

SHORT COMMUNICATION

Mapping of a novel viviparous unstable mutant of maize (*vp12*)

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ABSTRACT

A new viviparous mutant of maize (*Zea mays* L.), associated with genetic instability and designated *viviparous-12* (*vp12*), was identified in a synthetic Tuxpeño adapted to tropical regions. In the present work, the linkage group of this new locus was determined. Progenies of inbred line L477 segregating for the *vp12* mutant were crossed with *waxy*-marked reciprocal translocation stocks. The phenotypic frequencies of the *wx* and *vp12* mutants were analyzed in F₂ progenies. The results demonstrated that the *Viviparous-12* locus of maize is located on the long arm of chromosome 6.

INTRODUCTION

Mutations affecting seed development have been valuable in identifying genes involved in the control of maturation phase and seed development. In maize (*Zea mays* L.), these mutations include nine known loci that control seed dormancy, may exhibit precocious germination, and are named viviparous (Robertson, 1955; McCarty and Carson, 1991). Most of the maize viviparous mutants have been shown to affect levels of the phytohormone abscisic acid in the developing seed and to block carotenoid synthesis (Robertson, 1975; Neill *et al.*, 1987). The *viviparous-1* (*vp-1*) mutant embryos have reduced sensitivity to abscisic acid and blocked anthocyanin synthesis in aleurone and embryo tissues (Robertson, 1955). The *vp-1* gene has been cloned. It encodes a putative transcriptional activator that is involved in the expression of genes during seed maturation (McCarty *et al.*, 1989, 1991; Quatrano *et al.*, 1993; Vasil *et al.*, 1995).

A new viviparous gene of maize named *viviparous-12* (*vp12*) (Maluf, 1991) has been identified in a Tuxpeño variety adapted to tropical regions. This mutant is recessive, lethal, and is characterized by precocious germination of the embryo, lemon endosperm, albino seedlings, and genetic instability. It segregates in a Mendelian pattern as a single locus. Deficiency in the photosynthetic activity of *vp12* mutant has been demonstrated (Pereira *et al.*, 1994). Somatic genetic instability has been observed as yellow revertant sectors in the endosperm and green revertant stripes in albino leaves. Among the diverse viviparous mutants associated with alteration in endosperm color and albino seedlings in maize, *vp12* seems to be unique in its association with genetic instability (Maluf, 1991).

A new locus has classically been located and mapped by use of several techniques that are well established in maize. Once a gene has been located on a chromosome, its mapping by current techniques, including molecular markers, is accelerated. In maize, the series of reciprocal translocation stocks developed by Anderson (1956), with breakpoints closely linked to the *waxy* (*wx*) endosperm marker gene, have been widely used by geneticists to determine the placement

of genes on chromosomes. These translocation stocks are homozygous for the *waxy* (*wx*) gene and for a translocation having one of its break points on chromosome 9, linked to the *waxy* locus (Laughnan and Gabay-Laughnan 1994). Other break points are distributed among the other nine chromosomes. Crossing-over is suppressed in the regions of translocations, increasing the efficiency of linkage detection. These translocations are monitored by the *waxy* marker, a recessive endosperm mutant that blocks the synthesis of amylose, and produces starch composed entirely of amylopectin. The resulting mutant phenotype is an easily recognizable waxy endosperm.

MATERIAL AND METHODS

The *vp12* mutant, first identified in the synthetic Asteca-Prolífico, has been introduced to inbred line L477. Both genotypes were derived from Tuxpeño, a dent endosperm race of maize adapted to the coastal region of the Gulf of Mexico. Allelism tests demonstrated that *vp12* is not allelic to other viviparous mutants previously described in maize (Maluf, 1991). Plants of inbred line L477, segregating for the *vp12* mutant, were initially crossed as female parents with a series of homozygous *waxy*-marked reciprocal translocation stocks. These translocation stocks were provided by the Maize Genetics Cooperation Stock Center, University of Illinois, USA. A total of 17 translocation stocks were used, with one of the break points at chromosome 9 and the other breakpoints distributed among the other nine chromosomes of maize. About 2/3 of the L477 plants used in the crosses with the *waxy* tester stocks were expected to be heterozygous for the *vp12* mutant. The resulting F1 plants were self-pollinated. Frequencies of *wx* and *vp12* mutants were analyzed in a segregating F2 generation. The F2 kernels were classified according to the four expected endosperm phenotypes: vitreous-yellow, vitreous-lemon, waxy-yellow, and waxy-lemon. Chi-square tests were used to test for a Mendelian segregation pattern, expecting ratios of 9:3:3:1 of the above mentioned endosperm phenotypes.

RESULTS AND DISCUSSION

The waxy-marked reciprocal translocation technique used in this work involved analysis of phenotypic frequencies of *wx* and *vp12* mutants in F2 progenies derived from crosses with the 17 tester stocks. The total number of kernels analyzed for segregations

of yellow and lemon endosperm color within vitreous and waxy phenotypes in each one of the 17 crosses varied from 886 to 1595, distributed among seven to 11 ears. Frequencies of about 50% abortion of F2 seeds were observed in all progenies. It is known that in plants heterozygous for a reciprocal translocation, typically about 50% of the cells resulting from meiosis carry a chromosomal imbalance that is expressed as spore abortion (Brink, 1927; Burnham, 1930; Laughnan and Gabay-Laughnan, 1994). Therefore, the observed abortion was to be expected as a consequence of chromosomal imbalance in the products of meiosis.

Significant discrepant ratios for inheritance of yellow and lemon endosperm within vitreous and waxy phenotypes (Table I) were observed only in F2 progenies resulting from crosses involving interchange points between chromosome 9 and the long arm of chromosome 6 (*wx1* T6-9b[6L.1;9S.37]). Segregation ratios in these progenies revealed a clear linkage between waxy/lemon phenotypes and between vitreous/yellow phenotypes. This linkage was expected to occur in those crosses involving the chromosome 9 reciprocal translocation with the *vp12* linkage group. It is known that spores carrying balanced chromosome complements contain both interchanged or both normally arranged homologs (Burnham, 1930; Laughnan and Gabay-Laughnan, 1994). As a consequence, a complete artificial linkage between the

Table I - Determination of linkage group of the *vp12* gene of maize. Segregation of yellow and lemon endosperm within vitreous and waxy phenotypes in F2 kernels resulting from selfing of F1 plants heterozygous for the *vp-12* locus and for the waxy reciprocal translocation tester (*wx1* T6-9b[6L.1;9S.37]), with a homozygous interchange point of reciprocal translocation between chromosome 9 and the long arm of chromosome 6.

Ear	Vitreous kernels		Waxy kernels		χ^2
	Yellow	Lemon	Yellow	Lemon	
1	115	4	1	20	74.29*
2	117	2	1	17	74.58*
3	128	3	1	31	110.35*
4	109	1	2	29	104.42*
5	102	3	1	16	63.62*
6	109	3	1	19	72.17*
7	134	2	1	34	123.99*
8	96	6	1	24	76.12*
9	70	5	2	9	32.16*
10	79	17	1	8	25.15*
Total	1,059	46	12	207	708.76*

*Significantly different, at the 1% level, from a 9:3:3:1 ratio, demonstrating the linkage between waxy/lemon phenotypes and between vitreous/yellow phenotypes, and mapping the *vp12* locus to the long arm of chromosome 6.

two interchange regions as well as between the corresponding regions on their normal homologs is established in crosses where balanced chromosome complements are transmitted. This complete linkage between points of nonhomologous chromosomes is a direct consequence of nontransmission to progeny of other combinations of these points, present in sterile spores. Inferences regarding the positions of gene loci in the physical chromosomes are possible because the interchange points of reciprocal translocations in the physical chromosomes can be correlated with positions in the linkage maps (Anderson, 1956; Patterson, 1994). Therefore, the significant deviations of the expected phenotypic frequencies in F2 progenies derived from crosses between L477 (*Vp12/vp12*) with this specific reciprocal translocation stock revealed that the *Viviparous-12* locus of maize is located on the long arm of chromosome 6. The non-significant deviation values of F2 seeds resulting from crosses involving interchange points between chromosome 9 and the short arm of chromosome 6 (*wx1 T6-9a[6S.79;9L.4]*) indicate that the *vp12* gene is distant from the *wx* marker in this translocation (Table II). This result gives further evidence that the *vp12* is located on the long arm of chromosome 6. Since *vp12* is located near the breaking point of the translocation (*wx1 T6-9b[6L.1;9S.37]*), it is expected that this gene would be close to the RFLP markers *umc379*, *umc59*, *zda204*, *umc172*, *npi373* and *emp1*.

Table II - Determination of linkage group of the *vp12* gene of maize. Segregation of yellow and lemon endosperm within vitreous and waxy phenotypes in F2 kernels resulting from selfing of F1 plants heterozygous for the *vp-12* locus and for the waxy reciprocal translocation tester (*wx1 T6-9a[6S.79;9L.4]*), with a homozygous interchange point of reciprocal translocation between chromosome 9 and the short arm of chromosome 6.

Ear	Vitreous kernels		Waxy kernels		χ^2
	Yellow	Lemon	Yellow	Lemon	
1	116	39	35	13	0.30 ns
2	104	25	21	9	5.84 ns
3	55	34	29	8	9.33 *
4	99	19	34	13	6.02 ns
5	108	33	32	6	3.01 ns
6	94	40	34	17	4.35 ns
7	98	22	31	4	6.52 ns
Total	674	212	216	70	0.81 ns

ns, Not significant at the 5% level, demonstrating a 9:3:3:1 Mendelian segregation pattern and no linkage between the endosperm phenotypes.

* Significant at the 5% level, demonstrating a non-random deviation from the 9:3:3:1 Mendelian segregation pattern.

As the linkage group of *vp12* gene in maize genome has been now determined, high resolution mapping by use of the restriction fragment length polymorphism method should be relatively straightforward. Further studies in association with cloning of the *vp12* sequence would be a feasible approach to assess the role of this locus in seed developmental responses, and to achieve molecular characterization of its genetic instability.

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RESUMO

Mutantes vivíparos em milho têm sido alvo de interesse para estudos de regulação dos processos de desenvolvimento e maturação da semente. Um novo mutante vivíparo de milho, associado com instabilidade genética e denominado *vivíparo-12* (*vp12*), foi identificado em sintético Tuxpeño adaptado a regiões tropicais. No presente trabalho, o grupo de ligação desse novo loco foi determinado. Progenies da linhagem endogâmica de milho L477 segregantes para o mutante *vp12* foram cruzadas com estoques de translocações recíprocas marcadas com o mutante de endosperma *waxy*. As frequências fenotípicas dos mutantes *wx* e *vp12* foram analisadas nas progenies F2. Os resultados demonstraram que o loco *Vivíparo-12* de milho está localizado no braço longo do cromossomo 6.

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