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Full Length Article

Induced Genetic Variability for Fatty Acids and Oil Contents in Chickpea (*Cicer arietinum*)

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Abstract

The experimental material for the present study comprised of 94 selected induced mutants of two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi × kabuli introgression (CH40/91). The oil contents and saturated (palmatic and stearic acid) and unsaturated fatty acid (oleic, linoleic and lenolenic acid) contents were determined in four chickpea genotypes. The range of oil content was recorded 3.60 to 6.90% in desi variety Pb2000 followed by 3.43 to 8.48% in C44, 3.27 to 6.90% in Pb-1 and 3.50 to 5.93% in CH40/91. The highly significant increase in oil contents was observed in mutants with pink stem (8.48%) in C44. and in large leaf mutant (7.32%) of Pb-1. In desi × kabuli introgression genotype CH40/91, highly significant increase in oil content was noted in mutant of small leaf (5.93%). Out of 25 mutants of Pb2000 and 31 mutants of C44, increase in palmatic acid above the control was observed only in 11 mutants. Out of 27 mutants of Pb-1 and 11 mutants of CH40/91, increase in palmatic acid was observed in 16 and 10 mutants respectively. Early type mutants in Pb2000 (CM72/02 and CM137-01) and desi × kabuli introgression genotype CH40/91(CM1534/01) had high palmitic acid, stearic acid, oleic acid and linoleic acid, which supports their role of tolerance against frost. These mutants showed tolerance for frost in the field. The presence of high amount of fatty acids in plants may be used as selection criteria for frost tolerance in chickpea. Majority of the mutants of desi × kabuli introgression (CH40/91) showed increased in saturated as well as unsaturated fatty acids content simultaneously as compared to the mutants of three desi and kabuli types mutants. In conclusion, improvement of saturated and unsaturated fatty acid contents in desi × kabuli introgression lines of chickpea through induced mutation is not useful approach for heath point of view.

Keywords: Chickpea; Unsaturated and saturated fatty acids; Gamma irradiation; Ethylmethane sulphonate (EMS)

Introduction

Chickpea (*Cicer arieatinum* L.) is main rabi crop grown commercially in rainfed areas of Pakistan and is a good source of cheap vegetable protein, however, its production is under the constant threat of diseases and pests (Akhtar *et al*., 2008, 2009, 2011; Sarwar *et al.*, 2012). It is the only single major winter crop grown on about 96% rainfed sandy Thal areas of the country. The chickpea seed has protein contents in the ranges of 16.7 to 30.6% in desi and 12.6 to 29.0% in kabuli types which is 2–3 times higher than cereal grains (Wood and Grusak, 2007). The energy values of desi chickpea have been reported at 14–18 MJ/kg (334–437 kcal/100 g) and 15–19 MJ/kg (357–446 kcal/100 g) for kabuli types. The kabuli types normally have fairly higher energy values than desi types when grown under identical conditions due to a smaller seed coat component (WHO/FAO, 2003).

The fat (lipid) content of pulses varies in different species. Most species contain about 1% fat, while groundnut and soyabean, have 30 and 49%, respectively (FAO, 1968). The total fat contents of desi and kabuli types chickpea ranges from 2.9 to 7.4% and 3.4 to 8.8%, respectively which are rather higher for pulses (Wood and Grusak, 2007). The fat content, besides contributing to the energy needs, provides the essential supply of fatty acids for human beings. The chickpea and soybean contains linolenic acid, which is an omega–3–fatty acid. The fatty acid is currently being studied for its ability to reduce the risk of heart disease and cancer (Ofuya and Akhidue, 2005). The total fat content of chickpea mainly consists of polyunsaturated (62–67%), mono-unsaturated (19–26%) and saturated (12–14%) fatty acids (Wood and Grusak, 2007). Small amount of fat in chickpea is generally of the valuable category (mono-unsaturated and polyunsaturated) rather than saturated fats that have been associated to heart and circulatory diseases. A low amount of saturated fatty acid content in chickpea is the natural endowment for the deprived people.

The fat content in chickpea fulfilled the nutritional requirement of malnutrition of poors. In addition to this, the acyl chains of phospholipids and glycolipids of saturated and unsaturated fatty acids play an important role in the chilling resistance in chickpea plants during winter season (Lehninger, 1977; Vigh *et al*., 1998). The acyl lipid desaturation in the plasma membrane increases with increased frost tolerance in chickpea. The presence of more

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unsaturated lipid in the plasma membrane may prevent the cell lysis at low temperature (Bakht *et al.*, 2006). Frost tolerance of the plasma membrane results from a specific reduction in linoleic acid and an increase in linolenic acid, rather than from a general non-specific desaturation. Fatty acids are also present in the epicuticular wax of legume seeds, which contains 32.4% fatty acid whereas a wax extract of chickpea contains 5% fatty acid.

In spite of intensive breeding efforts, chickpea yields have not shown any considerable improvement during the past decade, thus leading to sharp increase in its price and reduction in per capita availability. Improvement of qualitative traits in the existing high yielding chickpea cultivars could be one of the possible methods of achieving the minimum requirement of 60 g per capita per day of protein (Anonymous, 1990). Although through conventional breeding, the improvement in qualitative traits can be achieved but it is painstaking and time consuming process, however the most useful and less time consuming techniques for improvement of fatty acid and oil content in crop plants is induced mutation (Banerjee and Swaminathan, 1966; Hameed *et al.*, 2012).

In the present study, genetic variability produced through induced mutagenesis (Shah *et al.*, 2010, 2011) has been characterized for the seed chemical composition (oil contents, saturated and unsaturated fatty acids) of selected morphological mutants of four chickpea genotypes.

Materials and Methods

Plant Material

The experimental material for the present study comprised 94 selected induced mutants of two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi × kabuli introgression (CH40/91). Genetic variability was induced in two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi × kabuli recombinant genotype (CH40/91) through gamma irradiation (200, 300, 400, 500 and 600 Gy) and ethyl methane sulphonate (EMS) (0.2, 0.3 and 0.4%) (Shah *et al*., 2008). The first set consisted of 56 induced mutants of desi genotypes (25 belonging to desi genotype Pb2000 and 31 from C44) along with their respective parents. The second set consisted of 27 mutants of kabuli genotype (Pb-1) and 11 of desi × kabuli introgression line (CH40/91) with their respective parents. The detail of doses and characteristics of induced mutants is presented in Table 1. The mutants were selected on the basis of diverse morphological mutations in growth habit, foliage morphology, flower color, disease reactions (resistant and susceptible) and seed color for qualitative studies.

Fatty Acid Analysis

Seed crude fat was determined according to standard procedures (AOAC, 1984). Triglycerides separated by TLC were converted to fatty acid methyl esters suitable for gas chromatography by the methanolic sulfuric acid esterification process, (AOAC, 1984 method 28.055).

Gas Liquid Chromatography

The methyl esters prepared by the above method were chromatographed isothermally on Gas Chromatograph (Perkin-Elmer Model 3920) with a flame ionization detector (FID) and attached to an integrator (Shimadzu- Japan- CR-4A). The samples were injected with a microliter syringe (SGE-Australia) with a rapid thrust. Calculation was based on area basis of the individual ester peak and then calculated the response factor (Rf) of each standard and finds the intensity of esters in unknown samples.

Identification of Fatty Acids

The chromatogrm produced by the integrator gave the retention times of every fatty acid methyl ester (FAME) present in the samples along with the concentration and area under peak. A table of retention time for all FAMEs was constructed by taking means of triplicate analyses for quick identification of fatty acids. Firstly the data for the retention time were obtained by determining the retention times of standard mixtures of pure FAMEs e.g. C6:0, C8:0, C12:0, C14:0, C16:0, C18:0, C18: 1, C18:2, C18:3, C20:0, C22:0 and C24:0. Then using a graphical procedure that involved plotting the logs of relative retention time against the chain length of homologous series of FAMEs (Ackman, 1969), four parallel lines were drawn which related molecular structure to retention times for homologous of saturated, monosaturated, diunsaturated and polyunsaturated (PUFA) acids. Equivalent chain length (ECL) or carbon number (CN) values for both saturated and unsaturated long chain fatty acids were also assessed for the provisional identification of peaks. The methods of identification and standard ECL values are given by Christie (1973) and Ackman, (1969).

Statistical Analysis

Data were subjected statistical analysis by using *t*-test. Each experiment was performed in triplicate and the results were expressed as average values. Difference between mean proteins of control and each mutant were compared at $p \geq 0.05$ and 0.01.

Results

Induced Variability for Total Oil Content

The range of oil content was recorded 3.60 to 6.90% in desi variety Pb2000 followed by 3.37 to 8.48% in C44, 3.03 to 7.32% in Pb-1 and 3.50 to 5.93% in CH40/91 (Table 1). The highly significant increase in oil contents was observed in gigas and early mutant (6.90%) followed by stipulated leaf (6.53%) and significant increase in prostate growth habit (6.22%) in desi genotype Pb2000 (Table 1).

Table 1: Fatty acid (Saturated and Unsaturated) and oil content in M_3 morphological mutants

Mutant	Mutagenic dose used	Character	Fatty acid content						Oil Content (%)
			Saturated		Unsaturated			Total conc. (%)	
			PA (%)	SA (%)	OA (%)	LNA (%)	LLA (%)		
Pb2000	Control	Semi erect	12.11	0.32	19.94	66.03	0.88	99.28	5.70±0.09
CM54/01	300Gy	Broad leaf	11.28	0.59	21.71	64.85	0.92	99.35	5.02±0.03*
CM87/01	300Gy	Spreading	13.97	0.95	26.05	58.40	0.68	100.05	4.00±0.02**
CM94-1/01	300Gy	Bold seed	11.88	0.73	26.00	60.45	0.60	99.66	4.93±0.05**
CM134/01	300Gy	Spreading	14.07	0.88	27.00	57.00	0.71	99.66	3.80±0.06**
CM137/01	300Gy	Early and Gigas	12.18	0.58	22.55	63.61	1.18	100.00	6.90±0.08**
CM143/01	300Gy	Long leaf	11.55	0.50	22.17	64.68	0.79	99.69	4.73±0.03**
CM149/01	400Gy	Prostate	12.29	0.76	26.00	60.25	0.70	100.00	6.22±0.01*
CM188-1/01	400Gy	Pink color foliage	11.36	0.94	30.0	56.90	0.40	99.6	3.92±0.04**
CM236/01	400Gy	EBL, V.bold seed	13.40	1.00	25.35	58.92	1.33	100.00	4.00±0.01**
CM269/01	400Gy	Round pod.	10.89	0.53	20.47	65.80	1.27	98.96	5.50±0.08^{NS}
CM270/01	400Gy	Long pod	15.29	0.73	25.00	56.81	2.16	99.99	4.77±0.03**
CM24-2/02	300Gy	Thick stem	15.12	0.99	32.71	49.46	1.38	99.66	5.04±0.01**
CM27/02	300Gy	White flower	11.70	0.70	22.70	64.50	0.94	99.84	4.97±0.03**
CM36-1/02	300Gy	late maturity	10.70	1.01	23.10	64.70	0.90	102.71	4.90±0.03**
CM43-2/02	300Gy	Tall	12.24	0.42	28.22	58.22	0.69	100.01	5.57±0.02^{NS}
CM54-3/02	300Gy	Round pod	11.10	0.43	21.22	66.08	0.55	99.38	4.33±0.02**
CM72/02	300Gy	Early	13.68	0.88	20.88	64.60	0.59	99.75	4.30±0.03**
CM87-1/02	300Gy	Compact	11.88	0.87	23.87	63.32	0.44	99.51	3.83±0.04**
CM54-4/02	300Gy	Wilt susceptible	11.62	0.87	19.00	66.55	1.47	99.51	4.00±0.03**
CM231/02	0.3% EMS	Wilt Resistant	13.58	0.35	19.15	66.72	0.55	99.55	4.08±0.03**
CM238-1/02	0.3% EMS	Pink flower	11.04	0.66	19.66	68.43	0.69	99.82	3.60±0.03**
CM238-2/02	0.3% EMS	White flower	15.02	0.75	20.71	61.36	1.85	99.69	4.24±0.04**
CM248/02	0.3% EMS	Lax, Narrow leaf	11.01	0.69	21.69	66.58	0.45	99.73	4.97±0.03**
CM449/02	0.3% EMS	Coriander type leaf	15.24	0.79	24.50	57.30	2.26	100.1	4.61±0.03**
CM520-1/02	0.4% EMS	Long stipulate leaf	11.79	0.40	19.34	67.58	0.72	99.83	6.53±0.03**
C44	Control		12.50	0.76	19.76	59.74	0.67	99.67	5.77±0.02
CM381/01	500Gy	V. tall, multi branches	11.96	0.37	26.00	61.12	0.55	100.00	4.20±0.02**
CM416/01	500Gy	Small pod	10.86	0.81	22.81	65.46	0.66	99.73	5.23±0.05*
CM418-1/01	500Gy	Double pod	10.96	0.26	26.20	62.10	0.49	100.0	4.00±0.02**
CM415/01	500Gy	Long leaf	12.02	1.04	24.20	61.81	0.55	99.62	4.03±0.04**
CM422/01	500Gy	Bold seed, bold pod	12.47	0.19	24.11	62.58	0.48	99.83	6.10±0.06*
CM438-1/01	500Gy	Broad leaf	11.79	0.30	21.97	65.10	0.85	100.01	4.87±0.02**
CM489/01	600Gy	Long pod	13.01	0.83	22.49	62.05	1.28	99.66	4.70±0.03**
CM509/01	600Gy	Palm shaped leaf	11.79	0.33	27.57	59.83	0.29	99.81	5.53±0.04^{NS}
CM553/01	600Gy	White fl., white seed	12.19	1.08	18.77	65.07	2.64	99.75	5.44±0.03*
CM563/01	600Gy	Prostate	11.33	0.33	27.70	60.27	0.38	100.00	6.00±0.15^{NS}
CM633/01	0.3% EMS	Leaf type	13.83	0.94	23.08	59.78	2.02	99.65	4.48±0.31**
CM661/01	0.3% EMS	Small leaf	11.71	0.49	22.49	65.80	0.80	100.00	3.40±0.03**
CM820/01	0.3% EMS	Lax	12.51	0.65	21.02	64.46	0.97	99.61	4.77±0.04**
CM926/01	0.3% EMS	thick stem	11.53	0.61	24.00	63.10	0.45	99.69	3.50±0.03**
CM946-1/01	0.4% EMS	Small leaflet	11.59	0.43	22.69	64.10	0.76	99.57	4.87±0.02**
CM1020-1/01	0.4% EMS	Light color, dwarf	12.47	0.38	25.00	61.57	0.26	99.68	5.50±0.03^{NS}
CM1116/01	0.4% EMS	Pink stem	12.41	0.71	23.72	62.80	0.36	100.00	8.48±0.06**
CM 1103/01	600Gy	Upright Bushy	13.95	0.87	23.28	59.56	1.92	99.58	4.67±0.09**
CM 546/01	500Gy	Compact -round leaf	13.93	1.34	22.12	60.80	1.49	99.68	4.50±0.02**
CM 1553/01	600Gy	Bipinnate compound leaf	12.30	0.63	22.06	63.96	0.80	99.75	3.37±0.01**
CM 3191/01	500Gy	purple zigzag stem	11.04	0.41	28.00	59.80	0.40	99.65	3.43±0.02**
CM 3649/01	500Gy	Gigas	13.30	0.69	22.50	62.66	0.83	99.98	4.5±0.03**
CM 3744/01	500Gy	Dwarf, Bushy	14.31	1.05	24.87	58.36	0.98	99.57	7.00±0.03**
CM 3531/01	500Gy	Yellow tips	11.63	0.54	24.7	62.27	0.66	99.8	4.00±0.03**
CM693-1/01	0.3% EMS	Blue Flower	14.01	0.84	22.54	59.54	2.98	99.91	3.95±0.02**
CM818-5/02	600Gy	Erect	13.54	0.60	18.20	66.60	0.91	99.85	4.40±0.03**
CM846-4/02	600Gy	Thick stem	12.63	1.08	19.68	66.33	1.05	99.68	4.53±0.02**
CM914-1/02	0.3% EMS	Spreading erect	12.54	0.75	18.62	66.46	1.30	99.67	5.77±0.02^{NS}
CM951-2/02	0.4% EMS	v. minute stem	11.59	0.71	20.71	67.41	0.41	99.71	3.53±0.02**
CM966/02	0.4% EMS	Dark purple stem	11.13	0.71	22.54	64.60	0.87	99.85	4.83±0.01**
CM966-1/02	0.4% EMS	VBL, bold seed	11.63	0.45	22.97	64.10	0.75	99.90	4.54±0.02**
Pb-1	Control		11.42	1.04	27.39	61.2	1.75	98.93	4.50±0.02

Table 1: Continued

Table 1: Continued

Mutant	Mutagenic dose used	Character	Fatty acid content						Oil Content (%)
			Saturated		Unsaturated			Total conc. (%)	
			PA (%)	SA (%)	OA (%)	LNA (%)	LLA (%)		
CM1411/01	0.2% EMS	Pink flower	21.94	1.73	24.61	40.10	2.51	90.89	3.96±0.03**
CM1476/01	200Gy	Blight Tolerant	11.54	0.67	21.06	65.41	0.91	99.59	3.27±0.01**
CM1507/01	200Gy	Blight Tolerant	11.80	0.46	23.54	63.25	0.87	99.92	3.03±0.04**
CM1782/01	300Gy	Heavy bearing, multibranches	10.78	0.28	20.28	67.90	0.89	99.85	4.03±0.04**
CM1964-1/01	300Gy	Spreading	13.39	0.36	20.48	64.53	1.24	100.00	6.03±0.02**
CM2029/01	0.2%EMS	Dark color	15.08	0.92	24.55	57.42	1.69	99.66	4.50±0.05^{NS}
CM2043/01	0.2%EMS	Bushy	11.08	0.45	22.95	64.56	0.78	99.82	4.23±0.02*
CM1410/92	0.2%EMS	Broad leaf, compact	11.47	0.77	27.00	59.49	0.98	99.71	4.03±0.04**
CM2071/01	0.2%EMS	thick stem	11.96	0.59	21.69	64.20	1.55	99.99	6.57±0.03**
CM2073/01	0.2%EMS	Bold, semi spreading	10.68	0.74	33.00	54.71	0.88	100.01	4.08±0.02*
CM2075/01	0.2%EMS	Small leaf, compact	11.27	0.75	22.77	63.93	1.10	99.82	4.17±0.02*
CM2076-2/01	0.2%EMS	SL, curved midrib	14.0	1.8	33.64	48.36	1.76	99.61	3.96±0.02**
CM2078/01	0.2%EMS	Light color, semi erect	14.31	1.14	32.19	50.74	1.63	100.01	5.02±0.03**
CM2164/01	0.3%EMS	Compact	15.30	1.35	25.29	56.28	1.43	99.65	4.00±0.03**
CM2267/01	0.3%EMS	Gigas type	13.22	0.70	21.70	64.05	1.03	99.97	6.90±0.05**
CM39/02	300Gy	Large pod	10.09	0.88	26.00	60.53	1.13	98.63	5.40±0.05**
CM 249/01	0.2%EMS	Double pod	13.47	0.89	26.00	58.49	0.94	99.79	7.07±0.03**
CM95/01	200Gy	Tall	10.49	0.84	24.5	62.53	1.4	99.76	5.33±0.04**
CM1461/01	0.1% EMS	Early	10.65	0.63	26.03	61.23	1.56	100.10	4.76±0.03*
CM444/02	300Gy	H.Wilt Resistant	11.65	0.84	23.00	64.08	0.44	100.01	4.23±0.02**
CM446/02	300Gy	H.Wilt Resistant	11.29	0.91	22.6	63.68	0.49	98.97	4.97±0.03**
CM 439/02	300Gy	Large Leaf, suspt. to beetle	11.65	1.11	23.84	64.08	0.44	99.60	7.32±0.03**
CM252/01	200Gy	Longer bold pod	11.54	0.47	24.36	62.55	1.08	100.00	3.67±0.03**
CM 432/02	300Gy	Dwarf, Small leaf	10.15	0.55	25.77	61.31	1.33	99.11	5.30±0.02**
CM1285-1/02	300Gy	Erect	15.71	1.32	30.19	50.94	1.52	99.68	5.33±0.03**
CM1362/02	300Gy	Fasciated stem	11.36	0.73	22.66	63.89	0.91	99.55	4.05±0.03**
CM1678/02	300Gy	Bold seed	10.92	0.85	26.77	60.71	0.75	100.00	4.03±0.02**
CH40/91	Control		11.13	0.32	27.00	60.88	0.67	100.00	4.68±0.01
CM1158-9/01	0.4% EMS	Extra Broad L, Bold seed	11.69	0.49	22.56	64.66	0.78	100.2	4.25±0.02*
CM1504/01	200Gy	Vigorous	14.08	0.78	27.89	56.25	0.76	99.76	5.00±0.03*
CM1509/01	200Gy	Bushy, Open canopy	11.48	0.39	26.40	61.58	0.69	100.5	4.73±0.02 ^{NS}
CM1523/01	200Gy	Broad leaf	12.00	0.40	25.70	61.16	0.56	99.82	5.00±0.03*
CM1534/01	200Gy	Early	12.36	0.64	21.29	65.30	0.86	100.45	4.44±0.02*
CM1584/01	300Gy	Blight tolerant	11.33	0.41	24.89	62.44	0.59	99.66	5.25±0.05**
CM1587/01	300Gy	Blight tolerant	11.55	0.47	25.89	61.56	0.67	100.14	5.57±0.03**
CM1590/01	300Gy	Blight tolerant	12.80	0.59	21.29	64.37	0.80	99.85	4.20±0.03**
CM1615/01	300Gy	Blight tolerant	11.84	0.82	24.80	61.67	0.68	99.81	5.00±0.06*
CM1631/01	300Gy	Bold pod	11.06	0.71	23.09	64.3	0.8	99.96	3.50±0.04**
CM1663/01	300Gy	Small leaf	11.23	0.56	24.74	62.33	0.76	99.62	5.93±0.02**

*****Mean value is significantly different at P≥ 0.05 from respective controls

**Mean value is highly significantly different at P≥ 0.01 from respective controls

NS non-significant

The highly significantly low amount of oil content was observed in pink flower (3.6%), spreading (3.80%) and compact leaf (3.83%) in Pb2000. All other mutants had oil content in the range of 4.00 to 5.57%. Improvement in oil content was recorded only three out of 25 mutants compared to the parent genotype Pb2000.

In desi genotype C44, the highly significant increase in oil content was observed in pink stem (8.48%) and bushy mutant (7.0%). A significant increase in oil content were observed in bold pod (6.1%) (Table 1). The minimum oil content was recorded in bipinnate leaf (3.37%), closely followed by small leaf (3.40%), zigzag stem (3.43%), thick stem (3.50%). Same was the case in minute stem (3.53%) and blue flower (3.95%) mutants. All other mutants showed oil content in the range of 4.00 to 5.77%. Out of 31 mutants, improvement for oil content was recorded only in four mutants compared to the parent C44 (5.77%).

In kabuli genotype Pb-1, the highly significant increase in oil content was observed in large leaf (7.32%), double poded (7.07%), gigas (6.90%), thick stem (6.57%), spreading growth habit (6.03%), large pod (5.40%), erect/tall (5.33%), dwarf (5.30%), light color (5.02%) and wilt resistant mutant (4.97%). A significant increase in oil content was recorded only in early (4.76%) mutant (Table 1). The reduced amount of oil content was observed in blight tolerant mutants (3. 03 and 3.27%), longer bold pod (3.67%), pink flower and curved midrib (3.96%) mutants. All other mutants had oil content in the range of 4.00 to 4.23%. Out of 27 mutants, 12 showed improved oil content as compared with its parent genotype Pb-1 (4.50%).

It was mentioned here that mutant with maximum oil content (large leaf having 7.32%) was highly susceptible to stored pest i.e., beetle that may be due to thin outer wall of seed coat with high amount of oil content of kabuli genotype. This susceptible trait of seed may be used as morphological marker for the detection of high amount of oil content in chickpea genotype in future.

In desi × kabuli introgression genotype CH40/91, highly significant increase in oil content was noted in small leaf (5.93%) and blight tolerant (5.57, 5.25). A significant increase in oil content was found in broad leaf and vigorous (5.00%) mutants. The minimum oil content was observed only in bold pod mutant (3.50%). Oil content in all other mutants varied from 4.20 to 4.44%. Out of 11 mutants, seven displayed improved oil content compared to the parent genotype CH40/91 (4.68%).

Induced Variability for Saturated and Unsaturated Fatty Acid Content

Saturated (palmatic and stearic acid) and unsaturated fatty acid (oleic, linoleic and lenolenic acid) contents were determined in four chickpea genotypes (Table 1). In selected mutants of desi genotype Pb2000, palmatic acid varied from 10.70 to 15.29%, stearic acid 0.35 to 1.0%, oleic acid 19.0 to 27.0%, linoleic acid 49.46 to 68.43% and lenolenic acid 1.40 to 2.26%. Out of 25 mutants of Pb2000, increase in palmatic acid above the control was observed in 11 mutants, while stearic acid increased in all mutants, oleic acid increased in 21 mutants, linoleic acid increased in five mutants and lenolenic acid (%) increased in nine mutants. A highest percentage of oleic acid was observed in thick stem (32.71) followed by pink foliage color (30.0) and tall (28.33) mutants over control (19.94). A higher percentage of linoleic acid was observed in pink flower (68.43) followed by stipulated leaf (67.58) and wilt resistant (66.72) mutants compared to control (66.03). A higher percentage of lenolenic acid was observed in coriander leaf type (2.26) followed by long pod (2.16) and white flower (1.85) mutants compared to control (0.88).

The palmatic acid in selected mutants of desi genotype C44 ranged from 10.86 to 14.31%, stearic acid 0.19 to1.34%, oleic acid 18.20 to 28.0%, linoleic acid 58.36 to 67.41% and lenolenic acid 0.26 to 2.98% (Table 1). Out of 31 mutants of C44, increase in palmatic acid over control was observed in 11 mutants, stearic acid in 10 mutants, olieic acid in 28 mutants, linoleic acid in 26 mutants and lenolenic acid in 17 mutants. A higher percentage of oleic acid was observed in zigzag stem (28.00) followed by prostate (27.70) and palm shaped leaf (27.57) mutants compared to control (19.76). A higher increase in linoleic acid was observed in minute stem (67.41) followed by erect growth habit (66.60) and spreading erect (66.46) mutants over respective controls (59.74). The higher percentage of lenolenic acid was observed in blue flower (2.98) followed by white flower (2.64) and leaf type (2.02) mutants compared to control (0.67).

The palmatic acid in selected mutants of kabuli genotype Pb-1 ranged from 10.09 to 21.94% followed by stearic acid 0.36 to1.80%, oleic acid 20.48 to 33.64%, linoleic acid 40.10 to 67.90% and lenolenic acid 0.44 to 2.51%. Out of 27 mutants of Pb-1, increase in palmatic acid over the control was observed in 16 mutants, increase of stearic acid in 6 mutants, olieic acid in 4 mutants, linoleic acid in 14 mutants and lenolenic acid only in 2 mutants. The higher percentage of oleic acid content was observed in curved midrib (33.64) followed by semi-spreading (33.00) and light color (32.19) mutants over control (27.39). A higher percentage of linoleic acid was observed in multibranches stem (67.90) followed by blight tolerant (65.41) and bushy (64.56) mutants compared to control (61.2). A higher percentage of lenolenic acid content was observed in pink flower (2.51) followed by white flower (2.64) and curved midrib (1.76) mutants compared to control (1.75).

The palmatic acid in selected mutants of desi × kabuli introgression genotype CH40/91 ranged from 11.06 to 12.80% followed by stearic acid 0.39 to 0.82%, oleic acid 21.29 to 27.89%, linoleic acid 56.25 to 65.3% and lenolenic acid 0.59 to 0.86%. Out of 11 mutants of CH40/91, increase in palmatic acid over the control was observed in 10 mutants, stearic acid in all mutants, oleic acid in one mutant, linoleic acid in 10 mutants and lenolenic acid in 8 mutants. A greater increase in oleic acid (%) was observed in vigorous mutant (27.89) compared to control (27.00). A higher increase of linoleic acid was observed in early (65.30), extra broad leaf (64.66) and blight tolerant (64.37) mutants over respective control (60.88). A highest percentage of lenolenic acid was observed in early flower (0.86) followed by blight tolerant and bold pod (0.80) mutants compared to control (0.67). A genetically stable mutant with an increase of 3.4% linolenic acid was identified by Wilcox *et al.* (1984) in soybean. This mutant was similar to its parent (Century) in days to maturity, plant height and resistance to lodging.

Discussion

A wider range of oil contents was observed in desi genotypes as compared to kabuli and desi × kabuli introgression mutants. The oil content among the desi parents ranged from 5.70 to 5.77% as compared to 4.75 to 4.80 among their mutants (Table 2). The mean oil content between the kabuli and desi × kabuli introgression parent ranged from 4.50 to 4.68% as compared to 4.79 to 4.81 oil content among their mutants. The overall oil contents were higher in the mutants of desi × kabuli introgression genotype CH40/91 (4.81%) followed by desi C44 (4.80%), kabuli Pb-1 (4.79%) and desi Pb2000 (4.75%) (Table 2). Oil content in the selected mutants of desi × kabuli introgression genotype CH40/91 and kabuli genotype Pb-1 were higher than parent means, whereas the mutants of desi genotypes Pb2000 and C44 had lower oil contents than their parent.

Table 2: Range, mean and coefficient of variation (CV) for oil contents in desi, kabuli and desi × kabuli introgression chickpea genotypes and their selected morphological mutants

Group	Oil content range (%)	Mean oil content (%)	CV (%)
Desi			
Pb2000 (parent)	5.70	5.70±0.09	3.2
Pb2000 (mutants)	3.60 -6.90	4.75±0.06	0.6
C44 (parent)	5.77	5.77±0.01	0.5
C44 (mutants)	3.43-8.48	4.80±0.07	1.4
Kabuli			
Pb-1(parent)	4.50	4.50±0.02	0.8
Pb-1 (mutants)	3.27 - 6.90	4.79±0.03	0.6
Desi × Kabuli introgression line			
CH40/91(parent)	4.68	4.68±0.01	0.5
CH40/91 (mutants)	3.50 - 5.93	4.81±0.02	0.4

Table 3: The percentage (%) of selected morphological mutants having higher oil content than their parents of desi, kabuli and desi × kabuli introgression chickpea genotypes

Genotypes	Total no. of mutants studied	No. of mutants with higher oil content	% of mutants with higher oil content
Desi			
Pb2000	25	3	12.0
C44	31	4	12.9
Total	56	7	12.5
Kabuli			
Pb-1	27	12	44.4
Desi × Kabuli introgression line			
CH40/91	11	7	63.6

In general, the mutants of desi × kabuli genotype (63.6%) showed relatively increased oil content compared to kabuli (44.4%) and desi (12.5%) type (Table 3). The literatures on the induction of genetic variability for the improvement of oil content in chickpea are scarce, whereas several reports on oil seed crops such as cotton (Bhat and Dani, 1993), soybean (Yu *et al*., 1995) and sunflower (Ferna´ndez-Martı´nez *et al.*, 1997) are available. In oilseed crops, the increased oil yield is achieved by increasing seed or fruit yield (Robellen, 1990). Several cultivars with higher yield and oil content have been developed with the help of induced mutations in different oil seed crops. In soybean mutant Longfu 81-9825, the negative correlation between protein content (44%) and the oil (21%) content had been broken (Wang *et al*., 1993). A high oleic acid mutant (HOM), containing 40% of oleic acid compared to 27% in parent cultivar MACS 450, was produced from a treatment with 200 Gy γ-rays and 0.15% EMS (Patil *et al*., 2009).

Reduction in saturated and increase in unsaturated fatty acid content is the improvement of nutritionally superior traits, which can increase the consumer preference by reducing the risk of heart diseases (Ofuya and Akhidue, 2005). In the present study, an increase in unsaturated and decrease in saturated fatty acids content in most of the promising mutants of three chickpea genotypes were observed, which may be a preliminary success in genetic manipulation of desired characters. The reduction in the palmatic acid (%) and increase in the linoleic acid (%) in desi genotype Pb2000 was observed in pink flower CM238/02, stipulated leaf CM520-1/02, round poded mutant CM54-3/02, wilt susceptible CM54-4/02 and narrow leaf CM248/02 as compared to parent. The increase in palmatic acid (%) and linoleic acid in desi genotype Pb2000 was observed only in wilt resistant mutant CM231/02. A reduction in the palmatic acid (%) and an increase in the linoleic acid in desi genotype C44 were observed in minute stem CM951-2/02, small poded CM 416/01, small leaf CM661/01, broad leaf CM 438-1/01 and white flower CM553/01. Increase in both palmatic acid (%) and linoleic acid in desi genotype C44 was observed in erect mutant CM818-5/02, spreading erect CM914-1/02 and thick stem CM846-4/02. A major reduction in palmatic acid (%) and higher increase in the linoleic acid in kabuli genotype Pb-1 was observed in multibranches CM1782/01 and blight tolerant CM1476/01. A greater increase in palmatic acid (%) and linoleic acid in kabuli genotype Pb-1 was observed in spreading mutant CM1964-1/01, thick stem CM2071/01, large leaf CM439/02 and gigas (CM2267/01).

Reduction in saturated fatty acids as compared to their parent was observed in desi × kabuli introgression genotype CH40/91. Out of 11 mutants, 10 showed an increase in the percentage of palmatic acid, stearic acid, linoleic acid and lenolenic acid at the same time. Increase in linoleic acid and decrease in palmitic acid was observed only in blight tolerant mutant CM1590/01. In the present study, there was a strong reverse relationship between oleic and linoleic acids in most of the mutants. This supports the hypothesis of sequential desaturation as the method of formation of unsaturated fatty acids in soyabean oil (Wilcox *et al.*, 1984). Lower stearic and low palmitic acid content observed in the

present study was comparable to that of sunflower (Miller and Vick, 1999). The induced genetic alteration for fatty acid by reducing the saturated fatty acid content of oil would benefit the sunflower industry through increased consumer preference for a low saturated sunflower product. Bhatia *et al*. (1999) reviewed mutation-breeding work for quality improvement in oilseeds. Mutant with altered fatty acid composition have been isolated in soybean, rapeseed, sunflower, linseed and minor oil crops. New cultivars having altered fatty acid composition have been released in rapeseed, sunflower and linseed. In linseed, fatty acid mutants have been utilized to develop 'linola' cultivars that yield edible oil suitable for human consumption with low (65-76%) linolenic acid content (Green and Dribnenki, 1994; Dribnenki *et al.*, 1996). Wang *et al.* (1993) reported high variation (1.2–6.0 times) of the fatty acid composition in promising mutant to that of the control in soybean. They suggested that 0.2% EMS was effective for inducing beneficial mutations in soybean fatty acids. Thus in the present study the important desirable characters, which had been achieved through induced mutation include high yield, grain quality, early maturity, disease resistance, improved plant type and better protein, oil and fatty acids. These mutants can be directly used as variety if they possess the desirable traits or they can be employed as germplasm in the cross breeding program.

In the present study, the response of mutants of desi × kabuli introgression genotype CH40/91 for the improvement of fatty acid was different as compared to the mutants of desi and kabuli genotypes (Pb2000, C44 and Pb-1). In most of the mutants of desi × kabuli introgression genotype (CH40/91) had improvement in saturated (palmitic acid and stearic acid) as well as unsaturated fatty acids (oleic acid, linoleic acid and lenolenic acid) content. This is contrary to the hypothesis of sequential desaturation as the method of formation of unsaturated fatty acids in soybean oil observed (Wilcox *et al.*, 1984). While in the mutants of remaining three genotypes (Pb2000, C44 and Pb-1), the increase in unsaturated and decrease in saturated fatty acids content in most of the promising mutants were observed. Increased amount of saturated fatty acid in the chickpea ultimately reflects the nutritionally important traits of chickpea which are harmful attributes regarding the human health. It may be recommended from the present work that the mutation breeding studies in desi × kabuli introgression genotype for the improvement of fatty acid content is not beneficial.

It was also observed that early mutants in desi genotype Pb2000 (CM72/02 and CM137-01) and desi × kabuli introgression genotype CH40/91(CM1534/01) have high palmitic acid, stearic acid, oleic acid and linoleic acid, which support the concept of their role in the frost resistance (Lehninger, 1977; Vigh *et al*., 1998). Bakht *et al.* (2006) noted an increase in fatty acids in plasma membrane of chickpea on exposure to frost. In the present study enhanced rate of fatty acid was also observed in most of the mutants and particularly early mutants. This indicated that these mutants might have resistance for frost or low temperature. The presence of high amount of fatty acids in plants may be used as selection criteria for frost resistance in chickpea. The early genotypes in the present study flowered and set seed at low temperature (at >5^{o}C) in the month of December. Further, it had long reproductive phase (from end of December to mid March) that resulted in increased yield.

In conclusion, a range of genetic variability for qualitative traits such as oil contents, saturated and unsaturated fatty acid may be created by the use of induced mutations. Improvement in the nutritionally superior traits like unsaturated fatty acid and reduction in saturated content in the chickpea is the consumer preference and suitable for reducing the risk of heart diseases. But the mutants of desi × kabuli introgression genotype have no good quality of unsaturated fatty acids and are not beneficial for use as food. On the other hand early mutants with high contents of oil or fatty acid were good for frost and have resistance for low temperature. The presence of high amount of fatty acids in plants may be used as selection criteria for frost resistance in chickpea.

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