Short Research Article

Understanding The Significance of Raffinose Family Oligosaccharides in Seed Physiology

ABSTRACT: -

Seed vigour and longevity are critical agronomic attributes that significantly impact crop yield and the global economy. Seed longevity, a measure of seed viability, plays a crucial role in gene bank management, affecting seed regeneration and recycling. The irreversible process of seed deterioration involves a complex biochemical network, altered metabolism, and damage to membranes, DNA integrity, mitochondria, proteins, and antioxidative machinery. Raffinose family oligosaccharides (RFOs) have emerged as key components for enhancing seed vigour and longevity. RFOs serve diverse functions in plants, including abiotic and biotic stress tolerance, regulation of seed germination, desiccation tolerance, and overall seed health. Studies indicate that RFOs contribute significantly to seed vigour and longevity by implementing roles such as cytoplasmic vitrification, water substitution, and osmoprotection in dried seeds. These compounds are abundant in the seeds of various crops, particularly in the legume family, and are also present in roots and specialized storage organs. The glassy state formed by RFOs plays a vital role in protecting cells from reactive oxygen species (ROS), enhancing enzyme stability, and preventing conformational changes in proteins. Additionally, inhibiting RFO degradation delays germination, highlighting the importance of RFOs in early germination events and seedling establishment.

Keywords: *α-galactosides, flatulence, galactinol synthase, prebiotic carbohydrates, grain legume crops, Glassy State, ROS*

1. INTRODUCTION

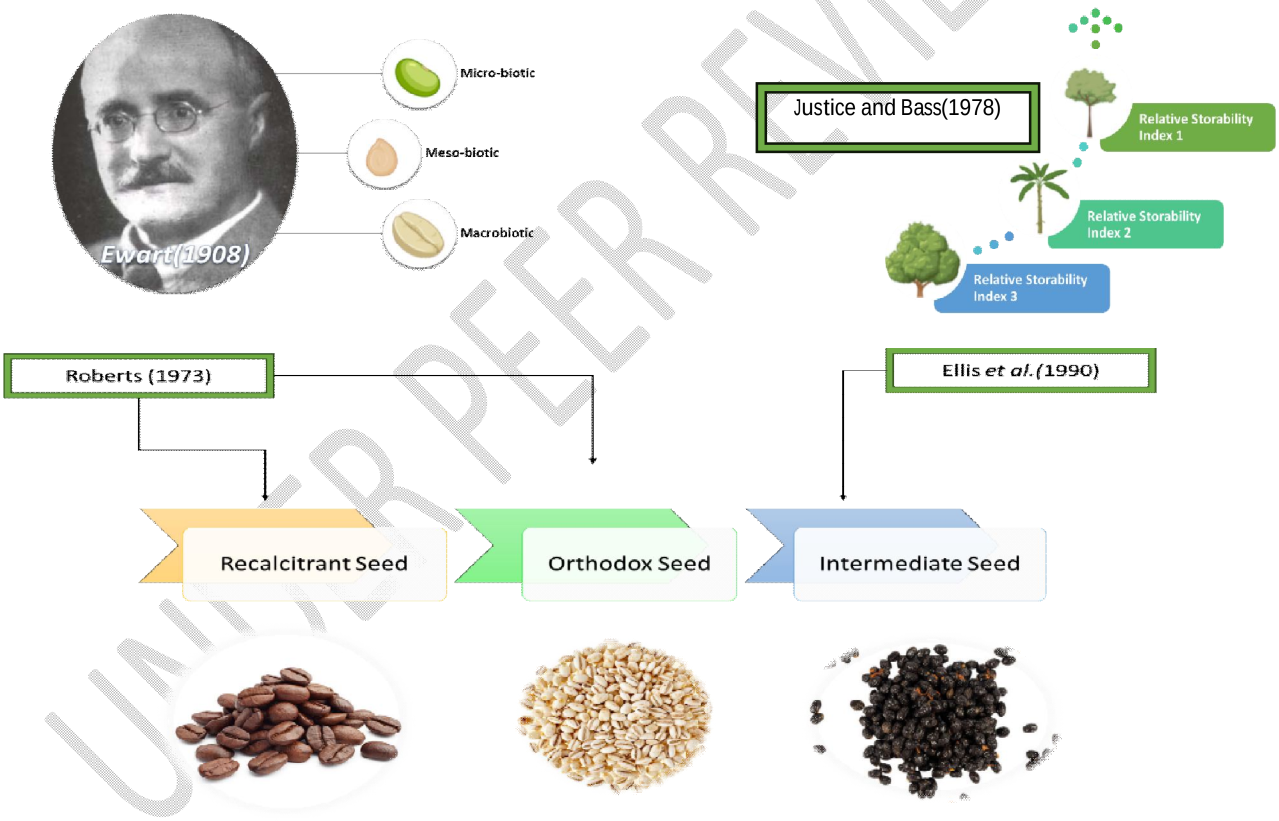
Ensuring the preservation of seed viability and vigour from the first crop planting to the subsequent sowing, whether it is within the same planting season or beyond, is crucial for the success of agriculture and the subsequent crop yield. Like all living beings, seeds likewise undergo the process of ageing, gradually decreasing their strength and capacity to survive until they eventually perish. Seed life encompasses the duration from the first growth stages to the point where seeds are no longer viable in a dry storage environment. The greatest lifespan achievable by seeds of a certain species may vary significantly from one another under similar conditions in the storeroom. Seed storability refers to the ability of seeds to stay viable during storage, and it is an important characteristic for preserving agricultural goods and germplasm in crops. Seed storability is a multifaceted trait influenced by hereditary and environmental variables throughout the process of seed production, development, and post-harvest.

1.1 Significanceofthe Seed Storability Research

Seed storability is described as the capacity to stay alive throughout storing and is a critical particularity for agricultural product and germplasm preservation in crops Variation among rice accessions that emerge from various eco-geographic locations. For instance, Indica-type seeds retained their viability longer than Japonica-type seeds

- Adaptive Advantage
- Major Advancement to Gene Banking
- Conservation of inheritable Diversity
- Successful Planning for the future season
- To study the effect of temperature and moisture contenton Storage Condition

1.2 fig 1 :Classification of Seeds Based on Longevity



1.3 Rules for Determination ofSeed Longevity:

a. Harrington's Thumb Rule

5% Reduction in Storage Temperature And 1% Reduction in Moisture Seed Longevity is Doubled (Provided Storage Temperature Range of 0-50 degree C and Moisture Range of 5-14%)

b. James Rule:

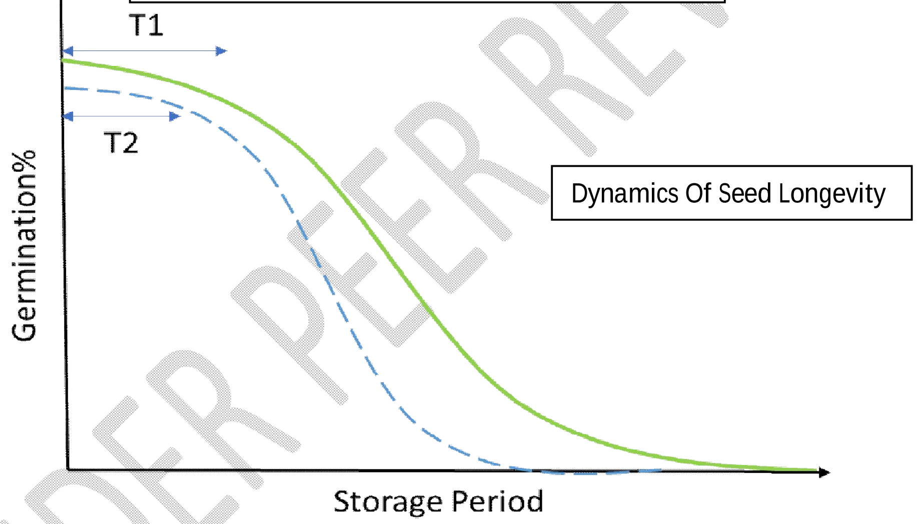
The temperature in Degrees Fahrenheit + Relative Humidity = Should not Exceed 100

1.4 Dynamics Of Seed Longevity:

- For all practical reasons, a Seed lot will be regarded rather than an individual seed The viability curve of a seed lot at each time is typically sigmoid indicating a normal distribution
- The viability curve of a seed batch at each time is generally sigmoid showing a normal distribution

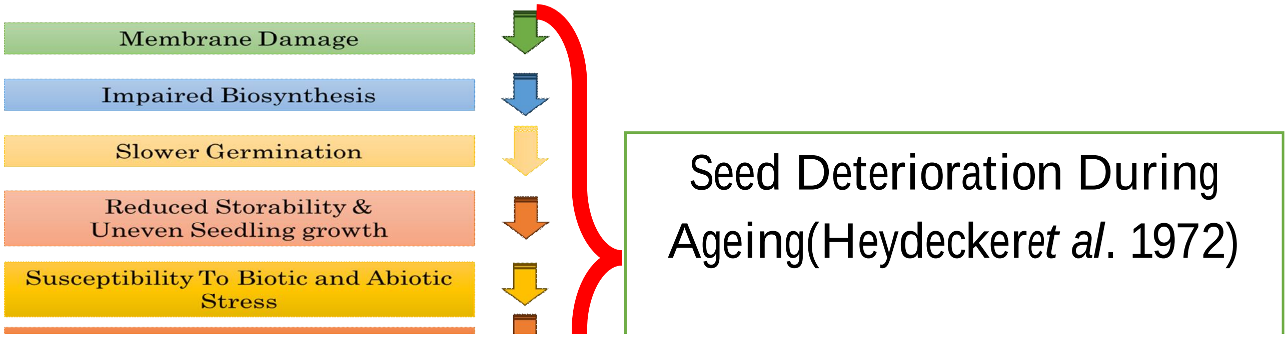
- In this T is the length after which the seed of a specific variety demonstrates quick and observable reduction in germination
- Tomato is an excellent storer (T1) as opposed to onion (T2) seen in the figure
- These results demonstrate that despite the storage environment, disparities exist in lifespan across species stored under the same setting (Singhal, N.C.,2009)

Fig 2 :Classification of Seeds Based on their Longevity



1.5 Concept of SeedAgeing

Seed ageing is a frequent physiological process during storage. It is a natural irreversible process that happens and evolves together with the lengthening of the seed storage period. Ageing rates of plant Anhydrobiotes are significantly reliant on the water content and temperature of storage, circumstances (Singhal, N.C., 2009)



Seed Deterioration During Ageing(Heydecker*et al*. 1972)

Fig 3 :Physiological Changes occurring while seed deteriorates by ageing

2. STORABILITY OVERVIEW and KEY FACTORS REGULATING SEED STABILITY IN STORAGE:

2.1 Seed Factors:

The preservation potential of the orthodox seeds has been related to the cellular protection mechanism. After seed development, orthodox seeds undergo the maturation processes, accompanying a fall in moisture level and stoppage of their metabolic activities. The dry condition is regarded as quiescent, which assists in seed storage potential. The defensive mechanisms imposed during late seed maturation include the accumulation of antioxidants, non-reducing carbohydrates, and protective proteins such as late embryogenesis abundant (LEA)proteins, heat shock proteins (HSPs) and lipocalins. The nuclear DNA is packed denser and chlorophyll is destroyed. Seed storage protein proteins function as key targets of oxidation, assisting in buffering reactive oxygen species (ROS) created during dry storage. Since chlorophyll levels can be measured extremely sensitively using their fluorescent qualities, chlorophyll fluorescence levels of individual seeds may be used as a marker for seed maturity for those seeds that contain chlorophyll throughout development, as most do. The lower the degree of fluorescence, the greater the level of seed maturity (Ranganathan, U., & Groot, S. P. C.,2023)

2.2 Role of Chemical Protectants in Various Tissues:

The principal harmful factor during seed storage is oxidative stress generated by ROS. Molecular and enzymatic antioxidants are there for crucial in seed lifespan. Under dry circumstances, enzymes cannot reach the ROS due to reduced molecular mobility within the cytoplasm. Under these arid circumstances, seeds depend on low molecular weight antioxidants like tocochromanols (tocopherols and tocotrienols), ascorbate (vitamin C) and glutathione. Seeds are generally rich in tocopherols, especially vitamin E (alfa-tocopherol), which are lipophilic antioxidants, that play a crucial role in preventing oxidation of storage and membrane lipids. Vitamin E-deficient Arabidopsis mutants have a drastically lower seed life. Ascorbate and glutathione are the principal water-soluble antioxidants contained in seeds. At the low water content of dry seeds, enzymatic renewal of the antioxidants is not feasible and ultimately the antioxidant pool will become depleted. Exposing seeds to a higher RH environment, or via imbibition, will enable enzymatic ROS scavenging activity by glutathione-reductase, superoxidedismutases, peroxidases and catalases. These enzymes also play a role in the regeneration of molecular antioxidants. Research with barley seeds indicated that tocopherol and glutathione levels drop with seed ageing, both under dry gene banks to rampage and under controlled degradation at 45°C and 75%RH.

During seed development, the cells acquire sucrose and raffinose family oligosaccharides (RFOs), which are raffinose, stachyose and verbascose. These sugars have been hypothesized to have a function in the establishment of the glassy state and consequently enhancing seed life. Galactinol is the direct precursor of raffinose and a positive genetic link between seed galactinol concentration and lifespan has been revealed for Arabidopsis, tomato and cabbage, while its significance was verified by reduced longevity exhibited by seeds from Arabidopsis galactinol synthase mutants. In that research Arabidopsis and tomato seed lifetime was evaluated at 85% RH and 40°C, whereas the cabbage seeds had been kept in paper bags at 20°C without RH regulation. Galactinol has also been proven to offer protection against oxidative stress in Arabidopsis leaves.

Late embryogenesis abundant proteins (LEAs) and heat shock proteins (HSPs) are generated near the conclusion of seed maturation. They serve a function in seed survival, via stability of the glassy cytoplasm, preserving structural proteins, condensation of chromatin and disassembly of thylakoids in chloroplasts.

The seed coat is a maternal tissue which covers the embryo and nutritive tissues, producing both a physical and physiological layer of protection. The seed coat cells become dead at the conclusion of the seed development. Metabolites collected during seed development regulate the content and structure of the seed coat and influence the chemical and mechanical protection of the seed and its lifetime potential. The polyphenols contained in the seed coat include flavonoids, lignins and lignans. During early seed development, polymeric colourlesschemicals collect in the vacuoles of the innermost layer or endothelial cells. Later during desiccation, they are oxidized into a brown hue by polyphenol oxidase to create flavonoids termed flavonols. The flavonoids function as antioxidants and scavenge the ROS finally lowering the oxidative stress. In rapeseed (Brassica napus) the dark-pigmented seeds survive longer under accelerated aging circumstances. Peroxidation of flavonoids accumulating in seed coats may produce browning and impaired water permeability of the seed coat. The proanthocyanidins(PAs) (also known as condensed tannins) found in the seed coat may also have antibacterial effects, so creating a chemical barrier against infections by fungi. In cowpea (Vigna unguiculata), Pas was also shown to be harmful to bruchid larvae and inhibited their infestation.

Lignin is a polymer of monolignol units which are plentiful in flax seeds. It is believed to preserve the seeds from mechanical stress alongside having antioxidant qualities. Defence-related proteins which increase in testa of Arabidopsis and soybean (Glycine max) include polyphenoloxidases, peroxidases and chitinases

2.3 Glassy State of Cytoplasm:

- During late seed development, drying leads to the transition of cell cytoplasm from a fluid condition to glass viscosity, thereby disturbing the usual crystal matrices.
- The glassy state has an exceptionally low molecular mobility that permits the halting of cell metabolism and stability of cellular components.
- This in turn decreases the deteriorative processes and leads to the extension of the seed lifetime.
- The glass phase transition is critical for the physiological state of the seeds and the responsiveness to external factors (Nadarajan, et al., 2023)

2.4Temperature:

- Seeds decay quicker in higher temperature circumstances, as they enhance the rate of chemical oxidation. For this reason, gene banks are urged to dry and preserve their important germplasm at sub-zero temperatures
- Raising the temperature reduces the Tg at which the glass phase transition occurs
- Seeds with an intermediate seed storage behaviour may withstand desiccation but are vulnerable to storage at sub-zero temperatures. An example is seeds from oil palm (Ranganathan, U., & Groot, S. P. C.,2023)

2.5Oxygen:

- The cause for deuteriation in dry conditions is Oxidation. The greater the oxygen conc. increasing the ageing of seed.
- In a dry storage environment, Molecular Oxygen is the major generator of ROS
- The internal environment of the seed is abundant with metal ions such as Fe3+, Cu2+, Zn2+
- Interaction between the Mol. Oxygen with the Co-factors (Fe, Zn, Cu) resulting in the creation of ROS

2.6Moisture Content, Water Activity:

The major cause for seed degradation during storage of orthodox seeds is dampness. Water is crucial for most chemical and enzymatic processes. Oxidation of lipids, proteins and nucleic acids, key building elements of living organisms, is increased by moisture, oxygen and temperature. In the oily or lipophilic component, degradation is caused bythe oxidation of the unsaturated fatty acids in the oil bodies and membranes. In the non-oily, or hydrophilic component, degradation is mostly due to oxidation of proteins, DNA and RNA and cross-linking of macromolecules. A first rule of thumb for the quantitative effects of humidity on seed ageing was developed by Harrington (1972), saying that when the seed moisture content is between 5% and 14%, each 1% drop in seed moisture doubles the shelf life of the seeds.

While studying the impact of moisture on seed ageing, a clear distinction should be established between seed moisture content and water activity or storage relative humidity (RH). Traditionally seeds were defined by data on their moisture content for the seed trade. Also, seed technologists were employed to determine the humidity level of the seeds in moisture content, either on a fresh or dry-weight basis. However, the seed moisture content does not specify the availability of water in the non-oily component of the seeds, and consequently, the degradation processes and rates at which these reactions are taking place. Imagine castor bean (Ricinus communis) seeds having 50% oil and 10% moisture content on a wet basis, which will entail a moisture level of 20% in the non-oily section. In contrast, seeds from a common bean (Phaseolus vulgaris L.) that contain just 2% oil would experience the same total seed moisture content only around 10% moisture in their non-oily section. As a result, the physiological circumstances of both seeds will vary, despite their equal seed moisture content. Seed oil content does not only change across crops but does so fluctuate between types and production circumstances. In the food sector, it is usual to utilize water activity (aw) to measure the moisture state of goods, particularly seeds. When in equilibrium with the humidity of the surrounding air, the law is more or less directly connected to the relative humidity RH, be it that aw is stated between 0 and 1.0 and the RH in percentages. At the inaugural Seed Longevity Workshop of the International Society of Seed Science (Wernigerode, Germany,5–8 July 2015) it was determined that for research on seed ageing, it is best to compare seeds based on their aw or equilibrium RH (eRH) instead of their moisture content. (Ranganathan, U., & Groot, S. P. C.,2023). According to the Seed Viability Equation, seed life prolongs with decreasing seed moisture levels, although this is limited. In fact, at extremely low moisture levels, under so-called ultra-dry storage circumstances, which equals eRH values below roughly 15–20%, seed destruction might be quicker.

3. ROLE OF RAFFINOSE IN SEED:

3.1 Overview of Raffinose Family Oligosaccharides

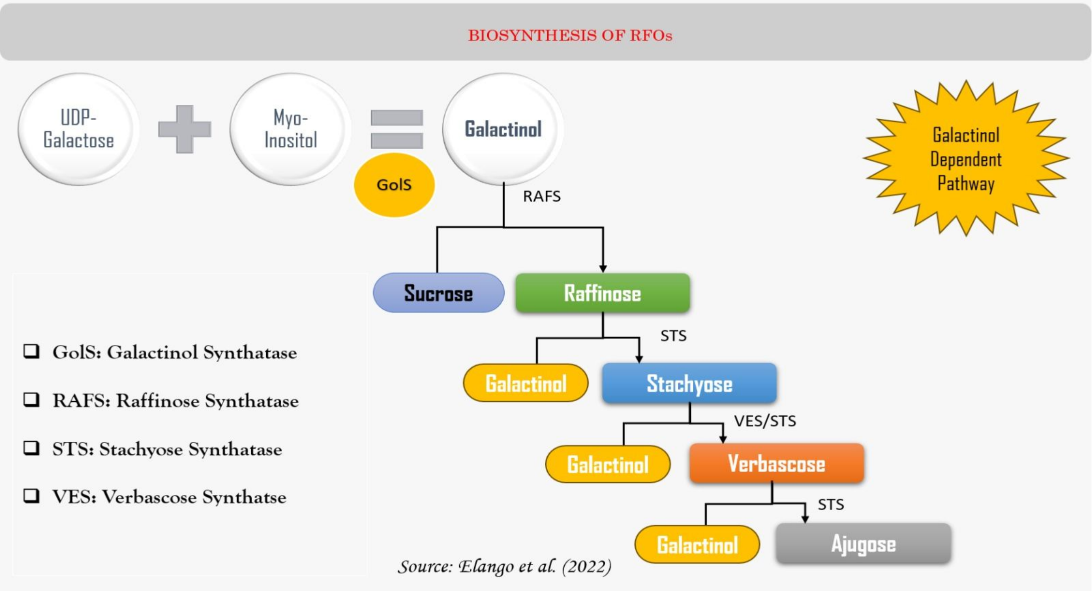
- Soluble carbohydrates ranked second to sucrose in their distribution in higher plants They are plentiful in the seed of many crops, notably in the legume family, e.g., soybean (*Glycine max*), lentil *(Lens culinaris)*, and chickpea *(Cicer arietinum)*. They are also found in roots and specialized storage organs such as tubers.
- Raffinose family oligosaccharides are α-D-galactosides of sucrose, a di-saccharide. They also exist in forms such as raffinose, stachyose, verbascose, and ajugose, belonging to trisaccharide, tetrasaccharide, pentasaccharide, and hexasaccharide groups, respectively. From the structural standpoint, they are termed α-galactosyl derivatives of sucrose. Raffinose

comprises galactose, glucose, and fructose. Stachyose contains two α-D galactose units, one α-D-glucose unit, and one
β-D-fructose unit.

- Due to the absence of alpha-galactosidase to breakdown RFOs they are neither absorbed nor digested in the upper gastrointestinal tract of humans and are stored in the large intestine of the human digestive system (Elango et al., 2022)

The Raffinose family is comprised of

- Raffinose
- Verbascose
- Stachyose
- Ajugose



Source: Elango et al. (2022)

Fig 4 :Biosynthesis of RFO's through Galactinol's Dependent Pathway

4. BIOSYNTHESIS OF RFOs

Next to sucrose, RFOs are soluble carbohydrates that are largely present in higher plants. RFOs are often present in the seeds of numerous agricultural plants, especially those belonging to the Leguminosae family, such as *Cicer arietinum* (chickpea), *Lens culinaris* (lentil), and *Glycine max* (soybean). They may also be collected in roots and specialized storage organs like leaves and tubers. RFO concentrations in Stachys sieboldii (Chinese artichoke) tubers and photosynthesizing *Ajuga reptans* leaves, for example, range from 25 to 80% of their dry weight. RFO production is catalysed by α-galactosyl transferases, which drive the successive transfer of galactosyl moieties to the sucrose. In RFO biosynthesis, galactinol synthase *(GolS)* is the most critical enzyme and the step it catalyses as it creates galactinol, which functions as a galactosyl donor for the production of other members of RFOs. Raffinose, the first element of RFOs is a trisaccharide generated by the activity of raffinose synthase (RAFS), which employs sucrose and galactinol as substrates. Similarly, stachyose is formed following the transfer of galactosyl moiety to raffinose by stachyose synthase (STS). Further transfer of the galactosyl moiety onto this chain will yield higher RFOs members such as verbascose and ajugose. The transfer of galactinol to stachyose leads in verbascose synthase (VES) mediated verbascose production. Further, transfer of galactinol to verbascose via STS leads to ajugose. In the above-mentioned RFO biosynthesis process, galactinol acts as a donor for galactosyl in each stage, so this method of RFO biosynthesis is referred to as galactinol-dependent pathway. However, another major enzyme, galactan-galactosyltransferase (GGT), is engaged in the galactinol-independent pathway, which is less prevalent than the first and has only been recorded in two Lamiaceae species: *Coleus blumei* and *Ajuga reptans.* The GGT catalyses the transfer of the galactosyl moiety from one RFO molecule to another, resulting in the synthesis of a higher RFO member. For instance, via the action of GGT, the transfer of a galactosyl moiety from one stachyose to another stachyose would result in the formation of verbascose and raffinose. In this approach, the GGT not only creates higher members of RFO but also controls the concentration of cellular RFO By the action of α-galactosidases RFOs are digested to sucrose and galactose. Sucrose degradation into fructose and glucose by invertase or to UDP-glucose and fructose by sucrose synthase. Galactose is initially phosphorylated by the ATP-dependent galactokinase to generate galactose-1-P (Gal-1P). Gal-1-P is further digested by two separate routes, one of which is the Lelior pathway and the other one is an alternate pathway in plants. In the Lelior pathway, hexose-1-P uridylyltransferase transfers the UMP from UDP-glucose to galactose-1-phosphate resulting in UDP-galactose, with the release of glucose-1-phosphate. However, in plants, galactose-1-phosphate is digested by an alternate mechanism. Pyrophosphorylase transforms galactose-1-phosphate and UTP to UDP-galactose and PPi. Subsequently, the NAD-dependent UDP-galactose-4-epimerase transforms UDP-galactose to UDP-glucose. The RFO biosynthesis pathway in plants includes various main enzymes like*GolS*, RAFS, STS, and VES whose modification in the crops leads to the acquisition of different stressors as well as other plant metabolic processes (Elango *et al.,* 2022)

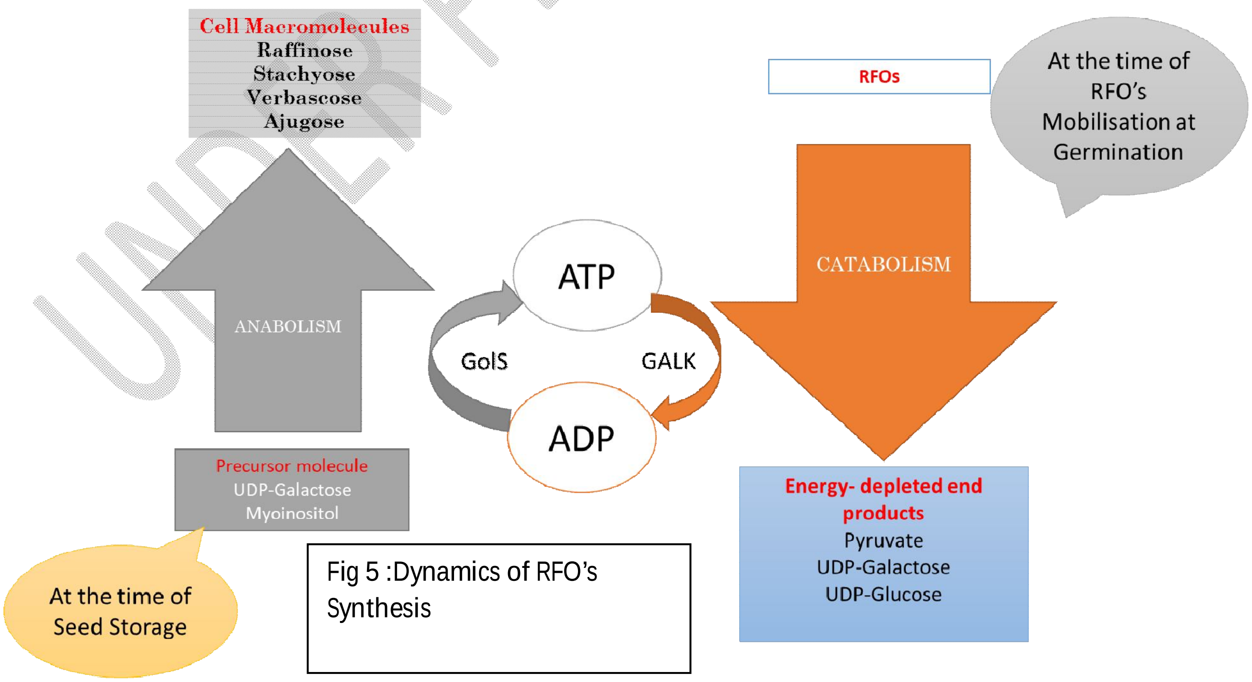


Fig 5 :Dynamics of RFO's Synthesis

5. GENETICS OF RFOs:

GolS Gene found in *Arabidopsis thaliana* and *Cicer arietinium*. *Raf* and *Sta*genes are found in *A. thaliana. Alpha-Gal* genes are found in Beech, *A. thaliana*

5.1 Process of Regulation of Seed Vigour and Longevity

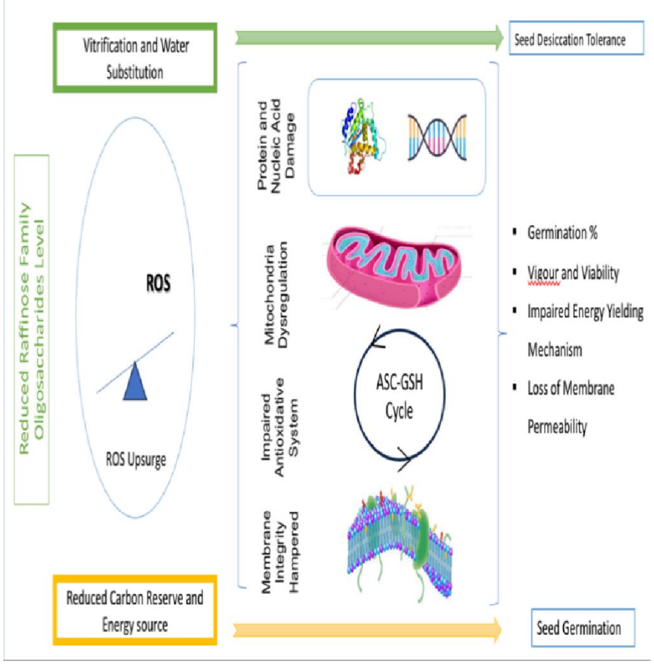
Dry seeds should be dispersed from the mother plant to enable plant generation continuance. When seeds develop, major physiological, biochemical, and physical changes occur, culminating in the capacity to survive in harsh circumstances. Dehydration occurs toward the conclusion of the maturation period in plant seeds, resulting in the buildup of potentially protective chemicals, especially soluble sugars such as RFOs and sucrose, as well as LEA proteins. The LEA proteins, in cooperation with the soluble sugars, contribute to the maintenance of protein and membrane structural integrity under dry environments by generating a glassy state that inhibits deteriorative processes. As seeds spread and mature, they lose water, a process known as ‘desiccation’, to prepare for survival in harsh or unfavourable settings. Non-reducing sugars such as sucrose and RFOs may develop in seeds and prevent desiccation at that time to avoid injury, and various research has demonstrated that RFOs play a function in desiccation tolerance. For instance, sucrose and RFOs, accumulate in the seeds of *Erythrina speciosa*, a Brazilian native tree, before major changes in water content and are re-allocated from vacuole reserves to the cytosol late in seed development. It has been proposed that they could aid in preserving the liquid crystalline condition of the cellular membranes in the dry state and are favourably connected with desiccation resistance as well as with seed life. On the other hand, the Brazilwood seeds usually display orthodox behaviour and survive the desiccation throughout their maturation owing to the formation of sugar alcohols such as galactopinitol-A, galactopinitol-B, ciceritol, and lipids. Broadly, two RFO mechanisms have been hypothesized to govern the desiccation process in seeds. The first process is referred to as ‘vitrification’. This is the situation of a cell solution that has become highly viscous due to water loss. The cell solution has the properties of a plastic solid in this situation. It is in regulation of cell stability, cellular collapse prevention, and hydrogen bonding preservation. LEA proteins, HSPs, and RFOs cause the vitrification state. The second is known as ‘water substitution’, in which RFOs’ hydroxyl groups may replace water molecules inside the cell and retain the hydrophilic interactions essential for the stability of native macromolecules and membrane structure during dehydration. Similarly, these RFOs have a major influence on seed germination, often protect embryos from desiccation during seed development, and improve seed life under harsh circumstances. However, sugars are generally believed to work as signalling molecules or as osmoprotectants; yet, their role and accumulation, especially RFOs, have been intensively explored in regulating and affecting seed vigour and lifespan. Like, RFOs have been proven to help sucrose in keeping membrane integrity by preventing lipid crystallization and aging processes. Sucrose is the most abundant sugar in maize seeds, but its quantity did not correspond with better storage; rather, raffinose as a mass fraction of total sugars demonstrated a large and favourable link with seed vigour and lifespan. In soybeans likewise, it has been proven that an increase in RFO to sucrose ratio as well as alterations in RFO metabolism genes including GolS and RAFS has been favorably related to seed maturity, vigour, and lifespan. Additionally, variations in soluble sugar content, notably RFOs, have been related to seed vigour and germinability in Arabidopsis and other species as well. Although the quantity and kind of RFOs that accumulates during seed development differ per species. For example, maize, Arabidopsis, and lettuce collect more raffinose than any other RFOs like stachyose and verbascose whereas castor bean accumulates more raffinose and stachyose but not verbascose. In contrast, galactinol and myoinositol levels have been reported excessively high in seeds across various species. Legumes are the major crops that acquire the greatest RFOs in their seeds. Alpha-galactosides (α-Gal), sucrose-1,6-galactosyl derivatives, are one of the principal complex sugars found in leguminous seeds. Furthermore, RFO accumulation and associated α-GAL activity are connected to ripe and developing chickpea seeds. When compared with the control, preventing RFO breakdown mediated by α-Gal with 1-deoxygalactonojirimycin (DGJ) reduced seed germination by roughly 25% in pea plants. The accumulation of galactinol, and sucrose, occurred during the early stages of chickpea pod formation, whereas the raffinose, and stachyose, accumulate during the later stages of seed maturity, which indicated the accumulation of the higher-order RFOs pathway during seed maturation. However, interestingly, some studies revealed that low RFO genotypes of soybean and chickpea did not display delayed germination, indicating that RFOs had no substantial role in increasing seed germination.

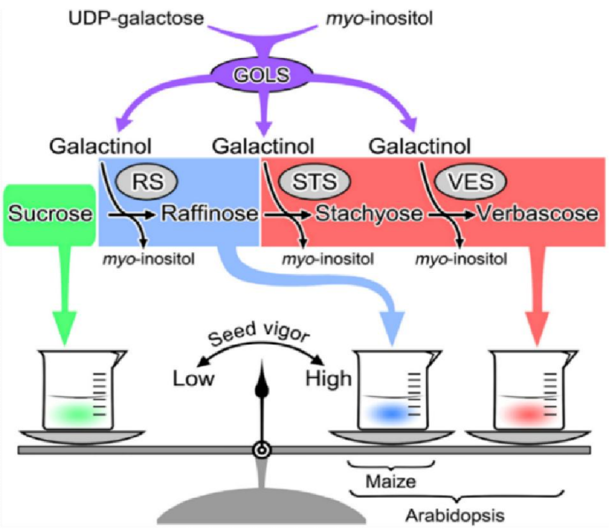
Similarly, galactinol production by GolS from Myo-inositol and UDP-galactose is seen as an important regulatory step in RFO biosynthesis. Carbon partitioning between sucrose and raffinose saccharides is controlled by GolS. The researchers presented a QTL mapping in tomatoes that discovered a co-locating QTL on chromosome 2 for galactinol concentration and seed longevity. The data demonstrated a positive connection between galactinol concentration and seed longevity across the three species tested, Arabidopsis, cabbage, and tomato, suggesting that this link occurs throughout Brassicaceae and beyond. They employed a reverse genetics technique to study the role of GolS enzymes in seed lifespan, utilizing T-DNA knockout lines in genes encoding enzymes of this system such as GolS1, GolS2, STC, and α-GAL, as well as Arabidopsis overexpresses of the cucumber GolS2 gene. The gols2 mutant and the gols1 gols2 double mutant demonstrated the lowest seed galactinol content, resulting in the shortest seed life. One more recent work has identified the up-regulation of GolS1 in the degradome sequencing after long-term storage and controlled degradation of Nicotiana and Oryza sativa seeds, which further ties the RFO metabolism genes to seed lifetime. Collectively, RFOs are virtually ubiquitous in the plant world and may be found in a variety of vegetative organs and seeds. RFO accumulation in response to several biotic and abiotic stimuli shows that RFOs have a role in stress adaptation in addition to serving as a signaling molecule and osmoprotectants during seed development and lifespan. In addition to its participation in stress adaptation, GolS is implicated in alkaloid tolerance and may have a function in cotton male fertility, fiber quality, and seed development.

Because GolS is a major regulatory enzyme in RFO production, many classic and innovative transgenic techniques have been investigated to generate plants that are more resistant to stress as well as seed vigor and lifespan. The role of RFOs in plant health is presently being contested, however, it has various consequences (Salvi *et al*., 2022)

6. Multifunctional Role of RFOs in Plant Health:

RFOs support the cell membrane under dehydration stress by inserting themselves inside the lipid head groups of the membrane bilayer and increase RFOs during desiccation and stability of membrane phospholipids. RFOs under abiotic stress conditions function as osmolytes to maintain cell turgor and act as an antioxidant against reactive oxygen species Even though RFOs are derived from an extended metabolic pathway of inositol, they aren’t directly involved in plants’ stress amelioration under natural conditions, unlike other products derived from the same pathway (Loewus and Murthy, 2000; Sengupta et al., 2012). A subsequent increase of RFOs (especially raffinose) has been observed in several cases of abiotic stresses such as heat, cold, salinity, or drought (Santarius and Milde, 1977; Bachmann et al., 1994; Taji et al., 2002; Pennycooke et al., 2003; Panikulangara et al., 2004; Nishizawa- Yokoi et al., 2008; Peters and Keller, 2009; Peters et al., 2010). However, there is not much literature defining the particular functional functions of RFOs in abiotic stress tolerance. Several additional compounds (e.g., sucrose and proline) with recognized functions in abiotic stress amelioration also tend to concentrate under similar circumstances. Reports have also revealed that the genetic deletion of biosynthetic enzymes linked with RFOs does not harm plants severely (Panikulangara et al., 2004), providing more proof of the aforementioned fact. On the contrary, several research argues that RFOs do have the advantageous features of a suitable solute. For example, research by Hincha et al. (2003) reveals that RFOs protect the cell membrane under dehydration stress by inserting themselves into the lipid head groups of the membrane bilayer. Farrant (2007) offered more confirmation to this fact by linking the occurrence of a rise in RFOs during desiccation with the stability of membrane phospholipids. Moreover, their long oligomeric length may favourablyaffect protective liposomes (Cacela and Hincha, 2006) and may operate as a free radical scavenger (Nishizawa-Yokoi et al., 2008). Furthermore, several reports suggest that the accumulated RFOs under abiotic stress conditions function as osmolytes to maintain cell turgor and act as an antioxidant against reactive oxygen species (Nishizawa- Yokoi et al., 2008; van den Ende and Valluru, 2008; Bolouri- Moghaddam et al., 2010; Stoyanova et al., 2011; van den Ende et al., 2011; Peshev et al., 2013). Galactinol synthase *(GolS*) is a crucial enzyme that is involved in the manufacture of RFOs (Saravitz et al., 1987) and is known to be associated to abiotic stress (Sengupta et al., 2015). Therefore, genetically altering the expression of *GolS*genes may reveal considerable information regarding the role of RFOs in mediating response to abiotic stressors. These investigations have been carried out largely in Arabidopsis thaliana or tobacco (Nicotiana tabacum) plants, since they tend to boost galactinol and raffinose content in response to abiotic stressors (Taji et al., 2002; Zhuo et al., 2013; Himuro et al., 2014; Shimosaka and Ozawa, 2015; Gu et al., 2016). Multiple isoforms of *GolS* have been found in diverse plant species so far; each is generated under varied situations of abiotic stressors. It has been observed that out of seven known *GolS* genes from Arabidopsis thaliana, AtGolS1 and AtGolS2 were stimulated by drought, salt, or heat stress. In contrast, AtGolS3 from the same genome were activated by cold stress (Taji et al., 2002). Over-expressing or knocking off these genes may be made use of for the research of RFO physiology. Studies by Taji et al. (2002) and Panikulangara et al. (2004) indicated that over-expression of these genes increased the accumulation of galactinol (Gol), raffinose (Raf), and stachyose (Sta) and subsequently enhanced the plant's tolerance level to drought, salt, or cold stress. Panikulangara et al. (2004) also established that AtGolS1 mutant plants fail to collect heat stress-induced Gol and Raf, suggesting that *AtGolS1* may be the essential *GolS* isoform responsible for heat stress-induced Raf or Gol accumulation. However, research by Peters et al. (2010) using a double mutant; reported that despite the better accumulation of *GolS1* in *GolS2* mutants, they remain hypersensitive to water stress, display fast loss of water and decrease enzymatic activity. Hence demonstrating they are drought-hypersensitive. Such discoveries addressing the fact that Arabidopsis neither stores nor transports RFOs indicate the participation of diverse biosynthetic pathways that are provided by different*GolS* isoforms. Likewise, cold temperature tolerance was obtained by overexpressing the Medicago falcata *Gols* (MfGolS1) gene in tobacco (Zhuo et al., 2013). Valluru and van den Ende (2011) elucidated the function of galactinol in signalling RFOs to modulate stress responses, including a signal in response to pathogen infection. Thus, illustrating the involvement of RFOs in defense against biotic stress.*GolS* promoted the expression of defence-related genes such as PR1a, PR1b, and NtACS1 in tobacco during Botrytis cinerea and *Erwinia carotovora* infection (Kim et al., 2008). Also, Gol promotes the salicylic acid (SA) signalling following pathogen infection and ultimately switches on the PR1a gene expression to regulate disease development (Couée et al., 2006). RFOs (primarily *GolS*and*RafS*) have W-box cis-elements in their promotors, controlled by ABA-inducible WRKY (Wang et al., 2009). This shows a probable involvement of RFOs in SA and ABA signalling under biotic and abiotic stressors. Figure 3, displays the importance of RFOs in plant health, including their involvement in seed germination, seed development, desiccation tolerance, and biotic and





abiotic stress tolerance (Elango *et al*., 2022).

Fig 6 :Multifunctional Role of RFO's

7. SUMMARY AND WAY FORWARD

Raffinose family oligosaccharides might be used as beneficial meals. Its multi-functional advantages are still yet to be appreciated in human and animal well-being. RFOs favorably influence the gut microbiota, large intestines, and colon health and might be employed as therapeutic agents to alleviate inflammation, diabetes, allergies, etc. RFOs are regarded the major candidate for flatulence in humans and animals. Hence, for the crops with high RFOs, notably grain legumes, adoption in the food and feed system is substantially affected owing to the flatulence issue. Therefore, we need to find the correct balance of RFO concentration in crops to promote them as functional foods. Still, the correct concentration of RFOs essential for human well-being remains the field to be researched further. Moreover, except for Japan, other countries of the globe have yet to certify RFOs as functional foods. Over the last 50 years, seed storage science has advanced from anecdotal 'Thumb Rules' to empirically-based advances in biochemistry, genomics and biophysics, which have increased the understanding of how seeds can attain cytoplasmic solidification upon drying, how the properties of these intracellular glasses are related to the kinetics of ageing and how seed ageing is characterised by the random and continuous oxidative degradation of proteins, lipids and nucleic acids, which cannot be quenched by the cells' antioxidant machinery. Seed storage biology is a complex and interdisciplinary discipline including seed physiology, biophysics, biochemistry and multi-omics (genomics, transcriptomics, proteome, metabolomics, Ionomics and phenomics) technologies. Knowledge advancement and technology development in these fields have helped us to better understand and predict seed storage behaviour and to optimise the storage environment that prolongs viability during storage, supporting agriculture, ex-situ conservation and the sustainable use of seeds (Buitink& Leprince, 2008) (Nadarajan et al., 2023)

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