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Storage induced biochemical changes regulate seedling Vigour and seed longevity in finger millet (*Eleusine coracana*)

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Abstract

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a nutritionally rich, climate-resilient cereal crop that plays a significant role in ensuring food and nutritional security, particularly in the semi-arid regions of Africa and Asia. Despite its importance, seed deterioration during storage remains a major challenge affecting seed viability, vigour, and field establishment. Seed ageing is primarily associated with oxidative damage, membrane deterioration, and disruption of cellular metabolism, leading to poor germination and reduced seedling growth. Therefore, understanding the physiological and biochemical mechanisms underlying seed longevity is essential for developing genotypes with improved storage potential. The present study was undertaken to evaluate the physiological and biochemical responses of two finger millet genotypes, GPU-28 and GPU-67, exhibiting differential rates of ageing under accelerated ageing (AA) conditions. The study demonstrated significant genotypic variation in seed longevity and tolerance to accelerated ageing. The superior physiological performance, higher seed viability, and lower oxidative damage observed in GPU-28 indicate that this genotype possesses stronger cellular defence mechanisms against ageing-induced deterioration than GPU-67. These findings suggest that physiological traits, together with biochemical markers such as TTC, MDA, and MG, provide reliable indicators for assessing seed longevity and identifying ageing-tolerant genotypes. GPU-28 can therefore serve as a valuable genetic resource for breeding programmes aimed at improving seed storage stability, seed vigour, and crop establishment in finger millet.

Keywords: Finger millet, seed longevity, accelerated ageing, seed vigour, oxidative stress

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a small-seeded, climate-resilient cereal that plays an important role in ensuring food, nutrition, and feed security for millions of people in Sub-Saharan Africa and South Asia. It is widely cultivated in countries such as Eritrea, Ethiopia, Kenya, Uganda, Tanzania, India, Nepal, China, and Sri Lanka ^[1]. Global production is estimated at approximately 28 million tonnes with an average productivity of 0.75 t ha⁻¹ ^[2]. Among the minor millets, it ranks third after pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*) ^[3]. Despite its agronomic and nutritional importance, finger millet remains one of the world's neglected and underutilized crops, often referred to as a "forgotten African crop" ^[4]. Africa is the largest producer of finger millet, while India contributes nearly 18% of global production ^[5].

Finger millet is valued for its exceptional nutritional composition, containing high levels of dietary fibre (18%), calcium (0.38%), and phenolic compounds (0.30-3.0%), making it nutritionally superior to major cereals such as maize, wheat, rice, sorghum, and barley ^[6]. Its rich phytochemical profile provides several health benefits, including antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, hypocholesterolemic, and anti-cancer properties, highlighting its potential as a functional food and nutraceutical ^[1, 6-9]. Furthermore, its gluten-free nature, low glycaemic index, and easy digestibility make it an ideal dietary choice for individuals with coeliac disease, gluten intolerance, and diabetes ^[10]. Despite its high nutritional value, finger millet contains anti-nutritional compounds such as phytates, tannins, and oxalates that reduce nutrient bioavailability. However, processing techniques including soaking, germination, fermentation, decortication, puffing,

and thermal treatments effectively lower these compounds while improving nutrient availability, digestibility, and product quality [8, 11]. Consequently, finger millet has excellent potential for the development of nutritious value-added foods and beverages that can enhance dietary diversity and food security.

Seed longevity and vigour are determined by a combination of physiological and biochemical processes that are progressively affected during seed ageing. Accelerated ageing is a widely used method to simulate natural seed deterioration and evaluate the storage potential of different genotypes. Physiological traits such as germination percentage, root length, and shoot growth provide reliable indicators of seed vigour and early seedling establishment, while biochemical markers including tetrazolium chloride (TTC) reduction, malondialdehyde (MDA) content, and methylglyoxal (MG) accumulation reflect changes in cellular viability, membrane integrity, oxidative damage, and metabolic activity during ageing. Therefore, the combined assessment of these physiological and biochemical parameters enables a comprehensive evaluation of seed deterioration mechanisms and helps identify genotypes with superior seed longevity and tolerance to storage-induced oxidative stress.

Recent research on finger millet has advanced in areas such as agronomic management, crop improvement, genomics, breeding, post-harvest processing, nutritional enhancement, and industrial utilization. However, significant research and development gaps remain in Eritrea, where cultivation is largely traditional, with limited mechanization, low adoption of improved varieties, and inadequate agronomic practices. Furthermore, studies on the crop's genetic diversity, germplasm characterization, conservation, and breeding are still limited, and Eritrea's contribution to global finger millet production remains under-recognized. Strengthening research, breeding programmes, and value-chain development is essential to fully exploit the potential of finger millet as a climate-resilient, nutritionally rich, and economically important crop.

Materials and Methods

Seeds of genotypes GPU28 and GPU67 were subjected to controlled accelerated ageing in order to simulate deterioration and to evaluate genotypic differences in

seedling vigour and biochemical response. For the accelerated ageing treatment, seeds were incubated in a humidity-controlled environment at 45 °C and ~100% relative humidity for durations of four, six and eight days; 100% RH was maintained by placing seeds above a water reservoir inside a desiccator kept within a temperature-regulated incubator. Following each ageing interval, seeds were either germinated under standard conditions to obtain seedlings or processed directly for biochemical analyses. Seed and seedling samples were assayed for cellular respiration-linked dehydrogenase activity using the triphenyl tetrazolium chloride (TTC) reduction test: tissue samples were incubated in 0.1% (w/v) TTC solution, and the formation of red formazan (reflecting NADPH-dependent dehydrogenase activity and respiratory viability) was quantified spectrophotometrically after extraction. Lipid peroxidation, an indicator of oxidative membrane damage caused by ageing, was quantified by measuring malondialdehyde (MDA) equivalents using the thiobarbituric acid (TBA) reactive substances method: samples were homogenized in trichloroacetic acid (TCA), reacted with TBA reagent, heated to drive the condensation reaction, cooled, and the absorbance of the resultant chromogen was read to calculate MDA concentration against appropriate blanks and standards. In addition, methylglyoxal (MG), a cytotoxic α -oxoaldehyde that accumulates under stress and contributes to carbonyl stress, was determined after protein removal using perchloric acid precipitation and subsequent derivatization with o-phenylenediamine (or 1, 2-phenylenediamine) to form the corresponding quinoxaline adduct, which was quantified spectrophotometrically (or by HPLC where higher specificity was required). All biochemical determinations were performed with appropriate controls and replicates, and results were expressed per unit fresh weight (or protein) to allow comparison across genotypes and ageing durations; these assays together provide complementary measures of viability (TTC), oxidative membrane damage (MDA/TBA), and carbonyl stress (MG), thereby enabling a mechanistic interpretation of the reduced vigour observed in aged seeds and seedlings of the contrasting genotypes.

Results and Discussion

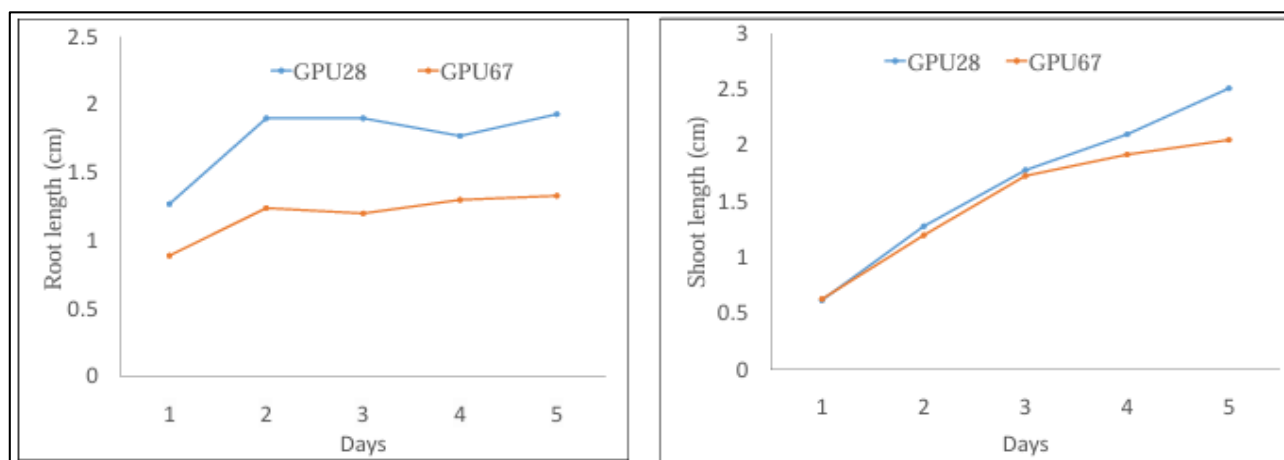


Fig 1: Shoot and Root growth of the genotypes under normal condition

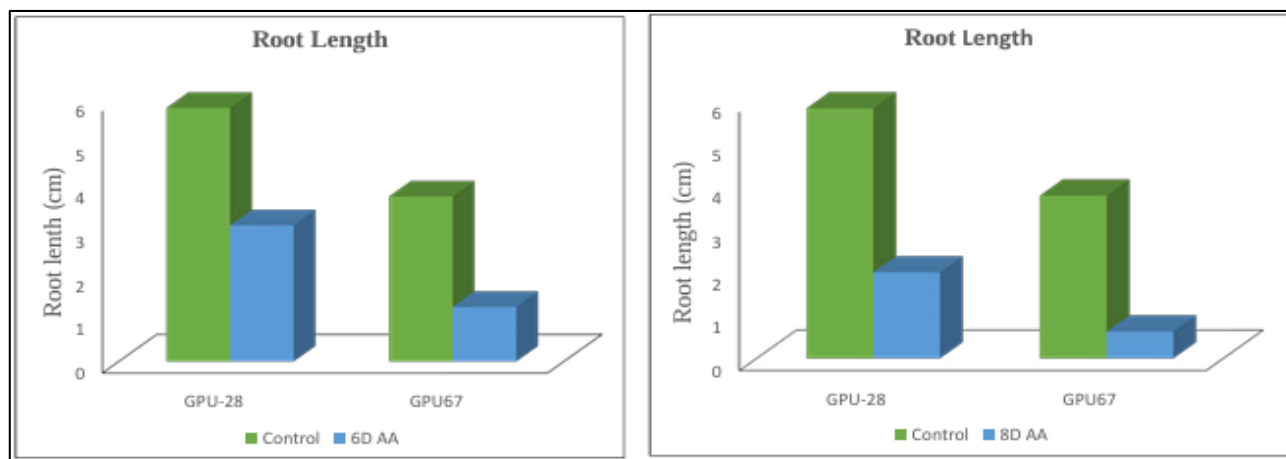


Fig 2: Root length of the genotypes under accelerated ageing (AA) conditions

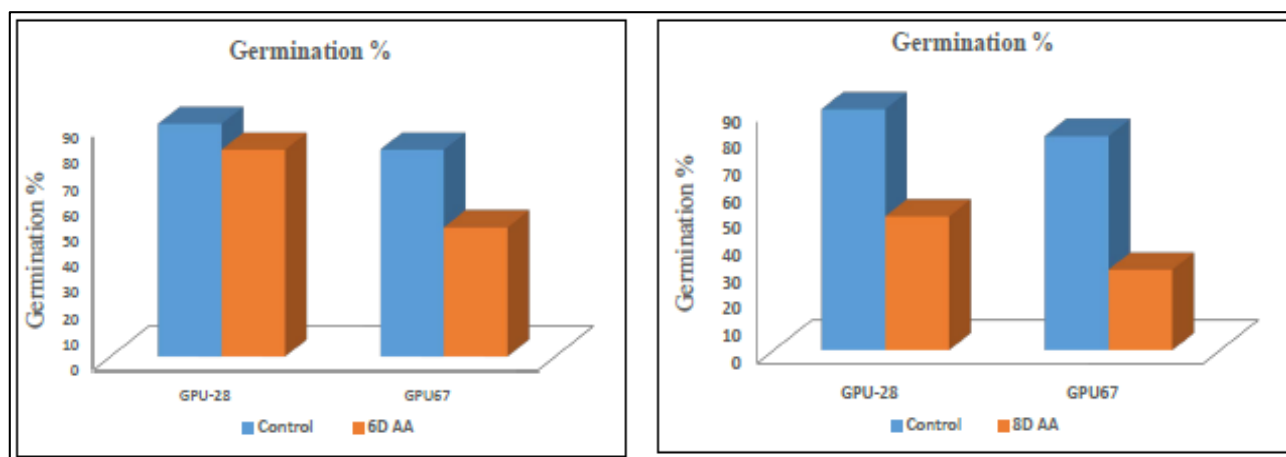


Fig 3: Germination Percentage of the genotypes under accelerated ageing (AA) and control conditions

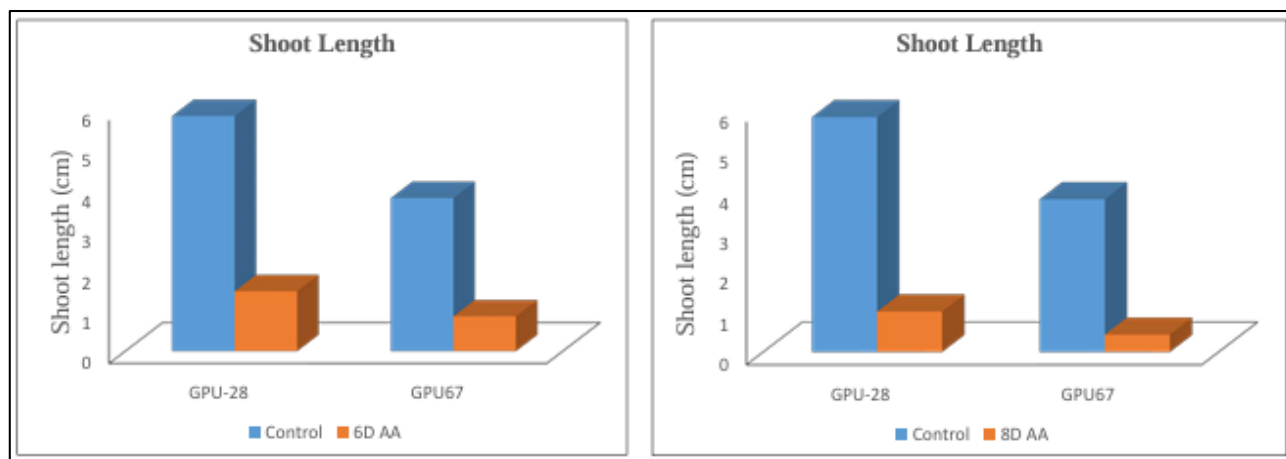


Fig 4: Shoot length of the genotypes under accelerated ageing (AA) and control conditions

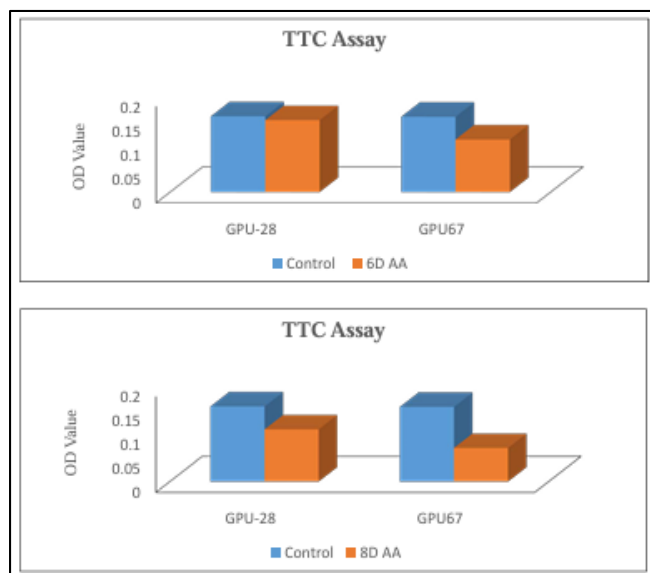


Fig 5: TTC Assay of the genotypes under accelerated ageing (AA) and control conditions

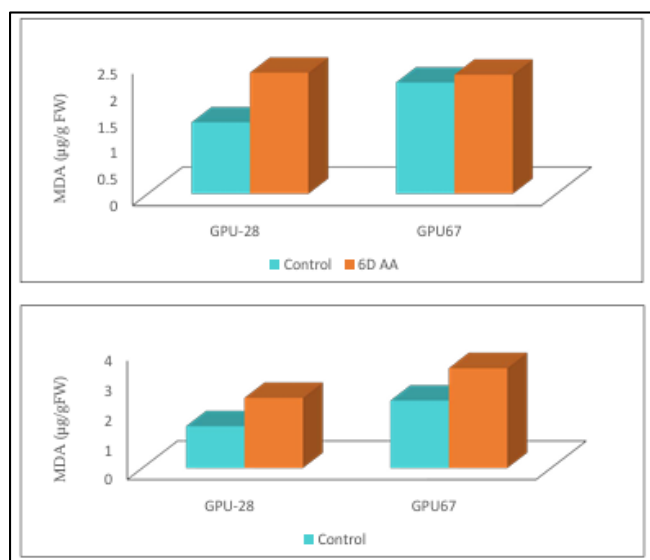


Fig 6: MDA Assay of the genotypes under accelerated ageing (AA) and control conditions

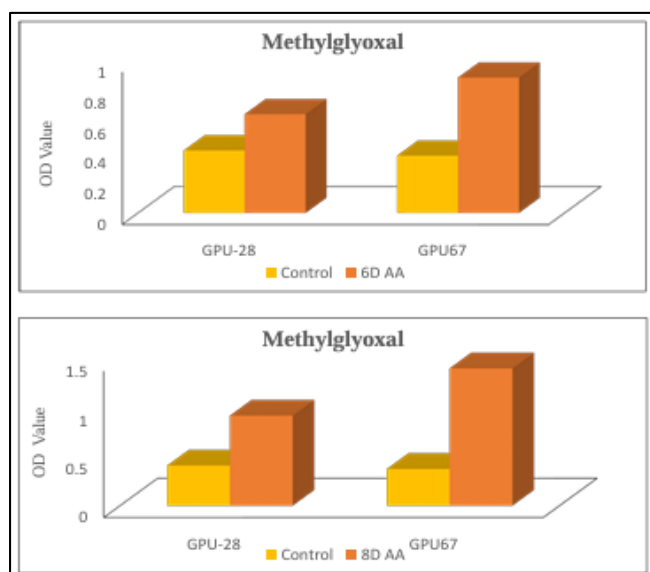


Fig 7: Methylglyoxal Assay of the genotypes under accelerated ageing (AA) and control conditions

Shoot and Root growth

Under control conditions, GPU-28 exhibited greater root and shoot growth than GPU-67 throughout the five-day observation period, indicating superior seedling vigour (Fig. 1). Root length increased rapidly during the initial days, while shoot length showed a gradual increase in both genotypes. The consistently higher root and shoot growth of GPU-28 suggests better early seedling establishment and more efficient utilization of seed reserves than GPU-67 [13-15].

Root length

Accelerated ageing (AA) significantly reduced root length in both genotypes, and the reduction became more pronounced with increasing ageing duration (Fig. 2). Under control conditions, GPU-28 recorded a root length of approximately 6.2 cm, compared with 4.2 cm in GPU-67. After 6 days of AA, root length declined to 3.5 cm in GPU-28 and 1.7 cm in GPU-67, while 8 days of AA further reduced root length to 2.4 cm and 0.6 cm, respectively. The greater retention of root growth in GPU-28 indicates superior seed longevity and tolerance to ageing. In contrast, the marked reduction in GPU-67 suggests greater susceptibility to oxidative stress, membrane deterioration, and depletion of seed reserves, which impair cell division and seedling establishment [16-18]. These results demonstrate that root length is a reliable indicator of seed vigour and ageing tolerance, highlighting GPU-28 as a promising genotype for improving seed longevity in finger millet.

Germination Percentage

Accelerated ageing (AA) significantly reduced germination in both finger millet genotypes, with the reduction increasing from 6 to 8 days of ageing (Fig. 3). Under control conditions, GPU-28 recorded higher germination (92%) than GPU-67 (82%). After 6 days of AA, germination declined to 82% in GPU-28 and 55% in GPU-67, while 8 days of AA further reduced germination to 55% and 35%, respectively. The higher germination retained by GPU-28 indicates superior seed longevity and ageing tolerance. In contrast, the greater decline in GPU-67 suggests enhanced oxidative stress, membrane deterioration, lipid peroxidation, and reduced metabolic activity during storage. Similar genotypic differences in germination and seed longevity have been reported in finger millet and other cereals, where accelerated ageing progressively decreases seed viability through oxidative damage and depletion of seed reserves [16-19]. These findings identify GPU-28 as an ageing-tolerant genotype with better storage stability.

Shoot Length

Accelerated ageing markedly reduced shoot length in both genotypes, with a greater reduction observed after 8 days of ageing (Fig. 4). Under control conditions, GPU-28 recorded a shoot length of approximately 6.0 cm, whereas GPU-67 measured 4.0 cm. After 6 days of AA, shoot length decreased to 1.8 cm in GPU-28 and 1.0 cm in GPU-67, and after 8 days of AA, it further declined to 1.2 cm and 0.5 cm, respectively. Despite the reduction, GPU-28 consistently maintained greater shoot growth than GPU-67, indicating superior seed vigour and tolerance to ageing-induced deterioration. The decline in shoot length is associated with oxidative stress, membrane damage, enzyme inactivation, and depletion of seed reserves, which impair cell division

and elongation during seedling establishment. Similar responses have been reported in finger millet and other cereals under accelerated ageing conditions [16-19]. Thus, the greater shoot growth retained by GPU-28 further confirms its superior seed longevity and potential as a donor genotype for breeding programmes aimed at improving storage tolerance.

Tetrazolium Chloride (TTC) Assay

The TTC assay showed a progressive decline in seed viability in both finger millet genotypes following accelerated ageing (AA), as indicated by reduced optical density (OD) values (Fig. 5). Under control conditions, GPU-28 and GPU-67 exhibited similar TTC values (0.18-0.19 OD). After 6 days of AA, TTC values declined to approximately 0.17 in GPU-28 and 0.14 in GPU-67, while 8 days of AA further reduced them to 0.13 and 0.10, respectively. The higher TTC reduction retained by GPU-28 indicates greater dehydrogenase enzyme activity, better membrane integrity, and superior seed viability than GPU-67. In contrast, the greater decline in GPU-67 suggests enhanced oxidative stress, mitochondrial dysfunction, and membrane deterioration, resulting in accelerated seed ageing. Similar reductions in TTC activity and seed viability under accelerated ageing have been reported in finger millet and other cereals [16-19]. These findings confirm the superior storage potential and seed longevity of GPU-28.

Malondialdehyde (MDA) Assay

Accelerated ageing significantly increased MDA content in both genotypes, with a greater increase observed after 8 days of ageing (Fig. 6). Under control conditions, GPU-28 recorded lower MDA content ($1.5 \mu\text{g g}^{-1}$ FW) than GPU-67 ($2.3 \mu\text{g g}^{-1}$ FW). After 6 days of AA, MDA increased to approximately 2.5 and $2.6 \mu\text{g g}^{-1}$ FW in GPU-28 and GPU-67, respectively, and further increased after 8 days of AA to 2.8 and $3.8 \mu\text{g g}^{-1}$ FW. The lower MDA accumulation in GPU-28 indicates reduced lipid peroxidation and better protection against oxidative damage, whereas the higher MDA levels in GPU-67 reflect greater membrane deterioration and susceptibility to seed ageing. Similar increases in MDA during accelerated ageing have been reported in finger millet and other cereals, where lipid peroxidation is closely associated with loss of seed vigour and viability [16-20]. Thus, the lower MDA content in GPU-28 further confirms its superior seed longevity and tolerance to storage-induced deterioration.

Methylglyoxal (MG) Assay

Accelerated ageing (AA) significantly increased methylglyoxal (MG) accumulation in both finger millet genotypes, with a greater increase observed after 8 days of ageing (Fig. 7). Under control conditions, GPU-28 and GPU-67 exhibited low MG levels (approximately 0.45-0.50 OD). After 6 days of AA, MG increased to approximately 0.70 OD in GPU-28 and 1.00 OD in GPU-67, while 8 days of AA further increased MG levels to 1.00 OD and 1.50 OD, respectively. The consistently lower MG accumulation in GPU-28 indicates superior tolerance to ageing-induced oxidative stress and better seed longevity than GPU-67. Methylglyoxal is a reactive cytotoxic by-product of glycolysis, and its accumulation during seed ageing causes protein glycation, enzyme inhibition, membrane damage, and disruption of cellular metabolism. The higher MG levels

observed in GPU-67 suggest greater oxidative damage and a less efficient glyoxalase detoxification system, whereas the lower MG accumulation in GPU-28 indicates more effective detoxification of reactive carbonyl compounds and better maintenance of cellular integrity during storage. Similar increases in MG during seed ageing have been reported in cereals and model plants, where MG is recognized as an important biochemical marker of oxidative stress [21, 22]. Likewise, finger millet studies have demonstrated that ageing-tolerant genotypes maintain lower MG accumulation and higher seed vigour than susceptible genotypes [16, 17]. Therefore, the lower MG content in GPU-28 further confirms its superior storage potential and resistance to seed deterioration compared with GPU-67.

Conclusions

The present study demonstrated significant genotypic variation in seed longevity and tolerance to accelerated ageing in finger millet (*Eleusine coracana*). Accelerated ageing adversely affected all physiological and biochemical parameters, with the severity of deterioration increasing from 6 to 8 days of ageing. Physiological assessments revealed progressive reductions in germination percentage, root length, shoot length, and overall seedling growth, indicating a decline in seed vigour during storage. Among the two genotypes, GPU-28 consistently outperformed GPU-67, maintaining higher germination, greater root and shoot growth, and better seedling establishment under both control and ageing conditions. Biochemical analyses further supported these findings, as GPU-28 retained higher tetrazolium chloride (TTC) activity, reflecting superior seed viability and respiratory metabolism, while exhibiting significantly lower malondialdehyde (MDA) and methylglyoxal (MG) accumulation than GPU-67. Reduced MDA and MG levels indicate lower lipid peroxidation, improved membrane integrity, and greater tolerance to oxidative stress during seed ageing. In contrast, GPU-67 exhibited rapid deterioration characterized by reduced seed viability, impaired seedling growth, and increased oxidative damage. Overall, the integration of physiological and biochemical parameters proved effective for evaluating seed longevity and ageing tolerance. The superior performance of GPU-28 identifies it as a promising genotype with enhanced storage stability and seed vigour, making it a valuable genetic resource for breeding programmes aimed at improving seed longevity, storage tolerance, and crop establishment in finger millet.

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