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Composition of Seeds Obtained from Non-nodulating and Nodulating Peanut Lines and Cultivars[1]

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ABSTRACT

The composition of seeds obtained from non-nodulating and nodulating peanut lines was studied. Peanut samples from the non-nodulating genotype were low in oil and protein, high in α-amino nitrogen, soluble carbohydrates, and the amino acids lysine, alanine, methionine, and threonine as compared to its nodulating parental lines and 'Florunner'. No differences however, were noted in iodine value of the oil. Peanuts from the non-nodulating line were also found to have higher activities of the hydrolytic enzymes leucine aminopeptidase and acid phosphatase.

Key Words: *Arachis hypogaea* L., Seed quality, Amino acids, Nitrogen fixation, Protein, Non-nodulating.

The peanut (*Arachis hypoagea* L.), a legume, is capable of forming a symbiotic relationship with rhizobia, whereby nodulation occurs and nitrogen is fixed (13). Gorbet and Burton (9) recently reported a non-nodulating peanut genotype. This non-nodulating genotype was first noted in F_3 progeny of a cross between two normal nodulating parents, PI 262090 and 487A-4-1-2, both virginia botanical type peanuts. The non-nodulating genotype failed to nodulate under field conditions and also in growth chamber tests even in the presence of rhizobium peanut inoculant. There are also reports of non-nodulating genotypes in soybean [*Glycine max* (L.) Merr.] and several other leguminous species (5,8,14,15).

It was further observed by Gorbet and Burton (9) that the non-nodulating plants showed N starvation symptoms toward maturity. It was not known if there are differences in the composition of peanut seeds obtained from the non-nodulating and nodulating plants.

This paper reports the results of analyses of peanut samples obtained from a non-nodulating peanut genotype, its two nodulating parental lines, and Florunner, a commercial cultivar.

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Materials and Methods

Peanuts from an F_8 non-nodulating breeding line, its two normal nodulating parents (PI 262090 and 487A-4-1-2) and the commercial cultivar Florunner were grown at the Agricultural Research Center, Marianna, Florida, during the 1980 growing season. The entries were planted in single row plots 91.4 cm apart and 6.1 m long at the rate of 16 seeds-row. Sound mature kernels were lyophilized, ground into a meal and stored at -20 C. Oil content of the ground peanut meal was determined by the Soxhlet extraction method (1). To determine iodine value the oil obtained by the Soxhlet extraction was slowly heated to 60 C to remove acetone and then tested by the Hanus method (1). The protein content of the defatted meal was determined by the micro-Kjeldahl method (1). The nitrogen value was multiplied by 5.46 to convert to protein. The amino acid composition of peanut proteins was obtained by hydrolyzing the defatted meal with 6N HCl at 110 C for 18h, followed by analysis on a JEOL-6AH amino acid analyzer (11). α-Amino nitrogen and soluble carbohydrates were extracted from the defatted peanut meal (100 mg) with 30 ml of the mixture of methanol:choroform:water (60:25:15, v/v/v), according to the method of Young et al., (18). α-Amino nitrogen was determined by the method of Yemm and Cocking (17) and the soluble carbohydrates were analyzed by the method of Yemm and Willis (16). Leucine aminopeptidase and acid phosphatase activities were determined as follows: Fifty mg of defatted peanut meal was extracted with 10 ml of cold 25 mM Tris-HCl buffer (pH 8.2) containing 10 mM 2-mercaptoethanol. The extract was centrifuged (20,000g for 20 min at 4 C) and the resulting supernatant was used for enzyme analysis. Leucine aminopeptidase activity was determined by incubating 5 µl extract (in triplicate) with 0.5 ml L-leucyl β-naphthylamine [(0.02%) w/v in 0.1 M sodium phosphate, pH 7.1] at 37 C. After 15 min, the reaction was terminated with 0.5 ml of 2N HCl and the released β-naphthylamine was measured as described earlier (3). For determination of acid phosphatase activity 5 µl enzyme extract was incubated with 0.5 ml substrate (20 mM p-nitrophenyl phosphate) at pH 4.9. After 10 min incubation at 37 C, the reaction was stopped by adding 1 ml of 1N NaOH and the released p-nitrophenol was measured at 410 nm (4).

Results and Discussion

The oil content of peanuts from the non-nodulating line was significantly lower than that of the two nodulating parental lines or of the commercial cultivar Florunner

(Table 1). Although no significant differences in oil content were observed between the two nodulating lines and Florunner, the non-nodulating line had approximately 10% less oil. The oil contents reported here were consistent with the previous findings for several nodulating peanut lines and cultivars (11,12).

Table 1. Composition of peanut seeds obtained from non-nodulating and nodulating peanut lines.%

Peanut Line or Cultivar	Biochemical parameters		
	Percent Oil	Percent Protein	Iodine Value
Non-nodulating	46.2b*	16.4b	88.9a
PI 262090	48.4a	26.2a	87.4a
487A-4-1-2	50.8a	24.9a	88.6a
Florunner	51.4a	23.2a	91.0a

*Within a column, means followed by the same letter are not significantly different at 5% level of significance using Duncan's Multiple Range Test.

The total protein content was found to be significantly lower in the non-nodulating line (Table 1). Peanuts from the non-nodulating line had approximately one third less protein than the parental lines and Florunner. Although a negative correlation between oil and protein content in peanuts has been reported (12), peanut samples obtained from the non-nodulating line were low in both oil and protein. The nodulating parental lines and Florunner however did show a negative relationship between oil and protein content (Table 1).

The iodine value is related directly to the relative degree of unsaturation of the fatty acids in penaut oil (7). No significant differences were observed in iodine values of peanut samples obtained from the genotypes studied. Iodine values were within the range of 86.8 to 98.7 previously reported by Cobb and Johnson (7).

The α-amino nitrogen, which represents the amount of free amino acids in the seed, was present in significantly higher amounts (150 to 180%) in the non-nodulating line than in the nodulating genotypes (Table 2). This is an interesting observation since plants without nodules are dependent on nitrate reduction as the sole source of reduced nitrogen while the nodulated plants can derive nitrogen from both nitrate reduction and nitrogen fixation (10). Lower amounts of protein and greater amounts of α-amino nitrogen in the non-nodulating line suggest that this line may be less efficient in protein synthesis than the nodulating lines. The soluble carbohydrate content of the non-nodulating line was higher (28 to 30%) than the nodulating lines (Table 2).

Table 2. α-Amino nitrogen and soluble carbohydrate content of seeds from non-nodulating and nodulating peanut lines.

Peanut Line or Cultivar	α-Amino nitrogen	Soluble Carbohydrates
	g/100g defatted meal	
Non-nodulating	1.09a*	6.67a
PI 262090	0.40b	5.20b
487A-4-1-2	0.37b	5.27b
Florunner	0.36b	5.37b

*Within a column, means followed by the same letter are not significantly different at 5% level of significance using Duncan's Multiple Range Test.

The total amino acid composition of the defatted peanut meal following hydrolysis is presented in Table 3. Seeds obtained from the non-nodulating line were significantly higher in alanine, lysine, threonine, and methionine, and lower in leucine as compared to the nodulating parental lines and Florunner. The other amino acids did not show significant differences and were within the range previously reported by Pancholy et al., (12).

Table 3. Amino acid composition of peanut seeds obtained from non-nodulating and nodulating peanut lines.

Amino acid	Non-nod	PI262090	487A-4-1-2	Florunner
	g amino acid/100g amino acids			
Lysine	5.05a*	4.08c	4.50b	4.52b
Histidine	2.62a	2.83a	2.94a	2.59a
Arginine	11.63a	10.60a	11.01a	11.24a
Aspartic acid	11.78a	11.92a	11.96a	12.05a
Threonine	5.25a	4.82b	3.99c	4.03c
Serine	4.47a	4.42a	4.34a	4.13a
Glutamic acid	19.50a	19.68a	19.76a	19.85a
Proline	3.89a	4.50a	4.29a	4.38a
Glycine	5.87a	5.63a	5.73a	6.11a
Alanine	4.18a	3.88a	3.69b	3.76b
Cystine	Trace	Trace	Trace	Trace
Valine	4.42a	4.47a	4.21a	4.06a
Methionine	1.11a	0.94a	0.74b	0.76b
Isoleucine	3.53a	3.63a	3.84a	3.92a
Leucine	5.85b	6.76a	6.84a	6.93a
Tyrosine	3.43a	3.67a	3.75a	3.63a
Phenylalanine	4.92a	4.96a	4.30a	4.26a

*Across a column, means followed by the same letter are not significantly different at 5% level of significance using Duncan's Multiple Range Test.

Hydrolytic enzyme activities, such as leucine aminopeptidase and acid phosphatase, which reflect seed metabolic activity, were much higher (20 to 64% and 93 to 250%, respectively) in the non-nodulating line (Table 4) than in the nodulating lines. Acid phosphatase hydrolyzes phosphate esters, while leucine aminopeptidase is an exopeptidase which cleaves peptide bonds from the N-terminal end of the protein. These enzymes contain sev-

Table 4. Leucine aminopeptidase and acid phosphatase activities in seeds from non-nodulating and nodulating peanut lines.

Peanut Line or Cultivar	Leucine aminopeptidase	Acid phosphatase
	units*/g defatted meal	
Non-nodulating	6,480a**	3,433a
PI 262090	5,446b	1,760b
487A-4-1-2	3,940d	986c
Florunner	4,580c	1,680b

*One unit Leucine aminopeptidase activity is defined as the amount of enzyme required to liberate in 30 min sufficient β-naphthylamine to yield a color product having an absorbance of 1 at 580 nm.

*One unit Acid phosphatase activity is defined as the amount of enzyme required to liberate in 10 min sufficient p-nitrophenol to yield a color product having an absorbance of 1 at 410 nm.

**Within a column, means followed by the same letter are not significantly different at 5% level of significance using Duncan's Multiple Range Test.

eral isozymes and are active during peanut seed maturation and initial stages of germination (2,6).

High concentration of α-amino nitrogen, soluble carbohydrates, and higher metabolic enzymic activity found in the non-nodulating line suggest that this line is not deficient in photosynthate and precursors of protein synthesis. Hence, it appears that the non-nodulating line may contain certain factors that limit protein synthesis. Alternatively, the protein synthesis mechanism of the non-nodulating line may be inefficient and unable to utilize the available precursors for protein synthesis.

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