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PATTERNS OF FATTY ACID DEPOSITION DURING DEVELOPMENT OF SOYBEAN SEED*

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Abstract—Fatty acid deposition in developing soybean seeds of high- and low-linolenic acid cultivars show a great deal of similarity In all cultivars, the greatest change in fatty acid content occurs during the first half of seed formation The amount of linolenic acid is highest during the very early stage of seed formation and the relative amount decreases thereafter Linolenic acid content in mature soybean seed is inversely proportional to that of oleic acid

INTRODUCTION

Linolenic acid (18 3 (9, 12, 15)) is an undesirable component of soybean oil The high degree of unsaturation in this acid leads to flavor reversal or the production of off-flavor components Normally, 18 3 comprises 8% of total soybean fatty acids Presently, there is considerable interest in developing improved soybean cultivars containing reduced levels of 18 3

The biosynthesis of fatty acids (FA) in plants has been investigated by many workers [1] Reubel *et al* [2], Rochm and Privett [3], and Privett *et al* [4] have shown that synthesis of unsaturated FA predominates in early phases of developing soybean seed From 24 to 40 days after anthesis, 30% of the total lipids are synthesized, and FA composition undergoes a rapid change from predominantly unsaturated to saturated FA By seed maturation, the 18 3 content decreases by at least 50% Linoleic acid (18 2 (9, 12)) synthesis in subcellular systems from yeast [5] and from safflower seeds [6] occurs by desaturation of oleic acid (18 1 (9)) In plant leaf tissue 18 2 and 18 3 arise by successive desaturations of 18 1 [7, 8] It has been shown that homogenates from soybean cotyledons, at a very early stage of development [9], are capable of producing 18 2 and 18 3 from oleoyl-CoA The activity of the desaturases decreased with soybean cotyledon development and the activities were irregular and decreased with time Information on patterns of 18 3 synthesis in various genotypes (e g low and high 18 3 producers) as a function of development might be helpful to identify possible site(s) of regulation Another objective of this study was to compare patterns of FA synthesis by immature soybean embryo *in vitro* with those from developed seeds

RESULTS

Patterns of fatty acid deposition during seed development

Developing soybean seeds were harvested between 15 and 65 days after flowering (DAF) for measurement of wt and FA concentration Fresh wt was recorded immediately following harvest, while dry wt was measured after 72 hr at 70° Prior to 15 DAF, the extremely small seed size made measurement impractical, while seeds had approached maturity by 65 DAF Even at 15 DAF, when seeds averaged 13 mg fr wt, moisture content of 92% resulted in low seed dry wt and difficulty in FA extraction The growth of seeds, measured by fr wt, dry wt and extractable FA was observed to occur in four distinct phases (1) a period of acceleration of fr and dry wt gains, occurring between 15 and *ca* 25 DAF (190 mg fr wt), (2) a period of linear increases in fr wt, dry wt and FA content beginning at 25 DAF and continuing through 40 DAF (490 mg fr wt), (3) deceleration of wt increases and protein gains between 40 and 50 DAF (600 mg fr wt), followed by, (4) seed dehydration and cessation of FA increases as seeds matured

Nine experimental lines of soybean were field-grown in 1980 and 1981 and the patterns of FA deposition in developing seeds were determined Variations of FA developmental patterns observed in these lines between 1980 and 1981 were very small Therefore, only patterns of FA deposition in developing seeds during 1981 of two experimental lines, '9656' and '9686', and two commercial lines, 'Wells' and 'Wayne', are shown (Fig 1) The mole percentage of stearic acid (18 0) remained the same throughout seed development for all lines examined Myristic acid (14 0) was usually high in very small embryos, (fr wt less than 100 mg) but decreased to a low level in 200 mg embryos In the case of line 9656, 14 0 was again fairly low in small embryos, peaked in content at 150 mg, and then declined

Palmitic acid (16 0), which is *ca* 10 mol % of the FA in small embryos, increased slightly with development, followed by a small decrease after 200 mg fr wt

The amount of 18 3 in the smallest embryos was *ca* 10 mol %, but increased with seed development until the seeds were *ca* 150 mg in fr wt In some cultivars, 18 3 reached more than 20 mol % ('9656', Fig 1A, 'Wayne', Fig 1D) In all seeds analysed, 18 3 content decreased when the fr wt of seeds exceeded 200 mg

The predominant FA in mature soybeans are 18 1 and 18 2 The relative amounts of these two acids increase during the first half (250 mg fr wt) of seed development

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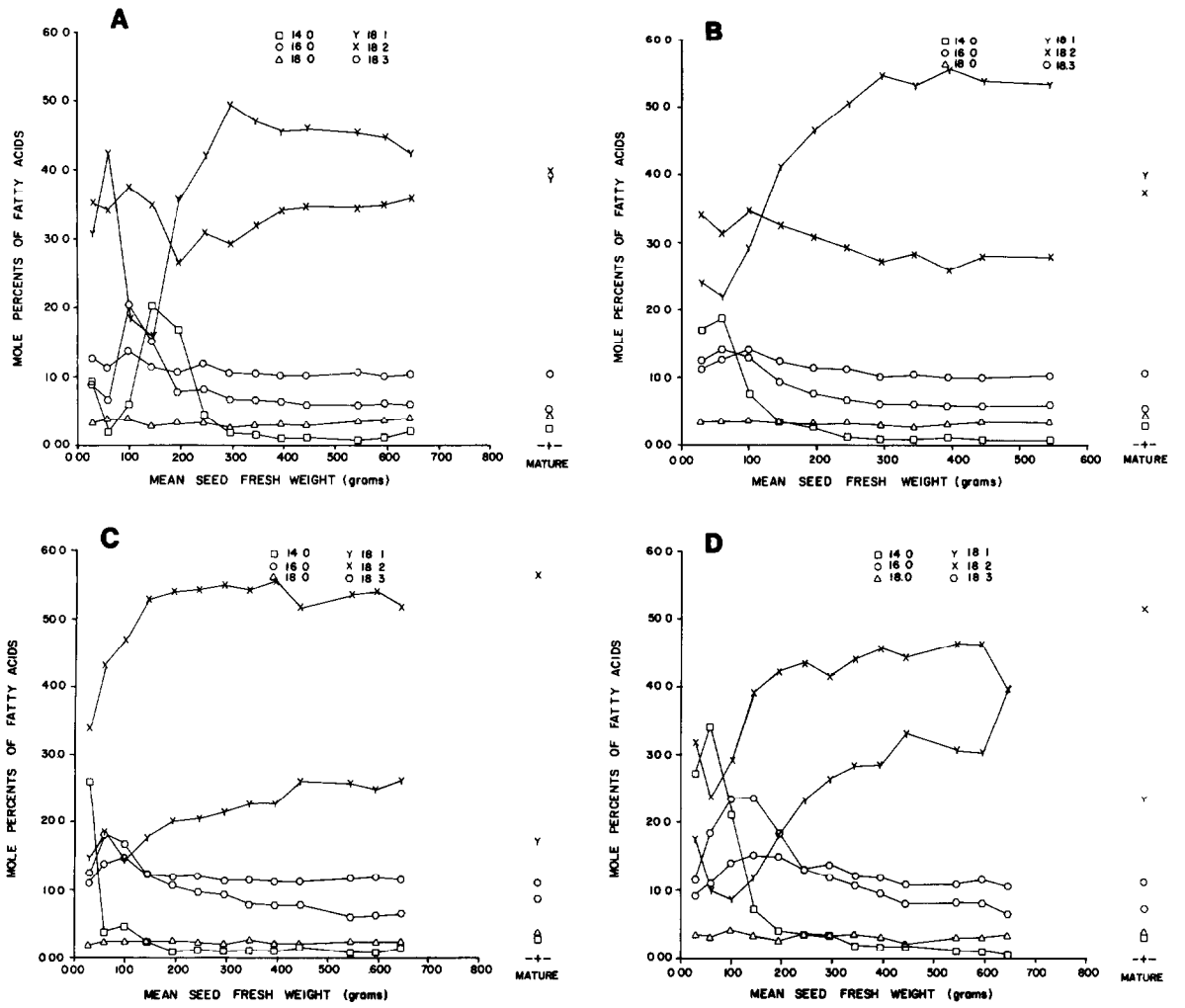

Fig 1 Changes in fatty acid content (mol %) of (A) 9656, (B) 9686, (C) Wells and (D) Wayne embryos during seed development, respectively

and remain at a near constant proportion through maturation Mature seeds of '9686' and '9656' contained *ca* the same ratio of 18 1 to 18 2 (0 95 to 1 08) (Table 1) In the four commercial cultivars analysed, 18 2 was present at twice the level of 18 1 However, the ratio of 18 2 to 18 3 remained *ca* the same for all soybean lines analysed

Embryo culture

Dry wt accumulation by 'Wayne' zygotic embryos (data not shown) was rapid for 18 days and then ceased after 30 days in culture It is likely that nutrients became a limiting factor during incubation In contrast to field-grown

Table 1 Fatty acid composition of 1981, field-grown soybeans near Lafayette, IN

	Fatty acid content (mol %)						Ratio of	
							18 1	18 2
Cultivar	14 0	16 0	18 0	18 1	18 2	18 3	18 2	18 3
O9686	2 81	10 39	4 15	39 53	36 50	4 93	1 08	7 4
N9686	2 57	10 51	4 33	40 03	37 54	5 03	1 06	7 5
O9656	2 21	10 20	3 96	39 11	39 34	5 19	0 99	7 6
N9656	2 55	10 27	4 27	37 79	39 73	5 19	0 95	7 6
'Wayne'	3 01	11 07	3 77	23 40	51 43	7 32	0 45	7 0
'Wells'	2 80	11 05	3 78	17 29	56 32	8 76	0 31	6 4
'Cutler'	3 01	10 73	3 72	18 12	56 30	8 12	0 32	6 9
'Union'	2 72	11 11	3 39	20 67	54 50	7 61	0 37	7 2

embryos, 14 0 accounts for over 60% of the FA in the smallest *in vitro* zygotic embryos (Fig 2) However, this could be a characteristic of very immature embryos as the mean fr wt of *in vitro* embryos was 17 4 mg, whereas the smallest field-grown embryos had a mean fr wt of 20 mg The content of 14 0 remained higher in embryos grown *in vitro* than in somatic embryos throughout the culture period The content of 18 3 rose from 3 to *ca* 9 mol % after 12 days in culture and remained constant thereafter The accumulation pattern of FA in '9656' embryos and '12309' embryos was similar to that of 'Wayne' (data not shown)

Comparison of FA accumulation by 'Wayne' embryos developing *in vivo* or *in vitro* (Figs 1D and 2) indicated significant differences in the accumulation of certain FA Patterns of accumulation of 18 2 and 18 3 *in vitro* were similar to those *in vivo* However, accumulation of 18 1 *in vitro* was very dissimilar to the *in vivo* system

DISCUSSION

Patterns of FA deposition in developing soybean seeds by high- and low-linolenic acid cultivars were similar (Fig 1) With the exception of 14 0, deposition of saturated FA (18 0 and 16 0) remained similar during seed development 14 0 usually was high in small seeds and decreased with development

The C_{18} unsaturated fatty acids undergo major changes in content during seed development Relative contents of 18 1 and 18 2 usually were minimal in seeds of less than 100 mg fr wt The relative proportion of these two acids greatly increase with seed development, particularly during the first half in gain of seed fr wt The relative content of 18 3 usually reaches a peak at 100–200 mg fr wt and then declines

In the early stages of seed development, the relative amount of 18 3 acid declines while those of 18 1 and 18 2 increase The high amounts of 18 3 acid detected at the early stages of seed development agree with the data of Stymme and Applequist [9] for soybean, and that of Ichihara and Noda [10] for safflower From these results we conclude that the rate of synthesis of 18 3 declines while that of 18 1 and 18 2 acid increases during seed development If 18 2 is a precursor for 18 3 synthesis, it appears that the amount of desaturation of 18 2 would limit 18 3 production during seed development However, it was also observed (Table 1) that the ratio of 18 2 to 18 3 in mature seeds of soybean lines did not change significantly That is, soybean lines with low levels of 18 3 also had reduced levels of 18 2, with a concomitant increase in 18 1 Therefore, we suggest that soybeans with low levels of 18 3 are limited in the enzyme system which desaturates 18 1 to 18 2 Future research will attempt to elucidate whether the two desaturation steps, viz 18 1 → 18 2 and 18 2 → 18 3, are enzymatically regulated during seed development Information on the differential regulation of desaturation of FA would be useful in developing a soybean line containing low levels of 18 3

Developmental patterns of growth and FA accumulation in zygotic embryos of soybean grown *in vitro* are genotype-dependent FA accumulation by *in vitro* embryos can differ both in patterns of accumulation during development as well as in concentration of individual fats accumulated Because of the reduced fr wt gains of embryos *in vitro*, it appears that additional manipulations are necessary to establish a tissue culture system amenable to study FA biosynthesis in embryos developing *in vitro*

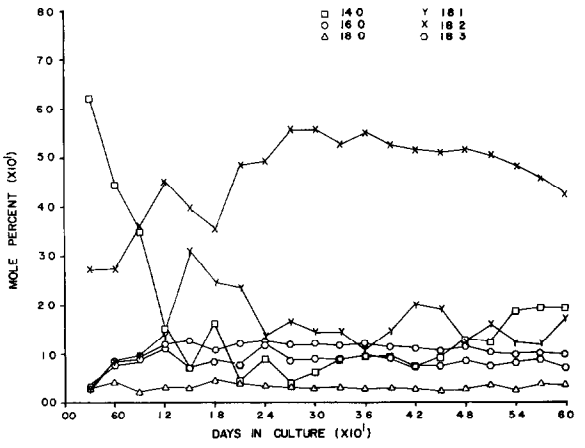

Fig 2 Changes in fatty acid content (mol %) of 'Wayne' embryos with time in culture

EXPERIMENTAL

Seed development Nine cultivars of soybean (*Glycine max* L Merr) varying in 18 3 content from 4 to 10% were field-grown near Lafayette, IN during 1980 and 1981 Developing, green pods were randomly selected from 7 5 m rows several times during the growing season and seeds were divided into size classes on the basis of fr wt (size groups in mg fr wt as follows 0–20, 21–40, 41–80, 81–120, 121–170, 171–220, 221–270, 271–320, 321–370, 371–420, 421–470, and 471–520) FA contents are plotted against these fr wts Lyophilized beans were ground in a mortar with a pestle, and lipids were extracted in $CHCl_3$–MeOH (2 1) with 15 0 added as an internal standard Extracted lipids were saponified with 0 5 M NaOH in MeOH and Me esters of FA prepared using BF_3 in MeOH [11] Duplicate samples taken from at least 10 developing embryos were analysed by GC Mature soybean seeds were ground in a Wiley mill to pass a 40-mesh screen and the resultant flour extracted, saponified, methylated and analysed as described above FA in the extracts were identified and quantified using authentic standards

Embryo culture Patterns of FA accumulation in zygotic soybean embryos cultured in Gamborgo's B5 medium without auxin were determined over a 60-day culture period Embryos from two low 18 3 experimental lines (9656 and 12309) and a commercial variety (Wayne) which accumulates relatively high levels of 18 3 were used in this study

Zygotic embryos, 2 2–3 mm in length, were isolated from fruits or plants grown in a greenhouse Embryos were cultured in 5 ml Gamborg B5 medium for 60 days on a Rollodrum apparatus (1–5 rpm) Incubation was at 26° and 1 5 klx illumination was provided for 16 hr per day Three embryos were sampled at 3-day intervals, lyophilized and prepared for GC analysis as before

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