MATURITY AND SEED QUALITY IN SUNFLOWER: PHYSIOBIOCHEMICAL ASSESSMENT THROUGH ACCELERATED AGEING.

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ABSTRACT

Physiological maturity (PM) in a dwarf cultivar (Modern) and a tall cultivar (EC-68414) of sunflower was found to be attained 30 and 36 days respectively after 50 to 60% completion of anthesis. Though seedling dry weight and yield per plant did not vary when plants were raised from such seeds after storage for six months with that of harvest-mature seeds, their germinability and rate of germination were somewhat low. Under accelerated ageing, germinability and rate of germination of PM seeds declined earlier in comparison to harvest-mature seeds. Activities of hydrolysing and oxidising enzymes as well as of dehydrogenase activity increased more during early days of accelerated ageing in harvest-mature seeds. On the other hand decrease of phospholipid content and increased lipid perodixation were noted earlier in PM seeds undergoing accelerated ageing. Incorporation of ^{32}P in phospholipid and nucleic acids during germation was also less in PM seeds when compared with harvest-mature seeds.

INTRODUCTION

At the time of physiological maturity (PM) sunflower seeds attain maximum dry weight, oil content and oil quality (Anderson, 1975; Brown, 1978). In spite of high moisture content at PM, harvesting of seeds is recommended to avoid biotic and climatic risks and also for the loss of oil quality (Robertson *et al.*, 1978; Gambhir and Anand, 1981). PM seeds however pass through a desiccation phase prior to harvest maturity (HM). Hence harvesting at PM might affect their programmed maturation process. Anderson (1975) proposed that for commercial harvesting, complete development of the embryo in the seed as judged by its viability is necessary. Therefore, keeping in view the aspects of germinability and viability, the present investigation was undertaken to compare the physiology and biochemistry of seeds harvested at both PM and HM stages.

MATERIALS AND METHODS

Capitulae of sunflower cultivars (*Helianthus annuus* L. *cv.* Modern and EC-68414) which initiated flowering on the same day were tagged when 60% of the florets had undergone anthesis. Sampling of heads (capitulae) was started 18 days after flowering (DAF) and continued at intervals of 6 days starting from the first week of January to the end of February (1979 and 1980) until 48 DAF. Ten heads were harvested at each date and the seeds were taken from the outer 6 rings for analysis.

Moisture content and 100-seed weight were measured after drying the harvested samples at 70°C for 72 hours. Ten g of seed were taken from each harvest and analysed for oil content extracted using Soxhlet apparatus. The attainment of maximum seed dry weight was determined following the 6 x 100 seed dry weight and the stage of physiological maturity (PM) was selected as one which did not differ significantly from the harvest mature samples (HM) of 48 DAF.

Sun dried seed lots (moisture content about 7%) from the PM and HM samples were stored in airtight containers for 6 months. Initially tests for germination (ISTA, 1976) and the rate of germination (Kotowski, 1926) of stored seeds were done. Dry weight of 7 day-old seedlings was taken after drying at 70°C.

For accelerated ageing of PM and HM seed lots, methods described by Gelmond *et al.*, (1978) were followed. Percentage and rate of germination was recorded at 0, 4, 8 and 12 days of accelerated ageing. From the same seed lots, electrolyte content of leachate was recorded by conductivity test after 8 hours of soaking.

Amylase and protease activities were measured following Punjabi and Basu (1978) and Biswas and Choudhuri (1978) respectively. For lipase, enzyme preparation was made following Longenecker and Haley (1935) and estimation were assayed according to Biswas and Choudhuri (1978) and Kar and Mishra (1976). Dehydrogenase activity of the aged seeds was measured following Rudrapal and Basu (1979).

The extraction of phospholipid from the embryo axes was done following Beutalmann and Kende (1977) and phospholipid phosphorus was estimated by the methods of Jaffe and Galston (1966). Malondialdehyde (1, 3-propandial), a peroxidation product, was measured from the embryo axes by the methods of Stewart and Bewley (1980).

For analysing ^{32}P incorporation, embryo axes from PM and HM seeds were asceptically separated from each set and were soaked for 12 hours in citrate buffer (pH 6.5) labelled with ^{32}P (^{32}P-orthophosphoric acid, specific activity 5.4 mci/ml) at 25°C. Phospholipids and nucleic acids were extracted from the ^{32}P treated embryo axes following Beutalmann and Kende (1977) and Cherry (1962) respectively. The radioactivity was measured in GM counter.

Each experiment was replicated six times in two consecutive years and the average data were statistically analysed (Duncan, 1955).

RESULTS

At 18 DAF the moisture content was 54 per cent in Modern and 62 per cent in EC-68414. A gradual decline in moisture content was noted thereafter to 15 per cent in Modern and 19 per cent in EC-68414 at 48 DAF. Maximum seed dry matter occurred at 30 DAF in Modern and at 36 DAF in EC-68414. No significant increase in seed weight occurred to 48 DAF hence the samples taken on days 30 and 36 were considered to be physiologically mature (PM). At 48 DAF the capitulae were completely brown and seed harvested at this stage were considered as harvest mature (HM). The oil content reached its plateau 6 days prior to PM in both cultivars (Table 1).

Table 1. Moisture content, dry weight and oil content of seeds of two sunflower cultivars harvested at different days after flowering (DAF)[a].

Cultivars	DAF	Moisture content (%)	100 seed dry weight (g)	Oil content (%)
Modern	18	54ef	4.3a	34.2a
	24	50e	5.2b	40.8bc
	30	42d	6.5cd	40.1b
	36	27b	6.1c	40.3b
	42	18a	6.3c	40.6b
	48	15a	6.2c	40.2b
EC-68414	18	62g	4.0a	32.3a
	24	57fg	5.9bc	38.8b
	30	53e	7.1d	43.2c
	36	44d	8.2e	43.0c
	42	32b	8.4e	42.6c
	48	19a	8.3e	42.4c

a Means in a vertical column followed by the same letter do not differ significantly at the 5% level (Duncan's Multiple Range Test).

Germination percent and the rate of germination were low in PM seeds of both the cultivars. In PM seeds this was 83 and 86 per cent in Modern and in EC-68414 as against 100 and 98 per cent in HM seeds of the two cultivars. Dry weight of seedlings grown from PM and HM seeds as well as yield from such seeds were similar (Table 2).

Table 2. Germination behaviour, seedling dry weight and field performance of physiologically mature (PM) and harvest mature (HM) seeds.[a]

Cultivars	Seed Type	Germination	Rate of germination (%)	Seedling dry weight (g)	Yield/ Plant (g)
Modern	PM	93a	49.7a	69a	48.4a
	HM	100b	59.2b	73a	49.8ab
EC-68414	PM	86a	52.6a	63a	52.7bc
	HM	98b	57.9b	68a	54.2c

a Means in a vertical column followed by the same letter do not differ significantly at the 5% level (Duncan's Multiple Range Test).

Germination percentage and the rate of germination decreased significantly with accelerated ageing at 8 days in PM seeds but at 12 days in HM seeds (Table 3). Leaching of electrolytes increased in PM seeds at 8 days and HM seeds at 12 days of ageing.

Table 3. Effect of accelerated ageing on germination, rate of germination and electrolyte leaching of PM and HM seeds of sunflower.a

Ageing days	Germination (%)		Rate of germination (%)		Electrolyte leaching (μMohs)	
	PM	HM	PM	HM	PM	HM
Modern						
0	83.7d	100.0b	50.2d	59.5b	317a	310a
4	85.2d	99.6b	51.9d	61.2b	323a	306a
8	74.5bc	96.4b	47.0c	58.9b	484c	322a
12	68.2b	79.2a	42.2b	49.4a	562d	496c
EC-68414						
0	87.4d	98.3b	52.9d	58.2b	393b	370ab
4	88.9d	99.0b	52.4d	60.4b	389b	383b
8	76.0c	95.6b	46.2c	57.7b	497c	389b
12	60.3a	72.0a	39.3a	47.8a	590d	550d

a Means in a vertical column followed by the same letter do not differ significantly at the 5% level (Duncan's Multiple Range Test).

Activities of amylase, protease and lipase increased at 4 days of ageing in both PM and HM seeds but the increase of protease activity was greater. These declined thereafter but the extent of decrease was more in PM seeds. Under ageing high catalase activity was maintained up to 8 days and then declined (RW18Table 4). Maximum activities of the enzymes peroxidase and dehydrogenese were noted after 4 days of ageing but again these declined with increase in ageing days. In all the cases, however, higher activity was noted in HM seeds.

Table 4. Effect of accelerated ageing of PM and HM seeds on activities of amylase (mg starch hydrolysed/ g/30 min.), protease (mg protein hydrolysed /g/hr), lipase (per cent hydrolysis of olive oil/g/hr), catalase (enzyme units/g/hr), peroxidase (enzyme units/g/hr) and dehydrogenase (OD of formazan extract).[a]

Ageing Days	Amylase		Protease		Lipase		Catalase		Peroxidase		Dehydrogenase	
	PM	HM	PM	HM	PM	HM	PM	HM	PM	HM	PM	HM
Modern												
0	61.7d	64.6e	46.6b	47.3bc	0.15c	0.17b	26.9b	28.3b	9.9b	11.6b	0.22bc	0.23b
4	62.6d	97.7f	106.0d	190.8f	0.19d	0.47c	53.4de	92.7cd	17.4e	30.4c	0.33f	0.49e
8	46.7c	56.7cd	56.4c	150.4d	0.09b	0.21b	48.9cd	88.5c	4.2a	8.2a	0.27de	0.29d
12	29.0b	36.8b	34.4a	36.2a	0.07ab	0.08a	21.3a	20.0a	4.2a	5.5a	0.18a	0.18a
EC-68414												
0	60.9d	62.4de	51.2bc	51.4c	0.18cd	0.19b	27.7b	29.6b	9.2b	9.4ab	0.25cd	0.25bc
4	63.2d	112.4g	110.4d	176.6e	0.24e	0.52b	57.7e	97.9d	15.8c	26.6c	0.38f	0.53f
8	44.2c	51.2c	47.2b	142.5d	0.07ab	0.17b	44.0c	92.4c	4.8a	7.7a	0.29e	0.27cd
12	22.3a	29.4a	37.9a	41.7ab	0.04a	0.06a	19.0a	21.5a	4.6a	4.4a	0.20ab	0.15a

a Means in a vertical column followed by the same letter do not differ significantly at the 5% level (Duncan's Multiple Range Test).

Phospholipid content of the embryo axes of PM seeds declined after 4 days but in HM after 8 days. Malondialdehyde content was more in the embryo axes of PM seeds at 8 days of ageing but in HM seeds an increase was noted at 12 days (Table 5). Embryo axes of 4-day aged PM and HM seeds showed maximum incorporation of ^{32}P in phospholipid and nucleic acids, followed by a decline, but incorporation in the embryo axes was more in HM seeds compared to the PM seeds.

Table 5. Accelerated ageing effect on changes in phospholipids and malondialdehyde content and ^{32}P incorporation in phospholipids and nucleic acids in the embryo axes of PM and HM seeds.[a]

Ageing days	Phospholipids (mg/g d. wt.)		Malondialdehyde (mg/g d. wt.)		^{32}P incorporation (cpm/g d.wt.)			
					Phospholipids		Nucleic acids	
	PM	HM	PM	HM	PM	HM	PM	HM
Modern								
0	0.882d	0.902b	0.41a	0.40a	6.190e	7.260b	22.990d	24.480c
4	0.865d	0.889b	0.41a	0.42a	7.200f	9.580c	23.870e	27.980e
8	0.794c	0.881b	0.74b	0.45a	5.100cd	7.360b	21.290b	23.970c
12	0.689a	0.787a	0.83c	0.73b	4.860bc	5.550a	20.100a	16.670b
EC-68414								
0	0.943e	0.956c	0.38a	0.37a	6.140e	7.220b	22.240c	23.990c
4	0.938e	0.951c	0.40a	0.37a	7.620f	9.100c	23.100d	26.910d
8	0.855d	0.947c	0.77b	0.41a	5.360d	7.870b	21.030b	23.790c
12	0.749b	0.795a	0.88c	0.76b	4.200a	4.890a	20.470a	15.550a

a Means in a vertical column followed by the same letter do not differ significantly at the 5% level (Duncan's Multiple Range Test).

DISCUSSION

Anderson (1975) reported that PM in Peredovik variety of sunflower occurred at 40% moisture content when oil content also reached its maximum. Time of PM in other cultivars is also reported (Browne, 1977; Robertson *et al.*, 1978). The present study showed that under the same environmental conditions PM was achieved at 30 DAF in Modern (dwarf) and at 36 DAF in EC-68414 (tall) cultivars. In both the cultivars, however, oil content reached its peak value at least 6 days prior to PM and such results are in agreement with the observation of Dorell (1978). But in PM the percentage and rate of germination were low. PM seeds of the cultivars were harvested at 42 and 44 per cent interval moisture level which decreased to 15 — 19 per cent in plants in case of HM seeds through a period of about 12 days. Harvesting of PM seeds, therefore, deprived them from going through slow desiccation in plants. Gradual water loss allows for controlled changes in the configuration of macromolecules of the seeds which favoured their stability or which facilitated limited biochemical reactions and hence increased their resistance (Bewley, 1979). Perry (1969a) stressed that when seeds are harvested in the immature stage or have prematurely experienced drying, this could seriously weaken a prospective plant. Under accelerated ageing, in the present case, germinability of PM seeds declined within 8 days when this remained unaffected in HM seeds. Decreased rate of germination and increased electrolyte leaching was earlier in PM seeds of both the cultivars. PM seeds, therefore, were found to be more susceptible under adverse storage condition.

Activities of the enzymes analysed increased up to 4-day period of accelerated ageing in both PM and HM seeds, the increase being more in HM seeds. Enzyme activity after 8 days of ageing was found to decline but the extent of the decline was more in PM seeds. Through initial dry matter contents of PM and HM seeds were similar, the former passed through a rapid dehydration. Such dehydration might affect the membrane protein, including enzymes, thereby decreasing their initial potentiality. In fact desiccation-induced reduction in macromolecules is well documented (Bewley, 1979).

Abdul-Baki and Anderson (1973) showed that the embryonic axes of a vigorous seed lot had greater synthetic capacity than the less vigorous ones under the same germinating environment. In the present study, nucleic acids and phospholipids biogenesis capacity of embryo axis increased at 4 days of accelerated ageing. Biogenesis of both the macromolecules declines after 8 days but in this case also synthetic capacity were much less in PM seeds. Under accelerated ageing, PM seeds therefore were found to be metabolically less active. Accelerated ageing resulted in a decline of membrane phospholipid content in seeds (Koostra and Harrington, 1969). Increased lipid peroxidation has been shown to occur in deteriorating soybean embryo axes (Stewart and Bewley, 1980). Phospholipid content declined significantly in PM and HM seeds of both the cultivars under accelerated ageing and such decline was associated with increased lipid peroxidase. In both PM and HM seeds such changes occurred only during post ageing days when viability declined.

Though harvesting of seeds at PM has been shown to have several advantages, this physiobiochemical study indicated some detrimental features. If changes occurring during the drying phase of seed are a preprogrammed part of the development, harvesting at PM might affect the maturation of the embryo. In the present study PM seeds were found to be inferior to HM seeds with respect to viability and germinability and under accelerated ageing. Hence in determining actual time of harvesting the question of complete ripening of the embryo should also be considered.

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IRRIGATED SUNFLOWER PRODUCTION FROM THE RED-BROWN SOILS OF NORTHERN VICTORIA.

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ABSTRACT

The irrigation areas of Northern Victoria produce half of Victoria's sunflower output. The product is a high quality oil with a low linoleic acid content. The soils in the irrigation areas of Northern Victoria are predominantly red-brown duplex types. A relatively fertile but shallow (100 mm) topsoil with an impermeable subsoil. The soil becomes structureless when irrigated and cement hard when dry. To overcome the problems of crop establishment and irrigation, growers have, with the aid of a vigorous extension service by the Department of Agriculture, Victoria, evolved a technique of obtaining yields in excess of 3 tonne per hectare with an oil percentage as high as 50%. The major techniques involved are the adoption of sowing hybrid cultivars with a precision planter on the flat at a plant density of 100,000 per hectare. Secondly, to irrigate frequently at 7 — 10 day intervals from bud stage to maturity, maintaing adequate soil moisture in a limited capacity shallow soil.

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