determine whether the two methods would be equally effective in generating high-yielding and MSV-resistant maize inbred lines.

## MATERIALS AND METHODS

### Germplasm and Generations

Two crosses were made between maize inbred lines CML321 (susceptible) and CML202 (resistant) and CML384 (susceptible) and CML202 to generate $F_1$ hybrids, CML321 × CML202 and CML384 × CML202, respectively. Their $F_1$ generations were backcrossed to the recurrent parents (CML321 and CML384) and also self-pollinated to generate both $BC_1F_1$ and $F_2$ progenies for each of the two crosses, respectively, resulting in four populations. These were further advanced to $BC_3F_1$ and $F_4$ generations, respectively, under both MAS and CS (Table 1).

### Conventional Selection

For CS, each of the four populations was planted in screening nurseries. In the first generation of selection ($BC_1F_1$ and $F_2$), each population was planted in three blocks, made up of 42 rows each with 15 plants row$^{-1}$. Two bordering blocks were planted with a susceptible hybrid to enhance natural virus infection (i.e., served as spreader rows). Pedigree selection was applied to generate $BC_2F_1$ and $F_3$ progenies, which were further advanced to $BC_3F_1$ and $F_4$. In each cycle, only plants with severity scores of 1 and 2, indicating high MSV resistance, were selected. Plants with a rating of 0 were not selected because plant immunity could not be separated from disease escape. A selection intensity of 10% was then achieved by discarding plants with undesirable agronomic traits. Disease incidence (%) was recorded and severity was rated (Bosque-Perez and Alam, 1992) as follows: 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% leaf area affected) with no yield.

## Marker-Assisted Selection

The major QTL *msv-1*, conditioning resistance to MSV disease, was targeted for transfer into susceptible backgrounds using MAS. The *Msv-1* is a well-characterized QTL in the genetic background of CML202 (Welz et al., 1998; Pernet et al., 1999a, 1999b). The *Msv-1* is found on the short arm of chromosome 1 within bin 1.04 and explains between 43 and 67% of the total phenotypic variation for MSV resistance (Pernet et al., 1999b). One simple sequence repeat (SSR) marker, *umc*1917, was employed in all the genotyping procedures. This was selected from a panel of 32 SSR primer pairs recommended by CIMMYT's Applied Biotechnology Centre because it was found to be consistently polymorphic and codominant. The *umc*1917 was the only marker that was very consistently polymorphic and codominant, and fortunately it amplifies within the region of the QTL in bin 1.04.

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 of 5-m length and 0.3-m plant spacing within rows. Plant genotyping was done on 200 plants for each population using *umc*1917 marker at $F_2$ and $F_3$ generations, and at $BC_1F_1$ and $BC_2F_1$ generations. Representative leaf samples were picked from 10 plants for each genotype at 4 wk from planting. Genomic DNA was extracted using cetyltri-methylammonium bromide protocol adopted from CIMMYT laboratory protocol (CIMMYT, 2003). A touch-down PCR procedure was adopted with annealing temperatures of 64 to 54°C. The PCR products were separated in 4% (m/v) MetaPhor agarose gels (3 MetaPhor:1 Seakam agarose; Cambrex, Charles City, IA) and 1× TBE (8.9 mM Tris-base, 88.95 mM boric acid, and 2.85 mM EDTA, pH 8.3) buffer at 120 V for 2 h, and visualized under ultraviolet following ethidium bromide staining of gels. For each population the numbers of homozygous dominant, heterozygous, and homozygous recessive individuals were tallied. Selection for advancement was done in two stages: first, all the plants homozygous or heterozygous for the QTL were selected, and then the number of plants selected was reduced based on agronomic traits to achieve a 10% selection intensity for each population.

**Table 1. Means of maize streak virus disease (MSD) incidences and standardized area under disease progress curve (AUDPC) values for MSD severity on 24 maize lines derived by conventional and marker-assisted selection (MAS) methods in Uganda.**

| Lines within population | Population | Breeding method | MSD incidence | Standardized AUDPC for MSD severity |
|---|---|---|---|---|
| | | | % | |
| 1 | $BC_3F_1$ of CML321 × CML202 (Population 1) | MAS with backcrossing | 83.3 | 2.4 |
| 2 | | | 70.8 | 1.5 |
| 3 | | | 29.2 | 1.2 |
| 4 | $BC_3F_1$ of CML384 × CML202 (Population 2) | MAS with backcrossing | 65.9 | 1.4 |
| 5 | | | 60.4 | 1.6 |
| 6 | | | 33.3 | 1.2 |
| 7 | $F_4$ of CML321 × CML202 (Population 3) | MAS with selfing | 52.1 | 1.3 |
| 8 | | | 85.4 | 1.9 |
| 9 | | | 91.7 | 2.3 |
| 10 | $F_4$ of CML384 × CML202 (Population 4) | MAS with selfing | 97.9 | 2.7 |
| 11 | | | 35.4 | 1.3 |
| 12 | | | 50.0 | 1.5 |
| 13 | $BC_3F_1$ of CML321 × CML202 (Population 5) | Conventional backcrossing | 95.2 | 2.3 |
| 14 | | | 68.8 | 2.4 |
| 15 | | | 89.6 | 2.3 |
| 16 | $BC_3F_1$ of CML384 × CML202 (Population 6) | Conventional backcrossing | 75.0 | 2.0 |
| 17 | | | 75.0 | 1.9 |
| 18 | | | 89.6 | 2.2 |
| 19 | $F_4$ of CML321 × CML202 (Population 7) | Conventional selfing | 54.2 | 1.4 |
| 20 | | | 52.1 | 1.4 |
| 21 | | | 56.8 | 2.0 |
| 22 | $F_4$ of CML384 × CML202 (Population 8) | Conventional selfing | 100.0 | 2.2 |
| 23 | | | 97.9 | 2.3 |
| 24 | | | 97.9 | 2.6 |
| CML202 | Resistant check | Inbred line | 37.5 | 0.7 |
| CML321 | Susceptible check | Inbred line | 100 | 3.2 |
| CML384 | Susceptible check | Inbred line | 100 | 3.0 |
| Mean | | | 72.00 | 1.93 |
| *P* | | | *** | *** |
| SED | | | 4.57 | 0.51 |
| $LSD_{0.05}$ | | | 9.17 | 1.03 |
| CV (%) | | | 32.10 | 32.60 |

***Significant at 0.001 probability level.

## Evaluation of Lines for MSV Resistance Per Se

To compare the relative efficiency of MAS and CS, lines at $BC_3F_1$ and $F_4$ generations were drawn from the eight populations basing on resistance to MSV and their agronomic qualities. Three lines were selected from each of the four breeding populations in MAS method and three other lines from the corresponding populations under CS approach. The resistant and the two susceptible parents were included as checks, giving a total of 27 lines. For the ease of discussion, the eight populations and the lines were designated Populations 1 through 8 and Lines 1 through 24, respectively (Table 1). At the seedling stage, the lines were artificially inoculated with leafhoppers as described by Bosque-Perez and Alam (1992).

## Evaluation of Lines in Testcrosses

To evaluate the performance of lines from different selection methods, Lines 1 to 24 (Table 1) were top-crossed to two single cross testers, CML442 × CML312 (Tester A) and CML444 × CML395 (Tester B), used at CIMMYT's midaltitude station in Zimbabwe. The tester CML442 × CML312 is intermediate maturity, while CML444 × CML395 is late maturing. Both testers are widely used in tropical and midaltitude maize programs in east and southern Africa. Because of drought, some of the crosses generated few seed, which were not adequate for the field evaluation trials. Twenty-four crosses (14 lines × Tester A

and 10 lines × Tester B) yielded enough seeds for evaluation at Namulonge (1150 m above sea level [asl]), Masaka (1250 m asl), and Iganga (1081 m asl) in tropical Uganda. Six standard cultivars comprising a susceptible check Hybrid1, two resistant open-pollinated cultivars (OPV1 and OPV4), and three popular hybrids (Hybrid2, Hybrid3, and Hybrid4) were included. Experiments were laid out as randomized complete block designs with two replications. Plants were spaced at 0.75 and 0.50 m between and within rows, respectively. The susceptible check Hybrid1 was also planted in border plots. Fertilizer was applied at the rate of 30 kg P $ha^{-1}$ and 45 kg N $ha^{-1}$ at all three locations. Standard cultural practices such as hand-weeding were followed to keep the fields clean of weeds. Diseases were monitored and data were collected on MSV at weekly intervals for 6 wk. Grain yield (t $ha^{-1}$) was determined immediately after harvest and adjusted to 15% moisture content.

## Data Analysis

Data from artificially inoculated lines collected at several dates were used to calculate area under disease progress curves (AUDPCs) (Campbell and Madden, 1990). The AUDPC was standardized by dividing the data by the total time of the epidemics, and was subjected to ANOVA in GenStat (Payne et al., 2007). 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 the testcrosses were also subjected to ANOVA in GenStat. Standardized AUDPC values were computed for MSV severity over 6 wk. The AUDPC data were transformed using inverse logit as follows: Inverse logit = $c/[1 + \exp(-x)]$, where $x$ is the data value and $c$ is a constant at a specified value of 10. The final results presented are in untransformed format. Yield and AUDPC data were subjected to additive main effects and multiplicative interactions analysis to determine genotype × environment interaction effects. Spearman's rank correlation analysis was also applied on yield data. 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 AUDPC data. A line × tester analysis to establish combining ability effects of the lines was not performed because some crosses did not yield adequate seeds for field evaluation; hence, data were not balanced.

## Analysis of Cost of MAS and CS at Two Early Generations of Selection

Field costs for CS were computed from a total of 588 breeding rows in the first selection cycle and from 420 rows in the second cycle of selection, while field cost of MAS was computed from 168 breeding rows in each selection cycle. Costs of laboratory procedures were calculated from 800 plant samples (200 plants $population^{-1}$) in the first MAS and 400 plant samples (100 plants $population^{-1}$) in the second round of MAS.

Costs of field operations performed during the two cycles of selection were computed from payment vouchers. Prices of laboratory consumables and chemical inputs used in field operations for both MAS and CS were obtained from receipts and supplier quotations (pro forma invoices). Capital costs were not included because all the research was done in an existing fully functional biotechnology laboratory at Makerere University in Uganda. Overhead cost (bench fee) of 7% was used, based on the rate at Makerere University, where the laboratory work was conducted. Reagents such as *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 materials used for each sample in PCR amplification. Technician labor cost was determined from their monthly salary rates. The following assumptions and considerations were made during cost computation for both MAS and CS:

Suitable markers were already available;
The analysis was done in an established laboratory, so costs of capital equipment were not included;
Scientists were paid their fixed monthly salaries at the rate of government institutions in Uganda;
Students were paid fixed monthly stipend and no additional payment for extra time spent in the field or laboratory;
Foreign currency exchange rate of 1800 Uganda shillings to US$1.00 was used.

For practical purposes, the same number of plants could not be used in MAS and CS. The CS would require more plants for effective selection, whereas fewer plants are used in MAS. Therefore, the cost per plant was calculated and used to facilitate fair comparison of costs associated with each method.

# RESULTS

## MSV Resistance of Lines Per Se

There was significant ($P < 0.001$) variation in percentage incidence of MSV between populations (Table 1). Significant variation was also observed among lines across populations and among lines within each population, and the disease incidence on all lines evaluated ranged from 29.2 to 100% (Table 1). Three out of 24 lines evaluated had disease incidence levels lower than that of CML202 (resistant check). Percentage of incidences varied on lines within populations. In $BC_3F_1$ (of CML321 × CML202) Population 1 from MAS, the best line had the lowest incidence (29.2%) and the worst line had the highest incidence of 83.3%. In the corresponding $BC_3F_1$ of CML321 × CML202 (Population 5) from CS, the best line had incidence of 68.8% and the worst line had incidence of 95.2%. Lines from $F_4$ of CML384 × CML202 with MAS and CS showed incidences ranging from 35.4 to 97.9% and from 97.9 to 100%, respectively.

Significant variation for AUDPC was observed within and across populations. The AUDPC data for the experimental lines ranged from 1.2 to 2.7. The smallest AUDPC was observed for the resistant check CML202, while the susceptible checks CML321 and CML384 displayed the largest AUDPC (Tables 1 and 2). For populations from MAS, AUDPC variation on lines within $BC_3F_1$ progenies of CML321 × CML202 (Population 1) was from 1.2 in the best line to 2.4 in the worst line. In $F_4$ progenies of

CML384 × CML202 (Population 4), the lines had AUDPC ranging from 1.3 in the best line to 2.7 in the worst line. $BC_3F_1$ progenies of CML384 × CML202 (Population 2) showed the least variation in AUDPC among the populations from MAS. Lines from $F_4$ progenies of CML321 × CML202 (Population 7) from CS showed the highest variations in AUDPC values. The best lines from Population 7 had AUDPC value of 1.4 and the worst lines had AUDPC value of 2.0.

The highest incidences were recorded in $F_4$ progenies of CML384 × CML202 (Population 8) followed by $BC_3F_1$ progenies of CML321 × CML202 (Population 5) (Table 2). The lowest disease incidences were 53.2 and 54.3%, recorded on $BC_3F_1$ progenies of CML384 × CML202 (Population 2) and on $F_4$ progenies of CML321 × CML202 (Population 7), respectively. The average incidence was higher in populations under CS (79.3%) than in those under MAS (62.9%). Higher variation was 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 2).

Average AUDPC for populations from MAS ranged from 1.4 to 1.8. Marker-assisted selection with backcrossing and selfing showed similar mean AUDPC of 1.5 and 1.8, respectively, while a range of 1.6 to 2.4 was observed for AUDPC on populations under CS (Table 2). The AUDPC for populations from MAS were lower than those from corresponding populations under CS methods. There was relatively less variation for AUDPC on lines within populations from CS methods than variation on lines from populations from MAS. The standard deviations for AUDPC for lines within populations from MAS varied, ranging from 0.2 to 0.8, while the standard deviations within populations from CS ranged from 0.1 to 0.3. There was a strong positive phenotypic correlation between disease incidence and severity ($r = 0.732$; $P \leq 0.001$).

## Time-Course Analysis of MSV Incidence and Severity

Analysis of MSV incidence against time showed that there was significant variation for disease incidence between populations ($P < 0.001$). Time-course of disease incidences and disease symptom development showed differences between populations from MAS and CS methods. Disease assessment at 1 wk after inoculation showed no symptoms. From the fourth week after inoculation, disease incidence remained constant (Fig. 1A and B). Symptom severity of MSV also varied significantly among the different populations ($P < 0.01$). There was a general increase in severity over time (Fig. 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 × time nor severity × time interaction effects were observed ($P > 0.05$).

## Testcross Evaluation for MSV Resistance and Yield

Analysis of variance for AUDPC for crosses showed that there were significant differences between crosses ($P = 0.021$) and also between locations ($P = 0.001$). There was no significant crosses × location interaction effects ($P > 0.05$) for AUDPC. The AUDPC was generally low and ranged from 0.6 to 2.1 among the crosses (Table 3). All crosses showed lower AUDPC than the susceptible check (AUDPC = 3.2). Except for the resistant check Hybrid2, which had AUDPC value of 0.7, 13 crosses had higher resistance to MSV than the other standard hybrids. Eight out of the best 13 crosses involved lines generated by MAS (Table 3).

Analysis of variance for yield results show that there were significant differences among the crosses ($P < 0.001$)

**Table 2. Pooled means of maize streak virus disease (MSD) incidence and area under disease progress curve (AUDPC) for maize lines within populations derived by conventional and marker-assisted selection (MAS) methods in Uganda.**

| Population | Method of selection | Lines | MSD severity | | MSD incidence | |
|---|---|---|---|---|---|---|
| | | | AUDPC | SD | % | SD |
| Population 1 ($BC_3F_1$ of CML321 × CML202) | MAS with backcrossing | 1, 2, 3 | 1.7 | 0.6 | 61.1 | 28.3 |
| Population 2 ($BC_3F1$ of CML384 × CML202) | MAS with backcrossing | 4, 5, 6 | 1.4 | 0.2 | 53.2 | 17.5 |
| Population 3 ($F_4$ of CML321 × CML202) | MAS with selfing | 7, 8, 9 | 1.8 | 0.5 | 76.4 | 21.3 |
| Population 4 ($F_4$ of CML384 × CML202) | MAS with selfing | 10, 11, 12 | 1.8 | 0.8 | 61.1 | 32.7 |
| Population 5 ($BC_3F_1$ of CML321 × CML202) | Conventional backcrossing | 13, 14, 15 | 2.3 | 0.1 | 84.5 | 13.9 |
| Population 6 ($BC_3F_1$ of CML384 × CML202) | Conventional backcrossing | 16, 17, 18 | 2.4 | 0.2 | 79.6 | 8.4 |
| Population 7 ($F_4$ of CML321 × CML202) | Conventional selfing | 19, 20, 21 | 1.6 | 0.3 | 54.3 | 2.4 |
| Population 8 ($F_4$ of CML384 × CML202) | Conventional selfing | 22, 23, 24 | 2.0 | 0.2 | 98.9 | 1.2 |
| CML202 (resistant check) | | | 0.7 | | 37.5 | |
| CML321 (susceptible check) | | | 3.2 | | 100 | |
| CML384 (susceptible check) | | | 3.0 | | 100 | |
| *P* | | | *** | | *** | |
| Mean | | | 1.9 | | 72.0 | |
| SED | | | 0.3 | | 10.9 | |
| $LSD_{0.05}$ | | | 0.6 | | 21.9 | |
| CV (%) | | | 32.6 | | 32.1 | |

***Significant at 0.001 probability level.

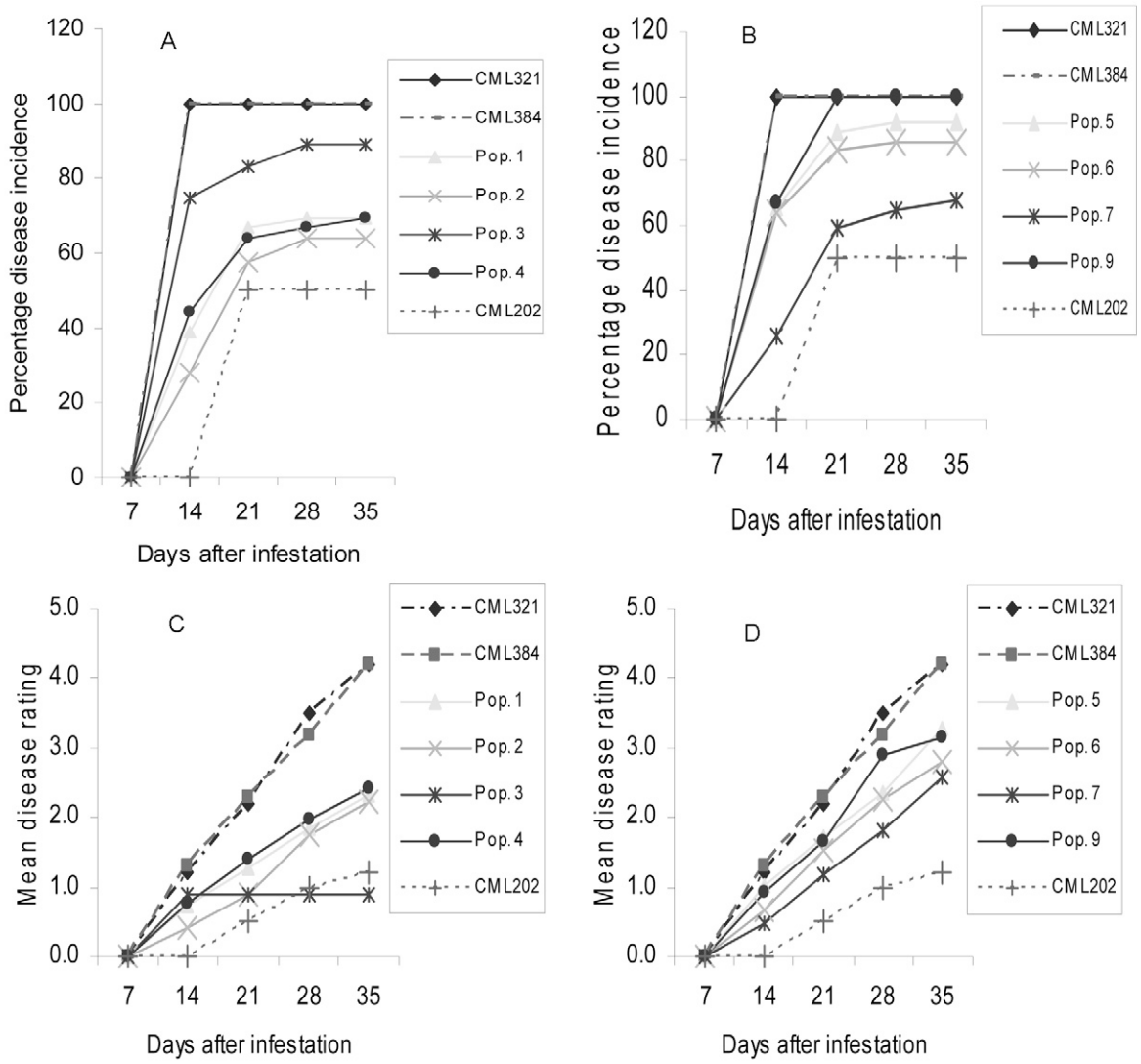


Figure 1. Percentage of maize streak virus disease (MSD) incidence in maize in Uganda on (A) Populations (Pop.) 1, 2, 3, and 4 from marker-assisted selection (MAS) and on (B) Populations 5, 6, 7, and 8 from conventional selection methods. Time-course of MSD severity in maize on (C) Populations 1, 2, 3, and 4 from MAS and on (D) Populations 5, 6, 7, and 8 from conventional selection methods.

and locations ($P < 0.001$), but the crosses × location interaction effects were not significant ($P > 0.05$). Because there were no significant interactions between crosses and locations, only the pooled mean yields for crosses across locations are presented (Table 3). Mean yields over three locations ranged from 3.92 to 9.24 t ha$^{-1}$. Five crosses performed better than the best standard (Hybrid3). The best standard yielded 7.56 t ha$^{-1}$ and the best five crosses had yields which ranged from 8.01 to 9.24 t ha$^{-1}$. Ten crosses had mean yields above the overall mean for all the crosses evaluated. Five of those crosses involved lines generated by MAS and the other five were from lines generated by CS. For the lines crossed with Tester A, line 22 showed the highest yield, followed by lines 17 and 9. With respect to Tester B, line 12 showed the highest performance, followed by lines 17 and 1. In general, higher yields were observed for crosses with Tester A (5.07–9.24 t ha$^{-1}$) than with Tester B (3.92–8.91 t ha$^{-1}$). Spearman's rank correlation coefficient for yield across locations was positive and significant ($r = 0.687$; $P = 0.001$).

## Analysis of Cost of MAS vs. CS

Results show that cost of CS for MSV during the first and second cycles of selection was 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 4). Costs per row of CS were about US$3.96 and US$4.66 for first and second cycles of selection, respectively. Costs incurred in the first and second cycles of laboratory procedures without field cost were US$1541.75 and US$678.34 (Table 5). Cost of molecular analysis using one SSR marker without the cost of field activities was US$1.82 per plant sample. Among laboratory requirements for molecular analysis, reagent costs contributed the largest proportion (60.86%) of the total laboratory procedure costs. The cost of field activities of MAS

Table 3. Mean yield and area under disease progress curve (AUDPC) of maize streak virus disease severity of 30 experimental crosses of maize lines derived by conventional and marker-assisted selection over three locations in Uganda.

| Crosses | Population | Breeding method | Yield† | AUDPC‡ | Rank mean | Rank SD |
|---|---|---|---|---|---|---|
| | | | t ha$^{-1}$ | | | |
| 13 × Tester A | $BC_3F_1$ of CML321 × CML202 | Conventional | 5.07 | 1.6 | 10 | 8.1 |
| 14 × Tester A | $BC_3F_1$ of CML321 × CML202 | Conventional | 6.11 | 0.6 | 14 | 9.5 |
| 15 × Tester A | $BC_3F_1$ of CML321 × CML202 | Conventional | 6.41 | 2.1 | 27 | 1.7 |
| 17 × Tester A | $BC_3F_1$ of CML384 × CML202 | Conventional | 8.54 | 1.0 | 23 | 8.4 |
| 18 × Tester A | $BC_3F_1$ of CML384 × CML202 | Conventional | 5.67 | 1.1 | 13 | 7.5 |
| 20 × Tester A | $F_4$ of CML321 × CML202 | Conventional | 6.20 | 1.0 | 12 | 6.7 |
| 21 × Tester A | $F_4$ of CML321 × CML202 | Conventional | 7.56 | 1.1 | 24 | 5.3 |
| 22 × Tester A | $F_4$ of CML384 × CML202 | Conventional | 9.24 | 1.8 | 28 | 1.5 |
| 23 × Tester A | $F_4$ of CML384 × CML202 | Conventional | 6.26 | 0.8 | 15 | 5.2 |
| 13 × Tester B | $BC_3F_1$ of CML321 × CML202 | Conventional | 6.48 | 1.1 | 13 | 8.1 |
| 14 × Tester B | $BC_3F_1$ of CML321 × CML202 | Conventional | 6.88 | 0.8 | 12 | 7.6 |
| 17 × Tester B | $BC_3F_1$ of CML384 × CML202 | Conventional | 8.01 | 2.0 | 19 | 6.8 |
| 2 × Tester A | $BC_3F_1$ of CML321 × CML202 | MAS | 5.53 | 1.6 | 9 | 5.5 |
| 3 × Tester A | $BC_3F_1$ of CML321 × CML202 | MAS | 6.54 | 0.7 | 14 | 10.1 |
| 5 × Tester A | $BC_3F_1$ of CML384 × CML202 | MAS | 6.05 | 1.2 | 13 | 4.9 |
| 6 × Tester A | $BC_3F_1$ of CML384 × CML202 | MAS | 6.69 | 1.0 | 18 | 11.3 |
| 8 × Tester A | $F_4$ of CML321 × CML202 | MAS | 6.40 | 1.9 | 14 | 7.6 |
| 9 × Tester A | $F_4$ of CML321 × CML202 | MAS | 8.15 | 0.9 | 22 | 11.6 |
| 1 × Tester B | $BC_3F_1$ of CML321 × CML202 | MAS | 7.46 | 0.8 | 22 | 7.0 |
| 12 × Tester B | $F_4$ of CML384 × CML202 | MAS | 8.91 | 2.1 | 24 | 6.5 |
| 3 × Tester B | $BC_3F_1$ of CML321 × CML202 | MAS | 5.35 | 1.7 | 8 | 7.4 |
| 4 × Tester B | $BC_3F_1$ of CML384 × CML202 | MAS | 3.92 | 0.8 | 1 | 0.6 |
| 6 × Tester B | $BC_3F_1$ of CML384 × CML202 | MAS | 6.15 | 1.8 | 14 | 7.8 |
| 8 × Tester B | $F_4$ of CML321 × CML202 | MAS | 5.37 | 1.8 | 9 | 3.8 |
| Hybrid2 | Resistant check | | 5.87 | 0.7 | 12 | 6.2 |
| Hybrid3 | Popular check | | 7.56 | 1.2 | 23 | 4.5 |
| Hybrid1 | Susceptible check | | 6.93 | 3.2 | 19 | 3.6 |
| Mean | | | 6.51 | 1.4 | | |
| $LSD_{0.05}$ | | | 2.21 | 1.1 | | |
| CV (%) | | | 29.60 | 19.8 | | |

†Data significant at $P < 0.001$.

‡Data significant at $P < 0.05$.

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 (Table 6). Comparison based on selection cycles showed that total costs of MAS were less than the costs for CS. The MAS incurred costs of US$1999.31 and US$1135.90 in the first and second selection cycles, while CS incurred costs of US$2322.58 and US$1955.60 in the respective selection cycles (Table 7). Total cost of MAS (field plus laboratory costs) was US$2.00 plant$^{-1}$, with the largest proportion of costs incurred during laboratory procedures (Table 7). The results showed that the cost of one plant under CS for MSV was US$0.29; thus, on a single-plant basis costs of CS were significantly lower than costs of MAS (Table 7). However, the overall cost over the two breeding cycles for MAS was US$3135.21 (total of 336 breeding rows) while that of CS was US$4278.18 (with a total of 1008 breeding rows), indicating that MAS was cheaper than CS by US$1142.97.

# DISCUSSION

## Comparison of Performance of Lines Generated by MAS vs. CS

When comparing resistance to MSV 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 minimized and, hence, the low incidences of disease observed were attributed to resistance. This view is also

**Table 4. Field costs for two generations of conventional selection for maize streak virus disease resistance in maize in Uganda.**

| Item | First generation | | Second generation | | Mean row$^{-1}$ |
|---|---|---|---|---|---|
| | Cost for 588 rows | Cost row$^{-1}$† | Cost for 420 rows | Cost row$^{-1}$ | |
| | US$ | | | | |
| Chemicals | | | | | |
| Fertilizer | 62.52 | 0.11 | 41.68 | 0.10 | 0.10 |
| Pesticides | 75.24 | 0.13 | 50.16 | 0.12 | 0.12 |
| 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 | 0.19 | 0.20 |
| Harvesting bags | 17.70 | 0.03 | 11.80 | 0.03 | 0.03 |
| Seed packets | 49.98 | 0.09 | 33.32 | 0.08 | 0.08 |
| Other supplies | 52.50 | 0.09 | 35.00 | 0.08 | 0.09 |
| Labor | | | | | |
| 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 labor | | | | | |
| Spreader rows labor | 61.32 | 0.10 | 40.88 | 0.10 | 0.10 |
| Planting | 120.00 | 0.20 | 80.00 | 0.19 | 0.20 |
| Weeding | 101.82 | 0.17 | 67.88 | 0.16 | 0.17 |
| Pollination | 196.98 | 0.34 | 131.32 | 0.31 | 0.33 |
| Termite control | 25.02 | 0.04 | 16.68 | 0.04 | 0.04 |
| Harvesting | 79.98 | 0.14 | 53.32 | 0.13 | 0.13 |
| Seed processing | 37.50 | 0.06 | 25.00 | 0.06 | 0.06 |
| Subtotal | 2170.64 | 3.69 | 1827.66 | 4.35 | 4.02 |
| Overhead | 151.94 | 0.27 | 127.94 | 0.31 | 0.29 |
| Grand total | 2322.58 | 3.95 | 1955.60 | 4.66 | 4.31 |

†5-m-long rows with plants at spacing of 0.3 m within rows.

supported by the highly significant correlation between disease incidence and severity.

Among all populations, disease symptoms progressed at slower rate and the final percentage of infection was also lower than that of the susceptible checks. A corresponding lower percentage of incidence was also observed on the populations than on the susceptible checks. This confirmed that 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 among lines within population were less in populations from CS than in populations from MAS. The variations observed between lines among the MAS populations could be attributed to the fact that MAS selected for lines with fixed QTL for resistance and those that were probably still heterozygous for the QTL. In general, MSV incidence and severity were lower in lines drawn from MAS than those from CS methods. The results showed that MAS was more efficient than CS in selecting for resistance to MSV. These findings are in agreement with reports that MAS is more efficient than CS in breeding for MSV resistance (Huang et al., 1997; Chen et al., 2000; Yousef and Juvik, 2001). However, low resistance in populations from CS methods might have resulted because of the elimination of 0 scores because of possible confounding with escapes. A high positive correlation coefficient ($r = 0.732$; $P < 0.001$) between MSV incidence and severity was observed, suggesting a form of resistance probably by nonpreference. It has been reported that low incidence is associated with resistance due to nonpreference by the leafhopper (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 to the nonpreference.

Resistance to MSV 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 extremely susceptible materials can be achieved faster with selfing. Selections through selfing have produced lines with resistance levels higher than those of the donor parents (Pixley et al., 1997). The study indicated that higher selection gain is realized when lines are derived from a segregating $F_2$ population than from a population backcrossed to the inferior parent under CS. In contrast, results show that the efficiency of MAS was the same in MAS with backcross and MAS with selfing. All lines from the MAS method were selected for the presence of a major QTL that is partially dominant and accounts for 45% of the phenotypic variations (Welz et al., 1998; Pernet et al., 1999a).

The testcrosses showed consistency in their expression of MSV resistance across locations, indicating stable resistance, which is consistent with findings by Flett et al. (1997). Their field evaluation of hybrids over six seasons showed that the relative resistance of the hybrids was stable. However, our results contradict findings by Dintinger et al. (1997) who reported significant genotype × environment interaction effects for MSV resistance. Results also show that neither incidence nor disease severity affected yield of testcrosses because correlations of yield with both disease incidence ($r = -0.0895$; $P = 0.7462$) and severity ($r = 0.0868$; $P = 0.8132$) were not significant. This was because the lines were fairly resistant and because of the partial dominant nature of resistance, the crosses were also resistant to MSV.

Yields of the testcrosses differed significantly but no significant cross × location interaction effects were observed, suggesting that hybrids were generally stable across the locations. The environment main effect was largely significant, with average yields of 7.21, 5.37, and

Table 5. Laboratory costs for two generations of simple sequence repeat marker-assisted selection for maize streak virus disease resistance in maize in Uganda.

| Components | First cycle | Second cycle | Two cycles | Cost per data point |
|---|---|---|---|---|
| | —— US$ —— | | % | US$ |
| Reagents | 944.09 | 406.97 | 60.86 | 1.09 |
| Liquid nitrogen | 43.75 | 18.75 | 2.82 | 0.05 |
| Extraction buffer | 42.00 | 20.00 | 2.79 | 0.05 |
| *Taq* polymerase | 280.00 | 120.00 | 18.02 | 0.33 |
| dNTPs | 37.80 | 16.20 | 2.43 | 0.04 |
| Primer(s) | 4.20 | 1.80 | 0.27 | 0.00 |
| Metaphor agarose | 462.88 | 198.38 | 29.79 | 0.54 |
| Seakam agarose | 53.76 | 23.04 | 3.46 | 0.06 |
| Ethidium | 2.90 | 1.40 | 0.19 | 0.003 |
| DNA ladder | 16.80 | 7.20 | 1.08 | 0.02 |
| Supplies | 296.80 | 127.20 | 19.10 | 0.35 |
| Pipette tips | 179.90 | 77.10 | 11.58 | 0.21 |
| Eppendorf tubes | 77.00 | 33.00 | 4.95 | 0.09 |
| PCR† tubes | 39.90 | 17.10 | 2.57 | 0.05 |
| Technical assistance (labor) | 200.00 | 100.00 | 13.51 | 0.25 |
| Subtotal | 1440.89 | 633.97 | 93.46 | 1.71 |
| Overhead | 100.86 | 44.37 | 6.54 | 0.11 |
| Grand total | 1541.75 | 678.34 | 100.00 | 1.82 |

†PCR, polymerase chain reaction.

Table 6. Field costs for two generations of simple sequence repeat marker-assisted selection for maize streak virus disease resistance in maize in Uganda.

| Item | First generation | | Second generation | |
|---|---|---|---|---|
| | Cost for 168 rows† | Cost row$^{-1}$ | Cost for 168 rows | Cost row$^{-1}$ |
| | —— US$ —— | | | |
| 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 |
| Labor | 47.28 | 0.28 | 47.28 | 0.28 |
| Technical assistance | 27.78 | 0.17 | 20.85 | 0.17 |
| Field supervisor | 12.50 | 0.07 | 20.85 | 0.07 |
| Tractor operations | 7.00 | 0.04 | 7.00 | 0.04 |
| Field activities labor | 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 |
| Subtotal | 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 |

†5-m-long rows with plants at spacing of 0.3 m within rows.

6.94 t ha$^{-1}$ at Namulonge, Masaka, and Iganga, respectively, possibly due to differences in the levels of disease pressure and altitude of the sites (data not shown). Namulonge, Iganga, and Masaka also differ in soil fertility, rainfall amount, and distribution, and the Masaka site is characterized by acidic soils.

Most lines from CS combined high yield and high stability as indicated by high-ranking values and low standard deviations across the locations, confirming their stability. However, it was apparent that lines from MAS showed more variable performance than lines under CS as indicated by high values of standard deviation. During CS, plants are also indirectly selected for their adaptation to the environment, while MAS methods are conducted independent of the environmental effects because selection is solely based on the presence of the marker. The performance of lines from MAS should be confirmed by planting them in environments of their potential deployment. This suggests that the MAS method could be improved if it is integrated with field evaluations so that adaptable lines are identified.

Evaluation of testcross progenies identified potential lines that can eventually be used for hybrid production in Uganda. 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$^{-1}$, and five of these testcrosses involved lines from MAS and the other five were from CS. These ten lines that performed well in testcrosses also showed high resistance to MSV, with AUDPC ranging from 1.2 to 2.4. These results indicate potential three-way cross hybrids that could be further tested for eventual release in MSV-prone environments in Uganda. The 10 outstanding inbred lines will be advanced by selfing and by further backcrossing to produce new lines and MSV-resistant versions of CML384 and CML321, respectively, for possible use in hybrids production in Uganda.

## Comparison of Cost of MAS vs. CS

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 CS will involve a trade-off between money and time (Morris et al., 2003). This study has shown costs associated with CS and MAS for MSV resistance during two cycles of selection. Despite the fact that fewer plants were raised in the second cycle, CS costs increased from US$3.96 row$^{-1}$ in the first cycle to US$4.66 row$^{-1}$ in the second selection cycle because the costs of transport, technical assistance, and other overhead expenses remained constant. The cost per row would be reduced if screening involved a larger population and the

Table 7. Costs comparison between marker-assisted selection and conventional selection methods for maize streak virus disease resistance in maize in Uganda.

| Cost category | Conventional selection | | Marker-assisted selection | | | |
|---|---|---|---|---|---|---|
| | First selection cycle (588 rows) | Second selection cycle (420 rows) | First selection cycle (168 rows) | | Second selection cycle (168 rows) | |
| | Field cost | Field cost | Field cost | Laboratory cost | Field cost | Laboratory cost |
| Total selection cost (US$) | 2322.58 | 1955.60 | 1999.31 | | 1135.90 | |
| | | | 457.56 | 1541.75 | 457.56 | 678.34 |
| Cost per plant (US$) | 0.26 | 0.31 | 0.18 | 1.82 | 0.18 | 1.82 |
| Total cost per plant (US$) | | | 2.00 | | 2.00 | |
| Avg. cost per plant (US$) | 0.29 | | 2.00 | | | |
| Cost per row (US$) | 3.95 | 4.66 | 11.90 | | 6.76 | |
| Avg. cost per row (US$) | 4.31 | | 9.33 | | | |

overhead costs are spread thin. Marker-associated costs per cycle were the same at US$1.82 sample$^{-1}$ for both cycles. This could be reduced further if a smaller breeding population was considered for selection. Results also indicate the need to identify ways of reducing costs of laboratory reagents, which accounted for a greater proportion of the cost of MAS. Bulk purchase of reagents, for example, can be considered in order to exploit the economies of scale. All the reagents are expensive because they are imported into Uganda, and this might be the case in most developing countries. Previous findings by Collard (as cited in FAO, 2004) suggested that equipment, consumables, and infrastructure were among the major costs of MAS.

Comparison of CS and MAS methods is a big challenge, especially given that in CS breeders consider cost per row, while in MAS costs are calculated per sample or data point. The difference in methodology or units used does not allow for a fair economic comparison of the two methods. As a result, we standardized the units in both CS and MAS by costing on a single-plant basis, and clear differences were observed. Marker-assisted selection, which cost US$2.00 plant$^{-1}$, was more expensive than CS (US$0.29 plant$^{-1}$) for MSV resistance in Uganda. This showed that MAS was 6.89 times more expensive than CS. However, the costing 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 undervalued.

To reduce breeding costs, MAS can be applied at one stage to select plants fixed for the traits and continue with CS for other agronomic traits. This approach involves selecting plants at an early generation with a fixed, favorable genetic background at specific loci, conducting single large-scale MAS, while maintaining as much as possible the allelic segregation in the rest of the genome (Ribaut and Betran, 1999). This would require a large population for selection, with implications for costs. The results of this study implicitly showed that costs depended on the population sizes; hence, any change in population size would change the breeding costs in the same direction. Therefore, by using a combination of MAS and CS approaches, in which a small population is used in MAS in the early generations, and CS is used in the advanced generations with fewer plants, would reduce breeding costs significantly in contrast to the use of CS alone. The desired recombinants could be obtained with <168 plants in the $F_2$ generation, which were used in both cycles of MAS in the current study (Huhn and Piepho, 2003). Studies by Chao and Ukai (2000) also suggested that 42 to 56 plants could be effective in identifying the desired genotypes by indirect section of flanking markers. With CS, >500 plants in the $F_2$ generation might be required to identify the desired recombinants. This is clearly reflected by the overall costs, which were significantly reduced for MAS than for CS in both breeding cycles (Table 7). Molecular MAS also derives advantage over CS because it permits screening even in the absence of the disease, thus cutting down on time and saving resources in the long run. Although in the current study we did not test the breeding costs with respect to the time spent to fix the MSV-resistance trait, it is a given fact that the CS is most likely to take longer than MAS to generate a new product (Ribaut and Hoisington, 1998). With an appropriate marker, the recurrent parent can be recovered in about two to three generations of backcrossing with MAS (Xu and Crouch, 2008) compared with four to seven generations with CS (Ribaut and Hoisington, 1998). Therefore, costs of CS could be expensive in the long term (De Koning, cited by FAO, 2004) and time consuming, especially when selection is delayed because of absence of adequate disease infection. The costs of artificial inoculation can be inflated by maintenance of large cultures of leafhoppers (*C. mbila*) that transmit the disease.

Other costs could not be computed with high precision. For example, labor for the technician, temporary labor and field supervision, and tractor operations were hired and at a rate different from that of the National Agricultural Research Organization in Uganda. Because research was conducted by a student, the costs of the scientist's time as reflected by Dreher et al. (2000) were not

done in the current study. This may not have a large effect on the overall costs, as the scientist's salary rate can be considered to be fixed such that the costs of MAS and CS would vary proportionately. Although the labor cost, especially for DNA extraction, was reported among the major costs (Williams, as cited in FAO, 2004) this is bound to vary with countries and in Africa labor might still be relatively cheap and might not amount to a great proportion of the costs of MAS.

## CONCLUSION

The MAS was more efficient than CS because average incidence of MSV was lower in populations under MAS (64.8%) than in those under CS (79.3%). A similar trend was observed for AUDPC, confirming superiority of MAS in breeding for MSV resistance; but both methods were effective in obtaining lines with higher resistance than susceptible checks. Five lines generated by MAS and another five from CS displayed high yield levels and MSV resistance that were superior to standard hybrids, suggesting that both methods were equally effective in identifying high-yielding lines. Costs of MAS and CS varied depending on the units for comparison. Considering the total costs of selection for MSV resistance, the costs of CS were higher in both first (US$2322.58) and second (US$1955.60) cycles than the costs of MAS in first (US$1999.31) and second (US$1135.90) cycles. However, when comparing costs per row CS (US$4.31) was cheaper than MAS (US$9.33) by about twofold; whereas, after standardizing costs per plant to facilitate appropriate economic comparison, CS was shown to be 6.89 times cheaper than MAS. The largest proportion of costs of MAS was from laboratory consumables. The overall cost over the two breeding cycles for MAS was US$3135.21, while that of CS was US$4278.18, indicating that MAS was cheaper than CS by US$1142.97. This implied that 26.7% of the breeding budget could be saved by adopting MAS, which was realized by using fewer plants or rows under MAS than under CS in Uganda. Possibly, costs of MAS can be further reduced by using a relatively smaller breeding population than the one used in the current study and by seriously considering ways to significantly reduce the costs of laboratory operation, which accounted for >60% of the operating costs of MAS. Results show that both CS and MAS were effective in generating lines with MSV resistance because disease incidence was high and it is easy to rate lines for MSV. However, if there was no disease or the incidence was low or occurrence was sporadic, then CS would not have been as effective as MAS because selection might not be possible due to absence or inadequate disease pressure. Thus, in a full-scale breeding program under CS, additional financial resources are invested in infrastructure and personnel (to maintain insect cultures) to sustain breeding progress under CS. Overall, our results suggested that, when the laboratory facilities are established and the appropriate molecular markers are available, use of MAS in breeding for MSV resistance would be recommended.


### Acknowledgments

We are grateful to The Rockefeller Foundation for supporting this research. We also thank CIMMYT for providing maize germplasm for the study. Assistance by Dr. K.V. Pixley in generating the populations, at CIMMYT in Zimbabwe, is acknowledged.


### References


Barrow, M.R. 1992. Development of maize hybrids resistant to maize streak virus. Crop Prot. 11:267–271.

Barrow, M.R. 1993. Increasing maize yields in Africa through the use of maize streak virus resistant hybrids. Afr. Crop Sci. J. 1:139–144.

Bosque-Perez, N.A. 2000. Eight decades of maize streak virus research. Virus Res. 71:107–121.

Bosque-Perez, N.A., and M.S. Alam. 1992. Mass rearing of *Cicadulina* leafhoppers to screen for maize streak virus resistance. IITA, Ibadan, Nigeria.

Bosque-Pérez, N.A., S.O. Olojede, and I.W. Buddenhagen. 1998. Effect of maize streak virus disease on the growth and yield of maize as influenced by varietal resistance levels and plant stage at time of challenge. Euphytica 101:307–317.

Bouchez, A., F. Hospital, M. Causse, A. Gallais, and A. Charcosset. 2002. Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics 162:1945–1959.

Campbell, C.L., and L.V. Madden. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, New York.

Caulfield, M.J. 1997. Genotype reaction to streak and mottle virus in maize. p. 33. *In* ARC-Grain Crops Institute (ed.) Maize Streak Dis. Symp. ARC, Nelspruit, South Africa.

Chao, Y.T., and Y. Ukai. 2000. Sample size required for marker assisted selection in improving quantitative traits of self-fertilizing species. Euphytica 116:87–94.

Chen, S., X.H. Lin, C.G. Xu, and Q. Zhang. 2000. Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci. 40:239–244.

CIMMYT. 2003. Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory. 3rd ed. CIMMYT, Mexico, D.F.

Dekkers, J.C.M., and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural populations. Nat. Rev. Genet. 3:22–32.

Dintinger, J., B. Reynaud, P. Letourmy, J.L. Marchand, and M. Peterscmitt. 1997. A multilocation cultivar trial in Africa to evaluate efficiency and stability of complete resistance to MSV originating in Le Réunion. *In* Proc. Int. Maize Streak Dis. Symp., 1st, Hazyview, Mpumalanga, South Africa. 9–11 Sept. 1997. Available at http://www.mcb.uct.ac.za/msv/msv_session_6.htm (verified 8 Jan. 2009). ARC, Nelspruit, South Africa..

Dreher, K., M. Khairallah, J.-M. Ribaut, K. Dreher, and M.L. Morris. 2003. Cost of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. Mol. Breed. 11:221–234.

Dreher, K., M. Khairallah, J.-M. Ribaut, S. Pandey, and G. Srinivasan. 2000. Is marker assisted selection cost-effective

compared to conventional plant breeding methods? The case of quality protein maize. *In* Annu. Conf. of the Int. Consortium on Agric. Biotechnology Res., 4th, Ravello, Italy. 24–28 Aug. 2000. Available at www.cimmyt.org/research/economics/map/research_results/other_tech/marker_assisted.pdf (verified 8 Jan. 2009). ICABR, Ravello, Italy.

FAO. 2004. Summary document of the FAO e-mail conference: "Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries." Available at http://www.fao.org/biotech/logs/C10/summary.htm (verified 15 Dec. 2008). FAO, Rome.

Fehr, W.R. 1987. Principles of cultivar development. Vol. 1. Theory and technique. McGraw-Hill, New York.

Flett, B.C., W. du Toit, and J. van den Berg. 1997. Tolerance and resistance of maize (*Zea mays*) hybrids naturally infested with maize streak virus. *In* Proc. Int. Maize Streak Dis. Symp., 1st, Hazyview, Mpumalanga, South Africa. 9–11 Sept. 1997. Available at http://www.mcb.uct.ac.za/msv/msv_session_6.htm (verified 8 Jan. 2009). ARC, Nelspruit, South Africa.

Frisch, M., M. Bohn, and A.E. Melchinger. 1999. Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Sci. 39:1295–1301.

Frisch, M., and A. Melchinger. 2001. Marker-assisted backcrossing for simultaneous introgression of two genes. Crop Sci. 41:1716–1725.

Frisch, M., and A.E. Melchinger. 2005. Selection theory for marker-assisted backcrossing. Genetics 170:909–917.

Gu, W.K., N.F. Weeden, J. Yu, and D.H. Wallace. 1995. Large-scale, cost-effective screening of PCR products in marker-assisted selection applications. Theor. Appl. Genet. 91:465–470.

Huang, N., E.R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett, and G.S. Khush. 1997. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95:313–320.

Huhn, M., and H.P. Piepho. 2003. Determining the sample size for co-dominant molecular marker-assisted linkage detection for a monogenic qualitative trait by controlling the type-I and type-II errors. Theor. Appl. Genet. 106:840–845.

Kairo, M.T.K., P.K. Kiduyu, C.J.M. Mutinda, and L.T. Empig. 1995. Maize streak virus: Evidence for resistance against *Cicadulina mbila* Naude, the main vector species. Euphytica 89:109–114.

Mesfin, T., and N.A. Bosque-Perez. 1998. Feeding behavior of *Cicadulina storeyi* China (Homoptera: *Cicadellidae*) on maize cultivars susceptible or resistant to maize streak virus. Afric. Entomol. 6:185–191.

Moreau, L., S. Lemarie, A. Charcosset, and A. Gallais. 2000. Economic efficiency on one cycle of marker-assisted selection. Crop Sci. 40:329–337.

Morris, M.L., J.-M. Ribaut, K. Dreher, and M. Khairallah. 2003. Money matters (II): Costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Mol. Breed. 11:235–247.

Payne, R.W., D.A. Murray, S.A. Harding, D.B. Baird, and D.M. Sourtar. 2007. Introduction to GenStat for Windows. 10th ed. VSN International, Hemel Hempstead, U.K.

Pernet, A., D. Hoisington, J. Franco, M. Isnard, D. Jewell, C. Jiang, J.-L. Marchand, B. Beynaud, J.-C. Glaszmann, and D. Gonzalez de Leon. 1999a. Genetic mapping of maize streak virus resistance from the Mascarene source: I. Resistance in line D211 and stability against different virus clone. Theor. Appl. Genet. 99:524–539.

Pernet, A., D. Hoisington, J. Franco, M. Isnard, D. Jewell, C. Jiang, J.-L. Marchand, B. Beynaud, J.-C. Glaszmann, and D. Gonzalez de Leon. 1999b. Genetic mapping of maize streak virus resistance from the Mascarene source: II. Resistance in line CIRAD 390 and stability across germplasm. Theor. Appl. Genet. 99:540–553.

Pixley, K.V., D.C. Jewell, A. Schechert, and G. Welz. 1997. Recent maize streak disease research at CIMMYT-Zimbabwe. *In* Proc. Int. Maize Streak Dis. Symp., 1st, Hazyview, Mpumalanga, South Africa. 9–11 Sept. 1997. Available at http://www.mcb.uct.ac.za/msv/msv_session_6.htm (verified 8 Jan. 2009). ARC, Nelspruit, South Africa.

Ragot, M., and D.A. Hoisington. 1993. Molecular markers for plant breeding: Comparisons of RFLP and RAPD genotyping costs. Theor. Appl. Genet. 86:975–984.

Reyes-Valdés, A.H. 2000. A model for marker-based selection in gene introgression breeding programs. Crop Sci. 40:91–98.

Ribaut, J.M., and J. Betran. 1999. Single large-scale marker-assisted selection. Mol. Breed. 5:531–541.

Ribaut, J.M., and D. Hoisington. 1998. Marker-assisted selection: New tools and strategies. Trends Plant Sci. 3:236–239.

Thomas, W.T.B. 2003. Prospects for molecular breeding of barley. Ann. Appl. Biol. 142:1–12.

Timothy, D.H., P.H. Harvey, and C.R. Dowswell. 1988. Development and spread of improved maize cultivars and hybrids in developing countries. USAID, Washington, DC.

Welz, H.G., A. Schechter, A. Pernet, K. Pixley, and H.H. Geiger. 1998. A gene for resistance to the maize streak virus in the African CIMMYT maize inbred line CML202. Mol. Breed. 4:147–154.

Willcox, M.C., M.M. Khairallah, D. Bergvinson, J. Crossa, J.A. Deutsch, G.O. Edmeades, D. González-de-León, C. Jiang, D.C. Jewell, J.A. Mihm, W.P. Williams, and D. Hoisington. 2002. Selection for resistance to southwestern corn borer using marker-assisted and conventional backcrossing. Crop Sci. 42:1516–1528.

Xu, Y., and J.H. Crouch. 2008. Marker-assisted selection in plant breeding: From publications to practice. Crop Sci. 48:391–407.

Yousef, G.G., and J.A. Juvik. 2001. Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Sci. 41:645–655.

Yu, K., S.J. Park, and V. Poysa. 2000. Marker-assisted selection of common beans for resistance to common bacterial blight: Efficacy and economics. Plant Breed. 119:411–415.