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DIFFERENCES IN THE FATTY ACID COMPOSITION OF SOYBEAN SEED PRODUCED IN NORTHERN AND SOUTHERN AREAS OF THE U.S.A.

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Key Word Index—*Glycine max*; Leguminosae; soybean; oil quality; environmental effects.

Abstract—The fatty acid composition of soybean (*Glycine max*) seeds was sensitive to the influence of both genotype and environment. To quantify their relative environmental sensitivities, four soybean cultivars, Wells, Wayne, Cutler and Union, plus two experimental lines, 9656 and 9686, developed for lower concentrations of linolenate, were grown in Indiana (northern area) and Mississippi (southern area) in 1981. Seeds produced in the two environments were analysed for total oil content and for fatty acid composition. Seeds produced in the southern area were slightly higher (20% vs 16%) in oil content, but weighed significantly less than those produced in the north. The oil content per seed of seeds produced in the south was only 51% of the oil content of seeds produced in the north. Seeds produced in the south were significantly lower in both myristate and linolenate, but significantly higher in oleate than seeds produced in the north. Genotype by environment interactions for many oil quality measurements were largely attributable to the responses of the lines 9656 and 9686. These results indicate that higher environmental temperature reduced the linolenate concentration of soybean oil.

INTRODUCTION

The biosynthesis of fatty acids in plants has been investigated by many workers [1]. Several reports [2–6] have shown that synthesis of unsaturated fatty acids predominates in the early phases of soybean (*Glycine max*) seed development. Between 24 and 40 days post-anthesis, 30% of the total lipids are synthesized, and the fatty acid composition is predominantly from oleic (18:1) and linoleic (18:2) acids [7]. Before seed maturation, the linolenate (18:3) content (mole percentage) of the seed oil decreases by at least half.

18:2 synthesis in subcellular systems from *Torulopsis utilis* [8] and from safflower (*Carthamus tinctorius*) seeds [9] occurs by desaturation of 18:1. In plant leaf tissue, 18:2 and 18:3 arise by successive desaturation of 18:1 [10, 11]. It has been shown that homogenates from soybean cotyledons, at a very early stage of development [6], are capable of producing 18:2 and 18:3 from 18:1-CoA. The activity of the desaturases decreased with soybean cotyledon development and the activities were irregular and decreased with seed development.

Soybean germplasm has been developed with differing levels of unsaturated fatty acids in the seeds [12, 13]. Comparisons can now be made of the amounts and rates of deposition of 18:3 in normal and low-18:3 lines. Using these genotypes, we recently showed [7] that the 18:3 content of mature soybean seeds was inversely related to the 18:1 content. Since earlier work [14] had shown environmental sensitivity of soybean oil compositions, we evaluated the fatty acid compositions of these genotypes to determine whether each responded similarly to environmental conditions. To establish an environmental contrast against which we could measure a potential genotype by environment interaction, we produced seeds in the north and at three locations in the south during the same growing season (1981). These seeds were analysed for fatty acid composition.

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RESULTS AND DISCUSSION

Seeds produced in northern and southern areas were analysed for seed dry weight, oil concentration and oil content (Table 1). Seeds produced in the south were slightly higher in oil concentration, but weighed significantly less than those produced in the north. Therefore, the amount of oil per seed for those produced in the south was only *ca* 50% of the amount of oil per seed for seeds produced in the north.

The fatty acid compositions of mature soybeans were significantly influenced by the area in which the seeds were produced (Table 2). 18:1 and 18:2 together comprised *ca* 75% of the total fatty acid complement, which is consistent with previous reports [4, 13, 15, 16]. There were, however, significant differences among the experimental lines for the degree of response to the production area and for the fatty acids that indicated this response. For cultivars Wells, Wayne, Cutler and Union, there were no significant differences between production areas for either 18:1 or 18:2 content (mole percentage). Conversely, the two lines selected for low 18:3 content produced significantly less 18:1 and more 18:2 if they were grown in the north than if they were grown in the south. All lines produced significantly more myristate (14:0) and 18:3 if they were grown in the north. Similarly, all lines but 9686 produced significantly less palmitate (16:0) if grown in the north, but these differences were minor in all cases.

Table 1. Seed weight and oil content of soybean seeds produced in northern and southern areas

	Seed wt (mg)			Oil concn (g/kg)			Seed oil content (mg/seed)		
Genotype	IN		MS	IN		MS	IN		MS
Wells	388	**	133	169	**	211	65.8	**	28.3
Wayne	380	**	162	161	**	209	61.3	**	33.9
Cutler	386	**	180	159	**	205	61.4	**	36.9
Union	412	**	173	178	*	205	73.1	**	35.4
9656	405	**	154	170	**	209	68.6	**	32.2
9686	391	**	157	136	**	191	53.0	**	30.1

*,**Indicate significant difference between means, at the 0.05 and 0.01 levels, respectively.
IN, = Indiana; MS = Mississippi.

Differences between production areas for stearate (18:0) content were significant only for the cultivar Wells and the genetic lines 9656 and 9686; in these cases, the 18:0 contents of the oils were higher if the seeds were produced in the north. In all cases, the changes in 18:3 content were lower in seeds produced in the south.

In a previous report [7] we described the inverse relationship between the 18:3 and 18:1 contents in soybean oil. The results obtained during this study reinforce this relationship, particularly for the breeding lines 9656 and 9686. The 18:1 content of the oil produced by these lines was *ca* 39% when they were grown in the north, but it was over 46% when they were grown in the south. This significant difference in 18:1 concentration was accompanied by compensatory differences in 18:2 and 18:3 concentrations. The compositional contrasts in soybean oils produced in the two production areas indicate that considerable environmental sensitivity may exist for the enzymatic activities responsible for the final composition of the oil.

We suggested earlier [7] that the low-18:3 lines had reduced desaturase activity that converted 18:1 to 18:2. Therefore, we calculated the 18:1 to 18:2 and the 18:2 to 18:3 ratios of oils of each genotype, in each production area (Table 3). For the genetic lines 9656 and 9686, the 18:1 to 18:2 ratio was significantly higher and appeared to be more environmentally sensitive than that for any cultivar. Conversely, although the 18:2 to 18:3 ratio of these two lines was higher than that for most cultivars, it appeared to be less sensitive to the effects of the area in which the seeds were produced. The patterns indicate that the desaturase activities of these lines differed from those of the commercially grown cultivars.

The 18:1 to 18:2 ratios of high-18:3 lines were between 0.31 and 0.49, while those of the low-18:3 lines were 0.99 and 1.40. These ratios were lower for seeds produced in the north than for those produced in the south. The 18:2 to 18:3 ratios of seeds produced in the north averaged 7.1, while they averaged 8.6 if produced in the south. The relative differences between production areas were less pronounced for 9656, and were not significant for 9686, reflecting the relatively higher 18:2 to 18:3 ratio of these lines when grown in the North. These data show that the increased accumulation of 18:1 in soybean seeds produced in the south was accompanied by reduced accumulation of 18:2 and 18:3.

The fatty acid compositions of the soybean oils were analysed by analysis of variance (Table 4). In all cases, the effects of both cultivar and production area were highly significant. The cultivar by production area interaction was significant only for 14:0, 18:0 and 18:2. For each of these fatty acids, the significance of the interaction was largely accounted for by the differential response of the two lines that had been selected for low 18:3 concentrations.

Hawkins *et al.* [15] recently reported that tropical environments could be validly used in the selection of genotypes with particular oil compositions. They, however, compared the relative classification of genetic lines produced in Iowa in the summer with that of the same lines produced in Puerto Rico in the winter. Our data, in which seeds were produced in the north and in the south during the same summer season, would indicate that there may be an important caveat to the general pattern that Hawkins *et al.* [15] reported. The significant cultivar by production area interaction identified for several fatty acids and for the ratios between them (data not shown) indicates that genetic selection, by which lines 9656 and 9686 were developed, apparently produced markedly different environmental sensitivities of the desaturase activities of the developing seeds.

A major contrast between northern and southern production areas would exist for the ambient temperature in which the seeds developed. This difference indicates that seeds produced in the south were exposed to higher average daily temperatures than those grown in the north. The data summarized in Table 5 show that the daily average minimum temperatures in the southern area was hotter by 5.8°, 5.5° and 5.3° for June, July and August, respectively. The daily average maximum temperatures were hotter by 4.5°, 4.3° and 5.8° for June, July and August, respectively. The soybeans grown in the northern area received 30 cm of rainfall during these 3 months, however, compared to 19.9 cm in the southern area.

The oil compositional differences between production areas would therefore be attributable to the differential activities of desaturase enzymes during seed formation. In 1957, Howell and Collins [17] described the response of soybean oil compositions to differences in ambient temperature during seed development. They found lower concentrations of both 18:3 and 18:2 in the oil of each of three cultivars grown at elevated temperatures. Slack and Roughan [16] described the rapid alteration of soybean oil composition due to temperature shifts. Similarly,

Table 2. Fatty acid compositions of soybeans produced in northern and southern areas

	Fatty acid (mole percentage)																	
	14:0			16:0			18:0			18:1			18:2			18:3		
Line	North		South	North		South	North		South	North		South	North		South	North		South
Wells	2.80	**	1.12	11.05	*	11.48	3.78	**	3.34	17.29		19.33	56.32		57.80	8.76	**	6.92
Wayne	3.01	**	1.44	11.07	**	12.15	3.77		3.94	23.40		25.40	51.43		51.36	7.32	**	5.72
Cutler	3.01	**	1.31	10.73	**	11.44	3.72		3.58	18.12		20.14	56.30		56.57	8.12	**	6.96
Union	2.72	**	1.53	11.11	**	11.77	3.39		3.24	20.67		23.21	54.50		54.31	7.61	**	5.94
9656	2.21	**	1.25	10.20	*	10.68	3.96	**	3.48	39.11	**	46.40	39.34	**	34.42	5.19	**	3.86
9686	2.81	**	1.44	10.39		10.66	4.15	**	3.55	39.53	**	46.84	36.50	*	33.43	4.93	*	4.07

*,**Compositional difference between production zones (northern vs southern) is significant; $P = 0.05$, $P = 0.01$, respectively.

Table 3. Ratios of oleate, linoleate and linolenate from soybean seeds produced in northern and southern areas

	18:1 to 18:2			18:2 to 18:3		
Line	North		South	North		South
Wells	0.31		0.33	6.43	**	8.35
Wayne	0.45		0.49	7.03	**	8.98
Cutler	0.32		0.35	6.93	**	8.13
Union	0.37		0.43	7.16	**	9.14
9656	0.99	*	1.35	7.58	*	8.92
9686	1.08	*	1.40	7.40		8.21

*,**Indicate significant difference between means, at the 0.05 and 0.01 levels, respectively.

Tremolieres *et al.* [18], in agreement with Canvin [19], described the effects of temperature and light levels on the accumulation of unsaturated fatty acids by sunflower (*Helianthus annuus*) and rape (*Brassica napus*) seeds, each of which responded differently to the alterations of the environment. Our compositional data indicate a pattern of fatty acid alterations consistent with the rapid alterations described by Slack and Roughan [16], and with the pattern described by Tremolieres *et al.* [18] for sunflower seeds.

Our data indicate a degree of environmental sensitivity that might be expected when producing soybeans outside their areas of adaptation. A significant interaction with production area was found for certain fatty acids in genotypes selected for specific compositional patterns. This indicates that caution should be exercised in the choice of winter nursery sites, particularly if genetic selections are to be made for seed compositional criteria. These data also indicate that the fatty acid composition of soybean cultivars might be controlled to a certain degree by the selection of a growing location. This might have a significant impact on the choice of seed production sites if, for example, specific compositional patterns were associated with seed quality and/or storage life.

EXPERIMENTAL

Four cultivars of soybean (*Glycine max* L. Merr.) and two experimental genotypes varying in 18:3 content from 4 to 9% were field-grown near Lafayette, IN (northern) and in three locations near Stoneville, MS (southern) in 1981. A randomized complete block field plot design was used within each production area. Seeds were harvested at maturity and cleaned by removal of dirt, hulls and immature seeds. Soy flour was prepared by grinding seeds in a Wiley mill to pass a 40-mesh screen. Lipids were extracted from the flour in $CHCl_3$–MeOH (2:1) and 15:0 was added as internal standard. Extracted lipids were saponified with 0.5 M NaOH in MeOH and Me esters of fatty acids were prepared using BF_3–MeOH [20]. Triplicate samples were analysed by GC [20]. Fatty acids were identified and quantified using standards of 14:0, 16:0, 18:0, 18:1, 18:2 and 18:3.

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Table 4. Analyses of variance of the fatty acid compositions of soybeans produced in the north and in the south

		Fatty acid					
Source	*df*	14:0	16:0	18:0	18:1	18:2	18:3
Zone	1	17.935**	3.151**	0.700*	134.40**	10.5	17.851**
Error *a*	4	0.042	0.065	0.070	5.53	2.07	0.430
Cultivar	5	0.199**	1.466**	0.254**	804.04**	597.28**	12.660**
Cv × zone	5	0.129*	0.128	0.125**	10.69	8.67**	0.195
Error *b*	22	0.032	0.048	0.012	5.05	2.08	0.142

*,**Indicate significance at the 0.05 and 0.01 levels, respectively.

Table 5. Climatological data for Lafayette, Indiana and Stoneville, Mississippi during the summer of 1981

	Temperature (°)								Rainfall (cm)	
	IN				MS					
Month	Min	Min (avg)	Max	Max (avg)	Min	Min (avg)	Max	Max (avg)	IN	MS
June	9.4	16.4	33.9	27.7	17.2	22.2	35	32.2	5.7	8.2
July	9.4	17.3	33.9	28.4	17.8	22.8	37.8	32.7	10.4	7.8
August	8.3	15.8	30.6	26.9	17.2	21.1	37.8	32.7	15.9	3.9

The data were obtained from the National Oceanic and Atmospheric Administration for each location. avg: Average.

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