NOTE

Ninhydrin Color Test for Screening Modified Endosperm *Opaque*-2 Maize[1]

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The recessive gene *opaque*-2 (o_2) has been used to improve protein quality in maize (*Zea mays* L.). This gene improves the nutritional quality by increasing the lysine and tryptophan content of the endosperm (Mertz et al 1964). *Opaque*-2 maize has not been widely used because of the lower grain yield of o_2 hybrids. When maize is used directly in the human diet, the chalky appearance of o_2 endosperm, insect infestation, and poor grit yield is unacceptable (Gupta et al 1970, Lambert et al 1969, Loesch et al 1976, Pinstrup-Anderson 1975). Selection of genes that modify the texture of o_2 endosperm to produce a harder kernel is a possible solution to the production problems associated with o_2 maize. Development of o_2 breeding materials with modified endosperm requires a rapid assay method to determine whether material is modified o_2 or contaminated by normal maize. A rapid ninhydrin test was developed by Mertz et al (1974) to differentiate between normal and o_2 genotypes. Misra et al (1975) modified the ninhydrin assay for determining the free amino acid content of double endosperm mutants involving o_2 and other endosperm mutants. The ninhydrin assay has not been used on a range of modified endosperm o_2 materials to determine whether the assay can differentiate between varying degrees of modification and normal maize. The purpose of this research was to determine whether the ninhydrin assay could be used to differentiate varying degrees of o_2 endosperm modification from nonmutant maize.

MATERIALS AND METHODS

Materials assayed were whole kernels of seven inbred lines of maize with their nonmutant and o_2 versions. In addition, four breeding populations were used. Three had the o_2 gene homozygous with varying degrees of endosperm modification. They were Syn. D. O. mod. o_2, Elite Syn. mod. o_2 and ETO/Ill. HE. mod. o_2. The normal endosperm population was Syn. D.O.

The ninhydrin assay developed by Misra et al (1975) was used with the following modifications: 1, ground samples suspended in water were shaken at room temperature for 1 hr instead of 20 min; 2, samples were centrifuged at 2,000 rpm for 4 min rather than 12,000 rpm for 10 min; 3, the supernatant (0.2 ml) was placed in a test tube, and 1 ml of ninhydrin reagent added. All other procedures were the same as those described by Misra et al (1975).

Grain protein content was determined using near-infrared reflectance procedures developed by Hymowitz et al (1974). Lysine content of the ground whole kernel samples was determined using 2,4,6, trinitrobenzene-1-sulfonic acid (TNBS) procedure developed by Obi (1982). The assay has been used in our laboratory for routine lysine determinations of breeding materials for several years. The method correlates well (r = 0.81*) with the amino acid analyzer. The free amino acid contents measured by the ninhydrin assay are reported as grams of L-leucine per 100 g of protein; lysine content is reported as grams of lysine per 100 g of protein.

The amount of endosperm modification was rated on a 100-kernel sample of whole grain using an eleven-class scale of 0.0–5.0 (0.0–0.5–1.0–1.5–, etc.). Class 0 had 5% or less of the endosperm area of the sample modified (ie, all opaque); class 0.5, 5–10%; class 1.0, 10–20%, and class 5, 90–100% modified (ie, all hard or vitreous).

RESULTS AND DISCUSSION

Results for free amino acids and lysine content of seven normal and o_2 versions of maize inbreds are presented in Table I. The mean difference in normal and o_2 inbreds was 6.38. The o_2 inbred Va36 had the lowest free amino acid content (5.08), and Mo17 had the highest of both the nonmutant and o_2 inbreds. Va35 o_2 had the second highest free amino acid level of 10%. The lysine content of the normal and o_2 inbreds varied. Free amino acid content was not correlated with lysine content for the seven genotypes assayed. These results agree with those of Mertz et al (1974).

TABLE I
Free Amino Acid and Lysine Content of Seven Normal and *Opaque*-2 Inbreds

Inbred	Percent Free Amino Acid[a] (g/100 g protein)		Percent Lysine[b] (g lysine/100 g protein)	
	Normal	*Opaque*-2	Normal	*Opaque*-2
B37	2.09[c]	5.38	2.53	4.57
Mo17	3.00	19.94	2.83	4.79
N28	2.51	8.36	3.27	6.10
Oh43	1.88	5.83	2.39	4.79
R802	1.60	5.10	2.13	5.50
Va35	2.02	10.00	2.50	3.37
Va36	1.98	5.08	1.93	3.81
Mean	2.15	8.53	2.51	4.70
Difference	6.38[d]		2.19[d]	

[a] Grams L-leucine/100 g protein. Range of total protein = 9.1–16.4%.
[b] Grams lysine/100 g protein.
[c] A single determination was made on each sample.
[d] Student's *t* test at $P > 0.05$.

TABLE II
Free Amino Acid and Lysine Content of Normal and *Opaque*-2 Germ Plasm

Germ Plasm	Percent Free Amino Acid[a] (g/100 g protein)	Percent Lysine[b] (g lysine/100 g protein)
Normal inbreds	2.15[c]	2.51
Opaque-2 inbreds	8.53	4.70
Synthetic D.O. mod. o_2[c]	5.48	3.11
Synthetic D.O. normal[c]	1.98	2.20
Elite Syn. mod. o_2[c]	5.36	3.31
ETO/Ill. HE. mod. o_2[c]	6.44	3.23
	Means	
Normal	2.07	2.36[e]
Opaque-2	5.76[d]	3.22

[a] Grams L-leucine/100 g protein. Range of total protein = 10.6–14.1%.
[b] Grams lysine/100 g protein.
[c] Mean of seven and 10 or more samples.
[d] Student's *t* test significant at $P > 0.05$.
[e] Student's *t* test significant at $P > 0.05$.

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TABLE III
Free Amino Acid and Lysine Content of Modified Endosperm Classes for Two Modified *Opaque*-2 Populations

Endosperm[a] Class	Syn. D.O. mod o_2 Percent Free Amino Acid[b]	Syn. D.O. mod o_2 Percent Lysine[c]	Elite Syn. mod. o_2 Percent Free Amino Acid	Elite Syn. mod. o_2 Percent Lysine[c]
0.0	8.09[d]	4.15	7.02	4.28
0.5	6.97	3.74	5.10	3.62
1.0	6.21	3.92	5.88	3.98
1.5	6.89	3.57	6.34	...
2.0	7.32	3.37	6.68	3.60
2.5	8.72	3.27	7.16	3.45
3.0	5.89	3.37	5.77	3.49
3.5	6.32	3.36	6.23	3.88
4.0	6.37	3.23	5.96	3.66
4.5	5.35	3.06	5.85	3.29
5.0	4.72	3.04	4.27	3.00
Mean	6.62	3.46	6.02	3.63
LSD[e]	ns	0.36	ns	0.47

[a] Scale 0–5.0; 0 = 5% or less of endosperm area modified; 5.0 = 90–100% area modified.
[b] Grams L-leucine/100 g protein. Range in protein = 9.0–12.7%.
[c] Grams lysine/100 g protein.
[d] Each value the mean of five samples.
[e] Least significant differences at $P = 0.05$.

The ninhydrin assay was used on heterogeneous breeding materials to determine whether the assay could differentiate normal from modified endosperm o_2 material. The results (Table II) show that the ninhydrin assay could detect differences in free amino acid content of normal and modified endosperm o_2 germ plasm. The three modified endosperm o_2 populations had a mean value of 5.76 vs 2.07 (g L-leucine/100 g protein) for the normal endosperm types (Table II). The lysine content was not correlated with the free amino acid content in this material. The ninhydrin assay for free amino acids is a good rapid method for distinguishing modified endosperm o_2 types from normal maize.

The ninhydrin assay was used on two modified endosperm o_2 populations to determine the variation in free amino acid and lysine content as it relates to the degree of endosperm modification. The mean free amino acid content of the two populations were 6.62 and 6.02 (Table III). The range in free amino acid content for the eleven endosperm classes were 4.72–8.72 and 4.27–7.16 for Syn. D.O. mod. o_2 and Elite Syn. mod. o_2, respectively. No significant differences were observed for the free amino acid content of the different modified endosperm classes within each population. Significant differences for lysine content of the different classes within each population were observed. The free amino acid content was negatively correlated with endosperm modification in Syn. D.O. mod. o_2 ($r = -0.65^*$) but not in Elite Syn. mod. o_2. Endosperm modification was negatively correlated with lysine content in Syn. D.O. mod. o_2 ($r = -0.92^{**}$) and Elite Syn. mod. o_2 ($r = -0.74^{**}$). The free amino acid content was not significantly correlated with lysine content in these two populations.

The data show that the ninhydrin assay can be used to monitor modified endosperm o_2 breeding materials for the presence of the o_2 gene, but the test cannot be used to select within a modified o_2 population for types having the most lysine.

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