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Physiological Impacts of Seed Ageing on Germination and Seedling Vigour in Brassica Species

Baldwin Meitankeisangbam[1], Thokchom Brien Singh[2], Yanglem Mansi Devi[3], Samjetsabam Chanulembi Devi[4]

[1]Young Professional, Seed Division, The Green Foundation, Imphal (India)
[2]Cluster Coordinator & Scientist, Integrated Organic Farming Demonstration Project, The Green Foundation, Imphal (India)
[3]Young Professional, Soil Division, The Green Foundation, Imphal (India)
[4]Young Professional, Crop Improvement Division, The Green Foundation, Imphal (India)

Abstract
Seed ageing significantly affects the physiological quality, germination capacity, and seedling vigour of *Brassica* species, which critical challenges for global agriculture. This study examines the effects of ageing on germination and early growth in six *Brassica* varieties under controlled and field conditions. Ageing processes, including increased accumulation of reactive oxygen, lipid peroxidation, and protein oxidation, impair cellular membrane integrity, as reflected by increased electrical conductivity (EC) values in aged seeds. Germination rates declined significantly, with aged seeds showing a 72.5% germination rate for Potsangbam Yella compared to 95.5% in fresh TS-36 seeds. Similarly, the seedling vigor index (SVI) for aged PM-28 seeds decreased to 66.20, compared to 94.81 in fresh TS-36 seeds. TS-36 exhibited strong tolerance to ageing, whereas Potsangbam Yella showed the greatest decline in seed quality. These results enhance the importance of optimized storage practices and highlight non-destructive EC testing as a practical tool for seed viability evaluation. The findings have significant implications for advancing seed storage protocols, enhancing breeding strategies, and promoting agricultural sustainability.

Keywords: seed aging, germination, seedling vigour, *Brassica* species, reactive oxygen species, electrical conductivity, seed longevity, varietal resilience

Introduction
Seed ageing is an unavoidable, progressive process that significantly impacts seed quality, agricultural productivity, and global food security. It leads to reduces germination potential, seedling vigour, and the capacity for successful crop establishment—factors essential for agricultural resilience and success. The physiological decline associated with aging is predominantly characterized by alterations in cellular and biochemical integrity, especially affecting the cell membrane. These changes impair germination and early seedling growth, diminishing seed viability and vigour and ultimately reducing the production of healthy seedlings. These challenges are particularly pronounced in *Brassica* species, which include

oilseed and vegetable crops, all of which contribute significantly to global agricultural systems (Basra et al., 2003; Ellis et al., 2006).

Brassica crops are especially vulnerable to ageing due to their relatively short shelf-life and high sensitivity to storage conditions, particularly fluctuations in temperature and humidity. Under suboptimal storage environments, *Brassica* seeds experience rapid declines in viability, directly affecting crop yields and food production. Despite their agronomic importance, research into the physiological mechanisms underlying seed ageing in *Brassica* species remains limited, especially when compared to staple crops such as maize, soybean, and rice. Studies on these other crops have extensively documented how lipid peroxidation, protein degradation, and oxidative stress caused by reactive oxygen species (ROS) contribute to ageing-associated declines in seed quality (Walters et al., 2020; Schwember et al., 2018). For instance, Walters et al. (2020) linked maize seed ageing to oxidative stress and membrane degradation, while Schwember et al. (2018) identified ageing-induced electrolyte leakage as a reliable indicator of reduced seed viability in soybean. While these studies provide valuable insights, they underscore the need for research specifically tailored to *Brassica* species, given their economic and ecological significance.

In *Brassica* seeds, ageing is similarly associated with ROS accumulation, lipid peroxidation, and protein oxidation, which together compromise cellular membrane integrity. Rajjou and Debeaujon (2008) identified ROS-mediated damage as a major factor in seed viability loss across oilseed species, highlighting the vulnerability of cell membranes to oxidative degradation Further, Choudhury and Khan (2017) demonstrated the utility of electrical conductivity (EC) as a rapid, non-destructive tool for evaluating membrane integrity, showing that elevated EC values in leachates from aged seeds correlate strongly with reduced germination capacity. While EC has shown promise as an indicator tool, its application has often been confined to a narrow range of species or storage conditions, leaving gaps in understanding its utility across the diverse *Brassica* genus.

This study aims to bridge these gaps by examining the physiological effects of seed ageing on germination and seedling vigour across a range of *Brassica* varieties. By using EC measurements to assess membrane integrity, the research seeks to establish quantitative links between ageing and seed viability loss in six *Brassica* species. Moreover, standardised germination tests and seedling vigour assessments will further illuminate the broader physiological impacts of ageing, enabling detailed comparisons across varieties.

Materials and Method:

Experiments were conducted during the Rabi season at Pandit Deen Dayal Upadhyay Institute, Manipur, and laboratory tests followed ISTA guidelines at ICAR Seed Technology Laboratory. The study investigated the physiological effects of seed ageing on germination and seedling vigour in six Brassica varieties: PM-28, M-27, TS-36, TS-38, Potsangbam Yella, and Kakching Yella. A factorial randomized block design with four replications was employed, comparing fresh seeds with 24-month-old seeds to explore ageing impacts. Soil analysis confirmed clay-loam characteristics with moderate fertility, and climatic conditions ranged from 5.5°C in winter to 32.5°C in summer.

Physiological traits were evaluated using standard germination tests, seedling vigor assessments, and electrical conductivity (EC) measurements. EC was determined by soaking seeds in deionized water, measuring leachates to assess membrane integrity. Accelerated ageing and cold tests simulated adverse storage and environmental conditions to measure germination resilience. Quantitative traits, including

emergence speed, plant height, and seed yield, were recorded, facilitating detailed inter-varietal comparisons.
Statistical analyses included ANOVA for factorial experiments to discern the effects of seed age and varietal differences. Results were interpreted using LSD and standard error calculations, providing a robust framework for understanding the physiological decline associated with seed ageing. This comprehensive approach integrates laboratory and field methodologies to elucidate ageing impacts and inform strategies for enhancing seed storage and crop productivity.

Result

Germination Rates in Fresh and Aged Seeds

The germination rates for fresh and aged seeds were analyzed across six Brassica varieties, as summarized in Table 1 and visually represented in Figure 1. The data indicate a consistent reduction in germination rates among aged seeds in comparison to their fresh counterparts, with some variations observed across different varieties. Fresh TS-36 seeds exhibited the highest germination rate at 95.5%, demonstrating superior seed quality and viability, whereas aged Potsangbam Yella seeds showed the lowest germination rate at 72.5%, underscoring the detrimental effects of seed aging on germination capacity.
This observed trend of reduced germination aligns with findings of Cosme and Kent (1989), who documented that seed aging diminishes seed viability primarily due to physiological changes, including increased membrane permeability. These changes compromise the seeds' ability to retain essential nutrients and support cellular functions during germination, leading to reduced germination rates. A statistical analysis performed to evaluate the significance of these differences confirmed that the variations in germination rates between fresh and aged seeds were statistically significant, with an SE(m) of 0.853 (1.004) across varieties and 0.492 (0.580) between conditions. These findings highlight the impact of seed aging on germination rates and underscore the necessity of optimal storage practices to preserve seed viability.

Table-1: Effect of ageing on seed germination of seeds in different varieties of rapeseed and mustard.

Varieties	Germination		
	Fresh seed	Aged seed	Mean
PM 28	89.50(71.22	79.50(63.10	84.50(67.16
M 27	92.50(74.29	83.50(66.06	88.00(70.17
TS 36	95.50(79.46	85.50(67.65	90.50(73.55
Kakching yella	91.00(72.82	80.00(63.45	85.50(68.14
Potsangbam yel-	84.50(66.90	72.50(58.38	78.50(62.64
TS 38	87.00(68.90	76.50(61.01	81.75(64.96
Mean	**90.00(72.26**	**79.58(63.27**	
Fac-	**A**	**B**	**A X B**
SE(m	**0.853(1.004**	**0.492(0.580**	**1.206(1.420**
C.D (0.5)	**2.465(2.902**	**1.423(1.676**	**3.483(4.100**

Mean of four replications in each treatment, *Value in parenthesis are Arc sine transformation, A-varieties, B-Condition (Fresh and Aged seed)

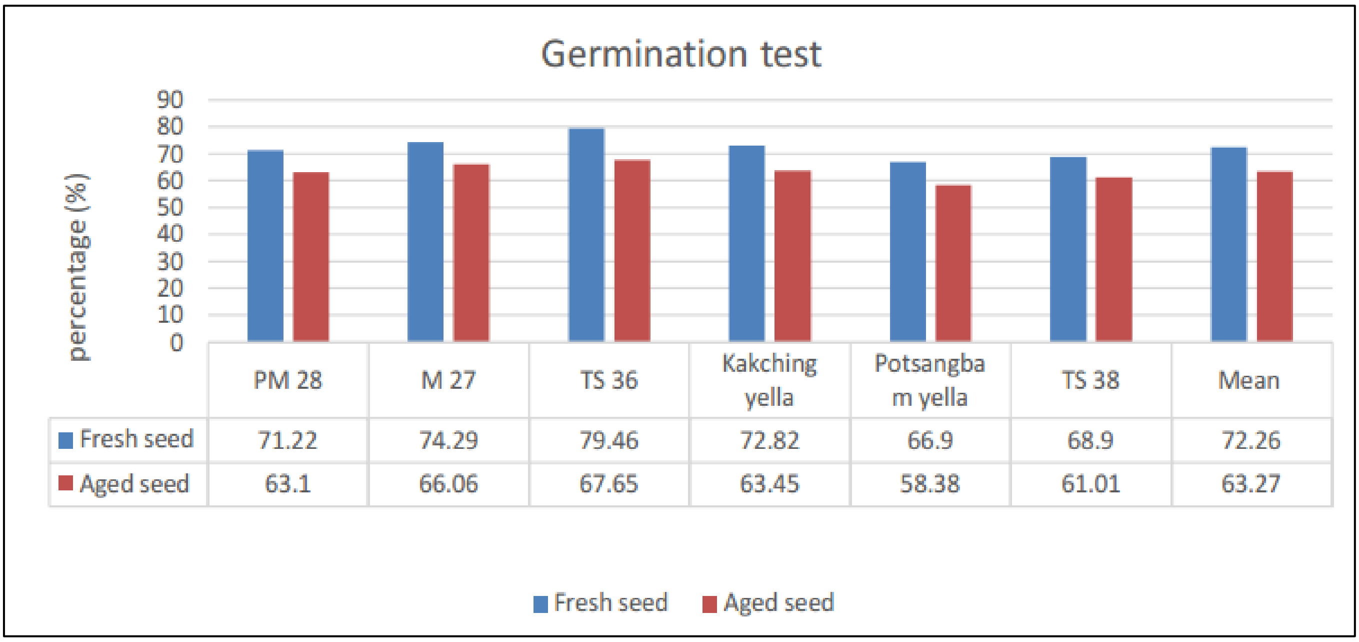

Figure-1: Effect of ageing on seed germination of seeds in different varieties of rapeseed and mustard. (Transform value)

Seedling Vigour Index

The Seedling Vigour Index (SVI) for both fresh and aged seeds was calculated and displayed in Table 2 and Figure 2, revealing significant differences between the two groups. Fresh seeds consistently showed higher vigour, with PM-28 and TS-36 achieving the highest SVI values, indicating healthy root and shoot growth. This suggests that fresh seeds possess greater reserves of readily available nutrients and maintain more efficient cellular processes, which support early seedling development. On the other hand, aged seeds, particularly from varieties such as Potsangbam Yella and TS-38, showed marked reductions in SVI, signalling the adverse effects of aging on nutrient uptake and seedling growth.

The reduction in seedling vigour in aged seeds can be attributed to the degradation of membrane integrity, which is essential for nutrient transport and cellular homeostasis. When membrane integrity declines, as evidenced by increased membrane permeability, the seeds lose their ability to regulate osmotic balance, essential for efficient water and nutrient uptake during germination and early growth phases. To further investigate the relationship between membrane integrity and seedling vigour, electrical conductivity tests were conducted. These tests showed that aged seeds exhibited higher electrical conductivity values, indicating compromised membrane structures. Such an increase in conductivity correlates with weakened membrane function, as described by Hussein et al. (2012), which disrupts the osmotic balance necessary for water absorption, ultimately leading to diminished seedling vigour and growth potential.

Table-2: Effect of ageing on vigour index of seeds in different varieties of rapeseed and mustard.

Varieties	Vigour index		
	Fresh seed	Aged seed	Mean
PM 28	73.23(58.83	66.20(54.44)	69.72(56.63)
M 27	80.94(64.08	71.91(57.98)	76.42(61.03)
TS 36	94.81(76.88	87.12(68.97)	90.97(72.93)
Kakching yella	72.55(58.41	67.77(55.39)	70.16(56.90)
Potsangbam yel-	71.44(57.67	64.90(53.66)	68.17(55.66)

TS 38	69.24(56.31	63.03(52.52)	66.14(54.41)
Mean	**77.03(62.03**	**70.16(57.16)**	
Fac-	**A**	**B**	**A X B**
SE(m	**0.777(0.522**	**0.448(0.301)**	**1.099(0.738)**
C.D (0.5)	**2.245(1.508**	**1.296(0.871)**	**3.173(2.133)**

Mean of four replications in each treatment, *Value in parenthesis are Arc sine transformation, A-Varieties, B-Condition (Fresh and Aged seed)

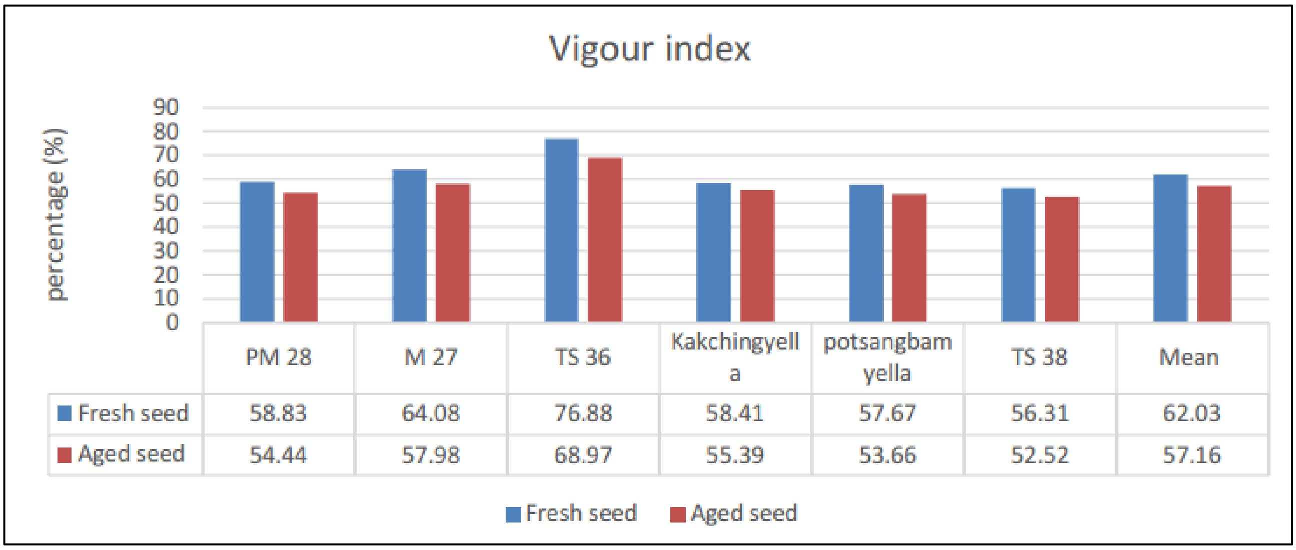

Figure-2: Effect of ageing on vigour index of seeds in different varieties of rapeseed and mustard. (Transform value)

Germination Percentage Analysis

Germination percentage analysis across the six Brassica varieties (PM-28, M-27, TS-36, Kakching Yella, Potsangbam Yella, and TS-38) provided additional insights into the impacts of aging. The fresh seeds displayed significantly higher germination percentages compared to aged seeds. For fresh seeds, germination rates varied from 84.50% (with a standard error of 66.90%) to 95.50% (79.46%), while for aged seeds, germination percentages ranged from 72.50% (58.38%) to 85.50% (67.65%). Among fresh seeds, TS-36 achieved the highest germination rate at 95.5%, whereas Potsangbam Yella displayed the lowest germination rate among aged seeds at 72.5%.

The statistical analysis underscored the significant differences in germination percentages between fresh and aged seeds, with an SE(m) of 0.853 (1.004) across varieties and 0.492 (0.580) between conditions, confirming that seed aging substantially impacts germination potential. These results reinforce the notion that seed aging leads to a decline in seed quality and viability, as reflected in both lower germination percentages and reduced seedling vigour. The findings from this study contribute to a broader understanding of how physiological changes associated with seed aging, particularly those affecting membrane integrity and nutrient retention, detrimentally impact germination and early growth in Brassica species.

Discussion

This study highlights the pronounced effects of seed aging on germination rates and seedling vigour across six varieties of *Brassica*, demonstrating a significant reduction in viability and vigour in aged seeds compared to fresh ones. Consistent with existing research, these findings underscore that seed aging compromises physiological functions, notably by increasing membrane permeability and cellular leakage, which impairs germination capacity (Walters et al., 2020). TS-36 displayed relatively high resilience, retaining a substantial portion of its germination potential, while Potsangbam Yella exhibited the steepest decline. These variations align with established seed aging models, where oxidative stress and membrane degradation are central factors driving seed deterioration. The observed correlation between higher electrical conductivity (EC) values and decreased seed vigor further supports the use of EC as a practical, non-destructive metric for seed quality assessment, confirming its reliability as indicated by earlier studies (Choudhury & Khan, 2017).

While the results largely align with prior research on seed viability loss through aging, the observed resilience of TS-36 suggests potential genetic factors that might contribute to its robustness against oxidative damage and membrane deterioration. Prior studies, such as Rajjou and Debeaujon (2008), have reported similar varietal differences in aging responses, hinting at underlying genetic variations that influence tolerance to oxidative stress. The resilience seen in TS-36 may reflect enhanced antioxidative defense mechanisms or membrane stability, which could offer valuable insights for breeding programs aimed at developing more resilient crop varieties. Conversely, the marked vulnerability of Potsangbam Yella reinforces the role of genetic composition in seed longevity, suggesting a need for further molecular and biochemical studies to identify markers and pathways associated with increased resilience to aging.

The findings have significant implications for seed conservation, agricultural productivity, and storage practices. The observed decline in germination rates and seedling vigour with aging underscores the necessity for optimized, variety-specific storage protocols. The integration of EC measurement as a seed quality indicator could provide seed banks and agricultural enterprises with an efficient, low-cost method for monitoring seed quality, potentially reducing waste and improving seed lot management. The varietal differences in aging resilience also point to physiological and genetic factors that could be targeted to enhance seed longevity, contributing to theoretical models in seed biology that explain the genetic basis of oxidative stress responses in seeds. These insights hold potential for broader applications, including in other crops with genetic similarities to *Brassica*, advancing agricultural resilience by improving seed viability during storage.

This study also challenges the traditional, one-size-fits-all approach to seed storage, revealing the variability in aging responses among different *Brassica* varieties. In regions where *Brassica* species are central to both food and oil production, implementing storage protocols tailored to specific varieties could enhance both agricultural productivity and food security. Furthermore, the study advocates for the development of rapid, cost-effective seed quality assessment tools, especially valuable in resource-limited settings where maintaining seed longevity can be critical. Future research should consider expanding the scope to include *Brassica* varieties from diverse environmental and climatic origins, offering a more comprehensive perspective on seed aging responses across different ecotypes and improving the applicability of findings to diverse agricultural systems.

Conclusion

This study highlights the significant impacts of seed aging on germination and seedling vigor across six *Brassica* varieties, showing that viability and growth potential decline markedly over time. The use of electrical conductivity (EC) as a non-destructive measure of seed membrane integrity proved effective in demonstrating how increased EC values correlate with decreased cellular stability and vigor in aged seeds. The findings reveal noteworthy varietal differences in aging resilience, particularly with TS-36 showing high retention of germination potential and Potsangbam Yella experiencing substantial declines, indicating that genetic factors play a vital role in aging tolerance. These results underscore the potential for breeding programs to enhance seed longevity in *Brassica* species through targeted selection of resilient genetic traits.

The study's contributions are valuable for both theoretical and practical aspects of seed science. By validating EC as a reliable, rapid metric for assessing seed aging in *Brassica*, this research offers a practical tool for seed banks and agricultural industries tasked with maintaining seed viability in storage. The findings advance our understanding of the physiological effects of aging on *Brassica* seeds, providing actionable insights for optimizing storage practices to prolong seed shelf life, thereby supporting food security. The observed varietal differences in aging response further open avenues for breeding programs to focus on enhancing traits associated with long-term viability, enabling the development of robust cultivars better suited for extended storage.

In conclusion, this study emphasizes the importance of adaptive storage strategies and innovative seed quality assessment methods in preserving seed viability. As sustainable agriculture becomes increasingly critical, insights from this research can guide future efforts to improve crop resilience and efficiency in seed management. Continued exploration of the genetic and physiological factors underlying seed aging will be essential for building sustainable agricultural systems, contributing to global food stability and strengthening resilience against environmental challenges.

Recommendations

To address the challenges of seed aging in *Brassica* species, agricultural practitioners and seed storage managers should adopt optimized storage protocols specific to each variety, as demonstrated in this study. Routine electrical conductivity (EC) testing is recommended as a non-destructive, early-detection tool for seed quality, allowing for proactive measures to prevent quality loss during storage. For varieties like TS-36, which showed resilience to aging, less stringent storage conditions may be suitable, whereas varieties like Potsangbam Yella, prone to rapid viability loss, should be kept in controlled, low-humidity, and moderate-temperature environments to maximize shelf life. Future research should focus on identifying biochemical and genetic markers associated with aging resistance and expanding trials to include diverse *Brassica* varieties and other oilseed crops for broader applicability. Policy initiatives should support the integration of EC testing into seed certification programs to uphold high seed quality standards. Additionally, funding policies for research into aging-resistant cultivars would foster agricultural resilience and food security, especially in regions dependent on *Brassica* crops for their economic and nutritional value.

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