

Conversión of Sugarbeet Primary Trisomic Types into Annual and Male-sterile Condition

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ABSTRACT

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The sugarbeet primary trisomics recently isolated present three specific problems which may difficult their use in genetic analysis: (1) difficulty in hand emasculation and artificial hybridization; (2) the presence of the S^f self-fertility allele; and (3) the biennial growth habit. To overcome these problems, systems for the conversion of the isolated trisomic types into annual and Mendelian male-sterile condition, and for the maintenance of the converted series are developed.

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INTRODUCTION

Primary trisomics provide a means to associate linkage groups and genes with their respective chromosomes in diploid species because the presence of an additional chromosome modifies the expected Mendelian ratio for genes on that chromosome. All nine sugarbeet primary trisomic types have been recently isolated in the progeny of inbred autotriploid plants (ROMAGOSA, 1982). However, these sugarbeet primary trisomics present some specific problems which may difficult their use in genetic analysis. The objective of this work is to design a way to overcome the specific problems of these sugarbeet trisomics by converting all primary types into annual and male-sterile condition.

PROBLEMS IN PRIMARY TRISOMIC ANALYSIS IN SUBGARBESET

Sugarbeet is a species where artificial hybridization is specially difficult. Anthers must be removed from flowers just ready to open and before anther dehiscence. Extreme care must be taken not to touch the stigma during anther removal. This difficulty in artificial pollination is more acute in the inbred lines where trisomics have been isolated. They all are highly, almost obligate, self-fertile. If enough hybrid seed is to be produced for genetic analysis, male-sterile or self-incompatible materials should be developed. Trisomics have been isolated in two isogenic lines, one being homozygous annual and the other highly inbred biennial. Some trisomic types are present only in the biennial condition. In order to reduce the length of the reproductive cycle, seedlings have to be exposed to prolonged cool temperatures for at least 4 months for the hard bolting biennial line and under continuous light. (GASKILL, 1952). Plants of the annual genotype have to be kept under these condition for only 4 to 6 weeks, in order to synchronize the flowering time of the different trisomic types at any time of the year. It appears, therefore, desirable to incorporate also the annual character in all isolated trisomic types in order to reduce the length of the reproductive cycle from 8 or 9 months to 5 or 6.

Cytoplasmic and genetic male-sterile annual equivalents of one line that has rendered most trisomic types, NB1, is now available (McFARLANE, 1981). The cytoplasmic male-sterile annual equivalent of the NB1 is not developed; but, since both biennial CMS and annual type 0 (maintainer) are available, it would not take much effort to develop. No pollen transmission of the extra chromosome has been detected

in these primary trisomics, although data were not sufficient to allow a decisive conclusion (ROMAGOSA, 1982). If pollen transmission of the extra chromosome is detected, CMS annual trisomics could be easily developed to be used in genetic analysis.

The self incompatibility system present in this species also has to be considered for the future use of the trisomics. According to LARSEN (1977), it consists of four gametophytic S-loci with two alleles at each locus with complementary interaction, i.e. four S-genes in the pollen have to be matched in the pistil to cause incompatibility. In addition, an allele for self-fertility enables production of highly self-compatible inbreds like the ones used to isolate the present trisomic types. Studying this S^f allele, SAVITSKY (1952) reported on a cross between a self-fertile and a self-incompatible genotype: all F_1 individuals were self-fertile. That was interpreted by a dominant S^f allele. Not only were all F_1 self-fertile but also when selfed, all following generations consisted of only self-fertile individuals. When the F_1 was crossed as female to a self-incompatible plant, among the offspring 77 were self-fertile and 80 self-incompatible. These crosses agreed with the gametophytic hypothesis: only pollen grains carrying the S^f allele are self-compatible. No allelic relationship among the S^f and any other hypothesized allele is known. The presence of this allele in these trisomics has an important bearing in their future use in genetic analysis. The F_1 between one self-fertile trisomic and a generally self-incompatible genetic marker will be fertile but in the F_2 generation there will not be Mendelian segregation for S^f allele and for any gene linked to it. Instead of a 3:1 or 8:1 ratios for disomic or trisomic segregations respectively, upon selfing all plants will be self-fertile, that is a all: none ratio of self-fertile to self-incompatible plants for both diploid and trisomic plants will be observed. Therefore, until the S^f allele is located, extreme care has to be taken to study F_2 generations. The backcross method of primary trisomic analysis is recommended when working with the linkage group where the S^f gene is located.

CONVERSION OF TRISOMIC TYPES TO ANNUAL AND MALE-STERILE CONDITION

As mentioned, McFarlane recently released the annual and Mendelian male-sterile equivalent of the NB1. Both characters are monogenic and located in linkage groups I and III, respectively (SMITH, 1980). The annual condition, B, is dominant over the biennial growth habit,

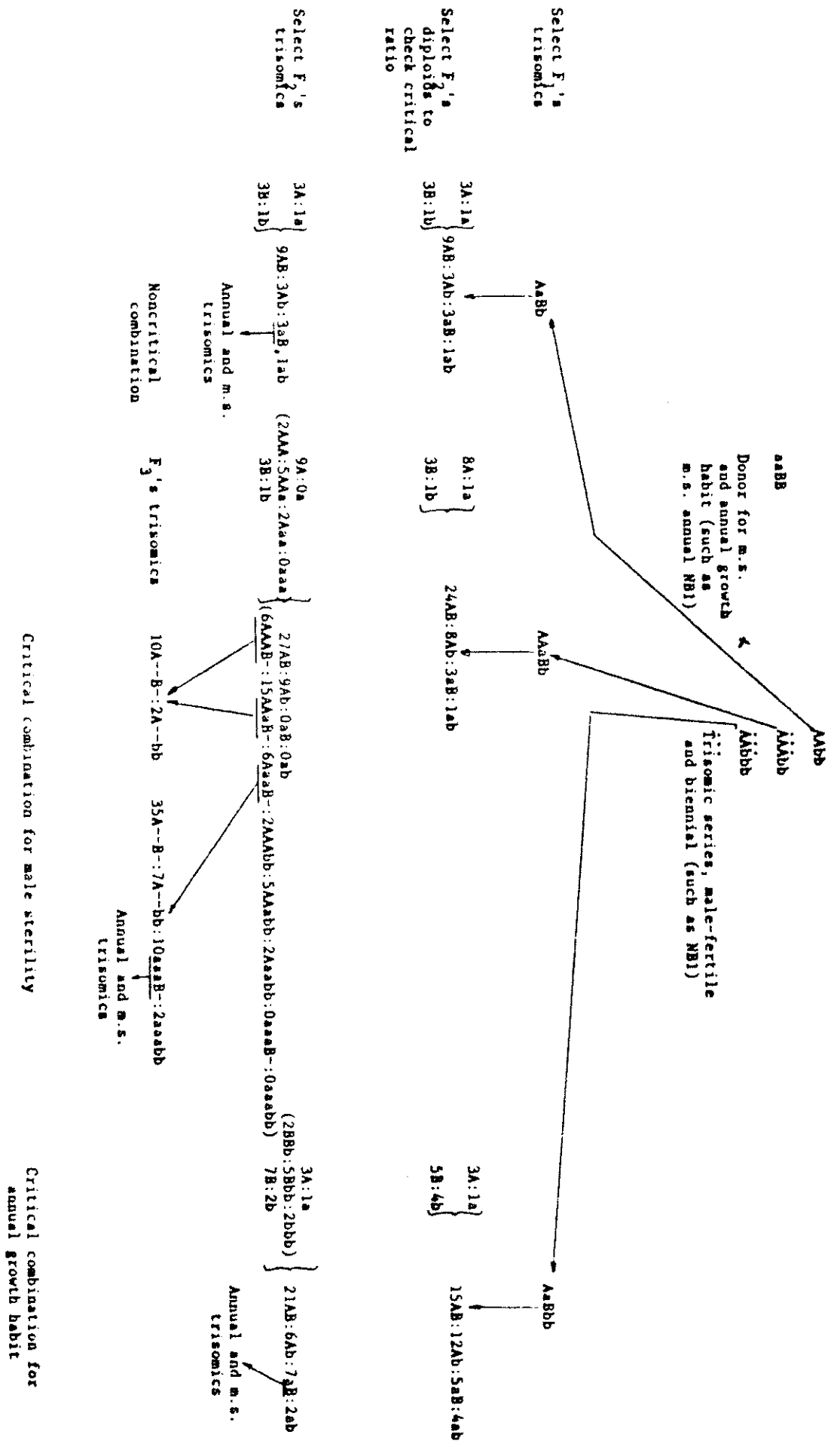


FIG. 1. —Procedure for trisomic conversion to annual and male-sterile condition, assuming pollen transmission of the extra chromosome.

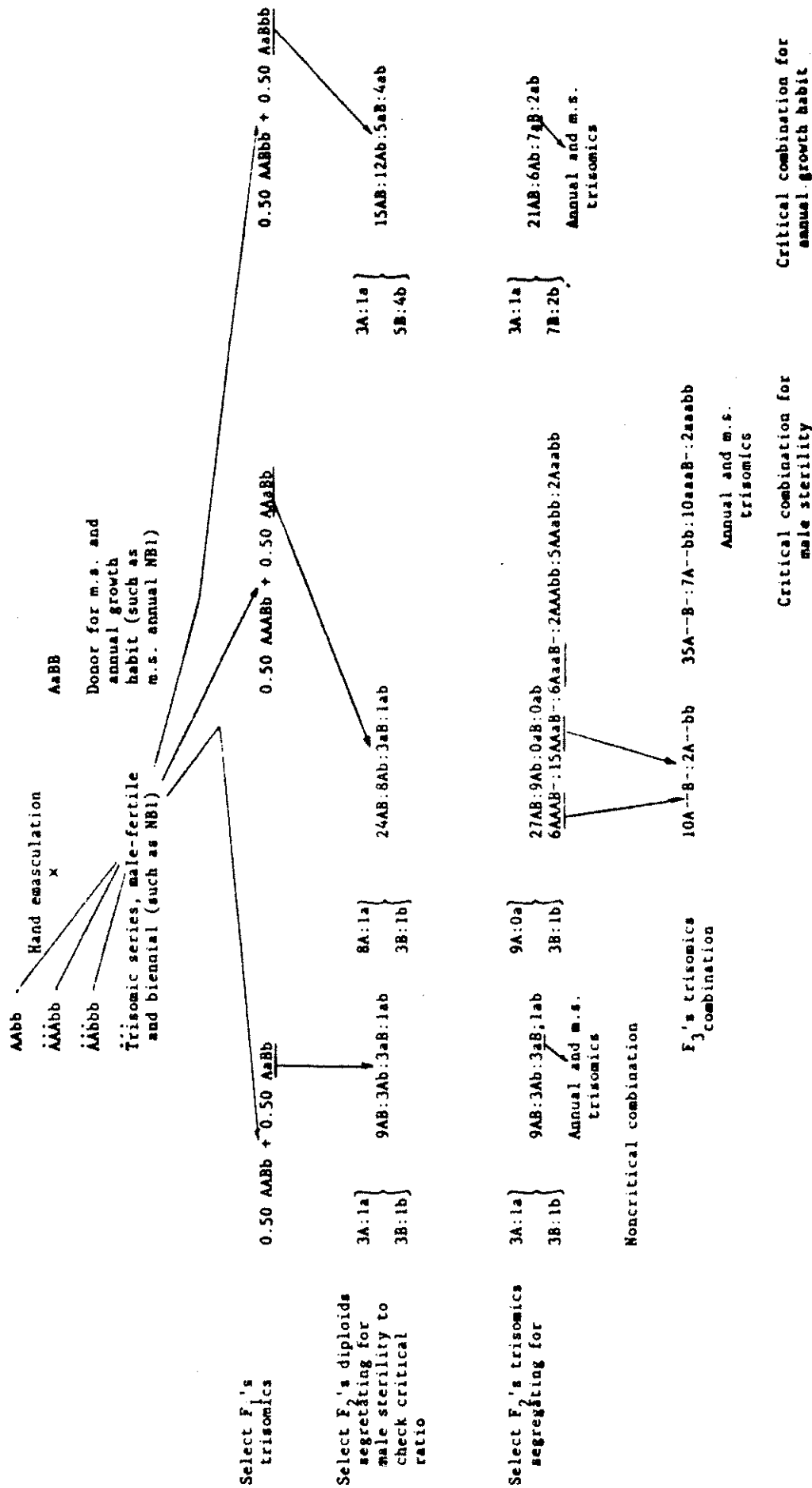


FIG. 2. —Procedure for trisomic conversion to annual and male-sterile condition, using the trisomic plants as female parents.

b. The genetic male-sterility, *a*, is recessive to the pollen-fertility, *A*. The process of converting all trisomic types into the annual and genetic male-sterile condition, will at the same time, permit the assignment of two known linkage groups I and III, to two different trisomic types. Schemes of the procedure appear in Figs. 1 and 2. The process represented in these two schemes are the backcrossing technique to incorporate on dominant and one recessive gene simultaneously in trisomic plants. According to the phenotypic ratios observed in the segregating F_2 and F_3 generations, we will be able to determine the chromosomes in which the genes, and therefore the linkage groups I and III, are located. The expected segregation ratios on these figures, were determined based on the chromosome segregation type of trisomic inheritance.

The procedure, in fully equipped greenhouses, may be summarized as follow:

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| Year 1. January. | Plant seed harvested on the biennial trisomics. |
| February. | Select, by chromosome counts, only trisomic plants. |
| March. | Take the trisomic seedlings into photothermal induction. |
| April. | Plant the donor for male-sterility and annual habit. |
| May. | Photothermal induction of male-sterile and annual plants. |
| June. | Remove all plants from photothermal induction to seed production. |
| July. | If no pollen transmission of the extra chromosome is detected, then hand emasculation of the trisomics and subsequent cross to an heterozygous for male-sterility is need. (Fig. 2). |
| August. | Harvest F_1 seed. |
| September. | Plant F_1 seed. |
| October. | Check chromosome constitution of F_1 hybrids. |
| November. | Photothermal induction of the F_1 trisomics. |
| Year 2. January. | Remove trisomics from photothermal induction to seed production. |

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- February. At this time if the trisomics are not true hybrids, that is, if they were biennial, then, they will not bolt.
- April. Harvest F_2 seed.
- May. Plant F_2 seed.
- June. Chromosome counts on F_2 seedlings.
- July. Photothermal induction of all F_2 plants.
- August. Remove F_2 plants to seed production.
- September. The double recessive *bb* biennial plants will not bolt at this time. The diploid sibs will facilitate at this stage the distinction between the disomic and the trisomic ratios. In 7 trisomic types both phenotypic ratios, for male-sterility and annual growth habit, will be the same for the diploid and trisomic fractions and close to the expected 3:1 disomic ratios. They will be the trisomic types for chromosomes not carrying linkage groups I and III. The ratio of bolters to nonbolters will be close to 5:4 and 7:2 for the diploid and trisomic fractions respectively of the F_2 plants involving the chromosome where the annual growth habit is located. Upon bolting, we will also be able to determine the ratio of male-sterile and fertile phenotypes. In the critical combination for this character the diploid and trisomic fractions will be close to 8:1 and 9:0, respectively. The process of converting the trisomic types to annual and male-sterile condition will at this point be finished except for the trisomic type which carries the male-sterility gene. Unless double reduction occurs, no annual and male-sterile trisomics will be isolated in the F_2 . All annual trisomics will be pollen fertile. It is necessary to isolate the F_3 for this specific trisomic type.
- Year 2. October. Harvest F_3 seed.
- November. Plant F_3 seed.

- December. Check chromosome number of F_3 seedlings. Isolate F_3 trisomics.
- Year 3. January. Photothermal induction of F_3 trisomics.
- March. Remove from photothermal induction to seed production.
- May. The double recessive, bb, will not bolt at this time.
- In the F_3 population of the trisomic type for the chromosome where the gene for male-sterility is present, the ratio of annual and male-sterile trisomics to annual and pollen fertile will be 1:19.25. If a large enough population is isolated, a significant number of annual and male-sterile trisomics will be detected.
- June. Harvest F_4 seed.

MAINTENANCE OF THE ANNUAL AND MALE STERILE TRISOMICS

Annual and male-sterile trisomics will be actually isolated in the segregating F_2 and F_3 populations. However, most of them will be heterozygous for the annual character. As a first step for the maintenance of these genetic stocks, we will need to isolate homozygous trisomics for the annual growth habit. The process will be easily made by selfing segregating male-fertile annual trisomics from the F_2 or F_3 population and selecting the pollen-sterile homozygous annual trisomics from the progenies not segregating for the biennial character. Selfing of male-fertile annual trisomics for the chromosome where the annual gene is located, i.e. genotypes AABBB, AABBBb, AABbb, AaBBB, AaBBb and AaBbb, and assuming no double reduction, will allow to distinguish only the Bbb from the BBb and BBB genotypes. If true triplex are desired, another generation of selfing, keeping records of the pedigrees, will be necessary.

Once the homozygous for the annual growth habit are isolated, the best way to maintain these trisomics stocks will be to cross them with their male-fertile diploid sibs, they will have the AaBB genotype. 50% of the trisomics will be pollen-fertile and 50% pollen-sterile. We will discard the pollen fertile-fertile plants for trisomic analysis, so that all crosses will be made with male-sterile trisomics and no hand-emasculation will be needed.

RESUMEN

Al utilizar los trisómicos de remolacha recientemente aislados en la localización cromosómica de genes aparecen tres problemas principales: (1) dificultad en la castración y polinización artificial, (2) presencia del alelo S^f de autofertilidad; y (3) carácter bianual que retarda significativamente el ciclo reproductivo. Para solucionar en alguna medida estos problemas, se describen sistemas de conversión de la citada serie trisómica en anual y androestéril genética, así como técnicas de mantenimiento de la misma.

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