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Biochemical evaluation of dent corn (*Zea mays* L.) genotypes cultivated under rainfed conditions in the hills of north western Indian Himalayan state of Jammu and Kashmir

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Abstract: The aim of present study was to investigate protein, oil and fatty acid composition in 11 maize (*Zea mays*) genotypes collected from diverse locations in the hills of north western Indian Himalayan state of Jammu and Kashmir in order to get an idea about the extent of variability in these biochemical traits in the local germplasm. The study revealed significant variation in these quality traits. The protein content among the genotypes ranged from 10.7% to 18.7% while oil content varied between 2.26% and 4.80%. Higher protein content in some of the genotypes especially IC-0617877 (18.7%) and IC-0617880 (17.6%) is noteworthy. The saturated fatty acids of palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and unsaturated fatty acids of oleic (C18:1), linoleic (C18:1) and elaidic (C18:1) were detected and quantified in these genotypes. Considerable variation has been recorded in fatty acid composition; 13.8-33.4% for palmitic acid, 21.5-48.1% for linoleic acid, 19.2-39% for oleic acid, 0.2-2.4% for elaidic acid, 2.5-8.5% for stearic acid and 0.1-6.6% for arachidic acid. Higher oleic acid content recorded in all the genotypes excepting IC-0617881 is a useful trait. Strikingly, highest oil (4.80%), palmitic acid (33.4%), stearic acid (8.5%) and arachidic acid (6.6%) contents have been recorded in this remarkably cold tolerant genotype with reddish yellow seeds containing moderate protein content of 13.8 %. The appreciable variation in these quality traits could be exploited in breeding programmes for improvement of this crop and opening up new opportunities for its food and industrial end uses.

Keywords: Fatty acids, Genotypes, Oil content, Protein content, *Zea mays* L.

INTRODUCTION

Maize (*Zea mays* L.) belonging to the family Poaceae, is the third largest cereal crop after wheat and rice cultivated worldwide and used as food, feed and as raw material for various industrial applications like production of starch, beverages, glue, industrial alcohol and fuel alcohol. Domestication of maize is believed to have taken place some nine thousand years ago in the Balsas River region in western Mexico from tropical teosinte, *Zea mays* ssp. *parviglumis* (Matsuoka *et al*., 2002, Doebley, 2004). Mexico has the highest diversity of maize germplasm (Ortega, 2000). The ultimate expression of maize domestication and subsequent diffusion was its diversification into numerous landraces, each of which has acquired distinct genetic and morphological characteristics mainly due to local adaptation and human selection (Vigouroux *et al*., 2008). It is now a key source of food and livelihood for millions of people in many countries of the world (Enujeke, 2013). Corn flour, corn oil, cornflake, corn syrup, popcorn, rice corn and corn soap are some popular corn products. United States, China and Brazil are the top three maize producing countries in the world producing approximately 563 of the total 717 million metric tonnes per year (Ranum *et al*., 2014). In India, which is its sixth largest producing country, maize was introduced probably during the seventeenth century by the Portuguese and now stands up as the third cash crop after wheat and rice with about 24 % of the maize produced used as human food, 16 % as animal feed, 44 % as poultry feed while about 16 % is utilized as an industrial raw material where most of the maize is processed through wet milling, producing starch as the major product, and oil, bran and gluten meal as by-products (Sanjeev *et al*., 2014). About two million tonnes of maize is used in the starch industry in the country yielding about 70,000 tonnes of maize oil an-

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nually. This figure is expected to rise to 8 million tonnes of maize by 2050 in the starch industry, which can yield about 0.3 million tonnes of oil (Hegde, 2012). The quality of the maize kernel which is widely used in both human and animal feeding is considered to be mainly related to its protein and oil concentrations. A typical maize hybrid contains 8-15% protein (Reynolds *et al*., 2005), and most of these proteins consist of prolamins. The oil concentration of widely used maize hybrids varies between 3.5-5% (Lambert, 2001, Laurie *et al*., 2004, Sanjeev *et al*., 2014); corn having oil content of more than 6% is regarded as high oil corn (Rajendran *et al*., 2012). Maize germ (embryo) is an oil-rich portion of the kernel containing 80-84% of total oil content followed by 12% in aleurone and 5% in endosperm (Rajendran *et al*., 2012). The quantity of germ in the kernel and oil in the germ is genetically controlled and varies widely (Becker, 2007). Among several corn related products, corn oil is an emerging one, increasingly becoming popular among edible oils owing to its unique health related benefits. Typical dent corn oil is tasteless and odorless containing 61.9% linoleic acid, 24.1% oleic acid, 11.0% palmitic acid, 2.0% stearic acid, 0.7% linolenic acid, and traces of other fatty acids (White *et al*., 2007).

In the north western Indian Himalayan state Jammu and Kashmir, maize is second most important crop after rice and is a staple food of tribal population (Najeeb *et al*., 2012). The crop is generally grown under rainfed conditions on marginal lands (1076 mm mean annual rainfall) particularly in hilly terrains, singly or intercropped with pulses such as common beans and green gram. Currently, the crop in the state is grown on an area of 309 thousand hectares with production of 461 thousand tonnes and productivity of 1495 kilograms/hectare against country level statistics of 9233 thousand hectares, 23673 thousand tonnes and 2564 kilograms/hectare respectively (GEOFIN, 2016). Even in presence of several high yielding varieties (Shalimar1, JMC 3), some landrace populations of maize are still popular in the farmers fields throughout the state owing to their better grain quality in terms of taste, better fodder quality, wider adaptability to local conditions, resistance to draught, cold, insects and pests, early maturity and low input requirement (Najeeb *et al*., 2012). The main aim of the present study was to evaluate protein content, oil content and fatty acid composition in maize genotypes collected from remote and hilly areas of the state. Assessment of extent of genetic variance existing in these quality traits in the local *Zea mays* germplasm may be helpful in developing nutritionally improved cultivars having industrial value as well.

MATERIALS AND METHODS

Plant material: Eleven genotypes of *Zea mays* L. collected from farmer fields during September/October 2016 in diverse hilly locations across the north western Indian Himalayan state of Jammu and Kashmir (32°17′ - 36°58′ N latitude, 73°26′ - 80°30′ E longitude) have been used in the present investigation (Table 1). At least seven cobs of each of these genotypes were randomly collected from the fields. Diversity was observed in cob length (cms), seed color, number of seed rows per cob and 100-seed weight (g) in these genotypes. After proper drying one major set of seeds was deposited in the National Gene Bank at ICAR-National Bureau of Plant Genetic Resources New Delhi for conservation and the other set was used for investigation of quality traits of protein content, oil content and fatty acid composition in the kernels. Seeds were selected from the central portion of the cobs as suggested by White and Weber (2003) to minimize the variance of oil content based on position on a cob.

Estimation of protein content: Crude protein was determined in 100 mg of finely ground kernel samples by the micro Kjeldahl method as described by Pearson (1976). The percentage crude protein content in three independent samples in each genotype was calculated as %N multiplied by the coefficient 6.25.

Estimation of oil content: Corn oil content was measured by TD NMR [Time-domain (TD) NMR bench top system (Bruker)]. The measurements were done in triplicate in each of the genotypes.

Determination of fatty acids: The oil was extracted from each sample using Soxhlet extractor following the method of AOAC (1990). 10 g sample was extracted with n-hexan at 70^{o}C for 6 hours. Extracted oil was stored at 4^{o}C to be used in fatty acid analyses by GC-FID (The Gas Chromatography-Flame Ionization Detector) carried out on Perkin Elmer Auto system XL GC equipped with FID). The oils were converted to corresponding fatty acid methyl esters (FAMEs) as follows:

The fatty acids methyl esters (FAMEs) were prepared by refluxing the oil with methanol:toluene:sulfuric acid (20:10:1) for 30 min following the literature procedure (Hammond, 1993). GC analysis of FAMEs were carried out on a Agilent 4890D Gas Chromatogram equipped with a flame ionization detector (FID) using a polyethylene glycol coated FSCAP column (30 m x 0.25 mm x 0.25 μm film thickness; Supelcowax).Hydrogen was used as the carrier gas at column head pressure of 20 psi. Each sample (0.2 μL) was injected into the injection port of the GC using a split ratio of 50:1. Temperature of the injector and detector was kept at 250^{o}C.Compound separation was achieved following a linear temperature program of 160^{o}C (1 min), 160 to 240^{o}C (2^{o}C/min), 240^{o}C (10 min), so the total run time was 51 minutes. Each sample was analyzed twice in GC. Peaks were identified by co-elution of standard methyl ester samples procured from Sigma-Aldrich in the same GC conditions. GC/MS utilized a Perkin Elmer auto system XL GC interfaced with a Turbo mass Quadrupole mass spectrometer based on the above oven temperature program. Injector, transfer line and source temperatures were 250 °C; ionization energy 70 eV; helium at 10 psi constant pressure; and mass scan range 40-500 amu. Characterization was achieved on the basis of retention time, elution order, calculated relative retention index using a homologous series of n-alkanes (C_{10}-C_{32} hydrocarbons, Polyscience Corp. Niles IL), mass spectral library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition).

Statistical Analysis: Pearson correlation coefficients for the relationships between all these quality traits were calculated using Statistical Package for the Social Sciences (SPSS) Software, version 16.

RESULTS AND DISCUSSION

Significant differences were observed for protein and oil contents in *Zea mays* genotypes during the course of our present study (Table 2). The protein content ranged from 10.7% to 18.7% while oil content varied between 2.26% and 4.80%. Highest and appreciable protein contents have been recorded in two genotypes IC-0617877 (18.7%) and IC-0617880 (17.6%). The saturated fatty acids of palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and unsaturated fatty acids of oleic (C18:1), linoleic (C18:1) and elaidic (C18:1) are the six fatty acids which have been detected and measured during our present study in maize genotypes cultivated in north western Indian Himalayan state of Jammu and Kashmir (Table 3). Considerable variation has been recorded in these fatty acids; 13.8-33.4% for palmitic acid, 21.5-48.1% for linoleic acid, 19.2-39% for oleic acid, 0.2-2.4% for elaidic acid, 2.5-8.5% for stearic acid and 0.1-6.6% for arachidic acid. Higher oleic acid content has been found in all genotypes excepting IC-0617881. Interestingly, highest oil (4.80%), palmitic acid (33.4%), stearic acid (8.5%) and arachidic acid (6.6%) contents have been recorded in this remarkably cold tolerant genotype with reddish yellow seeds containing moderate protein content of 13.8 %.

High yield is one of the main breeding goals in maize and substantial work has been done on improvement of yield as well as quality traits (Egesel *et al*., 2013). The typical maize kernel, on dry weight basis is composed of 61-78% of starch, 6-12% of proteins, 3.1-5.7% of oil, 1.0-3.0% of sugar and 1.1-3.9% of ash (Watson, 2003). In five different and recent studies on maize genotypes across the world protein content has been found to vary between 9.4-13%, 11.02-13.02%, 11.02-15.31%, 8.05-11.43% and 6.27-8.06%, while oil content has been found to vary between 3.32-4.70%, 2.56-5.57%, 4.39-9.71%, 3.80-5.02% and 2.7-5.2% respectively (Egesel *et al*., 2011, Aliu *et al*., 2012, Sanjeev *et al*., 2014, Scrob *et al*., 2014, Chibuike *et al*., 2015). In India, higher contents of oil (9.71%) and protein (15.31%) have been observed in sweet corn samples and attributed to shriveled grain texture because of an

Table 1. *Zea mays* genotypes and their collection sites in the north western Indian Himalayan state of Jammu & Kashmir used in the present study.

S. No.	Accession number	Place of collection	District	Latitude	Longitude
1	IC-0617874	Bhalra Bhaderwah	Doda	33°01′	75°41′
2	IC-0617875	Bidha	Kishtwar	33°19′	75°55′
3	IC-0617876	Gool Gulabgarh	Ramban	33°15′	75°00′
4	IC-0617877	Balot Bhaderwah	Doda	33°01′	75°42′
5	IC-0617878	Bidha	Kishtwar	33°19′	75°55′
6	IC-0617879	Batote	Ramban	33°07′	75°19′
7	IC-0617880	Ghowari Ghatha Bhaderwah	Doda	32°59′	75°42′
8	IC-0617881	Gulistan Tral	Pulwama	34°03′	75°04′
9	IC-0617882	Gutroo Tral	Pulwama	34°02′	75°05′
10	IC-0617883	Karnah	Kupwara	33°09′	76°15′
11	IC-0617884	Kralpora	Kupwara	34°29′	74°07′

Table 2. Protein and oil contents of *Zea mays* genotypes collected from north western Indian Himalayan state of Jammu and Kashmir.

S No.	Genotype	Protein content (%)	Oil content (%)
1	IC-0617874	15.6±0.5	2.26±0.07
2	IC-0617875	15.2±0.3	4.02±0.03
3	IC-0617876	15.7±0.7	3.48±0.19
4	IC-0617877	18.7±0.4	4.40±0.09
5	IC-0617878	13.6±0.8	3.47±0.07
6	IC-0617879	12.7±0.7	3.57±0.16
7	IC-0617880	17.6±0.4	4.10±0.02
8	IC-0617881	13.8±0.1	4.80±0.09
9	IC-0617882	12.2±0.7	4.58±0.19
10	IC-0617883	14.1±0.5	3.44±0.04
11	IC-0617884	10.7±0.6	3.37±0.03
	LSD at P=0.05	0.18	0.96

Table 3. Fatty acid composition **(%)** of *Zea mays* genotypes collected from north western Indian Himalayan state of Jammu and Kashmir.

S. No.	Genotype	Palmitic acid	Linoleic acid	Oleic acid	Elaidic acid	Stearic acid	Arachidic acid
1	IC-0617874	13.8	48.1	33.6	0.9	2.8	0.3
2	IC-0617875	26.3	22.2	37.4	2.4	6.5	1.4
3	IC-0617876	14.6	42.6	37.3	0.7	4.0	0.2
4	IC-0617877	14.7	44.8	34.7	0.9	3.8	0.2
5	IC-0617878	16.1	41.4	38.9	0.2	3.2	0.1
6	IC-0617879	14.5	44.6	36.5	0.6	2.5	0.2
7	IC-0617880	14.9	41.2	39.0	0.8	2.9	0.2
8	IC-0617881	33.4	21.5	19.2	1.1	8.5	6.6
9	IC-0617882	23.1	28.1	36.1	1.4	4.6	5.7
10	IC-0617883	15.3	42.0	37.1	0.9	2.9	0.2
11	IC-0617884	14.7	43.5	36.6	0.7	2.8	0.2

Table 4. Pearson correlation coefficients of fatty acid, protein and oil contents of *Zea mays* genotypes.

	Palmitic acid	Linoleic acid	Oleic acid	Elaidic acid	Stearic acid	Arachidic acid	Protein content (%)	Oil content (%)
Palmitic acid	1.000							
Linoleic acid	-.958**	1.000						
Oleic acid	-.718*	.495	1.000					
Elaidic acid	.604*	-.726*	-.111	1.000				
Stearic acid	.960**	-.903**	-.743**	.596	1.000			
Arachidic acid	.860**	-.797**	-.716*	.377	.770**	1.000		
Protein content (%)	-.159	.161	.063	.093	.018	-.270	1.000	
Oil content (%)	.651*	-.683*	-.383	.340	.635*	.660*	.103	1.000

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

increased embryo to kernel ratio (Sanjeev *et al*., 2014) which is in consonance with our study. The higher protein content in some of the genotypes especially IC -0617877 (18.7%) and IC-0617880 (17.6%) is quite appreciable and thus may serve as promising donors for this trait in maize breeding programmes. Maize kernel quality is considered to be mainly related to protein and oil concentration. Although higher oil content is a desirable trait in corn as it provides higher energy to the consumers, the oil quality in terms of fatty acid composition plays a more important role in human nutrition. Owing to its numerous health benefits, non-traditional corn oil is gaining importance in the international market; development of high oil corn with desired composition is now receiving increasing attention in India as the country is the third largest importer and consumer of edible oils (Ambika *et al*., 2012). Development of superior high oil inbred lines for commercial use is one of the major goals of today's maize improvement programmes. High oil maize with ideal fatty acid composition will offer maize growers higher profits through the Indian markets. Fatty acid contents of 9.2-12.7% (palmitic acid), 1.46-2.14% (stearic acid), 23.3-35.4% (oleic acid), 50.8-62.4% (linoleic acid) and 0.42-0.66% (arachidic acid) have been reported in normal maize oil (Egesel *et al*., 2011). In India, Sanjeev *et al*. (2014) have recorded 12.61-16.22% palmitic acid, 2.63-6.04% stearic acid, 33.54-46.61% oleic acid and 33.00-44.65% linoleic acid contents in the oil obtained from several normal and specialty maize genotypes. Thus, our findings on protein and oil content as well as fatty acid composition in the present study essentially corroborate these reports. However, in the genotypes IC-0617881 and IC -0617882 high arachidic acid content of 6.6% and 5.7% respectively has been found; much higher than reported in maize earlier. The average arachidic acid content of peanuts is 2.3% which is considered as richest source of this fatty acid. Hence these maize genotypes may serve useful donor parents for breeding genotypes rich in arachidic acid. Further, barring a single genotype IC-0617881, higher oleic acid content has been found in all other genotypes which is a useful trait. Oleic acid which has some advantages over the other fatty acids in terms of cooking and health is normally found in corn oil at a level of about 25%, and genotypes with higher levels of this fatty acid are economically more valuable (Mikkilineni and Rocheford, 2003). The high content of unsaturated fatty acid in maize oil is the main factor in its high quality (Ozcan, 2009).

Correlation coefficients between various traits studied have been given in Table 4. No correlation has been found between crude protein content and any of the fatty acids or overall oil content. However, oil content showed a significant positive correlation with palmitic, linoleic, stearic and arachidic acid contents. Regarding correlations among different fatty acids, it has been found that palmitic acid showed significant negative correlation with oleic acid (r= -0.718) and linoleic acid (r= -958) but a positive correlation with elaidic, stearic and arachidic acids. Similarly, linoleic acid has been found to be negatively correlated with elaidic, stearic and arachidic acids, while oleic acid showed a negative correlation with stearic and arachidic acids. On the other hand stearic acid has been found to be positive correlated with arachidic acid content. Considering the correlations among various fatty acids, it appears that all the fatty acids seldom contribute to that total oil production and some of them serve as precursors for compounds other than oil. Our results are thus in contrast with those of Ignjatovic-Micic *et al*. (2015) who have found significant positive correlations between maize protein and oil contents and non-significant correlation between oil content and principal fatty acids. Moreover, their study has revealed a positive correlation between palmitic and oleic acids.

Conclusion

In the present investigation, although only 11 local maize genotypes were studied but results have indicated a considerable variation existing among the genotypes not only in crude protein content but also in oil content and fatty acid composition. The genotypes identified for higher contents of protein: IC-0617877 (18.7%) and IC-0617880 (17.6%), palmitic acid: IC-0617881 (33.4%) and IC-0617875 (26.3%), stearic acid: IC-0617881 (8.5%) and IC-0617875 (6.5%) and arachidic acid: IC-0617881 (6.6%) and IC-0617882 (5.7%) may serve as promising donors for improvement of these traits in maize breeding programmes. Therefore, the local maize germplasm needs to be characterized for such quality traits on large scale for corn improvement strategies which would open up new opportunities for food and industrial end uses of this crop, ensuring nutritional and livelihood security of the tribal people in the region.

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