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Some Genetic and Biochemical Characteristics of a New Source of Maize Opaque-2 Mutant

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Summary. The opaque kernels separated from the F_1 of crosses of our opaque-2 strains with normal inbred lines contained 45, 50 and 74 percent more lysine in the whole kernel than the translucent kernels from the same ear. The opaque kernels from these crosses contained almost the same level of lysine as the parental opaque-2 strains.

Some of our opaque-2 strains contain 63 to 122 percent more lysine than the tested dent or flint normal inbred lines. In comparison with opaque-2 Purdue, four opaque-2 strains had almost the same lysine content and a strain labelled as SP-1 No. 15 contained 25 percent more lysine and 73 percent more tryptophan. Some of our opaque-2 strains contained 55 to 100 percent more tryptophan in the whole kernel than opaque-2 Purdue.

In contrast to lysine content, the transmission of the tryptophan content from opaque-2 strains to opaque kernels from their crosses shows variability.

We found weak positive correlation (r = +0,3057) between lysine and tryptophan content in opaque-2 kernels.

Our opaque-2 strains had a higher lysine content in the endosperm than did opaque-2 Purdue 22.1—36.6% more, and compared with normal lines they had 109.5—142.6% more.

It is apparent that a new source of opaque-2 mutant gene, which has the same genetic and biochemical characteristics as the opaque-2 mutant discovered by Mertz, Bates and Nelson (1964), has been found in a completely new, genetically divergent, strain derived from a large number of populations.

Introduction

Because of the exceptional interest in genetic, physiological and other aspects, intensive work is going on into transforming normal inbred maize lines into versions with opaque-2 and floury-2 endosperm, and the development of new lines. The opaque-2 and floury-2 mutants discovered by Nelson, Mertz and Bates (1964, 1965) are used as stock. Although expression of the allele opaque-2 depends upon the genotype of the line with normal endosperm, from the theoretical and practical standpoint a new source of the opaque-2 gene would be significant for the genetic divergence of the material.

This paper reports the genetic and biochemical characteristics of a new source of maize opaque-2 mutant.

Material and Methods

In the course of breeding inbred lines by the ear to row method, in a synthetic produced in 1963 from 10 populations and varieties SP-(1. mixture Chalqueno × domestic varieties and lines, 2. Timok yellow flint, 3. Ruma dent, 4. Yellow dent with long seed, 5. Šid dent, 6. Vukovar dent, 7. Mates multi row dent, 8. Yellow dent, 9. Red multi-rowed dent, 10. Long-eared yellow dent) we found, in 1966, two ears with opaque-2 seeds (Pešev, 1971). These two ears were labelled SP-9/2-ln and SP-1 No. 6. The others were sister lines that differed only in shape and colour of kernel.

In 1968 the opaque-2 strains, SP-9/2-ln, SP-1 No. 6 and SP-1 No. 4, were crossed with inbred lines of normal endosperm, A-632, W64A, WF-9, and with Yugoslav (ZP) flint inbred lines T-536/I-2 with 13.58% of protein. The kernels from the parental inbred lines and the kernels of the F-1 generation from their crosses were analyzed, the kernels from the same ear being separated into those with pronounced opacity of the endosperm and those with translucent-normal endosperm on the other side. For comparison, opaque-2 mutant Purdue and opaque-2 267 were obtained from the Plant Breeding Station, Clermont-Ferrand, France.

The kernels analyzed were mature, air-dried and had been produced by self-pollination. Protein content (nitrogen × 6,25) was analyzed by the micro-Kjeldal method. The amino acids in 100 mg of whole kernels and of endosperm were determined by HD-1200E automatic aminoacid analyzer. The tryptophan was estimated by Spies and Chambers' method modified by Strelkov and Kholodov (1968).

Results and Discussion

The population from which we produced the first two opaque-2 ears included a Yugoslav Timok yellow flint variety containing 2.65 g lysine, 1.45 g methionine and 0.44 g tryptophan/100 g of protein, almost twice as much as the other 5 Yugoslav varieties analyzed, and 11.56 percent protein (Pavličić and Jelenić, 1970). The Mexican Chalqueno is classified as a high lysine variety with 194 mg/gN and 333 mg/100 g of seed (Telo *et al.*, 1965). This synthetic, then, was made up from 10 populations and varieties which were divergent in geographical origin, seed type, raw protein content, lysine, tryptophan, etc.

The amino-acid and protein content in whole kernels of the normal (nonmutants) and opaque-2 strain, and in some of their crosses' kernels (separated into opaque and translucent, normal endosperm) in the F_1 are shown in Table 1.

In the F_1 generation progeny from the crossing (and backcrossing) of our opaque-2 strains with normal maize, opaque-2 kernels and kernels with a normal endosperm separated markedly. In the F_0 generation the kernels from the crossing had a normal endosperm. Kovacs *et al.* (1971) pointed out that in the heterozygote, $F_0\ O_2 \times Fl_2$, a lysine content corresponding to that of normal maize was found.

The opaque kernels separated from the F_1 of our opaque-2 strains (SP-9/2-1, SP-1 No. 4 and No. 6) crossed with normal inbred lines (WF-9, W 64A and A-632) contained 45, 74 and 50 percent more lysine than the normal, translucent kernels from the same ear. The opaque kernels from these crosses contained almost the same level of lysine as the opaque-2 strains. The lysine content of the translucent kernels of the F_1 generation from the same crosses is a little higher than in the normal homozygous inbred lines. Mertz *et al.* (1964) found that opaque-2 kernels contained 69 percent more lysine than the normal kernels from the same ear.

It follows, then, that our strains had the same critical genetic and biochemical properties of endosperm and lysine content as the opaque-2 mutant discovered by Mertz, Bates and Nelson.

Our opaque-2 strains contained 68 (Sp-1 No. 6), 88 (SP-1 No. 4) and 63 (SP-9/2-1) percent more lysine than the normal inbred lines (A-632, W-64A and WF-9) crossed with them. The three other opaque-2 non-crossed strains (SP-9/2-1, SP-1 No. 14 and No. 15) contained 82, 79 and 122 percent more lysine, respectively, than the normal flint inbred line T-536/I-2, with 13, 58 percent protein content. In comparison with opaque-2 Purdue, our opaque-2 strains, SP-1 No. 4, SP-1 No. 14 and SP-9/2-1n, had almost the same lysine content, while SP-9/2-1 and SP-1 No. 15 contained 2 and 25 percent more lysine, respectively. This indicates the amount of variability in lysine content in this source of maize opaque-2 and the possibility of breeding for this property.

Our maize opaque-2 strains and the opaque kernels from their crosses with normal inbred lines contained less glutamic acid, alanine, leucine, tyrosine and phenyl-alanine, but more lysine, arginine, aspartic acid, methionine and glycine (except in SP-1 No. 6) than the kernels with normal endosperm. When compared with the results of Mertz *et al.* (1964), our opaque-2 strains showed the same decreases or increases in the characteristic amino acids compared with the normal translucent kernels.

According to Mertz *et al.* (1964), the major reason for these changes in amino acids content is the synthesis of protein which is accompanied by a reduction in the ratio of zein (a low lysine-tryptophan protein) to glutelin in the opaque-2 mutant endosperm.

The tryptophan content has received the attention of investigators into the complex problems of modifying the protein quality of maize. Compared with opaque-2 Purdue (with 0.28% tryptophan/16 N), our opaque-2 strains, SP-9/2-1 (0,56), SP-1 No. 15 (0,50), SP-1 No. 4 (0,54) and SP-1 No. 14 (0,44), contained more (100 to 55 percent) tryptophan, while SP-1 No. 6 and SP-9/2-1n had similar levels.

It is interesting to note that opaque kernels separated from ears of crosses of opaque-2 strains with normal inbred lines did not the contain the same tryptophan level as the opaque-2 strains used in the crosses (except in the cross SP-1 No. $6 \times$ A-632 F_1O_2). In contrast with the lysine content, the transmission of tryptophan content from opaque-2 strains to the opaque kernel shows variability. On the other hand, the translucent kernels from the same crossed ear contained the same level of tryptophan as the normal inbred lines used in the crosses. These normal inbred lines (A-632, W-64A and WF-9) contained more tryptophan than did Opaque-2 Purdue and our two opaque-2 strains (SP-1 No. 6 and SP-9/2-1n) used in these crosses.

Lambert and Alexander (1968) found a frequency of about one spontaneous mutation in every 344,000 female gametes of dominant allele at the opaque-2 locus for four homozygous inbred lines (B_{37}, C_{103}, M_{14} and W-64A).

We have found a weak positive correlation (r = = + 0,3057) between lysine and tryptophan content for the 11 opaque-2 strains and crosses with opaque kernels analyzed here.

Alexander *et al.* (1971) pointed out that it has been assumed by many breeders that if lysine is increased by selection a parallel increase in tryptophan will also occur. However, they have shown that strict parallelism between lysine and tryptophan does not always occur (our results show this also) because globulin and glutelin are groups of heterogeneous proteins varying in amino acid composition and action of some modifiers. According to Specht (quoted by Alexander *et al.*, 1971), the correlation between lysine and tryptophan is not different from zero in synthetic opaque-2 varieties.

It is interesting that the SP-1 No. 15 opaque-2 strain contains high lysine and tryptophan at the same time.

From the genetic and breeding standpoint, it is interesting to see the results obtained by crossing on opaque-2 mutant with normal lines having an increased content of total proteins. With this aim, opaque and translucent-normal kernels obtained by crossing one of our opaque-2 strains (SP-9/2-1n) with a normal flint line (T-536/I-2) of increased total protein content were analyzed.

The content of total proteins was found to be increased by 0,7—1% compared with that of the parental opaque-2 line (Table 2). The content of amino acids in opaque kernels from this cross was found to show the same decrease or increase in relation to that of normal-translucent kernels as in the results given in Table 1.

These results confirm the data obtained by Nelson (1965) from crossing an opaque-2 mutant with Ill. H. P. strain.

Mertz *et al.* (1964) have emphasized that the main characteristics of the opaque-2 mutant are the increase in lysine content and the changes in the amino acid composition of the endosperm. Compared to

Table 1. *Protein and Amino Acids Content in Whole Kernels of Opaque-2 Strains, Normal Inbred Lines and Opaque and Translucent Kernels of Their Crosses (Amino Acid g/16 gN)*

Material	Protein %	Lysine	Histidine	Arginine	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrozine	Phenyl-alanine	Tryptophan
SP-1 No. 6 O_2	9.14	4.07	2.47	4.71	11.27	3,78	3.09	14.92	5.82	4.01	5.86	5.59	0.71	3.21	7.33	2.54	3.52	0.29
SP-1 No. 6 × A632 O_2	9.65	4.09	2.74	5.19	7.45	3.58	3.01	14.47	7.62	4.11	5.69	4.45	0.74	3.37	8.19	2.64	3.82	0.24
SP-1 No. 6 × A632 +	9.06	2.75	2.36	3.73	6.66	3.17	3.77	19.61	8.40	5.54	7.19	4.11	1.03	4.00	12.42	3.02	4.78	0.42
A632 +	10.50	2.43	2.40	3.83	6.44	3.51	3.40	19.51	7.59	3.16	7.09	7.55	1.33	3.55	12.37	3.25	4.39	0.43
SP-1 No. 4 O_2	9.57	4,46	2.79	5.18	16.26	3.60	3.07	14.37	5.77	4.38	5.26	5.40	0.46	4.26	5.46	2.70	3.64	0.54
SP-1 No. 4 × W64A O_2	10.93	4.34	2.80	5.78	10.28	3.48	2.95	15.88	7.02	4.09	5.53	5.85	0.56	2.32	7.88	2.67	3.57	0.31
SP-1 No. 4 × W64A +	10.26	2.43	2.49	3.24	7.20	3.52	2.03	20.33	7.86	2.71	7.35	5.28	0.91	3.73	12.99	3.57	4.52	0.36
W64A +	12.02	2.36	2.43	3.48	7.25	3.44	3.41	20.57	8.17	3.28	7.26	5.05	0.96	3.65	12.79	3.57	4.38	0.37
SP-9/2 — 1 n O_2	10.55	4.32	3.04	5.84	10.25	4.08	3.69	17.69	7.37	4.70	6.30	5.34	1.13	3.65	9.22	3.09	4.12	0.26
SP-9/2 — 1 n × WF9 O_2	10.04	4.24	2.72	5.67	11.78	3.85	2.82	15.58	6.79	4.02	5.40	5.35	0.70	3.06	7.74	2.68	3.46	0.50
SP-9/2 — 1 n × WF9 +	9.92	2.94	2.51	3.59	7.01	3.46	3.65	19.83	8.42	3.23	7.56	5.36	0.69	4.16	13.36	3.62	4.97	0.42
WF9 +	10.49	2.65	2.68	4.05	7.68	3.78	3.28	17.65	8.96	3.05	6.69	5.57	0.93	3.50	11.96	2.51	4.19	0.35
SP-1 No. 15 O_2	10.33	5.67	2.67	5.31	12.90	4.60	3.17	14.05	6.31	4.23	5.08	3.43	0.69	3.13	6.86	2.43	1.93	0.50
SP-1 No. 14 O_2	9.52	4.55	2.59	5.61	9.48	3.66	3.14	13.67	6.06	4.08	5.43	4.87	0.80	3.15	7.22	2.68	3.49	0.44
SP-9/2 — 1 O_2	8.72	4.62	3.02	5.27	13.11	4.80	3.61	15.97	6.68	4.53	6.09	5.49	0.84	3.45	8.29	2.83	3.89	0.56
T-536/I — 2 +	13.58	2.32	2.30	3.43	7.52	3.48	3.93	20.55	8.67	3.13	7.23	5.11	0.98	3.66	13.33	4.90	4.77	0.20
O_2 Purdue	10.21	4.52	2.69	6.40	9.89	3.86	3.19	18.70	7.59	5.13	5.35	4.85	0.92	3.16	7.16	9.73	3.38	0.28
O_2 No. 267	8.94	4.79	3.18	6.05	9.90	3.81	3.42	17.23	6.70	5.06	6.39	4.91	0.86	3.74	9.00	2.91	4.11	0.32

Table 2. *Protein and Amino Acids Content in Whole Kernels of an Opaque-2 Strain, a Normal Yugoslav (ZP) Inbred Line and Opaque and Translucent Kernels from Their Crosses (Amino Acid g/16 gN)*

Material	Protein %	Lysine	Histidine	Arginine	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrozine	Phenyl-alanine	Tryptophan
SP-9/2 — 1 n O_2	10.55	4.32	3.04	5.84	10.25	4.08	3.69	17.69	7.37	4.70	6.30	5.34	1.13	3.65	9.22	3.09	4.12	0.26
SP-9/2 — 1 n × T-536/ I-2 O_2	11.26	4.42	2.87	5.59	11.68	4.17	3.41	17.18	8.40	6.96	6.39	5.90	0.86	3.52	8.61	2.84	3.70	0.28
SP-9/2 — 1 n × T-536/ I-2 +	11.66	3.07	2.92	4.02	7.89	3.44	3.68	21.42	8.98	3.65	8.04	5.10	0.74	4.19	13.28	3.62	5.11	0.48
T-536/I-2 +	13.58	2.32	2.30	3.43	7.52	3.48	3.93	20.55	8.67	3.13	7.23	5.11	0.98	3.66	13.33	4.90	4.77	0.20

Table 3. *Protein and Lysine Content in Endosperm of Opaque-2 Strains, Normal Inbred Lines and Opaque and Translucent Kernels of Their Crosses*

Material	Protein (N×6.25) %	Lysine g/100 g of dry	Lysine g/16 gN
SP-1 No. 6 O_2	7.70	0.280	3.64
SP-1 No. 6×A-632 O_2	9.12	0.266	2.92
SP-1 No. 6×A-632 +	9.65	0.129	1.34
A-632 +	10.41	0.166	1.60
SP-1 No. 4 O_2	7.94	0.321	3.93
SP-1 No. 4×W64A O_2	8.91	0.332	3.72
SP-1 No. 4×W64A +	9.83	0.269	2.74
W64A +	10.46	0.169	1.62
SP-9/2-ln O_2	9.39	0.350	3.73
SP-9/2-ln×WF9 O_2	9.95	0.293	2.94
SP-9/2-ln×WF9 +	9.84	0.224	1.98
WF9 +	9.94	0.177	1.78
SP-1 No. 14 O_2	8.51	0.286	3.36
SP-9/2-1 O_2	7.76	0.316	4.07
SP-1 No. 14/1 O_2	7.72	0.285	3.69
O_2 Purdue	9.28	0.276	2.98

opaque-2 Purdue, which has 2.98 g/16 gN of lysine in the endosperm, our opaque-2 strains had (Table 3) more lysine – from 22,1% (SP-1 No. 6) to 31,9% (SP-1 No. 4) more. One line (SP-9/2-1), which was not used in crossing, contained 36,6% more lysine than opaque-2 Purdue.

Our opaque-2 strains were shown to have more lysine in the endosperm than the normal lines, they were crossed with (WF-9 and W-64A, respectively – from 109,5% (SP-9/2-1n) to 142,6% (SP-1 N°4) more.

The opaque kernels obtained by crossing opaque-2 strains with normal lines were found to have approximately the same (SP-1 No. 4×W 64A), or a somewhat lower lysine content than the opaque-2 lines, but a significantly higher content than the normal lines.

It is apparent that a new, probably independent, source of the opaque-2 mutant gene, which has the same genetic and biochemical characteristics as the opaque-2 mutant discovered by Mertz *et al.* (1964), has been found in a completely new, genetically divergent, strain derived from a large number of populations.

Zusammenfassung

Die vorgenommenen Prüfungen haben gezeigt, daß undurchsichtige Körner, die nach F_1-Kreuzung unserer Opaque 2-Linien mit Normallinien ausgelesen wurden, 45, 50 und sogar 74% mehr Lysin im ganzen Korn als normale durchsichtige Körner desselben Maiskolbens enthalten. Der Lysingehalt der undurchsichtigen Körner aus diesen Kreuzungen entspricht annähernd dem der Opaque 2-Elternlinien.

Einige unserer Opaque 2-Linien enthalten 63 bis 122% mehr Lysin als die untersuchten normalen Inzuchtlinien. Im Vergleich zu Opaque 2 – Purdue weisen unsere 4 Opaque 2-Linien einen etwa gleichen Lysingehalt auf, während die SP-1 No. 15 25% mehr Lysin und 73% Tryptophan enthält. Unsere Opaque 2-Linien zeigen im ganzen Korn 55 bis 100% mehr Tryptophan als Opaque 2 – Purdue.

Im Unterschied zum Lysingehalt zeigt die Übertragung des Tryptophangehalts von Opaque 2-Linien auf undurchsichtige Körner bei den Kreuzungen Variabilität.

Wir fanden eine schwach positive Korrelation (r = + 0,3057) zwischen dem Lysin- und Tryptophangehalt bei den Opaque 2-Körnern.

Unsere Opaque 2-Linien hatten mehr Lysin im Endosperm als Opaque 2-Purdue, und zwar 22,1 bis 36,6% bzw. 109,5 bis 142,6% im Vergleich zu den normalen Linien.

Offensichtlich ist eine neue Quelle für das mutante Opaque 2-Gen in einer ganz neuen, genetisch unterschiedlichen Synthese einer großen Anzahl von Populationen entdeckt worden. Die neue Quelle hat dieselben genetischen und biochemischen Merkmale wie die Opaque 2-Mutante, die von Mertz, Bates und Nelson (1964) aufgefunden wurde.

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