African Journal of Biotechnology Vol. 6 (14), pp. 1623-1628, 18 July 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

*Full Length Research Paper*

# The use of simple sequence repeats markers to study genetic diversity in maize genotypes resistant to gray leaf spot disease

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Accepted 19 February, 2007

**Genetic diversity in maize (*Zea mays* L.) is an important tool for progress in selection for traits of interest. The objective of this study was to screen genotypes for presence of markers linked to plant defense against fungal diseases, and to study the genetic diversity in gray leaf spot resistant maize genotypes. Forty-one genotypes comprising of collections from Kenya, International Maize and Wheat Improvement Centre, the International Institute for Tropical Agriculture and South Africa were identified to be resistant to gray leaf spot in Kenya in 2004. The genotypes were analyzed for variability by using twenty-eight microsatellite markers covering the entire maize genome. The results indicated diversity among lines for selected markers. Based on the diversity tree, ten clusters were observed. All genotypes occurred in clusters, except for genotypes EC573- (R12) $C_8S_3$-14-1, REGN 99/6, H623 and VHCY. The data indicated that, at least one line in each cluster showed a relationship in a locus with a corresponding parent. Occurrence of related lines also implied that there were common alleles that could have contributed to the gray leaf spot resistance.**

**Key words:** SSRs markers, diversity, gray leaf spot, genotype resistance.

## INTRODUCTION

Genetic gain during selection is a function of a genotype possessing favorable alleles for important traits, which in turn depends on genetic diversity (Fehr, 1987). For effective management of genetic diversity, germplasm collections need to be characterized and classified into heterotic groups. Presence of favorable alleles is sometimes difficult to detect in the conventional and phenotypic evaluation of germplasm. One of the underlying reasons is that conventional maize breeding programs are heavily dependent on the phenotypic expression of traits of economic importance such as resistance to foliar diseases. Resistance breeding is however dependent on the genetics of resistance, which may be difficult to understand due to their quantitative nature of inheritance. Marker assisted selection (MAS) and DNA finger printing techniques have been effectively used to increase the efficiency of conventional breeding by reducing the time of varietal development (Dreher et al., 2000; Welz and Geigerb, 2002). These techniques utilize molecular markers linked to quantitative trait loci (QTLs) that confer resistance to diseases.

In Kenya, gray leaf spot caused by *Cercospora zeae-maydis* (Theon and Daniels) results in significant yield losses of between 10 and 25% at any one season, especially in the western part of the country. In susceptible genotypes, yield losses of up to 60% are not uncommon. Gray leaf spot disease is difficult to control through conventional means such as chemical sprays, cultural, and physical measures. The most feasible method to manage the disease is through resistance breeding. However, inheritance of resistance to gray leaf spot has been found to be quantitative in nature, with small additive effects. QTLs for gray leaf spot have been identified in chromosomes 2, 3, and 4 (Bubeck et al., 1993), but the sizes and effects of these QTLs tend to differ with genotypes. Gray leaf spot resistance was initially addressed through conventional breeding (Ininda et al., 2004), but levels of resistance in most commercial genotypes are quite low,

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**Table 1.** Description of Forty-one maize populations used in this study.

| Line Code | Pedigree | Type |
|---|---|---|
| S539-10-2-2-1 | (EM11-133/Tzi3//E11-133/AO76)-x-39-10-2-2-1 | INBRED |
| S558-2-2-1-1 | (EM12-210/CML197//E12-210/OSU23i)-x-58-2-2-1-1 | INBRED |
| S558-2-2-2-1 | (EM12-210/CML197//E12-210/OSU23i)-x-58-2-2-2-1 | INBRED |
| S558-2-2-3-1 | (EM12-210/CML197//E12-210/OSU23i)-x-58-2-2-3-1 | INBRED |
| S558-3-4-1-1 | (EM12-210/CML197//E12-210/OSU23i)-x-58-3-4-1-1 | INBRED |
| S558-4-2-1-1 | (EM12-210/CML197//E12-210/OSU23i)-x-58-4-2-1-1 | INBRED |
| S580-17-2-1-1 | (EM12-210/Tzi3//E12-210/AO76)-x-80-17-2-1-1 | INBRED |
| REGN 29-5 | EA REGNUR 29-5 | INBRED |
| REGN 36-1 | EA REGNUR 36-1 | INBRED |
| POOL A 6-3-1 | Tuxpeno Sesquia EMBU | INBRED |
| EC 573 LINE 93 | EC573(R12)C8S3-93 | INBRED |
| EC573 LINE 14 | EC573(R12)C8S3-14-1 | INBRED |
| KSII LINE 38 | KS11(R11)C11S3-38 | INBRED |
| S496-21-1-1 | (E12-210/AO76//E12-210/CML197)-x- 96-21-1-1 | INBRED |
| S496-6-1-1 | (E12-210/A076//E12-210/CML197)-x- 96-6-1-1 | INBRED |
| S496-15-1-1 | (E12-210/AO76//E12-210/CML197)- x-96-15-1-1 | INBRED |
| Reg 99/48-1 | EA REGNUR 99/98-1 | INBRED |
| Reg 99/96-1 | EA REGNUR 99/96-1 | INBRED |
| Z168-11,Z276-4-1 | SZSYNKITII-F2 | INBRED |
| Z419-5,Z443-3-1 | SYN[Kitale/Tuxp-GLS]F2 | INBRED |
| Z426-43,Z387-4-1 | [CML 197/N3//CML206]-X-32-1-1-2-B-B | INBRED |
| Z468-2-1 | [MSR123 X II37TN-9-2-4-X-3/LZ956441]-B-1-5-5-B-4-B-B-B-B | INBRED |
| Z427-42,Z376-49-1 | [MSRXPOOL9]C1F2-205-1(OSU23i)-5-3-X-X-1B//EV7992/V8.. | INBRED |
| EM 11-133 | | INBRED |
| TZ i3 | | POP |
| A076 | | POP |
| OSU23i | | INBRED |
| CML 197 | | INBRED |
| EM12-210 | | INBRED |
| TUXPENO SEQUIA | | POP |
| POOL A | | POP |
| H614 | | HYBRID |
| H627 | | HYBRID |
| H623 | | HYBRID |
| PHB3253 | | |

most of them being very susceptible. To pyramid genes for high levels of resistance to gray leaf spot, diverse sources of resistance have been identified (Ininda et al., 2004). What is lacking is identity and locations of different genes in the diverse genotypes and molecular description of sources of resistance to gray leaf spot, for effective utilization in breeding programs.

## MATERIALS AND METHODS

### Genetic material

Forty-one genotypes (lines and populations) from the Kenya Agricultural Research Institute (KARI), International Maize and Wheat Improvement Centre (CIMMYT), East African Regional Disease Nursery (EA REGNUR), Grain Crop Institute (South Africa), and the International Institute for Tropical Agriculture (IITA) were used in the study. Twenty-three genotypes were selected from a set of eighty populations previously screened for resistance to gray leaf spot. Fourteen populations were identified as parents that contributed the gene for resistance to gray leaf spot and appear in the pedigree of the inbred lines. Four genotypes were hybrids used as susceptible checks (Ininda et al., 2004) as shown in Table 1.

### Field trials

The experiments were conducted in Kakamega (1585 m above sea level) in 2004. This site has environmental conditions that favor gray leaf spot development under field conditions. Materials were

**Table 2.** Microsatellite markers and the respective bins used for characterization of genotypes.

| Number | Marker name | Bin |
|---|---|---|
| 1 | umc1568 | 1.02 |
| 2 | bnlg2086 | 1.04 |
| 3 | phi109642 | 2.03-2.04 |
| 4 | umc1065 | 2.06 |
| 5 | Phi127 | 2.08 |
| 6 | umc1736 | 2.09 |
| 7 | umc2102 | 3 |
| 8 | umc2258 | 3.02-3.03 |
| 9 | bnlg1605 | 3.07 |
| 10 | umc1399 | 3.07 |
| 11 | umc1288 | 4.02 |
| 12 | umc2281 | 4.03 |
| 13 | umc1652 | 4.04 |
| 14 | umc2216 | 5.06 |
| 15 | umc1072 | 5.07 |
| 16 | phi112 | 7.01 |
| 17 | phi034 | 7.02 |
| 18 | phi328175 | 7.04 |
| 19 | umc1406 | 7.05 |
| 20 | umc1712 | 8.05 |
| 21 | umc2210 | 8.05 |
| 22 | umc1698 | 9.02 |
| 23 | phi022 | 9.03 |
| 24 | umc1570 | 9.04 |
| 25 | umc1380 | 10 |
| 26 | umc1318 | 10.01 |
| 27 | umc1152 | 10.02 |
| 28 | umc1196 | 10.07 |

planted in a randomized complete block design with three replicates. Each plant was artificially inoculated with gray leaf spot causative agent, *Cercospora zeae-maydis* at the 6-leaf stage and severity of disease scored by rating leaves at mid-plant height using the percent leaf area affected (PLAA) scale (Smith, 1989). This assigns a PLAA score on a scale of 1-5, where 1 = very small necrotic lesions on leaves, 2 = light necrosis covering <40 percent of plant; 3 = moderate necrosis on leaves 60% of leaf area; 4 = severe necrosis on about 80% of leaf area; 5 = very severe necrosis on more than 90 percent of leaf area or dead plants. Leaf damage data were collected for two other foliar diseases as well, northern corn leaf blight (NCLB) caused by *Exserohiluim turcicum* and common rust (CR) caused by *Puccinia sorghi* using the same scale.

### SSR analysis

DNA was extracted for all the forty-one samples using a modified CTAB procedure (Saghai-Maroof et al., 1984). DNA quality and concentration was checked using agarose gel electrophoresis and spectrophotometry respectively. Dilutions were made to a uniform stock concentration of 0.3 µg/µl for amplification. The genotypes were analyzed for variability by using twenty-eight microsatellite markers covering the entire maize genome. The positions of the primers were obtained from maize GDB website, and synthesized by Sigma Genosis. Poly-merase chain reaction (PCR) was done for all the 28 primers (Table 2). After PCR, the products were resolved by metaphor gel and capillary electrophoresis using ABI genotyping machine. For the gel separated fragments, alleles were scored as A for the upper fragment and B for the lower fragment, AB for both, and C for any extra fragment. Null alleles were scored as 0. For the capillary electrophoresis, allele sizes were extracted using genemapper software, and frequency of homozygosity, evidence for scoring errors due to stuttering and large allele dropout were estimated using macrochecker program. Data was analyzed by use of the PopGene Version 1.32 and tools for genetic population analysis (TFPGA) Soft Wares using MO17 and B73 as controls.

## RESULTS AND DISCUSSION

Variability in disease scores was obtained for all the 41 genotypes evaluated (Table 3). The scores ranged between 1.5 and 2.5 for gray leaf spot and between 1.5 and 3.0 for turcicum leaf blight and common rust. All genotypes were better for foliar diseases compared to the one check PH3253 that showed a score for gray leaf spot and turcicum leaf blight of 3.5. The most resistant genotypes showed a score of 1.5 for gray leaf spot. All parents showed a score of <2.5 for gray leaf spot, turcicum leaf blight and common rust. The results indicated diversity and polymorphism among lines for selected markers. Based on the diversity tree, 10 dendo-groups were observed (Figure 1). Except for genotypes EC573-(R12) C8S3-14-1, REGN 99/6, H623 and VHCY, where each of the genotype occurred in a unique group, the other lines occu-rred in clusters. The data shows at least one line in each cluster showed a relationship in a locus with a corresponding parent. The cluster E was the cluster with the largest number of genotypes. This cluster had both lines and parents. Parental genotypes CML197, EM12-210, OSU23i, Tuxpeno Sesquia, TZi35 occurred in this cluster. The data shows that the parents and inbreds showed resistance to the fungal diseases (gray leaf spot, turcicum leaf blight and common rust), thus inbreds obtained alleles for disease resistance form the parents. The results indicate different genotypes portray diversity location of genes for resistance to gray leaf spot and turcicum leaf blight. An excellent source for gray leaf spot resistance is found in local inbred lines such as Ec573 (R12)-$C_8S_3$-14. This data indicates sources of gray leaf spot and turcicum leaf blight are available locally and from other sources and hence the possibility of gene pyramiding for stable resistance to gray leaf spot and other diseases in Kenyan hybrids. Studies by other workers have found QTLs on chromosome 2 to have direct effects on selection for gray leaf spot resistance (Bubeck et al., 1993). Pyramiding gray leaf spot resistant genes from different sources is possible based on the fact that the different parents are in different groups as indicated by the dedogram Table 4. Hence for dedogram group 'G', G.1.1 and G.1.2 are possible parents. This is agreeable to the fact that the

**Table 3.** Disease scores for forty one genotypes evaluated at Kakamega in 2004.

| Genotype | Description | GLS | E.T. | RUST |
|---|---|---|---|---|
| EM11-133 | Parent | 2.0 | 2.0 | 1.5 |
| Tzi3 | Parent | 2.5 | 3.0 | 1.5 |
| AO76 | Parent | 1.5 | 2.5 | 1.5 |
| OSU23i | Parent | 2.5 | 2.0 | 1.5 |
| CML197 | Parent | 1.5 | 2.5 | 2.0 |
| EM12-210 | Parent | 1.5 | 2.0 | 1.5 |
| TUXPENO SESQUIA | Parent | 2.0 | 2.5 | 2.0 |
| Ec573(R12)$C_8S_3$-14 | Inbred | 2.0 | 2.0 | 1.5 |
| REG99/6 | Inbred | 1.5 | 2.0 | 1.5 |
| POOL A 6-3 | Parent | 2.5 | 2.0 | 2.0 |
| REG 36-1 | Inbred | 1.5 | 2.0 | 1.5 |
| REG99-6 | Inbred | 1.5 | 2.0 | 1.5 |
| CML202 | Parent | 2.0 | 2.0 | 1.5 |
| CML204 | Parent | 2.0 | 3.0 | 1.5 |
| CML206 | Parent | 2.0 | 4.5 | 1.5 |
| Tzi35 | Parent | 1.5 | 2.0 | 1.5 |
| VHCY | Parent | 2.0 | 3.5 | 2.5 |
| $S_5$39-10-2-2-1 | Inbred | 1.5 | 2.5 | 2.0 |
| $S_5$ 58-2-2-1-1 | Inbred | 2.0 | 2.5 | 2.0 |
| $S_5$ 58-2-2-2-1 | Inbred | 2.0 | 2.0 | 2.0 |
| $S_5$ 58-2-2-3-1 | Inbred | 2.0 | 2.0 | 1.5 |
| $S_5$ 58-3-4-1-1 | Inbred | 2.0 | 2.5 | 2.0 |
| $S_5$ 58-4-2-1-1 | Inbred | 2.0 | 2.5 | 2.5 |
| $S_5$ 80-17-2-1-1 | Inbred | 1.5 | 2.5 | 2.5 |
| REG 29-5-1 | Inbred | 1.5 | 2.0 | 1.5 |
| POOL A-6-3-1 | Inbred | 2.5 | 2.0 | 2.0 |
| Ec573(R12)$C_8S_3$-93-2 | Inbred | 1.5 | 1.5 | 1.5 |
| Ec573(R12)$C_8S_3$-14-1 | Inbred | 1.5 | 2.5 | 1.5 |
| $S_4$ 96–21-1-1 | Inbred | 1.5 | 2.0 | 2.0 |
| $S^4$ 96–6-1-1 | Inbred | 1.5 | 2.0 | 2.0 |
| $S_4$ 96–15-1-1 | Inbred | 1.5 | 3.0 | 2.0 |
| REG99/96-1 | Inbred | 2.0 | 2.5 | 1.5 |
| Z419-5 Z443-3-3 | Inbred | 2.5 | 2.5 | 1.5 |
| Z426-43 Z387-4-1 | Inbred | 2.0 | 2.5 | 2.0 |
| Z468-2-1 | Inbred | 2.5 | 3.0 | 1.5 |
| Z468-2-2 | Inbred | 2.5 | 2.5 | 1.5 |
| Z427-43 Z376-49-1 | Inbred | 1.5 | 2.0 | 1.5 |
| H614 | Check | 1.5 | 2.0 | 1.5 |
| H627 | Check | 1.5 | 2.0 | 2.0 |
| H623 | Check | 2.5 | 2.5 | 1.5 |
| PH3253 | Check | 3.5 | 3.5 | 2.0 |

*P=Parents, I=Inbred line, C=Check, GLS= Gray leaf spot, E.T.=Turcicum leaf blight, Rust=Common.

line $S_4$ 96-6-1-1 has the parent AO76 in its pedigree (Ininda et al., 2004). The dedogram group 'I' has CIMMYT materials, hence source of resistance is likely to be a line from CIMMYT (Pool 9A or CML197). Another interesting observation was the line $S_4$ 96-6-1-1 in the cluster G, where the parents AO76 belong, while the

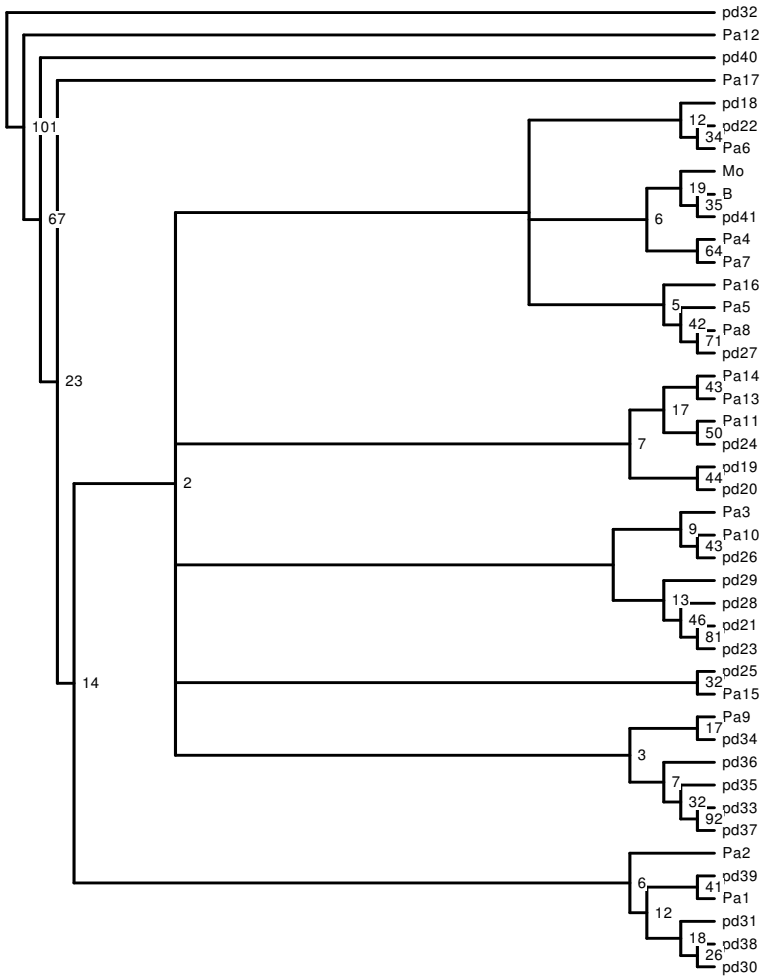


**Figure 1.** Dedogram showing classification of genotypes for twenty-eight microsatellite primers (see Table 4 for key to the dedogram).

**Table 4.** Key to dedogram in Figure 1.

| ID | Name in the tree | Dendro group | Population | Source |
|---|---|---|---|---|
| 28 | pd32 | A | EC 573-(R12) C8S3-14-1 | KARI |
| 12 | pa12 | B | REGN 99/96 | EA REGNUR |
| 40 | pd40 | C | H 623 | KARI |
| 17 | pa17 | D | VHCY | South Africa |
| 18 | pd18 | E.1 | $S_5$ 39 -10-2-2-1 | KARI |
| 22 | pd22 | E.1 | $S_5$ 58-3-4-1-1 | KARI |
| 6 | pa6 | E.1.2 | EM 12-210 | KARI |
| 42 | Control | E.2.1 | Mo17 | USA |
| 43 | Control | E.2.2 | B73 | USA |
| 41 | pd41 | E.2.2 | PHB 3253 | PIONEER |
| 4 | pa4 | E.2.3 | OSU 23i | CIMMYT-Ohio State |
| 7 | pa7 | E.2.3 | TUXPENO SEQUIA | CIMMYT |

**Table 4.** Contd.

| | | | | |
|---|---|---|---|---|
| 16 | pa16 | E.3.1 | TZ i35 | IITA |
| 5 | pa5 | E.3.2 | CML 197 | CIMMYT |
| 8 | pa8 | E.3.3 | EC 573 (R12) C8S3-14 | KARI |
| 31 | pd27 | E.3.3 | $S_4$ 96-15-1-1 | KARI |
| 13 | pa13 | F.1 | CML 202 | CIMMYT |
| 14 | pa14 | F.1 | CML 204 | CIMMYT |
| 11 | pa11 | F.2 | REGN 99/48 | EA REGNUR |
| 24 | pd24 | F.2 | $S_5$-80-17-2-1-1 | KARI |
| 19 | pd19 | F.3 | $S_5$ 58-2-2-1-1 | KARI |
| 20 | pd20 | F.3 | $S_5$ 58-2-2-2-1 | KARI |
| 3 | pa3 | G.1.1 | A O76 | South Africa |
| 10 | pa10 | G.1.2 | POOL A 6-3 | CIMMYT |
| 30 | pd26 | G.1.2 | S5 96-6-1-1 | KARI |
| 32 | pd29 | G.2.1 | REGN 99/96-1 | EA REGNUR |
| 25 | pd28 | G.2.2 | REGN 29-5-1 | EA REGNUR |
| 21 | pd21 | G.2.3 | $S_5$ 58-2-2-3-1 | KARI |
| 23 | pd23 | G.2.3 | $S_5$ 58-4-2-1-1 | KARI |
| 15 | pa15 | H | CML 206 | CIMMYT |
| 29 | pd25 | H | $S_4$ 96-21-1-1 | KARI |
| 9 | pa9 | I.1 | REGN 36-1 | EA REGNUR |
| 34 | pd34 | I.1 | Z 426-43, Z 387-4-1 | CIMMYT |
| 36 | pd36 | I.2 | Z 468-2-2 | CIMMYT |
| 35 | pd35 | I.3.1 | Z 468-2-1 | CIMMYT |
| 33 | pd33 | I.3.2 | Z 419-5, Z 443-3-3 | CIMMYT |
| 37 | pd37 | I.3.2 | Z 427-43, Z 376-49-1 | CIMMYT |
| 2 | pa2 | J.1 | TZ i3-S2 | IITA |
| 1 | pa1 | J.2 | EM 11-133 | KARI |
| 39 | pd39 | J.2 | H 627 | KARI |
| 27 | pd31 | J.3.1 | EC 573- (R12) C8S3-93-2 | KARI |
| 26 | pd30 | J.3.2 | POOL A 6-3-1 | CIMMYT |
| 38 | pd38 | J.3.2 | H 614 | KARI |

sister line $S_4$ 96-15-1-1 is in cluster E where CML197 belong. This is expected since the parent AO76 and CML197 were initial parents in the constitution of original populations of the two lines. Based on these study, breeders would be able to capture more diversity, and constitute varieties with polygenic resistance to gray leaf spot. More markers are however necessary to come up with conclusive results.

## ACKNOWLEDGEMENTS

The authors would like to thank Dr. Rebecca Nelson, Cornell University and The Rockefeller Foundation. The research was supported by Grant No. 2003 FS 041.

### REFERENCES

Bubeck DM, MM Goodman, WD Beavis, D Grant (1993). Quantitative trait loci controlling resistance to gray leaf spot in maize. Crop Sci. 33: 838-847.

Dreher K, Khairallah M, Pandey S (2000). Is marker assisted selection cost effective compared to conventional plant breeding methods? The case of Quality Protein Maize. Paper presented at the Fourth Annual Conference of the International Consortium on Agricultural Biotechnology Research (ICABR). 'The economics of Agricultural Biotechnology'. Held in Revello, Italy from 24-28 August, 2000.

Fehr WR (1987). Principals of cultivar development. V.1.Theory and Technique. McGraw-Hill, Inc. ISBN 0-07-020345-8.

Ininda J, Ajanga S, Odongo OM (2004). Evaluation of diverse tropical maize genotypes for resistance to gray leaf spot and turcicum leaf blight in Kenya. East African Agricultural and Forestry Journal. Submitted, December, 2004.

Saghai-Maroof MA, KM Soliman, RA Jorgensen, FW Allard (1984). Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA 81: 9014-8018.

Welz HG, Geigerb HH (2002). Principles of marker-assisted selection 1. Qualitative traits. In http://nbpgr.delhi.nic.in/mmarker/s2-1Welz. Web page.