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Qualitative and quantitative changes in lipids of cowpea (*Vigna unguiculata*): Impact of changes in seed vigour

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ABSTRACT

Seeds of cowpea (*Vigna unguiculata* L. Walp.) varieties (V 240, V 578 and V 585) were subjected to controlled ageing experiment and subsequently were divided into high, medium and low vigour categories depicting 24, 72 and 120 hr ageing. The lipid content, fatty acid profile and lipid peroxidation determinations were performed. Changes in seed vigour impacted lipid content where the lowest lipid content was displayed by low vigour seeds being more drastic in V3 but moderate in V1 and V2. Regarding fatty acid composition, both palmitic and stearic acids contents significantly increased in low seed vigour category in V2 and V3; however, in V3, there was no significant difference between various vigour categories. Similarly, stearic acid content V1, V2 and V3 displayed significant increase in the low vigour categories; whereas, increased oleic acid content in the medium and low vigour lots was evident in V1 and V2 while in V3 where the low vigour lot exhibited significantly lower content as compared to the control. A comparatively smaller proportion of arachidic acid was evident which also increased significantly as quality dropped in V1 consistent increase was not observed in V2, V3. The content of two unsaturated fatty acids namely linoleic and linolenic acid was differential in varieties tested. Lipid peroxidation value of the three varieties exhibited a concomitant increase with decrease in vigour thus registering the highest value in the seeds with poorest seed quality.

Key words: Controlled ageing, Cowpea, Deterioration, Fatty acids, Seed vigour

Ageing which occurs over the time during storage is called natural ageing (Bewley and Black 1994). However, as the seed viability is affected by storage temperatures and Relative Humidity (RH), it can be accelerated artificially by subjecting them to elevated temperature and high RH (Copeland and McDonald 2001). Therefore artificial ageing is a useful procedure for studying the storage behaviour and changes in physiological and biochemical parameters under laboratory conditions. The process of seed deterioration is more pronounced in oil-rich seeds because of high susceptibility to peroxidation of polyunsaturated fatty acids present in them (Priestley and Leopold 1983) than the seeds which have low oil content.

Cowpea (*Vigna unguiculata* L. Walp.), an indigenous African annual legume, is commonly known as southern pea, black eye pea, crowder pea, lubia, niebe, coupe or frijole (Craufurd *et al.* 1996, Hall *et al.* 1997). It is a high protein (25%), fibre (6.3%) and starch-rich (50-60%) vegetable crop having good nutritional qualities (Ricardo 1985). The oil content of cowpea is relatively low on an average ranging between 2.48-3.03% (Mahadevappa and Piyara 1981, Onwuliri and Obu 2002). It is sensitive to low temperature (Ehlers and Hall 1997, Hall *et al.* 1997) and is adapted to warm and humid weather conditions (Craufurd *et al.* 1996, Hall *et al.* 1997).

Several reports explain the relationship between seed ageing, changes in the lipid and fatty acid contents as well as the extent of lipid peroxidation. But most of these are pertaining to oilseeds where changes are well expressed. Thapliyal and Connor (1997) reported positive and substantial correlation of seed vigour with lipid content. Seeds of broad bean (*Vicia faba* L.) showed a drop of approximately 20 to 30% in the lipid fraction when subjected to accelerated ageing; however pea (*Pisum sativum* L.) seed, showed opposite trend (Shahidul Islam *et al*. 2008). The fatty acid composition of different lipid fractions was almost unchanged during the ageing of seeds of peanuts (Pearce and Abdel Samad 1980). According to these authors, loss of control

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over sub cellular compartmentation or intracellular concentrations of metabolites, resulting from loss of membrane lipids, might have been the cause of the loss of viability. Theories of mechanisms of lipid oxidation indicate that fatty acids with two or more unsaturated bonds should be more labile and prone to form free radicals than more saturated acids.

The technique of accelerated ageing is useful for producing seeds of various vigour categories quickly for experimental purposes. In this paper we report the results of an experiment where seeds of three cowpea cultivars were artificially deteriorated by subjecting them to controlled deterioration to produce three different vigour categories. These were analysed for their lipid content, fatty acid profile and lipid peroxidation values with the objective to study the impact of loss of vigour on the changes in the lipid and fatty acids contents of cowpea seeds.

MATERIALS AND METHODS

The study was undertaken during 2009 to 2012 in seed physiology and biochemistry laboratories of National Bureau of Plant Genetic Resources, New Delhi. Seeds of cowpea varieties, V 240 (V1), V 578 (V2) and V 585 (V3), obtained from Division of Genetics, IARI were used in the present study. The seed moisture contents were determined using the High Constant Temperature Oven Method, after drying the coarsely ground seed at 130°C ± 1°C for 1 hour (ISTA 2007).

Calculated numbers of seeds were placed over moist towel paper impregnated with water to allow slow and uniform absorption of water. When the seeds had attained the targeted weight they were removed and wiped with dry paper towels, sealed in tri-layered aluminium foil packets and placed in an oven set at 45°C ±1°C for periods ranging from 24 to 168 hr (7 days) to allow accelerated ageing of the seeds. After the specific period of ageing the seeds were dried on the bench top to reduce the moisture content to original levels and kept packed in LAF pouches at 4°C until further used. All these seed lots were tested for various seed physiological parameters and from these data based on the performance of the seeds three vigour categories were identified viz. High vigour lot (24 hr controlled aged seeds), medium vigour lot (72 hr controlled aged seeds) and low vigour lot (120 hr controlled aged seeds). Seeds from the three categories were used for the analysis of fatty acid composition of the lipid fraction. Untreated seeds served as control.

Germination tests were performed in the laboratory, using 50 seeds in three replications by the 'Between paper method' (ISTA 2008), where seeds were equidistantly placed over wet paper towels and wrapped in wax paper followed by incubation in a seed germinator maintaining adequate humidity (over 90%) and a temperature of 25°C. Seedling evaluation and germination counts were taken on the 7th day (ISTA 2008). Germination percentage was recorded on the basis of normal seedlings.

Lipid peroxidation was evaluated by spectrophotometric measurements of malondialdehyde (MDA) levels, according to Heath and Packer (1968) with slight modification.

The oil was extracted from seeds by soxhlet extractor using petroleum ether (40-60). The oil content was determined according to AOAC Official Method 920.39.

Samples of cowpea seeds were freshly ground (Remi homogenizer) and weighed so that 40 mg oil was obtained when extracted with 10 ml solvent mixture consisting of chloroform: hexane : methanol (8:5:2 v/v/v). The extracts obtained were dried at 60°C in nitrogen gas for 30 min. Methyl esters of oil samples were prepared according to the method of Neff *et al*. (1994) with slight modifications.1µl of the hexane extract was injected into a highly polar HP Innowax capillary column of 30 m length (inner diameter: 0.32 m, film thickness: 0.5µm, split: 1:80). A Hewlett Packard gas chromatograph, model 6890 equipped with flame ionization detector (FID) was used. The injector and detector temperatures were 260°C and 275°C respectively. Oven temperature was programmed from 150°C holding at 1 min. to 210°C at the rate of 15°C/min., followed by 210°C to 250°C at the rate of 5°C/min. for 12 min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards, run under similar separation conditions, peak integration was performed applying HP3398A software.

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS). Means were compared using Duncan's multiplication range test (DMRT). Correlation coefficient was also done using the same software.

RESULTS AND DISCUSSION

Germination and vigour

Three cowpea cultivars were subjected to ageing to get three different vigour categories, viz. high, medium and low with the untreated lot serving as control. In V1 and V3 the trend was similar where the lot categorized as high vigour lot registered significantly higher germination and vigour index than the control lot while the medium and low vigour lots had conspicuously decreased germination and vigour index. In the variety V2 on the other hand the trend was slightly different in that the germination was significantly reduced but vigour index was on par with the control (Fig 1). The rate at which seeds age depends on their physiological status, genetic constitution as well as the capacity of the cultivar to withstand ageing conditions (Anderson and Gupta 1986, Priestly 1986, Roberts 1989, Kalpana and Madhavarao 1994). In the present study, it is evident that two cultivars V1 (brown seed coat) and V3 (cream seed coat) showed very good stress withstanding capacity while the variety V2 (very bold seeded and cream seed coat) exhibited least tolerance. The increase in germination in V1 and V3 could be due to an effect similar to priming during the first 24 hr when the

Table1 Fatty acid composition of three cowpea varieties of different vigour categories

Treatments	Fatty acids (%)					
	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
			V 240			
Control	23.765d	4.2793d	11.270 bc	38.453a	21.222a	1.2143b
High vigour	24.770c	4.5322c	11.054c	37.174b	20.360b	1.2933b
Medium vigour	24.883b	5.1280a	13.084a	35.464c	20.220b	1.4593a
Low vigour	26.983a	4.8706b	11.732b	34.914d	19.628c	1.4383a
			V 578			
Control	24.600b	4.3386b	9.6256b	35.088a	25.107a	1.175b
High vigour	24.667b	4.4240b	11.357a	34.437b	24.303ab	1.2963b
Medium vigour	25.182a	4.4536b	11.686a	33.676c	23.798bc	1.4293a
Low vigour	25.543a	4.8713a	12.481a	33.125d	22.983c	1.2563b
			V 585			
Control	25.727a	4.7906b	8.0183c	38.541a	21.592a	1.4150a
High vigour	25.263a	3.9896c	10.462a	37.653ab	21.693a	1.1873b
Medium vigour	26.611a	5.1020ab	8.9380b	37.239b	20.973a	1.4810a
Low vigour	26.520a	5.2253a	7.2416d	37.651ab	21.615a	1.4840a

Data presented are means of 3 replications. Data, followed by the same letter in a column, do not differ at the 5% probability level.

metabolic events had commenced within a few hours of exposure to high temperature and proceeded till the seeds were removed after a day and the deterioration had not set in yet. Such seeds can be expected to show enhanced seed quality in terms of germination and vigour due to repair processes that might have occurred during the initial stages of ageing.

Lipid content

The lipid content of all the three varieties exhibited changes parallel to the changes in vigour (Fig 2). As the vigour of the seeds decreased, the lipid content also declined the same being more significant in the medium and low vigour lots while in the high vigour lots it was on par with the control. Comparatively the decrease was more drastic in V3 and moderate in the other two varieties. In the varieties presently considered for the study, the oil percentage ranged from 2.92 to 3% in the control seed lots, and this decreased with decrease in the vigour and germinability. However exact quantitatively proportional reduction in the PUFA content was not observed in any of the varieties. The study involved analysis of lipids and fatty acids extracted from whole seeds. The small changes observed could have been due to the fact that changes in the lipids in the radical or plumule would be masked in such analysis as has been reported by Pearce and Abdul Samad (1980).

The variation in the total lipid content with variation in the vigour of the three cowpea cultivars is shown in the Fig 2. Lipid contents have been related to seed vigour in many seeds (Perl *et.al*. 1987). There was an overall decrease in the lipid content of the seeds of all three varieties as the seed vigour decreased. Decrease in total seed lipid content with germination and vigour loss has been reported by Worang *et al.* (2008) in *Jatropha* seeds, Puckaka and Kuiper (1988) in *Acer* seeds, Gidrol *et al*. (1989) in sunflower and by Freitas *et al.* (2006) in cotton seeds. Significant decrease in total lipid content with duration of storage has been reported for red gram and greengram varieties by Gopinath *et al.* (2011).

Fatty acid content

There was an overall increase in the major saturated fatty acids (Palmitic and stearic acid) along with oleic acid with seed quality depletion and this trend was similar for all the cultivars. On the contrary, the unsaturated fatty acids (Linoleic and linolenic acid) registered a significant decrease due to reduction in quality. This reduction was more pronounced in the V1 with respect to linoleic acid and V2 with respect to linolenic acid. Linoleic acid content decreased significantly in medium and low vigour lots of all the three varieties. Similar trend was observed for linolenic acid in varieties V1 and V2. However no significant decline in linolenic acid content was observed in V3, even in low vigour lot.

With decrease in quality significant increase in palmitic acid content was observed in variety V1 and V2, while in variety V3 no significant difference between various vigour categories in palmitic acid content was observed. A similar trend was observed for stearic acid content. In all the three varieties there was a significant increase in the stearic acid content in the low vigour categories. Increased oleic acid content in the medium and low vigour lots was evident in all varieties, the exception being V3 where the low vigour lot exhibited significantly lower content as compared to the control. Arachidic acid which was present in comparatively smaller proportion also increased significantly as quality

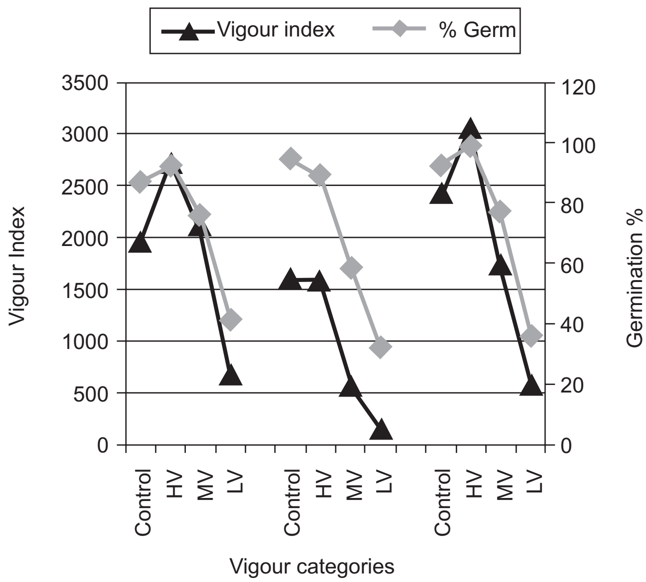

Fig 1 Vigour Index and germination percent of three cowpea varieties of different vigour categories

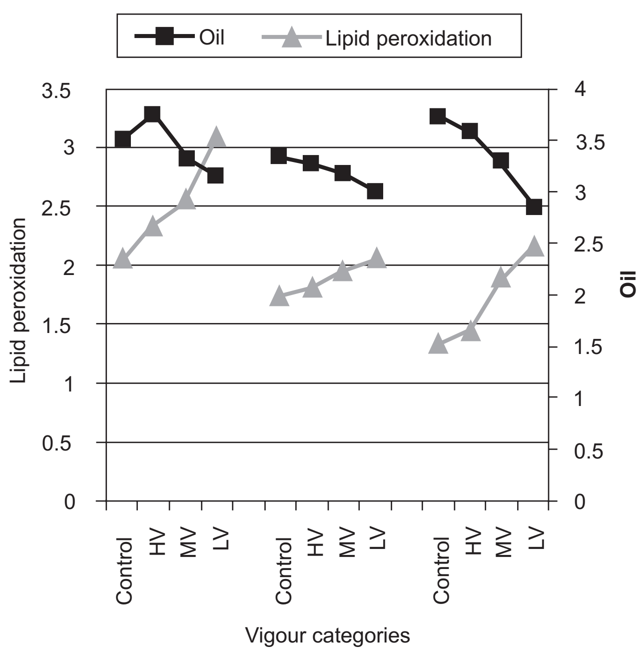

Fig 2 Lipid peroxidation and oil percent of three cowpea varieties of different vigour categories

dropped in variety V1 but in other two varieties the changes were not consistent.

The reports regarding loss of vigour and corresponding increase or decrease of the fatty acids are highly contradictory. Harman and Mattick (1976) found that the decrease in germination rate in aged pea seed was paralleled by a pronounced decline in linoleic and linolenic acid, whereas saturated and monoenoic fatty acids remained unchanged. Increase in oleic acid content as well as decrease in linoleic, linolenic acid content were observed over a period of six months of storage of red gram and greengram varieties by Gopinath *et al.* (2011). When seeds of soybean were subjected to ageing, the amount of total lipid from whole seed increased while the amount of unsaturated fatty acids showed decline (Priestley and Leopold 1983).On the other hand detectable changes were not observed in the fatty acid composition of storage lipids from whole seeds and axes of two soybean cultivars despite a considerable decline in seed vigour (Ferguson *et al*. 1990).

Corebeau *et al.* (2002), in sunflower, found that loss of viability was accompanied by an increase in the total lipid content which corresponded to a similar increase in the saturated as well as unsaturated fatty acids. The cause of such an increase has been hypothesized by them as due to relative stability of many of the enzymes involved in lipid biosynthesis. Besides, Douglas and Palag (1981) have observed that lipogenesis continues in various plant organs subjected to various stresses. The increase in the saturated fatty acids as well as less saturated oleic acids observed in the present study is probably due to selective synthesis of saturated fatty acids although unsaturated fatty acids showed a clear trend of decrease due to loss of vigour. Significant negative correlation between the saturated and unsaturated fatty acid content indicates the proportional shifts in the ratio of the two kinds of acids during quality changes in seeds of cowpea (Table 2).

Gidrol *et al.* (1989) have observed conspicuous decrease in the content of both saturated and unsaturated fatty acid with loss of viability in sunflower. Also there was accelerated formation of conjugated dienes at stressful condition but the viability remained high. In cowpea it has been observed that even in high vigour seeds irrespective of the varieties used, the lipid peroxidation values were significantly higher than control seeds. This increase could have been due to high temperature induced oxidation of non-specific lipids present in parts other than the membranes. Gidrol *et al.* (1989) have raised questions as to whether the peroxidation of the lipids occurs as a consequence of seed ageing or as a factor causing seed ageing. Perhaps investigating the ageing phenomenon and determining the products of peroxidation at frequent and shorter intervals might throw more light on these processes.

According to Trawatha *et al.* (1995) soybean seeds with highest linolenic acid content and lipoxygenase lost their physiological quality fastest during storage in unfavourable or extreme environmental (high temp and high moisture) conditions. In the present study the variety V2 which had the highest content of linolenic acid (Table 1) showed the fastest drop in seed quality. Medium vigour seeds of the variety registered a 1.4% decrease in this fatty acid content when the germination had fallen from 88.66 from the initial 94.66

Lipid peroxidation

A parallel change that was observed in the present study

Table 2 Pearson correlation coefficient association analysis for three varieties of cowpea

Variety	Characteristics	Correlation coefficient (r)
V1 (V 240)	VI and LP	–.754**
	VI and Oil	.870**
	LP and oil	–.744**
	LP and LA	–.898**
	LP and LnA	–.887**
	VI and LnA	.512*
	VI and LA	.572*
	Oil and LA	.744**
V2 (V 578)	VI and LP	–.959**
	VI and Oil	.860**
	VI and LA	.921**
	VI and LnA	.799**
	LP and Oil	–.903**
	LP and LA	–.979**
	LP and LnA	–.883**
	Oil and LA	–.875**
	Oil and LnA	.813**
V3 (V 585)	VI and LP	–.917**
	VI and Oil	.910**
	VI and LA	.218
	VI and LnA	.141
	LP and Oil	–.951**
	LP and LA	–.467
	Oil and LA	.297

VI, Vigour index; LP, lipid peroxidation; LA, linolic acid, LnA, linolenic acid

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

was the corresponding increase in the lipid peroxidation value as the vigour of the lots decreased. In all three varieties this trend was conspicuous and proportionate (Fig 2). There is a high probability that the reduction in the lipid content was brought about by active peroxidation of the membrane lipids in the present study. According to Lin and Pearce (1990) when seeds are damaged by improper storage conditions, lipid degradation reaction occurs and lipase and lipoxygenase are the two principle enzymes involved in the degradation of lipid in seeds. Lipid peroxidation has been shown as the most important factor causing loss of seed viability by many workers (Harman and Mattick 1976, Steward and Bewley 1980, Lima *et al*. 2010, Kaewnaree *et al*. 2011 and Wang *et al*. 2012).

The magnitude of changes observed in the germinability was substantial that these cannot be equated to small but significant changes observed in the lipid or fatty acid contents and more often the medium and poor vigour seeds seldom differed in their fatty acid content (Table 1). This could be due to the fact that cowpea seed is a non-oily seed and due to low lipid content, the free radical attacks may not be occurring at proportionate frequencies to cause corresponding quantitative changes in the lipids. Similar trend in fatty acid changes with vigour decrease has been reported by Thapliyal and Connor (1997), in *Dalbergia sissoo*, with very low lipid content. The increased lipid peroxidation value taken in conjunction with reduced germination or vigour index indicate that increased lipid peroxidation might be one of the causes of the low vigour of the poor quality seeds as has been observed by Kaewnaree *et al.* (2011) in sweet pepper seeds. Correlation studies revealed that the lipid peroxidation and vigour index, lipid peroxidation and oil content and lipid peroxidation and unsaturated fatty acids correlated negatively (Table 2). But the actual observed changes in the physiological parameters were several times greater than that occurring in the oil and/or fatty acid content and therefore pinpointing these as exclusive causal factors for seed quality loss may not be logically correct. There may be other factors simultaneously acting on the seed to bring down the germinability and vigour of cowpea seeds ultimately resulting in complete loss of quality. In artificially aged husk tomato seeds where the linoleic acid content was more than 80%, significant reduction in the germination and seedling emergence rate was observed without any effect on the fatty acid content while the respiratory rates correlated with the loss in seed quality thus indicating that loss of physiological quality in deteriorated seeds can be attributed to the reduction in respiratory activity (Gonzalez *et al.* 2010).

The study indicates the involvement of lipid peroxidation occurring with ageing and loss of vigour in cowpea seeds, since the decline in the fatty acid content is correlated to the increased lipid peroxidation values in all the genotypes used. Similar correlation between total lipids and lipid peroxidation is evident but these changes although significant are not proportional to the magnitude of physiological changes occurring due to ageing. Time course analysis of changes in vigour versus components of lipid metabolism including enzymes involved in fatty acid synthesis and degradation may throw some light on the role of these processes in the deterioration of cowpea and related grain legumes.

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