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Influence of Seed Membrane Integrity on Nutrient Retention in Aged Brassica Seeds

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Abstract:- This study examines the role of seed membrane integrity in maintaining nutrient levels specifically nitrogen (N), potassium (K), and calcium (Ca) in aged seeds of Brassica species. Seed longevity and the preservation of nutrients are essential for agricultural productivity, as the loss of nutrients during storage can reduce seed viability and negatively impact crop performance. Despite the global significance of Brassica crops, the association between membrane integrity and nutrient retention has not been extensively studied. In this research, six Brassica varieties were subjected to controlled ageing conditions to evaluate the effects of membrane degradation on nutrient levels. The findings show a significant decline in nutrient levels in aged seeds. Nitrogen retention decreased from 2.08%–2.81% in fresh seeds to 1.93%–2.23% in aged seeds, while calcium levels fell from 0.40%–1.03% to 0.38%–0.91% ($p < 0.05$). Potassium levels also decreased, from 0.64%–4.34% in fresh seeds to 0.53%–3.45% in aged seeds, but potassium showed comparatively better retention than nitrogen and calcium. However, the varieties M-27 and TS-36 show higher potassium retention, signifying that they have greater membrane stability. These results reveal the importance of membrane integrity in preserving nutrient levels, offering valuable insights specific to Brassica seeds. They also highlight the need for improved storage strategies, such as maintaining low humidity and temperature control, to minimize nutrient losses. Additionally, the findings suggest that osmopriming and breeding for enhanced membrane resilience could be effective strategies for improving nutrient retention. These approaches are essential for ensuring seed quality and promoting sustainable agricultural practices. Further research should explore broader Brassica varietal responses under diverse storage scenarios to refine and expand conservation practices.

Keywords:- *Seed Membrane Integrity; Nutrient Retention; Brassica Seeds; Aging; Nitrogen; Potassium; Calcium; Sustainable Storage.*

I. INTRODUCTION

Seed longevity and nutrient retention are essential for agricultural productivity, seedling vigour, and the nutritional value of crops, particularly for species like Brassica, which require prolonged storage. The process of seed aging involves complex biochemical and structural alterations, where membrane integrity is key to nutrient preservation and seed viability. Priestley (1986) identified oxidative damage as a fundamental contributor to seed aging, with membrane permeability playing a central role in the leaching of vital metabolites. Bewley et al. (2013) further demonstrated that increased membrane permeability due to aging contributes to the leakage of cellular contents, correlating with declines in seed vigour. This relationship between membrane stability and seed performance remains a critical area of focus, as maintaining membrane integrity has been linked to extended seed viability and enhanced germination rates (Roberts, 1973).

Further research on seed membrane stability highlights its protective function against oxidative stress, which is particularly influential in nutrient retention during storage. Bailly (2004) demonstrated that membranes serve as selective barriers that prevent nutrient leakage, maintaining internal homeostasis despite external stressors. A study by Walters et al. (2010) supported these findings by revealing that the accumulation of reactive oxygen species (ROS) in aged seeds exacerbates lipid peroxidation, thereby degrading membrane systems and accelerating nutrient loss. Additionally, Mira et al. (2019) observed that in seeds with compromised membrane integrity, essential nutrients like proteins, lipids, and minerals become increasingly susceptible to depletion, directly affecting seed quality. These studies underscore that membrane degradation is not only a consequence of seed aging but also a determinant of nutrient bioavailability critical for seedling establishment.

While research has broadly explored seed membrane degradation and nutrient loss, limited studies specifically address this relationship in *Brassica* species. Brassica species are globally cultivated and are known for their high nutritional value, contributing essential vitamins, minerals, and bioactive compounds to human diets. Yet, the prolonged storage periods often necessary for Brassica seeds present unique challenges, as membrane degradation associated with aging can result in nutrient loss, thereby compromising both seed viability and crop nutritional value (Finch-Savage & Bassel, 2016). Previous studies on Brassica have mainly focused on seed germination and general viability metrics, leaving a gap in understanding the precise mechanisms by which membrane-related nutrient retention influences seed quality over time.

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Addressing this gap is essential to optimize seed storage and conservation practices, particularly given the global reliance on Brassica crops. Although the impact of nutrient leakage and membrane deterioration have been independently explored, there remains a need for an integrated approach that specifically examines how membrane integrity affects nutrient retention in aging Brassica seeds. This understanding could inform enhanced storage methods, guide breeding programmes focused on membrane resilience, and support sustainable agricultural practices (Leprince et al., 2017).

This study aims to investigate the role of membrane integrity in nutrient retention within aged *Brassica* seeds, exploring the impact of membrane degradation on nutrient availability. Insights gained will enhance understanding of seed aging physiology, with implications for seed storage, crop quality, and sustainable agriculture.

II. MATERIAL AND METHODS

- *Experimental Design and Site*

The study was conducted at the Seed Technology Laboratory, ICAR-RC NEHR, Manipur, and the experimental field of Pandit Deen Dayal Upadhyay Institute of Agricultural Sciences, Utlou, Manipur, during the Rabi season. Six Brassica varieties, including M-27, TS-36, TS-38, PM-28, Potsangbam Yella, and Kakching Yella, were subjected to controlled aging. Field experiments followed a factorial randomized block design with four replications, testing fresh and 24-month-aged seeds. Laboratory analyses adhered to ISTA guidelines, focusing on membrane integrity and nutrient retention.

- *Seed Preparation and Aging Protocol*

Fresh seeds were stored under controlled aging conditions (41°C, ~100% relative humidity) for 5–7 days to simulate aging. Physiological assessments included germination tests, vigour index calculations, and electrical conductivity measurements. Total nitrogen, potassium, and calcium concentrations were estimated using the Kjeldahl method, dry ashing with flame photometry, and titration with EDTA, respectively.

- *Nutrient Analysis and Statistical Evaluation*

Nitrogen levels were measured by distillation after acid digestion, while potassium was quantified using a spectrophotometer after ash treatment with hydrochloric acid. Calcium determination employed complex metric titration with EDTA. Data were statistically analyzed using two-way ANOVA (FRBD) with least significant difference (LSD) at a 5% probability level to evaluate variations across treatments and varieties.

III. RESULT

- *Seed Membrane Integrity and Nutrient Retention*

- *Nitrogen Content*

In assessing nitrogen content, results indicated a decline in nitrogen retention across all varieties of Brassica seeds as they aged. The nitrogen concentration in fresh seeds varied between 2.08% and 2.81%, with the highest levels observed in the Kakching Yella variety (2.81%). Aged seeds showed a reduced nitrogen range of 1.93% to 2.23%, the lowest in variety M-27 (1.93%) and the highest in Kakching Yella (2.23%). Statistically, the effect of storage duration was significant for nitrogen content ($p < 0.05$), highlighting that membrane degradation over time impacts nitrogen retention in Brassica seeds.

- *Potassium Content*

Potassium content was analyzed in fresh and aged seeds, revealing a significant decrease over time, though results were not statistically significant ($p > 0.05$) when comparing fresh and aged seeds directly. Fresh seeds exhibited potassium levels from 0.64% to 4.34%, peaking in variety M-27. Aged seeds retained potassium at a reduced range, 0.53% to 3.45%, with TS-36 showing the lowest retention at 0.53%. Notably, the high potassium retention in M-27 suggests better membrane resilience in this variety under aging conditions.

- *Calcium Content*

Calcium retention displayed a similar trend, with fresh seeds ranging from 0.40% to 1.03% and aged seeds showing a decline, ranging between 0.38% and 0.91%. The highest calcium concentration was observed in fresh M-27 seeds (1.03%), and the lowest in aged Kakching Yella seeds (0.38%). Statistical analysis indicated a significant difference in calcium levels between fresh and aged seeds, confirming the effect of membrane integrity loss on calcium leaching over time.

Figures 1, 2 and 3 illustrate the effects of aging on nitrogen, potassium, and calcium content across Brassica varieties. Detailed table (Table-1) present means, standard errors, and confidence intervals for each nutrient under both storage conditions. Exact p-values for nitrogen and calcium reinforce the statistical significance of aging effects on nutrient retention, while non-significance for potassium suggests potential resilience for this element in certain varieties.

Nitrogen and calcium exhibited significant decreases in aged seeds across all varieties, with potassium showing less consistent results. These findings underscore the critical role of seed membrane integrity in preserving essential nutrients, particularly nitrogen and calcium, over storage periods. This data informs best practices for Brassica seed storage and highlights varietal differences in nutrient retention capabilities under aging stress.

IV. DISCUSSION

This study investigates the link between membrane integrity and nutrient retention in aged seeds of six Brassica varieties, focusing specifically on nitrogen, potassium, and calcium. The results consistently showed a marked decline in nutrient levels, with fresh seeds retaining significantly more nutrients compared to aged seeds, supporting the hypothesis that membrane degradation in aging seeds compromises nutrient preservation. Among the nutrients analyzed, potassium exhibited the largest decrease, underscoring its particular sensitivity to changes in membrane permeability. This finding highlights the critical role of membrane integrity in maintaining nutrient content and seed viability, with implications for both agricultural practices and seed storage strategies aimed at maximizing nutrient retention (McDonald and Wilson, 1979; Bentsink and Koornneef, 2008).

The observed nutrient loss in aged seeds appears to result from increased membrane permeability, which facilitates nutrient leaching, especially of potassium. This trend was most pronounced in varieties such as TS-36 and M-27, where potassium's solubility and high mobility within plant tissues likely contribute to its susceptibility to leaching when membrane integrity is compromised. Potassium's role in cellular osmotic regulation and enzyme activation may further exacerbate its loss under aging conditions. This supports a hypothesis that certain ions, depending on their role in cellular processes and solubility characteristics, are disproportionately affected by membrane degradation. While nitrogen and calcium also showed reduced levels, their roles in structural and metabolic compounds that are less prone to leaching may explain their relatively lower sensitivity compared to potassium (Ellis and Roberts, 1980; Walters, 1998).

These findings align with previous studies on membrane integrity and ion leakage, such as Parul et al. (2011), who demonstrated that the breakdown of membrane structure in stored seeds leads to increased potassium loss under thermal stress. This study extends the understanding of nutrient retention under aging conditions rather than temperature stress, showing that similar nutrient trends persist across various Brassica varieties. Recent research also emphasizes the role of lipid peroxidation and enzymatic degradation in membrane weakening, processes that are key in seed aging. By adding comprehensive, variety-specific data, this study advances existing literature on nutrient leakage, highlighting potassium as a particularly vulnerable nutrient in aged seeds (Rajjou and Debeaujon, 2008; Hay et al., 2008).

The findings have significant implications for both theoretical models of seed aging and practical applications. Theoretically, this research contributes to seed aging models by identifying potassium's vulnerability, which may prompt future investigations into ion-specific membrane transport dynamics in aging seeds. From a practical standpoint, these results can inform seed storage and conservation strategies, particularly for seed banks where nutrient preservation is essential. This study's insights could guide the selection of seed varieties with robust membrane integrity or lead to the development of treatments aimed at enhancing membrane stability to reduce nutrient loss during storage. These findings may also influence agricultural policy, advocating for optimized seed storage protocols to promote food security and biodiversity conservation (Bewley et al., 2013; Walters et al., 2005).

V. CONCLUSION

This study explored the impact of seed membrane integrity on nutrient retention in aged versus fresh seeds of *Brassica* species, specifically focusing on essential nutrients like nitrogen, potassium, and calcium. Conducted across six varieties of rapeseed and mustard, the research aimed to assess how storage-induced aging influences nutrient stability, providing insights into the physiological effects of membrane degradation on nutrient conservation. Results consistently showed that seed aging significantly reduces nutrient concentrations, with aged seeds exhibiting notably lower levels of nitrogen and calcium across all varieties, compared to fresh seeds. Although potassium levels declined as well, the reduction was statistically non-significant, suggesting that potassium may be relatively more stable under aging conditions. These findings affirm that membrane integrity is crucial for nutrient retention, as membrane deterioration facilitates nutrient leakage over time—a process influenced by storage conditions and aligned with previous findings on ion leakage and nutrient preservation in aged seeds.

The broader implications of this research underscore the importance of optimal storage conditions and careful seed handling in crop management practices. Nutrient retention in seeds is essential for maintaining seed viability and enhancing crop yields, particularly in regions where resource efficiency is critical to agricultural productivity. By highlighting the role of membrane integrity in nutrient retention, the study suggests that improving storage techniques could help mitigate nutrient loss, ultimately enhancing seed quality and supporting sustainable agricultural practices.

However, certain limitations of this study may affect the generalizability of its findings. This research focused on specific *Brassica* varieties and was limited to a single season and location, which may not fully capture the variability in nutrient retention across diverse environmental conditions or extended storage durations. Further studies are recommended to investigate nutrient retention in a broader array of seed varieties and environmental settings over prolonged storage periods, which would provide a more comprehensive understanding of seed aging and nutrient preservation mechanisms.

In conclusion, this research contributes to the field by highlighting the critical role of membrane integrity in nutrient retention, offering valuable insights for seed conservation and management strategies. As seed quality continues to underpin agricultural productivity, future studies focused on refining storage practices and developing protective treatments to enhance membrane stability will be essential. Such advancements could significantly optimize nutrient preservation, supporting resilient and sustainable agricultural systems.

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RECOMMENDATION

Brassica seeds, with notable declines observed in nitrogen, potassium, and calcium levels, particularly in varieties such as M-27 and TS-36. Based on these findings, we recommend maintaining low-humidity, temperature-controlled storage environments to minimize nutrient loss and support seed viability. Seed priming treatments, such as osmopriming, may further enhance membrane stability, particularly for potassium and nitrogen retention. Additionally, breeding programs could prioritize developing varieties with resilient membrane structures to reduce nutrient leakage, thereby improving seed quality for long-term storage. Researchers are encouraged to expand on these findings by exploring storage conditions and treatment options across various Brassica varieties and other crop seeds, while industry stakeholders might adopt advanced storage solutions, such as vacuum sealing and controlled atmospheres, to extend seed longevity. For policymakers, investments in seed storage infrastructure could support sustainable agriculture, especially in small-scale and rural settings, ultimately contributing to crop productivity and food security.

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Table 1 Effect of Ageing of Seeds of Different Varieties of Rapeseed and Mustard on Estimation of Nitrogen, Potassium and Calcium.

Varieties	Estimation of nitrogen %**			Estimation of potassium%**			Estimation of calcium %**		
	Fresh seed	Aged seed	Mean	Fresh seed	Aged seed	Mean	Fresh seed	Aged seed	Mean
PM 28	2.79(1.67)	2.23(1.49)	2.511(1.58)	0.91(0.94)	0.75(0.85)	0.83(0.90)	0.89(0.94)	0.63(0.79)	0.76(0.86)
M 27	2.08(1.44)	1.93(1.38)	2.009(1.41)	4.34(2.08)	3.45(1.85)	3.89(1.96)	1.03(1.01)	0.91(0.95)	0.97(0.98)
TS 36	2.42(1.55)	2.12(1.45)	2.276(1.50)	0.64(0.79)	0.53(0.72)	0.59(0.76)	0.57(0.75)	0.57(0.75)	0.57(0.75)
Kakching yella	2.81(1.68)	2.23(1.49)	2.526(1.58)	0.73(0.84)	0.61(0.77)	0.67(0.80)	0.40(0.63)	0.38(0.61)	0.39(0.62)
Potsangbam yella	2.32(1.52)	2.16(1.47)	2.245(1.49)	2.93(1.68)	2.84(1.67)	2.88(1.68)	0.92(0.96)	0.88(0.94)	0.90(0.95)
TS 38	2.09(1.45)	1.96(1.39)	2.031(1.42)	0.71(0.83)	0.69(0.83)	0.70(0.83)	0.98(0.98)	0.85(0.92)	0.91(0.95)
Mean	2.42(1.55)	2.10(1.44)		1.71(1.19)	1.48(1.12)		0.80(0.88)	0.70(0.83)	
Factor	**A**	**B**	**A X B**	**A**	**B**	**A X B**	**A**	**B**	**A X B**
SE(m)	0.088 (0.030)	0.051 (0.017)	0.125 (0.042)	0.180 (0.061)	0.104 (0.035)	0.254 (0.086)	0.037 (0.023)	0.021 (0.013)	0.053 (0.032)
C.D (0.5)	0.255 (0.085)	0.147 (0.049)	0.361 (0.120)	0.520 (0.176)	0.300 (0.102)	0.735 (0.249)	0.107 (0.066)	0.062 (0.038)	0.151 (0.091)

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

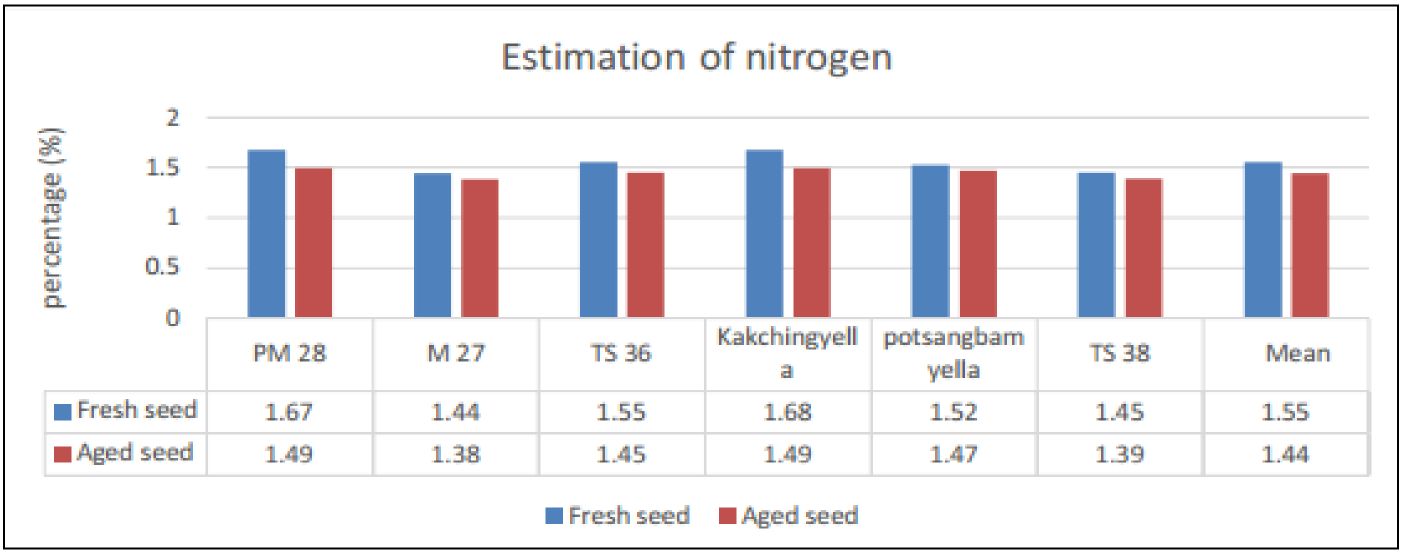

Fig 1 Effect of Ageing on Estimation of Nitrogen of Seeds in Different Varieties of Rapeseed and Mustard. (Transform value)

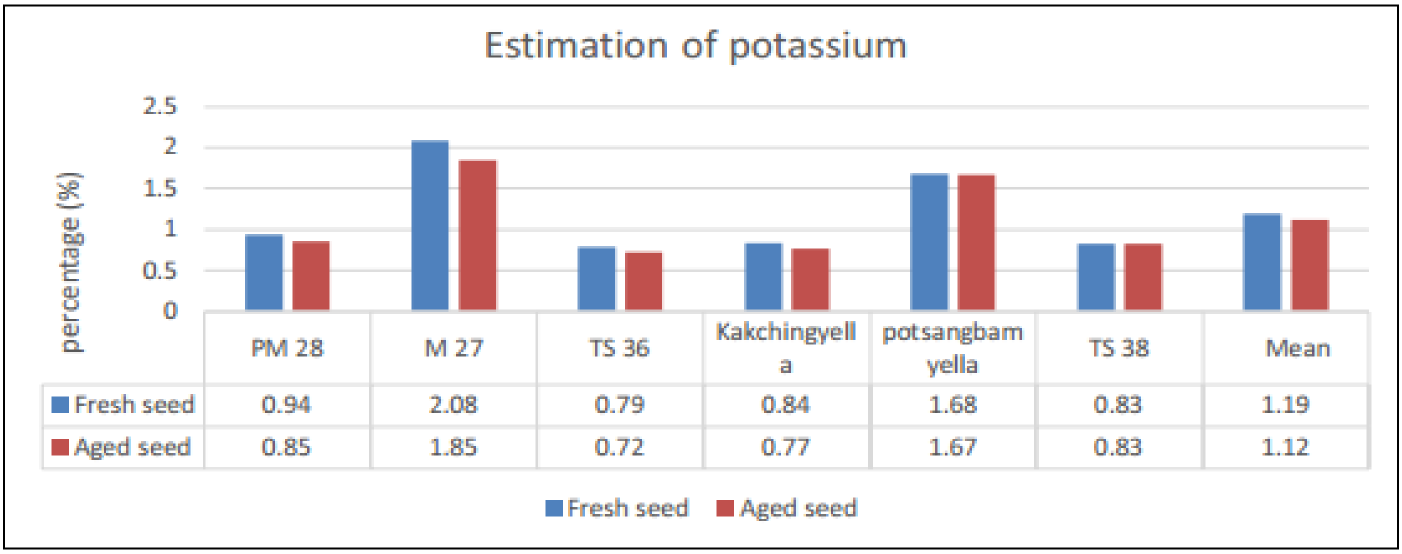

Fig 2 Effect of Ageing on Estimation of Potassium of Seeds in Different Varieties of Rapeseed and Mustard. (Transform value)

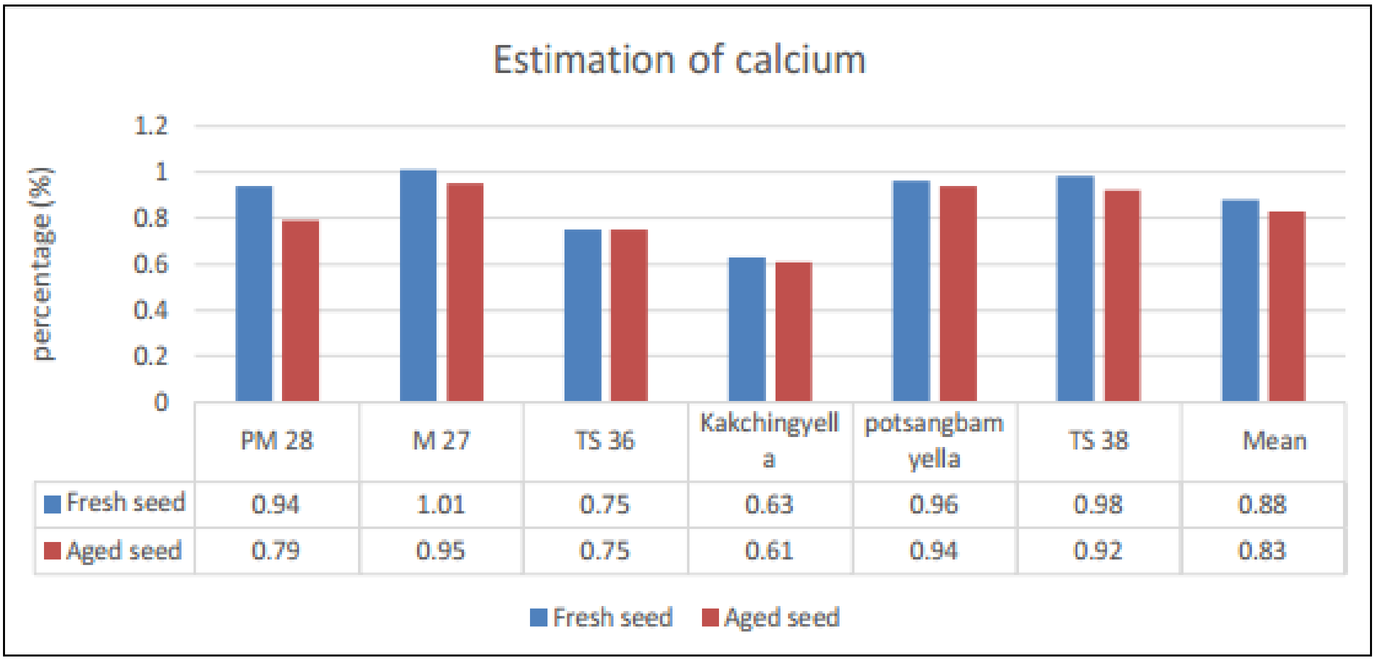

Fig 3 Effect of Ageing on Estimation of Calcium of Seeds in Different Varieties of Rapeseed and Mustard. (Transform value)