Figure 2. Effect of accumultated heat units on the average linoleic and oleic acid contents of five sunflower genotypes.

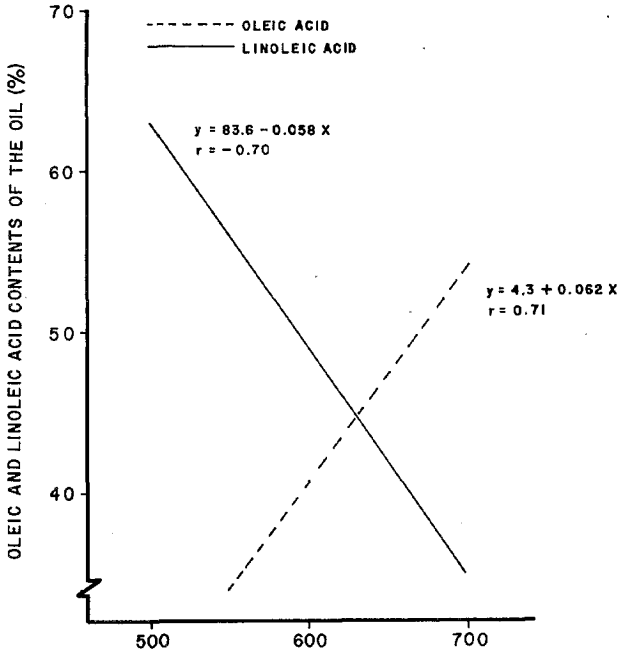
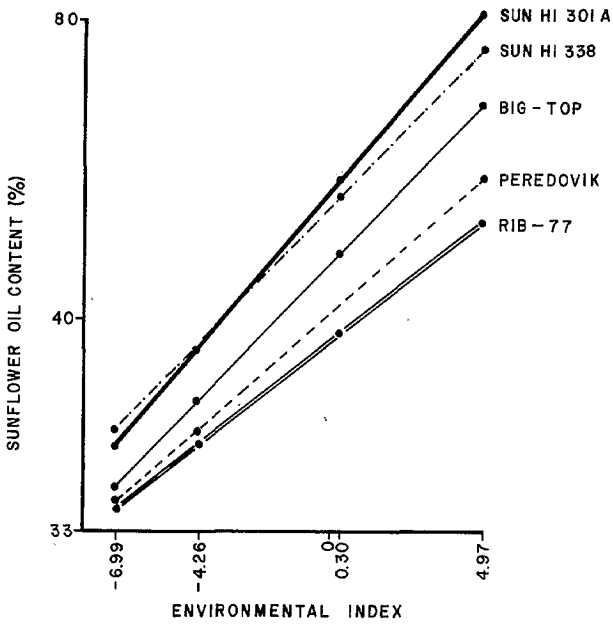


Figure 3. Performance of five sunflower genotypes in oil content.



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A TECHNIQUE FOR SELECTING SUNFLOWER GENOTYPES WITH TEMPERATURE-STABLE LINOLEIC ACID SYNTHESIS.

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ABSTRACT

High temperatures during seed development reduce the linoleic acid content of sunflower oil. An approach to this problem has been made using a new technique to investigate the effect of temperature on the *in vivo* synthesis of linoleic acid in sunflower cypselae (seeds). The technique involves growing developing seeds in agar medium containing nutrients and hormones. Seeds were taken from sunflower heads fourteen days after pollination and grown in the agar medium. After five days the oil content and dry weight of the embryos had doubled. The oil and storage tissue produced by embryos grown under these conditions appeared completely normal. The oil from these embryos was identical in composition to that from embryos taken from intact inflorescences. This technique is being used to screen sunflower genotypes for the capacity to synthesise high levels of linoleic acid over the range of temperatures experienced in sunflower-growing areas of Australia.

INTRODUCTION

Much of the sunflower oil produced in Australia is low in linoleic acid and does not meet the statutory level of 62% required for use in polyunsaturated products. The cause of this reduced level appears to be high temperature during seed development which depresses linoleic acid synthesis. Evidence from controlled temperature experiments indicates an inverse relationship between temperature during seed development and the degree of unsaturation of the fatty acids (Canvin, 1965; Harris *et al.*, 1978). This relationship has also been demonstrated in the field (Keefer *et al.*, 1976; Harris *et al.*, 1978; Goyne *et al.*, 1979).

In sunflowers, oil composition is under genetic control although there is also a strong environmental component (Putt *et al.*, 1969; Downey and McGregor, 1975). Selection has produced genotypes with the potential for higher linoleic acid levels, but these are thought to be no more stable to high temperature than standard genotypes. To guarantee the production high levels of linoleic acid in sunflower oil it will be necessary to select genotypes that are capable of high linoleic production at high temperature and thus will require an efficient screening technique. Because it is difficult to screen plants under uncontrolled temperature conditions in the field, a more reliable system involving the control of temperatures during seed development will be required. This is necessary because most of the synthesis of oil in developing sunflower seeds occurs over a 10 — 12 day period commencing about ten days after anthesis (Harris *et al.*, 1978). Also, virtually all the oil is contained in the axis and cotyledons of the developing embryo. If cypsellae (seeds) or naked embryos could be successfully grown *in vitro* during this phase of active oil aynthesis it should be possible to grow large numbers of embryos, representing individual genotypes, at an elevated temperature and, using a rapid oil assay, select those

that have the capacity to synthesize high levels of linoleic acid.

The study reported here was undertaken to develop an *in vitro* technique for culturing sunflower embryos during the stage of rapid oil accumulation to:
(a) study linoleic acid biosynthesis in the developing embryo.
(b) screen sunflower germplasm to identify genotypes with the ability to synthesize oil with high levels of linoleic acid at elevated temperatures comparable to those experienced during the seed development phase in the field.
(Work on the biosynthesis of linoleic acid is being undertaken in a co-operative programme by Dr. D.G. Bishop in the Division of Food Research, C.S.I.R.O., and will not be reported on in this paper.)

MATERIALS AND METHODS

Developing seeds used in the experiments were obtained from Sunfola 68-3 plants grown in ten litre pots in a heated glasshouse with daily maximum temperatures in the range of 25 — 35°C. Preliminary experiments were carried out to:
(a) identify the best stage of development at which to select seed for culture and to span the stage of rapid oil synthesis,
(b) determine the best way to remove and prepare seeds for growing in culture,
(c) devise the most suitable culture medium.

As a result of these experiments, the following procedure was adopted. Developing seeds were taken from the head 14 days (approx. 300 — 350 day-degrees) after pollination. The relevant segments of the inflorescence were sterilised in sodium hypochlorite and, using aseptic conditions, individual seeds were removed with a core of soft spongy inflorescence tissue. This contained vascular connections which provide a larger surface area for the uptake of water and nutrients from the culture medium. The developing seeds, plus supporting tissue, were placed in 10 ml of sterile culture medium containing minerals, sucrose, indole butyric acid (IBA) and growth factors (see Table 1) and incubated for five days in the dark at 22°C or for seven days in a range of temperatures from 15 — 27°C. Cultured seeds were compared at the end of the incubation period with seeds of equal age which were left to develop in the intact portion of the inflorescence (control seed).

Table 1. Constituents of culture medium.

Macronutrients (nM)	NH_4NO_3(10); KNO_3(10); NaH_2PO_4(1); $CaCl_2$(2); $MgSO_4$(1.5).
Micronutrients (μM)	H_3BO_3(50; $MnSO_4$(50); $ZnSO_4$(20); $CuSO_4$(0.1); $NaMoO_4$(0.1); $CoCl_2$(0.5); KI(2.5); $FeSO_4$(50); Na_2SO_4(450).
Growth Factors (μM)	Inositol (600); nicotinic acid (40); pyridoxine. HCl (6); thiamine HCl (40); biotin (1); D-Ca-pantothenate (5); ascorbic acid (10); choline chloride (10).
Amino Acids (μM)	L-cystein. HCl (120); glycine (50).
Hormone (μM)	Indole butyric acid (1.0).
Carbon Source	Sucrose (4%).
Agar	Difco-Bacto (0.8%).
	pH adjusted to 5.5

Total oil was extracted from developing embryos of both control and 'cultured' seed using a Folsch extraction (Folsch *et al.*, 1957) and the composition of the oil was determined using thin-layer chromatography. Fatty acid composition was determined using gas liquid chromatography. (Morrison and Smith, 1964; Bishop and Smillie, 1970).

The ultrastructural details of the storage tissue in the cotyledons of the control and 'cultured' embryos were examined using transmission electron microscopy. The tissue was fixed in gluteraldehyde, post-fixed in osmium tetroxide and embedded in Spurr's standard A resin (Spurr, 1969). Ultra-thin sections were counterstained with uranyl acetate and lead citrate (Reynolds, 1963) before examination.

RESULTS AND DISCUSSION

In preliminary experiments the most suitable material for use in the culture system was found to be intact seeds with vascular connections to a section of spongy head tissue. When samples of this type were incubated, embryos of all ages up to twenty days grew at approximately 70 — 80% of the rate of embryos grown on the head (control). Naked embryos were found to be unsuitable as they exhibited precocious germination, even when grown in total darkness. Embryos within intact seeds placed directly on the culture medium grew poorly. While the reason for this has not been closely examined, it is suggested that the vascular connection which crosses the otherwise impermeable pericarp did not permit sufficient uptake of nutrients and growth factors from the medium. Alternatively, it is possible that the elements of the vascular tissue of this fine connection may have been readily blocked, preventing uptake. The use of supporting spongy head tissue containing vascular tissue appears to have increased uptake as a result of the larger surface area of vascular tissue available to promote uptake.

The composition of the culture medium found to give the best growth of embryos is shown in Table 1. Tests showed that growth was not enhanced by higher concentrations of sucrose. The growth factors (Table 1) and IBA were found to be necessary for maximum oil synthesis. While some growth was recorded when other hormones were used (cytokinen, IAA and other synthetic auxins) the best response was achieved with IBA.

Table 2. Comparison of dry weight, oil content and fatty acids (oleic and linoleic) of developing embryos of sunflower when grown for either 5 days in the inflorescence or as detached seeds in a culture medium. Values represent the mean of 30 embryos.

Treatment	Dry Weight mg/embryo	Oil Content mg/embryo	Fatty Acid (%) Oleic	Linoleic
Initial Sample (14 days)*	8.8 ± 1.8	2.5 ±0.96	39.5	45.5
Head (Control) (19 days)*	20.6 ± 5.6	6.2 ±3.3	45.5	44.3
Cultured (19 days)*	16.2 ± 4.8	5.15 ±1.8	29.3	62.5

*days after pollination.

Although embryos of all ages grew well in the medium those at 14 days after pollination were used in studies of oil composition as the most rapid period of oil synthesis begins at this time (Harris *et al.*, 1978). The dry weight increase and oil content and composition of 'cultured' embryos are compared with head grown (control) embryos in Table 2. After five days' incubation in the culture medium, the dry weight had doubled and had reached 80% of the level of the control embryos. The analyses of oil content and composition indicate that, although the quantity of oil produced by 'cultured' embryos was somewhat less than that of the control, the composition was normal. In all cases, thin-layer chromatography revealed that the composition of extracted oil was predominantly triacylglycerol, with some polar lipid and traces of diacylglycerol. Fatty acid composition of cultured embryonic tissue was within the expected range (Table 2). The difference in the ratio of oleic to linoleic acid between the two groups of embryos possibly resulted from difference in the temperature regime in which they were grown. The 'cultured' embryos were maintained at a constant temperature of 22°C whereas those which developed in the head were taken from plants grown in a semi-controlled glasshouse in which daily mean temperatures fluctuated from 19°C to 29°C (mean 23°C). The decrease in linoleic acid percentage of 'cultured' embryos with increasing incubation temperature is shown in Table 3. Constant temperature above 21°C caused a marked reduction in linoleic acid and a corresponding increase in oleic adic. These results are similar to those of Canvin (1965) and Harris *et al.*, (1978).

Table 3. Fatty acid composition of embryos 'cultured' at different temperatures. Values represent the mean of 8 embryos.

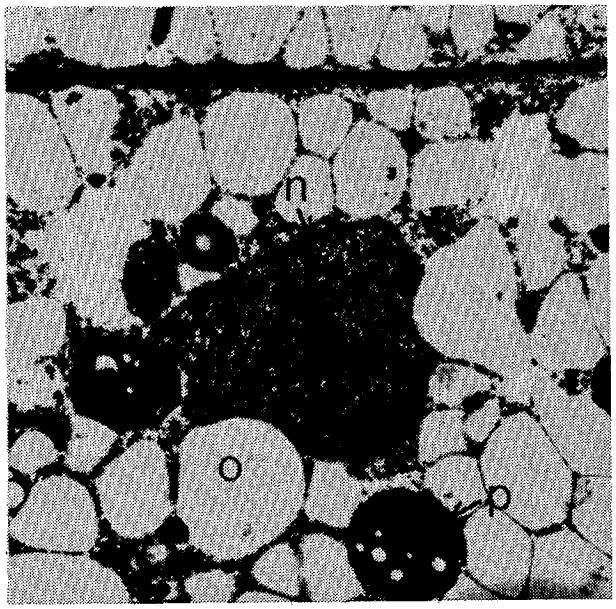
Incubation Temperature °C	Fatty Acid Composition (%)			
	Palmitic	Stearic	Oleic	Linoleic
15	4.0	3.1	14.4	78.6
18	5.0	6.2	18.8	70.7
21	3.6	5.2	29.3	62.0
24	5.4	4.6	38.2	51.9
27	6.5	4.4	52.3	36.9

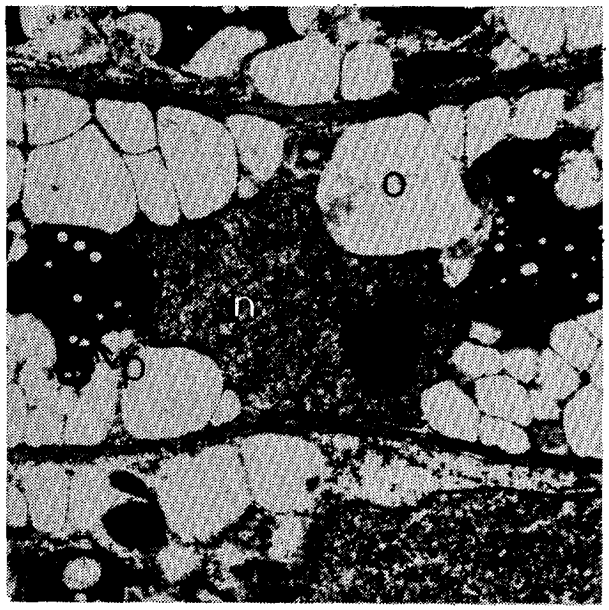
Ultrastructural studies of both control and 'cultured' embryos showed 'cultured' embryos to be essentially normal (Plates 1 and 2), although there is some suggestion of more protein bodies in the 'cultured' embryos. Further studies are being carried out to determine whether this difference is real, or whether it is an artefact of the preparation of these sections.

Plate 1. Control (head grown) embryo tissue.
n — nucleus, o — oil body, p — protein body.

Plate 2. 'Cultured' embryo tissue.
O — oil body, p — protein body.

(Magnification x 2000)





CONCLUSION

It has proved possible to use small numbers of seeds to carry out the analyses of oil composition using this technique. This is of particular importance to breeding programmes where usually only small quantities of seed on new genotypes are available. By taking seed from only part of a head for testing, it would be possible to allow the remainder of the head to mature and to be used for further breeding work.

Similarly, it has proved feasible to grow material in a range of temperatures to simulate the range of field environments which occur in sunflower-growing areas. This should provide an effective means of assessing the temperature response of genotypes for both the identification of high temperature stability and for the testing of subsequent material from breeding programmes.

ACKNOWLEDGEMENTS

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WATER REQUIREMENT OF SUNFLOWER IN A SEMI-ARID ENVIRONMENT.

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ABSTRACT

In Australia much of the sunflower (*Helianthus annuus* L.) production occurs under rainfed conditions; most crops experience moisture stress at some stage of development. An understanding of crop water use patterns is necessary to devise production strategies which minimize stress. Sunflower cultivars, Suncross 150 (early), 51 (mid) and 52 (full season), were grown on an alluvial grey cracking clay soil at Narrabri, N.S.W., during 1979/80 and 1980/81. Furrow irrigation supplemented rainfall, allowing a range of soil moisture availabilities to be imposed from pre-anthesis, anthesis and mid-seed development to physiological maturity. Total crop water use ranged from 246 to 957 mm, consumption increasing with maturity type. Peak daily water use was 1.2 times Class A pan evaporation but declined after ≅ 30 percent of extractable soil water had been used. All cultivars under rainfed conditions extracted water to a depth of greater than 1.8 metres and dried the soil to the same water content. Crops sown in early summer were unable to dry the soil to the same moisture contents as either early spring or late summer-sown crops. These results are relevant to the management of rainfed sunflower crops.

INTRODUCTION

In Australia the major area of oilseed sunflower (*Helianthus annuus* L.) is grown under rainfed conditions in the semi-arid regions. This area is characterised by high temperatures, high radiation, high vapour pressure deficits and high rainfall variability, conditions which enhance the probability that periods of low soil moisture availability will occur. The successful growth of a crop species in the semi-arid regions is dependent upon the total water supply from both fallow storage and rainfall during crop growth, the pattern of utilization of this water during growth, the response of the crop to moisture deficits at different growth stages and the efficiency with which water is utilized in the development of yield. Species or cultivars capable of stress avoidance by establishment of a deep root system, rapid phasic development and restricted water usage under conditions of limited soil water supply are considered to have the best adaptation to the conditions of erratic moisture supply and terminal drought which characterizes many semi-arid environments (Turner, 1981). Manipulation of the time of sowing so that the period of maximum daily water usage coincides with an interval of reduced evaporative demand represents a management strategy which can contribute to stress avoidance.

Several studies have examined the water requirement of the sunflower crop. Estimates of evapotranspiration range from 150 mm for moisture-limited rainfed crops (Alessi *et al.*, 1977; Anderson, 1979) to 930 mm for a crop grown under moisture non-limiting conditions (Muriel *et al.*, 1974). Total evapotranspiration for irrigated crops is usually between 650 and 800 mm (Talha and Osman, 1975; Browne, 1977; Unger, 1978). Daily evapotranspiration is closely related to plant growth (Paltineanu and Sipos, 1974; Anderson, 1979) with maximum rates up to 1.3 times Class A pan evaporation at maximum leaf area (Anderson, 1979). The timing of the onset of conditions which restrict water use is closely related to the size of the soil moisture reserves and the pattern of their replenishment. Extractable soil moisture is dependent upon soil type and depth of rooting of the species. Measured depths of soil utilized by the sunflower range from 1.5 metres (Browne, 1977) to greater than 2.5 metres (Unger, 1978) on deep clay soils.

The work reported in this paper was designed to examine the effect of cultivar maturity type on a) to total water use, b) the pattern of water use during crop development and c) the ability to utilize soil water reserves under conditions of varying moisture supply and evaporative demand.

MATERIALS AND METHODS

The experimental work was conducted on the New South Wales Department of Agriculture Research Station at Narrabri in north-western N.S.W. Plots of the sunflower hybrids, Suncross 150 (early maturity), Suncross 51 (mid maturity) and Suncross 52 (late maturity), were sown and irrigated as shown in Table 1. Where appropriate, furrow irrigation was used to prevent Extractable Soil Water (ESW) from declining below 40 percent of maximum storage in the root zone. In the spring 1979 sowing, additional treatments were managed to induce severe stress from either the pre-anthesis, anthesis, or seed fill phase of development to physiological maturity. Detailed description of the experimental treatments, phasic development and ESW and climatic conditions during growth have been presented by Harris *et al.*, (1982).