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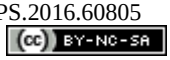
ISSN 2231-3354 

Total salinity stress on physico-chemical characterization of lecithin isolated from soya bean oil seeds grown in the coastal region of south, India

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ABSTRACT

Phospholipid is very essential in the balanced diet. The vegetarian people in the coastal area are habitant of using edible oil seeds as daily food grains. Salinity of water during cultivation decreases the accumulation of oil content (12-15%) in seeds. Present experiment was focused on total salinity and ionic stress on physiochemical characterization of extracted lecithin from soya bean oil under saline and non-saline cultivations. The experiment proves that the percentage of phospholipids in oil and lecithin is decreased by 1.02% and 8.08%, respectively under saline cultivation. The phospholipids of the lecithin were qualitatively identified by thin-layer chromatography (TLC) and high performance of liquid chromatography (HPLC). The R_f values for phosphatidyl-ethanolamine (PE), phosphatidyl-serine (PS), phosphatidyl-inositol (PI) and phosphatidyl-choline (PC) of samples were well related to the standard. HPLC spectrum is well resolved and the retention time (RT) is correlated the standard with high precision. Quantisation of phospholipids shows a variation in the average percentage of PC, PI, PS and PE as 17.925, 9.125, 5.9, 15.1 for saline cultivation and 22.25, 12.025, 8.525, 18.975 for non-saline cultivation. Average decrease in the percentage in saline cultivation is due to the total salinity and ionic (Na^+Cl^-) stress of water.

INTRODUCTION

Vegetable materials usually contain only small amounts of phospholipids, ranging from 0.3 to 2.5 wt. % (Wagner and Wolff, 1964).Phospholipids are complex lipids which contains one or more phosphate groups. Phospholipids are amphipathic in nature that is each molecule consists of a hydrophilic portion and a hydrophobic portion thus tending to form lipid bilayers (Dowhan and Bogdanov, 2002). In fact, they are the major structural constituents of all biological membranes, although they may be also involved in other functions such as signal transduction. The most abundant types of naturally occurring glycerol phospholipids are phosphatidyl-choline, phosphatidyl-ethanolamine, phosphatidyl-serine, phosphatidyl-inositol, phosphatidyl-glycerol and cardiolipin. The structural diversity within each type of phosphoglyceride is due to the variability of the head group, variability of the chain length and degree of saturation of the fatty acid ester groups. Phosphatidyl-choline Providesfree choline in the blood for the manufacture of acetylcholine which regulates digestive, cardiovascular and liver functions (Alvarez *et al.*, 1997; Spiers *et al.*, 1996). Phosphatidyl-choline and Phosphatidyl-ethanolamine (80:20) is essentialfor the production of stable liposomes, anti-spattering agent in margarine. Phosphatidyl-serine is essential to the functioning of all body cell, supports brain functions that decline with age, memory enhancer (Kidd, 1996). In case of pure vegetarians, it is so essential to make up the phospholipid content having vegetables and edible oil grains in their balanced diet. The plant sources of phospholipids are soybean (Wagner and Wolff, 1964), rapeseed (Sosulki, 1981), sunflower (Litinova *et al.*, 1971), cottonseed and peanut (Vijayalaxmi *et al.*, 1969), ricebran (Adhikari and Adhikari, 1986), palm, coriander, carrot (Goh *et al.*, 1982), papaya (Prasad *et al.*, 1987), olive, barley, cucurbit (Schneider, 1989), corn, karanza,

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castor bean (Paulose *et al.,* 1966), cocoa (Parsons *et al.,* 1969), neem (Prasad *et al.,* 1981), sesame, khakan (Prasad *et al.,* 1979), pear, quince, tobacco (Zlatanov *et al.,* 2000). Phospholipids are removed as by-product during the degumming process of vegetable oil refining.

Soybean seed is a major source of high-quality protein and oil for human consumption (Katerji *et al.,* 2001). The unique chemical composition of soybean has made it one of the most valuable agronomic crops worldwide (Thomas *et al.,* 2003). Its protein has great potential as amajor source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol (Essa and Al-ani, 2001). Growth, development and yield of soybean are the result of genetic potential interacting with environment. Protein and oil content is related with environmental factors like moisture, temperature *etc*.

The 2002 U.S. soybean crop had an average protein (35.5%) and oil (19.3%)content because of hot and dry weather conditions in those areas contributed to poor yields (Brumm and Hurburgh Jr, 2002). Soybean seed production may be limited by environmental stresses such as soil salinity (Ghassemi-Golezani *et al.,* 2009). Minimizing environmental stress will optimize seed yield (McWilliams *et al.* 2004). Soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many arid and semi-arid regions of the world (Meloni *et al.,* 2004). Salinity is a worldwide problem, affecting about 95 million hectares worldwide (Kazemghassemi-Golezani *et al.,* 2010). The UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed (Yan, 2008).

Most of the salt stresses in nature are due to Na^+ salts, particularly NaCl (Demirel, 2005). High salinity lowers water potential and induces ionic stress, and results in secondary oxidative stress. It severely limits growth and development of plants by affecting different metabolic processes such as CO_2 assimilation, oil and protein synthesis (Nasir khan *et al.,* 2007). Present work is mainly focused on the variation of phospholipids content due to salt tress of salinity of water during cultivation of soybeans.

MATERIALS AND METHODS

Chemicals

2-Propanol, n-hexane, acetic acid and chloroform with highest purity (HPLC grade) were procured from Hi-Media. Methanol, acetic acid, sodium acetate, per chloric acid, ammonium molybdate, aminonaphtholsulphonic acid, potassium dihydrogen phosphate, petroleum ether and acetone with highest purity (AR grade) were procured from S.D. Fine and Merck.

Collection of samples

Four varieties of soy bean seeds were collected from coastal and non-coastal region of Karnataka, Tamil Nadu and Andhra Pradesh (India) available in the weekend market. The seeds were dried under sun light first and then kept at 90°C (about three hours) in an oven. Then these are fine powdered and used for the extraction of oil. The samples were labelled as soya bean-a, soya bean-b, soya bean-c and soya bean-d for saline (coastal) cultivation and soya bean-a_o, soya bean-b_o, soya bean-c_o, soya bean-d_o for non-saline (non-coastal) cultivation.

Extraction of oil

50±0.5 g of seed powder is packed and stapled in a Whatman grade no. 42 filtered paper. The packet is inserted into the middle piece of soxhlet extractor. Oil was extracted using petroleum ether for three hours. Petroleum ether was recovered and the oil was dried 85°C in a preheated oven for one hour. Oil obtained is cooled in a desiccator and weighed for constant weight.

Extraction of Lecithin

5 ± 0.2 g of fresh oil was dissolved in analytical grade acetone and stirred well. The insoluble lecithin was filtered and flushed with N_2 gas. A feathery material was obtained which on drying in oven at 75-80°Cforms a reddish brown transparent solid. It was weighed for constant weight.

High Performance Liquid Chromatogram (HPLC) analysis of phospholipids

High Performance Liquid Chromatogram (HPLC) was recorded using Shimadzu LC-2010HT instrument series of wavelength range 190-600nm with bandwidth 8nm.Instrumental wavelength accuracy and wavelength reproducibility are ±1nm and ±0.1nm. Lichrosorb, Si-60, 10µm (C_{18}) column was saturated by 2-propanol and maintained at 30°C. 10µl of the sample in mobile phase was programmed for injection and the mobile phase n-Hexane: 2-propanol: acetate buffer (8:8:1, v/v) was pumped at rate of 2ml/min and chromatograms were recorded at 206 nm.

Quantization of lecithin

Sample was prepared by dissolving 0.1±0.005gm of extracted lecithin in chloroform according official methods and recommended practices of the American Oil Chemists Society (ACOS, 4thEdn 1990). Sample was prepared by dissolving 0.1±0.005gm of extracted lecithin in chloroform. Aliquot equivalent to 0.010±0.005gm was pipette into 30ml graduated test tubes and digested by adding 0.9ml 70 % per chloric acid at 80-90°C followed by 120°C and 150-180°C on sand bath. The colourless and clear solution in the test tube is cooled ad volume is made up to 2ml. To this 7.0ml of distilled water, 1.5ml of 2.5% ammonium molybdate and 0.2ml aminonaphtholsulphonic acid were added. The test tubes were placed in boiling water bath for exactly 7mins and cooled for 20mins. Then optical density was measured at 830nm using UV- Spectrophotometer against the blank.

Calibration curve was plotted using AR potassium dihydrogen phosphate solutions having 1to5µg of phosphorus.

$$\%\ of\ phospholipids = \frac{\text{A x 30.97 x 100}}{\text{W x 1000 x 1000}}$$

Where, A = Phosphorus content in µg from calibration curve
W = Weight of sample in g from the sample aliquot
30.97 = Converting factor for phosphorus into phospholipids

TLC analysis of phospholipids

TLC plates of size 20x20 cm 0.2mm silica gel coated glass plates were activated at 110°C for 1 hour. The developing solvent was Chloroform:methanol: Acetic acid: Water (25:15:4:2, v/v). Spots were identified by Iodine vapour and eluted by Chloroform.

An aliquot of 10µg of isolated lecithin dissolved in chloroform was spotted on TLC plates. Chromatogram was developed using the mobile phase Chloroform: Methanol: Acetic acid:Water (25:15:4:2, v/v). The condition was followed exactly as given by Skipski and others. The plates were dried at room temperature for 20 min after an average time of running for 1½ hours. Spots were identified by iodine vapour and encircled using sharp needle. When iodine vapour was completely evaporated, the silica gel was scrapped using razor, quantitatively transferred into centrifuging tubes and added 2ml chloroform. It was mixed well, centrifuged and the centrifugates were collected in labelled 30ml graduated test tubes. Extractions were repeated twice for each spots and collected together in test tubes. Finally the chloroform was evaporated and residues obtained were used for the determination of different phospholipids

RESULTS AND DISCUSSION

Determination oil content

Oil was extracted from four different variety soya bean seed powdered materials by soxhlet extraction method. Seed powdered material contains 0.9-1.5% moisture. Average percentage of oil recovery from soya bean seeds grown in coastal and non-coastal cultivation by soxhlet extraction method is reproducible and regressive. Oil content per seed decreases in the coastal cultivation due to salinity stress.

Table 1: Average percentage of oil content in different cultivation under saline and non-saline cultivation.

Variety of soya bean	Coastal Cultivation	
	Average weight (g)	Average Oil Content (%)
Soya bean-a	50.40	18.03
Soya bean-b	50.10	18.07
Soya bean-c	50.10	17.91
Soya bean-d	50.00	18.15
	Non Coastal Cultivation	
Soya bean-a_o	50.22	20.02
Soya bean-b_o	50.14	19.87
Soya bean-c_o	50.08	20.18
Soya bean-d_o	50.15	20.27

The Table1 correlates oil accumulation in soya bean seeds where coastal cultivation gives 18.04 per cent and that of non-coastal cultivation is 20.13%oil which relates literature value 2 1.38±0.6 % reported by American Soybean Association (Brumm and Hurburgh Jr, 2002). The recovery of oil shows linearity with regression R^2 equal 0.0667 for coastal and 0.5996 for non-coastal cultivation (Fig. 1).

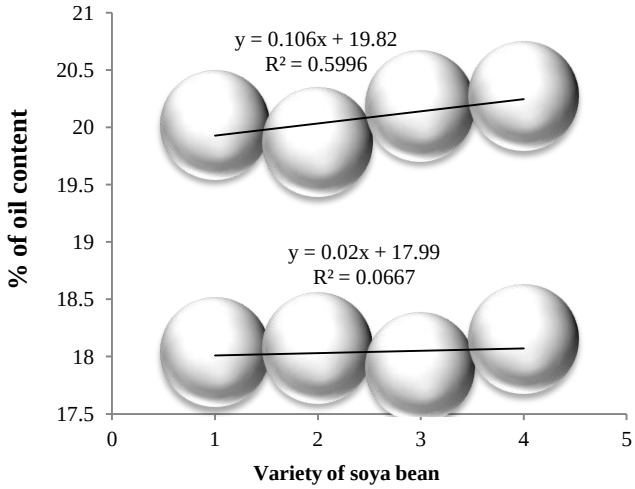

Fig. 1: Percentage of oil content soya bean for coastal and non- coastal cultivations.

Seeds from non-coastal cultivation have oil accumulation of 2.09% more than the seeds from coastal cultivation. This deterioration in oil content is caused by salt out effect of NaCl on soya proteins. Present work was focused on saline and non- saline condition of water for the cultivation of soya bean. Sea shore famers are dependents of saline water and faces saline stress.

Analysis oil and Lecithin for phospholipid content

Phospholipid content in oil and lecithin was determined by estimating the amount of phosphorus by per chloric acid digestion method using Official methods and recommended practices of the American Oil Chemists Society.

Amount of phosphorus in oil and Lecithin was estimated against sample aliquot weight. Table 2 lists the reproduce able results of the phospholipid content of soya bean oil and lecithin under saline, non-saline cultivation. The phospholipid content of oil and lecithin for soya beans in saline cultivation is relatively lesser compare to soya bean seed oil grown in non-saline cultivation.

Amount of phosphorus was determined by recording optical density using standard calibration curves (Fig. 2) thereby determining the percentage of phospholipid content of in and lecithin. Phospholipid content for oil and lecithin in saline (coastal) cultivation is 2.91% and 59.64% as compare to Non-saline (non-coastal) cultivation having 3.93% and 67.72%. These results reveal that the salinity of water is the main cause for deterioration of oil content of soya bean seeds. Saline stress on growing soya bean results in decrease of oil content and phospholipid content per seed.

Table 2: Amount of phosphorus, Average percentage of phospholipid in soya bean oil and lecithin under saline, non-saline conditions.

Variety of soyabean	Soya bean oil				Acetone Insoluble Matter (AIM)			
	Wt. in aliquot (g) $1x10^{-4}$	Optical Density	Phosphorus content in µg	Average % of phospholipid	Wt. in aliquot (g) $1x10^{-4}$	Optical Density	Phosphorus content in µg	Average % of phospholipid
Coastal cultivation								
Soya- bean-a	5.31	0.053	0.51	2.97	1.01	0.202	1.94	59.49
Soya- bean-b	5.60	0.054	0.52	2.84	0.99	0.200	1.92	60.10
Soya- bean-c	5.26	0.052	0.50	2.94	1.03	0.205	1.97	59.25
Soya- bean-d	5.69	0.055	0.53	2.88	0.98	0.197	1.89	59.73
Non-Coastal cultivation								
Soya- bean-a	4.48	0.051	0.49	3.39	0.89	0.203	1.95	67.90
Soya bean-b	4.58	0.053	0.51	3.45	0.95	0.213	2.05	66.98
Soya- bean-c	4.30	0.050	0.48	3.46	0.94	0.215	2.07	.68.03
Soya- bean-d	4.60	.054	0.52	3.50	0.90	0.206	1.98	67.96

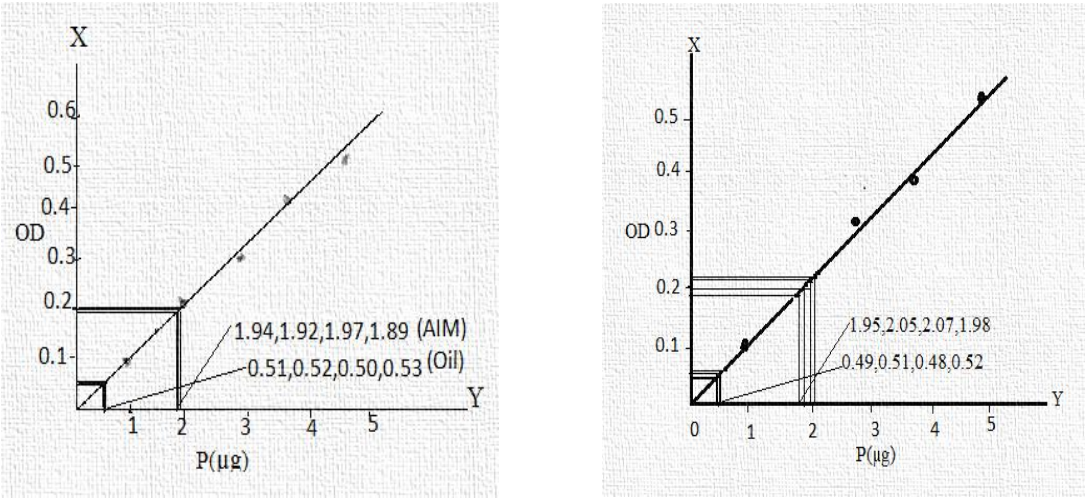

Fig. 2: Calibration curve for phosphorus content, in saline cultivation (a) and non-saline cultivation (b).

Qualitative identification of phospholipids Lecithin

Thin Layer Chromatography (TLC)

Fig. 3 gives one dimensional TLC of isolated lecithin (AIM) and the standard commercial lecithin of purity 35% against PC. The spots were identified as PE, PS, PI, and PC in comparison with the standard on exposing to iodine vapour in iodine chamber.

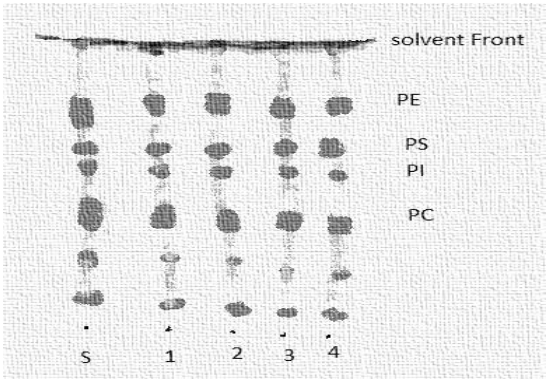

Fig. 3: TLC spots of phospholipids for standard and samples.

R_f values of each spot was determined as ratio of the distance travelled by solvent (solvent front) to the distance travelled by the spots and related with the standard.

Table 3 correlates the R_f of the samples and the standard. The R_f value of PE 0.912, PS 0.835, PI 0.794, PC 0.670 are well correlated with an average R_f value samples as 0.914, 0.834, 0.788 and 0.671. R_f values of sample are precisely correlated with the standard and have high accuracy with negligible deviation.

Table 3: R_f values of Phospholipids of standard and samples.

Phospholipids	R_f values				
	S	1	2	3	4
PE	0.912	0.918	0.912	0.906	0.918
PS	0.835	0.829	0.829	0.835	0.841
PI	0.794	0.788	0.788	0.794	0.782
PC	0.670	0.676	0.670	0.665	0.672

High Performance Liquid Chromatography (HPLC)

The identification of different phospholipids was further confirmed by HPLC. Fig. 4 gives relatively comparable chromatograms. Retention time of each phospholipid was well related and resolved. Table 4 lists retention time for each phospholipids and correlates the chromatograms of the standard and the samples.

Table 4: HPLC Retention time for PE, PA, PI and PC of soya lecithin.

Phospholipid	Retention Time (Standard) Min	Retention Time (Coastal cultivation) Min	Retention Time (Non-Coastal cultivation) Min
PE	4.64	4.65	4.63
PA	2.71	2.73	2.73
PI	3.59	3.61	3.62
PC	8.03	8.07	8.07

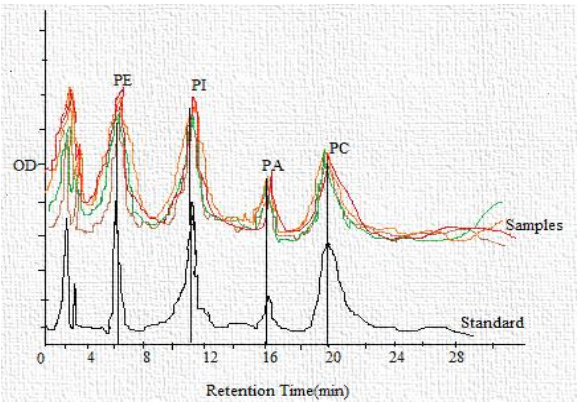

Fig. 4: HPLC of Soya Lecithin (a) Standard and (b) Samples from saline and non- saline cultivation.

Quantization of Phospholipids in Lecithin

An aliquot containing 10µg of each sample of lecithin in chloroform was spotted quantitatively on TLC plate and the spots were identified by iodine vapour (Fig. 5). Corresponding spots were marked using a sharp needle and silica gel was removed using razor after the complete elimination of iodine vapour adhere to the silica gel.

The phospholipids were quantitatively estimated by the experimental procedure. Table 5 summarises the relative percentage of PC, PI, PS and PE in the lecithin isolated from different variety of soya bean seeds obtained from saline and non-saline cultivations. Quantitative determination of Phospholipids by TLC gives an average percentage of PC, PI, PS and PE as 17.925, 9.125, 5.9, 15.1 for saline cultivation and 22.25, 12.025, 8.525, 18.975 for non-saline cultivation. The relative percentage of phospholipids in non- saline cultivation is higher than the saline cultivation.

The main cause for the decrease in the phospholipids content is the salinity and ionic (Na^+Cl^-) stress due to sodium chloride on accumulation of oil and phospholipids content. High concentration of total salt in water decreases the nitrogen fixation

Table 5: TLC quantisation of phospholipids of soya bean lecithin for saline and non-saline cultivation.

Soya bean	Wt. of PL in aliquot $1x10^{-4}$	Phosphorus in Phospholipids (µg)				Percentage of Phospholipids			
		PC	PI	PS	PE	PC	PI	PS	PE
Coastal Cultivation									
Soya bean-a	1.20	0.72	0.36	0.23	0.60	18.2	9.3	5.9	15.5
Soya bean-b	1.10	0.63	0.32	0.22	0.53	17.8	8.9	6.1	14.8
Soya bean-c	1.05	0.60	0.31	0.19	0.51	17.6	9.1	5.7	15.1
Soya bean-d	1.07	0.63	0.32	0.20	0.52	18.1	9.2	5.9	15.0
Non-Coastal cultivation									
Soya bean-a_o	1.04	0.75	0.41	0.29	0.63	22.2	12.1	8.5	18.8
Soya bean-b_o	1.12	0.82	0.43	0.30	0.70	22.6	11.9	8.3	19.3
Soya bean-c_o	1.14	0.81	0.45	0.31	0.69	21.9	12.2	8.4	18.7
Soya bean-d_o	1.20	0.86	0.46	0.35	0.74	22.3	11.9	8.9	19.1

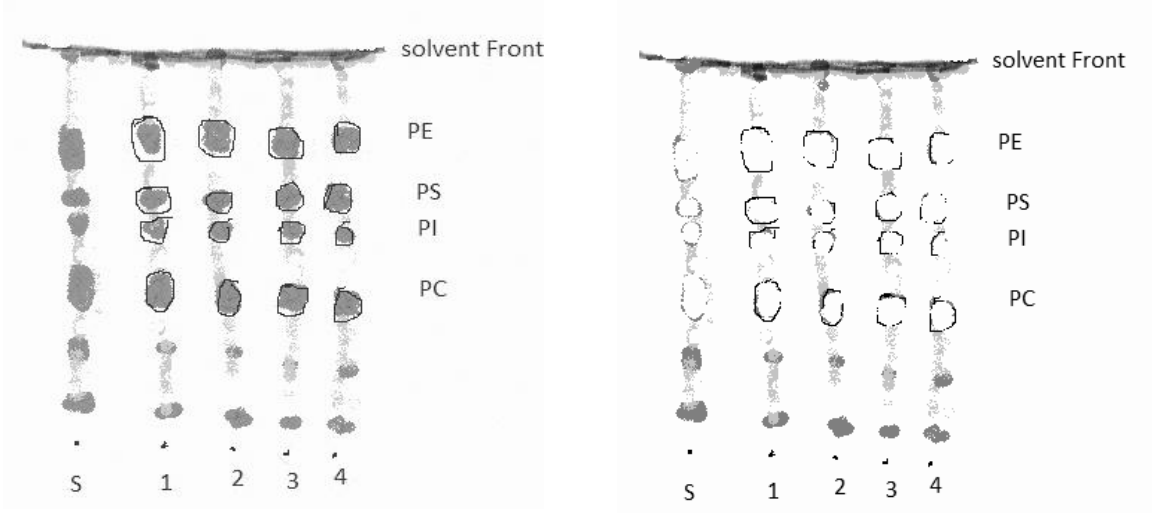

Fig. 5: (a) TLC spots for phospholipids after exposing to iodine vapour and (b) TLC spots for phospholipids after the removal of silica gel.

and salting out the protein accumulation. The overall results evidences the salinity stress adversely affects the accumulation of phospholipid percentage in the soya bean oil seeds.

CONCLUSION

Present work was focused on salinity stress for the cultivation of soya bean under saline and non-saline conditions. Four varieties of soya bean seeds from coastal and non-coastal cultivation were analysed for oil and phospholipid content. The experimental result proves that the non-saline (Non-coastal) cultivation have higher oil and total phospholipid content (20.09% and 67.72%) than the saline (coastal) cultivation (18.04% and 59.64%).The experiment was also conducted for qualitative identification of individual phospholipids (PC, PI, PS and PE) by one dimensional TLC and HPLC spectrum. The spectral results were well related with the standard and the quantisation of phospholipids by TLC gives that the individual phospholipid content for non-saline cultivation is higher than that of saline cultivation. In summary, the overall results contribute to novelist of the experiments that the total ionic and salinity stress causes a decrease in total and individual phospholipid contents of soya bean seeds.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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