Evaluation of Four *Brassica* Germplasm Collections for Fatty Acid Composition

D.L. Auld[a,*], K.A. Mahler[a] and D.J. LeTourneau[b]
[a]Dept. of Plant, Soil and Entomological Sciences and [b]Dept. of Bacteriology and Biochemistry, Univ. of Idaho, Moscow, Idaho 83843

A total of 1116 accessions of cultivated *Brassica* spp. from four collections were evaluated for fatty acid composition to identify variants which would be useful in plant improvement programs. The accessions included lines of *B. campestris* L., *B. napus* L., *B. oleracea* L. and *B. carinata* Braun obtained from the USDA collections at Ames, IA, Pullman, WA, and Geneva, NY, as well as the Centre for Genetic Resources-the Netherlands (CGN) at Wageningen, Netherlands. Fatty acid composition of each accession was determined by using gas chromatography. The range, mean, standard deviation and frequency distributions were calculated for the seven primary fatty acids found in the accessions from each collection.

Fatty acid profiles of the four species were very similar. Except for levels of erucic acid in the oilseed accessions of *B. campestris* and *B. napus*, only limited economic variation in fatty acid composition was evident. These data suggest that in these *Brassica* species the fatty acid profile has been tightly conserved. Estimated energy obtained by β-oxidation of a high erucic acid rapeseed (HEAR) oil was 13.8% higher than for a low erucic acid rapeseed (LEAR) oil. The higher energy levels of oils containing high levels of erucic acid may have played a significant role in survival and adaptation of these extremely small seed *Brassica* species. This may explain why such limited variation in fatty acid composition was found in these extensive and diverse collections.

Five species of rapeseed (*Brassica* spp.) have been used as sources of edible and industrial vegetable oils throughout much of the world (1). In 1986, rapeseed oil was the fourth most important source of edible vegetable oil, supplying in excess of 13% of the world supply (2). Genetic manipulation of fatty acid composition would allow increased utilization of rapeseed oil for both edible and industrial purposes (3,4).

Earlier research concentrated on characterizing genetic variation in fatty acid compositions available in wild species of the Brassicaceae (Cruciferae) (5-11). These studies showed tremendous variation in the relative concentration of the seven fatty acids commonly found in rapeseed. Some species also produced significant concentrations of hydroxy (10) and extremely long-chained fatty acids (9). Less extensive studies indicated the fatty acid composition of the six cultivated *Brassica* species was very similar. Characteristically, *B. campestris* L., *B. nigra* Koch, *B. oleracea* L., *B. Juncea* L. Coss., *B. napus* L. and *B. carinata* Braun had from 37-53% erucic acid (1,6). Of these six species, only *B. oleracea* has not been grown as an oilseed crop. In 1961 and 1964, Canadian scientists identified plants with low levels of erucic acid in *B. napus* (12) and *B. campestris* (13), respectively. These low erucic acid rapeseed (LEAR) lines produce a premium grade edible oil which has been extensively used throughout the world. The purpose of this research was to systematically screen four germplasm collections of *Brassica* to identify other significant variants in fatty acid composition.

*To whom correspondence should be addressed.

EXPERIMENTAL PROCEDURES

Seed collections. Seed of 364 accessions of *B. napus* and *B. campestris* were supplied by Bryce Abel, Regional Plant Intro. Stn., Iowa State University (Ames, IA). Seed of the 351 accessions of *B. oleracea* var. *capitata* were supplied by Rich Hannan, Western Regional Plant Introd. Stn., Washington State University (Pullman, WA). Seed of the 294 accessions of *B. oleracea* var. *botrytis* were supplied by Dr. Steve Kresovich, NE Regional Plant Introd Stn. (Geneva, NY). The seed of the 107 accessions of *B. carinata* were supplied by Dr. Louis van Soest, of the Centre for Genetic Resources the Netherlands (CGN) (Wageningen, Netherlands).

Fatty acid composition. Fatty acid composition of each accession was determined on a single 0.5 g sample of seed which had been received directly from the germplasm collection. Limited funding prevented the increase of the 1116 accessions in a common environment since incompatibility systems in several of the *Brassica* species would have required sibling hand pollinations. The seeds were ground with a mortar and pestle using silica sand as an abrasive. Oil was extracted from the ground seed with 4 ml of anhydrous ethyl ether. The oil solution was removed with a syringe using a synthetic cotton ball as a filter, and transesterified in 200 μl of 520% tetramethylammonium hydroxide in methanol. The sample was shaken and allowed to settle for 1 min before 2 ml of distilled water were added. The sample was shaken again and the phases allowed to separate. A 1 μl sample of the supernatant was injected by a Varian model 8000 Autosampler into a Varian Model 3700 Gas Chromatograph (GC) equipped with a flame ionization detector. The GC used a 3.05 m glass Supelco column containing 3% SP-2310 and 2% SP-2300 on a 100/120 mesh chromasorb WAW support maintained at 200° C. Helium was used as the carrier gas. Injector and detector temperatures were 250 and 300° C, respectively. A Varian Model 4290 integrator was used to determine relative concentrations of the major fatty acids.

A rapeseed standard (Supelco, Inc., cat. no. 4-7019) was injected as the control after every 19 accessions to ensure the accuracy of the analyses. Duplicate analyses were conducted on those accessions with unusual fatty acid profiles or limited integration areas to confirm initial results. Population distributions, means, ranges and standard deviations were calculated for the seven primary fatty acids for the accessions from each collection. Data taken on individual accessions were summarized in a separate report (14).

The potential energy derived by β-oxidation was estimated for the seed oils of both a winter LEAR cultivar ("Cascade") and a winter HEAR cultivar ("Bridger") grown at Moscow, ID during 1984-85 (15). It was assumed that both cultivars had an equivalent number of triacylglycerol molecules and that the slight difference in oil content was primarily due to the increased molecular weight of the longer chained fatty acids.

RESULTS AND DISCUSSION

The 364 accessions from Ames, IA and the 107 accessions of *B. carinata* have been grown primarily as oilseed crops. The palmitic acid concentration of the individual accessions of the USDA collection ranged from 1.6–9.2% and from 2.9–5.2% for the CGN collection (Table 1). Over 64% of the accessions in these two collections had concentrations of 2–4% of this saturated fatty acid (Fig. 1). The 351 accessions of *B. oleracea* var. *capitata* obtained from Pullman and the 294 accessions of *B. oleracea* var. *botrytis* from Geneva had slightly higher levels of palmitic acid (Table 1 and Fig. 1).

Stearic acid had a slightly smaller range of variation (0.0–3.6%) in all four collections (Table 1 and Fig. 2). The very low readings may reflect the difficulty of accurately measuring the extremely low concentration of minor fatty acids. The oils of rapeseed already contain lower levels of the saturated fatty acids (primarily palmitic and stearic acid) than any other commercial vegetable oil (1). Selection within the *B. napus* or the *B. campestris* accessions of the USDA-Ames germplasm collection could probably allow development of lines with less than 2% of palmitic acid and less than 1% stearic acid. However, selection in these populations for economic levels of these saturated fatty acids would be futile.

The three monounsaturated fatty acids (oleic, eicosenoic and erucic acids) comprised a significant proportion of the fatty acids found in the accessions from

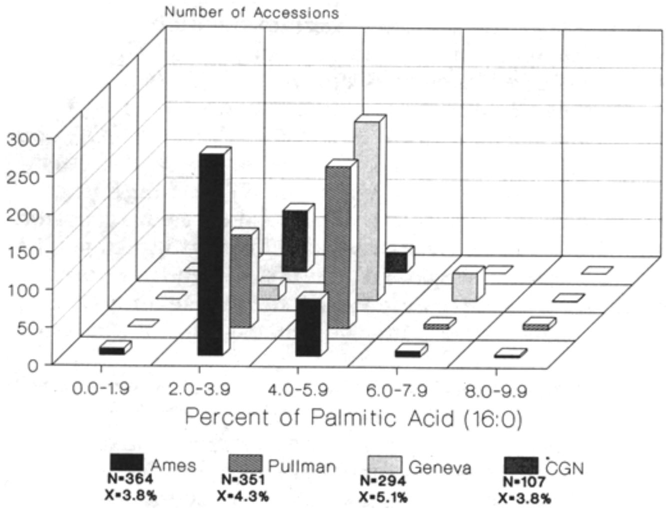

FIG. 1. Distribution of palmitic acid (16:0) concentration in 1116 accessions from four collections of *Brassica* germplasm.

TABLE 1

The Range, Mean and Standard Deviation of Concentration of the Seven Primary Fatty Acids in Accessions from Four Collections of Cultivated *Brassica* Species

Fatty acid	USDA-Ames (364 accessions)	USDA-Pullman (351 accessions)	USDA-Geneva (294 accessions)	CNG-Netherlands (107 accessions)
		Range of concentrations		
		% of methyl esters		
Palmitic	1.6 - 9.2	2.3 - 9.7	2.8 - 8.2	2.9 - 5.2
Stearic	0.0 - 3.6	0.0 - 1.9	0.0 - 1.6	0.5 - 1.4
Oleic	11.0 - 57.8	8.5 - 35.6	6.7 - 38.3	7.8 - 20.5
Linoleic	8.4 - 26.4	9.5 - 24.6	9.8 - 22.1	14.1 - 21.4
Linolenic	3.3 - 14.8	4.5 - 19.7	4.6 - 17.6	11.6 - 20.7
Eicosenoic	1.9 - 17.7	5.4 - 12.8	2.4 - 11.7	6.3 - 10.2
Erucic	0.7 - 54.3	15.6 - 56.1	25.7 - 62.9	29.7 - 48.6
		Mean concentration		
		% of methyl esters		
Palmitic	3.8	4.3	5.1	3.8
Stearic	1.4	0.7	0.6	0.9
Oleic	20.3	16.3	14.0	10.7
Linoleic	15.2	14.3	14.6	17.1
Linolenic	8.9	10.9	10.3	15.0
Eicosenoic	9.4	8.6	6.1	7.6
Erucic	39.7	43.7	48.7	42.6
		Standard deviation		
		% of methyl esters		
Palmitic	1.0	1.1	0.9	0.4
Stearic	0.4	0.6	0.3	0.1
Oleic	7.5	3.3	4.8	1.8
Linoleic	2.6	2.1	2.2	1.4
Linolenic	1.6	2.1	2.2	1.6
Eicosenoic	2.4	1.4	1.8	0.7
Erucic	10.0	5.2	7.1	2.9

all four collections (Table 1). Those accessions with low levels of erucic acid show almost directly proportional increases in the concentration of oleic acid. The maximum levels and greatest variation in oleic acid concentration in this study were observed in accessions from the Ames collection (Fig. 3). Recently developed (LEAR) cultivars commonly produce oils with more than 50% oleic acid (14). These high levels of oleic acid occur when the fatty acid elongation pathway responsible for the formation of eicosenoic and erucic acid is genetically blocked. As the concentration of erucic acid is reduced in rapeseed there is an almost direct increase in the concentration of oleic acid (3,16). Similar genetic blocks were not detected in the accessions from the other three collections. The 364 accessions of the Ames collection also had the greatest range in eicosenoic acid concentration (1.9–17.7%) (Table 1 and Fig. 4). Only the Ames collection had accessions with very low levels of erucic acid (Table 1 and Fig. 5). The erucic acid concentration ranged from 15.6–62.9% in the other three collections. Only 12 of the accessions had oils with less than 5% erucic acid (14). This reflects the large number of Ames accessions that were collected prior to 1961 when the gene(s) that reduce the level of erucic acid were first identified. Reduction of the level of erucic acid to less than 2% is controlled by a single gene in the diploid *B. campestris* (13,17) and by two genes in the allotetraploid *B. napus* (3,10,17–21). Other genes and/or multiple alleles also play a role in intermediate and very high levels of erucic acid (13,18,22). This level of variation was not found in the vegetable *Brassica* accessions or the *B. carinata* collection. Fourteen accessions from Geneva had greater than 60% erucic acid, but none of the lines exceeded the theoretical limit of 66% erucic acid found in many Brassicacae species (1,6). However, the Geneva collection may carry genes which could allow maximum expression of erucic acid within this limit.

The two primary polyunsaturated fatty acids found in rapeseed are linoleic acid and linolenic acid (1,6). The concentration of linoleic acid in accessions from their collections ranged from 8.4–26.4% while the concentration of linolenic acid ranged from 3.3–14.8% (Table 1, Figs. 6 and 7). Once again, the greatest variation occurred in the oilseed *Brassica* accession from Ames. Most of the accessions of *B. carinata* had slightly lower higher levels of polyunsaturated fatty acids than the other *Brassica* species (Figs. 6 and 7). Reduced and/or increased levels

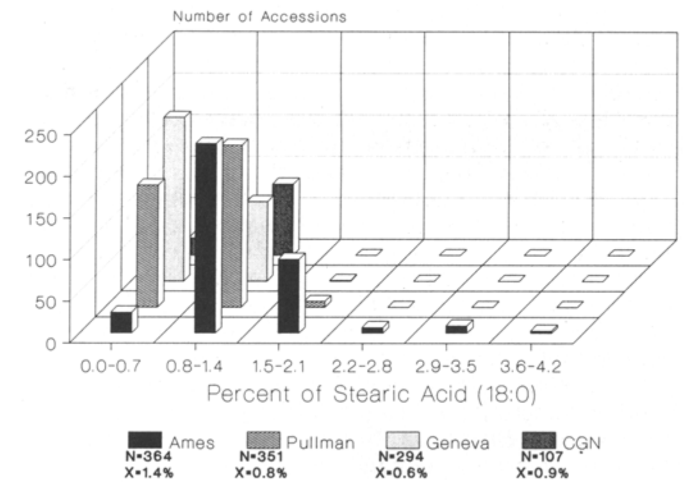

FIG. 2. Distribution of stearic acid (18:0) concentration in 1116 accessions from four collections of *Brassica* germplasm.

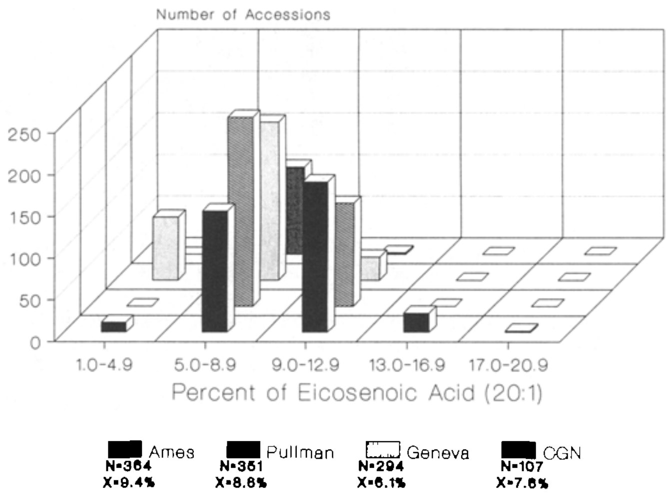

FIG. 4. Distribution of eicosenoic acid (20:1) concentration in 1116 accessions from four collections of *Brassica* germplasm.

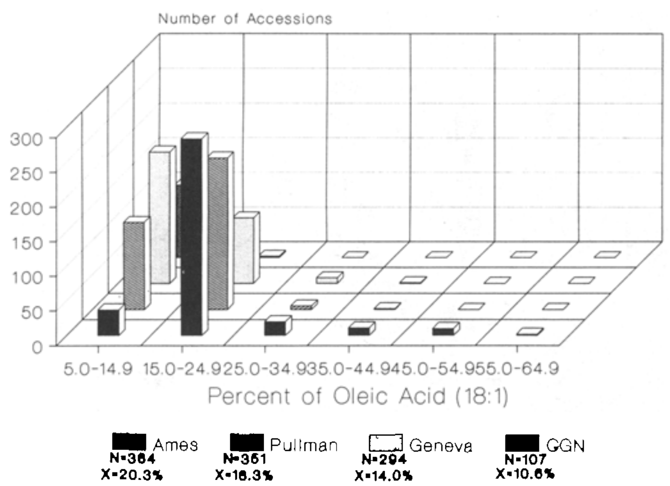

FIG. 3. Distribution of oleic acid (18:1) concentration in 1116 accessions from four collections of *Brassica* germplasm.

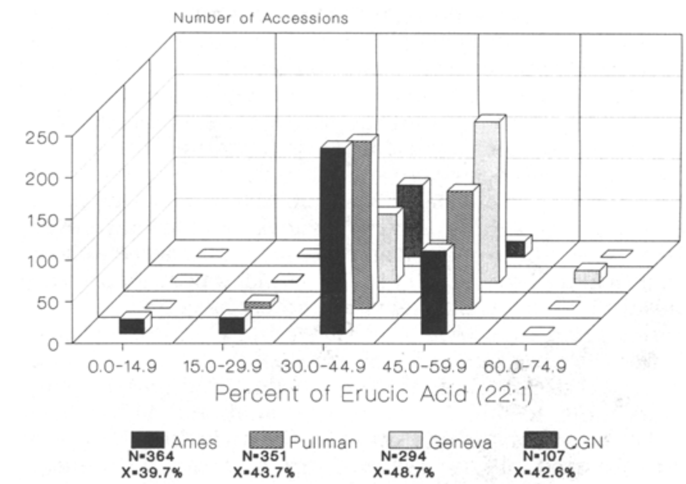

FIG. 5. Distribution of erucic acid (22:1) concentration in 1116 accessions from four collections of *Brassica* germplasm.

TABLE 2

Estimated Energy Derived by β-oxidation Content of Typical Low Erucic Acid (LEAR) and High Erucic Acid (HEAR) Winter Rapeseed Oils from Seed Grown at Moscow, Idaho (25)

Fatty acid	Molecular weight	Energy content	LEAR oil Fatty acid composition	LEAR oil Energy content	HEAR oil Fatty Acid composition	HEAR oil Energy content
	g	-ATP/Mole-	-% Methyl Ester-	-ATP/Mole %-	-% Methyl Ester-	-ATP/Mole %-
Palmitic	256	129	4.5	6.45	2.3	3.74
Stearic	284	146	1.6	2.34	0.5	0.88
Oleic	282	144	61.6	88.56	15.8	24.77
Linoleic	280	142	19.4	27.70	11.0	17.04
Linolenic	278	140	10.7	15.26	5.0	7.70
Eicosenoic	310	161	1.4	1.93	7.9	12.56
Erucic	338	177	0.3	0.35	57.0	95.58
Total			99.5	142.6	99.5	162.3

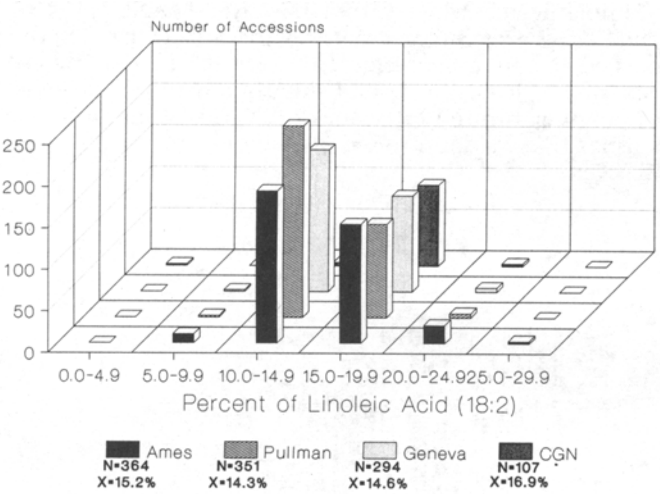

FIG. 6. Distribution of linoleic acid (18:2) concentration in 1116 accessions from four collections of *Brassica* germplasm.

of these two fatty acids could probably be achieved by using the variation expressed in this collection. Since the relative concentration of these two fatty acids is influenced by both environmental factors and the maternal genotype, selection of lines with differential concentrations of these fatty acids has been difficult (23–25).

The Ames oilseed accessions had the greatest variation in fatty acid composition, reflecting selection for this trait over the past 40 years (Table 1). The 107 accessions of *B. carinata* obtained from the Netherlands were originally collected in Ethiopia and are agronomically and botanically distinct from the USDA collections. Despite these differences, the fatty acid profiles of the *B. carinata* accessions were similar to those observed in the other *Brassica* species. The vegetable accessions of *B. oleracea* from Pullman and Geneva, which had never been screened for fatty acid composition, were almost identical to the oilseed types. It would appear that in the absence of artificial selection, most accessions of *B. napus*, *B. campestris*, *B. oleracea* and *B. carinata* have a fatty acid composition of approximately 4% palmitic acid, 1% stearic acid, 17% oleic acid, 15% linoleic acid, 10% linolenic acid, 8% eicosenoic acid, and 44% erucic acid. Accessions which vary significantly from this profile are probably the result of rare mutants in the fatty acid biosynthesis pathway which are expressed only during seed maturation (1). All of the low erucic acid rapeseed cultivars currently grown worldwide originated from only three sources (12,13,20). The absence of other major variants in fatty acid synthesis of *Brassica* in this study, as well as in the literature, indicate that fatty acid composition in these species has been tightly conserved.

To determine if HEAR oils could impart a selective advantage over LEAR oil, the energy derived by β-oxidation of both oils was calculated. The energy contents of the LEAR and HEAR oils were 142.6 and 162.3 ATP/Mole % respectively (Table 2). The oil in the LEAR seeds had 13.8% less ATP equivalents per mole percentage than the HEAR seeds. The increased energy content of the seeds containing HEAR oils suggests that fatty acid composition could have had an influence on the survival of extremely small seeded species such as rapeseed. The high melting point of triacylglycerols with more than two erucic acids probably limited the development of genotypes with erucic acid levels higher than 66%. This may explain why so little variation in fatty acid composition was found in the 1116 accessions of the four *Brassica* species evaluated from these divergent collections. Plant breeders selecting for differential fatty acid composition of the oilseed *Brassicas* would need to use other techniques to incorporate or induce additional variation.

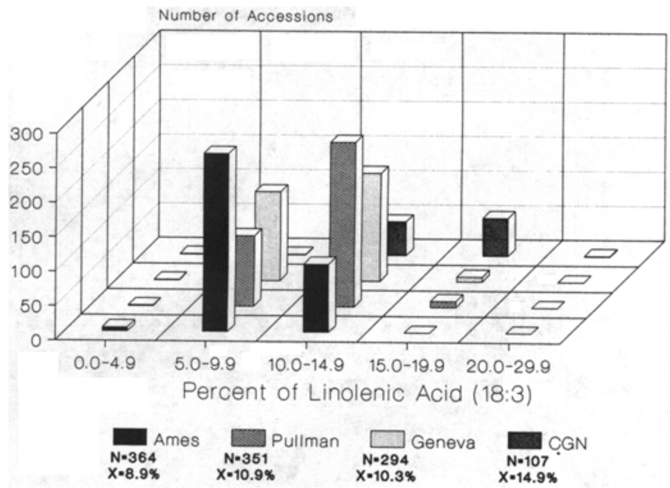

FIG. 7. Distribution of linolenic acid (18:3) concentration in 1116 accessions from four collections of *Brassica* germplasm.

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REFERENCES

1. Robbelen, G. and W. Thies, in *Brassica crops and wild allies*, edited by S. Tsunoda, K. Hinata, and Gomez-Campo, Japan Sci. Soc. Press, Tokyo, Japan, 1980, p. 253–283.
2. USDA Agricultural Statistics, 1986. p. 138.
3. Downey, R.K., *J. Am. Oil Chem. Soc. 41*:475–478 (1963).
4. Nieschlag, H.J. and I.A. Wolff, *Ibid. 48*:723–727 (1971).
5. Appelqvist, L.A., *Ibid. 48*:740–744. (1971).
6. Goering, K.J., R. Eslick and D.L. Brelsford, *Econ. Bot. 19*:251–256 (1965).
7. Kumar, P.R. and S. Tsunoda, *J. Am. Oil Chem. Soc. 55*:320–323 (1978).
8. Kumar, P.R. and S. Tsunoda, in *Brassica crops and wild allies*, edited by S. Tsunoda, K. Hinata, and Gomez-Campo, Japan Sci. Soc. Press, Tokyo, Japan, 1980, p. 235–252.
9. Mikolajczak, K.L., T.K. Miwa, F.R. Earle, I.A. Wolff and Q. Jones, *J. Am. Oil Chem. Soc. 38*:678–681 (1961).
10. Mikolajczak, K.L., C.R. Smith, Jr., and I.A. Wolff, *Ibid. 42*:939–941 (1965).
11. Miller, R.L., F.R. Earle, and I.A. Wolff, *Ibid. 42*:817–821 (1965).
12. Stefansson, B.R., F.W. Hougen, and R.K. Downey, *Can. J. Plant Sci. 41*:218–219 (1961).
13. Dorrell, D.G. and R.K. Downey, *Ibid. 44*:499–504 (1964).
14. Auld, D.L., K.A. Mahler and A.A. Voorhis, Idaho Agr. Exp. Stn. Misc., Series No. 141 (1988).
15. Zubay, G., *Biochemistry*. Addison-Wesley Publishing Co., Reading, MS, 1988, p. 603–607.
16. Craig, B.M., *Can. J. Plant Sci. 39*:437–442 (1961).
17. Jonsson, R., *Hereditas 86*:159–170 (1976).
18. Harvey, B.L. and R.K. Downey, *Can. J. Plant Sci. 44*:104–112 (1964).
19. Kondra, Z.P. and B.R. Stefansson, *Can. J. Genet. Cytol. 7*:500–510 (1965).
20. Krzymanski, J. and R.K. Downey, *Can. J. Plant Sci. 50*:345–346 (1969).
21. Stefansson, B.R. and F.W. Hougen, *Ibid. 44*:359–364 (1964).
22. Anand, I.J. and R.K. Downey, *Ibid. 61*:199–203 (1981).
23. Kondra, Z.P. and B.R. Stefanson, *Ibid. 50*:345–346 (1970).
24. Thomas, P.M. and Z.P. Kondra, *Ibid. 53*:221–225 (1973).
25. Tremolieres, H., S. Tremolieres, and P. Mazliak, *Phytochemistry 17*:685–687 (1978).

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