Theor Appl Genet (1994) 87:650–656

Chromosome labeling with transposable elements in maize

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Received: 2 March 1993 / Accepted: 3 May 1993

Abstract. Transposable elements randomly insert into a targeted locus at a frequency of 10^{-6} to 10^{-5}. The *En* element has been shown in previous studies to transpose more frequently into closely linked sites. Thus, it is appropriate to place an *En* element onto each of the 20 chromosome arms in maize to maximize tagging efficiency. This is called chromosome labeling for tagging purposes with transposons. After a chromosome arm has been labeled with a transposon, genes residing in that arm will have a greater chance to be tagged by the transposon. To date, all of the maize chromosome arms have been labeled with at least one of five *En*-containing alleles. The elements were linked to the arms using reciprocal translocations. The usage of these arm-labeled lines is discussed in the context of gene tagging.

Key words: *En* – Transposable elements – Chromosome labeling – Gene tagging – Maize

Introduction

Transposable elements have been successfully used for gene tagging and subsequent gene cloning in maize (Shepherd 1988; Döring 1989). Many genes in maize have been cloned through this technique, which was first used to clone the *bz* locus of maize with *Ac* (Fedoroff et al. 1984). Transposon tagging is based on the insertion of a transposable element into a gene. Transposable elements randomly insert into a specific locus at a frequency of 10^{-6}–10^{-5} (Peterson 1963, 1978). A total of approximately one hundred thousand to one million individuals must be screened to search for a single insertional mutant. The screening is labor intensive and makes transposon tagging a formidable task.

In previous studies (Peterson 1970; Nowick and Peterson 1981), the *En* transposable element was shown to insert more frequently into closely linked sites. This result agrees with that of Greenblatt's *Ac* studies with the *P-vv* allele (1974). These findings establish the basis of the current study, which was designed to place an *En* element into each of the 20 maize chromosome arms with the assistance of reciprocal translocations. The chromosomes will be "labeled" or "marked" by the *En* element, hence the term, "chromosome labeling". After an arm has been labeled with an element, genes residing in the arm will have a greater chance of receiving an element's visitation and, thus, be tagged more readily. In this way, chromosome labeling could considerably accelerate transposon tagging. To date, all of the arms have been labeled, though not all of the reconstructed arms are in a homozygous condition.

Materials and methods

Five *En*-containing alleles were used in the labeling (Table 1). Each of these alleles contains an autonomous *En/Spm* element, inserted within an exon or intron of the locus, that conditions the mutability at the locus. These alleles were chosen on the basis of their high mutability (early and frequent transpositions) and suitable locations in the genome for ease of relocation. High mutability is important for the purpose of tagging because a high excision rate of the donor allele results in more frequent insertions to nearby sites.

Communicated by F. Salamini
Correspondence to: P. A. Peterson
Journal Paper No. 15224 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project No. 3176

Translocation stocks were obtained from the Maize Genetics Cooperation Stock Center. In this study 23 reciprocal translocations were used. They are listed in Table 2.

There were two major concerns in selecting the translocations: the chromosomes involved and translocation break points (Burnham 1962; Longley 1958). The specific translocations were critically selected so that all of the chromosome arms, and as much of a specific arm as possible, could be targeted by relocating the elements. Reciprocal A-A translocations are identified readily by virtue of an accompanying semisterility (Burnham 1962). Just before the anthers shed, pollen is checked for semisterility with a field microscope. The pollen grains of fertile plants are a normal type (round, milky and opaque), while those plants with a translocation contain two kinds of pollen, a normal type and a sterile type (wrinkled, colorless or yellowish, and transparent). A pollen sample with approximately 1/2 normal: 1/2 sterile pollen grains is produced by a heterozygous translocation-carrying plant (Burnham 1962).

Table 1. *En*-containing unstable alleles used in labeling

Allele	Residing locus	Phenotype
a-m(papu)[a]	*A*	Colorless to pale and purple sectors
a2-m55064[b]	*A2*	Colorless to colored sectors
c2-m1[c]	*C2*	Colorless to colored sectors
c2-m826019[d]	*C2*	Colorless to colored sectors
wx-844[e]	*Wx*	Waxy to non-waxy sectors

[a] Peterson 1961, 1970, 1985a
[b] Peterson 1978
[c] McClintock 1967
[d] Peterson 1983
[e] Peterson 1985b; Pereira et al. 1985

Table 2. Reciprocal translocations used in the relocation of the *En* alleles

Translocation	Break point[a]	Mutable allele[b]	Target arm labeled
T1-3e	1L.58 3L.45	*a-m(papu)*	1L
T1-3 5597	1S.77 3L.48	*a-m(papu)*	1S
T1-4 8602	1S.41 4L.81	*c2-m*[c]	1S
T2-3d	2L.67 3L.48	*a-m(papu)*	2L
T2-3e	2S.76 3L.48	*a-m(papu)*	2S
T2-5 032-9	2L.40 5S.31	*a2-m55064*	2L
T3-5 4635	3S.44 5S.48	*a2-m55064*	3S
T3-7e	3L.25 7S.56	*a-m(papu)*	7S
T3-7 6466	3L.36 7L.14	*a-m(papu)*	7L
T3-8 043-14	3L.02 8S.40	*a-m(papu)*	8S
T3-8 4874	3L.28 8L.32	*a-m(papu)*	8L
T3-9b	3L.48 9L.53	*a-m(papu)*	9L
T3-10 036-15	3L.48 10L.64	*a-m(papu)*	10L
T4-5e	4S.41 5S.32	*a2-m55064*	4S
T4-6 033-16	4L.50 6S.90	*c2-m*[c]	6S
T4-6 8764	4L.32 6L.90	*c2-m*[c]	6L
T4-9b	4L.90 9L.29	*c2-m*[c]	9L
T4-10 073-8	4L.41 10S.74	*c2-m*[c]	10S
T5-9 8386	5L.87 9S.13	*wx-844*	5L
T5-10 031-18	5S.58 10S.55	*a2-m55064*	10S
T6-9 5454	6ctr. 9S.75	*wx-844*[d]	6S or L
T7-9 027-9	7L.61 9S.18	*wx-844*	7L
T9-10b	9S.13 10S.40	*wx-844*	10S

[a] From Burham 1962
[b] Peterson 1961, 1970, 1978
[d] Pereira et al. 1985
[c] Either *c2-m-1* or *c2-m826019*

Results

Labeling chromosome arms with a transposable element by the use of reciprocal translocations

The five *En*-containing alleles were linked to chromosome arms through the use of reciprocal translocations. Homologous pairing, followed by appropriate crossovers between the translocated segment and its counterpart, enables the relocation of an *En* allele to a targeted arm. An example of the general scheme for labeling is shown in Fig. 1, which illustrates the labeling of the long arm of chromosome 2 with the *a-m (papu)* allele using T2-3d as the translocation.

A line containing the *a-m(papu)* allele is first crossed with a specific reciprocal translocation line, which has the *A* allele, corresponding to *a-m(papu)*, yielding an F_1 hybrid (Season 1 of Fig. 1). At meiosis of this F_1 hybrid, four types of gametes are formed: N *a-m*, $T \approx A$, N *A*, and $T \approx$ *a-m*, (where *a-m* is an abbreviation of *a-m(papu)*, and the $\approx$ signal indicates the physical linkage between a translocation break point and an allele). These four genotypes can be differentiated by testing them with a recessive *a/a* tester (Season 2 of Fig. 1). The desirable $T \approx$ *a-m*/N *a* progeny individuals can then be selected and isolated. The selected individuals (spotted kernel and semisterile pollen) are selfed to obtain homozygotes (Season 3 of Fig. 1). The resulting spotted and fully fertile offspring are selfed again and tested on a N *a*/N *a* tester to confirm their homozygous translocation status (Season 4 of Fig. 1p).

The cross of a plant with a labeled homozygote (a homozygous translocation) to N *a*/N *a* should yield all semisterile progeny because all of the offspring will be heterozygous for the translocation. Similarly, when the same labeled homozygote is crossed to its parental, homozygous translocation line (Season 4 of Fig. 1n), normal offspring are produced because the genetic information is balanced. There are three criteria used for the verification of labeled homozygous plants: the two critical crosses and the mutability expression associated with the arm-labeled lines. For heterozygous labeling, semisterility and mutability are the two criteria used for verification.

As shown in Table 1 there are at least four arms (3L, 4L, 5S, and 9S) that already contain an *En/Spm* allele at a gene locus. The 16 chromosome arms that lack an already established *En* element are targeted for labeling. The 16 targeted arms are labeled in this study

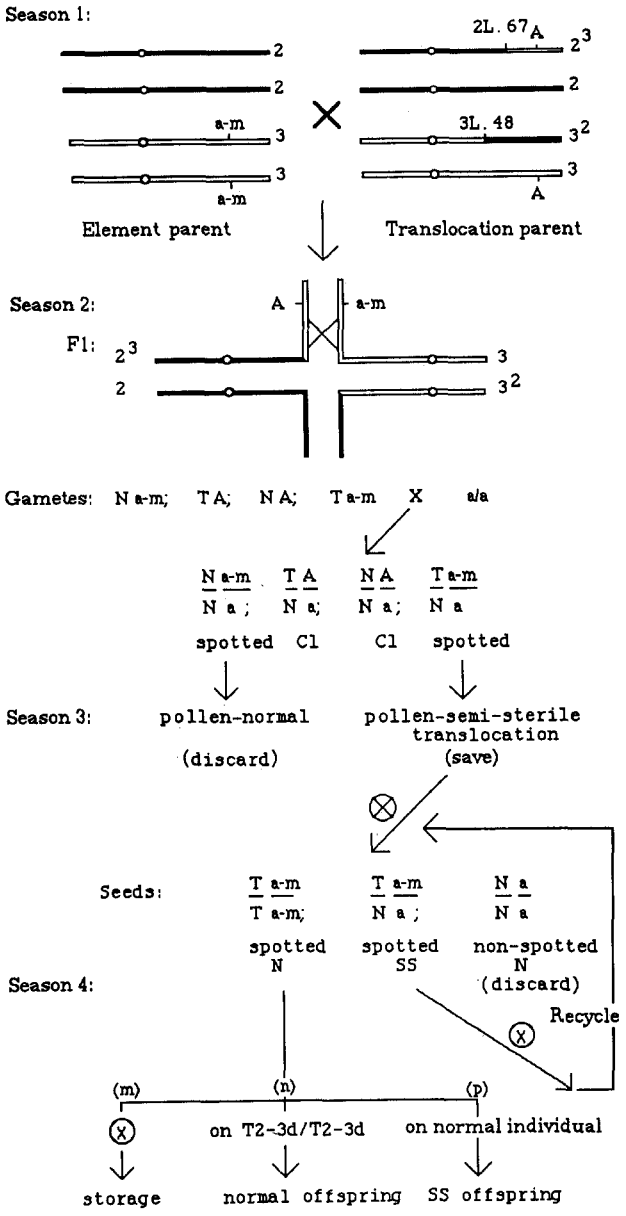

Fig. 1. Strategy for chromosome labeling by the crossing of an element-containing line to a translocation line. *Season 2* The F_1 is testcrossed; gametes of F_1. *Season 3* Self shown of selected testcross progeny. *Season 4* Crosses to isolate the homozygous translocation. *A* or *a* Dominant or recessive allele of *A* gene, *a-m a-m(papu)*, $T \approx$ *a-m* linkage between translocation break-point and *a-m(papu)*, *Cl* colored, *2* chromosome 2, 2^3 chromosome 2 with a translocated segment of chromosome 3

with at least one *En* containing allele by using one, and sometimes two, translocations (Table 3).

Confirming the homozygous labeled arms

Of the 16 arms 8 (1S, 2L, 2S, 6L, 6S, 7L, 8S, and 10L) are made homozygous for their labeling status, whereas the other 8 (1L, 3S, 4S, 5L, 7S, 8L, 9L, and 10S) are heterozygous. The eight arms labeled in homozygous condition are crossed to plants with normal pollen. All offspring of these crosses showed semisterility in pollen, confirming the homozygosity of the parents. Three translocations, T2-5 032-9, T3-10 036-15, and T4-6 033-16, for arms 2L, 10L, and 6S, respectively, were also crossed to their original homozygous translocation lines, all of which produced normal offspring as expected.

Table 3. The chromosome labeling status of transposons to chromosome arms

Arm	Mutable allele	Translocation	Labeling status
1S	*a-m(papu)*	T1-3 5597	homozygous
2L	*a-m(papu)*	T2-3 d	homozygous
	a2-m55064	T2-5 032-9	homozygous
2S	*a-m(papu)*	T2-3 e	homozygous
6L	*c2-m*	T4-6 8764	homozygous
6S	*c2-m1*	T4-6 033-16	homozygous
7L	*a-m(papu)*	T3-7 6466	homozygous
	wx-844	T7-9 027-9	homozygous
8S	*a-m(papu)*	T3-8 043-14	homozygous
10L	*a-m(papu)*	T3-10 036-15	homozygous
1L	*a-m(papu)*	T1-3 e	heterozygous
3S	*a2-m55064*	T3-5 4635	heterozygous
4S	*a2-m55064*	T4-5 e	heterozygous
5L	*wx-844*	T5-9 8386	heterozygous
7S	*a-m(papu)*	T3-7 e	heterozygous
8L	*a-m(papu)*	T3-8 4874	heterozygous
9L	*a-m(papu)*	T3-9 b	heterozygous
	c2-m	T4-9 b	heterozygous
10S	*c2-m*	T4-10 073-8	heterozygous
	a2-m55064	T5-10 031-18	heterozygous
	wx-844	T9-10 b	heterozygous

Double labeling – an example

The labeling of chromosome arm 2L with *a-m(papu)* and *a2-m55064* with T2-3d and T2-5 032-9 is shown in Fig. 2. The proximal 0.67 portion of 2L is labeled with *a-m(papu)* linked to translocation T2-3d, while the distal 0.60 portion of 2L is labeled with *a2-m55064* linked to T2-5 032-9. With both translocations, the 2L arm is fully labeled with either *a-m(papu)* or *a2-m55064*. In fact, the portion between 0.40 and 0.67 is labeled twice, as shown in Fig. 2. A very similar situation was also found with arm 7L, which was labeled with *a-m(papu)* and *wx-844* using T3-7 6466 and T7-9 027-9. Similar complementary labeling was also designed for arms 1S, 9L, and 10S, though the labeling condition of these arms had not been made homozygous.

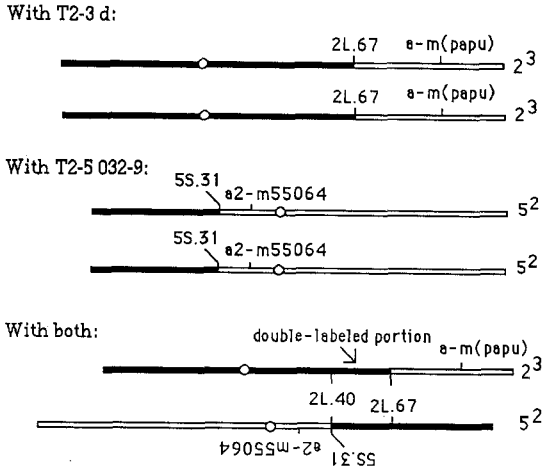

Fig. 2. An example of labeled chromosome arms: the labeling of 2L with *a-m(papu)* and *a2-m55064* using T2-3d and T2-5 032-9, respectively

Discussion

Transposable elements have significant value in genetic studies and plant breeding, especially in gene tagging (Peterson 1986) and in the evolution of organisms (Schwarz-Sommer et al. 1985). Element insertion in genes is readily identified by genetic analysis (Peterson 1981), and the inserted element in any gene can serve as a genetic or molecular marker. This has been proven in several instances (Döring 1989). If the element has been cloned and characterized, as with *En* (Pereira et al. 1985) and *Ac* (Fedoroff et al. 1983), it is then available for use as a molecular probe in corresponding procedures. The gene can then be cloned when the inserted element is used as a probe (Peterson 1991).

This gene-rescue procedure, called "transposon tagging", is ideal for cloning genes that do not have identifiable transcripts used in classical cloning methods, especially when the confirmatory revertant is available. This transposon-tagging approach was first exploited by Bingham et al. (1981) in cloning the *white* locus in *Drosophila*. The initial application of this procedure in maize was made by Fedoroff et al. (1984) in cloning the *bz* locus with *Ac*. With the availability of *Ac*, and after the isolation of other transposable elements, such as *En/Spm* and *Mu*, many genes in maize have been cloned using transposon tagging. These include *A1* (O'Reilly et al. 1985), *C1* (Paz-Ares et al. 1986; Cone et al. 1986), *C2* (Wienand et al. 1986), *P* (Lechelt et al. 1989), (Peterson and Schwartz 1986), *Bz2* (Theres et al. 1987; McLaughlin and Walbot 1987), *O2* (Schmidt et al. 1987; Motto et al. 1988), *R* (Dellaporta et al. 1988), *Vp* (McCarty et al. 1989a, b), *Sh2* (Bhave et al. 1990), *Y1* (Buckner et al. 1990), *A2* (Menssen et al. 1990), *Bt1* (Sullivan et al. 1991), and *HM1* (Johal and Briggs 1992).

The *En* transposable element system has been shown to transpose more frequently into closely linked sites (Peterson 1970; Nowick and Peterson 1981). Because a number of genes have an autonomously mutable *En* element (a locus with a functional element), this element is available for chromosome labeling. Thus, the *En* element is placed in linear continuity with the targeted gene in gene-tagging strategy. This procedure, called chromosome labeling, was documented only recently (Dash and Peterson 1989; Chang and Peterson 1991). After each of the 20 maize chromosome arms is labeled with an *En* element, any gene can be tagged by the element using an appropriate arm-labeled line, provided the gene is phenotypically detectable. A further requirement is that the element transposes at a high rate.

All of the 20 maize chromosome arms are now labeled with *En*, including the 16 labeled in this study. The other 4 arms, 3L, 4L, 5S, and 9S, already contained an *En* element. The portion of each arm labeled is shown in Table 3. Of the 16 arms labeled in this study, 1S, 2L, 7L, 9L, and 10S are so labeled as to adequately cover the whole arms. As for the others, a considerable portion of each arm is covered. Of the 16 targeted arms 8 were made homozygous with reference to their labeling status, and the rest are heterozygous or their homozygosity has yet to be proven (Table 3).

Procedures in the use of a labeled arm for gene tagging

The main purpose in labeling the chromosome arms is for their utilization in gene tagging. Labeling will enrich the tagging process. The basic procedure for using the arm-labeled lines to tag genes is shown in detail in Fig. 3.

As an example, the procedure for *Rp1* tagging includes the following steps:

Step 1. Original cross: introducing the target gene. For example, *Rp1* is crossed with a homozygous labeled line, i.e., *Rp1/Rp1 A2/A2* × *rp1* ≈ *a2-m/a2-m* (Cross 1). Now the line is heterozygous for *Rp1* and the element (Season 1 of Fig. 3).

Step 2. Test cross: isolating the coupling linkage between the element and the target gene, in our case, *Rp1* and *a2-m*. To distinguish among the different genotypes produced by the F_1 hybrid, the F_1 progeny form Cross 1 is crossed to a tester line, i.e., *Rp1 A2/rp1* ≈ *a2-m* × *rp1/rp1 a2/a2* (Cross 2) (Season 2 of Fig. 3).

Step 3. Selecting the correct genotype and self to achieve homozygosity. From the test-cross progeny of Cross 2 in Step 2, *Rp1* ≈ *a2-m/rp1 a2* (spotted, resistant

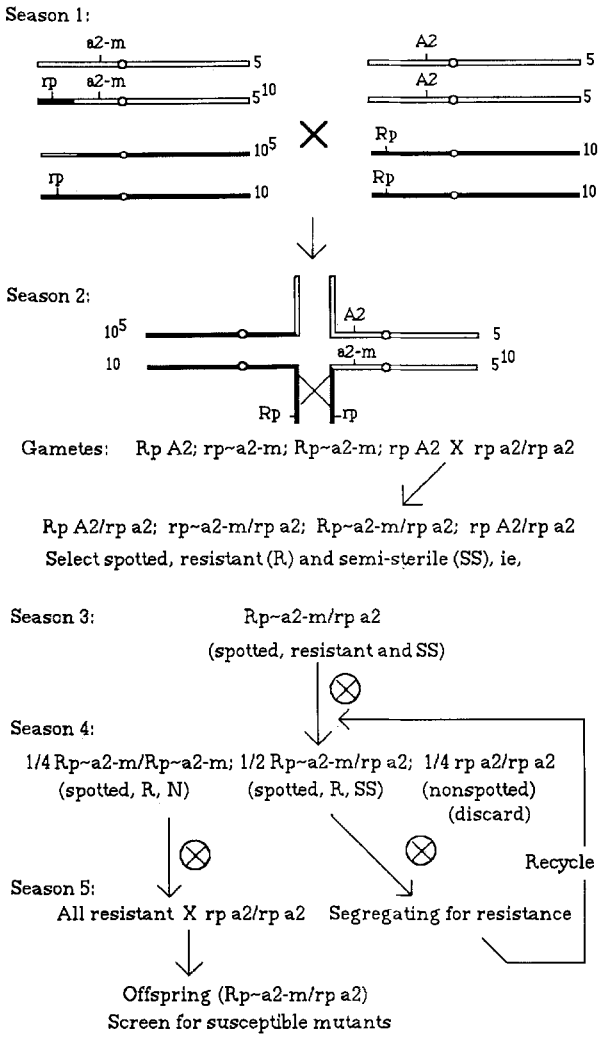

Fig. 3. Strategy for gene tagging using an arm-labeled line. *Rp* or *rp* dominant or recessive allele of *Rp1* gene, *A2* or *a2* dominant or recessive allele of *A2* gene, *a2-m a2-m55064*, *Rp* ~ *a2-m* linkage of *Rp* with *a2-m55064*, *5* chromosome 5, 5^{10} chromosome 5 with a translocated segment of chromosome 10

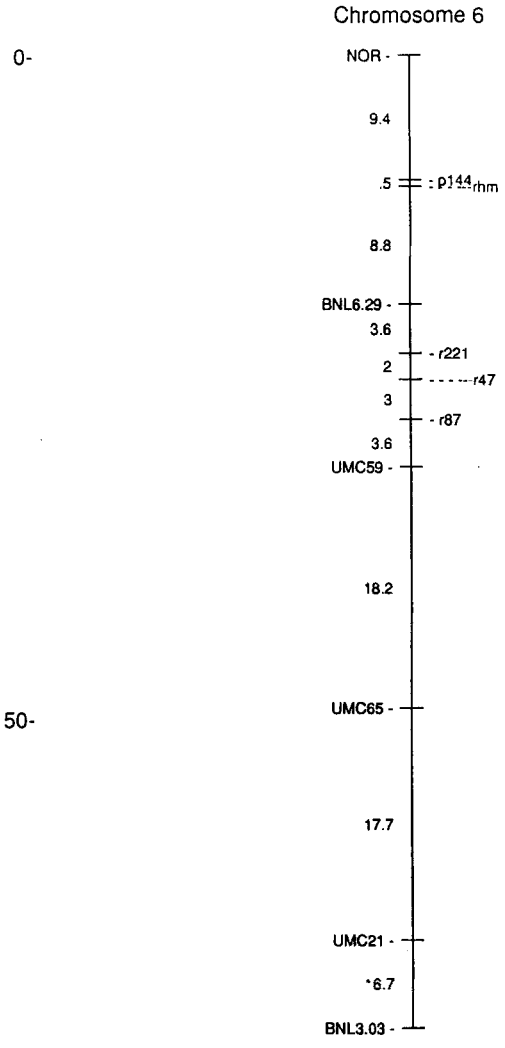

Fig. 4. The RFLP map of the *rhm* region (after Zaitlin et al. 1993)

and semisterile) is selected and selfed (Season 3 of Fig. 3).

Step 4. Selecting and amplifying the correct genotype from the progeny of Step 3. The correct genotype *Rp1* ≈ *a2-m/Rp1* ≈ *a2-m* (spotted, resistant and pollen-normal) is correctly identified and selected in this step (Season 4 of Fig. 3). The amplification step is optional. If enough seed can be obtained, the correct genotype can be crossed to the tester described in Step 5, instead of being selfed.

Step 5. Producing seed for screening. The lines with the genotype *Rp1* ≈ *a2-m/Rp1* ≈ *a2-m*, amplified in Step 4, are crossed to a recessive tester, (*rp1 a2/rp1 a2*), of the target gene to develop seed for screening, i.e., *Rp1* ≈ *a2-m/Rp1* ≈ *a2-m* × *rp1/rp1 a2/a2* (Season 5 of Fig. 3). The correct genotype for screening is *Rp1* ≈ *a2-m/rp1 a2*.

Step 6. Screening for mutants. With *Rp1* tagging, the mutants have the phenotype of the recessive allele *rp1*, i.e., susceptible to common rust.

As described in these procedures, the development of appropriate seed for targeting a gene with a labeled chromosome arm takes four or five seasons. However, if the arm-labeled line has a dominant allele for a gene to be tagged, a direct cross between the labeled line and a recessive tester will achieve the final construct for screening. In the latter case, the necessary seed for screening is developed in only one season. The tagging of *rhm* with *c2-m*, using T4-6 033-16, can be taken as an example. The *rhm* gene conditions resistance to the southern leaf-blight caused by *Helminthosporium maydis* (Hooker et al. 1978) and is located close to the

centromere of chromosome 6 (Fig. 4). The dominant allele *Rhm* of the gene determines susceptibility, while the recessive homozygote *rhm/rhm* determines resistance to the disease. The use of the translocation T4-6 033-16 enables us to bring the *c2-m* mutable allele to 6S.90, indicating that 90% of the proximal portion of 6S will be labeled by the allele. Since most breeding and genetic lines have the genotype *Rhm/Rhm*, the labeling of the arm will result in the genotype $c2\text{-}m \approx T \approx Rhm/c2\text{-}m \approx T \approx Rhm$. This is the final construct needed for developing seed for screening. Only one cross is needed, $c2\text{-}m \approx T \approx Rhm/c2\text{-}m \approx T \approx Rhm \times rhm/rhm \rightarrow c2\text{-}m \approx T \approx Rhm/N\ rhm$.

The efficacy of this translocation-element labelling procedure is currently being tested. Several mutants with be targeted at arms that are labeled. This will provide a test of this procedure outlined in these pages.

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Note added in proof

In addition to the autonomous En/Spm transposable elements listed in Table 1, the *o2-m20* allele on chromosome seven (7s) Schmidt et al. (1987) can also be used. This transposon was recently used to target the *In* locus (Peterson 1993)

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