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Characterization of diverse provitamin: A rich inbreds for *lipoxygenase* (LOXs) gene governing oxidative deterioration in maize

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Abstract
Post-harvest deterioration of the quality of maize grain has become a serious problem worldwide. LOXs are large group of monomeric dioxygenase proteins having detrimental role on quality parameters in maize grains. Here, a comprehensive analysis was carried out on physicochemical parameters of *LOX* gene in diverse maize inbreds. *In-silico* analysis revealed differential expression of *LOX* genes in different seed parts like ear, embryo, embryo sac and endosperm. Variation was also found for sub-cellular localization of the protein. Of the 16 inbreds screened in the study through enzymatic assay, LOX activity varied from 2.63 to 9.36 units/min/mg of protein, with mean of 5.09 units/min/mg of protein. VQL1-PV was found to have the highest lipoxygenase activity, whereas HKI323 recorded the lowest LOX activity, offering potential scope in multivitamin staking in bio fortification programme along with high oil content. Identification of contrasting genotypes with differential LOX activity would help in future genetic studies.

Keywords: Maize, lipoxygenase, oil, provitamin A, storage

1. Introduction
Maize is the third most important cereal crop in the world next to the rice and wheat. Maize offers diverse end uses and of the total maize produced in India, 10% is used for human consumption, 59% for poultry and animal feed, 17% for industrial purposes and 14% for export and other purposes (Yadav *et al*., 2016) [1]. Maize grain contains 70 to 75% starch, 8–10% protein, and 4–5% oil (Boyer and Hannah 2001) [37]. Among the total oil, 80–84% is located in the embryo, 12% in the aleurone layer whereas around 5% of oil is present in the endosperm (Singh *et al.*, 2014) [2]. Corn oil is used as salad oil, cooking oil and in margarine; andit was preferred over other vegetable oils because of its stability during storage and cooking (Singh *et al.*, 2014) [2]. Normal corn inbreds generally possess high amount of starch and provides around 4% of oil, whereas high-oil corn (HOC) inbreds were identified containing more than 6% of oil (Lambert 2001) [3]. Three QTLs were identified responsible for high oil content in maize (Zhang *et al.*, 2008) [4]. Although, maize inbreds are varying in their oil content, yield of the oil from maize germ was also influenced by cooking methods used for extraction (Moreau *et al.*, 2005) [5].

As a third most important crop, maize was also bred for provitamin-A (pro A) to combat the vitamin A deficiency (VAD) (Muthusamy *et al*., 2014; Zunjare *et al.*, 2018) [6, 7]. Pro A carotenoids play a vital role for normal growth and development in humans (Burt *et al*., 2011; Zhai *et al*., 2016) [8, 9]. There are different types of carotenoids, of which β-carotene, α-carotene and β-cryptoxanthin serve as the precursor for synthesis of vitamin-A in humans (Tang 2010) [10]. Harvest Plus, a CGIAR initiative on development of micronutrient dense crops has made efforts in development and release of several bio fortified varieties in various crops in collaboration with the other crop based CGIAR institutions like CIMMYT and IITA etc. About 11 pro A-rich hybrids and/or open pollinated varieties (OPVs) developed by CIMMYT, Mexico were released in African countries like Malawi, Zambia and Zimbabwe. Around 15 proA-rich OPVs, developed by International Institute of Tropical Agriculture (IITA), Ibadan, were released in Nigeria, Ghana and DR Congo (www.harvestplus.org). One pro A synthetic has also been released from Ghana (CSIR-CRI, Honampa).

So far, more than 40 pro A maize cultivars including synthetics, single-cross hybrids, and three-way hybrids have been released in many African countries like DR Congo, Ghana, Malawi, Mali, Nigeria, Rwanda, Tanzania, Zambia, and Zimbabwe (Andersson *et al.,* 2017) [11]. Besides, 64 synthetics and 74 pro A enriched hybrids were under extensive testing in 14 African countries (Manjeru *et al.,* 2017) [12]. Research efforts at ICAR-Indian Agricultural Research Institute (IARI), New Delhi has led to the successful introgression of the favourable allele of *crtRB1 and lcyE* into elite parental inbreds using marker-assisted selection (MAS) (Muthusamy *et al*., 2014; Zunjare *et al*., 2018) [6, 7].

However, there was an adverse effect of high oil content on the pro A compounds resulting from the action of LOX enzyme due to production of free radicals. LOXs are large group of monomeric lipoxygenase proteins containing non-heme, non-sulphur, iron cofactor that oxidize a wide range of carotenoid compounds in a non- specific manner (Chedea and Jisaka2013) [13]. A total of 13 *LOX* genes were reported in maize genome showing oxygenase activity (Ogunola *et al.,* 2017) [14] whereas 64, 11 and 8 putative *LOX* genes in cotton (Shaban *et al.,* 2018) [15], tea plant (Zhu *et al.,* 2018) [15] and pepper (Sarde *et al.,* 2018) [17], respectively were reported through genome wide gene family studies. Five Rosaceae species namely pear, apple, peach, mei and woodland strawberry were investigated through genome wide studies and 23, 36, 16, 18 and 14 *LOX* genes were found in the five species, respectively (Li *et al*., 2014) [18]. LOX catalyze the oxidation of polyunsaturated fatty acids (PUFA) like linoleic, linolenic and arachidonic acid at 9- or 13-carbon position to yield free radicals and hydro peroxides (Borrego and Kolomiets 2016) [19]. Theyalso play a negative role for colour, off-flavour and antioxidant status of plant based food. During bread making lipoxygenase activity promotes greater loss of carotenoids (Leenhard *et al.,* 2006) [20]. Down-regulation of LOX enzyme activity promotes the retention of carotenoids during post-harvest storage in biofortified rice indicating LOX as an effective tool to minimize post-harvest and economic losses during storage (Gayen *et al.,* 2015) [21]. It was reported that storage tolerance of rice seeds improves through TALEN based mutagenesis of *LOX3* through the creation of null mutant (Ma *et al.,* 2015). In India, Directorate of Soybean Research, Indore developed first lipoxygenase free soybean genotypes (NRC109 and NRC110) which have higher consumer preference and acceptability due to their good storability (Kumar *et al.,* 2013) [23]. By taking everything in consideration, maize inbreds should be bred for multi-vitamins along with high oil content. Considerable efforts have been made for staking multivitamin enriched maize inbreds (Naqvi *et al.,* 2009) [24].

Therefore, identification of null mutant for LOX in the Indian maize germplasm will be remunerative to breed for inbred showing low lipoxygenase mediated oxidation during post-harvest stora*g*e. Considering the growing significance of maize as food and feed; identification of the genotypes with low lipoxygenase activity in maize grain assumes great importance in the current scenario. Comprehensive *in-silico* was also undertaken for studying different physicochemical properties of the LOX proteins along with their molecular weight, amino acid contents. As LOX proteins were working better under a certain pH range (Niu *et al*., 2015) [25], iso-electric point was determined for all 13 LOX proteins. Enzymatic study was carried out to identify the inbred lines showing lower activation of lipoxygenase activity. Identification of the genotype with low LOX activity can be used as recipient parent for staking multivitamin along with high oil and reduce antagonistic effect of LOX on high pro A in combination with high oil.

2. Materials and methods

2.1 Plant material

A set of 16 inbreds were selected for the study and the panel comprised of 11 high pro A inbreds and 5 inbreds with low pro A. The 11 high pro A includes the inbreds developed through marker-assisted selection at Maize Genetics Unit, IARI viz. VQL1-PV, VQL2-PV, V335-PV, V345-PV, HKI161-PV, HKI163-PV, HKI193-1-PV, HKI193-2-PV, HKI1105Q+PV, MGU-PV101 (Muthusamy *et al*., 2014; Zunjare *et al.,* 2018) [6, 7] and the pro A donor named HP465-30 received from CIMMYT-Harvest Plus. The five low pro A inbreds used in the study are VQL1, V335, HKI161, HKI1105 and HKI323.

2.2 *In-silico* prediction of optimal pH for lipoxygenase enzyme assay

The sequence and molecular weight of different LOX protein were obtained from Ensembl Plant database (https://plants.ensembl.org/index.html; Zerbino *et al*., 2017). The retrieved sequences were analysed for the presence of conserved domains in the sequences using Pfam database (https://pfam.xfam.org/; Finn *et al*., 2015) through HMMER program using hidden Markov Models (Eddy 2004). The Ex PASY Prot Param tool (https://web.expasy.org/protparam; Gasteiger *et al*., 2005) was used to determine the physicochemical parameters of the protein viz. amino acid length, molecular weight of the protein, iso-electric point of the protein (pI), instability index, grand average of hydropath city (GRAVY) and number of negatively and positively charged amino acids in protein of the retrieved protein sequences. The optimum pH for majority of lipoxygenase enzyme as obtained from Ex PASY Prot Param tool was used further for enzymatic assay. The CELLO server v2.5 (cello.life.nctu.edu.tw; Yu *et al*., 2006) and Target P 1.1 Server (www.cbs.dtu.dk/services/TargetP/; Emanuelsson *et al*., 2000) were used to determine the corresponding sub-cellular localization of all the proteins.

2.3 Enzyme extraction from fresh seed

Maize kernels (1 g) from sixteen different inbreds were collected immediately after harvest and ground using mortar and pestle under ice cold condition using 5 ml of 0.2 M phosphate buffer; and was centrifuged at 15000 rpm at 4ºC for 15 minutes. Supernatant from each of the inbreds were collected separately and immediately stored in -20ºC for further use. For maintaining the pH of the phosphate buffer used for crude enzyme extraction (pH 7.0), 61.50 mL of 1M $K_2HPO_4$was added with 38.50 mL of 1M KH_2PO_4.For studying the enzymatic activity pH of the buffer was maintained at 6.0 by adding 61.50 mL of 1M $K_2HPO_4$with 38.50 mL of 1M KH_2PO_4 and final volume of 500 mL was made by adding double-distilled water. Buffer solution was freshly prepared immediately before the enzyme assay.

2.4 Preparation of standard curve and quantification of protein

Protein content of sample material was determined by Bradford method (Bradford 1976) on the basis of absorbance of the protein at 595 nm in UV spectrophotometer (Bench Top Labsystem, USA). For preparation of the standard curve, crystalline bovine serum albumin (BSA) (Himedia, Mumbai) was used as a standard.

2.5 Preparation of substrate solution for enzyme assay

140 µl of linolenic acid (SRL Pvt Ltd., Mumbai), 140 µl of Tween-20 (SRL Pvt Ltd., Mumbai), were mixed in 5 ml of double distilled water. The mixture was dissolved by addition of 600 µl of 1.0M NaOH to make it transparent. Final volume of 12.5 mL was made by adding double distilled water. The substrate solution was prepared freshly to avoid the instability of the substrate mixture.

2.6 Lipoxygenase enzyme assay

Lipoxygenase activity was measured by observing increasing absorption spectrum at 234 nm due to conjugated double bond formation during the reaction. The reaction was performed by adding 100 µl of substrate solution and 1.4 mL of sodium phosphate buffer (200mM; pH at 6.0) in 1cm cuvettes. pH of the solution was determined on the basis of pI value of majority of the paralogous LOX proteins as shown in 3.4.2. Majority of the protein show active pI value at acidic pH as shown by Prot Param tool (https://web.expasy.org/protparam; Gasteiger *et al*., 2005) and as also reported by Niu *et al.*, (2015) [15] in maize. The spectrophotometer was set to zero at 234 nm using this mixture and then 100 µl of enzyme extract was added to it and stirred gently. Immediately it was transferred to spectrophotometer for taking reading at 6 second intervals for duration of 144 seconds. Absorbance value at 144 second was taken for analysis. All the experiments were carried out at room temperature (28ºC).

3. Results and discussion

3.1 Database search for expression profiling of the LOX gene family

Thirteen different *LOX* genes were reported in maize by Ogunola *et al.,* (2017) [14]. Length of the amino acid and molecular weight of the protein of the individual *LOX* gene was obtained from Ensembl Plants database. Expression profile of the *lipoxygenase* genes in different plant parts was also searched in Expression Atlas database. It was found that *LOX* genes possessed differential expression in several seed part of maize like ear, embryo, embryo sac, and endosperm. Expression level was high in ear and embryo as compared to the embryo sac and endosperm (Table 2). The paralogous genes expression also varied on the basis of whole transcriptome. It was found that *LOX10* had the highest expression profile (1255 TPM) in whole trasncriptome followed by *LOX2* (200 TPM). However *LOX3* had the highest expression level (115 TPM) in the embryo, as compared to other *lipoxygenase* gene, whereas in the embryo sac and endosperm *LOXs* genes had substantially low expression (Table 2). A comprehensive analysis in tea plant (*Camellia sinensis*) reviled that *CsLOX5*, *6* and *9* have predominant expression in seeds, flowers and roots, respectively using RNA sequencing, quantitative reverse transcription–PCR and Northern blot analysis (Zhu *et al.* 2018) [15]. Expression study in pepper (*Capsicum annuum*) revailed that 13-*LOXs*expression was more dynamic as compared to 9-*LOXs* both in response to exogenous Jasmonic acid (JA) application and to thrips feeding (Sarde *et al*., 2018) [17]. Splice variants of the maize *LOX* genes were taken from Ensembl plants database. *LOX3, LOX4* and *LOX2* had the highest number of transcripts (15, 15 and 14) as compared to other *LOX* genes (Table 2). Alternatively splicing of six *CsLOX* genes were validated in tea plant and were dynamically regulated through time and varied in relative abundances understresses (Zhu *et al.,* 2018) [15].

Maize embryo is rich in oil content as compared to other part of the seed and *LOX* genes mainly act upon the fatty acid like linoleic acid, linolenic acid, arachidonic acid (Borrego and Kolomiets 2016) [19]. Thus free radical production will be more in seed embryo due to the higher activity of *LOX3* gene as compared to the other *LOXs*. Hence, the *in-silico* analysis suggest that identification of inbreds with less LOX activity, particularly LOX3 would be of much important to breed for improving grain quality after storage. A total of 64 putative LOX genes were identified through genome wide gene family study in four cotton species (*Gossypium hirsutum, G. barbadense, G. arboreum,* and *G. raimondii)* and functional characterization using virus induced gene silencing (VIGS) approach of two candidate genes (*Gh LOX 12* and *GhLOX13*) revealed their increased sensitivity to salinity stress in target gene-silenced cotton (Shaban *et al*., 2018) [15]. A total of 11 *Cs LOX* genes were reported in tea plant (*C. sinensis*) and found to have role during development and in response to abiotic or biotic stresses in tea plants (Zhu *et al*., 2018) [15]. Using *in-silico* approaches, a total of eight *LOX s*were identified in pepper and phylogenetic analysis categorized these eight *LOXs* in two classes viz., four *LOXs*(*CaLOX1*, *CaLOX3*, *CaLOX4* and *CaLOX5*) as *9-LOXs* and four (*CaLOX2*, *CaLOX6*, *CaLOX7* and *CaLOX8*) as 13-*LOXs* (Sarde *et al*., 2018) [17]. Although 13 *LOX* genes were found in maize genome, their role with respect to the storability is still unknown making a scope for further study.

Table 1: Details of different *lipoxygenase* (*LOX*) genes in maize

Gene name	Gene ID	Length(AA)	Mol. Weight (Dal)	Chr.
ZmLOX1	Zm00001d042541	185	19893.71	3.06
ZmLOX2	Zm00001d042540	697	79180.92	3.06
ZmLOX3	Zm00001d033623	867	96734.52	1.09
ZmLOX4	Zm00001d033624	887	100363.64	1.09
ZmLOX5	Zm00001d013493	887	100356.96	5.0
ZmLOX6	Zm00001d002000	892	97395.23	2.02
ZmLOX7	Zm00001d025524	929	103603.70	10.04
ZmLOX8 Tassel seed1	Zm00001d003533	941	105306.72	2.04
ZmLOX9	Zm00001d027893	968	108271.97	1.02
ZmLOX10	Zm00001d053675	905	102055.14	4.09
ZmLOX11	Zm00001d015852	449	48234.79	5.04
ZmLOX12	Zm00001d041204	850	95992.19	3.04
ZmLOX13	Zm00001d031449	956	105332.77	1.0

AA: Amino Acid; Dal: Dalton; Chr: Chromosome

Table 2: Expression level of different *lipoxygenase genes* (*LOXs*) in maize seed

S. No.	Gene	Ear (TPM)	Embryo (TPM)	Embryo Sac (TPM)	Endosperm (TPM)	Whole organism (TPM)	Number of transcript
1	*LOX1*	3	0.5	bc	bc	96	7
2	*LOX2*	18	2	2	2	200	14
3	*LOX3*	5	115	bc	3	64	15
4	*LOX4*	17	12	5	0.9	25	15
5	*LOX5*	46	18	1	1	109	8
6	*LOX6*	12	3	0.6	1	128	8
7	*LOX7*	bc	3	-	bc	2	2
8	*LOX8*	1	2	4	bc	6	2
9	*LOX9*	12	14	-	bc	15	4
10	*LOX10*	1	0.7	bc	bc	1255	6
11	*LOX11*	5	2	bc	0.8	95	8
12	*LOX12*	bc	bc	bc	1	18	3
13	*LOX13*	bc	bc	bc	bc	5	2

LOX: *Lipoxygenase*; TPM: transcript per million; bc: below cutoff

Table 3: Summary of the domains of the different Lipoxygenase (LOX) protein

Gene	pI	Instability index	GRAVY	Cellular localization	*(-)ve AA*	*(+)ve AA*	Description of the domain
ZmLOX1	7.86	10.99	0.014	Chloroplastic (1.189), Extra-cellular (1.189)	15	16	PLAT/LH2 domain(58-165)
ZmLOX2	6.16	44.51	-0.448	Cytoplasmic (2.205)	88	79	Lipoxygenase (4-675)
ZmLOX3	5.76	33.93	-0.321	Cytoplasmic (2.795)	106	89	Lipoxygenase (171-844), PLAT/LH2 domain (51-158)
ZmLOX4	6.18	42.53	-0.446	Cytoplasmic (2.990)	117	104	Lipoxygenase (177-863), PLAT/LH2 domain (58-164)
ZmLOX5	6.35	39.91	-0.430	Cytoplasmic (3.040)	115	105	Lipoxygenase (177-863), PLAT/LH2 domain (58-164)
ZmLOX6	5.61	39.04	-0.292	Cytoplasmic (1.559), Chloroplast (1.101)	114	95	Lipoxygenase (212-312), Lipoxygenase(345-875)
ZmLOX7	6.69	51.76	-0.420	Mitochondrial (2.020)	117	113	Lipoxygenase (225-912), PLAT/LH2 domain (137-212)
ZmLOX8	6.82	53.16	-0.443	Mitochondrial (2.048)	120	117	Lipoxygenase (232-924), PLAT/LH2 domain (135-219)
ZmLOX9	8.71	50.69	-0.328	Mitochondrial (2.083), Nuclear (1.140)	110	119	Lipoxygenase (237-692), Lipoxygenase (706-951) ,PLAT/LH2 domain (139-224)
ZmLOX10	6.11	42.01	-0.388	Cytoplasmic (1.951), Chloroplastic (1.247), Mitochondrial (1.003)	123	108	Lipoxygenase (220-888), PLAT/LH2 domain (116-207)
ZmLOX11	11.89	71.82	-0.994	Nuclear (3.313)	41	84	No hit found
ZmLOX12	8.21	48.08	-0.440	Mitochondrial (1.681)	104	107	Lipoxygenase (165-830), PLAT/LH2 domain (50-152)
ZmLOX13	6.27	51.69	-0.328	Chloroplastic (2.306)	116	107	Lipoxygenase (269-939)

pI: Iso electric point; GRAVY: Grand Average of Hydropath city; AA: Amino Acid

3.2 *In-silico* analysis of LOX protein

Protein sequences of the LOX protein were taken from Ensembl Plants database and conserved domains of the proteins were confirmed by Pfam through HMMER using hidden Markov Models. All the lipoxygenase protein contain PLAT or lipoxygenase domain, however no domain was found in case of *LOX11* in the HMMER programme. Summary of the domain of the protein has been tabulated in the table 3. The Prot Param tool was used to determine the physicochemical parameters of the protein. The amino acid length, molecular weight of the protein, iso-electric point of the protein, instability index, grand average of hydropath city (GRAVY) and number of negatively and positively charged amino acids in protein of lipoxygenase protein has been tabulated (Table 3). Sub-cellular localization of the proteins as determined by CELLO v2.5 and Target P 1.1 Server was also tabulated, among which four genes (*Zm00001d002000*, *Zm00001d027893*, *Zm00001d015852* and *Zm00001d031449*) are translocate in the mitochondria and three genes (*Zm00001d025524*, *Zm00001d003533* and *Zm00001d053675*) were translocate in chloroplast, whereas it could not predict the localization of the other protein at a threshold limit. Zhu *et al.* 2018 [15] in tea reported that CsLOX2 and CsLOX9 proteins were localized in chloroplasts and the cytoplasm, respectively by tagging with green fluorescent protein (GFP) fusion proteins. There was a considerable variation in size and molecular weight of the Zm LOX protein. It was found that majority of the lox enzyme worked better at slightly acidic pH whereas ZmLOX1, ZmLOX9, ZmLOX11and Zm LOX 12 worked under alkaline pH. On this basis, pH of the buffer was maintained at acidic level for enzymatic study.

3.3 Quantification of enzymatic activity

Standard curve was prepared using BSA and the diluted samples were taken for the preparation of the standard curve with a regression of 0.994 which implied its reliability for further analysis. The prepared standard curve was used for determination of protein concentration of the unknown samples. Specific activity of the protein was retrieved from OD change at 144 sec duration corresponding to total protein concentration of the genotype. The total protein concentration along with their absolute and specific activity of the LOX

protein in a set of genotypes is presented in Table 4. Among the 16 inbreds screened in the study, lipoxygenase activity varied from 2.63 to 9.36 units/min/mg of protein, with a mean of 5.09 units/min/mg of protein. VQL1-PV was found to have the highest lipoxygenase activity i.e. 9.36 units/ min/ mg of protein, followed by VQL2-PV (6.85 units/ min/ mg of protein), V335-PV (6.77 units/ min/ mg of protein), VQL1 (6.71 units/ min/ mg of protein) and HKI161 (6.18 units/ min/ mg of protein) (Table 5). Inbreds, HKI323 (2.35 units/ min/ mg of protein), HP465-30 (2.63 units/ min/ mg of protein), HKI1105Q+PV (2.81 units/ min/ mg of protein) recorded low LOX activity. Enzymatic activity of lipoxygenase (LOX) gradually increased from day two of germination in green gram seeds and reached at optimum level on day 4 and declined thereafter in germinating (Deshpande *et al*., 2015) [34]. Cloning of the full length of lipoxygenase cDNA (*VrLOX*) was done from green gram germinating seedlings and enzymatic assay revealed more LOX activity at day 3 at pH 6.5 (Kotapati *et al*., 2015) [35]. *In-silico* analysis predicted the putative functions of two *13-LOXs* namely*CaLOX6* and *CaLOX7* in pepper in the biogenesis of green leaf volatiles which were involved in defence indirectly (Sarde *et al*., 2018) [17]. Down-regulation of lipoxygenase enzyme activity promotes the higher retention of carotenoids during post-harvest storage in bio fortified rice indicating *LOX* as an effective tool to minimize huge post-harvest and economic losses during storage (Gayen *et al*., 2015) [21]. Hence, inbreds with high pro A and low LOX activity like HP465-30 and HKI1105Q+PV can be used as a potential donor germplasm in the maize proA bio fortification programme. Free radicals was produced due to action of the *LOX* genes which act on wide range of carotenoids to cleave them (Chedea and Jisaka 2013) [13]. Co-oxidation of carotenoids compounds was also reported in tomato fruits due to production of free radicals (Allen and Gramshaw 1996) [36]. Thus this study would help in developing high oil and proA rich maize genotypes with less LOX activity, so as to prevent the oxidative loss of proA in the maize kernel. However, functional validation of the results is required for further confirmation.

Table 4: Specific activity of lipoxygenase enzyme in a set of maize inbreds

S. No.	Genotype	Protein concentration (µg/ml)	Specific activity (Units/min/mg of protein)
1	VQL1-PV	560.45	9.36
2	VQL2-PV	479.00	6.85
3	V335-PV	550.60	6.77
4	V345-PV	560.28	5.88
5	HKI161-PV	782.66	4.20
6	HKI163-PV	710.04	4.18
7	HKI193-1-PV	881.93	3.06
8	HKI193-2-PV	829.21	4.93
9	HKI1105Q+PV	948.22	2.81
10	MGU-PV-101	678.97	4.53
11	HP465-30	827.68	2.63
12	VQL1	654.52	6.71
13	V335	627.39	5.78
14	HKI161	677.05	6.18
15	HKI1105	629.64	5.27
16	HKI323	601.30	2.35

4. Conclusion

Differential expression of various *LOX* genes in different parts of the seed as revealed by the *in-silico* analysis identified *LOX3* as the potential gene with highest activity in embryo. The study also identified variation for sub-cellular localization of the protein and other physic-chemical properties. Inbreds with low *LOX* activity and high pro A would serve as a potential donor in the maize bio fortification programme. Identified inbreds can also be used for multi-vitamin staking along with high oil.

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6. References

1. Yadav OP, Prasanna BM, Yadava P, Jat SL, Kumar D, Dhillon BS *et al*. Doubling maize (*Zea mays*) production of India by 2025 - Challenges and opportunities. Indian Journal of Agricultural Sciences. 2016; 86(4):427-434.
2. Singh N, Vasudev S, Yadava DK, Chaudhary DP, Prabhu KV. Oil improvement in maize: potential and prospects. In Maize: Nutrition Dynamics and Novel Uses. Springer, New Delhi, 2014, 77-82.
3. Lambert R. High-oil corn hybrids. In: Hallauer A (ed) Specialty corns, 2nd edn. CRC Press, Boca Raton, 2001, 131–154.
4. Zhang J, XQ Lu, XF Song, JB Yan, TM Song, JR Dai *et al*. Mapping quantitative trait loci for oil, starch, and protein concentrations in grain with high-oil maize by SSR markers. Euphytica. 2008; 162(3):335-344.
5. Moreau RA, Johnston DB, Hicks KB. The influence of moisture content and cooking on the screw pressing and prepressing of corn oil from corn germ. Journal of the American Oil Chemists' Society. 2005; 82(11):851-854.
6. Muthusamy V, Hossain F, Thirunavukkarasu N, Choudhary M, Saha S, Bhat JS *et al*. Development of β-carotene rich maize hybrids through marker-assisted introgression of β-carotene hydroxylase allele. PLoS one. 2014; 9(12):113583.

7. Zunjare RU, Hossain F, Muthusamy V, Baveja A, Chauhan HS, Bhat JS *et al*. Development of Biofortified Maize Hybrids through Marker-Assisted Stacking of β-carotene hydroxylase, lycopene-ε-cyclase and opaque2 Genes. Frontiers in Plant Science. 2018; 9:178.
8. Burt AJ, Grainger CM, Young JC, Shelp BJ, Lee EA. Impact of postharvest handling on carotenoid concentration and composition in high-carotenoid maize (*Zea mays* L.) kernels. Journal of Agricultural and Food Chemistry. 2010; 58(14):8286-8292.
9. Zhai SN, Xia XC, He ZH. Carotenoids in Staple Cereals: Metabolism, Regulation, and Genetic Manipulation. Frontiers in Plant Science. 2016; 7:1197.
10. Tang G. Bioconversion of dietary provitaminA carotenoids to vitamin A in humans. American Journal of Clinical Nutrition. 2010; 91(5):1468-1473.
11. Andersson MS, Saltzman A, Virk PS, Pfeiffer WH. Progress update: crop development of biofortified staple food crops under Harvestplus. African Journal of Food, Agriculture, Nutrition and Development. 2017; 17(2):11905-11935.
12. Manjeru P, Biljon AV, Labuschagne M. The development and release of maize fortified with provitamin a carotenoids in developing countries. Critical Reviews in Food Science and Nutrition. 2017. https://doi.org/10.1080/10408398.2017.1402751.
13. Chedea VS, Jisaka M. Lipoxygenase and carotenoids: A co-oxidation story. African Journal of Biotechnology. 2013; 12(20):2786-2791.
14. Ogunola OF, Hawkins LK, Mylroie E, Kolomiets MV, Borrego E, Tang JD *et al*. Characterization of the maize lipoxygenase gene family in relation to aflatoxin accumulation resistance. PLoS One. 2017; 12(7):0181265.
15. Shaban M, Ahmed MM, Sun H, Ullah A, Zhu L. Genome-wide identification of lipoxygenase gene family in cotton and functional characterization in response to abiotic stresses. BMC genomics. 2018; 19(1):599.
16. Zhu J, Wang X, Guo L, Xu Q, Zhao S, Li F *et al*. Characterization and alternative splicing profiles of lipoxygenase gene family in tea plant (*Camellia sinensis*). Plant and Cell Physiology. 2018; 59:1765–1781.
17. Sarde SJ, Kumar A, Remme RN, Dicke M. Genome-wide identification, classification and expression of lipoxygenase gene family in pepper. Plant molecular biology. 2018; 98(4-5):375-387.
18. Li M, Li L, Dunwell JM, Qiao X, Liu X, Zhang S. Characterization of the *lipoxygenase* (*LOX*) gene family in the Chinese white pear (*Pyrus bretschneideri*) and comparison with other members of the Rosaceae. BMC genomics. 2014; 15(1):444.
19. Borrego EJ, Kolomiets MV. Synthesis and functions of jasmonates in maize. Plants. 2016; 5(4):41.
20. Leenhardt F, Lyan B, Rock E, Boussard A, Potus J, Chanliaud E *et al*. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during bread making. Journal of Agricultural and Food Chemistry. 2006; 54(5):1710-1715.
21. Gayen D, Ali N, Sarkar S, Datta S, Datta K. Down-regulation of *lipoxygenase* gene reduces degradation of carotenoids of golden rice during storage. Planta. 2015; 242(1):353-363.
22. Ma L, Zhu F, Li Z, Zhang J, Li X, Dong J *et al*. TALEN-based mutagenesis of *lipoxygenase* (*LOX3*) enhances the storage tolerance of rice (*Oryza sativa*) seeds. PLoS ONE. 2015; 10(12):0143877.
23. Kumar V, Rani A, Rawal R, Husain SM. Lipoxygenase-2-free Indian soybean (*Glycine max* L.) genotypes. Current Science. 2013; 104:586-587.
24. Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J *et al.* Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proceedings of the National Academy of Sciences. 2009; 106(19):7762-7767.
25. Niu L, Li D, Liu C, Liu F. Comparison of lipoxygenase activity characteristics in aqueous extracts from milk-stage sweet corn and waxy corn. Food Science and Biotechnology. 2015; 24(3):867-873.
26. Zerbino DR, Achuthan P, Akanni W, Amode MR, Barrell D, Bhai J *et al*. Ensembl 2018. Nucleic Acids Research. 2017; 46(1):754-761.
27. Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL *et al*. The Pfam protein families database: towards a more sustainable future. Nucleic Acids Research. 2015; 44(1):279-285.
28. Eddy SR. What is a hidden Markov model? Nature Biotechnology. 2004; 22(10):1315-1316.
29. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the Ex PASy server. In The Proteomics Protocols Handbook, 2005, 571-607.
30. Yu CS, Chen YC, Lu CH, Hwang JK. Prediction of protein subcellular localization. Proteins: Structure, Function, and Bioinformatics. 2006; 64(3):643-651.
31. Emanuelsson O, Nielsen H, Brunak S, Von Heijne G. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. Journal of Molecular Biology. 2000; 300(4):1005-1016.
32. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72(1-2):248-254.
33. Papatheodorou I, Fonseca NA, Keays M, Tang YA, Barrera E, Bazant W *et al*. Expression Atlas: gene and protein expression across multiple studies and organisms. Nucleic Acids Research. 2017; 46(1):246-251.
34. Deshpande T, Padma AS, Sukumaran MK, Rajani D. Enzymatic Activity Levels of Lipoxygenase (LOX) in Germinating Green Gram Seeds (*Vigna radiate*). Int. J Curr. Microbiol. App. Sci. 2015; 4(8):621-628.
35. Kotapati KV, Palaka BK, Anangi R, Kedam T, Ampasala DR. Cloning and characterization of lipoxygenase gene from germinating seedlings of green gram (*Vigna radiata* L.). Indian Journal of Plant Physiology. 2015; 20(4):345-352.
36. Allen CL, Gramshaw JW. The co-oxidation of carotenoids by lipoxygenase in tomatoes. Special Publication-Royal Society of Chemistry. 1996; 197:32-37.
37. Boyer CD, Hannah LC. Kernel mutants of corn: Specialty Corns. Second Edition CRC press, 2001, 13-44.
38. Li M, Li L, Dunwell JM, Qiao X, Liu X, Zhang S. Characterization of the *lipoxygenase* (*LOX*) gene family in the Chinese white pear (*Pyrus bretschneideri*) and comparison with other members of the Rosaceae. BMC genomics. 2014; 15(1):444.