CHAPTER 5. THE VARIABILITY IN FATTY ACID COMPOSITION OF SUNFLOWER OIL

5.1 INTRODUCTION

The problem of low linoleic acid contents in Australian sunflower oil and the need for new 'temperature - stable' genotypes has been mentioned previously (Section 2.6). To successfully breed these new genotypes sufficient variability must exist in the fatty acid composition of the present cultivars. Several researchers have reported variation in fatty acid composition between heads within a line and between seeds within a head (Putt *et al.*, 1969; Kinman, 1971; Zimmerman and Fick, 1973). However, there is very little information available on whether the high linoleic acid levels reported are capable of being maintained under high temperature conditions.

The previous two chapters have described the development of a technique that should facilitate the investigation of genetic variation in fatty acid composition of sunflower by allowing groups of seeds to be easily exposed to high temperatures during rapid oil synthesis. Using this technique and a population of field grown sunflowers the question of variability of fatty acid composition and the maintenance of this variability was examined in the cultivar Sunfola 68. Therefore experiments were carried out to

a) examine the variability of fatty acid composition of oil from different heads.
b) to examine the variability in the fatty acid composition of oil from individual seeds within a head; and
c) to attempt to make selections from the cultivar Sunfola 68 capable of producing high levels of linoleic acid even when high temperatures are experienced during seed development.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Procedures

5.2.1 Experiment 1. Variation in the Fatty Acid Composition of Oil from Field-grown Heads and Embryos (Kernels)

A row of sunflowers was planted at the Myall Vale Research Station of the Department of Agriculture at Narrabri, N.S.W on 26th November 1981. Approximately half the heads were flowering by the 9th February 1982. The heads were allowed to cross-pollinate naturally and were then covered in gauze bags to minimize bird damage. At physiological maturity (when heads were yellow and bracts were turning brown) sixty-six heads were harvested and allowed to dry in a glasshouse. The material was examined in two ways. Fifty heads were selected to be analysed. A sample of ten seeds was taken from the outer third of each head, and the kernels removed. A composite sample of oil from each head was obtained by extracting the oil from these ten kernels. This oil was then analysed to give the 'mean' (composite) fatty acid composition of the head.

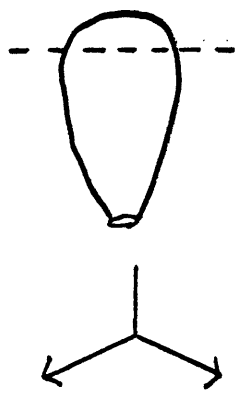
Fifty individual seeds were also sampled from the outer third of two selected heads. The abaxial part of the seed (approximately one third of the whole seed) was removed with a sharp scalpel (diagram 5.1). The pericarp was removed from the cut off portion of the seed and the oil was extracted from the cotyledonary material. This oil was then analysed for fatty acid composition to determine whether variability in fatty acid composition exists between individual embryos within a head matured under high temperature conditions.

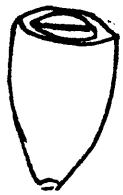
5.2.1.2 Experiment 2. Variation in the Fatty Acid Composition of Oil from Embryos of Cultured Seeds

Plants of the standard cultivar were grown under field conditions at Armidale, N.S.W. Prior to anthesis the heads were bagged. Brush pollination

DIAGRAM 5.1

Removal of the Abaxial Section of the Sunflower Seed





some seeds
germinated between
moist filter paper

cotyledon
material

pericarp

oil extraction
Fatty Acid Composition

was carried out only once when the outer third of the head had receptive stigma. At approximately 300 day-degrees after pollination four heads were harvested and disinfested and seeds were taken from each head for culturing. Seeds from two of the heads were incubated at 18°C while the seeds from the remaining two heads were incubated at 27°C. When the heat sum at both incubation temperatures had reached 200 day-degrees, embryos were removed from the cultured seeds and the oil of each embryo was analysed for fatty acid composition.

5.2.1.3 Experiment 3. Selection for a Sunflower Genotype with Stable High Linoleic Acid

Two heads having high linoleic acid percentages in their oil were chosen from the field grown material from experiment one. Individual seeds were selected from the outer third of each of these heads. Similarly to the procedure used in experiment one, approximately one-third of the abaxial part of the seed was removed and the cotyledonary material contained in this portion of seed was analysed for the fatty acid composition of its oil (diagram 5.1). The remaining two thirds of the seed (containing the growing point or axis) was germinated between moist filter paper in an incubator at 21°C and then planted out individually in 30cm pots in the glasshouse. When the resulting plants reached anthesis they were self-pollinated. This was performed by collecting the pollen from a single head with a soft brush and reapplying this pollen to the receptive stigma in the head. After pollination the plants were removed to a hot glasshouse (mean day/night temperatures 32/23°C). When the heat sum reached approximately 700 day-degrees after pollination the heads were harvested. The oil composition of this material was examined in two ways. Firstly a composite sample of ten seeds from each head was removed and analysed for fatty acid composition. Individual seeds from promising heads were also selected and the kernels of these seeds were analysed individually for fatty acid composition again by taking approximately one third of the

abaxial portion of the cotyledons for analysis.

5.3 RESULTS

5.3.1 Variation in Fatty Acid Composition of Field-grown Heads and Kernels

The fatty acid composition of a composite oil sample from each of the fifty field-grown heads was determined (Table 5.1a and Figure 5.1a). The range of linoleic acid percentages between the individual heads was 23.8% to 60.1%. The oleic acid content of the heads showed a corresponding range of values - 28.0% to 65.0%. Despite the wide range of values obtained for linoleic and oliec acid percentages for individual heads the mean values for the fifty heads were 34.9% linoleic acid and 56.1% for oleic acid. The saturated acids, palmitic and stearic acid, also showed some variation in oil from different heads (Table 5.1a).

The fatty acid composition of individual embryos (kernels) within a single head showed similar variation in the four fatty acids to that seen between heads (Tables 5.1b,c and Figures 5.1b,c). In one head (N12) the linoleic acid percentage of the oil from individual embryos ranged from 26.2% to 49.7% with oleic acid ranging from 45.9% to 63.7%. The mean value for the linoleic acid percentage of the fifty individual embryos was 34.9% while the composite value obtained from the extraction of ten embryos from the same head (as determined for variation between heads) was 35.0%.

The other head examined (N25) gave slightly different results. The range of linoleic acid percentages between individual embryos within this head was 41.4% to 68.0% with the corresponding range for oleic acid being 23.1% to 48.2% (Table 5.1c and Figure 5.1c). Again there was good agreement between the mean value for the linoleic acid from the fifty individual embryos and value for the composite sample of ten embryos analysed from this head - 55.9% compared to 56.1%.

TABLE 5.1

Fatty acid composition of oil of sunflower seeds grown during mid-summer at the Myall Vale Agricultural Research Station, Narrabri, N.S.W.

a) Variation in fatty acid composition of composite kernel samples (10 embryos) of fifty heads.

b) Variation in the fatty acid composition of the oil of individual kernels of a head (N12) with low linoleic acid content.

c) Variation in the fatty acid composition of the oil of individual kernels of head (N25) with a high linoleic acid content.

	Fatty Acid	Mean ± S.D. (% t.f.a.)	Range (% t.f.a.)
a)	palmitic	4.4 ± 0.7	3.1 - 6.4
	stearic	4.7 ± 1.1	2.5 - 7.2
	oleic	56.1 ± 6.8	28.0 - 65.0
	linoleic	34.9 ± 7.1	23.8 - 60.1
b)	palmitic	4.0 ± 0.7	1.9 - 5.9
	stearic	3.4 ± 1.2	1.8 - 5.2
	oleic	55.3 ± 4.4	45.9 - 63.7
	linoleic	37.2 ± 5.1	26.2 - 49.5
c)	palmitic	4.8 ± 0.5	3.5 - 5.8
	stearic	4.8 ± 0.7	3.1 - 6.5
	oleic	33.1 ± 8.3	23.1 - 48.2
	linoleic	55.9 ± 7.1	41.4 - 68.0

FIGURE 5.1

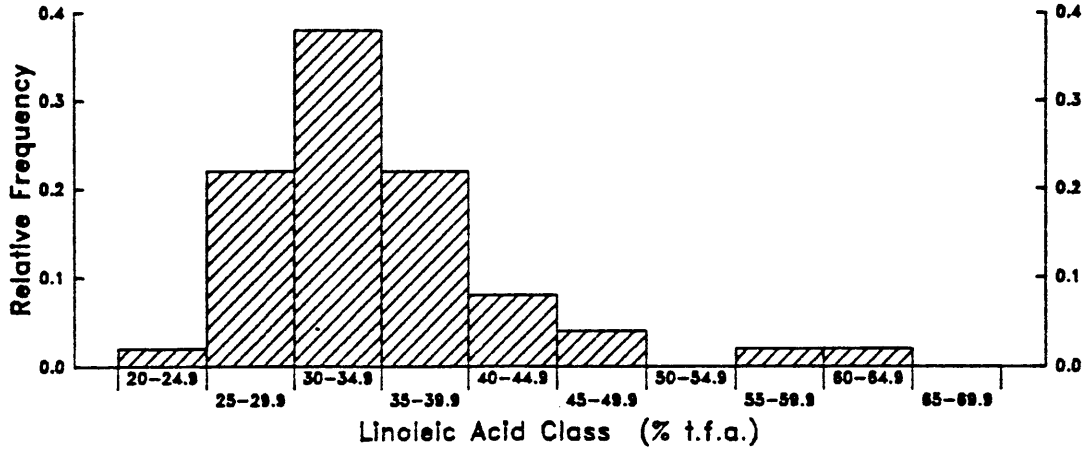
Linoleic acid percentages of oil from sunflower seeds grown during mid-summer at the Myall Vale Agricultural Research Station, Narrabri, N.S.W.

a) Variation of linoleic acid percentages of oil from composite samples from fifty heads.

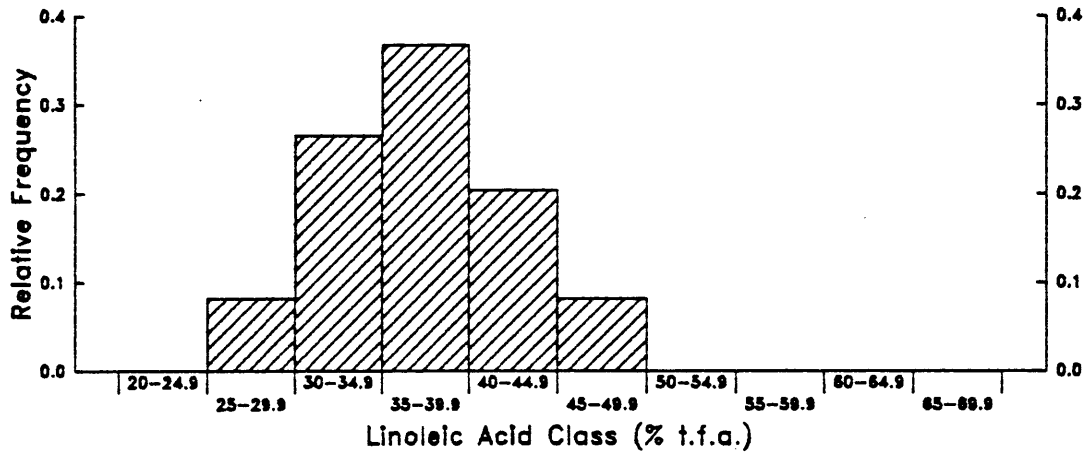
b) Variation of linoleic acid percentages of oil from individual kernels of a head N12 with low linoleic acid content.

c) Variation of linoleic acid percentages of oil from individual kernels of a head N25 with high linoleic acid content.

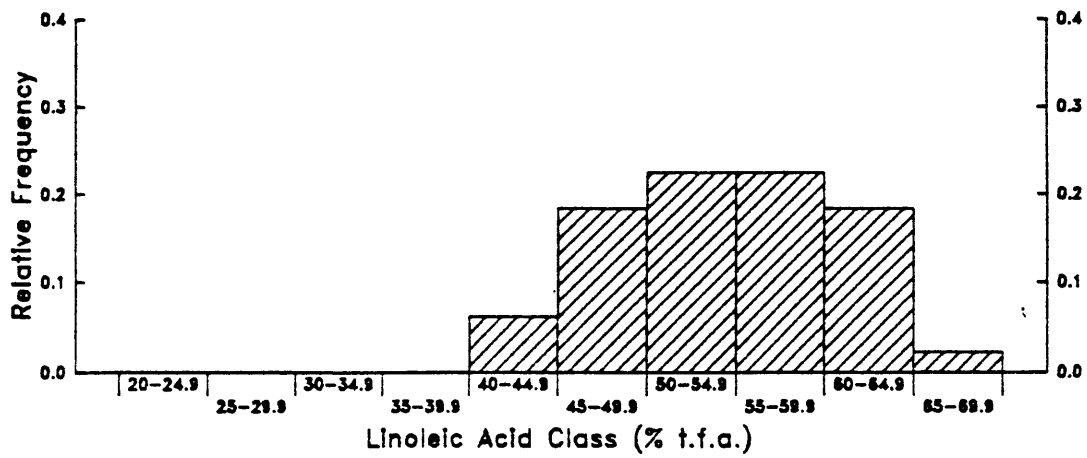
a) Heads grown at Narrabri



b) Head number N12



c) Head number N25



5.3.2 Variation in the Fatty Acid Composition of Oil from Embryos of Cultured Seeds

The results from the culture experiments were similar to the results obtained for field-grown material. At 27°C the linoleic acid percentages of the oil of indivdual embryos from the two heads (A and B) ranged from 8.8% to 64.6% and 7.8% to 57.0% respectively, with oleic acid values ranging from 21.3% to 72.2% and 26.7% to 78.8%, (Table 5.2 and Figure 5.2a). At 18°C the range of variation in linoleic acid from the two heads (C and D) was 27.2% to 78.2% and 25.4% to 82.0%, with the corresponding oleic acid ranges being 12.6% to 61.3% and 8.8% to 60.7%, (Table 5.2 and Figure 5.2b). The saturated acids, palmitic and stearic, also showed some variation in percentage at both temperatures (Table 5.2).

5.3.3 Selection for a Sunflower Genotype with Stable High Linoleic Acid

Individual seeds from the Narrabri head number 25 (N25) with linoleic acid percentages in the range of 61% to 68% were selected, grown out and self-pollinated. The linoleic acid levels in the composite oil samples extracted from these heads ranged from 27.8% to 51.2% (Table 5.3). Individual seeds were selected from the head with the highest linoleic acid percentage (51.2%; head N25-2) and the individual kernels of this head were analysed for fatty acid composition. Linoleic acid values ranged from 34.5% to 74.4% for the individual kernels (Table 5.4).

Individual seeds from another Narrabri head (N49) were also selected and grown out and the resulting plants self-pollinated. Although the selected parent seed had linoleic acid values of 62-72% the progeny heads gave linoleic acid percentages for composite oil samples of 32.5% to 52.0% (Table 5.3). These results are similar to the results obtained for the other head, N25, examined.

TABLE 5.2

Variation in the fatty acid composition of the oil from individual embryos of seeds cultured at 27°C or 18°C

Temperature °C	Head	Fatty Acid	Mean ± D.S. (% t.f.a.)	Range (% t.f.a.)
27	A	palmitic	5.3 ± 0.9	3.8 - 6.6
		stearic	7.7 ± 3.0	3.4 - 13.5
		oleic	60.1 ± 16.1	21.3 - 72.2
		linoleic	26.7 ± 16.1	8.8 - 64.6
27	B	palmitic	4.4 ± 1.5	1.1 - 7.1
		stearic	6.8 ± 2.9	1.6 - 13.6
		oleic	56.9 ± 18.1	26.7 - 78.8
		linoleic	31.7 ± 17.8	7.8 - 57.0
18	C	palmitic	5.3 ± 0.9	3.2 - 7.0
		stearic	5.0 ± 3.0	1.5 - 13.4
		oleic	36.5 ± 15.2	12.6 - 61.3
		linoleic	52.9 ± 16.2	27.2 - 78.2
18	D	palmitic	6.6 ± 1.4	3.4 - 10.5
		stearic	4.3 ± 2.2	1.8 - 10.9
		oleic	33.8 ± 15.3	8.8 - 60.7
		linoleic	55.7 ± 15.3	25.4 - 82.0

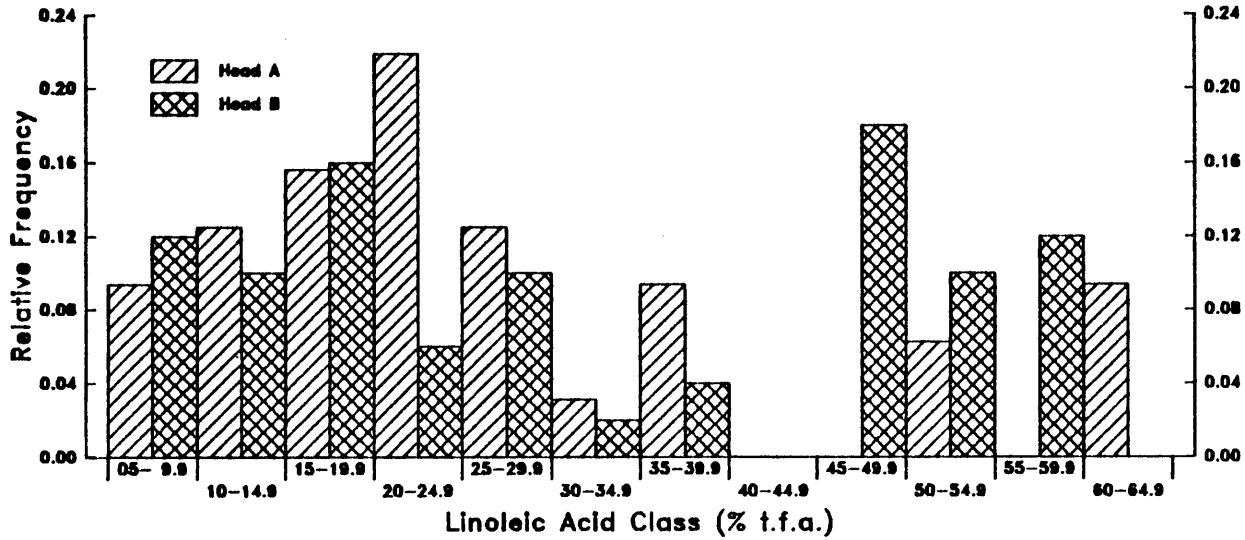
FIGURE 5.2

Linoleic acid percentages of oil from embryos cultured at 27°C or 18°C

a) Head A and Head B, 27°C

b) Head C and Head D, 18°C

a) 27 ° C


Head A
Head B
Relative Frequency
0.24
0.20
0.16
0.12
0.08
0.04
0.00
05– 9.9
10–14.9
15–19.9
20–24.9
25–29.9
30–34.9
35–39.9
40–44.9
45–49.9
50–54.9
55–59.9
60–64.9
Linoleic Acid Class (% t.f.a.)

b) 18 ° C

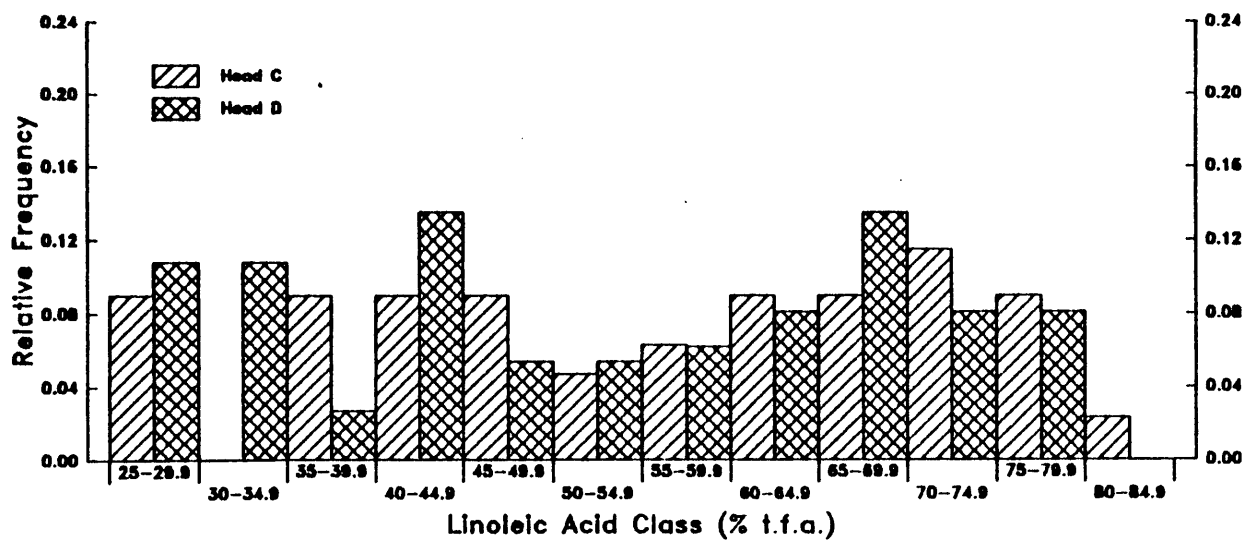

Head C
Head D
Relative Frequency
0.24
0.20
0.16
0.12
0.08
0.04
0.00
25–29.9
30–34.9
35–39.9
40–44.9
45–49.9
50–54.9
55–59.9
60–64.9
65–69.9
70–74.9
75–79.9
80–84.9
Linoleic Acid Class (% t.f.a.)

TABLE 5.3

Linoleic acid percentages of kernels from selections from head N25 and head N49

	Linoleic Acid (% t.f.a.)			
	Head N25		Head N49	
Seed No.	Original Seed	Progeny	Original Seed	Progeny
1	66.6	47.7	70.1	38.3
2	68.0	51.2	63.7	45.0
3	66.2	27.8	68.3	50.2
4	62.4	33.3	64.6	43.4
5	63.3	37.2	70.3	52.0
6	66.7	29.9	72.4	44.7
7	63.3	31.6	66.2	45.7
8	67.7	40.7	71.0	33.0
9	65.9	32.9	67.8	32.5
10	61.8	28.7	65.6	48.4
11	-	-	58.7	53.0
12	-	-	67.7	43.2
13	-	-	69.5	48.4
14	-	-	62.5	48.9
15	-	-	63.7	36.0
16	-	-	67.8	46.5
17	-	-	65.2	36.3
18	-	-	70.3	41.6

TABLE 5.4

Linoleic acid percentages of kernels from individual seeds of the selfed head N25-2

Seed Number	Linoleic Acid (% t.f.a.)
1	74.4
2	72.3
3	69.9
4	65.9
5	59.7
6	59.5
7	57.9
8	57.1
9	50.8
10	49.7
11	41.3
12	41.0
13	36.9
14	35.4
15	34.6
16	34.5

5.4 DISCUSSION

There were marked differences in fatty acid composition between heads of the cultivar Sunfola 68-2 grown at Narrabri. These differences are due to a combination of both environment and genotype. Not all heads commenced flowering on the same date but most heads commenced flowering over approximately one week. The heads experienced slightly different environmental conditions during development but the mean maximum temperature for the period was 31.3°C with a mean minimum temperature of 20.8°C. Therefore differences in fatty acid composition mostly express differences in genotype although some influence of environment, particularly temperature, cannot totally be ruled out. The differences in the fatty acid composition between heads were not confined to the unsaturated fatty acids, as palmitic and stearic acid also showed considerable variation between heads. Whether this variation is related to the variation of the unsaturated fatty acids is not known. This variation however might be of significance if selection for the modification of the saturated fatty acid content of sunflowers is ever required.

With so much variability in fatty acid composition shown to exist between individual embryos within a head, it is not unreasonable to question the validity of using a sample size of only ten embryos to determine a composite value for linoleic and oleic acid for a head. Fifty individual embryos were extracted and analysed for fatty acid composition and the mean values for the FAC calculated from these fifty determinations. Also a sample of ten embryos was taken from the same part of the head (the outer third) and extracted to give one oil sample (composite sample) which was analysed. The values obtained for the two samples were almost identical (see Section 5.3.1). It was therefore concluded that the sample size of ten embryos was adequate for the composite determination of fatty acid composition.

A marked difference in fatty acid compostion was also found between individual embryos (kernels) within a head. Seeds for these analyses were taken from the outer third of the sunflower head so that, since pollination would have taken place over more than one day, the seeds would have experienced slightly different temperature conditions. These differences in fatty acid composition between kernels within a head also can be expected to be due primarily to genotype. However in the culture experiments reported in this chapter, the uncertainties relating to the differences in time of flowering are removed. Therefore since the variability in fatty acid composition in the culture experiments is similar to that reported for individual embryos within a field grown head, the genotypic effect is confirmed.

The variation in fatty acid composition recorded was similar to that reported by Putt *et al.*, (1969), Kinman, (1971) and Zimmerman and Fick, (1973) all of whom found wide variation in fatty acid composition both among heads within a line and among seeds within a head. Since these results seem to offer proof that genetic variability exists in the sunflower, considerable scope appears to exist, for the selection of sunflower genotypes with modified fatty acid composition and the culturing technique offers a tool for this to be carried out.

The attempt to select for high linoleic acid production at high temperatures was initially disappointing. Single seeds with high linoleic acid (produced under high temperature conditions in the field) when grown into plants under high temperature conditions gave heads that had moderate to low levels of linoleic acid. However, when individual seeds from these heads were analysed there were some seeds present that did have high linoleic acid levels. Obviously much more work needs to be carried out before a proper assessment can be made, but the results presented in this chapter clearly indicate that genetic variation exists. Therefore the opportunity to select for a modified fatty acid composition in sunflower does exist.

The control of the fatty acid composition of the oil of the sun-

flower embryo is still being investigated. Putt *et al.*, (1969) postulated that the fatty acid composition was controlled mostly by the genotype of the embryo while Mill, Fick and Credeno (1977) (cited by Fernandez-Martinez and Knowles, 1982) reported that the ratio of oleic and linoleic acid is determined primarily by the maternal parent. However, Fernandez-Martinez and Knowles, (1982) have reported that both embryo genotype and the maternal parent can influence the fatty acid composition of the sunflower embryo. The wide variation in values obtained for the fatty acid in the results presented in this chapter seem to suggest that the embryo genotype and not just the maternal parent influences fatty acid composition of the embryo.

In order to carry out further work on the selection of stable high linoleic acid genotypes from the cultivar Sunfola 68-2 it will be necessary to determine which is the source of greatest variation - between head variation or variation among seeds within a head. This would involve a fairly large experiment but the information provided would indicate which direction a breeding programme will need to take to produce a sunflower cultivar with the capacity to produce high levels of linoleic acid regardless of the temperature conditions experienced during seed development.

CHAPTER 6. APPLICATION OF THE TECHNIQUE OF ISOLATED SEED CULTURE

6.1 INTRODUCTION

Chapter four described the development of a technique that involves culturing isolated sunflower seeds during their development. This technique could be useful in two areas. As previously suggested (Section 2.6) this technique could be valuable in determining the response of the fatty acid composition of oil from sunflowers of differing genotype to temperature.

The technique could also be used to study the biosynthesis of unsaturated fatty acids and its regulation in the developing sunflower embryo. Harris *et al* (1978) found linoleic acid (and oleic acid) percentage in oil from field-grown sunflowers to be linearly related to mean minimum temperature during development. A similar relationship has been found by Robertson *et al* (1978a). More evidence of the influence of night temperature on linoleic acid synthesis has also been presented by Rochester and Silver (1983). Sunflower plants were placed in two different growth conditions, both with the same day temperatures but with different night temperatures (28/22°C or 28/15°C). After exposure to $^{14}CO_2$ the oil of these sunflowers was extracted and the differences in the labelling of the fatty acids showed that there was increased synthesis of linoleic acid at the lower 'night' temperature. This appeared to support the conclusion from field trails that night temperature was the major environmental factor influencing fatty acid composition in sunflower seed oil. To further investigate both of these genetic and environmental effects, a series of experiments was carried out to:

a) determine the response of several inbred lines and hybrids formed from them to a range of temperatures during seed growth; and

b) investigate the effect of alternating day/night temperatures on the fatty acid composition of sunflower oil.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Procedures

Part A. Testing of Inbred and Hybrid Lines

Seed from several Sirosun restorer lines were obtained from A. Low, C.S.I.R.O., Griffith, N.S.W. Seed of a male sterile female A line Sirosun 89A was also obtained to make hybrids. The plants were grown in the field at Armidale, N.S.W. Prior to anthesis all heads were bagged. Restorer lines were self-pollinated by collecting pollen from one head and re-applying it to that head with a soft brush. Hybrids were formed by one brush application of the appropriate pollen to the male sterile heads.

When the heat-sum after pollination reached 300 day-degrees, the heads were harvested, disinfested and cultured. Fifteen seeds from each head were incubated at each of the temperatures - 15, 18, 21, 24, 27°C. When the heat sum at each incubation temperature reached 175 day-degrees, the embryos were removed from the seeds and the oil was extracted from ten embryos from each head at all temperatures and analysed for fatty acid composition.

Part B. The Effect of Alternating Temperature on Fatty Acid Composition

Plants were grown either in the field or in 30cm pots in the glass-house, and hand pollinated by the standard method. When the heat sum after pollination reached 300 day-degrees the heads were harvested, disinfested and cultured. The seeds were incubated at 27°C, and 18°C and at 27/18°C day/night temperatures. When the heat sum at each temperature reached 175 day-degrees the embryos were removed from the seeds and their oil analysed for fatty acid composition.

In the final experiment, plants were grown in 30cm pots in natural conditions until anthesis. After pollination half of the plants were transferred to a cool glasshouse (mean day/night temperatures 24/15°C) while the other half were transferred to a hot glasshouse (mean day/night temperatures

32/23°C). When the heat sum after pollination reached 300 day-degrees seeds were taken for culturing and the experiment proceeded as described above.

6.2.2 Statistical Analysis

The results of the final culture experiment were subjected to analysis of variance, and the Duncan's Multiple Range Test was used to determine the significance of differences between means.

Regression lines were fitted to the linoleic acid data for the inbred and hybrid experiment, and these lines were subjected to a test of parallelism.

6.3 RESULTS

6.3.1 Response of Inbred Lines and Hybrids to Temperature

Four inbred lines and four hybrids were cultured over a range of temperatures. The response of the four inbred lines to temperature is presented in Tables 6.1a-6.1d. The results for the four hybrids are presented in Tables 6.2a-6.2d. In all cases linoleic acid percentage decreased with incubation temperature. There was a corresponding increase in oleic acid. Regression lines fitted to the linoleic acid data are presented in Figures 6.1 and 6.2. All inbred and hybrids displayed a linear response in linoleic acid percentage to temperature. The saturated fatty acids, palmitic and stearic acid, showed no real trend with increasing temperature in either inbreds or hybrids.

6.3.2 The Effect of Alternating Temperature on Fatty Acid Composition of Sunflower Oil

A series of experiments was carried out examining the effect of constant or alternating temperature on the fatty acid composition of sunflower oil. The results are presented in Table 6.3. The response of fatty

TABLE 6.1

The fatty acid composition of embryos from Sirosun inbred lines cultured in a range of constant temperatures

a) Sirosun 117R

b) Sirosun 122R

c) Sirosun 152R

d) Sirosun 110R

	Temperature °C	Fatty Acid Composition (% t.f.a.)							
		Palmitic		Stearic		Oleic		Linoleic	
		A*	B	A	B	A	B	A	B
a)	15	6.2	7.7	8.1	4.0	16.7	16.0	69.0	72.3
	18	6.1	7.1	7.1	5.2	25.7	26.6	61.2	61.1
	21	6.3	7.0	8.9	6.1	31.3	34.3	53.4	52.4
	24	6.1	7.0	8.4	6.9	41.8	41.7	43.7	44.3
	27	6.7	7.1	10.1	7.0	49.3	52.8	33.1	33.9
b)	15	6.9	5.8	5.3	5.0	16.2	20.8	71.6	68.3
	18	6.9	5.7	4.2	6.2	24.2	27.5	64.7	63.0
	21	7.3	5.2	8.4	4.3	32.9	35.3	51.4	52.8
	24	6.2	6.3	5.6	5.5	40.8	42.5	47.4	45.7
	27	6.8	7.1	6.2	6.1	50.4	46.4	36.6	40.5
c)	15	6.9	6.6	4.8	7.1	24.9	26.9	63.4	59.3
	18	6.4	5.5	6.3	7.3	31.6	34.3	55.6	52.8
	21	6.0	6.5	8.7	8.1	43.9	39.7	41.4	45.9
	24	6.5	6.4	9.2	9.5	44.9	46.3	39.3	37.9
	27	6.9	6.4	8.5	8.0	49.9	50.8	34.8	34.8
d)	15	7.2	6.2	7.6	6.6	13.6	19.4	71.6	67.8
	18	6.9	6.8	7.8	11.4	23.2	27.9	62.1	54.0
	21	6.0	6.5	7.7	7.9	35.6	35.2	50.6	50.4
	24	5.8	6.0	5.9	7.5	50.4	42.1	37.9	44.4
	27	5.7	6.1	8.1	7.3	58.0	56.8	23.8	29.8

* A,B represent two different heads

FIGURE 6.1

The change in linoleic acid percentage of embryos from four inbred lines cultured over a range of temperatures

a) Sirosun 117R

b) Sirosun 122R

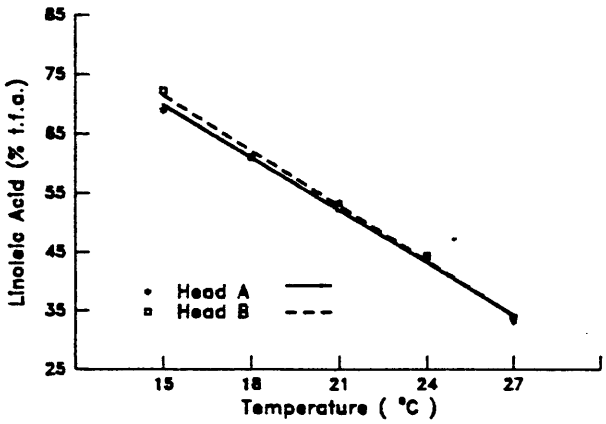
c) Sirosun 152R

d) Sirosun 110R

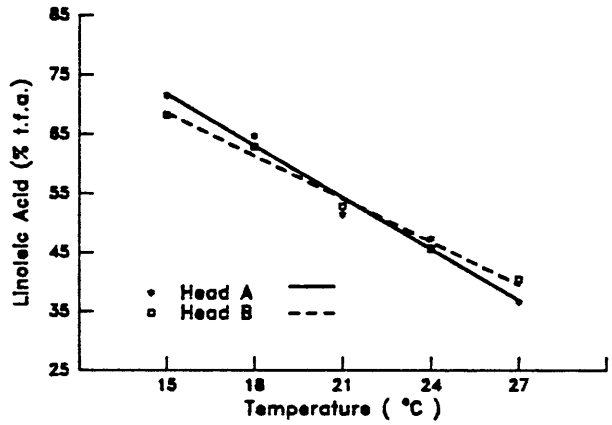
The regression lines are given by the equation linoleic acid = a + b temperature where the values of the coefficients are as follows

Line	Head	a	b	r^2
117R	A	114.59	-2.98	0.995
	B	118.32	-3.12	0.997
122R	A	98.35	-2.45	0.928
	B	90.87	-2.13	0.986
152R	A	115.45	-2.91	0.981
	B	105.09	-2.43	0.988
110R	A	133.06	-3.99	0.995
	B	109.20	-2.85	0.952

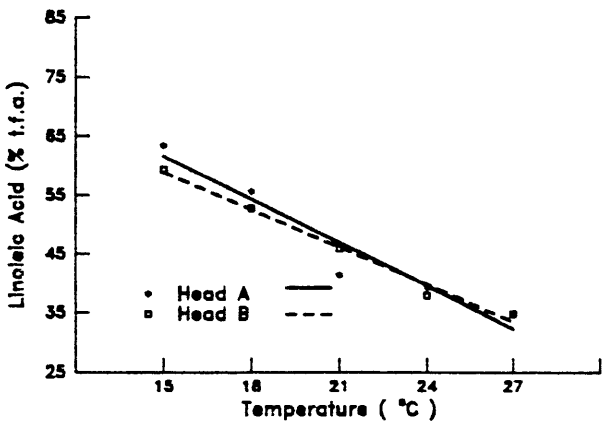
a) 177R



b) 122R



c) 152R



d) 110R

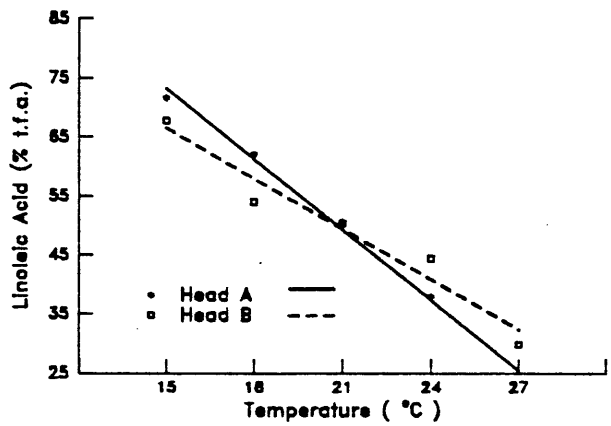


TABLE 6.2

The fatty acid composition of embryos from Sirosun hybrids cultured in a range of constant temperatures

a) male sterile Sirosun 89A x Sirosun 117R

b) male sterile Sirosun 89A x Sirosun 122R

c) male sterile Sirosun 89A x Sirosun 152R

d) male sterile Sirosun 89A x Sirosun 110R

	Temperature °C	Fatty Acid Composition (% t.f.a.)							
		Palmitic		Stearic		Oleic		Linoleic	
		A*	B	A	B	A	B	A	B
a)	15	8.5	7.8	4.0	3.7	10.6	9.0	77.0	79.5
	18	7.7	7.0	4.0	4.3	18.0	18.5	70.3	70.2
	21	7.0	8.0	6.4	4.4	34.2	24.1	52.4	58.5
	24	7.0	9.1	6.9	5.7	41.7	28.4	44.3	46.8
	27	7.1	7.9	6.8	5.1	44.9	35.8	41.2	39.9
b)	15	6.5	5.8	5.8	5.0	11.2	24.8	76.5	64.3
	18	7.6	5.2	8.0	4.3	19.0	27.3	65.4	63.0
	21	7.1	5.7	6.0	6.2	22.2	35.3	59.7	52.8
	24	6.4	6.3	6.6	5.5	34.7	42.5	52.3	45.7
	27	6.6	7.1	6.0	6.1	56.7	45.5	30.7	41.5
c)	15	6.8	8.2	4.8	4.9	13.0	11.5	75.4	75.4
	18	6.6	7.3	4.8	5.2	15.6	16.9	73.0	70.6
	21	8.5	7.6	7.1	5.1	22.8	26.4	61.6	58.0
	24	7.8	7.7	6.9	6.1	33.5	35.0	51.8	51.1
	27	8.9	7.9	7.8	6.1	45.1	44.6	38.2	41.4
d)	15	4.2	7.4	3.1	3.4	12.4	10.4	80.3	78.3
	18	6.5	7.8	5.5	4.9	26.1	11.8	62.0	75.6
	21	8.6	6.7	3.9	11.1	36.5	34.0	50.9	58.2
	24	6.5	7.5	8.9	4.9	45.4	39.5	39.2	48.1
	27	5.7	6.9	7.6	5.7	47.7	49.3	39.0	38.0

FIGURE 6.2

The change in linoleic acid percentage of embryos from four hybrids cultured over a range of temperatures

a) 89A x 117R

b) 89A x 122R

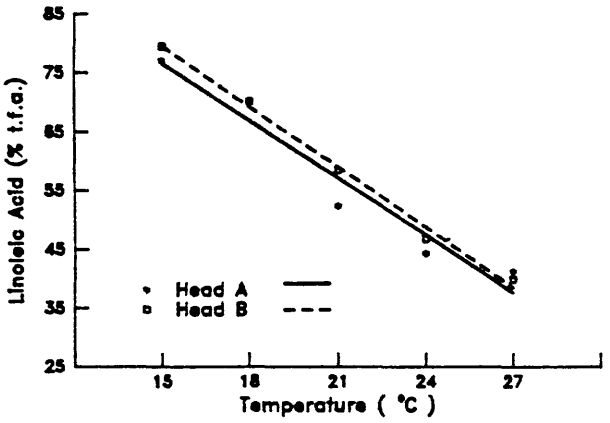
c) 89A x 152R

d) 89A x 110R

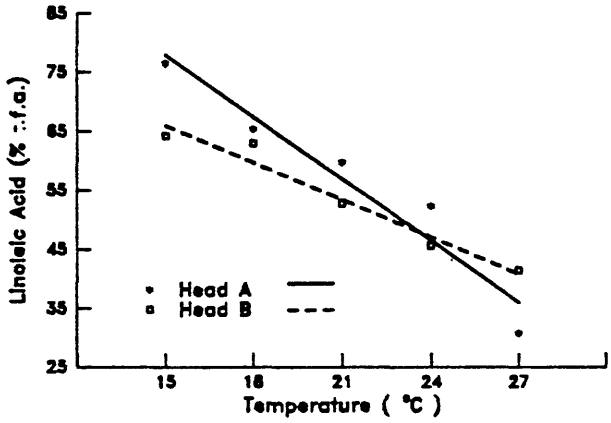
The regression lines are given by the equation linoleic acid = $a + b$ temperature where the values of the coefficients are as follows

Hybrid	Head	a	b	r^2
89A x 117R	A	125.36	-3.25	0.944
	B	130.80	-3.42	0.993
89A x 122R	A	130.21	-3.49	0.935
	B	97.49	-2.10	0.960
89A x 152R	A	126.92	-3.18	0.961
	B	120.55	-2.92	0.986
89A x 110R	A	128.06	-3.51	0.919
	B	135.31	-3.60	0.969

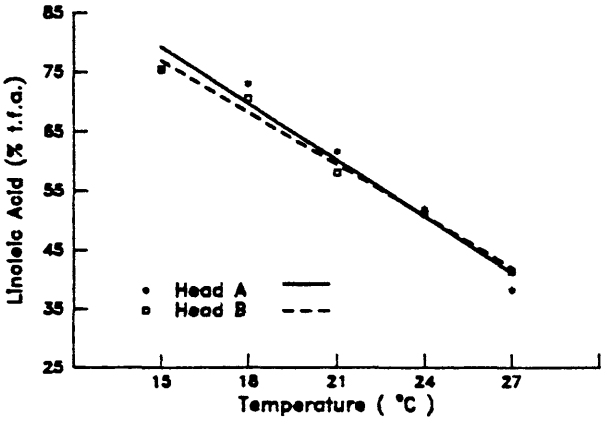
a) 89A x 117R



b) 89A x 122R



c) 89A x 152R



d) 89A x 110R

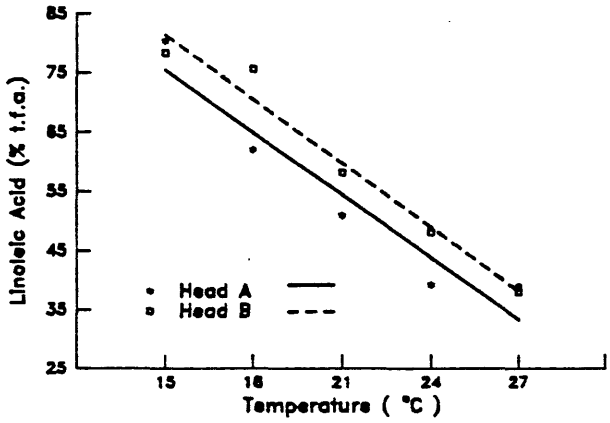


TABLE 6.3

The linoleic acid percentages of oil from sunflower embryos cultured in constant or alternating day/night temperatures

Temperature °C	Linoleic Acid (% t.f.a.)				
	A*	B	C	D	E
27	27.4	20.9	14.9	22.9	26.2
27/18	58.3	45.6	35.8	23.2	29.1
18	52.4	43.0	57.3	58.6	57.3

* A-E represent five different heads.

acid composition to the alternating temperature was varied. In two experiments the linoleic acid composition at 27/18°C was similar to that at 18°C. In two other experiments it was similar to that at 27°C and in the remaining experiment it had a value between the two constant temperatures.

In view of the different responses obtained in the previous experiments another experiment was carried out to attempt to clarify the situation. The only variable that could be detected between the treatment of the heads was the temperature conditions experienced after pollination but prior to culturing. Despite the evidence presented in chapter three, an experiment was designed to investigate the effect of the temperature conditions prior to culturing on the subsequent synthesis of oil. In this experiment three heads experienced 'high' temperatures and three 'low' temperatures after pollination and then were cultured in common temperatures. The results from these regimes are presented in Table 6.4. The weights of fatty acids were calculated from oil content and fatty acid percentages as set out in Appendix 3.2. There was no significant difference in the synthesis of fatty acids at all three temperatures due to different preculture temperature conditions. But there does appear to be a consistent trend for the linoleic acid percentage to increase with a corresponding decrease in oleic acid in the treatments which experienced low preincubation temperature. The alternating temperature treatment (27/18°C) had a similar effect on fatty acid synthesis to the highest constant temperature. The mean results have been presented in Table 6.4 but there was considerable variation in the response of each head to the three different temperatures.

6.4 DISCUSSION

6.4.1 Response of Inbreds and Hybrids

Lines fitted to the data for linoleic acid percentage verses incubation temperature (Figure 6.1) clearly demonstrated a linear decrease in linoleic acid with increasing temperature for both the inbred lines and for

TABLE 6.4

The effect of preculturing conditions on the fatty acid composition of oil from sunflower embryos cultured in constant or alternating temperatures. The results represent the mean of ten embryos from each of three heads

a) Fatty acid compositions expressed as percentages of the total fatty acids.

b) Fatty acid compositions expressed as the increase in individual fatty acids.

a) Temperature °C	Preculturing Conditions *	Fatty Acid Composition (% t.f.a.)			
		Palmitic	Stearic	Oleic	Linoleic
27	H	6.3±0.5	8.8±1.6	57.5±10.3	27.5±12.4
	L	6.9±0.5	6.8±0.6	40.8±9.0	45.6±9.0
27/18	H	5.8±0.7	9.0±2.4	53.1±6.8	32.1±8.6
	L	6.6±0.5	6.9±1.3	42.3±3.8	44.1±4.6
18	H	5.4±0.9	8.1±1.6	30.5±2.0	56.0±4.2
	L	6.6±0.6	6.7±1.4	22.0±4.3	64.6±5.0

b) Temperature °C	Preculturing Conditions	Increase in Fatty Acid Content (mg/10 embryos)			
		Palmitic	Stearic	Oleic	Linoleic
27	H	2.2±0.3	2.3±0.5	16.9±8.4	$18.4^{a'}$±11.5
	L	2.2±0.5	1.3±0.1	9.9±3.7	20.0^{a}±4.5
27/18	H	1.9±1.0	2.2±1.7	13.7±8.0	22.5^{a}±10.2
	L	2.5±0.9	1.9±0.5	13.9±3.9	22.3^{a}±3.1
18	H	1.2±0.6	1.4±0.5	-6.5±3.4	40.0^{b}±8.1
	L	1.2±0.6	0.9±0.6	-5.4±3.7	36.8^{b}±3.5

*'H' represents high temperature (32°/23°C)

'L' represents low temperature (24°/15°C)

' values followed by the same letter did not differ significantly ($p \leq 0.05$)

hybrids formed by crossing them with a restorer line. A test of the significance of the slope of the response showed that differences ($p \leq 0.05$) existed among the inbred lines. Although genetic variability exists between the inbred lines the sensitivity of all lines to temperature is such that these differences appear to have little biological significance. It is, however, of note that variability exists although no selection for temperature stability was made during the development of the inbred lines.

The temperature experiments with the inbred lines and hybrids emphasise the potential usefulness of the culturing system in screening material in breeding programmes. The technique allows the response of the genotype to temperature to be determined quickly and results were available before remaining field grown plants had matured.

6.4.2 The Effect of Alternating Temperature on the Fatty Acid Composition of Sunflower Oil

The varied response to alternating temperature (Table 6.3) was thought possibly to be due to the effect of different temperature conditions prior to culturing. Although the results were not statistically significant there is a consistent trend, in all three treatments, for linoleic to increase and oleic to decrease at the low preincubation temperatures. This may be a real effect although the results of this experiment cannot prove this hypothesis. It is possible that intra-head variability is sufficiently high in all treatments to prevent clarification of this effect. Both high and low pretreatments gave the same direction of response when the heads were cultured at any of the three temperatures. These results appear to further support the assumptions made previously (3.4.2) that the triacylglycerols synthesised at the beginning of the rapid phase of oil accumulation can undergo acyl exchange leading to a fatty acid composition that reflects the temperature conditions experienced after their synthesis. The results at 18°C in Table 6.4b actually show a decrease in oleic acid content from the start of the culture experi-

ment until the end of the incubation period. Since most of the oleic acid would have been bound in triacylglycerols, with some bound in diacylglycerol and phospholipid, oleic acid has either undergone acyl exchange reactions with linoleic acid or desaturation of bound oleate derivative has occurred.

The cause of the varied results in the culture experiment (Table 6.3) remains unclear since the differences between heads could not be explained by different temperature conditions prior to culturing. It is possible that the varied results could be due solely to the response of different genotypes to temperature. A large amount of variability in fatty acid composition was found when the cultivar Sunfola 68 was grown under high temperatures in the field (Chapter 5). Similarly, variation in the fatty acid composition of embryos from cultured seeds has also been demonstrated. Therefore the varied results could have simply occurred due to using five different Sunfola heads for the experiment.

These experiments show that the technique of isolated seed culture could be useful for the study of fatty acid synthesis and its regulation in developing sunflower seeds. This would be especially so in studies examining the effect of temperature on fatty acid composition. By including such compounds as radioactive tracers in the culture medium further information on the influence of temperature on fatty acid synthesis could possibly be obtained.

CHAPTER 7. ALTERNATIVE METHODS TO SCREEN FOR SUNFLOWER GENOTYPES WITH STABLE FATTY ACID COMPOSITION

7.1 INTRODUCTION

It has been mentioned previously (Section 2.6) that an adequate supply of sunflower oil with a high linoleic acid content will not be reliably available in Australia with existing sunflower genotypes. One method that could help screen for new genotypes with stable linoleic acid contents has been described in Chapter 4.

This method of isolated seed culture, whilst having been demonstrated to be useful for screening for temperature response (see Chapter 6), involves making sterile culture medium and the preparation of the tissue for culturing. This requires meticuluous attention to detail and is time consuming. A simpler, easier technique may be more appropriate for a large breeding programme, and, with this in mind, alternatives were explored.

One method that could prove useful is to 'culture' not individual seeds but detached sunflower heads in solution during exposure to a range of temperatures. This technique has been used successfully to grow spikes of wheat during the grain filling phase (McWilliam, pers. comm.). Although large controlled temperature facilities would be needed the preparation of the material should be both quicker and less demanding.

In another approach to selecting plants that produce high levels of linoleic acid, Downes and Tonnet (1982) found that relationships existed between the rate of germination at 4°C and the linoleic acid content of the seeds of the resulting plant. These researchers reported that, using this technique, selected plants and families were isolated that were capable of producing 70-80% linoleic acid under conditions in which the original population had an average linoleic acid content of 40%. It was postulated that early germinating seeds may themselves contain high linoleic acid contents. If this is so, then early germination of seeds at 4°C might be able to be used to select seeds with high levels of linoleic acid from

populations of seeds matured under high temperature conditions.

Therefore the series of experiments in this chapter were carried out to assess alternative methods of screening for sunflower genotypes with high levels of linoleic acid at high temperatures. The two methods investigated were:

a) the use of detached sunflower heads to assess the response of oil fatty acid composition to temperature; and

b) the detection of seeds with high linoleic acid, by their early germination at 4°C.

7.2 MATERIALS AND METHODS

7.2.1 Experimental Procedures

7.2.1.1 Detached Head Experiment

Sunflower plants were grown in the field at Armidale, N.S.W. The heads were bagged prior to anthesis and brush pollinated once when approximately one third of the stigma were receptive. When the heat sum from pollination reached 300 day-degrees the heads were cut from the plant under water, leaving a stem of approximately 45 cm in length. The heads were transferred to incubators set at either 21°C or 27°C, placed in a 1 litre beaker of water and supported by a retort stand. Lighting was provided by fluorescent lights for a twelve hour photoperiod. At this stage ten seeds were removed from each head and analysed for oil content and fatty acid composition. Every day approximately 1 cm of stem from the base of the detached head was cut away underwater. When the heat sum at each temperature reached 160 day-degrees, ten seeds from each head were removed and the embryos analysed for oil content and fatty acid composition of the oil.

7.2.1.2 Germination at 4°C

A random sample of seeds was taken from two heads grown at Narrabri under high temperature conditions (Experiment 1, Chapter 5). Seeds were

soaked in water for fours hours, placed in petri dishes between moistened filter papers and incubated at 4°C. Benlate (1% w/v) was applied to prevent fungal growth during the experiments. The seeds were examined daily and germination, taken to be when the radicle had reached a length of 5mm, was recorded. Following germination the embryos were removed from the pericarp and analysed for fatty acid composition.

7.2.2 Statistical Analysis

Regression lines were fitted to the data obtained from the germination experiments.

7.3 RESULTS

7.3.1 Detached Head Experiment

Preliminary experiments with detached heads revealed that heads grew better when placed in water rather than in water supplemented with minerals or sucrose (data not presented). Two detached heads were incubated at 21°C and three at 27°C. The increase in oil content of embryos from each head is presented in Table 7.1. The fatty acid composition of the oil from the five heads was determined (Table 7.2). The oil from the two heads incubated at 21°C had linoleic acid percentages of 63.2 and 75.7, whilst the three heads grown at 27°C had 42.6%, 31.5% and 11.5% linoleic acid in their oil.

7.3.2 Germination at 4°C

Preliminary experiments were carried out to determine the change in the fatty acid composition of the oil of sunflower seeds germinating and growing at 4°C. It was found that the linoleic acid percentage of the oil was altered only slightly (less than 1%) by germination and growth for up to twelve days at 4°C.

The results from the two germination experiments are presented in Figure 7.1. To test whether a relationship existed between linoleic acid percentage and the number of days to germination, an attempt was made to fit

TABLE 7.1

The increase in oil content of embryos from detached heads grown at two temperatures

Incubation Temperature °C	Head	Increase in Oil Content mg/10 embryos
21	A	73.6
	B	55.8
27	C	52.2
	D	58.9
	E	84.4

TABLE 7.2

The fatty acid composition of embryos from detached heads grown at 21°C or 27°C

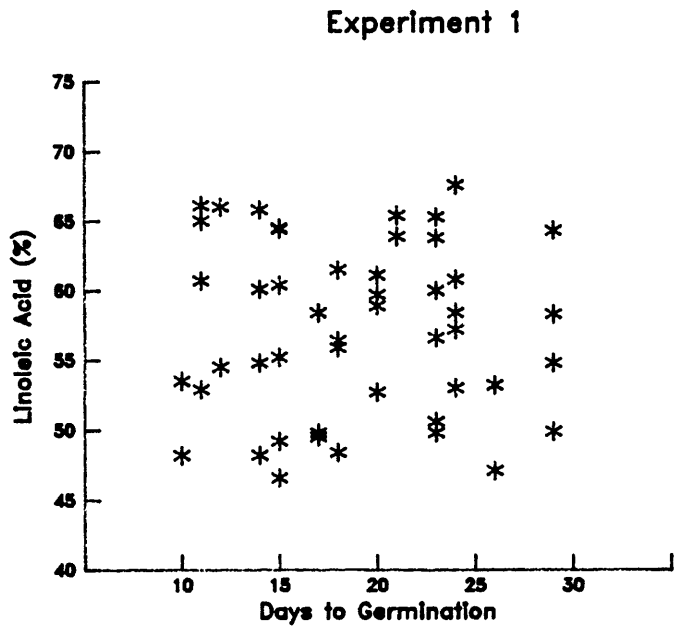
Temperature °C	Head	Fatty Acid Composition (% t.f.a.)			
		Palmitc	Stearic	Oleic	Linoleic
21	A	4.6	4.3	27.9	63.2
	B	5.7	1.3	17.3	75.7
27	C	3.7	2.3	62.5	31.5
	D	4.9	2.4	50.1	42.6
	E	2.5	3.5	82.5	11.5

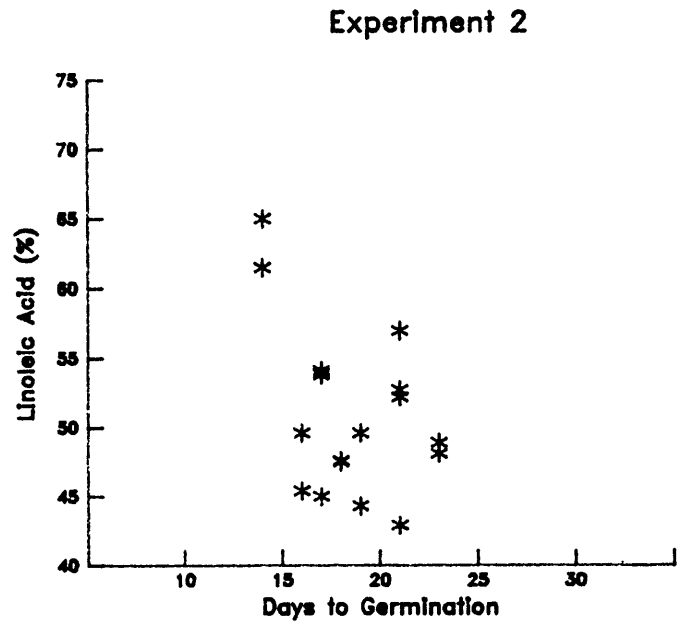
FIGURE 7.1

The relationship of linoleic acid percentage of embryos to the number of days needed to reach germination.

a) Experiment 1

b) Experiment 2


Experiment 1
Linoleic Acid (%)
75
70
65
60
55
50
45
40
10
15
20
25
30
Days to Germination


Experiment 2
Linoleic Acid (%)
75
70
65
60
55
50
45
40
10
15
20
25
30
Days to Germination

regression lines to the data but these lines could only account for a small percentage of the variation in the data (r^2 = 0.015, 0.156).

7.4 DISCUSSION

7.4.1 Detached Head Experiment

The detached heads grew well in water, with no sign of wilting up to a heat sum of 160 day-degrees. The increase in oil content of embryos from the detached heads indicates that growth was satisfactory (Table 7.1). The fatty acid composition of the oil from the heads at the two temperatures showed the expected trends. Head E, which had the greatest increase in oil content also had the lowest linoleic acid percentage of the heads grown at 27°C. The oil from the heads grown at the lower temperature had a higher linoleic acid content (63.2 and 75.7) than the oil from the heads grown at 27°C (11.5 - 42.6% linoleic acid). Individual heads at 27°C gave varied responses to temperature (Table 7.2) again reflecting the variability of the response of individual sunflower genotypes of the cultivar Sunfola to temperature (see Chapter 5).

Experiments with detached heads involved less time to set up than a typical culture experiment, but the stems of the sunflower heads had to be cut each day, otherwise wilting occurred, presumably due to blockages of the vascular bundles in the stem. A second disadvantage of the method is that seed from one head cannot be tested over a range of temperatures. Further, seed cannot be left to mature in the head to provide germinable seed for further generations.

However, the use of detached heads may have some application in testing the response of fatty acid composition of inbred lines to temperature. Here the variability between plants should be limited due to reduced heterozygosity and the response of a line to high temperature could be determined by placing a few heads from each line under high temperature conditions. This method could be used for a first screening and then be followed by use of

the culturing technique for more rigorous testing of lines giving a favourable response. Thus the method may have some application but is less flexible than the embryo culture technique.

7.4.2 Germination at 4°C

Two experiments were carried out to determine whether a relationship existed between the rate of germination of a seed at 4°C and its fatty acid composition. It was thought that this would provide a rapid means of detecting seeds with a high linoleic acid content from a large population of seed that had been subjected to high temperature conditions during maturation. Unfortunately no such relationship could be established.

Downes and Tonnet (1982) postulated that seeds germinated earlier because of a higher linoleic acid content. It is possible that this earlier germination of seed may be due to some other factor that, similarly to fatty acid composition, is also affected by maturation temperature. Another possible explanation of the failure to observe a relationship between linoleic acid and germination could be the cultivars used. Downes and Tonnet (1982) used a cultivar that had been selected for early germination under cold conditions. This may have led to some inadvertent previous selection that may be related to high linoleic acid content. It would be interesting if some of the Downes and Tonnet material could be examined using the culturing technique to determine its response to a wide range of temperatures, but no such material could be obtained for this study.

Germination in seeds of the cultivar Sunfola commenced over a wide range of days. Some seeds commenced germination at ten days, while others did not start until twenty days. Because the initiation of germination is such a complex event (Mayer and Marbach, 1981), other factors besides the linoleic acid percentage of the seed oil play an important role. There are, for example, strong indications that increased seed dormancy occurs when seeds mature under high temperatures (MacPherson pers. comm.). An alternative method could be to monitor the elongation of the radicle after

germination had occurred. This would overcome misleading results due to other factors influencing the time of germination.

Another approach to screening for high linoleic acid seed could involve the use of an oxygen electrode. Exploratory experiments were carried out in which segments of cotyledonary material were placed in an oxygen electrode and the rate of O_2 disappearance was recorded. The rate of O_2 disappearance was greater when embryo tissue from seeds with high linoleic acid contents was used than when tissue from seeds with low linoleic acid was used. Whether this difference is due to different rates of oxygen consumption by the tissues and hence a different rate of metabolism, or due to the action of a linoleate lipoxidase present that is liberated when the tissue is cut, remains unclear. However, it does offer the possibility of a simple system for detecting seeds with high linoleic acid contents without a long wet chemistry procedure. Unfortunately time was not available in the current project to pursue this line of investigation.

CHAPTER 8. INTEGRATING DISCUSSION

The overall aim of the experiments in this thesis were to examine the occurrence of sunflower genotypes with the ability to produce oil with a high level of linoleic acid and also to examine methods that could be used to aid in the selection of such genotypes.

The use of embryo culture as a technique to aid the selection of the desired genotypes had been proposed (Section 2.6). In order to define the time of seed development most suitable for the culture of embryos the phases in the development of the sunflower embryo and the effect of periods of high temperature during seed development on the final acid composition of the oil, were examined. Rapid oil accumulation was found to occur between 200 and 500 day-degrees after pollination in the developing sunflower embryo (Figure 3.1b). It had been hypothesised that it would be this rapid phase of oil accumulation that would be most sensitive to temperature, simply because this is when most of the oil is being synthesised. This idea has been partially substantiated by the results of the high temperature exposure experiment. Treatments which experienced high temperature during the 300 to 500 day-degree period differed significantly from the low temperature control (Table 3.2). In treatments that experienced high temperature for part of this period there was the suggestion of an effect of temperature on the final fatty acid composition of the seed.

The results of the experiments in Chapter three indicated that the ideal time to take sunflower embryos for culturing was between 300 and 500 day-degrees after pollination. At this stage of development the embryos would only have just commenced the phase of rapid oil accumulation, so there would not be a large amount of oil already present to mask subsequent changes in fatty acid composition. Also at this stage of development the embryo appears to be most sensitive to the influence of temperature.

Removing isolated seeds from sunflower heads at 300 day-degrees after pollination and placing them in culture medium allowed growth and oil syn-

thesis in the intact embryo. Although the rate of growth was slower than the growth of embryos remaining in the head, the composition of the oil of the 'cultured' embryos was normal consisting predominantly of the storage lipid triacylglycerol. The response of the fatty acid composition of the oil from 'cultured' embryos to temperature was as expected, with linoleic acid percentage decreasing with increasing temperatures (Figure 4.1). In electron microscope studies the ultrastructural detail of tissue from 'cultured' embryos was similar to that of embryos grown on the head. The technique of isolated seed culture appears to allow normal growth of the sunflower embryo in a way in which the embryo can be easily subjected to differing external influences, especially temperature.

The need to select for a sunflower genotype with stable high linoleic acid production has been discussed previously (Section 2.6). To assess whether sufficient variability existed in sunflower to make this selection, a population of the open pollinated cultivar Sunfola 68 was grown under high temperature conditions at Narrabri, N.S.W. There was marked variation in the levels of linoleic and oleic acids between composite samples from Narrabri heads and also between individual embryos (or kernels) from within these heads (Table 5.1). This variation in fatty acid composition could be partially due to environmental conditions but was primarily due to genotype. This genotypic effect was confirmed by examining the differences in fatty acid composition between individual embryos from cultured seeds from within a single head (Table 5.2). Again a marked variation in linoleic and oleic acid was found.

Since these results show that genetic variability exists in the sunflower, considerable scope appears to exist for the selection of a sunflower genotype with the capacity to produce high levels of linoleic acid over a wide range of growing temperatures. The culturing technique allows embryos from a single head to be grown over a range of temperatures. Therefore the technique offers a tool to enable such selections to be

carried out.

To test the effectiveness of the technique it was used to study the temperature response of four inbred lines and the hybrids formed from these lines and a restorer line (Figure 6.1 and 6.2). These inbred lines (and hybrids) all showed a decrease in linoleic acid with increasing incubation temperature. Although the slope of the response of individual inbreds to temperature was significantly different, indicating that genetic variability exists even in a population that had not been selected for temperature response, the sensitivity of all lines was such that these differences appear to have little or no biological significance.

The culturing technique has been successfully used to examine the temperature response of inbred lines and hybrids, confirming its use as a tool to facilitate the selection for temperature stable sunflower genotypes. Although the technique is fairly involved with the preparation of culture media and of aseptic plant material, it still has a place in a breeding programme, since it enables the embryos of a single head to be exposed to a wide range of temperatures. This is particularly useful for the small numbers of seeds that are normally available in breeding programmes.

Ideally the culturing technique would be used coupled to a more rapid screening method. Early germination at low temperature (Downes and Tonnet, 1982) and oxygen consumption of embryo tissue have been proposed as possible rapid screening methods (Section 7.4). Such methods would allow large numbers of seeds to be assessed rapidly and the more promising of these could then be examined in more detail using the culturing technique.

The required variability appears to exist in sunflower to select for temperature-stable linoleic acid synthesis. Attempts to select a genotype with such a trait was not successful. Selections were hampered by low self-fertility in the open pollinated cultivar Sunfola 68, which

made the yields of seed from selfed heads very low. Maturation of the selfed heads under high temperature conditions also resulted in seed with low germinability. This effect has also been noted in other cultivars matured under high temperature conditions (MacPherson pes. comm.). To successfully select the desired sunflower genotype from Sunfola 68 an intensive breeding programme would be required that could solve the problems of low self-compatibility and germinability. But even with these difficulties, initial selections have shown that it is a feasible objective to attempt to select a sunflower genotype with the capacity to synthesise high levels of linoleic acid over a wide range of temperatures.

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APPENDIX 3.1

OPERATIONAL SETTINGS OF THE GAS-LIQUID CHROMATOGRAPH

Temperature Settings

	Temperature °C
Inlet	210
Column oven	175
Detector oven	220
Outlet	230

Gas Flow Rates

Gas	Flow Rate (ml $min.^{-1}$)
Air	400
Nitrogen (carrier)	40
Hydrogen	60

APPENDIX 3.2

CALCULATION OF FATTY ACID WEIGHTS

This method of calculation only provides a reasonable estimate of fatty acid weight. The method of choice would have been by use of an internal standard such as a C17 fatty acid but as this was not possible weights were calculated as set out below.

1) Triacylglycerol content was taken as >99% of the oil, to simpify calculations.

2) Calculate average molecular weight of a fatty acid in a TAG molecule.

for example GLC trace gives C16:0 10%, C18:0 10%, C18:1 40% and C18:2 40%

$$\begin{aligned} AMW = {} & 10\% \times MW\ C18{:}0 = 10\% \times 239 = 23.9 \\ & + 10\% \times MW\ C18{:}0 = 10\% \times 267 = 26.7 \\ & + 40\% \times MW\ C18{:}1 = 40\% \times 265 = 106.0 \\ & + 40\% \times MW\ C18{:}2 = 40\% \times 263 = \underline{105.2} \\ & = \underline{261.8}\ g\ mole^{-1} \end{aligned}$$

3) Calculate MW of average TAG.

for example

$$\begin{aligned} MW\ of\ average\ TAG &= 3 \times AMW\ of\ fatty\ acid\ (as\ calculated\ above) \\ &= + MW\ of\ glycerol \\ &= 261.8 \times 3 + 89 \\ &= 874.4\ g\ mole^{-1} \end{aligned}$$

4) Calculate fatty acid weights from oil content

for example if oil content = 150mg

$$\text{then moles of TAG} = \frac{0.150}{874.4} = 0.17\text{mmole}$$

$$\text{then weight of glycerol} = 89 \times 0.17 \times 10^{-3} = 0.015\text{g}$$

therefore the weight of fatty acids = 0.150 - 0.015 = 0.135g

and the weight of individual fatty acids is

C16.0 = 10% x 0.135g = 13.5mg

C18:0 = 10% x 0.135g = 13.5mg

C18:1 = 40% x 0.135g = 54.0mg

C18:2 = 40% x 0.135g = 54.0mg

The GLC trace provides fatty acid results in % by weight and should be converted to moles % before a calculation like the one outlined above, but with only C16 and C18 fatty acids involved such a calculation would have made very little difference.

APPENDIX 4.1

PREPARATION OF STOCK SOLUTIONS FOR CULTURE MEDIA

Prestock Solutions

Solutions with the following concentrations were prepared

Prestock (P.S.) Number	Constituent	Concentration (mM)
1	$FeSO_4$	10
	Na EDTA	10
2	H_3BO_3	10
3	$ZnSO_4$	1.0
4	$CuSO_4$	1.0
5	$CuSO_4$	0.01
6	Na_2MoO_4	1.0
7	Na_2MoO_4	0.01
8	$CoCl_2$	1.0
9	KI	0.5
10	Nicotinic Acid	8.0
	Pyridoxine HCl	1.2
11	Thiamine	8.0
12	Biotin	0.2
13	D-Ca-Pantothenate	1.0
14	Ascorbic Acid	0.2
	Choline Chloride	0.2
	Glycine	1.0

Preparation of Culture Stocks

1) Preparation of 100X Mineral Stock

The following constituents were required to make 1 litre volumes

Stock Number	Constituents	Amount of Constituents Low	High
1	NH_4NO_3	40.025 g	80.050 g
	KNO_3	-	101.110 g
	$MgSO_4.7H_2O$	12.325 g	36.975 g
2	KH_2PO_4	1.3609g	-
	$NaH_2\ PO_4.2H_2O$	-	15.601 g
3	KCl	14.1664g	-
	$CaCl_2$	2.4404g	5.8808g
4	$FeSO_4/Na_2EDTA$	100ml P.S.1	500ml P.S.1
	Na_2SO_4	568.2mg	6.3923g
5	H_3BO_3	100ml P.S.2	309.2mg
	$MnSO_4.H_2O$	169.02mg	845.1mg
	$ZnSO_4.7H_2O$	100ml P.S.3	575.12mg
	$CuSO_4$	100ml P.S.5	10ml P.S.4
	Na_2MoO_4	100ml P.S.7	10ml P.S.6
	$CoCl_2$	10ml P.S.8	50ml P.S.8
	KI	100ml P.S.9	41.51mg

2) Preparation of 10X Growth Factors and Amino Acid Stock

The following constituents were dissolved in one litre of water

Constituents	Amount of Constituents
Inositol	1.0810g
Nicotinic Acid	50ml P.S.10
Pyridoxine HCl	
Thiamine HCl	50ml P.S.11
Biotin	50ml P.S.12
D-Ca-Pantothenate	50ml P.S.13
Ascorbic Acid	
Choline Chloride	500ml P.S.14
L-cysteine HCl	189.16mg

3) Preparation of 10X 6-auxin mix

6-auxin mix contained the following constituent

Constituents	Concentration (μM)
Indole Butyric Acid	10
Indole Acetic Acid	10
α-Naphthoxy Acetic Acid	10
α-Naphthalene Acetic Acid	10
$2_1$4-Dichlorophenoxy Acetic Acid	10
para-Chlorophenoxy Acetic Acid	10

All prestock and stock solutions were stored in a freezer (-18°C) and thawed when media were prepared.