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CHROMOSOMAL LOCATION OF GENES FOR LEAFINESS (Lfy1) AND SUSCEPTIBILITY TO NICOSULFURON (nsf1) IN MAIZE GENOME

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Agronomy

by

Orlando José Moreno
B. S., Universidad de Oriente, Venezuela, 1980
M. S., Mississippi State University, 1990
May 1999

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DEDICATION

To Maria, our mother

To Silvia, my wife

To our children; Johnnaisil, Orsilmar, Orfran and Silvia V.

And to men everywhere, who put their family first, but have an ideal.
ACKNOWLEDGMENTS

First of all, I would like to thank God and the Virgin Mary for giving me strength, perseverance and enthusiasm to accomplish this big task and challenge.

My utmost gratitude goes to my major professor, Dr. Manjit S. Kang, for his great support, advice, friendship, encouragement, leadership, and understanding throughout this study at Louisiana State University. I wish to express my appreciation to my dissertation committee members, Dr. Scott B. Milligan, Dr. H. J. Mascagni, Jr., Dr. John Kovar, Dr. Michael Stine, and Dr. Richard Story for their helpful comments and suggestions that made my work significantly better. I thank also Dr. James Board and Dr. Wojciech A. Krotoski for their encouragement and advice during the earlier phases of my graduate study. My deepest respect to you all.

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My deepest thanks to my family, Silvia Coromoto, Johnnaisil, Orsilmar, Orfran, and Silvia Verónica for their help, encouragement, and caring love throughout all my endeavors. To them I am forever indebted.

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ABSTRACT

Genes that may confer resistance to diseases, tolerance to plant stress, good stalk quality, high yield potential and/or other desirable attributes are important. The leafy gene (Lfy1 Lfy1) discovered in 1971 in maize (Zea mays L.) is known to produce much higher yields in hybrids that possess it. These hybrids suffer much less stalk lodging than those with the non-leafy (lfy lfy) gene. The location of the leafy gene to a chromosome arm could facilitate both its transfer from one genotype to another and its use in linkage studies, and aid in completing the maize linkage map. The purpose of this investigation was to identify the chromosome arm that carries the leafy gene by using waxy-marked reciprocal translocations. Inbred lines 371 and 957 containing the leafy gene were crossed with the translocation stocks and subsequently test crosses were produced. An examination of the probability values for these test-crosses revealed that the leafy gene in both inbreds is located in the long arm of chromosome 4.

The gene, nsf1, conditions susceptibility to nicosulfuron (Accent) herbicide in maize and it was discovered in 1992. Pinpointing its location to a specific chromosome arm could facilitate its use as a genetic marker in linkage studies and in other genetic experiments. B-A chromosomal translocations provide an efficient means of mapping recessive genes in a single generation. The objective was to identify the chromosome arm that carries the nsf1 gene via B-A translocational analysis. Fifteen homozygous B-A translocations that were resistant to nicosulfuron were each crossed as male parents to a susceptible inbred line...

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The F₁ seeds were field and greenhouse grown in 1997 and 1998. Data obtained after treating F₁ progeny with nicosulfuron and genetic analyses of these data revealed that the nsf1 gene occupies a locus on the short arm of chromosome 7.
INTRODUCTION

Maize (*Zea mays* L.) is an important crop in the United States, occupying about 28 to 32 million hectares annually. In 1996, U. S. farmers harvested 32 million hectares and produced 232 billion kilograms of maize, averaging 7,970 kg ha\(^{-1}\) (Corn Refiners Assoc., 1997). The United States' total use will hit 250 billion kilograms from 2001/02 on and is projected to reach 275 billion kilograms by 2005/06 (Corn Refiners Assoc., 1997). Maize has been a major crop in Louisiana for several years. Maize production area in this state increased from 155,925 hectares in 1986 to 211,815 hectares in 1997. Louisiana farmers produced 29 million kilograms of maize in 1997, which represented a 70.9% increase in area, an 86% increase in production, and an 8.6% increase in yield as compared with 1994 (Corn Refiners Assoc., 1997).

Maize breeding is a process of designing and pursuing a desirable end product (i.e., hybrid, synthetic, inbred line) that represents a compilation of desirable agronomic/economic traits. Obtaining as much genetic information as possible about important genes and their interactions improves the efficiency and probability of success in achieving an end product with the desired attributes. Construction of a detailed genetic map will make available information that breeders can use to manipulate and complement traits to maximum advantage.

**Gene for Leafiness in Maize (*Lfy1*)**

The extra-leaf trait (*Lfy1*), discovered in 1971 by Robert C. Muirhead of Hughes Hybrids, Inc. appears to confer resistance to stalk rots, good stalk quality,
high yield potential, and other desirable attributes to the germplasm which contains it (Shaver, 1983). The leafy materials are characterized by extra leaves above the ear, low ear placement, highly lignified stalk and leaf parts, and high yield potential (Shaver, 1983). This gene has not been used extensively, because it was patented by a private company, and therefore, restricted in use (Anonymous, 1986; Shaver, 1983). However, about 14 years ago, this gene was made available, under licensing, to public geneticists.

Several investigations have provided evidence for the numerous advantages conferred by the leafy gene. Because stalk quality is affected by rind strength and pith quality (Zuber and Kang, 1978), it was deemed important to study parenchyma cell death in stalk internodes of leafy maize (Dronavalli et al., 1992). A positive relationship of pith cell death to the spread of stalk rotting fungi had previously been established (Pappelis, 1957; Pappelis et al., 1971; Kang et al., 1974). Gorman et al. (1992) evaluated seven leafy synthetics for resistance to aflatoxin production and reported relatively low levels of aflatoxin accumulation in the leafy maize. Dwyer et al. (1995) examined mean stem carbohydrates for two leafy hybrids and a check, finding that the larger drop in stem carbohydrates in leafy hybrid two could be an indication of greater translocation to the grain during early-grain-filling period of this hybrid.

Following the discovery of the leafy gene and recognition of its attributes, it was deemed important to pinpoint its location on a chromosome arm. Such knowledge can be useful in developing a more complete genetic map and in
possibly cloning the gene. The location of the $Lfy1$ gene to a chromosome arm could facilitate its transfer from one genotype to another more efficiently. Incorporation of the $Lfy1$ gene through reciprocal chromosomal translocations or other means into materials adapted to different environments could possibly boost maize yields.

Chromosomal translocations that have easily distinguishable phenotypic expression (i.e., semi-sterility of the pollen and ovules) are reliable markers for linkage tests with unplaced genes. Reciprocal chromosomal translocations (interchanges) have been used effectively to locate genes for both qualitative and quantitative traits in maize (Burnham, 1966; Kang et al., 1979).

**Nicosulfuron Susceptibility Gene ($nsf1$)**

The nicosulfuron susceptibility trait is controlled by a single recessive gene ($nsf1$) (Kang, 1993). The simple inheritance pattern for the susceptibility implied that if both inbred parents of a cross were homozygous recessive ($nsf1 \ nsf1$), the resultant $F_1$ hybrid would be susceptible. The commercial release of such a susceptible hybrid could be disastrous to growers, seed companies, and herbicide manufacturers.

Following the discovery of the $nsf1$ gene, its chromosomal location was deemed to be an important objective to facilitate its use as a genetic marker in linkage tests and in other genetic studies. If such information is available, chromosomes or chromosome segments with the desired gene can be transferred into breeding populations through reciprocal translocations, as described by
Burnham (1966). Transfer of desirable segments by means of B-A translocations also is possible (Peterson and Wernsman, 1964; Robertson, 1967).

The most rapid and reliable methods for locating genes to chromosomes or chromosome arms make use of hemizygosity (heterozygosity with a deficiency). A recessive gene (e.g., nsf1) can be located through observations in the F1 progeny when hemizygosity is created. Dosage-dependent genes and codominant genes also can be recognized in hemizygous progeny and can be located in the immediate generation. Dominant genes (e.g., Ly1) require a second-generation progeny test, which is usually definitive because of failure in transmission of the deficiency or inviability of the homozygote (i.e., in the critical cross, when the dominant factor is hemizygous, no recessive will segregate from the hemizygote).

Several procedures that make use of hemizygosity can be applied, including B-A translocations, monosomies, maintainable deficiencies, and induced deficiencies (Coe et al., 1988).

The most efficient technique for chromosomal location of nsf1 gene in maize is the B-A translocational analysis. Roman and Ullstrup (1951) described the use of B-A translocations to determine in a single generation the chromosome arm on which recessive gene controlling endosperm or a plant trait is located. B-A translocations have been used to detect chromosomal regions controlling fatty acid composition of embryo oil (Shadley and Weber, 1985), and high levels of sucrose in the maize endosperm (LaBonte and Juvick, 1991). B-A translocations for 19 of the 20 chromosome arms of the A complement are now available, which can
uncover more than 90% of the known map length through hypoploidy (hemizygosity) (Coe et al., 1988).

Because of the importance of the Lfy1 and nsf1 genes, it was deemed essential to locate them to respective chromosomal arms, so that breeders and geneticists could use them more effectively. Therefore, the main objective of this research was to pinpoint (a) via reciprocal chromosomal analysis, the chromosome arm that carries the Lfy1 gene, and (b) via B-A translocations, the chromosome arm that carries the nsf1 gene.

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CHAPTER 1. CHROMOSOMAL LOCATION OF THE GENE FOR LEAFINESS (\textit{Lfyl}) IN MAIZE BY USE OF RECIPROCAL TRANSLOCATIONS

Introduction

\textbf{Discovery of the Leafy Gene and Its Importance}

Maize breeders are always interested in obtaining, characterizing, and using new germplasm that may have resistance to stalk and ear rots, exhibit good stalk quality, have high yield potential, and/or possess other desirable attributes. Germplasm containing the leafy gene (\textit{Lfy1}) appears to have that potential (Shaver, 1983; Gorman et al., 1992; Dwyer et al., 1995). The leafy-gene materials are characterized by extra leaves above the ear, low ear placement, highly lignified stalk and leaf parts, and high yield potential (Shaver, 1983).

Yield advantage of a hybrid possessing the \textit{Lfy1} gene over its normal counterpart has been shown to be as large as 60\% [234 bushels per acre (14,674 kg ha\(^{-1}\)) vs. 146 bushels per acre (9,156 kg ha\(^{-1}\)) at 30,000 plants per acre (74,100 plants per hectare)] (Anonymous, 1986; Shaver, 1986). Additional advantages of the \textit{Lfy1} gene hybrids are superior stalk quality, drought tolerance, prolificacy, and a more extensive root system (Anonymous, 1986; Shaver, 1985, 1986). Synthetics, inbreds, and hybrids containing the leafy (\textit{Lfy1}) gene have been developed by Cornnuts, Inc.

The extra-leaf trait (\textit{Lfy1}), discovered by Robert C. Muirhead of Hughes Hybrids, Inc. in 1971, is controlled by a single dominant gene (Shaver, 1983). This gene has not been used extensively, because it was patented by a private
company, restricting its use (Anonymous, 1986; Shaver, 1983). However, about 14 years ago, this gene was made available, under licensing, to public geneticists.

Factors such as plant stress and insect damage have been associated with Aspergillus flavus infection and aflatoxin contamination in maize (Zuber and Lillehoj, 1979; Widstrom et al., 1990; Darrah and Barry, 1991). To test the hypothesis that genotypes with greater tolerance to these factors could enhance resistance to aflatoxin, Gorman et al. (1992) evaluated seven leafy synthetics for resistance to aflatoxin production and reported relatively low levels of aflatoxin accumulation in the leafy maize.

Dwyer et al. (1995) examined mean stem carbohydrates in two leafy hybrids and a check. Mean stem carbohydrates showed a small increase during the first 10 to 12 days after pollination (DAP), followed by a decline before 35 DAP, which was largest in leafy hybrid two. Between 40 and 60 DAP, concentration in leafy hybrid one fell slightly, whereas that in leafy hybrid two and the check rose. The larger drop in stem carbohydrates in leafy hybrid two could be an indication of greater carbohydrate translocation to the grain during early-grain-filling period of this hybrid.

Crabbe (1996) reported the benefit of using the Lfy1 gene in combination with the old dwarf gene in areas generally considered too cool for maize production (eastern and western Canada). According to Crabbe (1996), inbreds produced good leaf area for light absorption and photosynthesis, which led to higher yield. Moreover, since the plant is shorter, its moisture requirements are
low. The new leafy hybrid had an unusually extensive root system, possibly because the extra leaves promoted root growth. The leafy gene may also reduce weed competition as the plant’s canopy shades out weeds quickly.

Because of the above advantages of the \textit{Lfy1} gene, locating it to a chromosome arm was considered to be a worthwhile objective. Knowledge of chromosomal location of the gene can be useful in developing a more complete genetic map and in possibly cloning the gene. Furthermore, the location of the \textit{Lfy} gene to a chromosome arm could facilitate its transfer from one genotype to another more efficiently. Incorporation of the gene through reciprocal chromosomal translocations or other means into materials adapted to different environments could possibly increase yield.

\textbf{Reciprocal Translocations and Their Use}

Reciprocal chromosomal translocations (interchanges) have been used effectively to locate genes for both qualitative and quantitative traits in maize (Burnham, 1966; Kang et al., 1979). Semi-sterility, which is a characteristic of plants heterozygous for most interchanges, is a clearly classifiable character and can be used as a marker.

Information on specific chromosome or chromosome arm carrying the \textit{Lfy1} gene is of significance from two standpoints: (a) from an academic standpoint, the location of this gene is useful in studying its linkage relationships with other genes on the same chromosome. The knowledge of its location should improve the linkage map of maize and facilitate its use as a marker in genetic studies, and (b)
transfer of desirable segment or arm carrying the \textit{Lfy1} gene can be accomplished via chromosomal translocations. Therefore, the main objective of this research was to locate the \textit{Lfy1} gene to a specific chromosome or chromosome arm through reciprocal chromosomal translocational analyses.

**Literature Review**

**Reciprocal Translocations and Their Consequences**

A translocation represents the transfer (as well as the result of such a transfer) of a chromosome segment from its original position to another position in the genome. The chromosomes involved in a translocation function normally if each possesses a single centromere (acentric and dicentric events generally fail). Reciprocal translocations are exchanges of segments between nonhomologous chromosomes. The genetic and cytological consequences of reciprocal translocations result in neither loss nor gain of genetic information. Rather, there is only a rearrangement of genetic material. The presence of a translocation does not directly alter viability of individuals bearing it. A translocation may produce a position effect, because it may realign certain genes in relation to other genes, and translocations can change the length of chromosomes involved in a translocation (Sybenga, 1992).

Homologues heterozygous for a reciprocal translocation undergo uncommon synapses during meiosis. If chiasmata are formed in each of the paired arms, a ring of four chromosomes forms at metaphase, and pairing results in a crosslike configuration. Chromosomes will segregate in three different ways.
during anaphase I of meiosis. In adjacent I disjunction, non-homologous centromeres go to the same pole and in adjacent II disjunction, homologous centromeres go to the same pole. In both cases, because of deficiencies and duplications complete gametic abortion occurs. In alternate disjunction, alternate centromeres go to the same pole, and gametes formed are normal as neither deficiency nor duplication of chromosomal segments occurs (Figure 1.1). As few as 50% of the progeny of parents heterozygous for a reciprocal translocation may survive; this condition, called semi-sterility, has an impact on the reproductive fitness of organisms, thus playing a role in evolution (Sybenga, 1992). The chromosomes involved in a ring of four are not inherited independently; the two normal chromosomes are inherited as one group and the two translocated ones are inherited as another group (Klug and Cummings, 1994).

The inheritance of a translocation can be treated in linkage tests as if semi-sterility were produced by a single dominant gene ($T$). The $T$ marker of translocation heterozygotes offers advantages over conventional genetic markers in studying inheritance and linkage relations of genes determining agronomic characters. In particular, the presence of interchange chromosomes does not modify the plant phenotype or affect classification of traits being studied. Also, the frequent inhibition of recombination at the translocation point increases the distance over which linkage tests are effective (Carlson, 1988).

Translocations have proven useful in the placement of new gene mutations on chromosomes. Two general methods for testing the linkage of new markers
to translocations have been proposed (Anderson et al., 1955; Burnham, 1966). In one protocol, classification of semi-sterility ($T$) is utilized. A second method depends on classification of an endosperm marker which is closely linked to $T$. For the latter method, a series of chromosome-9 translocations has been developed in which each $T$ site is linked to the waxy ($wx$) locus. With this series, classification of $Wx$ vs. $wx$ phenotype in test-crosses is similar to classification of semi-sterile vs. fertile. If a trait shows linkage to the $wx$ locus in tests with all or many of the translocations, it must be located on chromosome 9. Otherwise, linkage to only one of the translocations places the gene on a specific chromosome other than 9 (Carlson, 1988).

Reciprocal chromosomal translocations have been used effectively to locate genes for both qualitative and quantitative traits (Burnham, 1966; Kang et al., 1979). In pollen, semi-sterility (50% empty pollen grains) is recognized easily on a black, opaque surface with a 30x or higher magnification pocket microscope in the field. On the ear, irregular seed set is observed, resulting from the abortion of half of the ovules.

Scott and Grogan (1969) and Scott and Nelson (1971) used the gene-marked chromosomal translocations to locate recessive genes in maize: the gene for susceptibility to atrazine was indicated to be on the long arm of chromosome 8, and two genes conditioning resistance to Maize Dwarf Mosaic (MDM) virus were located on the two arms of chromosome 6. These results were corroborated by Findley et al. (1973), using inbreds Oh7 and Mo22. However, they found
Figure 1.1. Pairing and disjunction of translocation heterozygotes.
additional genes for resistance on the long arm of chromosomes 1, 2, and 10 as well. In the same experiment, a gene for MDM resistance was associated with the long arm of chromosome 7 in Mo22, but not in Oh7. Major genes for resistance in Oh7 were associated with both arms of chromosome 6 and the short arm of chromosome 8. In Mo22, evidence pointed to a major gene being on the short arm of chromosome 10. Findley et al. (1973) suggested that different genetic systems controlled resistance to the various virus strains comprising the MDM complex, but the systems probably had some genes in common.

Kang et al. (1979) distinguished between semi-sterile and fertile plants by visually classifying anthers. Semi-sterile plants were usually characterized by shriveled anthers, whereas fertile plants had non-shriveled, normal anthers. Verification by observing seed-set at harvest led to the conclusion that 95% of plants were correctly classified as fertile or semi-sterile with the anther method. This method appeared to be simple and less time-consuming than the commonly used, pocket microscope method for pollen classification.

Dempsey and Rhoades (1990) localized, via a set of wx-translocation series ($T$-$wx$), $Mrh$-37962 mutant (high-dot kernels) to the short arm of chromosome 5. Neuffer (1993) used the same technique to locate the dominant wrinkled plant mutant ($Wrp1$) on the long arm of chromosome 2. He found a significant linkage for three translocations involving chromosome 2, but not for any others. Data indicated that $Wrp1$ was located on the long arm of chromosome 2 between $wx1$ T2-9b and T2-9d breakpoints. Similarly, Neuffer and England (1994)
located a bright yellow ethyl methane sulfonate (EMS) induced dominant yellow-green plant mutant (Yg*-2448) on the short arm of chromosome 1.

Harper et al. (1995) localized Mlg*-1 (multiple ligule) gene by T-wx translocation mapping technique. They crossed mlg*-1 heterozygotes to a T-wx series and Mlg progeny were test-crossed to wx. Mlg*-1 was found to be linked to both 3S and 3L translocations. A DNA study was done, using a DNA fragment specific to a lg3 gene, to confirm the results. No recombinants were found between the Mlg phenotype and restriction fragment length polymorphism (RFLP) markers, suggesting close linkage, and probable allelism between lg3 and mlg.


Recently, Jackson and Hake (1998) mapped the dominant fascicled (Fas1) mutation that causes enlargement and splitting of the primary inflorescence apical meristem. Fas1 plants have hugely branched ears and split central tassel branches. Analysis of the F2 progeny from several different translocation crosses indicated that Fas1 was located on the long arm of chromosome nine. This result was confirmed by a two-point-linkage test using the rolled leaf gene (Rld1). In the
F₂, they found that Fas₁ and Rld₁ were in repulsion phase linkage, (38 +/+; 89 Fas₁/+; 85 +/Rld; 27 Rld/Fas individuals), indicating a map distance of approximately 27 cM between Rld₁ and Fas₁.

**Materials and Methods**

Twenty-one translocation stocks were obtained from the Maize Genetics Cooperation Stock Center at the University of Illinois-Urbana in 1996. The cytological information on the position of break points was taken from Longley (1961) (Table 1.1). Plants homozygous for the translocations and non-leafy (Ify₁ Ify₁) were crossed with leafy (Lfy₁ Lfy₁) inbred lines 371 and 957 from July 20 to August 10, 1996. Each of the semi-sterile, leafy F₁ plant was test-crossed to commercial hybrid DynaGro (DG) 5510A and inbred line L668, both non leafy, from July 10 to August 8, 1997 (Figure 1.2). Seed was produced for most test-crosses, representing 16 different chromosome arms and one centromere. All of these interchanges involved chromosome nine. Thirty seeds of each available test-cross were planted on June 1, 1998 at the Ben Hur Plant Science Farm, Baton Rouge, LA, on a Commerce silt loam (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent) soil, in a one-row plot of 6.1 m length, with 90 cm between rows. Four replications of each test-cross were grown. At anthesis, plants were classified, by anther examination, as semi-sterile or fertile. Semi-sterile plants were usually characterized by shriveled anthers, whereas fertile plants had non-shriveled anthers. The classification was verified by observing seed-set at harvest. Data were analyzed for each test-cross (all plants of each test-cross were
Parents: T4-9b

Inbred line 957

Gametes:

Test-cross

Figure 1.2. Diagram of the four products from a test-cross of a normal-leafy parent by a non-leafy plant, homozygous for the translocation T4-9b.
grouped to test for homogeneity). Differences between semi-sterile and fertile plants (pollen classes) for the leafy trait were tested for significance with the chi-square and the log likelihood ratio test statistics. Significant deviation from the 1:1:1:1 ratio (1 fertile:1 semi-sterile with respect to the translocation and 1 \textit{Lfy1 Ify1} : 1 \textit{Ify1 Ify1} for the gene) was expected for the critical test-cross (the one in which the gene of interest is identified). If there was no linkage between the non-leafy trait and semi-sterility (i.e., some non leafy plants were fertile and vice versa), I concluded that the two chromosome arms involved in the translocation did not carry the leafy gene.

To confirm the location of \textit{Lfy1} gene, I examined the results from another translocation involving only one of the chromosomes (specifically a chromosome arm) involved in the previously implicated translocation.

\textbf{Table 1.1. Reciprocal translocation stocks used (Longley, 1961).}

<table>
<thead>
<tr>
<th>Designation</th>
<th>Translocation</th>
<th>Breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{wx 30A}</td>
<td>T1-9c</td>
<td>1S.48-9L.22$^+$</td>
</tr>
<tr>
<td>\textit{wx 30B}</td>
<td>T1-9 (4995)</td>
<td>1L.19-9S.20</td>
</tr>
<tr>
<td>\textit{wx 30C}</td>
<td>T1-9 (8389)</td>
<td>1L.74-9L.13</td>
</tr>
<tr>
<td>\textit{wx 31B}</td>
<td>T2-9b</td>
<td>2S.18-9L.22</td>
</tr>
<tr>
<td>\textit{wx 31A}</td>
<td>T2-9c</td>
<td>2L.49-9S.33</td>
</tr>
<tr>
<td>\textit{wx 32A}</td>
<td>T3-9 (8447)</td>
<td>3S.44-9L.14</td>
</tr>
<tr>
<td>\textit{wx 32B}</td>
<td>T3-9 (8562)</td>
<td>3L.65-9L.22</td>
</tr>
<tr>
<td>\textit{wx 34C}</td>
<td>T4-9b</td>
<td>4L.90-9L.29</td>
</tr>
</tbody>
</table>

(Table con'd)
Results and Discussion

The mean observed number of semi-sterile and fertile plants in each test-cross corresponded with the 1:1 segregation ratio. This verified the transmission of the translocation breakpoints, which is important for the reciprocal translocation system to work (Table 1.2).

Table 1.2. Mean observed number of semi-sterile and fertile plants in each test-cross in 1998 planting.

<table>
<thead>
<tr>
<th>Test-cross</th>
<th>Semi-sterile</th>
<th>Fertile</th>
<th>Probability of fit to 1:1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T x 371) x L668</td>
<td>29</td>
<td>28</td>
<td>0.90-0.50</td>
</tr>
<tr>
<td>(T x 371) x DG 5510A</td>
<td>35</td>
<td>33</td>
<td>0.90-0.50</td>
</tr>
</tbody>
</table>

\(^{\dagger}\) short arm of chromosome 1, and long arm of chromosome 9, respectively.
\begin{table}
\centering
\begin{tabular}{lccc}
\hline
Genotype & \text{Frequency} & \text{Expected} & \text{Observed} \\
\hline
(T x 957) x DG 5510A & 20 & 25 & 0.50-0.10 \\
(T x 957) x L668 & 19 & 21 & 0.90-0.50 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{f} denotes reciprocal translocation.

A significant difference among test-crosses implied that the \( Lfy \) gene might be located in either chromosome involved in that specific translocation, specifically on one of the two chromosome arms involved in the translocation. Inspection of data indicated major deviations from a 1:1:1:1 segregation ratio in the test-crosses involving the translocations T3-9 (8447), T4-9b, T4-9 (5657), and T5-9a (Table 1.3). An examination of the breakpoints indicated that the gene conditioning leafiness (\( Lfy1 \)) could be located on one of the following arms: short arm of chromosome 3 or 9, long arm of chromosome 4, 5, or 9. Upon observing the probability values for these test-crosses individually, and comparing them with others involving the short arm of chromosome 9 and the long arm of chromosome 5 and 9, it was apparent that the test-crosses involving the long arm of chromosome 4 [T4-9b, and T4-9 (5657)] were the only ones, highly significantly different (Table 1.4). Therefore, we concluded that the gene conditioning leafiness (\( Lfy1 \)) in maize is located on the long arm of chromosome 4 (Figure 1.3).

Data revealed that some leafy plants were semi-sterile and some non-leafy were fertile in the critical test-crosses (Table 1.3). This was unexpected in an experiment that involves only one major gene. It was assumed that these slight discrepancies could be explained firstly, by the occurrence of crossing over between the translocation breakpoint and the gene. Crossing over in translocation
Figure 1.3. Cytological map of chromosome 4 of maize indicating the location of the leafy gene (Lfy1). The arms divisions, centromere (empty circle), and overall length are marked proportionally (number on left side). (modified from Neuffer et al., 1997).
heterozygotes is usually lower than for homologous regions in standard or normal chromosomes. This reduction in crossing over is produced by imperfect synapsis in pachynema. In some cases, translocation heterozygosity does not show any inhibitory effect on crossing over (Anderson et al., 1955; Maguire, 1968). A second cause of inconsistencies in the data, could be attributed to environmental effects. For instance, the uncommon drought stress that occurred in 1998, could have increased the experimental error.

The procedure used here to locate the gene for leafiness can be extended to obtain estimates of the type of gene action conditioning the trait. If the gene for leafiness were completely dominant and I did not detect any crossover or high experimental error, I would expect all semi-sterile plants to be non-leafy and those fertile plants to be leafy. On the other hand, if a gene for non-leafiness were dominant, I would expect all semi-sterile and fertile plants to be non-leafy.

A definitive location of Lfy1 on the long arm of chromosome 4 awaits linkage studies with known markers on this chromosome. Linkage between Lfy1 and phenotypic markers on 4L, such as glossy-3 (gl3), colorless aleurone (c2), and distal pale (dp1), or molecular markers such as UMC111, BNL323, and UMC52 (Coe et al., 1988) would provide data to accurately map Lfy1 on the chromosome.

A tight linkage between one of the recessive markers and the Lfy1 would provide a convenient way to identify leafy plants in segregating populations at the seedling stage. The recessive markers gl3, c2, and dp1 are identifiable at the seedling stage prior to making crosses. If these phenotypic markers are not linked
to the *Lfy1* gene; RFLPs, RAPDs, or isozymes could be used as molecular markers. They have advantage over phenotypic markers in that plants can be identified in heterozygous condition and are more numerous.

This study provided valuable information, about the location of the *Lfy1* gene in the maize genome, and has paved the way for new experiments to extend the limited current knowledge of this important agronomic/economic gene. With the location of the *Lfy1* gene known, its interaction with flanking markers could be studied, which might result in a better understanding of its effect on yield and other desirable traits.

**Table 1.3. Summary of semi-sterile and fertile plants and leafy and non-leafy plants observed in the test-crosses.**

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Breakpoint</th>
<th>Semi-sterile</th>
<th>Fertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leafy</td>
<td>Non-leafy</td>
<td>Leafy</td>
</tr>
<tr>
<td>T1-9c</td>
<td>1S.48-9L.22</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>T1-9(4995)</td>
<td>1L.19-9S.20</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>T1-9(8389)</td>
<td>1L.74-9L.13</td>
<td>32</td>
<td>61</td>
</tr>
<tr>
<td>T2-9b</td>
<td>2S.18-9L.22</td>
<td>47</td>
<td>70</td>
</tr>
<tr>
<td>T2-9c</td>
<td>2L.49-9S.33</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td>T3-9(8447)</td>
<td>3S.44-9L.14</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>T3-9(8562)</td>
<td>3L.65-9L.22</td>
<td>32</td>
<td>51</td>
</tr>
<tr>
<td>T4-9b</td>
<td>4L.90-9L.29</td>
<td>26</td>
<td>77</td>
</tr>
<tr>
<td>T4-9e</td>
<td>4S.53-9L.26</td>
<td>22</td>
<td>41</td>
</tr>
<tr>
<td>T4-9g</td>
<td>4S.27-9L.27</td>
<td>29</td>
<td>53</td>
</tr>
<tr>
<td>T4-9(5657)</td>
<td>4L.33-9S.25</td>
<td>27</td>
<td>78</td>
</tr>
</tbody>
</table>

(Table con'd)
Table 1.4. Probability values for the log likelihood ratio test statistics of homogeneity between semi-sterile and fertile plants for four sets of test-crosses to DG 5510A and L668.

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Breakpoint</th>
<th>371</th>
<th>957</th>
<th>371</th>
<th>957</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-9c</td>
<td>1S.48-9L.22</td>
<td>0.529</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-9 (4995)</td>
<td>1L.19-9S.20</td>
<td>0.479</td>
<td>0.254</td>
<td>0.472</td>
<td>0.276</td>
</tr>
<tr>
<td>T1-9 (8369)</td>
<td>1L.74-9L.13</td>
<td>0.155</td>
<td>0.708</td>
<td>0.881</td>
<td></td>
</tr>
<tr>
<td>T2-9b</td>
<td>2S.18-9L.22</td>
<td>0.105</td>
<td>0.822</td>
<td>0.272</td>
<td>0.331</td>
</tr>
<tr>
<td>T2-9c</td>
<td>2L.49-9S.33</td>
<td>0.537</td>
<td>0.557</td>
<td></td>
<td>0.402</td>
</tr>
<tr>
<td>T3-9 (8447)</td>
<td>3S.44-9L.14</td>
<td>0.820</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3-9 (8562)</td>
<td>3L.65-9L.22</td>
<td></td>
<td>0.117</td>
<td>0.856</td>
<td>0.511</td>
</tr>
<tr>
<td>T4-9b</td>
<td>4L.90-9L.29</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>T4-9e</td>
<td>4S.53-9L.26</td>
<td>0.533</td>
<td></td>
<td></td>
<td>0.907</td>
</tr>
<tr>
<td>T4-9g</td>
<td>4S.27-9L.27</td>
<td>0.383</td>
<td>0.119</td>
<td></td>
<td>0.962</td>
</tr>
</tbody>
</table>

*, ** Significantly different from the 1:1:1:1 ratio at the 0.05 and 0.01 probability levels, respectively.
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>T4-9(5657)</td>
<td>4L.33-9S.25</td>
<td>0.001</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>T5-9a</td>
<td>5L.69-9S.17</td>
<td>—</td>
<td>0.195</td>
<td>—</td>
</tr>
<tr>
<td>T5-9 (4817)</td>
<td>5L.06-9S.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T5-9 (8386)</td>
<td>5L.87-9S.13</td>
<td>0.889</td>
<td>0.382</td>
<td>—</td>
</tr>
<tr>
<td>T6-9 (4778)</td>
<td>6S.80-9L.30</td>
<td>0.279</td>
<td>0.213</td>
<td>—</td>
</tr>
<tr>
<td>T6-9 (4505)</td>
<td>6L.13-9ctr</td>
<td>0.552</td>
<td>0.658</td>
<td>—</td>
</tr>
<tr>
<td>T6-9 (8768)</td>
<td>6L.89-9S.61</td>
<td>—</td>
<td>0.308</td>
<td>—</td>
</tr>
<tr>
<td>T7-9a</td>
<td>7L.63-9S.07</td>
<td>0.265</td>
<td>0.140</td>
<td>0.264</td>
</tr>
<tr>
<td>T8-9d</td>
<td>8L.09-9L.16</td>
<td>0.096</td>
<td>0.951</td>
<td>0.338</td>
</tr>
<tr>
<td>T8-9 (6673)</td>
<td>8L.35-9S.31</td>
<td>0.290</td>
<td>0.160</td>
<td>0.392</td>
</tr>
<tr>
<td>T9-10b</td>
<td>9S.13-10S.40</td>
<td>0.201</td>
<td>—</td>
<td>0.210</td>
</tr>
</tbody>
</table>

— denotes no seed available.

References


CHAPTER 2. CHROMOSOMAL LOCATION OF THE NICOSULFURON SUSCEPTIBILITY GENE (nsfl) IN MAIZE BY USE OF B-A TRANSLOCATIONS

Introduction

Accent (active ingredient: nicosulfuron) \{2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide\} herbicide, manufactured by Dupont, was approved as a selective herbicide to control johnsongrass \(\text{Sorghum halepense}\) (L.) Pers.) in maize \(\text{Zea mays}\) L.). However, Kang (1993) discovered in his maize nursery in 1992 that not all field maize was resistant to the herbicide. He reported that of 223 public inbreds, 198 were resistant and 25 were susceptible to nicosulfuron. Furthermore, Kang's observation of 3 resistant to 1 susceptible segregation ratio in two F\(_2\) populations suggested that susceptibility to nicosulfuron was controlled by a single recessive gene. The new gene was named nsfl (Kang, 1993).

Following the discovery of a new gene, the next important step is to know its chromosomal location. Pinpointing the nsfl gene to a specific chromosome arm is important to facilitate its use as a genetic marker in linkage tests and in other genetic studies. If such information were available, chromosomes or chromosome segments with the desired gene could be transferred into breeding populations through reciprocal translocations, as described by Burnham (1966). Transfer of desirable chromosome segments by means of B-A translocations is also possible (Peterson and Wernsman, 1964; Robertson, 1967). Transformation
of susceptible plants via Particle Gun Bombardment (Howe and Smith, 1989; Christou et al., 1991) also should be possible.

An efficient technique for chromosomal location of recessive genes in maize, is the B-A translocational analysis. Roman and Ullstrup (1951) described the use of B-A translocations to determine in a single generation the chromosome arm on which a recessive gene controlling endosperm or a plant character is located.

B-A translocations involve an interchange between a supernumerary B chromosome and a member of the normal maize (A) chromosome (Roman, 1947) (Figure 2.1). B-A translocations are now available for 19 of the 20 chromosome arms in maize (Beckett, 1991); only the short arm of chromosome 8 does not have B-A translocation available at this time. It is estimated that the available B-A translocation can uncover recessive genes in more than 90% of the maize genetic map (Coe et al., 1988). The main objective of this research was to pinpoint, via B-A translocational analysis, the chromosome arm that carries the nsf1 gene.

**Literature Review**

**B-A Translocations**

Maize normally has 10 pairs of chromosomes, but supernumerary or extra chromosomes, known as B-type chromosomes, are of frequent occurrence in various commercial varieties and genetical cultures (Randolph, 1941). The B chromosome of maize has been studied extensively. The presence or absence of B chromosomes in plants of a maize population can only be determined
cytologically; that is, morphological effects of B chromosomes are not readily evident (Randolph, 1941). The B chromosome modifies crossing over among A chromosomes, alters timing of the mitotic cycle, and controls a system of B chromosome non-disjunction in the pollen (Carlson, 1994).

During microsporogenesis, the B chromosomes, if present, show non-disjunction at the second mitotic division of the microspore, giving one sperm nucleus in a pollen grain with two B chromosomes and one with no B chromosome (Figure 2.1). It is this cytological behavior of the B chromosome that lends itself to uncovering genes for both endosperm and plant traits through the resultant condition called hemizygosity.

Roman (1947) developed a series of translocations between the B chromosome and members of the normal (A) chromosome, combining the B with numerous markers. He showed that the A^B (A centromere) chromosome in a translocation was perfectly stable in the pollen, whereas the B^A (B centromere) retained the non-disjunction characteristic of the original B chromosome.

The B-A translocation technique offers an efficient system for determining, in the F_1, the chromosome arm on which recessive endosperm and plant factors are located (Roman and Ullstrup, 1951). The effective use of B-A translocations is based on the behavior of the B chromosome when transmitted through the male parent. The B chromosomes carry no alleles of genes found in the A chromosomes and are relatively inert (Randolph, 1941).
Roman (1947) established that the non-disjunction of the B centromere in the division of the generative nucleus of the microspore results in a pollen grain with two sperm nuclei that differ in their constitution with respect to the translocated A chromosome segment (one will be deficient - hypoploid, and the other will have an extra segment - hyperploid) (Figure 2.2).

Depending upon how these aberrant sperm nuclei unite with the egg and the polar nuclei of the embryo sac, two classes of seeds will result: (1) Seeds with an embryo hypoploid for the segment translocated to the B centromere and an endosperm hyperploid for this segment (Class I seed) and (2) seeds that are hyperploid in the embryo and hypoploid in the endosperm for this segment (Class II seed). A third type of seed will result from normal male and female gametes (Class III seed) (Figure 2.3). For plant traits, Class I seed (hypoploid embryo) is critically examined and analyzed, whereas for endosperm traits, Class II seed (hypoploid endosperm) is relevant.

B-A translocations are useful for a variety of genetic studies by virtue of their ability to produce viable male gametes either lacking part of a chromosome arm or carrying two copies. As a result, most recessive and codominant mutants, including RFLPs, amplified fragment-length polymorphism (AFLP), etc., are readily identified in the F₁, thus simplifying their assignment to respective chromosome arms (Beckett et al., 1997).

Researchers have used the B-A translocations approach to locate numerous genes in the maize genome. B-A translocation was used to identify
Figure 2.1. Production of a B-A translocation by breakage of a normal (A) chromosome at the sites indicated by arrows, followed by rejoining of broken ends (modified from Beckett, 1978).

Figure 2.2. Development of a pollen grain containing a B-A translocation (modified from Roman, 1947).
Parents:

Gametes:

F₁ seeds:

Class I seed
Hypoploid embryo

Class II seed
Hyperploid embryo

Class III seed
Normal

Figure 2.3. Diagram of three seed classes produced from a cross of a normal seed parent by a plant homozygous for TB-7Sc (modified from Roman and Ullstrup, 1951).
chromosomal regions controlling fatty acid composition of embryo oil (Shadley and Weber, 1985) and high levels of sucrose in the maize endosperm (LaBonte and Juvik, 1991). Song and Lu (1991) located a recessive opaque endosperm small germ gene (os) on the short arm of chromosome two; this conclusion was confirmed by use of wx-marked translocations. Scanlon et al. (1991) mapped a large number of defective kernel mutants (deks) to specific chromosome arms. These deks are useful in transposon tagging experiments of seed developmental loci.

Stinard (1991a) crossed known heterozygotes of mn3 (miniature-3: a viable miniature kernel mutant) with TB-6Lc and TB-6Sa but got only normal sized kernels, indicating that mn3 is proximal to the TB breakpoints on chromosome six. A new recessive mutant conditioning aleurone mosaicism (Crinkly-4, cr4) was uncovered by crossing it to TB-10Sc. To confirm the placement of this mutant to a chromosomal arm, 10 mosaic kernels from the TB-10Sc cross were planted in the field, and the resulting plants selfed and outcrossed to both oy and y9 testers. All 10 plants grown from mosaic kernels were hyperploids, confirming that this mutant is located on the short arm of chromosome 10 (Stinard, 1991b).

Albertsen et al. (1993) described and mapped the recessive mutant tassel-less (tls1) to the distal one third of chromosome 1L (long arm of chromosome 1). They confirmed the location of tls1 using the following RFLP probes on chromosome one: bnl8.10, bnl7.25, bnl8.29, php15058,php20557, and bnl6.32. Stinard and Schnable (1993) located the recessive gene o12 (opaque endosperm)
on the short arm of chromosome four distal to the TB-4Sa breakpoint. This gene conditions aleurone mosaicism. Stinard et al. (1993) located a new recessive etched endosperm kernel mutant (et2) on the short arm of chromosome two, distal to the TB-2Sa breakpoint.

Barkan et al. (1994), working with nuclear mutations affecting chloroplast biogenesis, found that the crp1 (nuclear mutation that causes the loss of cytochrome f/b6 complex and failure to accumulate monocistronic petB and petD mRNAs) was uncovered in crosses with TB-7Lb. Thus, they pinpointed it to the long arm of chromosome seven. The cps1 (nuclear gene required for chloroplast protein synthesis) was placed on the long arm of chromosome one. From an investigation using B-A translocations, Wright (1995) indicated that the gene conditioning high oleic maize oil (old) is located on the long arm of chromosome one. Similarly, Scanlon and Freeling (1995) demonstrated that the ns (narrow leaf and plant stature mutant narrow sheaths) duplicate factors are located on chromosome arms 3S (ns1) and 4L (ns2). They used molecular markers (RFLP) to locate the ns loci more precisely.

Barkan and Roy (1995) displayed mapping results of nuclear mutations affecting chloroplast biogenesis. They mapped the psb1 (mutation that causes the specific loss of photosystem II) to the long arm of chromosome six, and the psb2 (another mutation that causes the specific loss of photosystem II, which is not allelic to psb1) to the long arm of chromosome five.
Zhou and Li (1997) placed a new male sterile gene, which was tentatively called msx, on chromosome 4 with a recombination value of 15-20% with su1 (sugary gene). Stinard (1997) pinpointed gl7 (glossy gene) and b17 (virescent gene) on the short arm of chromosome 4. In a recent study, Stinard and Jackson (1998) applied the B-A translocation technique to locate a selected subset of the symbolized but unplaced mutants from the Maize Genetics Stock Center collection. They pinpointed the mutants l3, l4, oro2, pb4, v13, vp10, and vp12 from analyzing crosses involving: TB-6L, TB-7Sc, TB-1Sb-2L(4464), TB-5La, TB-5Sc, TB-10L(19), and TB-5La, respectively.

The translocations between the supernumerary B chromosome and the normal A chromosomes of maize have been a valuable tool for many manipulations in maize genetics. Marker systems relying on the anthocyanin pigment genes and their transposable element derivatives have been developed for the phenotypic recognition of these kernel types (Birchler and Alfenito, 1993). The mentioned systems utilized the B-A translocations as a male parent.

To expand the utility of the B-A translocations, Birchler and Guo (1997) described marker systems that permit the recognition of aneuploid kernels derived from transmission of extra chromosomes through the female parent. These systems rely on transposable elements in anthocyanin pigment genes that exhibit different patterns of transpositions depending on the dosage in the genotype.

Newton and Schwartz (1980), Evola et al. (1986), and Burr et al. (1988) demonstrated the usefulness of B-A translocations in mapping both isozymic and
RFLP loci in maize. Weber and Helentjaris (1989) determined the breakpoints of 24 different B-A translocations in their RFLP map. They identified the short and long arms of each chromosome, the chromosomal regions containing the centromeres, and correlated their RFLP map with the cytological and conventional genetic maps. Cheng and Lin (1998) used B-A translocations to isolate AFLPs on chromosome 10. They used two B-A translocations associated with the same chromosome arm but with different break positions (TB-10L19 and TB-10L32) to locate 47 AFLPs on the long arm of chromosome 10. Lin and Chang (1998) defined the map region of the centromere on chromosome one and nine using B-A translocations with the most proximal breakpoints on both arms of chromosome one and nine. The mapping strategy was based on the fact that the centromere is located between the breakpoints of the two most proximal translocations: one in the short arm and the other in the long arm of the same chromosome. The RFLP marker closest but distal to the translocation breakpoint on both arms defined the map region of the centromere. According to this study, the centromere was located between the bnl5.10 - umc20 marker intervals.

**Nicosulfuron and the nsf1 Gene**

Nicosulfuron is a sulfonylurea herbicide introduced to the U.S. maize market in 1990. It is one of the first selective postemergence herbicides that effectively control perennial and annual grasses as well as some broadleaf weeds in maize (Foy and Witt, 1990). As a member of the sulfonylurea family of herbicides, nicosulfuron inhibits the acetolactate synthase (ALS), the first common
enzyme of valine, leucine, and isoleucine biosynthesis, in susceptible plants (Chaleff and Mauvais, 1984; Ray, 1986).

Sulfonylurea herbicides are highly specific and have a wide weed spectrum of control (Neighbors and Privalle, 1990). This family of herbicides is used in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), maize, soybeans [*Glycine max* (L) Merr], and oilseed rape (*Brassica napus* L.), with additional crop selective compounds in cotton (*Gossypium hirsutum* L.), potatoes (*Solanum tuberosum* L.), and sugarbeet (*Beta vulgaris* L.) (Brown, 1990).

The primary basis for selectivity of sulfonylurea herbicides is differential rates of herbicide metabolism. Tolerant species rapidly detoxify sulfonylureas to herbicidally inactive products, whereas the metabolism is much lower and less extensive in susceptible species (Obrigawitch et al., 1990). Nicosulfuron was metabolized within 20 hours in maize (resistant), while there was no perceptible metabolism in johnsongrass (susceptible) even after 24 hours (Obrigawitch et al., 1990).

Nicosulfuron and primisulfuron (the two most important sulfonylureas used in maize) herbicides display differential selectivity between species, between varieties within species, and between herbicides within species (Burton et al., 1992; Diehl et al., 1993; Green and Ulrich, 1993). Herbicide metabolisms have been corroborated as the primary basis for these responses (Burton et al., 1992; Diehl et al., 1993); however, other factors have been implicated as possible contributors as well (Camacho and Moshier, 1991; Green and Ulrich, 1993).
Varietal susceptibility is an important factor for all herbicides. Most herbicides show varietal differences and even kill some plants in breeding populations. Over time, herbicides used in breeding programs and performance trials select against susceptible varieties and eliminate them from commercial production. Such a situation began with the sulfonylurea maize herbicides, given the many favorable characteristics and the rapid acceptance in many herbicide markets of this family of herbicides (Green and Ulrich, 1993; Green, 1998).

Weed researchers generally use maize hybrids in herbicide trials (Vidrine and Girlinghouse, 1991), assuming that results will apply to all types of maize, including inbreds. However, because recessive genes in inbred lines are masked by their dominant alleles in a heterozygous hybrid, susceptibility controlled by recessive gene(s) would remain undetected. The discovery of the nicosulfuron susceptibility gene in maize suggested a need for including both hybrids and inbred lines in herbicide trials before researchers and/or chemical companies recommend any herbicide for general use (Kang, 1993).

In the past, commercial herbicides were selected over time against susceptible lines in breeding populations and performance trials, which eliminated susceptible germplasm from production. However, today the faster introduction of new herbicides and hybrids and greater fragmentation of the market no longer ensures the elimination of susceptible germplasm within a product's life cycle. Maize breeders would achieve greater efficiency if they could transfer the nicosulfuron resistant gene into elite but nicosulfuron susceptible inbred lines. The
identification and use of inherent resistance will be the most efficient and least controversial solution for crop safety (Green, 1998).

**Materials and Methods**

A set of 15 B-A chromosomal translocations, and their corresponding testers were obtained from the Maize Genetics Cooperation Stock Center, at the University of Illinois-Urbana in 1996 (Table 2.1). I crossed plants homozygous for B-A translocations and resistant to nicosulfuron as male parents with a susceptible inbred line (L668) from July 20 to August 10, 1996. Each B-A translocation also was crossed to a known recessive tester to validate the occurrence of the deficient sperm that is necessary for the B-A translocation system to work. Each tester stock contained a recessive mutant marker allele with its locus beyond the breakpoint for a specific B-A translocation. Endosperms or embryos from the crosses that express the mutant phenotype in hemizygous condition allow verification of the transmission of a translocation. The details of the B-A translocational analyses are given by Beckett (1978, 1991).

Each (L668 x Translocation) cross resulted in three classes of seed on a pollinated ear. Since nssf controls a plant trait, I was interested in the hypoploid embryo (Class I seed) where the gene of interest is uncovered (hemizygous condition). The Class II seeds were the smallest in size, as they represented hypoploid endosperm (Figure 2.3). Thus, these seeds were easily removed. However, because Class I and Class III seeds are indistinguishable on the basis of seed size (both these categories represent normal size seed), I planted Class
I and Class III seeds on May 9, 1997, and on June 1, 1998, at the Ben Hur Plant Science Farm to distinguish between the two seed classes. Class I seeds produced hypoploid plants (shorter in stature and upright leaves) and Class III seeds produced plants of normal size (Table 2.2). The hypoploid plants represented hemizygosity for a portion of an A-type of chromosome. Nicosulfuron (46.2 g/ha) was applied as a postemergence herbicide when maize was at the 4-to-6-leaf stage. About 7-10 days later, susceptible maize plants exhibited visible symptoms of injury due to nicosulfuron (bronzing of leaves and retardation of plant growth). I visually classified the plants as resistant or susceptible. The F₁ progeny size, on the average, was 51 plants for each cross. This sample size was considered large enough to include some hypoploid plants in a cross. When the procedure is used to locate a recessive gene, all hypoploid plants (class I seed) from a critical cross (the one in which the gene of interest is uncovered) should be susceptible (nsf1) and all normal plants (class III seed) resistant (Nsfl nsf1). However, when a chromosome involved in a B-A translocation does not carry the gene, hypoploid plants should be expected to be resistant (Nsfl nsf1) to the herbicide. Similarly, all the normal plants should be expected to be resistant (Nsfl nsf1).

Table 2.1. B-A translocation stocks used (Beckett, 1991)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Arm (s) uncovered by translocation†</th>
<th>Testers‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-1Sb</td>
<td>1S.05—</td>
<td>vp5 or dek1§</td>
</tr>
</tbody>
</table>

(Table con'd)
<table>
<thead>
<tr>
<th>chromosome</th>
<th>break point</th>
<th>allele notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-1La</td>
<td>1L.20→</td>
<td>bz2 R1-scm2 or lw1</td>
</tr>
<tr>
<td>TB-1Sb-2L</td>
<td>1S.05-.53, 2L.28→</td>
<td>w3</td>
</tr>
<tr>
<td>TB-3Sb</td>
<td>3S.5→</td>
<td>Cl1 Clm1-3</td>
</tr>
<tr>
<td>TB-3La-2S</td>
<td>3L.10-.60, 2S.46→</td>
<td>y3 r2</td>
</tr>
<tr>
<td>TB-4Sa</td>
<td>4S.25→</td>
<td>su1 bt2</td>
</tr>
<tr>
<td>TB-5La</td>
<td>5L.1</td>
<td>Pr1 R1-scm2 or lw2</td>
</tr>
<tr>
<td>TB-6Sa</td>
<td>6S.5 (middle of nucleolus organizer)→</td>
<td>dek28</td>
</tr>
<tr>
<td>TB-6Lc</td>
<td>6L.11→</td>
<td>y1 su2</td>
</tr>
<tr>
<td>TB-7Sc</td>
<td>part of 7S</td>
<td>vp9 or sh6 or y8</td>
</tr>
<tr>
<td>TB-7Lb</td>
<td>7L.30→</td>
<td>o5</td>
</tr>
<tr>
<td>TB-8Lc</td>
<td>8L.24±.05→</td>
<td>pro1</td>
</tr>
<tr>
<td>TB-9Sb</td>
<td>9S.4→</td>
<td>c sh bz</td>
</tr>
<tr>
<td>TB-10Sc</td>
<td>10S.3 (or closer)→</td>
<td>y9</td>
</tr>
<tr>
<td>TB-10L (19)</td>
<td>10L, centromere→</td>
<td>r1</td>
</tr>
</tbody>
</table>

† Each break point is given as a decimal fraction of the distance from the centromere to the end of the chromosome arm; the arrow indicates that the portion of the arm beyond the break point is uncovered by the translocation.

‡ Kernel traits.

§ For description of gene symbols, see Mutants of Maize (Neuffer et al., 1997).

**Results and Discussion**

The seeds of Class I (hypoploid embryo, but hyperploid endosperm) and Class III (normal embryo and endosperm) being indistinguishable on the basis of their size, were classified after planting (Table 2.3). The genetic constitutions of the embryo and endosperm for Class I and Class III seeds or plants are illustrated in Figure 2.3. Class I seeds produced hypoploid plants that were shorter in stature and had upright leaves and Class III seeds produced normal, taller plants. For the critical B-A translocation, that is, the one in which the gene was uncovered...
because of the hemizygous condition for the portion of the chromosome carrying the gene, all hypoploid plants (nﬂ-) were expected to be susceptible to nicosulfuron as only the recessive allele, nﬂ, was present in them, whereas all the normal plants were expected to be resistant to nicosulfuron (Nsf1 nsf1). In a non-critical F1, that is, one in which the gene was located on a chromosome other than the one involved in that particular translocation, hypoploid plants were resistant, similarly, the normal plants also were resistant to nicosulfuron.

Inspection of the data (Table 2.3) indicates that the critical cross was L668 X TB-7Sc, as no resistant hypoploid plants were observed and all the normal plants were resistant. I believe that most of the susceptible maize plants that eventually died in the 1997 planting were hypoploids. To confirm that hypoploid plants did exist in this critical cross, 125 seeds (Class I and Class III) were planted in a greenhouse at the San Gabriel Research Station in 1997. Nine hypoploid plants were observed (7%). I also confirmed the presence of hyperploid plants by planting 75 Class II seeds (hyperploid embryos and hypoploid endosperm) and spraying them with nicosulfuron, that all plants were resistant to the herbicide, as would be expected in a critical cross. In the summer of 1998, the presence of hypoploid plants was confirmed in an untreated plot containing the same F1 progeny from the crosses of the B-A translocation series with inbred line L668 (Table 2.2).

Other crosses that showed somewhat similar results in 1997 as L668 x TB-7Sc were L668 x TB-3Sb and L668 x TB-1La. However, I think that there probably
were no hypoploid plants in these crosses because of a small sample size and consequently, no resistant hypoploid plants were observed. The data collected only showed the absence of hypoploid plants. The results were confirmed after obtaining additional data in the summer of 1998 (Table 2.2). Seeds from three new B-A translocations were included for the second year of planting. Since the pattern of segregation in the L668 x TB-7Sc cross is that of a critical one, I concluded that the nsf1 gene is located on the short arm of chromosome 7 (Figure 2.4).

A conclusive pinpointing of nsf1 on 7S will require other genetic studies, such as linkage test analyses (two-point or three-point linkage) using known phenotypic or molecular markers on this chromosome [i.e. vp9, v5, bnl15.40, or psu2 (bZip)]. However, data from B-A translocations are considered more reliable than recombinational data because B-A data are qualitative while recombinational data are quantitative in nature (Weber and Helentjaris, 1989). It is also advisable to back up this experiment by another procedure, such as chromosomal reciprocal translocational analysis, methodology described elsewhere (Kang et al., 1979).

Clearly, B-A translocations technique is an exceedingly powerful tool in mapping recessive genes. For instance, in RFLP mapping studies, B-A translocations provide much additional information (i.e., order of loci tightly clustered around the centromeric region, location of centromeres, etc.) about an RFLP map. Currently, certain probes analyzed using B-A translocations are used by several researchers as their RFLP maps are being constructed, and this will...
Figure 2.4. Cytological map of chromosome 7 of maize indicating the position of the nicosulfuron susceptibility gene (*nsfl*). The arms divisions, centromere (empty circle), and overall length are marked proportionally (number on the left side). (modified from Neuffer et al., 1997.)
enable them to identify the short and long arms of their linkage groups (Weber and Helentjaris, 1989).

The positions of the breakpoints on the cytological and conventional genetic maps are known for most B-A translocations (Hoisington et al., 1988). The determination of the breakpoints on the RFLP map allows these three maps (cytological, genetic, and RFLP maps) to be better correlated.

This research provides consistent information about the location of \textit{nsf1} gene. Comprehensive knowledge of gene location should permit efficient assembly of genotypes for improving desirable agronomic traits and help develop a marker system that enables identification of the \textit{nsf1} gene in segregating populations, taking advantage of the number of RFLP loci actually mapped in maize, which exceeds the number of morphological and biochemical (isozymic) loci mapped on the conventional maize genetic map (Coe, 1993). Ming et al. (1997) reported a total of 103 RFLP loci mapped, with total length 1624.7 cM and average interval between markers 15.8 cM in a study to map major genes conferring resistance to maize mosaic virus.

\textbf{Table 2.2. \textit{F}_1 plants from crosses of B-A translocations by inbred L668 in an untreated plot in 1998.}

<table>
<thead>
<tr>
<th>Translocation</th>
<th>No. of normal plants</th>
<th>No. of hypoploid plants</th>
<th>% Hypoploid plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB-1Sb</td>
<td>40</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>TB-3La-2S</td>
<td>36</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

(Table con'd)
<table>
<thead>
<tr>
<th></th>
<th>1997</th>
<th></th>
<th>1998</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hypop. plants</td>
<td>Normal plants</td>
<td>Hypop. plants</td>
<td>Normal plants</td>
</tr>
<tr>
<td>TB-1 La</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>TB-1 Sb</td>
<td>12</td>
<td>0</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Tb-1Sb-2L</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>TB-3 Sb</td>
<td>0</td>
<td>0</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>TB-3La-2S</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TB-4Sa</td>
<td>6</td>
<td>0</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>TB-5La</td>
<td>6</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>TB-6Sa</td>
<td>4</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>TB-6Lc</td>
<td>4</td>
<td>0</td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>TB-7Sc</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.3. Observed resistant and susceptible plants from Class I and Class III seeds following treatment with nicosulfuron at 4 to 6-leaf stage in 1997 and 1998.

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<tbody>
<tr>
<td>TB-7Lb</td>
<td>1</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>TB-8Lc</td>
<td>2</td>
<td>0</td>
<td>45</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>TB-9Sb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TB-10Sc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TB-10L19</td>
<td>9</td>
<td>0</td>
<td>39</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>L668§</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DG5510A¶</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

--- Denotes no seed available.
† Resistant hypoploid plants.
‡ Susceptible hypoploid plants.
§ Denotes susceptible control.
¶ Denotes resistant control.

**References**


Robertson, D. S. 1967. The use of $R_2^{scm}$ to facilitate the transfer of maize chromosome segments. J. Hered. 58:152-156.


GENERAL CONCLUSIONS

Reciprocal translocations and B-A translocations were used, respectively to identify chromosomal regions controlling leafiness (Lfy1) and nicosulfuron susceptibility (nfsf1) in the maize genome. The Lfy1 gene is located on the long arm of chromosome 4 and the nfsf1 gene occupies a locus on the short arm of chromosome 7.

The Lfy1 gene is known to produce much higher yield in hybrids that possess it and much less stalk lodging than those with the non-leafy counterpart allele. With the position of this important gene known, it can be better used in linkage analyses and for cloning purposes. Studies in maize have identified genetic variation attributable to unitary factors at perhaps one thousand or more loci, loosely defined. This finding should help develop a more saturated linkage map.

Following the assignment of the nfsf1 gene to a chromosome arm in the maize genome, its use as a genetic marker in linkage studies and in other genetic studies will be facilitated. The transfer of the resistant allele (Nsf1) to elite but nicosulfuron-susceptible inbred lines could be an obvious usage of this finding.

The use of reciprocal translocations and B-A translocations continues to offer advantages for locating genes for both qualitative and quantitative traits in crops in which a complete set of translocations is available. Translocational analyses are less expensive than molecular marker methods for locating genes.
The \textit{Lfy1} and \textit{nfl} genes condition two important agronomic/economic traits in maize. Additional research, such as: two or three-point linkage analyses, studies of the relationship of these genes with other genes, flanking molecular markers, and so forth should be conducted to better understand their effect on yield, disease resistance, stress tolerance, herbicide resistance and other important traits to determine the best way to use them for improving maize populations and increasing yield.
VITA

Orlando José Moreno was born on May 18, 1955, in Araure, Portuguesa State, Venezuela. He attended elementary school at Belarmino Láres Agriculture School and graduated from Technical High School of Agriculture at Turén, Portuguesa State. He enrolled at the Oriente University in 1974, where he earned the Ingeniero Agrónomo degree in May 1980. Upon graduation, he was employed by the Fondo Nacional de Investigaciones Agropecuarias (FONAIAP), a government institution for agricultural research, as a researcher in cereal breeding, in Araure. The author was invited in 1983 by the Centro Internacional de Agricultura Tropical (CIAT) located in Cali, Colombia, to participate in the first Latin American and Caribbean area meeting on rice weed control, where he presented a national report. In 1985, he returned to CIAT to enroll in a six-month rice breeding and genetics course and earned a Rice Breeder certificate. He also participated in the first South American Rice Breeders Workshop organized by CIAT in 1986 at Cali-Villavicencio, Colombia. In 1988, he enrolled in the graduate school at Mississippi State University, Starkville, and conducted his master's thesis under the direction of Dr. James Delouche in the Department of Agronomy in the area of seed technology and plant breeding. In 1990, he returned to Venezuela to continue his work at FONAIAP. He coordinated the sorghum breeding program from 1990 through 1995. In 1993, he received from the Japan International Cooperation Agency (JICA) an invitation to participate in a six-month training course on rice production and protection. He was awarded a Rice Specialist
certificate. In 1995, he embarked upon a doctoral degree program under the
direction of Dr. Manjit S. Kang in the Department of Agronomy in the area of maize
genetics. In 1998, he competed in the graduate student poster competition at the
Southern Branch of American Society of Agronomy annual meeting held at Little
Rock, Arkansas. He presented the following article:

Moreno, O. J., M. S. Kang, and G. Wang. 1998. Location of the nicosulfuron
ASA Annual meeting. Little Rock, AR.

He authored one review paper on an important worldwide maize problem:

Moreno, O. J., and M. S. Kang. 1999. Aflatoxins in maize: the problem and

He coauthored two additional refereed journal articles:

in exotic x adapted maize (Zea mays L.) germplasm for resistance to maize weevil.

Wang, G., M. S. Kang, and O. J. Moreno. 1999. Genetic analyses of grain-
filling rate and duration in maize. Field Crops Res. (In press).

He is a member of the American Society of Agronomy, Crop Science Society of
America, Sigma Xi-The Scientific Research Society, Gamma Sigma Delta Honor
Society of Agriculture, Venezuelan Engineers Society, and Venezuelan
Agronomists Society. He is currently a candidate for the degree of Doctor of
Philosophy in agronomy, which will be conferred in May 1999.
Candidate: Orlando Jose Moreno

Major Field: Agronomy

Title of Dissertation: Chromosomal Location of Genes for Leafiness (Lfly) and Susceptibility to Nicosulfuron (nsfl) in Maize Genome

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:
Feb. 12, 1999