POTENTIAL FOR SELECTION OF FATTY ACIDS ON A SINGLE SEED BASIS IN SUNFLOWER (*HELIANTHUS ANNUUS L.*)

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Summary

Fatty acid analyses of individual seeds (achenes) from within single heads of Hysun 30, Pervenets and CSB 101 showed considerable variation in linoleic acid content (2.2-76.0%) whilst the saturated fatty acids (palmitic plus stearic) remained virtually constant (~7-14%). Oleic acid levels varied inversely with those of linoleic acid. Fatty acid composition of a seed appeared unrelated to its position in the head. This indicated the influence of genetic rather than environmental factors.

Seeds were dehulled and cut transversely into thirds. The fatty acid composition of each third was similar, indicating that oil composition was reasonably consistent throughout the seed.

Removal of up to two thirds of the seed did not prevent germination of the remaining section. Thus, a simple screening technique based on a single seed rather than an average of a head or plot is available.

A procedure to analyse one half (tip) of the seed and to germinate the remainder is offered as a technique to allow a specific seed selected for fatty acid content to be grown through to maturity.

Introduction

Sunflower oil varies considerably in the degree of unsaturation. Such variation creates difficulties for manufacturers of vegetable oil based products.

Temperature during seed maturation influences the linoleic/oleic ratio (Keefer *et al.*, 1976; Harris *et al.*, 1978; Goyne *et al.*, 1979). The linoleic/oleic ratio decreases at higher temperatures. When planting times necessitate crop maturation under high summer temperatures, genotypes with stable high linoleic acid content (> 62%) would be desirable.

Putt *et al.* (1969), found genetic variability for stearic, oleic and linoleic acids among varieties of inbred lines which matured at the same time. Using single seed analyses, they found variability decreased with level of inbreeding.

Knowledge of the genetic control of fatty acid content is helpful in determining the breeding procedure. If fatty acid content depends upon the genotype of the embryo, then a single seed technique enables selection of segregating F_2 embryos on F_1 plants. Alternatively, if fatty acid content is maternally controlled, then a bulk sample of seed from F_1 plants could be used.

The literature on genetic control of fatty acids in sunflower reports differing results. Fernandez-Martinez and Knowles (1982) found that maternal control of

linoleic acid was more important than embryonic and that genes for high linoleic acid appeared to be dominant to genes for low linoleic acid. Miller and Zimmerman (1983) reported that high and low oleic acid appeared to be controlled by two or more embryonic genes acting independently and additively. Maternal influences were small. Fick (1984) and Urie (1984) reported high oleic acid content to be embryonically controlled by a single partially dominant gene or single dominant gene respectively. Genes with minor effects on oleic acid content may be present (Gassner, 1973) with significant maternal effects being noted by Fick (1984). Differences in genetic control may be related to the germplasm studied.

Zimmerman and Fick (1973) found that the linoleic acid and palmitic contents of the oil increased and the oleic acid content decreased from the perimeter toward the centre of the head. Because flowering takes seven to ten days to complete and starts at the perimeter and ends at the centre of the head, individual seeds may mature under different environmental conditions.

Several studies have reported single seed techniques for selection for fatty acids. Putt *et al.* (1969) used partial seed analysis but did not report the relationship between fatty acid composition of the embryonic and non-embryonic sections or report on the germination of sectioned seeds. Downes and Tonnet (1982) proposed the use of low temperature (4°C) germination as a screening technique, based on the theory that seeds lower in linoleic acid would be slower to germinate due to decreased mobility of the oil within the seed.

This paper describes analysis of a section of seed for fatty acid composition to enable selection of high oleic or high linoleic lines. The remainder of the seed can then be germinated and plants grown through to maturity. Preliminary findings of fatty acid variation among seeds within and among heads of three cultivars are reported.

Materials and Methods

Three cultivars, Pacific Hysun 30, Pervenets and CSB 101 were space planted at Hermitage Research Station (near Warwick, Queensland) on 16 September, 1982 to coincide seed development with mid-summer conditions. Plants were irrigated to avoid moisture stress. Hysun 30 is a single cross hybrid, Pervenets is a Russian open-pollinated cultivar reported to have high oleic acid content (Vick *et al.*, 1984) and CSB 101 is an inbred line selected for high linoleic acid content (Downes, pers. comm.).

At maturity, a number of single seeds from a head of each cultivar were dehulled and kernel cut transversely into thirds with a sterile scalpel. The oil from each third was then hexane extracted and analysed for fatty acid composition (Simpson and Osborne, 1978) to determine the consistency of composition throughout the seed. Twenty-eight seeds were analysed.

The radicle section (lower one third) of several other seeds was placed on wet cotton wool in an incubation oven at 25°C to observe germination.

To determine the influence of seed position upon fatty acid composition for each cultivar, seeds were randomly taken from positions across a head.

A representative sample of seed was removed from five or six heads of each cultivar, cleaned, and pressed to provide an oil sample which was analysed for fatty acid content.

Results and Discussion

Correlation coefficients of the tip and mid sections of the seed with the radicle section were high for all fatty acids (Table 1). Correlation coefficients for palmitic acid were lowest and this may reflect some variability among cultivars. For all fatty acids, the mid section was more highly correlated with the radicle section than the tip. The high correlation of fatty acid composition of tip and mid sections of the seed with the radicle section, enables single seed selection because embryos selected on the basis of composition of the tip or mid sections can be germinated and grown to maturity.

Table 1. Correlation coefficients (r^2 in brackets) for the tip and mid seed sections with the radicle section of 28 seeds for four fatty acids.

	Fatty Acid			
	Palmitic	Stearic	Oleic	Linoleic
Tip	0.71 (0.50)	0.87 (0.75)	0.94 (0.88)	.99 (0.99)
Mid	0.84 (0.70)	0.93 (0.87)	0.95 (0.90)	.99 (0.99)

% LA* (Radicle section) = 1.04 x % LA (Tip) + 2.54
= 1.03 x % LA (Mid) + 0.86

* LA = Linoleic acid.

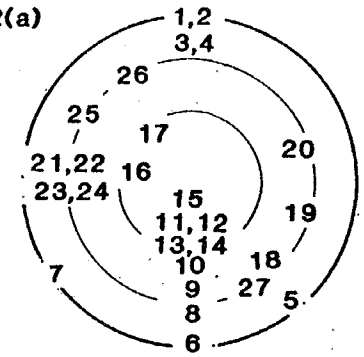
The radicle third was germinated without specialised treatment. Comparison with control seeds (not sectioned) showed that sectioned seeds tended to germinate faster - possibly due to more rapid moisture uptake. As development continued, those with more intact cotyledons were more vigorous. Although successful germination was found with only one-third of the seed, a larger section (for example, half) would produce more vigorous seedlings. Also, the fatty acid composition of a larger section may be more highly correlated with the embryo section. Dehulling is not required, but was used here to observe germination development. For large batches, sectioned seeds can be germinated using standard techniques, e.g., seeds are placed between two sheets of absorbent paper, a wet towel is placed on either side of the paper and then the towel and the paper are rolled up and placed in an incubation oven. Fungicide may be useful in reducing infection.

Fatty acid composition data and diagrams illustrating seed position within single heads of three cultivars are presented in Tables 2-4 and Figures 2(a)-4(a) respectively. These results indicate that large variation within a head is possible and that this variation appears unrelated to seed position, which is in contrast to the study by Zimmerman and Fick (1973). However, this may have resulted from limited and random sampling, which did not allow statistical analysis in this study. The considerable and apparently random variation among seeds within a head found for the unsaturated acid content in all cultivars, suggests the importance of genetic rather than environmental effects. Variability especially for unsaturated acids was found for composite samples of six seeds by Zimmerman and Fick (1973) who suggested even greater variability would occur for single seeds due to the lack of averaging of genetic effects.

The distributions of linoleic acid levels in seeds from one head of Hysun 30, Pervenets and CSB 101 are presented in Figures 2(b), 3(b) and 4(b) respectively.

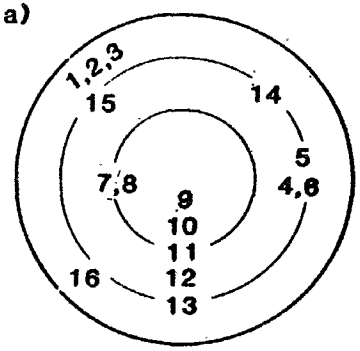
HYSUN 30

Fig 2(a)



PERVENETS

Fig 3(a)



CSB 101

Fig 4(a)

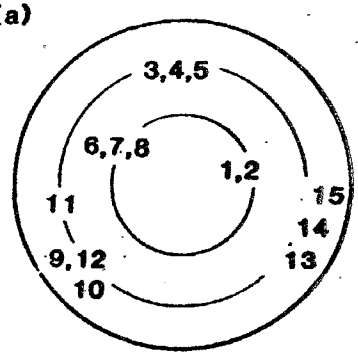


Table 2 Seed composition

No.	%Oleic	%Lin.	No.	%Oleic	%Lin.
1	23.1	64.1	15	25.4	62.2
2	31.8	58.1	16	17.2	69.7
3	42.2	45.3	17	24.9	63.0
4	38.8	49.7	18	49.0	39.4
5	49.6	39.2	19	51.6	36.9
6	59.6	30.7	20	39.1	48.8
7	21.0	67.0	21	32.0	56.0
8	57.4	32.1	22	33.2	54.5
9	47.0	42.6	23	35.4	50.6
10	47.7	40.8	24	37.3	50.7
11	42.4	46.7	25	23.2	63.1
12	47.5	41.1	26	27.1	59.9
13	43.6	43.7	27	47.5	41.7
14	55.9	33.3			

Table 3 Seed composition

No.	%Oleic	%Lin.	No.	%Oleic	%Lin.
1	87.5	4.0	15	91.0	1.6
2	36.1	52.0	16	89.7	2.4
3	89.1	3.5			
4	74.7	16.7			
5	17.0	73.7			
6	90.6	2.0			
7	37.5	46.1			
8	41.8	43.1			
9	37.1	51.5			
10	90.8	2.3			
11	90.5	3.1			
12	91.1	1.9			
13	87.8	3.8			
14	46.3	42.7			

Table 4 Seed composition

No.	%Oleic	%Lin.	No.	%Oleic	%Lin.
1	44.4	44.5	15	86.1	6.2
2	48.8	40.3			
3	81.4	11.0			
4	88.5	3.9			
5	87.7	4.9			
6	49.4	40.0			
7	89.2	3.4			
8	15.6	71.6			
9	71.3	19.6			
10	44.3	45.8			
11	59.1	31.8			
12	84.5	7.3			
13	83.3	9.3			
14	44.4	45.5			

Fig 2(b) Distribution of linoleic acid

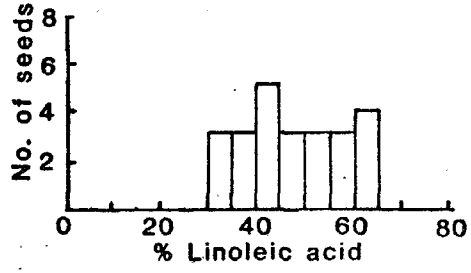


Fig 3(b) Distribution of linoleic acid

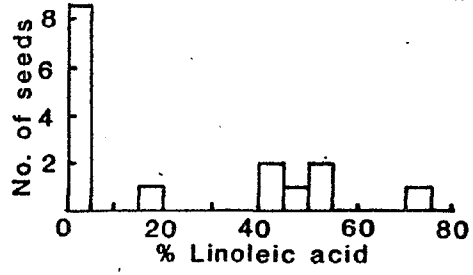
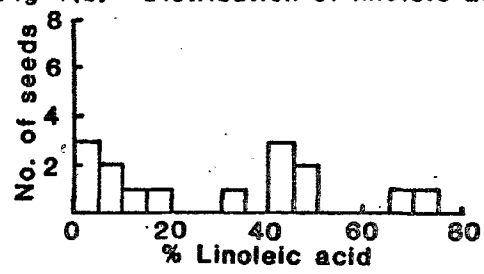


Fig 4(b) Distribution of linoleic acid



Although only a small number of seeds were analysed, differences in distribution were apparent. Hysun 30 showed a narrower range than either Pervenets or CSB 101. The distribution for Pervenets (Figure 3b) suggests skewness towards low linoleic acid values resulting in a low average level of linoleic acid.

Table 5. Fatty acid levels in single sunflower heads.

	Head No.	%Palmitic	%Stearic	%Oleic	%Linoleic
Hysun 30	1	6.3	4.8	48.0	40.9
	2	6.0	6.0	45.3	42.7
	3	5.4	5.7	44.5	44.4
	4	5.4	5.8	46.3	42.6
	5	5.5	5.9	47.0	41.6
	Mean (SD)	5.7 (.41)	5.6 (.48)	46.2 (1.4)	42.4 (1.3)
CSB 101	1	5.4	3.9	36.1	54.5
	2	5.3	4.2	50.1	40.4
	3	5.7	2.8	35.1	56.4
	4	6.4	5.2	36.0	52.4
	5	6.3	4.5	35.3	53.9
	6	5.4	3.5	47.5	43.6
	Mean (SD)	5.8 (.48)	4.0 (.83)	40.0 (6.9)	50.2 (6.6)
Pervenets	1	4.7	5.2	58.2	31.9
	2	4.8	4.8	53.0	37.4
	3	5.1	5.6	41.0	48.2
	4	4.3	2.9	81.9	10.9
	5	4.8	3.2	67.0	25.0
	Mean (SD)	4.7 (.29)	4.3 (1.2)	60.2 (15.3)	30.7 (13.9)

Considerable differences among heads within cultivars were measured for fatty acid composition (Table 5). Variability among heads for unsaturated acids was less for Hysun 30 than other cultivars and this may be due to the uniformity of F_1 plants. Greater genetic variability for unsaturated acids for Pervenets was expected because Pervenets is a heterogeneous, open-pollinated cultivar. Vick and Miller (1984) also found this cultivar to be variable. As Hysun 30 flowered 14 days later than CSB 101, lower linoleic acid content was expected due to seed maturation under higher temperatures.

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