



Rice lipases: a conundrum in rice bran stabilization: a review on their impact and biotechnological interventions

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Abstract

Rice is a primary food and is one of the most important constituents of diets all around the world. Rice bran is a valuable component of rice, containing many oil-soluble vitamins, minerals, and oil. It is known for its ability to improve the economic value of rice. Further, it contains substantial quantities of minerals like potassium, calcium, magnesium, iron and antioxidants like tocopherols, tocotrienols, and γ -oryzanol, indicating that rice bran can be utilized effectively against several life-threatening disorders. It is difficult to fully utilize the necessary nutrients due to the presence of lipases in rice bran. These lipases break down lipids, specifically Triacylglycerol, into free fatty acids and glycerol. This review discusses physicochemical properties, mechanism of action, distribution, and activity of lipases in various components of rice seeds. The phylogenetic and gene expression analysis helped to understand the differential expression pattern of lipase genes at different growth phases of rice plant. Further, this review discusses various genetic and biotechnological approaches to decrease lipase activity in rice and other plants, which could potentially prevent the degradation of bran oil. The goal is to establish whether lipases are a major contributor to this issue and to develop rice varieties with improved bran stability. This information sets the stage for upcoming molecular research in this area.

Keywords Lipase · *Oryza sativa* · Gene expression · Rice bran

Introduction

Almost half of the world's population consumes rice (*Oryza sativa*) as their staple food and is the second most important cereal crop following wheat and is cultivated in approximately 114 countries (Sharif et al. 2014). Besides,

in developing countries like India, the ever-increasing issue of the food crisis, due to fewer crop yields and increasing population; necessitates the fact that every part of available resources is utilized effectively. Therefore, to provide an ample amount of food to the entire population, the use of all the by-products produced in food processing and preparations should be formulated. Rice contains a tough siliceous hull or husk that surrounds the mature rice grain or

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the brown rice or caryopsis which is harvested typically as paddy. The caryopsis is comprised of two adapted leaves: the palea (dorsal) and the lemma (ventral) (Wu et al. 2016). Rice caryopsis comprises three genetically distinctive components: (i) the nucellus, pericarp (ovary wall), and testa (seed coat) which is part of the diploid maternal tissue (ii) the endosperm which is part of the triploid filial component and (iii) the diploid filial embryo. The outermost coating of the endosperm is morphologically and biochemically different from it and is recognized as the aleurone layer (Ram et al. 2020). Nucellus, pericarp, and testa are fused within and also with the seed. Every day, people worldwide consume white polished rice that is produced through two main processing steps in the rice paddy. The first step is shelling, de-husking, or dehulling, which removes the husk from the paddy. The second step is polishing or milling, which removes the embryo and bran (including the pericarp, testa, and aleurone layer) (Nambi et al. 2017). When white rice is processed from paddy, it results in a starchy endosperm that makes up 70% of the product. The remaining 30% is made up of rice hulls (20%), rice bran, and the germ or embryo (10%) (Al-Doury et al. 2018).

The major energy reserve in most of the oil-seed crops such as cereals like rice, oats, barley, and corn, are the storage lipids [stored in the form of triacylglycerols (TAGs)]. In most cereal plants, carbohydrates and proteins are stored in the endosperm; whereas TAGs and micronutrients are stored in the bran layer which is further used for oil extraction (Rondanini et al. 2019). Rice bran is a fine, powdered substance comprising the pericarp, aleurone layer, testa (seed coat), germ, and some portions of the endosperm among which the aleurone layer is known to be high in TAGs, proteins, vitamins, and mineral content (Gul et al. 2015). Several factors like the variety of rice, the treatment of rice kernel before the milling process, the type and degree of milling system, and fractionation processes used during the milling process determine the physical and chemical nature of the bran (Heiniö et al. 2016). The rice bran makes up about 8–10% of the grain's total mass and contains a variety of components. It consists of 34–62% carbohydrates, 15–20% lipids, 11–15% proteins, 7–11% crude fibre, 7–10% ash, and 9–11% water. Additionally, it contains several amino acids, vitamins, and minerals (Gul et al. 2015).

Rice bran oil (RBO) contains a well-balanced source of saturated (20% palmitic acid), monounsaturated (42% oleic acid), and polyunsaturated fatty acid (32% linoleic acid) respectively (Lai et al. 2019). The general make up of crude RBO is TAG (81–84%), diacylglycerols (2–3%), monoacylglycerols (1–2%), free fatty acids (FFA) (2–6%), wax (3–4%), glycolipids (0.8%), phospholipids (1–2%) and an unsaponifiable fraction (4%) (Punia et al. 2021a). The presence of a high amount of polyunsaturated fatty acids (PUFA) makes rice bran a healthy food. RBO contains

substances that might lower cholesterol absorption and elevate the elimination of cholesterol which in turn results in lowering cholesterol levels (Sohail et al. 2017). Various bioactive compounds like anthocyanins (found in pigmented rice varieties), phenolic acids, α -tocopherol, tocotrienols, γ -oryzanol, adenosine, etc. are also present in the rice bran and these bio-actives impart antioxidant, anti-inflammatory, anti-cholesterol, anti-diabetic, anti-carcinogenic, anti-biotic, anti-allergic, and immune-stimulatory properties (Garofalo et al. 2021; Tan et al. 2023). In addition, rice bran also contains 4-hydroxy-3-methoxycinnamic acid (Ferulic acid) (Aalim et al. 2019), which is an antioxidant and photoprotective compound. These health-beneficial properties of bran are attributed to various antioxidants as well as the ability of bran to alter the host microbiome and help to maintain and restore eubiosis (Sivamaruthi et al. 2023). Rice bran also plays an important role in the cosmetics, confectionery, and pharmaceutical industries, and the industries involved in the production of biofuels and bioethanol through the utilization of rice bran fatty acids (Pal and Pratap 2017). RBO is considered a 'Heart oil' and 'Wonder oil' owing to its low expense, easy accessibility, and elevated antioxidant properties. The presence of highly digestible proteins in rice bran also makes it a functionally effective food (Alauddin et al. 2017). Based on the available data and the prior knowledge of the plethora of nutrients contained in the byproducts of paddy processing, there is an imminent need to formulate ways in which the wastage of food in the form of post-harvest processing can be avoided and maximum benefits from the byproducts can be obtained.

India is a leading producer of RBO, responsible for nearly 75% of the world's production, which amounts to 1.2 million tons. Japan, Thailand, and China follow India in production (Nayik et al. 2015). However, only 0.3 out of 0.9 million tons of oil produced in India is considered valuable for edible purposes due to limited processing facilities. The remaining oil, with lower quality, is blended with other oils or used for vanaspati manufacturing (Nayik et al. 2015). Compared to the rice endosperm, the bran is considered nutritionally superior since the bran is a major reservoir for numerous vitamins, minerals, essential mineral oils, and other significant bioactive compounds (Ram et al. 2020). Nevertheless, rice bran is often under-exploited as a food product due to the instability of the bran after the milling process. The most important rationale for the existing failure of utilizing rice bran as a food component is the time lag between its manufacture and consumption and its instability during longer storage. The environment can also aggravate the rate of oil degradation and FFAs formation (about 5–7% per day and up to 70% per month). More than 5% FFA in bran oil is remarked to be rancid oil rendering it useless for extraction of edible oil and bran oil with more than 10% FFA is unhealthy for human consumption (Tiwari et al. 2016a, b).

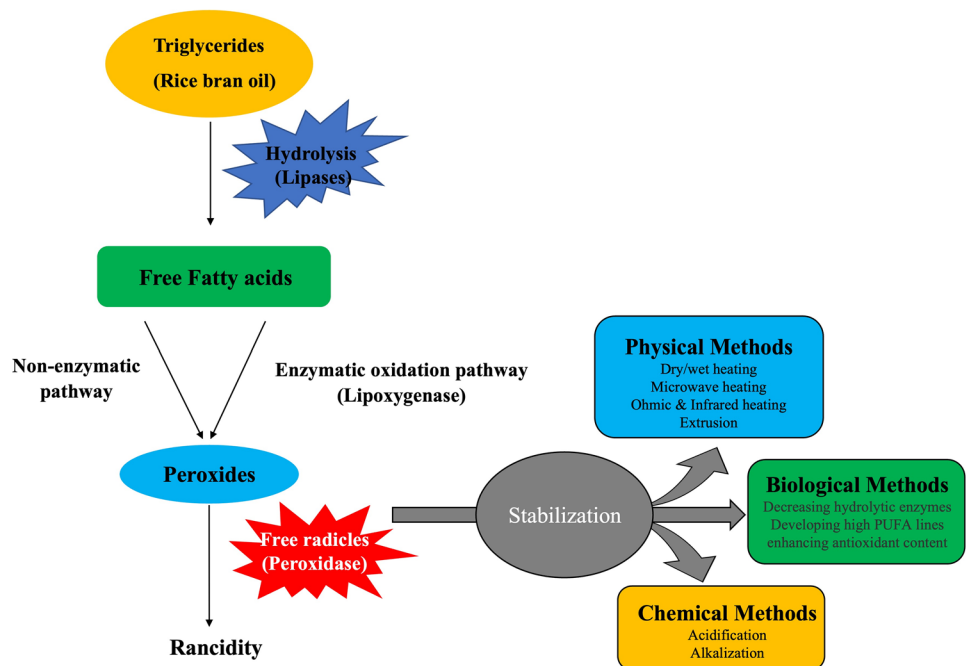
Various hydrolytic enzymes, such as lipases, lipoxygenases and peroxidases present in bran cause lipid degradation which in turn results in bran rancidity and instability (Viswanath et al. 2020; Bhunia et al. 2023). The TAG, which are the major component of RBO, are converted to FFA by the action of lipases, whereas the action of lipoxygenase turns TAG into various peroxides (Fig. 1). Peroxides are further converted into free radicals by the action of peroxidase enzymes (Suzuki et al. 1996). These peroxides and free radicals are the major culprits which negatively affect the flavour and reduce the stability of the bran.

Rice bran instability

The rice bran becomes rancid and inedible due to the reaction that occurs between the rice bran lipids and the highly reactive hydrolytic enzymes like lipases. This reaction leads to the degradation of lipids into FFA which is the major cause of the shortened life of freshly milled rice bran (Tiwari et al. 2016a, b). In rice bran, compartmentalized TAGs are stored as oil bodies in the aleurone layer of the bran fraction. However, during the process of milling individual cells are disrupted, which exposes the TAG reserve to highly reactive endogenous lipases which catalyze the hydrolysis of TAGs resulting in the release of non-esterified fatty acids (NEFAs). This hydrolytic activity tends to continue during the prolonged storage of milled bran, reaching up to 40–60% of NEFAs (Sinha et al. 2020). These liberated NEFAs are further subjected to oxidative degradation catalyzed by lipoxygenase (LOX) enzymes which results in

the production of hydroperoxides (Viswanath et al. 2020), which are the key metabolites responsible for the off-flavours of rice bran lipids. Rancidification is the process of full or partial hydrolysis or oxidation of fats and oils when exposed to air, light, moisture, or enzymatic action developing an unpleasant taste and odour. The rice bran deterioration can be characterized as hydrolytic rancidity and oxidative rancidity (Suzuki et al. 1996). In hydrolytic rancidity, the lipase enzyme catalyzes the reaction in which the triglycerides are hydrolyzed into glycerol and FFA which are further catalyzed by the LOX enzyme to act as a substrate for oxidative rancidity. In the oxidative rancidity process, the double bonds of the FFA are oxidized and the production of hydroperoxides of unsaturated fatty acids occurs. Several other compounds like aldehydes and ketones are also produced during this process (Maszewska et al. 2018). With the aid of several elements such as light, heat, oxygen, moisture, metals, and metalloids, the enzymatic oxidation pathway by LOXs and the non-enzymatic oxidation pathway, also known as autoxidation, progressed by free radical chain reaction (Fig. 1). The increase in acidity, decrease in the pH, and soapy flavour of the rice bran are all characteristic features of rancid rice bran. One possible way to curb the loss due to the degradation of rice grain during the storage of rice can be the removal of water content from the rice by processes like drying. In addition, storing the rice grains in a cool and dark place would also help in reducing the loss due to degradation. Schaal Oven Test and Rancimat test are performed to check the rancidity and stability of oils. In this process, the peroxide value, fatty acid value, anisidine value, and carbonyl values are calculated (Maszewska et al. 2018) and

Fig. 1 Schematic representation of hydrolytic and oxidative deterioration of rice bran oil, and different stabilization approaches



FFA content assessment is an alternative approach to check rancidity (Kim et al. 2014).

Rice bran stabilization

For rice bran stabilization, i.e., to reduce the activity of lipase within the bran and conserve the nutritional attributes of the rice bran, various conventional physical and chemical methods and non-conventional methods have been employed over the years with varying degrees of success (Punia et al. 2021a, b; Yilmaz 2023). Some of the physical methods employed are roasting and pelleting, microwave heating (Lavanya et al. 2019), ohmic heating (Dhingra et al. 2012), hot air drying (Bergonio et al. 2016), refrigeration (Yu et al. 2020), freeze-drying followed by dry-heating, autoclaving, pulsed electric fields (Qian et al. 2014), gamma irradiation, low-pressure cold plasma treatment (Chen et al. 2015), wet or moist heating by parboiling or steaming (Rocha-Villarreal et al. 2018), infrared heating (Irakli et al. 2018), extrusion cooking (Guevara-Guerrero et al. 2019), electron beam irradiation (Luo et al. 2019), toasting (Sapna et al. 2019). These methods have been shown to reduce the enzyme activity by 80–92% and stabilize the rice bran from 4 to 24 weeks depending upon the method and conditions used (Dubey et al. 2019). Similar to the physical methods several chemical treatments have also been used to manage enzymatic rancidity in rice bran which includes treatments with hydrochloric acid (HCl) (Akhter et al. 2015), the addition of salts—NaCl (Doblado-Maldonado et al. 2012), short-chain organic acids—a mixture of acetic acid and propionic acid (Gopinger et al. 2015), the addition of anti-oxidants—butylated hydroxytoluene and citric acid (Atapattu et al. 2013), Butylated hydroxyanisole, addition of succinic anhydride (Moin et al. 2016), ozone treatment (Zhu 2018), removal of FFA (Vaisali et al. 2015) and treatment by essential oil vapor—*Michelia alba* (Songsamoe et al. 2017). It is suggested that the recent advances in the RBO extraction approach including super/sub-critical CO₂, microwave-assisted subcritical H₂O, and ultrasound-assisted aqueous extraction methods are more effective and ecologically friendly over traditional extraction techniques and aid in making RBO an important commodity for the food and non-food industries (Punia et al. 2021a; b). Yu et al. (2020) suggested that among six heating and five non-heating treatments investigated, treatments with microwave, extrusion, steaming, –80 °C, ultraviolet irradiation, and infrared heating had higher possibilities in terms of stabilizing the rice bran. Extrusion and microwave processes were found to be suitable for large-scale with short-time industrial processing although with an increase in oil peroxide values and colour (Yu et al. 2020).

Other than the most common physical and chemical methods, stabilization of the rice bran through enzymatic

treatment where the use of protease to decompose lipase has also been extensively studied. The decomposition of lipase through protease can be achieved by mixing the rice bran with water and is maintained at a constant temperature to achieve irreversible inactivation of lipase (Laokuldilok and Rattanathanan 2014). It is suggested that this method of stabilization is simple and cost-effective and results in the selective targeting of enzyme action with mild reaction conditions and no limiting reagent residue and preservation of rice bran nutrients.

The stabilization of the rice bran using all these physical, chemical, and enzymatic methods has been reported to have varying degrees of success in terms of reducing lipase activity, prolonged storage, and maintaining nutrient content in rice bran (Liu et al. 2019). It is to be noted that stabilizing the rice bran is as important as the retention of nutritional constituents like antioxidants during the application of such stabilization procedures. For instance, among the physical methods, Thanonkaew et al. (2012) suggested that heat treatments can inactivate the lipase of rice bran, which can effectively destroy microorganisms, and maintain nutrient content in rice bran. The authors further stated that hot air heating is an efficient and economical method, suitable for use in small and medium-scale operations, and more importantly, domestic heating can be employed without deleterious changes to the nutritional profile in the bran (Thanonkaew et al. 2012). Similarly, Pradeep et al. (2014) reported that during steam stabilization, the contents of oryzanols, tocotrienols, and tocopherols in bran were higher and without significant loss of health-beneficial components in rice bran. The results suggested that the steaming treatment not only effectively denatures lipase activity but also significantly promotes the level of numerous important bioactive compounds due to the shift from bound to free status induced by the effect of heat-moisture treatment. In another method of physical stabilization, Dung (2014) used ohmic and microwave heating to achieve effective stabilization of rice bran with the addition of moisture. Both methods resulted in lower levels of lipase activity compared with the untreated samples, along with reduced FFA, high phenolic content, and antioxidant activity in the treated samples. Rafe and Sadeghian (2017) used extrusion for stabilizing the rice bran and it was observed that compared to the raw rice bran, the levels of phytic acid, protein, and vitamin E in stabilized rice bran were significantly reduced whilst lipids, vitamins B2, B3, B5, and folic acid remained unchanged, and a significant enhancement in the dietary fibre content. Irakli et al. (2020) evaluated the effects of dry-heating, infrared-radiation, and microwave-heating on the nutritional, antinutritional, functional, and bioactivity attributes of rice bran. Among the treatments, infrared radiation exhibited the strongest inactivation, resulting in 34.7% residual lipase activity. All the stabilization methods were found to be effective in the reduction

of antinutrients, including phytates, oxalate, saponins, and trypsin inhibitors. However, all heat treatments caused a significant decrease in vitamin E and total phenolics content in the rice bran.

Similar to physical methods, chemical methods have also been tried and tested and success in stabilizing the rice bran has been achieved. The most commonly used chemical methods include acidification of the bran through treatment with HCl and acetic acids, alkalization with NaCl, treatment with alcohols, etc. Such approaches change the pH of the bran which reduces the activity of various hydrolytic enzymes as well as prevents the bacterial infection in the bran. However, it has been suggested that the use of chemicals may contaminate the bran oil, and chemical stabilization may be more useful for treating rice bran which is ultimately used as animal feed (Gopinger et al. 2015). The efficacy of combining multiple treatments has also been assessed through the steaming or cocktail enzyme and lactic acid bacterial fermentation (Liu et al. 2017). Results showed that pre-treatment of rice bran using α -amylase led to enhanced total phenolics and flavonoids following the multi-treatment of fermentation and complex enzyme hydrolysis, with the highest increase in ferulic acid. It is proposed that phenolic esterase and carbohydrase are produced from lactic acid bacteria for hydrolyzing the ester bond between phenolics and cell wall components to release higher phenolic compounds. Further, the applied complex enzymes of glucoamylase, cellulase, and protease convert macromolecules to small molecules for promoting bacterial fermentation which results in softening the cell wall structure, which further stimulates the formation of free phenolic acids (Liu et al. 2017).

Laokuldilok and Rattanathanan (2014) compared the efficiency of five proteolytic enzymes (trypsin, chymotrypsin, papain, bromelain, and Flavourzyme) and compared them with thermally stabilized rice bran and analyzed the antioxidant content. Studies demonstrate enzymes trypsin, chymotrypsin, and papain showed a higher rate of hydrolysis than bromelain and Flavourzyme. The total phenolics content of enzymatically stabilized rice bran was found to be 94.10% higher than in raw and thermally stabilized rice bran and the γ -oryzanol content was also found to be 2.23- and 2.05-fold higher than that of raw and thermally stabilized rice bran compared to the enzymatically stabilized bran. Further analysis of lipase activity of two-month-old storage showed that lipase activity increased throughout raw rice brans. However, no changes were noted in the lipase activity of the enzyme after thermal treatment with significantly lower activity in enzyme-mediated stabilization. Papain enzymatic hydrolysis is recommended for stabilizing rice bran with high antioxidant content and storage stability (Laokuldilok and Rattanathanan 2014). Similarly, in a recent report, Rattanathanan et al. (2022), compared heat and enzymatic stabilization methods to assess the hydrolytic stability and

changes in antioxidant compounds of pigmented and non-pigmented rice during ambient storage. Both heat-stabilized (open steaming at ~ 100 °C) and enzymatically stabilized (papain hydrolysis) non-pigmented and black rice bran samples showed lower lipase activity during an 8-week storage time. The analysis of heat-stabilized samples revealed that with raw rice bran, there was a significant decrease in tocols, and anthocyanin contents, but a significant increase in γ -oryzanol was observed. However, all antioxidant compounds in enzymatically stabilized samples of both pigmented and non-pigmented increased significantly (Rattanathanan et al. 2022).

In this scenario, the biotechnological approach to reduce the rice bran instability is a promising endeavour to achieve a high degree of success compared to the traditional methods. Hence, the identification of rice bran-specific lipase genes and the pattern of their expression in the developing seeds would be critical to ascertain and evaluate all the lipase genes concerning their part in stimulating rancidity in rice bran. This review highlights the distinct approaches utilized to sustain the value and improve the shelf life of bran oil.

Physico-chemical properties and enzyme kinetics of lipases

Lipases can be categorized based on properties like specificity, selectivity, and the sources from which they are derived, like plants, animals, insects, and microbes (Sarmah et al. 2018). Although many sources of lipases are available, the microbial source has the highest application. Some of the microbial sources of lipases are *Aspergillus oryzae*, *Candida antarctica*, *Rhizomucor miehei*, etc. The plant sources of lipases other than rice are avocado, walnut, coconut, lentils, chickpea, oats, eggplant, etc. These enzymes differ from esterase which works solely on water-soluble carboxylic ester molecules and lipase specificities can be divided into different categories: (a) substrate specificity; (b) positional specificity or regioselectivity; (c) fatty acid specificity or typo-selectivity; and (d) stereospecificity (Barros et al. 2010). Due to the presence of strong alkyl groups on the surface of the lipases, they are considered to be strongly hydrophobic. To activate the lipases, they must be absorbed at the water-oil interface. The activity of the lipases is reduced in the aqueous solutions that contain soluble substrates. The lipases generally follows Michaelis-Menten kinetics and usually stable at room temperature with an optimum activity at 30–40 °C (Bhardwaj et al. 2001). Based on the source and origin of lipases, they have a mass ranging from 20 to 75 kDa, an enzymatic activity at pH 4.0 to 9.0, and a temperature from 30 to 70 °C bestowing thermostable behaviour of lipases (Castro et al. 2004). The production and rate of lipase

depend on various factors such as the configuration, type, and concentration of carbon and nitrogen sources, the presence of metal ions and chelating agents, and the suspended oxygen concentration (Patel et al. 2019). Additionally, the culture pH, growth temperature, aeration, and agitation also play a significant role in determining lipase production and its rate. Column chromatography on DEAE-Toyopearl was used for the purification of two lipases from *Rhizopus niveus*. Lipase I consist of two protein chains. The smaller protein is associated with a sugar moiety (A-chain) and the larger protein is of 34 kDa molecular weight (B-chain). These have an optimum pH of 6.0–6.5 and optimum temperature of 35 °C. On the other hand, Lipase II has a molecular mass of 30 kDa and comprises only a single protein chain that functions at pH 6.0 and 40 °C (Kohno et al. 1994). Studies have revealed that lipases exist in multiple variations, and the presence of Tween 80 and Tween 20 alters the distribution of these lipase forms in the culture medium (Chang et al. 1994). Due to these properties, lipases find their way into various promising industrial applications such as in organic chemical processing, oleochemical industry, detergent industry, pulp, and paper industry, textile industry, leather industry, agrochemical industry, food and dairy industry, pharmaceutical processing, synthesis of biosurfactants and biodiesel, in nutrition and cosmetics production (Andualema and Gessesse 2012). Based on observations, it appears that lipases in nature typically operate within a pH range of 4–9 and a temperature range of 30–70 °C. Additionally, their mass does not exceed 75 kDa.

Mechanism of action of lipases

In rice, multiple types of lipases, including TAG lipases, phospholipases D, and glycolipases have been reported. The hydrolysis of ester carboxylate bonds is catalyzed by lipases. This reaction releases the fatty acids and organic alcohols along with the catalyzing effects that it has on several esterifications (esterification, thioesterification, and amidation) and transesterification (interesterification, acidolysis, alcoholysis, and aminolysis) reactions (Supp Fig. 1). TAG lipases facilitate breakdown of ester bonds in TAGs, which leads to mobilization of TAGs and liberation of FFA, whereas phospholipase D break-down the ester bonds in phospholipids which releases phosphatidic acid (Melani et al. 2020). Whereas TAG lipases mainly work in TAG/oil degradation but phospholipase D are mainly involved in regulating membrane remodelling, thereby functioning in diverse biological responses such as nutrient uptake and stress responses (Deepika and Singh 2022). In contrast to the function of TAG lipases in TAG breakdown, phospholipase D plays an important role in TAG biosynthesis (Yang et al. 2017; Bai et al. 2020). Additionally, another type of

lipase, called GDSL esterase/lipases is differentiated from the above lipase enzymes by a characteristic of conserved GDSL motif GDSxxDxG (Shen et al. 2022). Catalysis by lipases is similar to serine proteases catalysis which is a two-step process. The first step is the rate-limiting acylation and fast deacylation which is initiated by the attack of the oxygen atom of the hydroxyl group of the nucleophile serine residue on the carbon atom of the lipid ester linkage carbonyl group. This process leads to the formation of a transient tetrahedral intermediate that is responsible for the hydrogen bonding between the negatively charged carbonyl oxygen atom and at least two backbone nitrogen atoms (one from the residue just behind the nucleophilic serine and the other one from the residue at the end of strand β 3) in the oxyanion hole. The nucleophilicity of the serine is enhanced by histidine. This process occurs when the histidine donates a covalent intermediate or acyl-enzyme intermediate which is followed by the esterification of the acid component of the substrate to the enzyme's nucleophilic serine residue and the proton is relocated to the ester oxygen of the susceptible bond which is cleaved and the alcohol component is released. The second stage is deacylation in which the activation of the water molecule occurs by the active site of the histidine. This happens when a proton from it results in the hydrolysis of the covalent intermediate. As a result, the hydroxide ion produced attacks the carbonyl carbon atom of the acyl group, which is covalently attached to the serine residue. This results in a temporary negatively charged tetrahedral intermediate, which is stabilized by interactions with the oxyanion hole. In the catalytic process, histidine gives a proton to the oxygen atom of the active serine residue, which breaks the bond between the serine and acyl component. As a result, the enzyme loses an acyl component through the "electrostatic catapult" mechanism. This mechanism involves electrostatic repulsion between the negatively charged carboxyl group and the negative electrostatic potential of the active site of serine. This process results in the enzyme returning to its original state, ready for another round of catalysis (Gerits et al. 2014).

Spatio-temporal distribution of lipases in different parts of the seeds of rice plant

In general, the majority of dry seeds lack lipases and are very likely to produce de novo after germination. The lipolytic activity is also not observed in non-germinated seeds and is rather found only post-germination. This also adds to the fact that drying or dry heating for increasing the shelf life of the rice or rice bran is practically contributing. The energy that is required by the plant for its growth during germination is majorly obtained from the enzymatic activity in the form of hydrolysis of compounds. Nevertheless,

enzymes are not always stimulated as soon as germination initiates (Guzmán-ortiz et al. 2019). Lipase activity can be noticed two days after imbibition when cotyledons appear from the seed coat on day 3 and lipase activity extends to a maximum at about day 5-6 and the number of total lipids decreases quickly (El-Kouhen et al. 2005). Triglyceride hydrolase is the most important lipase in germination which initiates the mobilization of the stored lipids or polysaccharides and delivers energy to support post-germination growth and development (Guzmán-ortiz et al. 2019). High levels of lipids are observed in the aleurone layer, low levels are observed in the basal endosperm, and lipids deposited in the cereal embryo and aleurone are the first to be mobilized during the early stages of germination before the starch breaks down into sugars in the endosperm (Barthole et al. 2012). Throughout germination, seed lipase activity was found to be at its maximum. In endosperm, 7–10 days after imbibition (DAI) shows the activity of lipase I which is responsible for the growth of the seedling and the mobilization of the fat during the post-germination. During the initial stages of germination, that is between the 1–7 DAI, lipase II shows the maximum that increases gradually. According to a recent report by Sinha et al. (2020), the storage lipids in the bran of germinating rice grains come primarily from TAG. The mobilization of these lipids begins four days after imbibition, while the expression of lipase and *LOX* genes is also observed during this process. Their gene expression analyses revealed that five genes (*OsLip1*, *OsLip9*, *OsLip13*, *OsLOX3* and *OsLOX14*) are predominantly expressed in the bran of germinating rice grains. Most of the lipases selected in this study, along with other lipases are analyzed through phylogenetic tree (Fig. 2). The authors concluded that the high lipase and LOX expression levels in bran tissues unveiled specific molecular targets for downregulation to achieve better bran shelf stability. Similarly, Bansal et al. (2021) analyzed 113 lipase genes present in the rice genome and identified 59 lipases which have lipase motif (GX SXG). Phylogenetic analysis among these 59 lipase genes helped to establish evolutionary relationship among these lipases (Fig. 2). During seed development, analysis based on the gene expression in different seed tissues, the authors were able to identify three bran-specific and three endosperm-specific lipases, and one lipase which expresses in both bran and endosperm tissues (Bansal et al. 2021). Further, it was suggested that the expression of these genes increases during seed maturation and decreases during seed germination.

During the initial stages of germination, lipid hydrolysis is carried out by a lipase. The lipase is most active in the endosperm between 8 and 10 days after initial germination. In roots, the root length is associated with the increased activity of lipase, being most active at 4–8 DAI and then consequently declining in its activity while in shoots, the activity of lipases is opposite to the roots. There is a

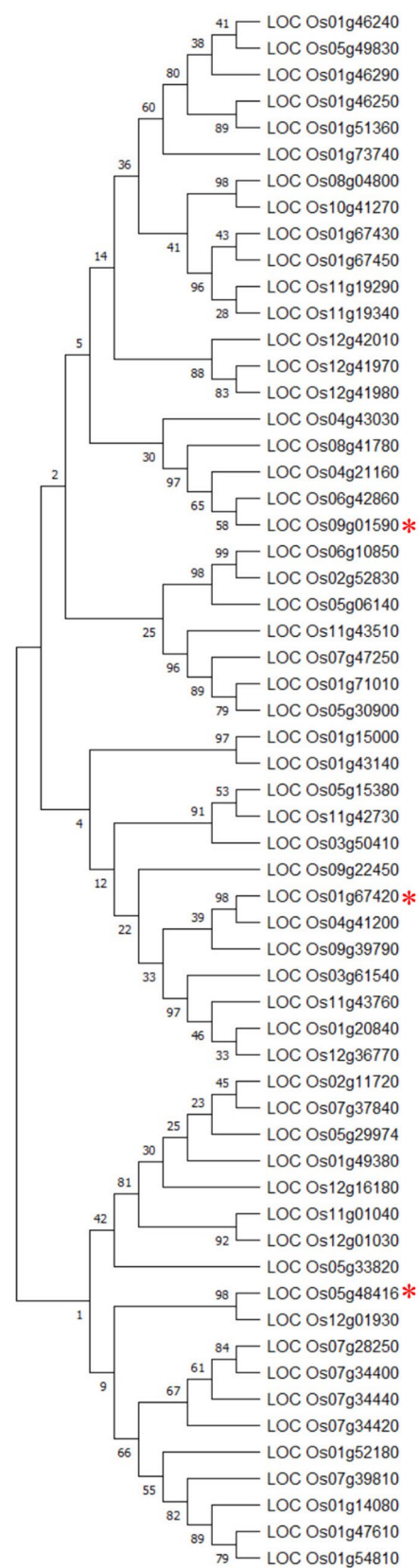


Fig. 2 The phylogenetic tree for selected lipases was constructed using protein sequences MEGA 7.0 software using the Maximum-likelihood method. The lipases marked with * are expressed in rice bran

marginal decline in lipase activity from 4 to 6 DAI and then progressively increases in the activity to a maximum of 10 DAI. In comparison to the endosperm, the lipolytic activity of roots is 3-fold higher while in shoots is only 0.5 fold and mainly lipase I is active during the post-germination in both roots and shoots (Vijayakumar and Gowda 2012). During germination, the radicle is the initial part to sprout from the seedling. This supports the notion that the primary lipase activity is present in the root or radicle. This could be due to the fact that the embryo supports the storage of lipids, which are broken down to produce energy and promote the growth of the radicle first. Additionally, the endosperm is composed mostly of starch, which may encase the lipids and hinder their hydrolysis (Guzmán-ortiz et al. 2019). Based on the information available, it appears that lipases play a vital role in providing nutrients for plant growth during seed germination. Additionally, various parts of both the seed and the plant exhibit differing levels of lipase activity throughout the different stages of growth. To better comprehend this idea, the expression patterns of 73 chosen lipase genes in various parts of rice plants were studied through the Genevestigator tool (<https://genevestigator.com/gv/>). During various growth stages of rice plants, the expression levels of these genes vary, as illustrated in Fig. 3.

The activity of lipases during different stages of development in rice

The development of flower initiates from the panicle initiation stage and develops in a stepwise fashion over 4–5 weeks unless the maturity stage is reached (Sinha et al. 2020). The shoots show almost constant lipase activity during the panicle development but the activity increases linearly from the heading and flowering stages to the milky and dough stages

and then reaches the maximum at the maturity stage. The mature grain shows almost 6-fold greater lipase activity than the panicle stage and depicts a 1.7-fold greater lipase activity as compared to the germinating endosperm. Lipase-I is primarily expressed throughout the initial developmental stages of seed like in the panicle whilst Lipase-II is practically entirely expressed during later stages of seed development *i.e.* from the milky stage to the maturity stage (Guzmán-ortiz et al. 2019). The developing rice depicts the increased lipase activity during the early stages of grain development, and it reaches the maximum at about 10–12 days after flowering (DAF). Following this, there is a decrease in the lipase activity which is completely declined by the end of the 24th DAF. Triglycerides, the most important components of non-starch lipids of brown rice, are examined to gather at higher levels with an increase in lipase activity through grain development, and this lipase offers energy by mobilizing lipid reserves throughout reproductive development. A presumed class III lipase gene, Extra Glume 1 (*EGI*), is also detected to be fervently expressed in the florescence primordia and synchronizes rice empty-glume fate and spikelet development (Li et al. 2009). Our analysis show that among all the 73 lipases analyzed, *LOC_Os01g49380* shows the highest expression in the embryo among the seed tissues (Caryopsis, embryo and endosperm) (Fig. 3). Of the three bran specific lipases *LOC_Os05g48416* was only expressed in caryopsis and reproductive tissues, whereas rest of the other two bran specific lipases were more equally expressed in vegetative and reproductive tissues (Fig. 3). Expression analysis of bran specific lipases (Fig. 2) in different seed development stages as well as during seed germination reveals that expression of these lipases increased with seed maturation and decreases with seed germination (Bansal et al. 2021). To sum up, lipase activity is present throughout the entire lifespan of rice plants. However, the specific type of lipase

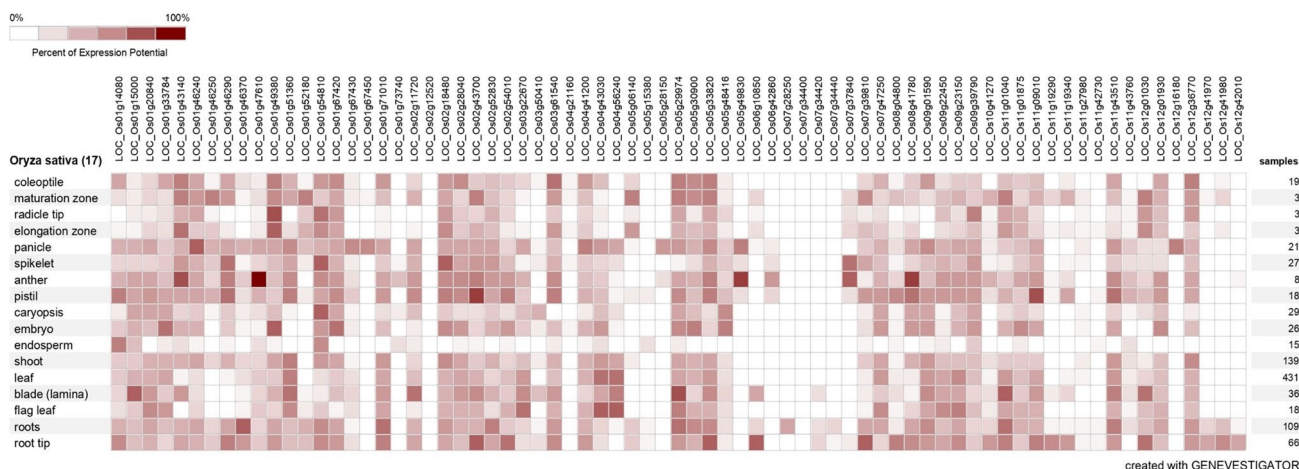


Fig. 3 Heatmap showing spatio-temporal expression pattern of lipase genes using expression data from Genevestigator tool

that is active varies depending on the stage of growth and development. Lipase I is observed to have higher activity in the initial stages while lipase II acts during the later stages of development. The activity gradually increases from the vegetative stage (seed germination stage) to the reproductive stage (panicle development stage) and ultimately to the ripening stage (mature stage).

Biotechnological approaches to reduce the activities of lipases

The physical and chemical methods used for stabilizing the rice bran can be effective only to a certain extent. Therefore, it is imperative to adopt certain other approaches to reduce the rancidity and effectively improve the quality and storage ability of the RBO and this can be done by suppressing the expression of the lipase genes. There can be several approaches that can be used to inactivate the enzymes such as lipase, lipoxygenase or peroxidases. This would reduce the rancidity and increase the storage ability of grains and seeds. Some well-known approaches are the denaturation of enzymes, rendering them inert, or creating environments that are unsuitable for enzyme activity such as low water content or a chemical environment that restricts the catalytic effect of the enzyme (Urban-Alandete, 2019).

Numerous genetic engineering strategies aimed at improving plant biomass and nutritional characteristics by targeting the TAG pathway have been previously reported (Table 1). Through the genetic manipulation of the key genes and regulators implicated in TAG biosynthesis, there has been an efficient development in the TAG buildup in oil-seeds and vegetative plant tissues (Xu et al. 2018). It can be observed that several methods can be used for silencing or decreasing the functionality of the genes to increase the stability and storage ability of the seeds. Numerous plant species including *Oryza sativa*, *Arabidopsis thaliana*, *Glycine max*, *Jatropha curcas*, *Physcomitrella patens*, *Helianthus*, *Lycopersicon esculentum*, *Zea mays*, *Arachis hypogaea*, *Brassica napus*, and *Sesamum indicum* have been used as target towards genetic manipulation of TAG biosynthesis (Table 1). Several methods including targeted mutagenesis using TALENs antisense technology, T-DNA insertional mutagenesis, gateway-based RNA interference technology, CRISPR-Cas9 mediated targeted mutagenesis, point mutation, cloning, site-directed mutagenesis, etc. can be used for the process of increasing the stability and storability of these grains.

Antisense or RNAi technology can be effectively utilized for achieving higher oxidative stability and storability of rice bran by silencing or down-regulation the expression of *LOX* and Fatty Acid Desaturase (*FAD*) genes (Tiwari et al. 2016a, b). The seed longevity and quality during the

storage period can be improved by suppression of *PLD α 1* (Phospholipase D alpha1) and *LOX* gene activity in the rice (Kaur et al. 2020a, b). In *Arabidopsis*, innumerable genes like *SDPI* (Sugar Dependent 1), *SDPIL* (SDP1-Like), *ATGLL* (Adipose Triglyceride Lipase-Like), and *CGI58* (Comparative Gene Identifier-58-Like) are engaged in TAG hydrolysis (Xu et al. 2018) and *CGI-58* positively controls the activity of PXA1 (Peroxisomal ABC-Transporter 1) protein which facilitates in β -oxidation metabolism and lipid homeostasis (Park et al. 2013). Mutation in these genes was caused by a decrease in TAG lipase activity and the peroxidation of unsaturated fatty acids, which led to a delay in the development of stale flavour during storage. Additionally, modifications were made to *FAD* genes in rice (Tiwari et al. 2016a, b), soybean (Pham et al. 2010; 2011), and rapeseed oil (Kaur et al. 2020a, b) to improve the oxidative stability and quality of seed oil. Effectively targeting DGAT (diacylglycerol acyltransferase), MGAT- (Monoacylglycerol acyltransferase), Oleosins, and transcription factors like *LEAFY*, *COTYLEDON1* and *WRINKLED1* can accelerate oil synthesis and accumulation (Barthole et al. 2012; Vanhercke et al. 2019).

One way to improve the storage life of rice grains is to manipulate the lipid biosynthesis pathway. This can be achieved through the development of low linoleic and high oleic rice lines, which decrease the amount of PUFAs that are targeted during oxidation. Another technique is to exploit null mutants of lipases and, which create non-functional enzymes. By employing these substrate-deficient and catalyst-deficient methodologies, we can upgrade the storability and lifespan of rice grains (Bollinedi et al. 2021). Improving the expression of endogenous antioxidants by targeting genes, such as the *VTE* gene (Vitamin E locus), is a lasting solution to reducing lipid oxidation and seed viability (Bollinedi et al. 2021). Additionally, quantitative trait loci (QTL) analysis of fatty acid metabolism is used to recognize candidate genes related to lipid metabolism and target them to improve the quality of seed oil (Bao et al. 2018). Analysis of QTLs to enhance storage capacity in rice, aiming the genes or QTLs to strengthen the innate antioxidant content in rice grain to terminate the lipid peroxidation (Bollinedi et al. 2021). Single Nucleotide Polymorphisms (SNPs) or allelic variation directs to variation in the protein motif and domain structure synchronizing the enzyme activity (Kaur et al. 2020a, b) and CRISPR-Cas9-mediated targeted mutagenesis to disrupt the *FAD* gene improving the fatty acid composition in RBO (Abe et al. 2018) are other molecular and genetic approaches to reduce rancidity and stabilization of rice bran. In the past, methods such as heating or chemical treatment have been used to reduce the activity of lipase in RBO, which improves its shelf life. However, these methods may not be effective in all situations and have less success rate. Alternatively, biotechnological approaches that target

Table 1 Genetic manipulation of the key genes and regulators in different plants involved in TAG biosynthesis for increasing stability and storability

S. No.	Gene	Gene description	Gene function	Targeted plant species	Study type	Method used	Remarks	References
1	<i>AtDAD1</i>	Defective in Anther Dehiscence1	Encodes a particular phospholipase A1 (PLA1) lipolytic enzyme which helps in Jasmonic Acid (JA) biosynthesis	<i>Arabidopsis thaliana</i>	Mutation in <i>DAD1</i>	T-DNA insertion	Enhanced the release of Linolenic acid	Ishiguro et al. (2001)
2	<i>WR1</i>	AP2/EREBP transcription factor WRINKLED1	Role in the regulation of seed storage metabolism	<i>Arabidopsis thaliana</i>	Overexpression of <i>WR1</i>	Ectopic expression of the WRINKLED1 cDNA	Increased seed oil content and accumulation of triacylglycerols in developing seedlings	Cernac and Benning (2004)
3	<i>LeL1D1</i>	Lipase homologous to DAD1	TAG lipase	<i>Lycopersicon esculentum</i>	Expression profile	Semi-quantitative RT-PCR	Involved in fat mobilization during post-germinative growth of the seedlings as a true lipase	Matsui et al. (2004)
4	<i>VTE1</i> and <i>VTE2</i>	<i>Vitamin E</i> loci	Lipophilic antioxidants and scavenge lipid peroxyl radicals	<i>Arabidopsis thaliana</i>	Disruption of <i>VTE1</i> and <i>VTE2</i> locus	T-DNA insertion	Enhanced lipid oxidation and reduced seed viability	Sattler et al. (2004)
5	<i>AtLip1</i>	<i>Arabidopsis thaliana</i> Lipase	Helps in TAG degradation	<i>Arabidopsis thaliana</i>	Knockout of <i>AtLip1</i>	T-DNA insertion in the third exon	No significant difference can be observed between wild type and mutant	El-Kouhen et al. (2005)
6	<i>AtSDPI</i>	Sugar-dependent	Patatin-like acyl-hydrolase domain and initiates oil breakdown in germinating <i>Arabidopsis</i> seeds	<i>Arabidopsis thaliana</i>	Deficiency of <i>SDPI</i>	A point mutation (Substitution)	Unable to hydrolyze TAGs	Eastmond, (2006)
7	<i>PLDα1</i>	Phospholipase D	Cleaves phospholipids to generate phosphatidic acid	<i>Arabidopsis thaliana</i>	Knockout and knockdown of <i>PLDα1</i>	T-DNA insertional mutagenesis	In <i>PLDα1</i> -knockdown seeds, more seed germination and oil stability after storage than <i>PLDα1</i> -knockout seeds	Devaiah et al. (2007)

Table 1 (continued)

S. No.	Gene	Gene description	Gene function	Targeted plant species	Study type	Method used	Remarks	References
8	<i>MDAR4</i>	Monodehydro Ascorbate Reductase	Protect membrane lipids and integral proteins from oxidative damage (SDP2 is the peroxisomal membrane isoform of MDAR4)	<i>Arabidopsis thaliana</i>	Deficiency of <i>MDAR4</i>	<i>Sugar-dependent2</i> (SDP2) mutant	Hydrogen peroxide causes oxidative damage to oil bodies	Eastmond, (2007)
9	<i>SDPIL</i>	SDP1-LIKE gene	TAG hydrolysis	<i>Arabidopsis thaliana</i>	Mutation of <i>SDPIL</i>	T-DNA insertion	Reduction in the activity of TAG lipase	Kelly et al. (2011)
10	<i>AtDLAH</i>	<i>Arabidopsis thaliana</i> DAD1-like acylhydrolase	Have phospholipase A1 activity for hydrolysis of phospholipids at the <i>sn</i> -1 position	<i>Arabidopsis thaliana</i>	Overexpression of <i>AtDLAH</i>	T-DNA insertion	Enhanced germination rate and viability of seeds with lower levels of accumulated lipid hydroperoxides, and higher levels of polar lipids	Seo et al. (2011)
11	<i>ZmLEC1</i> and <i>ZmWRI1</i>	<i>ZmLEAFY COTYLEDONI</i> and <i>ZmWRINKLED1</i>	Maize Transcriptional regulators	<i>Zea mays</i>	Overexpression of <i>ZmLEC1</i> and <i>ZmWRI1</i>	Direct cloning	Increased oil content and accumulation in maize kernels	Barthole et al. (2012)
12	<i>AtMGAT</i>	Monoacylglycerol acyltransferase	Catalyzes the synthesis of diacylglycerol, the precursor of triacylglycerol biosynthesis	<i>Arachis hypogaea</i>	Overexpression of <i>AtMGAT</i>	Site-Directed Mutagenesis	Increased triacylglycerol accumulation	Vijayaraj et al. (2012)
13	<i>AtMGAT</i>	Monoacylglycerol acyltransferase	exhibit both acyltransferase and hydrolase activities	<i>Arabidopsis thaliana</i>	Overexpression of <i>AtMGAT</i>	Site-Directed Mutagenesis	Decrease in overall phospholipids, particularly phosphatidylethanolamine, phosphatidylcholine, and lysophosphatidylcholine (LPC)	Vijayaraj et al. (2012)
14	<i>JcFAD2-1</i>	enzyme 1-acyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine delta 12-desaturase	responsible for the production of linoleic acid in plants	<i>Jatropha curcas</i>	Knockdown of <i>JcFAD2-1</i>	RNA interference	A dramatic increase of oleic acid (> 78%) and a corresponding reduction in polyunsaturated fatty acids (< 3%) in seed oil	Qu et al. (2012)

Table 1 (continued)

S. No.	Gene	Gene description	Gene function	Targeted plant species	Study type	Method used	Remarks	References
15	<i>PDATI</i>	Phospholipid:Diacylglycerol Acyltransferase 1	Role in Fatty Acids (FA) synthesis	<i>Arabidopsis thaliana</i>	Overexpression of <i>PDATI</i>	<i>Trigalactosyl-diacylglycerol-1 (igd1-1)</i> mutant	Enhances both FA and TAG synthesis in leaves and also the turnover of FAs	Fan et al. (2014)
16	<i>r9-LOX1</i>	Recombinant-9 Lipoxygenase 1	Catalyzes lipid peroxidation of seeds	<i>Oryza sativa</i>	Down-regulation of <i>r9-LOX1</i> gene	Gateway based RNAi technology	Enhances seed viability under storage conditions	Gayen et al. (2014)
17	<i>JcSDP1</i>	Sugar-dependent 1	TAG Degradation	<i>Jatropha curcas</i>	Deficiency of <i>JcSDP1</i> in transgenic <i>Jatropha</i>	RNA interference	Reduction in Free fatty acid (FFA) content in seeds and higher accumulation of total seed storage lipids	Kim et al. (2014)
18	<i>BnGLIP</i>	GDSL-like lipase	Involved in plant development, seed germination, plant morphogenesis, and pathogen defense	<i>Brassica napus</i>	Overexpression of <i>BnGLIP</i>	Expressed in <i>Nicotiana benthamiana</i>	Recombinant protein detected as a GDSL lipase which modulates lipid metabolism	Tan et al. (2014)
19	<i>JcDGATI</i> and <i>JcDGAT2</i>	Diacylglycerol acyltransferases	Play a critical role during TAG biosynthesis and accumulation in developing oleaginous seeds	<i>Jatropha curcas</i>	Overexpression of <i>JcDGATI</i> and <i>JcDGAT2</i>	Direct cloning	Enhanced the quantity of TAG biosynthesis, increase seed oil content and linoleic acid content in TAG	Xu et al. (2014)
20	<i>PAH1</i> and <i>PAH2</i>	Mg (2+)-dependent phosphatidic acid phosphohydrolase s	Repress phospholipid biosynthesis at the endoplasmic reticulum	<i>Arabidopsis thaliana</i>	Suppression of <i>PAH1</i> and <i>PAH2</i>	T-DNA insertion	Stimulates net phosphatidylcholine biosynthesis and proliferation in the endoplasmic reticulum by activating <i>CTP: Phosphocholine Cytidylyltransferase1</i>	Eastmond et al. (2010), Craddock et al. (2015)
21	<i>LOX3</i>	Lipoxygenase 3	Dioxygenation of polyunsaturated fatty acids	<i>Oryza sativa</i>	Knockout of <i>LOX3</i> Down-Regulation of <i>LOX3</i>	Targeted mutagenesis using TALENs Antisense technology	Enhances seed quality and storability Preserve rice grain quality during storage	Ma et al. (2015) Xu et al. (2015)

Table 1 (continued)

S. No.	Gene	Gene description	Gene function	Targeted plant species	Study type	Method used	Remarks	References
22	<i>OsLTP136</i>	Lipid transport protein	Regulate seed quality, seed development, and seed germination in rice	<i>Oryza sativa</i>	Down-regulation of <i>OsLTP136</i>	RNAi technology	Reduced-fat acid content resulted in delayed embryo development and decreased seed germination rate	Wang et al. (2015)
23	<i>LDAP1</i>	Lipid droplet-associated proteins	Store triacylglycerols in seeds	<i>Arabidopsis thaliana</i>	Overexpression of <i>LDAP1</i>	CLSM analysis	Compartmentalization and triacylglycerol degradation during post germinative growth	Gidida et al. (2016)
24	<i>AtMAGL8</i>	Monoacylglycerol lipase	Hydrolysis of monoacylglycerol (MAG) to fatty acid and glycerol	<i>Arabidopsis thaliana</i>	Overexpression of <i>AtMAGL8</i>	Expressed in <i>E. coli</i>	Involved in storage lipid breakdown during seed germination and associated with the surface of oil bodies	Kim et al. (2016)
25	<i>Os-FAD2-1</i>	$\Delta 12$ fatty acid desaturase (<i>FAD2</i>)	Converts oleic acid into linoleic acid	<i>Oryza sativa</i>	Knockdown of <i>Os-FAD2-1</i>	RNAi technology CRISPR/Cas9-mediated targeted mutagenesis	Increases proportion of nutritionally desirable oleic acid in TAG Improve the fatty acid composition in Rice bran oil	Tiwari et al. (2016a, b) Abe et al. (2018)
26	1-Butanol	Inhibitor of phospholipase D-dependent	Inhibit seed germination	<i>Arabidopsis thaliana</i>	Addition of 1-Butanol	<i>In-vitro</i> activity assay	Retardation of the mobilization of triacylglycerols and delay in seed germination production of the signalling molecule phosphatidic acid	Jia and Li (2018)
27	<i>AtOBL1</i>	Oil body lipase 1	Responsible for a major TAG lipase activity in seed extracts and pollen tube growth	<i>Arabidopsis thaliana</i>	Overexpression of <i>AtOBL1</i>	Site-directed mutagenesis	Increased in the release of all three FAs from TAG, diacylglycerol, and 1-monoacylglycerol	Müller et al. (2018)

Table 1 (continued)

S. No.	Gene	Gene description	Gene function	Targeted plant species	Study type	Method used	Remarks	References
28	<i>GmSDPI</i>	Sugar-dependent 1	Regulate the TAG content and fatty acid composition of soybean seeds during grain filling	<i>Glycine max</i>	Knockdown of <i>GmSDPI</i>	RNA interference	Enhanced the oil yield in soybean and inhibition of TAG degradation during seed development	Kanai et al. (2019)
29	<i>Oleosin-L</i>	Oil bodies	Stabilize the lipids and TAGs	<i>Sesamum indicum</i>	Overexpression of <i>Oleosin-L</i>	Biolistic co-transformation	More synthesis and accumulation of TAG in leaves	Vanhercke et al. (2019)
30	<i>PpGPAT9</i>	Glycerol-3-Phosphate Acyltransferase 9	Catalyzes the synthesis of lysophosphatidic acid (LPA) from a glycerol-3-phosphate and acyl-CoA	<i>Physcomitrel la patens</i>	Overexpression of <i>PpGPAT9</i>	Ectopic expression of transgenic <i>Arabidopsis</i>	Increased the total seed oil content with significantly higher levels of PUFAs in the transgenic <i>Arabidopsis</i>	Yang et al. (2019)
31	<i>GmABI3b</i> , <i>GmNFYA</i> and <i>GmFAD2-1B</i>	genes related to seed oil synthesis in soybean	Regulate soybean seed oil content	<i>Glycine max</i>	Screening of soybean seed oil-related candidate genes	WGCNA analysis	Controls oil and fatty acid content in soybean seeds	Yang et al. (2019)
32	<i>AHL4</i>	AT-hook motif-containing nuclear localized (AHL) protein	Regulates lipid mobilization and fatty acid b-oxidation during	<i>Arabidopsis thaliana</i>	Overexpression and knockout of <i>AHL4</i>	T-DNA insertion	<i>AHL4</i> suppresses lipid catabolism by repressing the expression of specific genes involved in TAG hydrolysis and fatty acid oxidation	Cai et al. (2020)
33	<i>OsPLDα1</i>	Phospholipase D alpha1 gene	A lipolytic enzyme that causes rancidity and 'stale flavor' in the oil	<i>Oryza sativa</i>	Allele mining at <i>OsPLDα1</i> locus	SNPs/InDels	Reduce rice bran rancidity	Kaur et al. (2020a, b)
34	<i>HaLPCAT</i>	Lyso-phosphatidyl choline acyltransferase	Catalyzes acylation of lysophosphatidyl choline (LPC) into phosphatidylcholine (phospholipid)	<i>Helianthus annuus</i>	Overexpression of <i>HaLPCAT</i>	Direct cloning	Increased the production of polyunsaturated linoleic acid	Mapelli-Brahm et al. (2020)

the genes involved in lipase activity and rancidity can be used. These approaches can involve silencing, blocking, or mutating the genes, and may be more efficient than traditional physical, chemical, and enzymatic methods.

Conclusion and future perspectives

Rice is the main staple food for a large portion of the world's population, making it essential to find ways to make the most out of this primary food source. This includes using every part of the grain efficiently while minimizing waste. However, rice bran's intrinsic factors, such as the presence of hydrolytic enzymes like lipases, can lead to short shelf life and quick rancidity of rice and rice-based products like RBO. Since lipases require water to function, there are limited physical and chemical techniques available to reduce the activity of lipases.

Moreover, different genes are responsible for the synthesis and function of multiple hydrolytic enzymes in rice, with varying concentrations and growth phases. Therefore, relying only on physical and chemical methods to decrease lipase activity is not reliable. To make the most out of rice, we must develop strategies to increase consumption while minimizing waste. The review discussed the use of different biotechnological methods for the proper usage of RBO. Different methods can be utilized to modify genes responsible for the formation/production of these hydrolytic enzymes by inducing mutations, knocking out or downregulating specific genes. These methods consist of antisense technology, T-DNA insertional mutagenesis, gateway-based RNA interference technology, CRISPR-Cas9 mediated targeted mutagenesis, and point mutation. These modifications aim to improve seed quality and storability, enhance seed germination and oil stability, increase the proportion of nutritionally beneficial oleic acid and TAG, improve fatty acid composition of oils, and boost fat mobilization during the post-germinative growth of seedlings.

This review comprehensively explores the structural and functional aspects of lipases and effective approaches for targeting specific genes responsible for producing these hydrolytic enzymes. Identifying the specific hydrolytic enzymes in rice bran is crucial for reducing rancidity and extending shelf life. In the future, different approaches can be used to target lipase genes for this purpose, but it is important to ensure that any down-regulation or knockout of these genes is non-toxic. While genetic modification doesn't always yield successful results, improving the efficiency of these approaches without increasing toxicity in the grains and systematically assessing nutrient contents in rice grains can be future goals.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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