Theor Appl Genet (1983) 65:123–128

Mucronate, Mc, a dominant gene of maize which interacts with *opaque-2* to suppress zein synthesis

F. Salamini, N. Di Fonzo, E. Fornasari and E. Gentinetta
Istituto sperimentale per la Cerealicoltura, Sezione di Bergamo, Via Stezzano 24, I-24100 Bergamo, Italy

R. Reggiani and C. Soave
Istituto Biosintesi Vegetali, C.N.R., Via Bassini 15, I-20133 Milano, Italy

Received December 30, 1982
Communicated by P. L. Pfahler

Summary. This paper describes a new dominant mutation of maize, *Mc,* which interferes in the endosperm with the synthesis of storage proteins. The mutant is characterized by an opaque phenotype; it reduces the deposition of zein and it increases the level of methionine. The mutation is specifically related to storage protein synthesis since soluble and insoluble carbohydrates are present at normal levels. The main interest of this mutant lies in its synergistic interaction with *opaque-2* in repressing zein synthesis. In the double mutant *o2Mc* the accumulation of zein is reduced to less than 10% of that of the normal endosperm. The control on zein synthesis exerted by the double mutant is at the level of production or stability of translatable zein mRNAs. The double mutant *o2Mc* germinates well offering the opportunity of using it in biochemical and molecular studies related to storage protein synthesis; the reduced endosperm weight of *o2Mc* negates its practical utilization in breeding maize for quality.

Key words: Maize endosperm – Zein – High-lysine genes – Storage proteins

Introduction

Zein, the storage protein of maize endosperms, is a mixture of polypeptides, encoded by at least four major families of structural genes (Soave et al. 1978, 1981, 1982; Valentini et al. 1979). Within the two families encoding the high molecular weight zeins (19–20Kd and 21–22Kd), subfamilies were identified by sequence analysis of cloned cDNA genes (Marks and Larkins 1982). In addition to sequencing, several other properties common to all zein polypeptides were used as criteria to describe and classify zeins: molecular weight, molecular charge, solubility, amino acid composition, cell compartimentation, tissue specificity (recently discussed in Soave and Salamini 1982). Moreover, the expression of zein structural genes is under the control of the so-called high-lysine genes whose mutated alleles depress the rate of zein deposition (Mertz et al. 1964; Nelson et al. 1965; McWhirter 1971; Ma and Nelson 1975; Salamini et al. 1979).

High-lysine genes of maize improve the nutritional value of the maize flours by decreasing the content of zein (Mertz et al. 1964; Nelson et al. 1965). Mutants of these loci reduce zein synthesis to different extents. For instance, four of these mutations show a degree of repression according to the following order: *o7* > *o2* > *fl2* > *De*-B30* (summarized in Soave and Salamini 1979). The synthesis of the SDS 19-20 and 21-22Kd zein families appear to be specifically controlled: *o7* and *o2* control both zein families but exhibit a preferential action respectively on SDS 19-20Kd and SDS 21-22Kd zeins; *Fl2* controls the accumulation of both components, while *De*-B30* inhibits only the SDS 22Kd zeins. The high-lysine mutants differ also in their degree of dominance: *o2* and *o7* are recessive, *fl2* is dose dependent, *De*-B30* is dominant.

Interaction among high-lysine mutants has been studied for the combination *o2-o7, o2-fl2, o7-fl2* (Di Fonzo et al. 1980; Fornasari et al. 1982). The *fl2* allele appears to be completely hypostatic to both *o2* and *o7,* while the interaction *o2-o7* is additive. In this paper we present data on a new mutant, *Mc,* which controls the rate of zein accumulation and shows an interesting synergistic interaction with the *o2* allele: in the double mutant *o2Mc* zein synthesis is almost completely supressed.

Table 1. Weight and total protein, non protein nitrogen and zeins content of normal and mutant endosperm during development and at maturity (+ + + + + + = B37 normal; + + *Mc* + + + = ♀ B37 normal × ♂ B37*Mc*; *McMc* + + + + = ♀ B37*Mc* × ♂ B37 normal; *McMcMc* + + + = B37*Mc*; + + + *o2o2o2* = B37 *o2*; *McMcMc o2o2o2* = B37 *o2Mc*)

Genotype	Endosperm weight (mg)				Protein per endosperm (mg)				Non protein nitrogen per endosperm (mg of protein equivalent)				Zeins[a] per endosperm (mg)			
	DAP[b]				DAP				DAP				DAP			
	21	28	35	M	21	28	35	M	21	28	35	M	21	28	35	M
+ + + + + +	51	113	128	200	8.0	15.2	16.9	22.7	1.1	1.1	0.7	0.9	2.9	7.5	9.6	11.8
+ + *Mc* + + +	55	104	128	198	7.9	13.6	16.6	24.5	0.9	0.9	0.6	1.2	3.0	6.9	9.1	13.0
McMc + + + +	51	91	121	161	7.7	11.9	14.4	17.5	1.0	0.9	0.5	0.8	2.8	5.6	7.7	9.4
McMcMc + + +	52	100	126	158	7.0	12.2	14.6	17.6	0.9	0.9	0.8	1.0	2.5	5.5	7.4	8.4
+ + + *o2o2o2*	41	96	137	160	5.5	10.4	12.7	13.8	1.9	3.4	3.1	2.2	0.4	1.7	3.5	4.1
McMcMc o2o2o2	50	99	117	122	6.9	10.9	11.4	9.8	2.6	4.3	3.3	1.8	0.3	0.8	1.1	1.1

[a] Fractions $Z_1 + Z_2$ of Landry and Moureaux (1970)
[b] DAP = days after pollination (M: maturity)

Materials and methods

The mutant Mucronate[1] (*Mc*) was isolated from a selfed ear of an *opaque-2* synthetic. In that ear the majority of seeds were modified toward a softer and lighter phenotype, different from that of standard *opaque-2* seeds. Genetic work, done using the modified kernels as starting material, permitted the separation from *opaque-2* of the homozygous *McMc* mutant. Phenotypically this mutant can be classified as opaque; its degree of opaque expression, however, is less than in *o1, o2, o6, o7, fl2* and *De*-B30*. The mutant allele is dominant to the wildtype. Allelism tests were run against the dominant or semidominant opaque types *fl1, fl2* and *De*-B30;* the F2s gave negative results and the mutation was then considered an allele of a new dominant opaque locus of maize. The mutant *Mc* was converted to the background of the inbred B37. The double recessive B37 *o2Mc* was then obtained by crossing B37 *Mc* by B37 *o2*, selecting in the F2 putative *o2Mc* phenotypes and progeny-testing them separately against B37 *o2* and *B37 Mc*.

B37 normal, B37 *o2*, B37 *Mc* and B37 *o2Mc*, were grown in 1980 at the farm of the Istituto sperimentale per la Cerealicoltura, Section of Bergamo. At flowering, plants were selfed. Crosses were also done between B37 *Mc* females and B37 normal males, and between B37 normal females and B37 *Mc* males. This gave endosperms with different doses of the *Mc* allele which were used to study the dosage effect of the mutant. Ears were harvested at 21, 28 and 35 days after pollination (DAP) and at maturity; unripe ears were frozen in liquid nitrogen, dehulled, the seeds stored at −20 °C and an aliquot at −80 °C.

Data on proteins, amino acids and carbohydrates were from lyophilized endosperms. Protein fractions were extracted from defatted meals as previously described (Di Fonzo et al. 1977). Nitrogen content was determined photometrically, following a micro-Kjeldhal digestion, by an indophenol method performed automatically by a Technicon AutoAnalyzer (Ferrari 1960). SDS-electrophoresis was performed according to Motto et al. (1979). Densitomer tracings of the zein electrophoretic patterns were made as already described in Vitale et al. (1982). Starch, sucrose and reducing sugars were determined following the methods of Thivend et al. (1972), Van Handel (1968) and Nelson (1944), respectively. For the other carbohydrates the procedure of Catravas (1967) as modified by Gentinetta et al. (1979) was adopted.

Total endosperm RNA was isolated as described by Wöstemeyer et al. (1980); the in vitro translation with the rabbit reticulocite lysate system was performed in 50 µl assay volumes as described by the supplier (NEN-DREIEICH, West Germany). The RNA was heated at 65 °C for 1 min prior to addition to the translation mixture. SDS gel electrophoresis of the translation products was performed according to Laemli (1970) and the gel was prepared for fluorography and autoradiography according to Laskey and Mills (1975).

Results

Seed weight, total proteins and protein fractions

Tables 1 and 2 present the results of kernel weight and protein traits. The two tables consider 6 genotypes, namely B37 normal, the two single mutant version of *o2* and *Mc*, the double mutant *o2Mc* and the two crosses between B37 normal and B37 *Mc*.

Considering that maize endosperm is a triploid tissue to which the female parent contributes with two chromosome complements, the comparison among the four genotype B37 normal, B37 normal × B37*Mc*, B37*Mc* × B37 normal and B37*Mc* allows one to evaluate *Mc* gene dosage effects. From the two tables the wildtype, *Mc* and *o2* alleles can be compared, and the effect of the two mutants alone or in combination can also be evaluated.

1 This genetic factor was called mucronate because of the phenotype observed when segregating within the *o2o2* ears: in the background of B37 the double mutant *o2Mc* phenotype shows a slightly collapsed endosperm with the tip of the embryo emerging

Table 2. Protein percentage of dry matter and protein fractions percentages of total proteins in normal and mutant endosperms during development and at maturity (genotypes as in Table 1).

Genotype	Protein percentage of dry matter				Protein fraction percentage of total proteins											
					Albumins and globulins				Zeins[a]				Glutelins plus insoluble fraction[b]			
	DAP[c]				DAP				DAP				DAP			
	21	28	35	M	21	28	35	M	21	28	35	M	21	28	35	M
+ + + +++	15.5	13.4	13.1	11.4	50.1	29.4	18.0	5.3	37.1	50.0	56.7	52.1	12.8	20.5	25.3	42.5
+ + *Mc* +++	14.2	13.0	12.9	12.3	47.6	27.3	17.9	7.3	38.5	50.4	55.0	53.1	13.9	22.2	26.6	39.6
McMc + +++	14.9	13.1	11.9	10.8	47.6	30.2	16.5	6.3	36.9	46.7	53.1	53.6	15.4	22.9	30.3	39.9
McMcMc +++	13.4	12.1	11.5	11.1	46.6	26.2	16.9	8.4	35.3	45.3	50.2	47.6	18.1	28.4	32.8	43.4
+ + + *o2o2o2*	13.2	10.7	9.2	8.5	79.8	69.1	41.9	21.9	8.4	16.4	27.7	29.7	11.7	14.2	30.3	48.3
McMcMc o2o2o2	13.7	10.9	9.7	8.0	84.1	72.6	51.3	27.0	4.2	7.3	9.5	10.1	11.6	20.0	39.2	61.9

[a] Fractions Z_1 and Z_2 of Landry and Moureaux (1970)
[b] Fractions $G_2 + G_3$ + insoluble proteins of Landry and Moureaux (1970)
[c] DAP = days after pollination (M: maturity)

Mutant *Mc*, with respect to the wildtype, has lighter endosperms, particularly at maturity, less protein per endosperm (at maturity 17.5 mg per endosperm as opposed to 22.7) while the protein percentage and the non protein nitrogen are unmodified, around 11% and 1.0% respectively. Zeins per endosperm are significantly less in the mutant (8.4 mg as opposed to 11.8 at maturity). Percent of albumins and globulins are slightly increased in *Mc* when compared to wildtype, but only at maturity. Glutelins, on the other hand, are higher in the mutant, particularly during development. Percentage of zeins at all developmental stages is lower in *Mc*.

Considering protein related traits, the *Mc* allele does not appear completely dominant over the wildtype: only when two doses of *Mc* enter into a cross can effects similar to those evident in *Mc* homozygous endosperms be observed.

Comparison between *Mc* and *o2* on seed and protein related traits shows deep differences. At all stages considered, total protein is higher in *Mc* than in *o2*. Moreover, compared to *Mc*, *o2* reveals its stronger repression of zein synthesis which is coupled with an increase in the non protein nitrogen per endosperm and of the percentages of albumins, globulins and glutelins.

Of particular interest is the behaviour of the double mutant *o2Mc* compared to the effects induced by *o2* and *Mc* alone. Seed weight and protein per endosperms are strongly reduced but only at later stages of development, while non protein nitrogen per endosperm and protein percentage on dry matter show levels similar to those of *opaque-2*. Zeins are severely reduced in the double mutant (at maturity 1.1 mg per seed in *o2Mc* vs 4.1 in *o2*, 8.4 in *Mc*, 11.8 in B37 normal and, as percentage on total protein, 10.1 in the double mutant, 29.7 in *o2*, 47.6 in *Mc* and 52.1 in B37 normal). In the double mutant, endosperm variations in the percentage of albumins and globulins and of glutelins are the opposite of those of zeins: the former increases to much higher levels than that of the wildtype or than that of the single mutant genotypes.

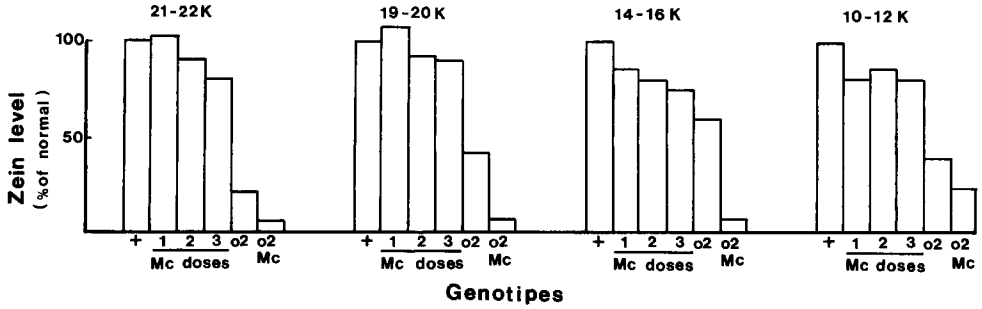

Fig. 1. Level of expression of different zein families in normal and mutant genotypes (+ = B37 normal; *Mc* = B37*Mc*; *o2* = B37*o2*; *o2Mc* = B37*o2Mc*). Partitioning of total zein in zein families was done after SDS-electrophoresis of total zein by densitometer tracings of the electrophoretic patterns

Figure 1 presents the effect of the various genotypes on the different size classes of zein. After separation of zein polypeptides by size, four main SDS families are easily recognized: the 21-22K, the 19-20K, the 14-16K and the 10-12K. Mutant *Mc* alone induces a slight inhibition of all 4 zein families. The recessive allele *o2* represses preferentially the accumulation of 21-22K zein polypeptides, but also the other three families are greatly affected. In *o2Mc* endosperms accumulation of the 21-22K, 19-20K and 14-16K zeins is almost completely nullified; only the 10-12K zeins are still synthesized to a certain extent.

Amino acids

The behaviour, at maturity, of the genotypes B37 normal, *o2*, *Mc* and *o2Mc* with respect to protein fractionation is mantained when the amino acid spectrum of total endospermic protein is considered (Table 3). Based on zein content the four genotypes fit into the following order: + > *Mc* > *o2* > *o2Mc*. The same order holds when single amino acids are considered. The percentage of those amino-acids which are very abundant in zeins, such as glutamate, proline, alanine and leucine, decreases according to the order + > *Mc* > *o2* > *o2Mc*, while aspartate, glycine, lysine and arginine, which are not found to any large extent in zeins, increase.

We emphasize the behaviour of *o2Mc* endosperms when the lysine level reaches 5.3%. The high level of methionine found in *Mc* compared to that of normal endosperm, is also worth mentioning.

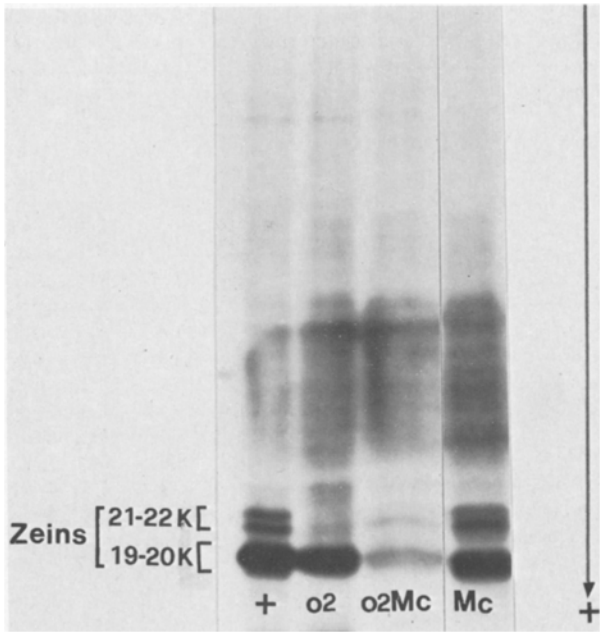

Fig. 2. In vitro translation products of RNA from B37 normal (+) and mutant endosperm (*o2* = B37*o2*; *Mc* = B37*Mc*; *o2Mc* = B37*o2Mc*). Electrophoresis of the ^{3}H-leucine labelled translation products of 30 DAP endosperm total RNA in a rabbit reticulocyte lysate was carried out on 15% SDS polyacrilamide gels. Translatable products of 10 and 14 Kd mRNA are not visible in the figure

Table 3. Amino acid content (% of total protein) of mature endosperms of normal and mutant genotypes (genotypes as in Table 1)

Amino acid	Genotype			
	+	*Mc*	*o2*	*o2Mc*
Aspartate-Asparagine	6.7	7.2	9.8	9.6
Threonine	3.7	3.9	4.0	4.4
Serine	5.2	5.9	5.2	5.7
Glutamate-Glutamine	18.8	17.2	15.9	13.3
Proline	11.3	10.1	9.4	9.8
Glycine	5.9	6.7	8.5	9.6
Alanine	11.3	11.0	9.4	9.1
Valine	5.6	5.8	5.7	6.3
Methionine	0.1	1.2	0.6	–
Isoleucine	3.5	3.5	3.3	3.3
Leucine	13.6	12.4	9.3	7.6
Tyrosine	1.9	2.0	2.0	2.0
Phenylalanine	3.8	3.8	3.6	3.4
Histidine	2.7	2.4	2.8	2.8
Lysine	2.5	3.2	4.5	5.3
Arginine	3.1	3.7	5.3	6.0
Recovery	93.2	91.0	88.7	73.2

Zein messenger RNAs

Total RNAs from thirty-day-old endosperms were extracted from B37 normal and from the mutant genotypes *o2*, *Mc*, *o2Mc*. The products of in vitro translation of these RNAs were studied by SDS-electrophoresis followed by fluorography and autoradiography (Fig. 2). As expected, the major translation products in the wild-type were the 21-22K and 19-20K zeins. The *opaque-2* mutant, as already known (Pedersen et al. 1980), interferes particularly with the 21-22K zein messenger RNAs. Mutant *Mc* does not substantially decrease the level of zein messenger RNAs; however its effect is dramatic when acting in the double combination with *o2*. In this case the translatable high molecular weight zein RNAs are only present in traces.

Carbohydrates

Soluble and insoluble carbohydrates have been determined in 35 DAP and mature endosperms of the genotypes B37 normal, *Mc*, *o2* and *o2Mc* (Table 4).

Table 4. Starch and sugar contents (% of dry matter) in normal and mutant endosperms at 35 DAP and at maturity (genotypes as is Table 1), (t = traces)

Genotype	Starch		Sucrose		Reducing sugars		Maltose		Fructose		Galactose		Xylose		Glucose	
	35	M	35	M	35	M	35	M	35	M	35	M	35	M	35	M
+ + + +++	73.5	78.9	1.99	0.29	0.34	0.17	0.028	0.009	0.153	0.084	t	0.041	t	0.013	0.278	0.093
+ + *Mc* +++	70.5	74.5	2.08	0.48	0.34	0.18	0.018	0.006	0.192	0.079	t	0.034	–	0.010	0.128	0.090
McMc + +++	75.5	77.3	2.00	0.33	0.33	0.09	0.012	0.005	0.113	0.070	t	0.020	–	0.011	0.117	0.083
McMcMc +++	74.2	77.8	2.10	0.44	0.38	0.20	0.014	0.006	0.183	0.093	0.128	0.061	t	0.012	0.128	0.109
+ + + *o2o2o2*	75.8	78.1	0.79	0.55	0.26	0.19	0.007	0.007	0.109	0.072	0.193	0.034	–	0.014	0.173	0.086
McMcMc o2o2o2	73.4	77.1	1.56	1.15	0.45	0.27	0.008	0.006	0.230	0.078	0.073	t	–	t	0.201	0.169

Starch content (% of dry matter) does not vary among genotypes. Sucrose level is higher than normal in *o2Mc*, both at 35 DAP and maturity. However *Mc* alone does not differ from wildtype. Reducing sugars seem slightly enhanced in *o2Mc* and reduced in *o2*. The disaccharide maltose and the monosaccharides fructose, galactose, xylose and glucose show no significant variations due to specific genotypes, with the exception of galactose which seems to reach a higher level than normal in *Mc*, *o2* and *o2Mc*.

Discussion

The mutant *Mc* belongs to the group of mutations which interfere with the synthesis of storage proteins in maize endosperm. It is characterized by an opaque phenotype and it negatively affects, though to a reduced extent, the deposition of zein. In the mutants *fl2* and *De*-B30* (Nelson et al. 1965; Salamini et al. 1979), it increases the level of methionine. This mutation does not affect zein synthesis by altering a primary route of carbohydrate metabolism, as occurs in the mutants *shrunken-2* and *brittle-2*. In the latter cases sucrose-starch interconversion is so affected by the absence of ADP-glucose pyrophosphorylase activity that high amount of the disaccharide accumulates (Tsai and Nelson 1966; Dickinson and Preiss 1969). This has not been observed in *Mc* endosperms confirming that the mutation is specifically related to storage protein synthesis or deposition.

Phenotypically the mutant allele appears dominant over the wildtype: segregations of 3 opaque to 1 normal seed have always been observed in selfed ears of heterozygous plants. This study shows, however, that the degree of dominance of *Mc* is not complete when protein parameters rather than kernel phenotype are considered. In the triploid endosperm one dose of the mutant *Mc* allele does not induce those effects conditioned by three doses. In this respect the mutant is similar to *fl2* which behaves as a semidominant trait showing dose-dependent effects on storage protein deposition (Di Fonzo et al. 1980). The main interest of this mutant lies in its synergistic interaction with *opaque-2* in repressing zein synthesis. In the double mutant *o2Mc* zein is less than 10% of that of the normal endosperms. This is unexpected considering the effects induced by *Mc* and *o2* alone and considering also the results of previous work done on the interactions among other mutants which reduce zein deposition. For instance the *fl2* mutation appears hypostatic to both *o2* and *o7;* in the combinations with these two recessive alleles only the effects of the latters are manifested (Di Fonzo et al. 1980; Fornasari et al. 1982). In the interaction *o2* and *o7*, on the other hand, a pattern of additivity emerges where a strong suppression of the synthesis of the two high molecular weight zein families is due to the *o2* suppression of the 21-22K family joined to that of *o7* on the 19-20Kd peptydes (Di Fonzo et al. 1979). In the case of the double mutant *o2Mc* the synergism of the two mutations leads to an almost complete suppression of zeins. We investigated the possibility that in the double mutant endosperms degradation of newly synthesized zein was taking place along with its synthesis. However it appears that the control exerted by the double mutant is at the level of production or stability of translatable zein mRNAs since synergism was observed also at the level of mRNAs translation. Furthermore this control is specific for zein messenger RNAs since: – 1) albumins, globulins and glutelins, on a per endosperm basis, are not altered in the double mutant; – 2) the in vitro translation patterns of total RNAs, except for the high M.W. zeins region, is very similar in all the genotypes; – 3) the double mutant *o2Mc* kernels have a good germinating capability indicating a normal development of the endosperm which can sustain a physiologically normal embryo (Salamini, unpublished data). The same is not the case of other genetic situations leading to an extreme repression of zein synthesis: in *o6*

the embryo develops in a short-lived seedling (Ma and Nelson 1975) while *o2o7* seeds seldom germinate (our personal observations).

The significant reduction in endosperm weight in the *o2Mc* endosperms, which is not coupled to apparent gross defects in the carbohydrate metabolism, may simply be explained by the already known sink effect of zein synthesis on carbohydrate accumulation (Tsai et al. 1978, 1980).

It can then be concluded that the unexpected interaction on zein protein deposition of *Mc* with *o2* generates a metabolic situation where zein synthesis is almost completely suppressed. This is coupled to a normal development of both the seed and the plant, offering the opportunity for using the double mutant in biochemical and molecular studies related to storage protein synthesis. Due to the reduction in seed weight and total protein content, the double mutant has no practical interest in breeding maize for quality. Of some interest, on the other hand, is the induction by *Mc* alone of a methionine content higher than normal.

References

Catravas GN (1967) New developments in automatic sugar chromatography. In: Technicon (ed) Automation in analytical chemistry. Mediad, New York, p 397

Dickinson DB, Preiss J (1969) Presence of ADP-glucose pyrophosphorylase in *shrunken-2* and *brittle-2* mutants of maize endosperm. Plant Physiol 44:1058–1062

Di Fonzo N, Fornasari E, Salamini F, Soave C (1977) SDS-Protein subunits in normal, *opaque-2* and *floury-2* maize endosperms. Maydica 22:77–88

Di Fonzo N, Fornasari E, Salamini F, Reggiani R, Soave C (1980): Interaction of the mutants *floury-2* and *opaque-7* with *opaque-2* in the synthesis of endosperm proteins. J Hered 71:397–402

Di Fonzo N, Gentinetta E, Salamini F, Soave C (1979) Action of the *opaque-7* mutation on the accumulation of storage products in maize endosperm. Plant Sci Lett 14:345–354

Ferrari A (1960) Nitrogen determination by continuous digestion analysis system. Ann NY Acad Sci 87:792–800

Fornasari E, Di Fonzo N, Salamini F, Reggiani R, Soave C (1982) *Floury-2* and *opaque-7* interaction in the synthesis of zein polypeptides. Maydica (in press)

Gentinetta E, Zambello M, Salamini F (1979) Free sugars in developing maize grain. Cereal Chem 56:81–83

Laemmli UK (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature 227: 680–684

Landry J, Moureaux T (1970) Hétérogénéité des glutélines du grain de mais: extraction sélective et composition en acides aminés de trois fractions isolées. Bull Soc Chim Biol 52: 1021–1037

Laskey RA, Mills AD (1975) Quantitative film detection of H^3 and C^{14} in polyacrylamide gels by fluorography. Eur J Biochem 56:335–341

Ma Y, Nelson OE (1975) Amino acid composition and storage proteins in two new high-lysine mutants in maize. Cereal Chem 52:412–419

Marks MD, Larkins BA (1982) Analysis of sequence micro heterogeneity among zein messenger RNA. J Biol Chem (in press)

McWhirter KS (1971) A floury endosperm, high-lysine locus on chromosome 10. Maize Genet Coop News Lett 45:184

Mertz ET, Bates LS, Nelson OE (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145:279–280

Motto M, Salamini F, Reggiani R, Soave C (1979) Evaluation of genetic purity in hybrid corn seed production through zein isoelectrophoretic patterns. Maydica 24:223–233

Nelson N (1944) A photometric adaptation of the Somogy method for the determination of glucose. J Biol Chem 153:375

Nelson OE, Mertz ET, Bates LS (1965) Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science 150:1469–1470

Pedersen K, Bloom KS, Anderson JN, Glover DV, Larkins BA (1980) Analysis of the complexity and frequency of zein genes in the maize genome. Biochemistry 19:1644–1650

Salamini F, Di Fonzo N, Gentinetta E, Soave C (1979) A dominant mutation interfering with protein accumulation in maize seed. In: IAEA (ed) Seed protein improvement in cereals and grain legumes, vol 1. IAEA, Vienna, pp 97–108

Soave C, Reggiani R, Di Fonzo N, Salamini F (1981) Clustering of genes for 20K zein subunits in the short arm of maize chromosome 7. Genetics 97:363–377

Soave C, Reggiani R, Di Fonzo N, Salamini F (1982) Genes for zein subunits on maize chromosome 4. Biochem Genet (in press)

Soave C, Salamini F (1979) High-lysine genes in maize: theoretical and applied aspects. Monografia di Genet Agrar 4:107–140

Soave C, Salamini F (1982) Zein protein: a multigene family of maize endosperm. Qualit Plant 31:191–203

Soave C, Suman N, Viotti A, Salamini F (1978) Linkage relationships between regulatory and structural gene loci involved in zein synthesis in maize. Theor Appl Genet 52:263–267

Thivend P, Mercier C, Guilbot A (1972) Determination of starch with glucoamylase. 6. General carbohydrate methods. In: Whister RL, Miller JN (eds) Methods in carbohydrate chemistry. New York, Academic Press, pp 104–128

Tsai CY, Huber DM, Warren HL (1978) Relationships of the kernel sink for N to maize productivity. Crop Sci 17: 399–404

Tsai CY, Huber DM, Warren HL (1980): A proposed role of zein and glutelin as N sinks in maize. Plant Physiol 66:330–333

Tsai CY, Nelson OE (1966) Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity. Science 151:341–343

Valentini G, Soave C, Ottaviano E (1979): Chromosomal location of zein genes in *Zea maize*. Heredity 42:33–40

Van Handel B (1968) Direct microdetermination of sucrose. Anal Biochem 22:280–283

Vitale A, Smaniotto E, Longhi R, Galante E (1982) Reduced soluble proteins associated with maize endosperm protein bodies. J Exp Bot 33:439–448

Wöstemeyer J, Behrens U, Merkelbach A, Müller, M, Starlinger P (1980) Translation of *Zea maize* endosperm sucrose synthase mRNA in vitro. Eur J Biochem 114: 39–44