

ICRISAT Public Disclosure

Title of Patent Application	Millet and Food Products with Reduced Lipase Activity, Genes and implementation thereof
Type of Filing	Priority Indian Patent Application No: 202141036329 PCT Application Number: PCT/US2022/074765
Date of Provisional Application Filing	Filed on August 11, 2021 (in India)
Name of Applicants	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) AND Pioneer Hi-Bred International, Inc.
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Territories of protection (through PCT application)	USA and India
Current status	<ol style="list-style-type: none"> 1. Patent Application No. 202141036329 was provisionally filed with Indian Patent Office on 11 August 2021. 2. Based on this provisional application, International Patent Application No PCT/US2022/074765 was later filed in the United States PCT Receiving Office on August 10, 2022. 3. The application is now published with publication number WO/2023/019172.

ICRISAT has adopted the CGIAR IA Principles which require that CGIAR Centers may register/apply for (or allow third parties to register/apply for) patents and/or PVP over the CGIAR Centers' respective Intellectual Assets if such IP protections are necessary for the further improvement of the Intellectual Assets or to enhance the scale or scope of impact on target beneficiaries, in furtherance of the CGIAR Vision. This disclosure is in fulfilment of the CGIAR IA Principles for the patent application filed by ICRISAT.

Brief background

Pearl millet is highly nutritious and a principal staple food crop for millions of people across Asia and Africa. Pearl millet is a highly nutritional source of carbohydrates, protein, vitamins, and minerals such as iron (Fe) and zinc (Zn). Besides being a good source of all beneficial elements, the grain is gluten-free and contains high amounts of antioxidants which have proven beneficial for human health and well-being. It has the potential to contribute to food and nutritional security, as it is a powerhouse of nutrients.

Pearl millet is an important food crop in developing countries and is known for having superior balanced nutrients compared to other cereals. The functional flour market is projected to reach USD 954 billion

by 2022 at a CAGR (Compound annual growth rate) of 7.8% from 2017 to 2022. Nonetheless, pearl millet has not been able to capture sufficient market due to the issue of rancidity. The rapid development of off-flavors and taste in pearl millet flour is the major hindrance to wider consumer acceptability and is also increasing the drudgery of users, especially women traditionally involved in food preparation. Post-harvesting and/or pre-milling techniques can reduce the rancidity and increase shelf life to some extent. However, these techniques have limitations on maintaining the quality of the micronutrients intact. Not only does this result in food waste, but the onset of rancidity due to degradation and oxidation of the several lipid molecules also destroys the good fats and vitamins and, in the long run, could lead to the development of health problems. Thus, there is a need to alleviate the rancidity problem and improve the shelf life of pearl millet flour without altering its other nutritional and phenotypic parameters.

Problem Statement

Over the last decade, pearl millet consumption and commercialization has declined in urban areas and in commercial outlets, leading to lower incentives for its cultivation by smallholder farmers. The consumers and processing industry is constrained due to rapid onset of rancidity in its milled flour. Among millets, pearl millet is the only known millet which has rancidity associated with flour. This problem makes a species-specific challenge for flour shelf life and maintaining quality. The low shelf-life quality of the flour is largely caused by lipid rancidity due to the activities of enzymes such as lipase, lipoxygenase, peroxidase, and polyphenol oxidase. Additional factors include microorganisms, oxygen, light, high temperature and high humidity storage conditions, can exacerbate the problem. The lipases which are involved in rancidity have a key role in seed viability and germination as well. Therefore, using lipases and similar metabolic pathway targets can pose an additional element of complexity. To address rancidity, efforts have been made to develop post-harvest processing and pre-milling techniques to inactivate the biological components that lead to accelerated rancidity. However, these mechanical and physicochemical techniques have not been very effective, and in fact have been shown to negatively influence the nutritional quality of both the micro- and macro-nutrients. Recently, combination of hydro, hydrothermal and thermal near infrared treatment of grains of this millet has increased the storage quality for 90 days, while preserving the functional properties. Whether bioavailability of nutrients and other inorganic salts *in vivo* will remain unchanged due to the above-mentioned treatment is not yet unknown. Also, it is well documented that malting which involves steeping, germination and drying is best treatment in terms of nutritional enhancement and anti-nutrient reduction compared to roasting or blanching (Sehga et al 1998; Adebisi et al 2016). The specific genetic factors to be targeted to improve the shelf life of pearl millet flour are unknown and hamper the ability of the crop breeders to develop genotypes that have enhanced shelf life. Therefore, to revive the importance of this nutri-cereal in dryland agriculture, further understanding of the biological processes that lead to the development of rancidity in pearl millet is urgently needed.

The present innovation mainly relates to identifying the candidate gene in millets associated with offsetting rancidity and the associated bad taste and enhancing the shelf-life stability of the pearl millet flour. To develop a genetic solution for this problem, alteration of the identified candidate gene expression or activity or specific polymorphisms plays a key role in lipid hydrolysis. It has shown the potential to stabilize the triacylglycerol (TAG) during the milling process, thereby adding value for breeding high-yielding hybrid pearl millet varieties with prolonged flour shelf life, enhancing the pearl millet's market value.

The work described in the patent application has the potential to greatly improve the keeping quality of pearl millet flour and thereby improve the effective utilization of this nutri-cereal in daily diets of smallholder farmers and their communities. It is expected to provide more stable varieties (with enhanced flour keeping quality) to farmers. Further work may be done to develop this technology in other seed derived flours and oils where rancidity is a problem.

Work to further optimize and/or expand the impact of the invention may include:

- Use it in a wider range of pearl millet genotypes including hybrids.
- Study other factors contributing to the rancidity observed in different genotypes that also can be minimized.
- Develop new allelic combinations that further lower rancidity in additional genotypes and

potentially in other cereals.

- Field studies to develop a more complete trait profile and confirm absence of associated undesirable traits in a range of genotypes.
- Additional evaluations of storage quality (duration and quantity) and nutritional value and bioavailability parameters.
- Testing application of the technique to other cereals.

Three to four years will be necessary to refine the method and identify the best possible allelic/mutant combinations, and from five to six years to test the mutants in the field.

Patent Application

- This Patent Application is based on research results produced by a public-private collaboration between ICRISAT and Pioneer Hi-Bred International, Inc. (a member of the Corteva Agriscience group of companies). The application identifies a set of genes in pearl millet that lowers rancidity and the associated bad taste and flavor, thus enhancing shelf-life stability of the pearl millet flour. Thus, the application provides a genetic solution to the problem discussed above and demonstrates that the inactivation of candidate enzymes involved in lipid hydrolysis can stabilize triacylglycerol (TAG) during the milling process, thereby adding potential value to both the economics and nutritional quality of pearl millet.
- The application discloses altered candidate genes and genetic polymorphisms that can be used for developing and breeding high-yielding hybrid pearl millet varieties with prolonged flour shelf life. The work provides better understanding of the rancidity mechanism, which is an underlying and essential trait for nutritional improvement in pearl millet flour. The application also describes chemical mutagenesis or gene editing/CRISPR-based approaches to develop new genetic materials that mitigate rancidity in elite milled pearl millet germplasm. Increasing the storage life of flour from this nutritious grain offers tremendous opportunities for primary and secondary processing, creating markets and enhanced profits for smallholder farmers in South Asia and Sub-Saharan Africa.
- This work will help to eliminate or minimize the problem of rancidity in pearl millet, an important staple crop of the semi-arid tropics.
- Access to the technology disclosed in the patent application including further innovations and varieties/hybrids developed for improving the shelf-life of milled flour will be extended to farmers, especially in the developing countries of South-Asia and Sub-Saharan Africa.
- As a non-profit international organization, ICRISAT promotes responsible technology transfer and intellectual property management in accordance with the CGIAR Principles on the Management of Intellectual Assets (“IA Principles”).
- This patent application conforms with the CGIAR Intellectual Assets Principles concerning intellectual property applications, i.e. necessary for the further improvement of the innovation or to enhance the scale or scope of impact on target beneficiaries, in furtherance of the CGIAR Vision.
- The pearl millet accessions utilized for developing this technology are from ICRISAT’s Genebank. ICRISAT will continue to comply with all obligations of the SMTA including benefit-sharing, where applicable.
- Future ICRISAT licensees will be bound by the benefit-sharing obligations under the SMTA. In addition, and as per ICRISAT’s policy, royalties paid by future licensees to ICRISAT shall be shared on a voluntary basis with the benefit-sharing fund of the International Treaty for Plant Genetics for Food and Agriculture.

Impact of the Invention

The research outcomes from this invention are expected to advance the development of pearl millet with enhanced flour shelf life, which will change the way this nutria-cereal is viewed by the processing industry and open up a larger market for this gluten-free flour that is currently underdeveloped. Thus,

this invention is likely to open new opportunities for enhancing the scope of its utilization as low GI, gluten-free flour, bakery & confectionary items, weaning foods, and dietary formulations for children as well as geriatrics etc. Additionally, this work will provide pearl millet breeders and biotechnologists with a toolbox to develop elite germplasm with enhanced processing quality. The longer shelf-life of nutritious pearl millet grain should also increase the opportunities for its primary and secondary processing, thereby creating markets and enhanced profits for smallholder farmers. Reducing rancidity in millet flour will reduce waste and create a value-added product for both rural and urban consumers.

Justification and Compliance note

This invention and patent application are the outcome of collaborative research activity between ICRISAT and Pioneer Hi-Bred International, Inc. (a member of the Corteva Agriscience group of companies), ICRISAT's discovery partner. ICRISAT has partnered with Corteva for collaborative research to utilize the seed knowledge and technical expertise that Corteva has in combination with the gene sequencing and pearl millet expertise that ICRISAT has. The collaborative research is resulting in creation of IP for both ICRISAT and its discovery partner, Corteva. The collaboration also ensures that the resulting IP rights comply with and fulfill the missions and mandates of both the entities. ICRISAT and Corteva will explore potential partnerships that can support further use and development of this technology. The knowhow and user rights of the patent will be shared with partners under appropriate licensing and Material Transfer Agreements. ICRISAT and Corteva have taken steps to safeguard smallholder farm use rights and widespread access to this technology.

This invention includes the identified mutated genes and the lines carrying those mutations leading to 'next-generation' material having lower rancidity (due to lower activity of lipase enzyme in millet flour) and higher shelf-life, thereby having potential to be used as starting material for various other products which might be healthier/nutritious as well. The ability to mill flour in bigger batches with longer shelf life will reduce the drudgery of regular milling by hand and the potential to mix millet flour with other flours for nutritional enhancement and to reduce the massive import costs of pure wheat flour in some countries, particularly in the Sahel regions and drylands more generally. Reducing rancidity in millet flour will reduce waste and create a value-added product for rural and urban consumers. ICRISAT and Corteva will devise a responsible dissemination strategy after the grant of the patent with appropriate partners in the countries where the technology will be used. The strategies to facilitate wider dissemination of the technology will be devised adhering to appropriate legal and seed production compliance systems with the commitment to benefit the smallholder farmers.

Access to the technology disclosed in this patent application will be extended to farmers, including use of the pearl millet or other varieties per se, especially in the developing countries of South- Asia and Sub-Saharan Africa. As a non-profit international organization, ICRISAT promotes responsible technology transfer and intellectual property management in accordance with the CGIAR Principles on the Management of Intellectual Assets ("IA Principles").

All ICRISAT's existing and proposed activities would strictly be in-line with the applicable laws. Applicants will not exercise its rights in ways that limit access to the original in-trust germplasm with ICRISAT as per FAO's International Treaty on Plant Genetic Resources for Food and Agriculture. ICRISAT will also take measures in making the technology available for emergency uses as per the CGIAR Principles on the Management of Intellectual Assets.