THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-34-December -2019

# Biochemical and molecular analysis of Indian durum wheat genotypes for lipoxygenase activity and yellow pigment content

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## ABSTRACT

*Wheat is a key crop mainly used for human consumption through a range of wheat based end products. Lipoxygenase activity is correlated with quality characters of wheat viz., physical characteristics of flour and colour of grain. Lipoxygenase activity (LOX) in wheat grains is related to unwanted discoloration of end products based on wheat. To increase end user acceptability, various strategies were adopted to screen genotypes with low LOX activity and high yellow pigment content (YPC). 33 different Indian durum wheat genotypes were analyzed for LOX activity and YPC. Further validation was done using PCR based STS markers viz. LOX16, LOX 18 and YP7A. The biochemical data reveals that highest LOX activity was found in wheat genotype A28 (29.17 units $min^{-1}$ $gm^{-1}10^3$) and lowest in genotype RAJ911 (0.09 units $min^{-1}$ $gm^{-1}$103). Further, high YPC was found in wheat genotype PDW233 (11.90 ppm) and lowest in case of HI7483 (3.29 ppm). A non-significant correlation was observed for LOX activity and YPC (r2=0.0018), indicating that yellow pigment content is independent of LOX activity. STS markers, LOX16, LOX18 and YP7A were used to find the presence of TaLox-B1a, TaLox-B1b and PsyA1 alleles in different durum wheat genotypes. Majority of wheat genotypes were found to possess TaLox-B1b that signifies low LOX activity. Further, 17 genotype amplified a fragment of 194-bp and have PsyA1a allele for high YPC. It can be concluded from present study that LOX16, LOX18 and YP7A are the proficient and stable markers for the evaluation of LOX activity and YPC and may be used in crop improvement programs.*

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*Keywords: Durum wheat, LOX, yellow pigment content, STS markers*

## Introduction

In India, the second most important winter cereal crop after rice is wheat. It serves as a source of high protein and energy as compared to the various other cereal crops. India ranks only second in the world in wheat production next to China. The world produces about 731.9 million tons of wheat annually out of which 80.8 million tons are produced by India itself which comprises 35% of the total annual production (National Agricultural Science Centre, New Delhi, 2016).

India contributes significantly in the production of durum wheat. Durum wheat (*Triticum turgidum* L. var. durum) is an allotetraploid species. Its peculiar features have made it the most promising raw material for pasta production. In addition to pasta, other products made from durum wheat flour are leavened and unleavened bread, couscous, burghul wheat etc. Wheat based end product quality is significantly influenced by flour colour. Flour colour in turn is dependent on the activity of enzymes like Polyphenol oxidase (PPO) and Lipoxygenase (LOX). Other documented durum wheat quality characteristics are kernel vitreousness, quality and content of protein as well as β-carotene content (Troccoli et al., 2000; Ficco et al., 2014).

The deep yellow colour of pasta, an important end-use quality trait, is contributed by the presence of carotenoid pigments in the grain (Borrelli et al., 1999, 2003, 2008; Troccoli et al., 2000, Elouafi et al., 2001, Cenci et al., 2004). Quality parameters such as wheat physical properties of flour and grain color are correlated with lipoxygenase activity. LOX is an enzyme which is responsible for catalyzing the oxidation of free and esterified linoleic and linolenic acids as well as the bleaching of carotenoid pigment (Faubion and Hoseney, 1981; Gelinas et al.,

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1998). In the last 5-6 years, eating habits of Indian youngsters is deviated more from having traditional wheat based food products like chapattis and bread to eating pasta and noodles. Further, as they contribute as the major percentage of end product consumers, so there is need to grow wheat varieties having low LOX activity and high yellow pigment content keeping in view the consumers' preference (Atienza et al., 2007). This issue is of extraordinary worry for food enterprises as it influences the wholesome quality, nutritional value and appearance and therefore thus diminishes the buyer's adequacy. Now a day's emphasis is thus given on development and selection of wheat cultivars with desirable quality traits. Developing wheat cultivars with low LOX activity utilizing proficient and stable markers is probably the most ideal approaches to decrease the bothersome and unwanted discoloration of the end product. Various efficient biochemical and molecular approaches can be used to characterize Indian wheat genotypes possessing low LOX activities in addition to having more yellow pigment content. Marker assisted selection is one of the reliable and reproducible approaches that is usually employed for the characterization purposes. An integrated approach can thus be employed for the better characterization of available Indian wheat germplasm for the abovementioned objective.

As of now, very few scientists have worked on estimation of LOX activity in wheat genotypes (Borrelli et al., 1999; Gokmen et al., 2007; Pastore et al., 2000; Fu et al., 2013). Literature shows that genes residing on homologous group 4 and 5 chromosomes are those which primarily governs the lipoxygenase activity (Verlotta et al., 2010; Feng et al., 2012). Out of various markers, STS markers for LOX genes (LOX 16 and LOX 18) and yellow pigment content viz., YP7A, YP7A-2, YP7B-1, YP7B-2 are the most reliable ones. These STS markers facilitate the selection of genotypes for low LOX activity and high YPC.

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Therefore, keeping in view the consumer preference for the quality of end products, the present study was thus planned for selection of wheat genotypes with low LOX activity and high yellow pigment content using biochemical assays and reliable markers, as marker assisted selection is the most reliable and time saving method for the above mentioned purpose.

## MATERIAL AND METHODS

Grain samples of 33 Indian durum wheat genotypes were used for the present study. The contribution of Germ Plasm Unit, Indian Institute of Wheat and Barley Research, Karnal, India for providing the seed samples is highly acknowledged. Lipoxygenase activity was determined using a method given by Geng et al. (2011) with slight modifications. Briefly, hydroperoxidation of substrate linoleic acid catalyzed by lipoxygenase was estimated by an increase in absorbance at 234 nm using spectrophotometer. For initiation, 0.2 ml of already prepared enzyme solution was injected into a cuvette having 50µl of substrate solution and 2.0 ml of phosphate buffer. 50µl of substrate solution mixed with 2 ml of buffer served as a blank. The amount of enzyme that brings about a change of one unit in absorbance at 234 nm per minute is taken to express one unit of LOX activity (Shiiba et al., 1991). Yellow pigment content in durum wheat seeds was determined by a protocol proposed by Blanco et al. (2011) with minor modifications. Further, CTAB method was followed for genomic DNA extraction (Doyle and Doyle, 1990).

### STS analysis

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The STS markers used for amplification of desired alleles for wheat LOX genes and YPC genes to study lipoxygenase activity and yellow pigment content, respectively, are given in Table 1.

Table 1: STS markers and primer sets for wheat LOX genes and YPC genes used in the present study.

| Markers | Primer sequence | Gene/allele size | Size | Reference |
|---|---|---|---|---|
| LOX 16 | Forward CCATGACCTGATCCTTCCCTT Reverse GCGCGGATAGGGGTGGT | TaLox-Bla | 489 | Geng et al., 2012 |
| LOX 18 | Forward ACGATGTGAGTTGTGACTTGTA Reverse GCGCGGATAGGGGTGC | TaLox-Blb | 791 | Geng et al., 2012 |
| YP7 A | Forward GGACCTTGCTGATGACCGAG Reverse TGACGGTCTGAAGTGAGAA TGA | PsyA1a | 194 | He et al., 2008 |
| | | PsyA1b | 231 | |

The PCR products of LOX DNA and YPC DNA were separated by electrophoresis on a 1.5% and 2% agarose gel, respectively. The bands were visualized under UV-transilluminator after staining with ethidium bromide.

## Result and Discussion

### Estimation of lipoxygenase activity

The activity of lipoxygenase has been investigated by number of research groups due to its significant involvement in natural pigment degradation in wheat flour and bleaching of pasta products produced from high LOX germplasm (Geng et al., 2011; Fu et al., 2013). Measuring LOX activity and selecting for low LOX germplasm in early generation has become an important objective in wheat breeding programs. LOX assay is predictive of bleaching in pasta products.

LOX activity in 33 Indian durum wheat genotypes was estimated. An increase in absorbance at 234nm $min^{-1}$ $gm^{-1}$ of protein under assay conditions is taken to express one unit of LOX activity. The LOX activity of 33 wheat genotypes is shown in Figure: 2 and 3. The range of LOX activity in 33 Indian wheat genotypes is found to be between 0.09 to 29.17 unit $min^{-1}$ $gm^{-1}10^3$. The highest LOX activity was found in wheat genotype A28 (29.17 unit $min^{-1}$ $gm^{-1}10^3$) and lowest LOX activity was observed in RAJ911 (0.09 unit $min^{-1}$ $gm^{-1}10^3$). It can be clearly depicted from Figure 2 and 3 that out of 33 wheat genotypes, 10 genotypes (DWR185, HD4502, HD4530, MACS2846, NIDW295, PDW215, RAJ1555, RAJ911,WH92, AKDW2997-16) showed low LOX activity, 12 genotypes ( WH896, PDW233, PBW34, NIDW295, N59, MACS1967, HI8381, HD4672, GW1, GW2, DWR1006, BAXI288-18) showed moderate LOX activity and rest of the 11 genotypes (A28, BIJAGA YELLOW, DWL5023, DWR137, GW1139.

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HI7483, HI8498, JNK-4W-184, JU12, MACS9, NIDW15) showed high LOX activity. The results revealed that 30% of durum wheat genotypes show significantly low LOX activity

Statistically significant differences ($p < 0.05$) were obtained among the tested wheat genotypes for LOX. Similar studies were done on 71 wheat genotypes for LOX activity by Simone et al. (2010), where the genotypes were identified for low LOX activity (Gokemen et al., 2007; Simone et al., 2010).

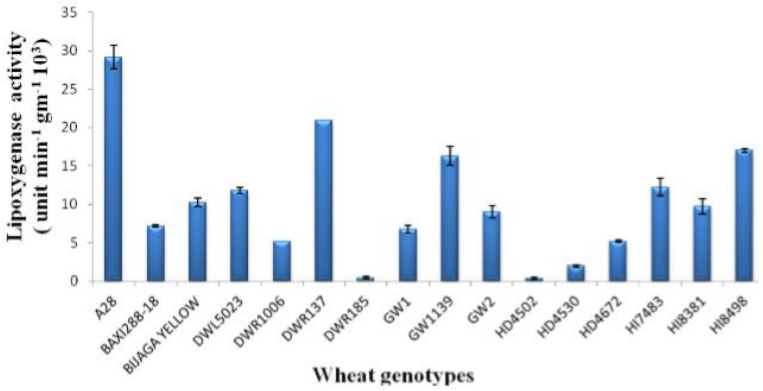


Figure 2: Lipoxygenase activity in different wheat genotypes.

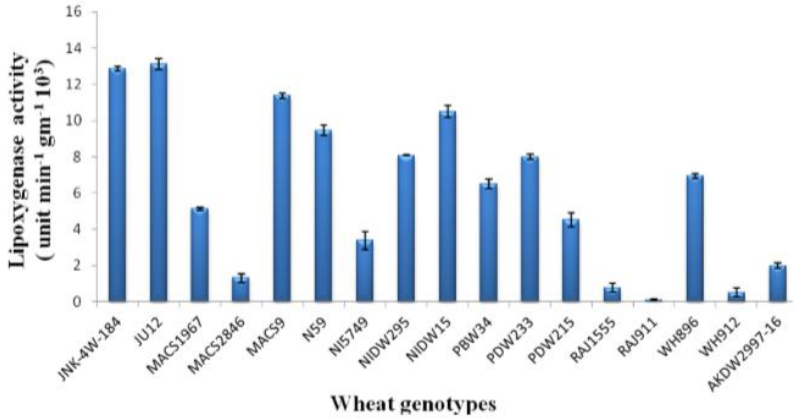


Figure 3: Lipoxygenase activity in different wheat genotypes.

Determination of Yellow pigment content

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The amount of yellow pigment content (YPC) in all wheat genotypes were chosen for study using AACC 14- 50 approved methods. The yellow pigment content in Indian durum wheat varieties is shown in Figure-4 and 5. Out of 33 wheat genotypes taken for study, the range of YPC is found to be between 3.29 to 11.90 ppm of wheat flour. The highest YPC was found in the extract of wheat genotype PDW233 where as lowest in case of HI7483 with 11.90 and 3.29 ppm, respectively. Among 33 wheat genotypes, 15 genotypes(DWL5023, DWR 137, DWR185, GW1139, GW2, HD4502, HD4530 HI7483, HI8498, JNK-4W-184, JU12, MACS2846, N59, PBW34, RAJ911) showed low YPC, 10(BAXI288-18, BIJAGA YELLOW, GW1 HI8381, MACS1967, NI5749, NIDW15, NIDW295, PDW215, RAJ1555) genotypes showed moderate YPC and rest of the 8 genotypes (A28, DWR1006, HD4672, MACS9, PDW233,WH896, WH912, AKDW2997-16) showed high YPC. (Figure-4 and 5). 24% of the total durum wheat genotypes showed high YPC content. Statistically significant differences ($p<0.05$) were obtained among the tested wheat genotypes for YPC. The range of mean values also varies greatly among the genotypes. Our results are in confirmation with the results obtained by Santra et al. (2006). They analyzed 71 different Indian wheat genotypes for YPC and the results revealed that most of the durum wheat genotypes have lower YPC in a range of 3-6 ppm with an average value of 4.5 ppm. The genotypes having high YPC are JNK-4W-184, MCS-9, PDW233, RAJ 6496, Bijapur 370-4. This study will enable wheat breeders in selection of parental lines for crossing in breeding program to develop high YPC cultivars.

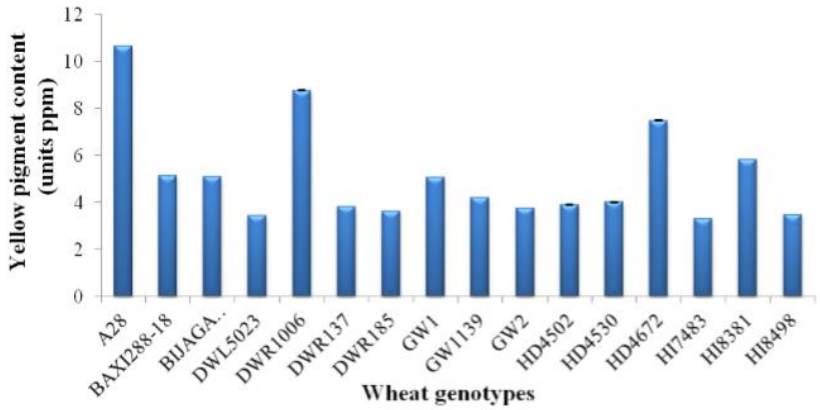


Figure 4: Yellow pigment content in different wheat genotypes.

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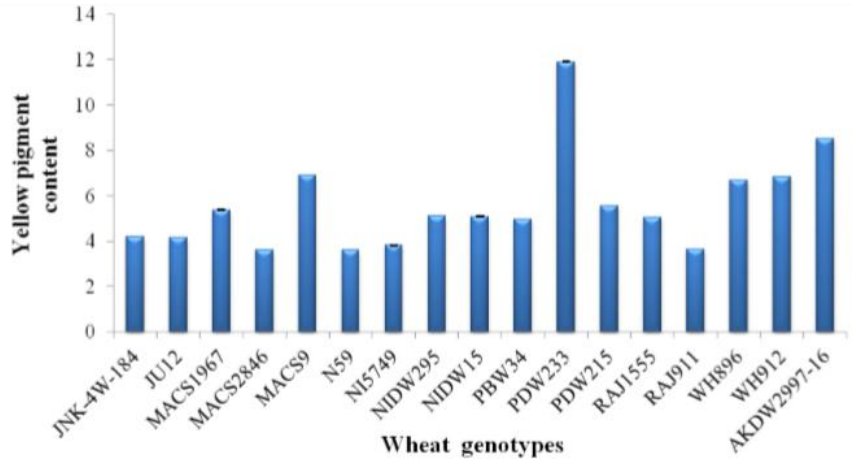


Figure 5: Yellow pigment content in different wheat genotypes.

To understand the relationship among LOX activity and yellow pigment content, correlation analysis was established based on Pearson correlation. Non significant correlation was observed for LOX activity and yellow pigment content (r2= 0.018), indicating that yellow pigment content is not dependent on LOX activity.

STS analysis

Application of functional markers is especially important for an accurate discrimination of different alleles in marker assisted selection. Many studies have independently shown that LOX activity is associated with homologous group 4 chromosomes and YPC is associated with homologous group 7 chromosomes. The functional markers for LOX genes on chromosomes 4BS and for YPC on chromosomes 7AL have been developed based on DNA sequences in Genbank. In the present study, two dominant complementary functional markers, LOX16 and LOX18 on chromosome 4BS and a co-dominant marker,YP7A on chromosome 7AL, were tested for their occurrence in 33 Indian durum wheat genotypes. The STS marker LOX16 amplified a 489-bp fragment corresponding to TaLox-B1a allele from cultivars with high LOX activity. In contrast LOX18 amplified a 791-bp fragment corresponding to TaLox-B1b allele from cultivars with low LOX activity. The functional marker YP7A yielded 194-bp with allele PsyA1a and 231-bp fragments with allele PsyA1b from cultivars with high and low YPC content, respectively (Figure-7).

It can be clearly depicted from Figure 6 that many of durum wheat genotypes amplified a fragment of 791-bp with LOX 18. The result signifies that majority of durum wheat genotypes taken for study has low LOX activity as is also shown by LOX assay. Our result in confirmation with the results obtained by Geng et al., 2012.The results revealed that majority of the durum wheat genotypes showed low LOX activity and similar kind of banding pattern was obtained. This further confirms that these kinds of markers can be used for the selection genotypes with low LOX activity and high YPC.

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In addition toYP7A some other studies were performed with wheat genotypes using dominant functional markers YP7B-2, YP7B-3, YP7B-4, YP2D-1 and co-dominant functional markers YP7B-1, YP7D-1, YP7D-2 in cultivars with low and high YPC.

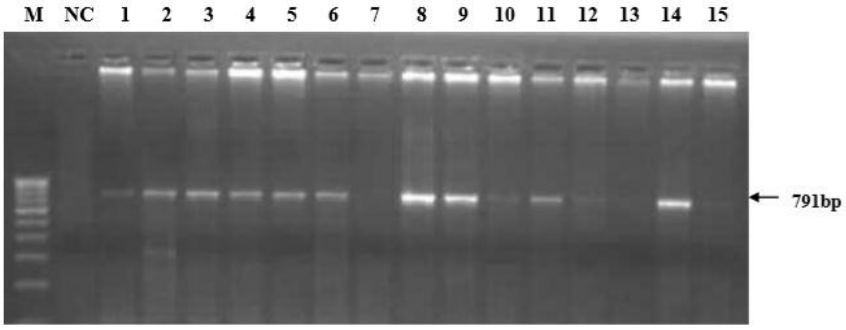


Figure 6: PCR fragments amplified by LOX18 in genotypes with low LOX activity. M DNA ladder 100-bp, NC-Negative control, 01-MACS9, 02-MACS1967, 03-PDW215, 04-PDW233, 05-RAJ911, 06PDW274, 07-RAJ1555, 08-DWR185, 09-WH896, 10-JU12, 11-GW1, 12-GW2, 13-DWR1006, 14WH912, 15-PDW291.

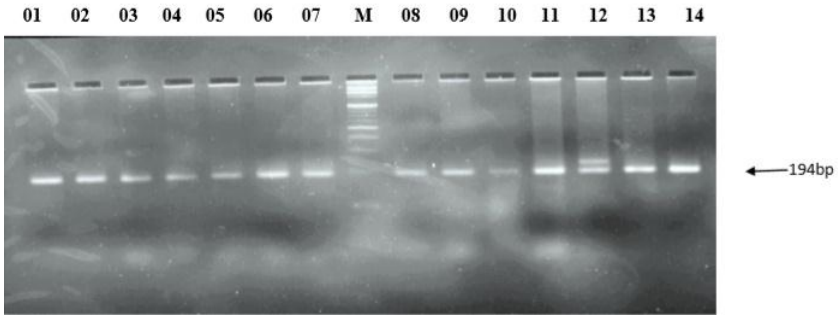


Figure 7: PCR fragments amplified by YP7A in genotypes with high alleles YPC .M ladder 100-bp, 01-PDW233, 02-WH896, 03-GW1139, 04-RAJ911, 05-MACS9, 06-WH912, 07-HI8496, 08PBW215, 09-HI8381, 10-DWL5023, 11-RAJ1555, 12-PBW34, 13-N59, 14-NIDW15

From the results it can be concluded that genotypes MACS9, MACS1967, PDW215, PDW233, RAJ911, PDW274, RAJ1555, DWR185, WH896, JU12, GW1, GW2, DWR1006, WH912 and PDW291 have TaLox-B1b allele and genotypes PDW233, WH896, GW1139, RAJ911, MACS9, WH912, HI8498, PBW215, HI8381, DWL5023, RAJ1555, PBW34, N59, NIDW15, DWR1006, HD4672 and AKDW2997-16 with PsyA1a alleles, which signifies low LOX and high YPC, respectively. It can be concluded from present study that LOX16, LOX18 and YP7A are the efficient and reliable markers for the evaluation of LOX activity and YPC and may be used in wheat breeding programs to improve the quality of wheat end products.

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