TETRASOMIC SEGREGATION FOR MULTIPLE ALLELES IN ALFALFA[1]

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Manuscript received December 3, 1980
Revised copy accepted February 8, 1982

ABSTRACT

Evidence of tetrasomic inheritance in alfalfa, *Medicago sativa L.* and *M. falcata* L., for multiple codominant alleles at three isozymic loci is reported in this study. The locus *Prx-1* governing anodal peroxidase and the loci *Lap-1* and *Lap-2* governing anodal leucine-aminopeptidase were studied by starch gel electrophoresis in seedling root tissue or seeds. The progenies from several di-, tri- or tetra-allelic plants belong to the species *M. sativa* and *M. falcata* and their hybrids were studied for the segregation of the three genes. In all cases, tetrasomic inheritance of chromosomal-type segregation was observed. In another progeny resulting from the crossing of two plants involving four different alleles at locus *Lap-2*, tetrasomic segregation with the possible occurrence of double reduction was observed. This study presents direct evidence of autotetraploidy and the existence of tetra-allelic loci in alfalfa. It also supports the concept that the species *M. sativa* and *M. falcata* are genetically close enough to be considered biotypes of a common species.

MOST of the information dealing with the biochemical genetics of polyploids has been obtained from allopolyploid species. ROOSE and GOTTLIEB (1976) have provided a good review of this field. In their study of Tragopogon they discuss the fitness advantages of this type of polyploid, due perhaps to fixed heterozygosity, which is likely to result in increased biochemical versatility.

For autotetraploids there is much indirect evidence on the tetrasomy and the possible origin of these species (HARLAN and DEWET 1975). For instance, in several species of fish, evidence of autotetraploidy is based on gene duplication, chromosome number, relative DNA value and residual tetrasomy (WRIGHT *et al.* 1980). In plants, evidence of autopolyploidy has been obtained mostly on the basis of segregation ratios for morphological characteristics and chromosome pairing. Alfalfa (*Medicago sativa* L. and *M. falcata* L.) is considered to be an autotetraploid ($2n = 4\times = 32$) (BARNES and HANSON 1967; BUSBICE, HILL and CARNAHAN 1972; BARNES *et al.* 1977; BINGHAM 1979). STANFORD (1951) presented the best documented evidence of autotetraploidy in this crop after demonstrating tetrasomic segregation for flower color in F_3 generations. The availability of electrophoretic markers in alfalfa representing various loci wtih multiple alleles (QUIROS and MORGAN 1981) provides a better insight into the genetic structure of this crop. This is facilitated by having both diploid and tetraploid

[1] This paper is dedicated to Professor CHARLES M. RICK in the year of his retirement.

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sources of alfalfa so that genes could be studied first in diploids and then in the more complex tetraploids. QUIROS and MORGAN (1981) reported the inheritance and linkage relations of genes governing the enzymes peroxidase and leucine-aminopeptidase for several accessions of diploid alfalfa. For most loci, multiple alleles of codominant expression were found. They segregated in a Mendelian fashion with ratios expected for monomeric enzymes. In the present study, I report the segregation of some of these alleles at three loci for peroxidase and leucine-aminopeptidase in tetraploid alfalfa. In all cases tetrasomic segregation was observed, confirming the autotetraploid nature of this crop.

MATERIALS AND METHODS

Horizontal starch-gel electrophoresis of crude extracts from root tissue or seeds was conducted as described by QUIROS (1981). Peroxidase (PRX) and leucine-aminopeptidase (LAP) were stained according to the techniques of HOYLE (1978) and BREWBAKER *et al.* (1968), respectively.

Mono, di- and tri-allelic parents: The S_1 progeny of three alfalfa plants of *M. sativa* cv. Tuna (79M 6–51, 79M 6–54, and 79M 6–57), Tuna, were scored for allozyme segregation in root tissue at three loci, *Prx-1*, *Lap-1* and *Lap-2*. The phenotypic effects of these genes have already been reported (QUIROS and MORGAN 1981). The progenies resulting from the selfing of two tetraploids of the alfalfa species *M. falcata* were scored for segregation at loci *Lap-1* and *Lap-2* in root tissue. These two plants, identified as HR 1–49 and FV 11–27, originated from the accessions UAG 110 and UAG 2296, respectively, which are part of the Medicago germ plasm collection kept at the University of Alberta.

Tetra-allelic parents. Tetra-allelic plants for loci *Prx-1* and *Lap-2* were obtained after crossing di-allelic or tri-allelic individuals carrying different alleles. Plant 80M 133–3 belonging to the species *M. falcata* (UAG 110) was obtained after crossing two plants of phenotypes LAP-2^3/LAP-2^5/LAP-2^6 and LAP-2^2/LAP-2^4. Plants 81M 10–3, 81M 10–27 and 81M 10–32, tetra-allelic for locus *Prx-1*, were selected from a cross betwen the *M. falcata* plant FV 11–27 of genotype *Prx-1*^{1}/*Prx-1*^{2}/*Prx-1*^{3}/*Prx-1*^{4} and the *M. sativa* plant 79M 6–51 of genotype *Prx-1*^{1}/*Prx-1*^{1}/*Prx-1*^{2}/*Prx-1*^{2}. Although plant FV 11–27 was tetra-allelic for locus *Prx-1*, this was not realized until it was crossed with 79M 6–51. Seeds obtained from the reciprocal cross of these two plants were assayed for LAP and scored for segregation at locus *Lap-2*.

Before crossing, flowers were emasculated by cutting the standard petal, tripping the flower carefully and removing the anthers by suction. No alcohol was used for the emasculations. Selfings were performed by rolling fully open florets with the fingers. The χ^2 test was used to assess the goodness-of-fit for all the progeny distribution to tetrasomic inheritance.

RESULTS AND DISCUSSION

The observed S_1 segregations from di-allelic parents at loci *Prx-1*, *Lap-1* and *Lap-2* in the progeny of the three *M. sativa* plants are shown in Table 1. These plants segregated according to the expected ratios for tetrasomic inheritance. The progeny from plants 79M 6–54 and 79M 6–57, both di-allelic for the genes *Lap-2* and *Lap-1*, respectively, segregated in a 3:1 ratio. Hence, the genotypes of these plants must have been *Lap-2*^{1}/*Lap-2*^{1}/*Lap-2*^{1}/*Lap-2*^{3} and *Lap-1*^{1}/*Lap-1*^{1}/*Lap-1*^{1}/*Lap-1*^{3}, respectively. It might be suggested that 3:1 ratio would be expected for disomic inheritance. QUIROS and MORGAN (1981) showed that these electrophoretic alleles were codominant. Therefore, the actual expected ratio for disomic inheritance would be 1:2:1.

TABLE 1

Tetrasomic segregation for two alleles at three loci in the S_1 progenies of four alfalfa plants

Plant	Parent genotype	Progeny phenotype	Observed distribution	Expected ratio	χ^2	p
79M 6–54	*Lap-2¹/Lap-2¹/Lap-2¹/Lap-2³*	LAP-2¹	11	1		
		LAP-2¹/LAP-2³	29	3	0.03	0.85
79M 6–57	*Lap-1¹/Lap-1¹/Lap-1¹/Lap-1³*	LAP-1¹	34	1		
		LAP-1¹/LAP-1³	84	3	0.72	0.45
FV 11–27	*Lap-1¹/Lap-1³/Lap-1³/Lap-1³*	LAP-1³	54	1		
		LAP-1¹/LAP-1³	137	3	0.93	0.30
79M 6–51	*Prx-1¹/Prx-1¹/Prx-1²/Prx-1²*	PRX-1¹	7	1		
		PRX-1¹/PRX-1²	175	34		
		PRX-1²	7	1	1.22	0.60

No homozygous individuals were found for the alleles *Lap-1*^{3} and *Lap-2*^{3} in the progenies of plants 79M 6–57 and 79M 6–54. The best explanation is that they segregated in a tetrasomic fashion. The progeny of plant 79M 6–51, heterozygous for phenotype PRX-1^{1}/PRX-1^{2}, segregated in the 1:34:1 ratio (Table 1 and Figure 1A). This is typical of tetrasomic inheritance for codominant alleles in duplex genotypes. Therefore, the genotype of this plant must have been *Prx-1*^{1}/*Prx-1*^{1}/*Prx-1*^{2}/*Prx-1*^{2}.

The progeny of the plants HR 1–49 and FV 11–27, both tri-allelic at locus *Lap-2*, segregated in the ratio 1:8:18:8:1 (Table 2), expected for tetrasomic inheritance involving three codominant alleles, *Lap-2*^{3} in duplex and *Lap-2*^{5} and *Lap-2*^{6} in simplex (Figure 1B). The presence of homozygous plants for *Lap-2*^{3} in the progeny of both plants indicates that this was the allele in duplex in the parental plants. In addition, plant FV 11–27 segregated in the 3:1 ratio for

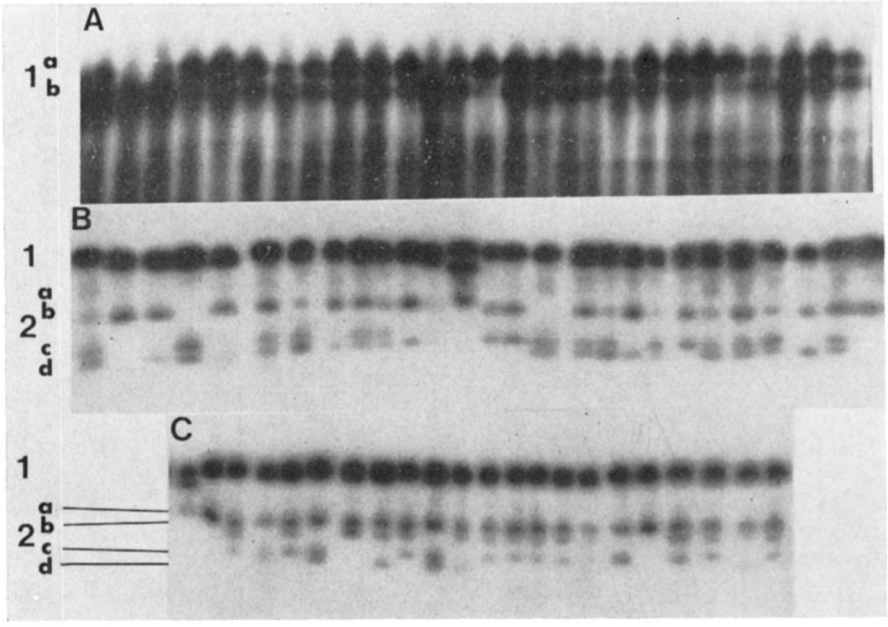

FIGURE 1.—Zymograms displaying the segregation of various alleles at loci *Prx-1* and *Lap-2*. Anodal direction is above.

A. Segregating progeny of plant 79M 6–51 with genotype *Prx-1*^{1}/*Prx-1*^{1}/*Prx-1*^{2}/*Prx-1*^{2}. From the left, plants 2 and 3 are homozygous for *Prx-1*^{2} (band 1b) and plant 14 is homozygous for *Prx-1*^{1} (band 1a). The rest of the plants are heterozygous for these two alleles. B. Segregating progeny of plant HR 1–49 with genotype *Lap-2*^{3}/*Lap-2*^{3}/*Lap-2*^{5}/*Lap-2*^{6} in the positions 2b, 2c and 2d, respectively. Plant 13 from the left was included as a control. It is homozygous for *Lap-2*^{1} (band 2a). *Lap* loci are numbered at margin. C. Segregating progeny from the crossing of plant 79M 6–51, homozygous for allele *Lap-2*^{1} (band 2a) by FV 11–27 of genotype *Lap-2*^{3}/*Lap-2*^{3}/*Lap-2*^{5}/*Lap-2*^{6} at positions 2b, 2c and 2d, respectively. Individual 17 from the left, with phenotype LAP-2^{1}/LAP-2^{5} probably originated by double reduction. Individuals 2 and 18 from the left, homozygous for *Lap-2*^{1}, originated by double reduction. Individuals 2 and 18 from the left, homozygous for *Lap-2*^{1}, originated from accidental selfing of plant 79M 6–51. The first plant from the left was used as a reference. *Lap* loci are numbered at margin.

TABLE 2

Tetrasomic segregation for three alleles at locus Lap-2 *in the* S_1 *progenies of three alfalfa plants*

Plant	Parent genotype	Progeny phenotype	Observed distribution	Expected ratio	χ^2	p
HR 1–49	*Lap-2³/Lap-2³/Lap-2⁵/Lap-2⁶*	LAP-2³	6	1		
		LAP-2³/LAP-2⁵	38	8		
		LAP-2³/LAP-2⁵/LAP-2⁶	80	18		
		LAP-2³/LAP-2⁶	26	8		
		LAP-2⁵/LAP-2⁶	4	1	3.16	0.55
81M 10–27	*Lap-2¹/Lap-2¹/Lap 2³/Lap-2⁵*	LAP-2¹	6	1		
		LAP-2¹/LAP-2³	46	8		
		LAP-2¹/LAP-2³/LAP-2⁵	86	18		
		LAP-2¹/LAP-2⁵	38	8		
		LAP-2³/LAP-2⁵	4	1	1.58	0.85
FV 11–27	*Lap-2³/Lap-2³/Lap-2⁵/Lap-2⁶*	LAP-2³	6	1		
		LAP-2³/LAP-2⁵	54	8		
		LAP-2³/LAP-2⁵/LAP-2⁶	97	18		
		LAP-2³/LAP-2⁶	34	8		
		LAP-2⁵/LAP-2⁶	5	1	4.90	0.32

Lap-1, which indicates that its genotype must have been *Lap-1*^{1}/*Lap-1*^{3}/*Lap-1*^{3}/*Lap-1*^{3} (Table 2).

The S_1 progeny from plant 80M 133–3 which was tetra-allelic for locus *Lap-2*, fitted well the expected ratio of 1:1:1:1:1:1:6:6:6:6:6 for tetrasomic inheritance (Table 3 and Figure 2A). The F_2 progenies resulting from selfing the F_1 plants 81M 10–3, 81M 10–27 and 81M 10–32, all tetra-allelic for locus *Prx-1*, and derived from the hybridization of the species *M. falcata* (plant FV 11–27) and *M. sativa* (plant 79M 6–51), produced the 11 segregating classes expected for tetrasomic inheritance (Table 4 and Figure 2B). Only the progeny of plant 81M 10–32 deviated significantly from the expected 1:1:1:1:1:1:6:6:6:6:6 ratio (Table 4). In this progeny, an excess of the di-allelic classes PRX-1^{1}/PRX-1^{3} and PRX-1^{2}/PRX-1^{4} was observed. The excess of individuals in these classes did not appear to be due to the effect of preferential pairing of the chromosomes from each species since alleles *Prx-1*^{1} and *Prx-1*^{2} originated from the *M. sativa* parent and the alleles *Prx-1*^{3} and *Prx-1*^{4} from the *M. falcata* parent. Since the resolution of the bands for this enzyme was good, it is unlikely that this deviation is due to scoring errors. The best fit for the expected segregation was obtained from the progeny of plant 81M 10–3. Although the progeny of plant 81M 10–27 did not deviate significantly from the expected segregation, there was an excess of in-

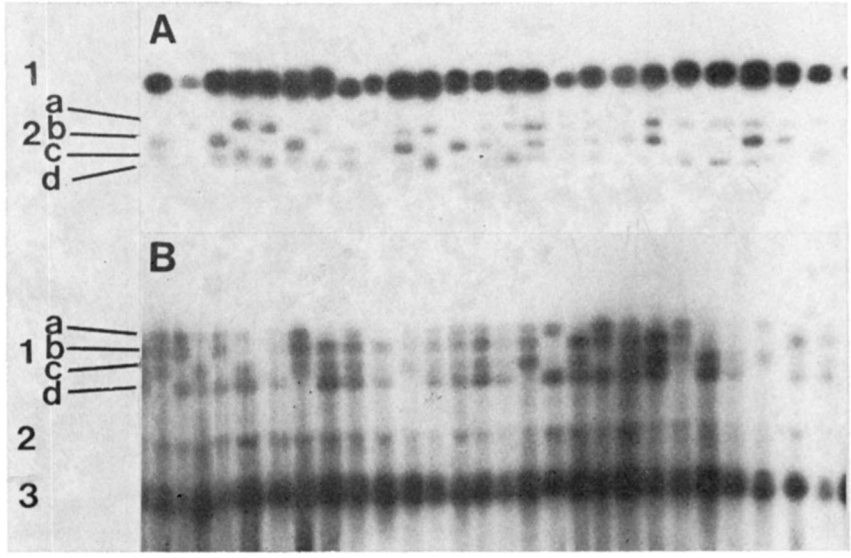

FIGURE 2.—Zymograms displaying tetra-allelic segregation for loci *Lap-2* and *Prx-1*. Anodal direction is above.

A. Segregating progeny of plant 80M 133–3 with genotype *Lap-2*^{2}/*Lap-2*^{3}/*Lap-2*^{4}/*Lap-2*^{5} in the positions a, b, c and d, respectively. Plants 8, 12, 15 and 16 from the left are tetra-allelic. The rest of the plants are di- or tri-allelic for the different allelic combinations. *Lap* loci are numbered at margin. B. Segregating progeny of plant 81M 10–27 with genotypes *Prx1*^{1}/*Prx1*^{2}/*Prx-1*^{3}/*Prx-1*^{4} in the positions b, c, d and a, respectively. Plants 9, 12 to 15 and 27 are tetra-allelic. The rest of the plants are di- or tri-allelic for the different allelic combinations. The three *Prx* loci are numbered at margin.

TABLE 3

Tetrasomic segregation for four alleles at locus Lap-2 *resulting from selfing a tetra-allelic alfalfa plant*

Plant	Parent genotype	Progeny phenotype	Observed distribution	Expected ratio	χ^2	p
80M 133–3	*Lap-2²/Lap-2³/Lap-2⁴/Lap-2⁵*	LAP-2²/LAP-2³	5	1		
		LAP-2²/LAP-2⁴	9	1		
		LAP-2²/LAP-2⁵	5	1		
		LAP-2³/LAP-2⁴	6	1		
		LAP-2³/LAP-2⁵	7	1		
		LAP-2⁴/LAP-2⁵	5	1		
		LAP-2²/LAP-2³/LAP-2⁴	31	6		
		LAP-2²/LAP-2³/LAP-2⁵	21	6		
		LAP-2²/LAP-2⁴/LAP-2⁵	41	6		
		LAP-2³/LAP-2⁴/LAP-2⁵	29	6		
		LAP-2²/LAP-2³/LAP-2⁴/LAP-2⁵	37	6	10.40	0.45

TABLE 4

Tetrasomic segregations for four alleles at locus Prx-1 *in the S_1 progenies of three tetra-allelic plants of identical genotype,* Prx-1^1/Prx-1^2/Prx-1^3/Prx-1^4

Phenotypic classes	Expected ratio	Observed progeny distribution 81M 10-3*	81M 10-27*	81M 10-12*	Total
PRX-1^1/PRX-1^2	1	10	7	6	23
PRX-1^1/PRX-1^3	1	9	7	14	30
PRX-1^1/PRX-1^4	1	6	4	7	17
PRX-1^2/PRX-1^3	1	7	6	4	17
PRX-1^2/PRX-1^4	1	7	7	12	26
PRX-1^3/PRX-1^4	1	4	1	4	9
PRX-1^1/PRX-1^2/PRX-1^3	6	30	33	39	102
PRX-1^1/PRX-1^2/PRX-1^4	6	37	43	32	112
PRX-1^1/PRX-1^3/PRX-1^4	6	29	31	33	93
PRX-1^2/PRX-1^3/PRX-1^4	6	39	20	31	90
PRX-1^1/PRX-1^2/PRX-1^3/PRX-1^4	6	35	44	29	108
	χ^2	7.96	16.78	21.28	27.80
	p	0.65	0.09	0.03	0.12

* Plant number.

dividuals in the tetra-allelic class and in the tri-allelic class PRX-1^1/PRX-1^2/PRX-1^4, and a deficiency of individuals in the class PRX-1^2/PRX-1^3/PRX-1^4. When this progeny was scored for *Lap-2* locus, which appears unlinked to the *Prx* loci, (QUIROS and MORGAN 1981) its segregation fitted well the expected tetrasomic ratio (Table 2). This may indicate that the possible deviations are restricted to the gene *Prx-1*. The fact that no significant deviations from tetrasomic inheritance were observed in two of three progenies from this hybrid, favors the concept that *M. sativa* and *M. falcata* are just different biotypes of a common species—a conclusion also reached by LESINS and LESINS (1979). When the data of the three families scored for *Prx-1* are pooled (χ^2 heterogeneity = 27.8, p = 0.12), the number of di-allelic individuals PRX-1^3/PRX-1^4 is deficient, and PRX-1^1/PRX-1^3 and PRX-1^2/PRX-1^4 individuals (Table 4) are in excess. This observation might suggest that a detrimental interaction exists between the *M. falcata* chromosome segments carrying the alleles *Prx-1*^{3} and *Prx-1*^{4}. The same trend was observed in the two tri-allelic classes carrying these two alleles. In the tetra-allelic class, however, the presence of the two different sets of chromosome segments seems to correct the possible detrimental effect. This provides support for the theory that the success of autotetraploid individuals depends on favorable allelic interaction (BUSBICE 1968; DUNBIER and BINGHAM 1975). BUSBICE (1968) pointed out in this regard that "epistatic gene action may be the important factor in zygote viability; recessive lethals in the homozygote state at one locus may mask the effects of viability genes at all other loci."

In general, all the progenies scored for the three loci, *Prx-1*, *Lap-1* and *Lap-2*, segregated according to the expected ratios for tetrasomic inheritance of codominant genes governing monomeric enzymes, assuming chromosomal segregation.

TABLE 5

Tetrasomic segregation for four alleles at locus Lap-2, *assuming double reduction. Progeny resulting from crossing two plants belonging to the species* M. sativa *and* M. falcata

Plant	Parental genotype	Progeny phenotype	Observed distribution	Expected ratio	χ^2	p
79M 6–51	*Lap-2¹/Lap-2¹/Lap-2¹/Lap-2¹*	LAP-2¹/LAP-2³	21	16		
		LAP-2¹/LAP-2³/LAP-2⁵	37	20		
×	×	LAP-2¹/LAP-2³/LAP-2⁶	32	20		
FV 11–27	*Lap-2³/Lap-2³/Lap-2⁵/Lap-2⁶*	LAP-2¹/LAP-2⁵/LAP-2⁶	16	10		
		LAP-2¹/LAP-2⁵†	3	3		
		LAP-2¹/LAP-2⁶†	2	3	3.90	0.60

* For maximum equation segregation.
† Segregation classes probably originating by double reduction.

However, the F_1 progeny resulting from crossing the plant 79M 6–51 and FV 11–27 seems to provide evidence for double reduction at locus *Lap-2* (Table 5 and Figure 1C). This cross involved four alleles with parent 79M 6–51 being monoallelic for *Lap-2*^{1} and the parent FV 11–27, tri-allelic with genotype *Lap-2*^{3}/*Lap-2*^{3}/*Lap-2*^{5}/*Lap-2*^{6}. The four phenotypic classes expected for chromosomal segregation were observed, namely LAP-2^1/LAP-2^3, LAP-2^1/LAP-2^3/LAP-2^5, LAP-2^1/LAP-2^3/LAP-2^6 and LAP-2^1/LAP-2^5/LAP-2^6, in the expected ratio 1:2:2:1 ($\chi^2 = 1.15$, $p = 0.75$). In addition, the phenotypic classes LAP-2^1/LAP-2^5 and LAP-2^1/LAP-2^6 were observed in lower proportions (Table 5). These two classes could have arisen by double reduction, resulting from the presence of two chromosomes in a gamete, carrying both the allele *Lap-2*^{5} in one case and *Lap-2*^{6} in the other. These chromosomes would have come from sister chromatids in the microspores of plant FV 11–27 after crossing over between the centromere and the locus *Lap-2*. Cytological examination of the pollen mother cells in plant FV 11–27 revealed occasional trivalents and quadrivalents, which is a prerequisite for double reduction. Although chromosomal nondisjunction or chromosomal loss cannot be ruled out as explanations for the presence of the two unexpected phenotypic classes in this progeny, these are nevertheless unlikely because no meiotic abnormalities were observed in plant FV 11–27. The possible occurrence of double reduction was also reported by STANFORD (1951) in his progenies segregating for flower color.

In conclusion, the present study provides further evidence of autotetraploidy in alfalfa based on the segregation of multiple alleles at three loci. It also proves the existence of tri- and tetra-allelic loci in this species. The advantage of this genetic system is the unequivocal detection of tetrasomic segregation in one generation. For morphological traits such as flower color, it often may be necessary to go to the F_3 generation for the same determination (STANFORD 1951). This study implies that new breeding methodologies utilizing the concept of maximum heterozygosity suggested by DUNBIER and BINGHAM (1975) and BINGHAM (1975) and BINGHAM (1979) have a genetic basis.

The author is indebted to CHARLES M. RICK and DONALD K. BARNES for reviewing the manuscript, to DORA G. HUNT for editorial work, to KENT KERBY and JEFF THURSTON for technical assistance. This work was supported by the Alberta Research Council grant DB102 to R. C. VON BORSTEL and grant NSERC GR2 to the president of the University of Alberta.

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Corresponding editor: R. L. PHILLIPS