Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 47 Issue 6 November 2026 pp. 1284-1293
DOI: http://doi.org/10.22438/jeb/47/6/MRN-6081

J. Environ. Biol., 2026
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Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Influence of packaging materials and storage conditions on root system architecture (RSA) of papaya (*Carica papaya* L.)

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Received: 22 May 2026 **Revised:** 02 July 2026 **Accepted:** 21 August 2026

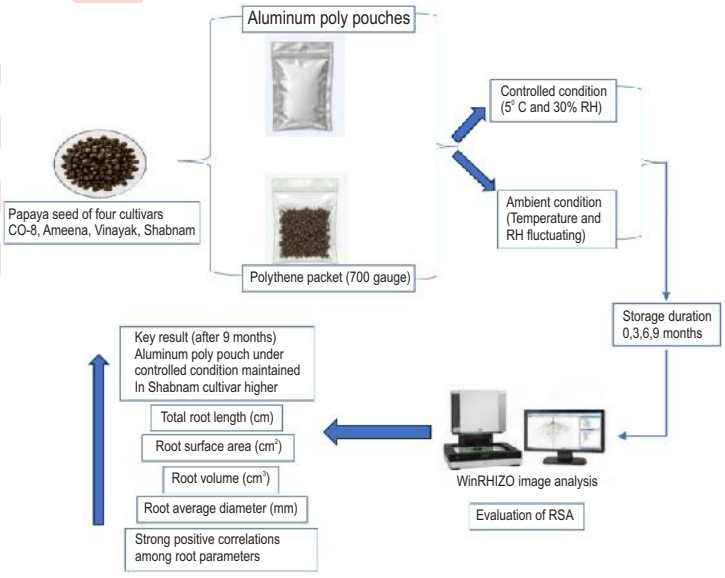
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Abstract

Aim: To evaluate the effects of packaging materials and storage conditions on root system architecture (RSA) of papaya (*Carica papaya* L.) seeds during prolonged storage, and identify optimal storage conditions for maintaining seedling vigour.

Methodology: Seeds of four papaya cultivars (CO-8', Vinayak, Shabnam, and Ameena) were packed in polythene packets or aluminium polypouches and stored under controlled conditions (5°C, 30% RH) or ambient conditions. RSA traits–total root length (cm), root surface area (cm²), root volume (cm³) and root average diameter (mm)–were assessed at 0, 3, 6 and 9 months of storage using WinRHIZO™ image analysis software, and inter–trait correlations were analysed using JMP statistical software. Data were evaluated using a factorial completely randomized design with Tukey's test at $p \leq 0.05$.



Results: RSA traits declined progressively with storage duration, deteriorating by 9 months under ambient conditions. Aluminium polypouches under controlled conditions consistently preserved higher RSA traits than polythene packets under ambient storage. 'Shabnam' showed the best storability (0 to 9 months, controlled storage): total root length 68.94 to 55.30 cm, root surface area 10.23 to 6.61 cm², root volume 0.130 to 0.100 cm³, and root average diameter 0.514 to 0.309 mm. 'Vinayak' in polythene packets under ambient conditions showed the sharpest decline: root length 32.13 to 18.49 cm, surface area 3.88 to 0.66 cm², volume 0.045 to 0.021 cm³, and diameter 0.311 to 0.104 mm. All four RSA traits were strongly and positively correlated with each another.

Interpretation: Aluminium poly pouches combined with controlled low-temperature storage effectively preserved RSA and seedling vigour in papaya during extended storage. The superior performance of the cultivar 'Shabnam' indicates inherent genetic variability in seed storability and ageing tolerance, offering useful insights for improving seed storage strategies and enhancing seedling establishment.

Key words: *Carica papaya,* Packaging material, Root system architecture, Seed storage, Seedling vigour

How to cite: Verma, B., K.K. Singh, S.K. Lal, D. Singh, S.R. Jacob, A. Anand, J. Prakash and R. Choudhary: Influence of packaging materials and storage conditions on root system architecture (RSA) of papaya (*Carica papaya* L.). *J. Environ. Biol.*, **47,** 1284-1293 (2026).

Introduction

Papaya (*Carica papaya* L.; 2n = 18) is the only cultivated and economically important species of the family Caricaceae. It is widely grown in tropical and subtropical regions due to its high nutritional, medicinal, and industrial value. Its cultivation is expanding because of its rapid growth, year-round fruiting, high productivity, and good economic returns (Chan and Tang, 2012). Beyond its desirable sensory attributes, papaya is nutritionally rich, providing essential vitamins and minerals, including carotene, thiamine, riboflavin and iron (Koul *et al.,* 2022). Papaya has an average productivity of 44.86 MT ha^{-1} in India. It is widely cultivated in Tamil Nadu, West Bengal, Madhya Pradesh, Bihar, Gujarat, Karnataka, Andhra Pradesh and Maharashtra, which are among the major papaya-producing states in the country (NHB, 2023). Despite this significance, maintaining seed quality during storage remains a persistent challenge, particularly in crops like papaya where seeds are highly sensitive to environmental fluctuations. Seed conservation in storage depends on maintaining viability over time, and desiccation tolerance is a key factor determining storage success, particularly in tropical species such as papaya (Silva and Lado, 2026).

Seed deterioration during storage is governed by a complex interplay of factors, including temperature, relative humidity, oxygen availability and storage duration, all of which collectively influence the physiological and biochemical stability (Gebeyehu, 2020; Corbineau, 2024). In this regard, the inherently hygroscopic nature of seeds further intensifies the problem, as continuous interaction with the surrounding environment results in fluctuations in moisture content that accelerate ageing processes and ultimately reduce vigour (Murthy *et al.,* 2003). Against this backdrop, the selection of appropriate packaging materials and storage conditions emerges as a critical intervention for mitigating seed deterioration. Moisture-proof and oxygen-restrictive packaging systems, such as aluminium polypouches, have been shown to effectively limit oxidative damage and maintain seed viability by minimizing moisture exchange with the environment (Hongand Kim, 2004; Netra *et al.,* 2015).

In contrast, permeable packaging materials may allow internal humidity to fluctuate in response to ambient conditions, thereby accelerating deterioration and diminishing seed performance (Bhuker *et al.,* 2023). Packaging materials play a decisive role in preserving seed quality by regulating moisture exchange, oxygen availability, and exposure to biotic and abiotic stresses. Seed deterioration is strongly influenced by seed moisture content and ambient relative humidity, both of which are directly affected by the permeability of storage containers (Dadlani *et al*., 2023). Complementing this, low-temperature storage conditions play a vital role by suppressing metabolic activity, reducing respiration rates and delaying biochemical degradation, ultimately extending seed longevity (Ramtekey *et al.,* 2022; Parmar *et al.,* 2023). While a substantial body of research has focused on seed viability and germination, comparatively limited attention has been directed towards understanding how storage conditions influence RSA, a critical determinant of seedling establishment and overall plant performance. RSA describes the spatial configuration of the root system in soil and is critical for plants to adapt to different environments (Zhu *et al.,* 2011; Bardgett *et al.,* 2014). In highly heterogeneous soil environments, RSA is considered more important than morphology for nutrients and water uptake (Fitter, 1987). It has been shown that RSA can facilitate plant adaptation to waterand nutrition deficit conditions (Bell and Sultan, 1999), resistance to disease and insect pest infection (Roman-Aviles *et al.,* 2004), andmediate-intraandinter specific competition pressure (Tosti and Thorup-Kristensen, 2010; Fang *et al.,* 2013; Belter and Cahill, 2015). Root traits such as total root length, root surface area, root volume and root average diameter are fundamental to water and nutrient acquisition and thus, serve as key indicators of seedling vigour (Waisel and Eshel, 2002; Lynch, 2007). RSA traits may serve vitally as more sensitive indicators of seed vigour than conventional germination percentage, since seeds with reduced vigour can achieve acceptable germination counts while producing seedlings with compromised root development. This disconnect between germination percentage and root growth potential means that seeds apparently meeting germination standards may still yield seedlings with reduced establishment ability and lower field performance (Finch-Savage and Bassel, 2016). Assessing RSA traits, therefore, provides a more mechanistic and functionally relevant measure of post-storage seedling quality. Environmental conditions during seed storage can exert lasting effects on these traits by altering seed physiological status and subsequent growth dynamics (Walters, 1998). In this context, acomprehensive understanding of how packaging materials and storage environments interact to influence RSA becomes essential for refining seed storage strategies and enhancing crop establishment. Therefore, the present study was undertaken to evaluate the combined effects of different packaging materials and storage conditions on the root system architecture of papaya cultivars during prolonged storage.

Materials and Methods

Experimental site and seed material: The experiment was conducted during 2024 at the National Phytotron Facility and the Division of Seed Science and Technology of the ICAR–Indian Agricultural Research Institute (IARI), New Delhi, India. Four papaya cultivars were selected to represent both public and private-sector breeding programmes in India, comprising one public-sector cultivar, 'CO-8' developed by Tamil Nadu Agricultural University (TNAU), and three commercially important private-sector cultivars, 'Vinayak', 'Shabnam' and 'Ameena', procured from VNR Seeds Company, Chhattisgarh. This selection encompassed a broad genetic diversity within commercially cultivated papaya germplasm and reflected variations in horticultural characteristics and market significance, thereby facilitating the evaluation of genotypic variability in seed storability and RSA traits during storage. A single authenticated seed lot per cultivar was used throughout the experiment to ensure uniformity and eliminate inter-lot variability. The

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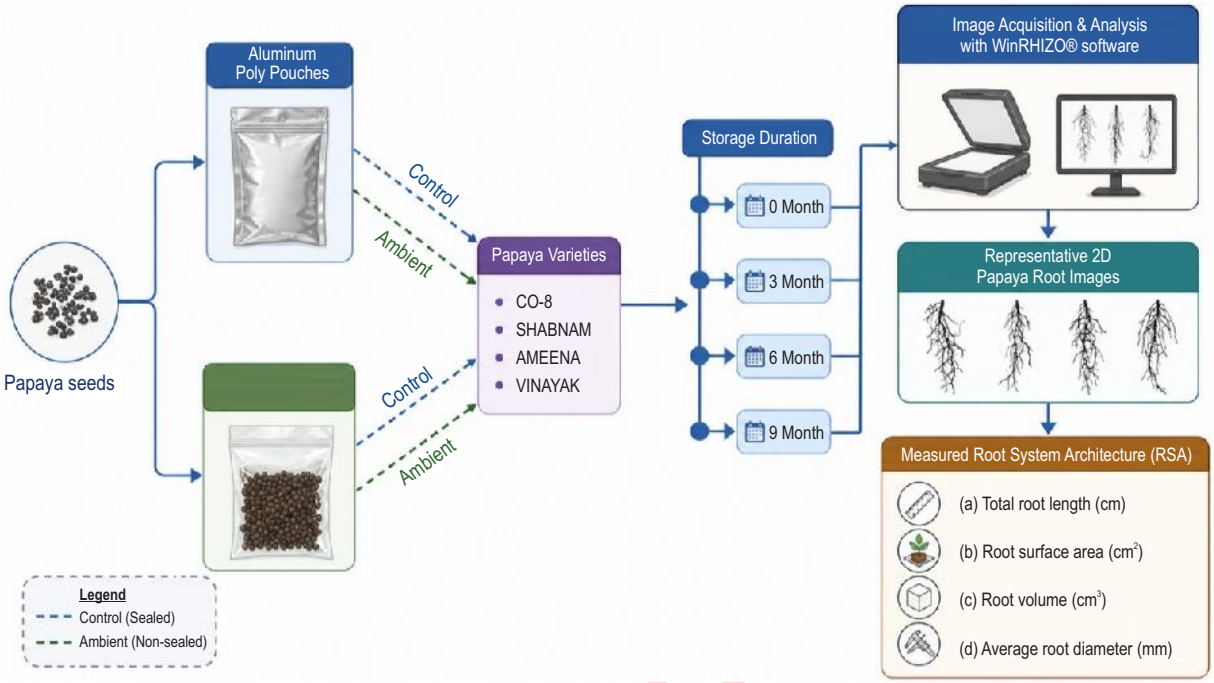


Fig. 1: Experimental details including varieties, storage period and packaging materials on root system architecture (RSA).

experimental layout adopted for the study is presented in Fig. 1.

Packaging materials and storage conditions: Dried seeds of each cultivar were packed in two types of packaging materials, namely polythene packet (700 gauge) and aluminum polypouches. Prior to packaging, the initial moisture content of the seeds was determined using the hot air oven method at 103 ± 2°C for 17 hr in accordance with ISTA (2023) Guidelines. The seed moisture content at the initiation of storage was uniformly maintained at 8.0% (fresh weight basis) across all four cultivars. The packaged seeds were stored under two storage environments: controlled and ambient conditions. Under controlled storage conditions the seeds were stored at 5°C and 30% RH in a precision walk-in cold storage chamber equipped with automated temperature and humidity controllers, maintaining environmental conditions within an accuracy range of ±0.5°C for temperature and ±2% for RH. Under ambient storage, the minimum temperatures ranged from 13-21°C, the maximum temperatures from 27-33°C and RH from 30-60%. RSA was evaluated at 0, 3, 6 and 9 months of storage to assess the influence of packaging material and storage environment on subsequent seedling performance.

Seedling raising and root trait measurement: For root evaluation, the seeds were sown in trays containing cocopeat mediumand maintained under controlled glasshouse conditionsat the National Phytotron Facility, ICAR-IARI, New Delhi. Seedlings were grown under an alternating temperature regime of 20°C (night) and 32°C (day), with 70% RH, a 12 hr light/12 hr dark photoperiod, and a light intensity of 700-800 klux. Twenty-five seeds per replication were used in each treatment. Seedlings were raised for 28 days after sowing before RSA measurements were recorded. After 28 days, five representative seedlings from each replication were carefully removed from the cocopeat medium without damaging the root system and were thoroughly washed under running tap water to remove adhering particles. Roots were scanned using a flatbed root scanner (Epson LA 1600) at a resolution of 400 DPI. Image analysis was performed using WinRHIZO™ software (Version 2012b, Regent Instruments Inc., Canada) with the threshold set between 50and 200 grey levels to accurately distinguish roots from background (Arsenault *et al.,* 1995; Bouma *et al.,* 2000). The parameters measured were total root length(cm), root surface area (cm²), root volume (cm³) and average root diameter (mm).

Statistical analysis: The experiment was analysed in a factorial completely randomized design (FCRD) with three replications. Mean values of the observations were subjected to statistical analysis using SPSS software. Treatment means were compared by Tukey's multiple comparison test at P≤ 0.05. Correlation analyses and figure generation were performed using JMP Pro statistical software (Version 16, SAS Institute Inc., USA).

Results and Discussion

The present investigation demonstrated that both packaging materials and storage conditions significantly influenced the RSA traits in papaya cultivars during storage. A progressive decline in all root parameters, including total root length, root surface area, root volume and root average diameter

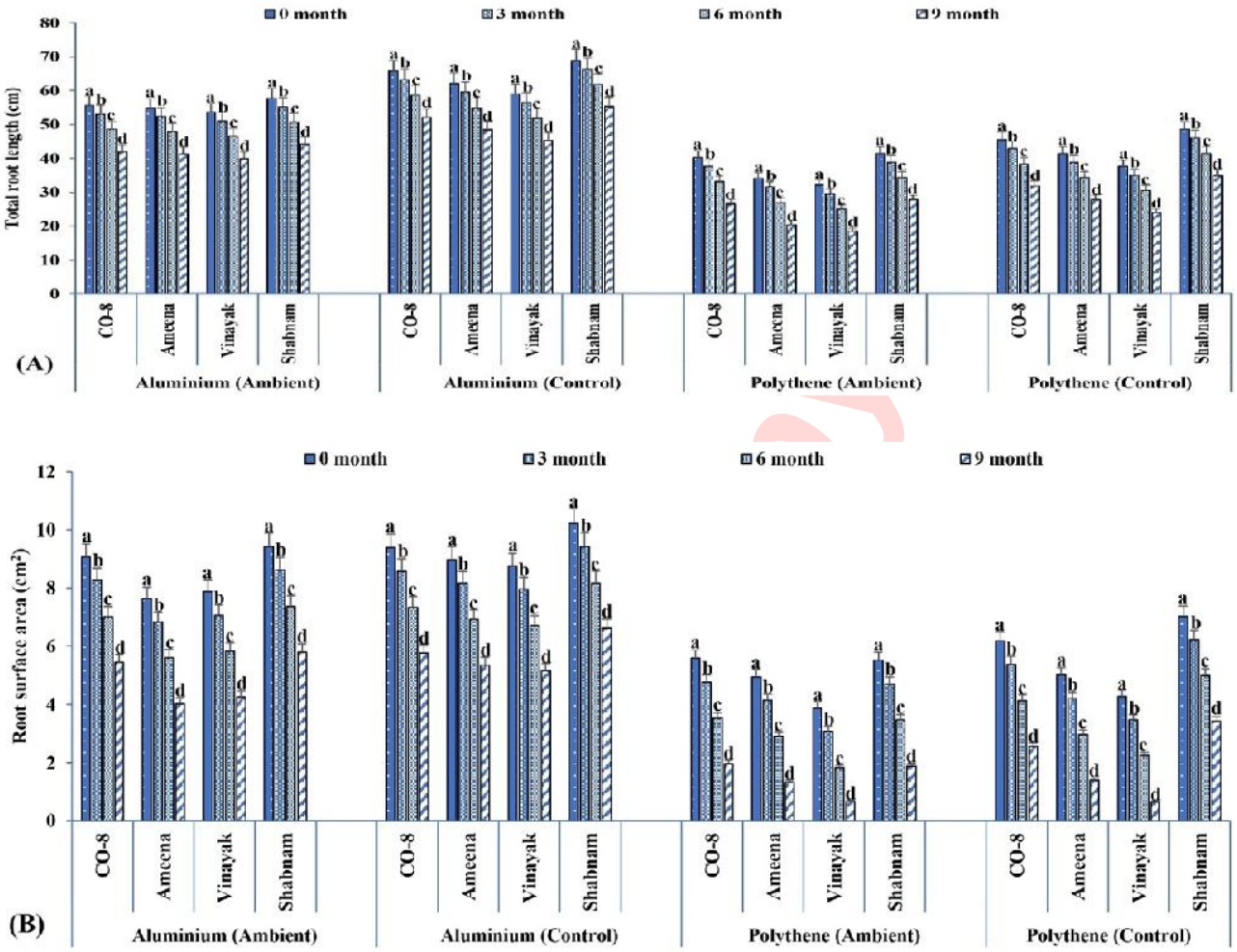


Fig. 2: Effect of packaging material and storage condition on (A) total root length (cm) and (B) root surface area (cm²) of four papaya cultivars evaluated at 0, 3, 6 and 9 months of storage. Values are means±SE of three replications; five seedlings were analysed per replication. Bars bearing the same letter do not differ significantly at P ≤0.05 (Tukey's test).

was observed with increasing storage duration, irrespective of cultivar, packaging material and storage environment. However, the magnitude of decline varied considerably among treatments, indicating the important role of storage conditions in maintaining seed vigour and subsequent root development.

Total root length declined gradually with increasing storage duration under all treatments, but seeds stored in aluminium poly pouches under controlled conditions retained significantly higher values throughout the storage period compared with seeds stored in polythene packets under ambient conditions. Among the cultivars, 'Shabnam' maintained the highest total root length, recording 68.94 cm at 0 months and retaining 55.30 cm even after 9 months of storage under controlled conditions in aluminium poly pouches($p \leq 0.05$) (Fig. 2A, 4). In contrast, the cultivar 'Vinayak' stored in polythene packets under ambient conditions exhibited a substantial reduction in total root length from 32.13 cm to 18.49 cm during same period ($p \leq 0.05$). Comparatively better retention of total root length in aluminium polypouches under controlled conditions may be attributed to reduced moisture fluctuation and lower metabolic activity, which collectively delayed seed deterioration and preserved seedling vigour. Similar observations regarding the beneficial effect of low temperature and moisture-proof packaging on seed quality preservation have been reported earlier (Hong and Kim, 2004; Netra *et al.,* 2015; Parmar *et al.,* 2023).

Root surface area also showed a declining trend with advancement of storage duration across all treatments (Fig. 2B, 4). Nevertheless, seeds stored in aluminium polypouches under controlled conditions consistently maintained greater root surface area compared with seeds stored under ambient conditions. Among the evaluated cultivars, 'Shabnam' again performed superiorly by retaining a (RSA) of 10.23 cm² at 0 months and 6.61

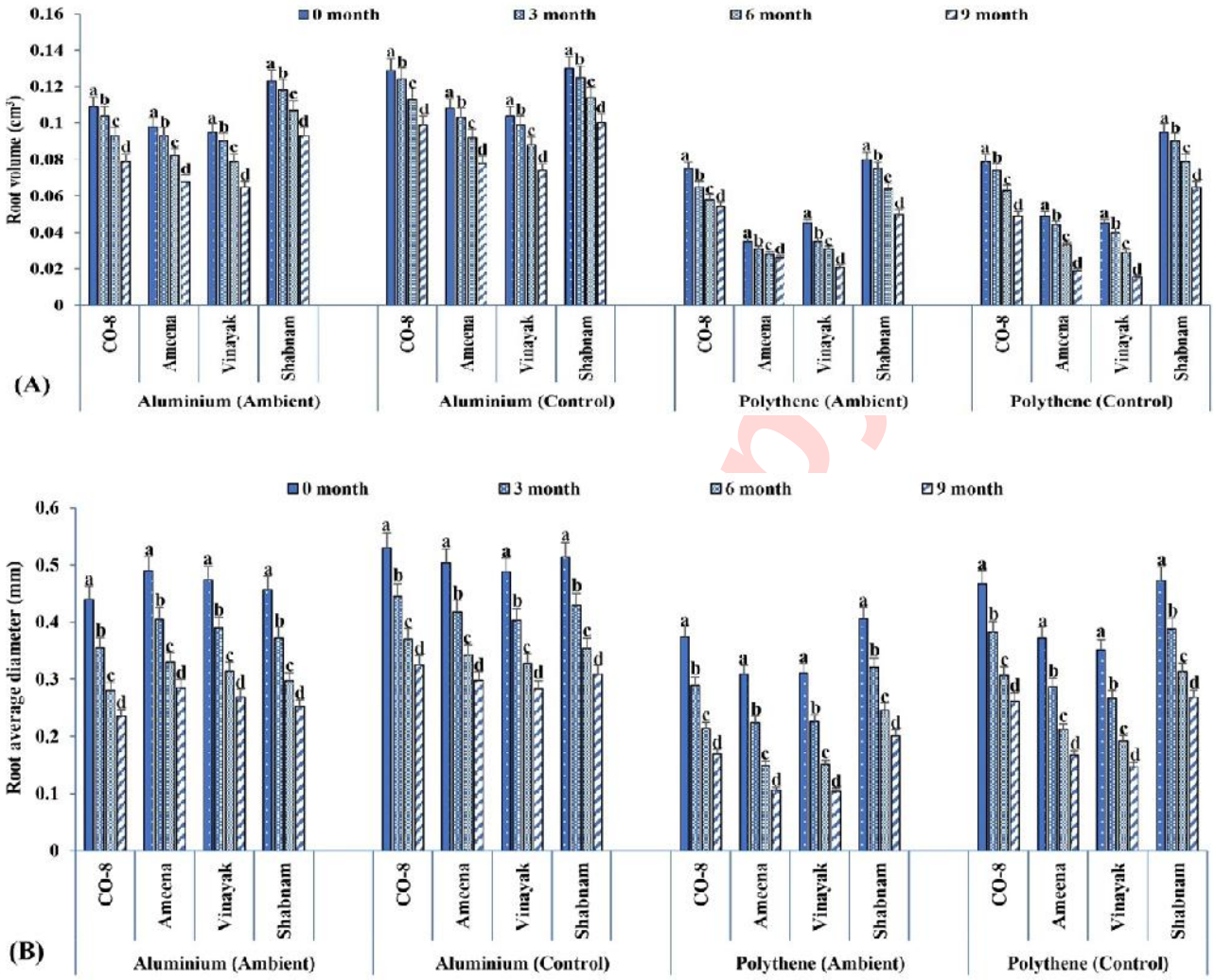


Fig. 3: Effect of packaging material and storage condition on (A) Root volume (cm3)and (B) Root average diameter (mm) of four papaya cultivars evaluated at 0, 3, 6 and 9 months of storage. Values are means ± SE of three replications; five seedlings were analysed per replication. Bars bearing the same letter do not differ significantly at P≤0.05 (Tukey's test).

cm² after 9 months of storage (p≤0.05). In contrast, the cultivar 'Vinayak' stored in polythene packets under ambient conditions exhibited a substantial reduction in (RSA) from 3.88 cm² to 0.66 cm² during the same period (p≤0.05). The higher RSA observed under controlled storage conditions indicates improved maintenance of root growth potential and enhanced ability for nutrient and water absorption. The reduction in RSA under ambient storage may be associated with accelerated ageing processes caused by fluctuations in temperature and relative humidity, which adversely affect membrane integrity and metabolic functions (Murthy *et al.,* 2003; Corbineau, 2024). The gradual decline in both total root length and root surface area with prolonged storage supports the general concept of seed ageing, wherein oxidative damage, membrane deterioration and enzymatic dysfunction collectively impair seedling establishment and root growth (Bailly, 2004). This reduction in root development may be explained by increased oxidative stress, which impairs the antioxidant defence mechanism and accelerates seed deterioration, since mitochondria are highly susceptible to oxidative damage, leading to a decline in root development under ageing conditions. Furthermore, progressive seed ageing causes structural deterioration of mitochondria, including vesiculation of the inner mitochondrial membrane, disappearance of cristae and dissociation of ATP synthase, resulting in reduced ATP production and impaired cellular metabolism (Xia *et al.*, 2015).

Root volume was significantly influenced by storage conditions, packaging materials, cultivars and storage duration (Fig. 3A, 4). A gradual reduction in root volume was observed with increasing storage period under all treatments. However, the decline was less pronounced in seeds stored in aluminium poly pouches under controlled conditions. Among the cultivars,

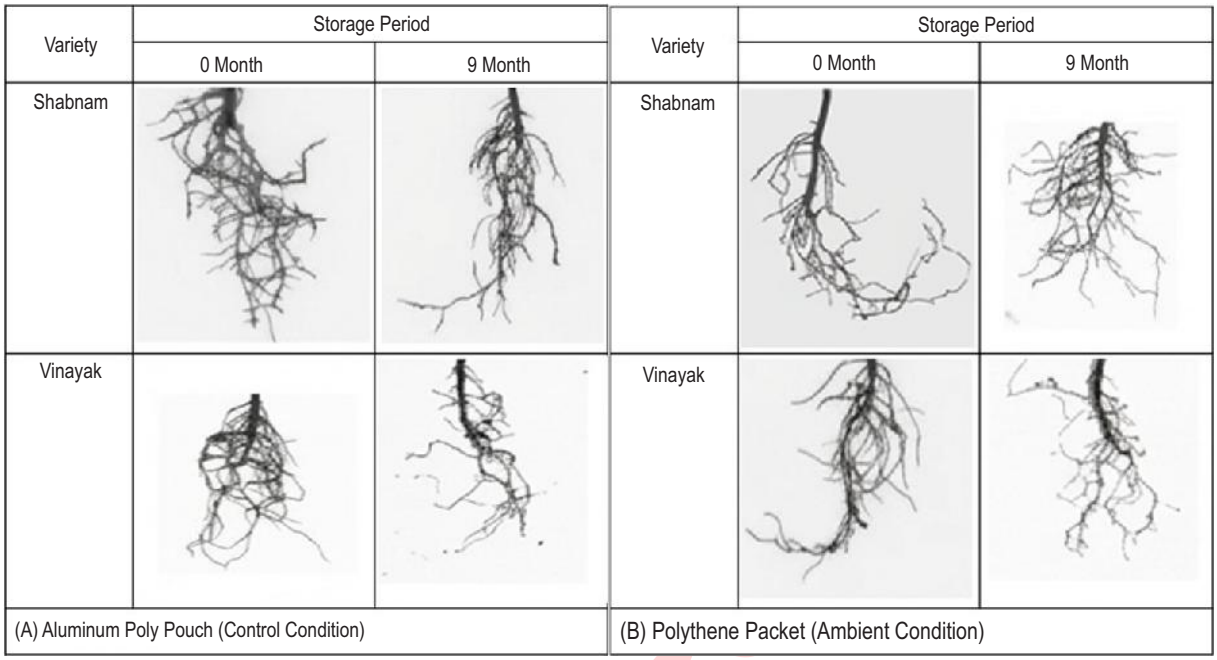


Fig. 4: Exhibition of root system architecture in different papaya varieties with respect to packaging materials, storage period and condition.

'Shabnam' maintained the highest root volume, decreasing marginally from 0.130 cm^3 at 0 months to 0.100 cm^3 after 9 months of storage ($p \leq 0.05$). In contrast, the lowest root volume was recorded in 'Vinayak' stored in polythene packets under ambient conditions, where values declined from 0.045 cm^3 to 0.021 cm^3 over the storage period ($p \leq 0.05$).

Similarly, root average diameter also decreased progressively with increasing storage duration across all treatments (Fig. 3B, 4). Seeds stored in aluminium polypouches under controlled conditions maintained significantly higher root average diameter compared with seeds stored under ambient conditions. Among the cultivars, 'Shabnam' again exhibited superior performance by retaining the highest root average diameter, decreasing marginally from 0.514 mm at 0 months to 0.309 mm during 9 months of storage ($p \leq 0.05$). In contrast, 'Vinayak' stored in polythene packets under ambient conditions showed a pronounced reduction from 0.311 mm to 0.104 mm ($p \leq 0.05$). The better preservation of toot volume and root average diameter under controlled conditions may be attributed to reduced respiration rates, slower metabolic degradation, minimized oxidative stress at low temperature and low relative humidity. Aluminium polypouches likely acted as an effective barrier against moisture ingress and oxygen exchange, thereby reducing lipid peroxidation and preserving membrane stability (Hong and Kim, 2004; Netra *et al.,* 2015).

Conversely, ambient storage conditions with fluctuating temperature and humidity accelerated seed deterioration through repeated hydration and dehydration cycles, leading to increased oxidative damage and loss of vigour (McDonald, 1999; Bhuker *et al.,* 2023). The superior performance of the cultivar 'Shabnam' across all RSA traits suggest the presence of inherent genetic variability in seed storability and tolerance to ageing. Such variability among cultivars may be associated with differences in antioxidant defence systems, membrane stability and reserve mobilization efficiency, which collectively contribute to maintenance of seed vigour during prolonged storage (Kaur *et al.,* 2021; Naghisharifi *et al.,* 2024).

Correlation analysis at 0 months of storage (Fig. 5) revealed strong and positive associations among all root system architecture traits. Total root length (cm) exhibited strong positive correlations with root surface area (cm^2)® =0.90), root volume (cm^3)® = 0.90) and root average diameter (mm) (r= 0.90), indicating a coordinated pattern of root development. As shown in Figs. 6, 7 and 8, these correlations persisted across subsequent storage intervals (3, 6 and 9 months), though a slight decline in correlation strength was observed with prolonged storage, reflecting gradual disruption of root trait coordination due to seed deterioration. The persistence of positive correlations throughout the storage period suggests that the fundamental relationships among RSA traits remained stable despite ageing-induced reductions in overall root growth. However, a slight decline in correlation strength was observed with prolonged storage, indicating a gradual weakening of trait associations due to seed deterioration. Similar coordinated relationships among root traits have been reported in previous studies (Waisel and Eshel, 2002; Lynch, 2007), where emphasis was paid on the importance of integrated root development for efficient nutrient and water uptake. A

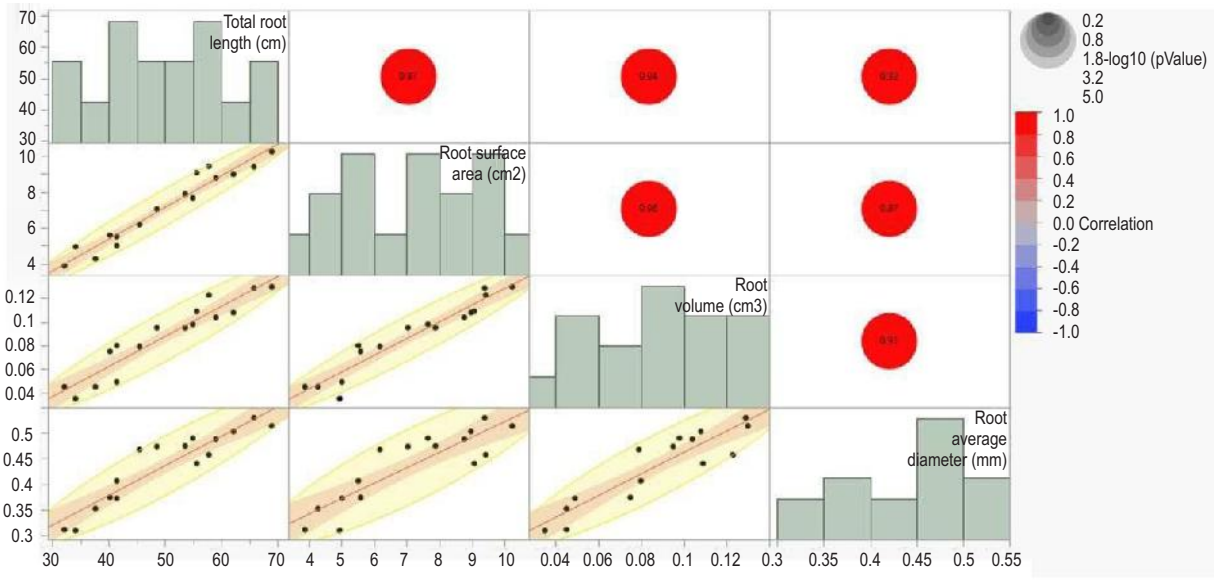


Fig. 5: Pearson correlation matrix among RSA traits-Total root length (cm), root surface area (cm²), root volume (cm³),and root average diameter (mm)-evaluated across all cultivars, packaging materials,and storage conditions at 0 months of storage.

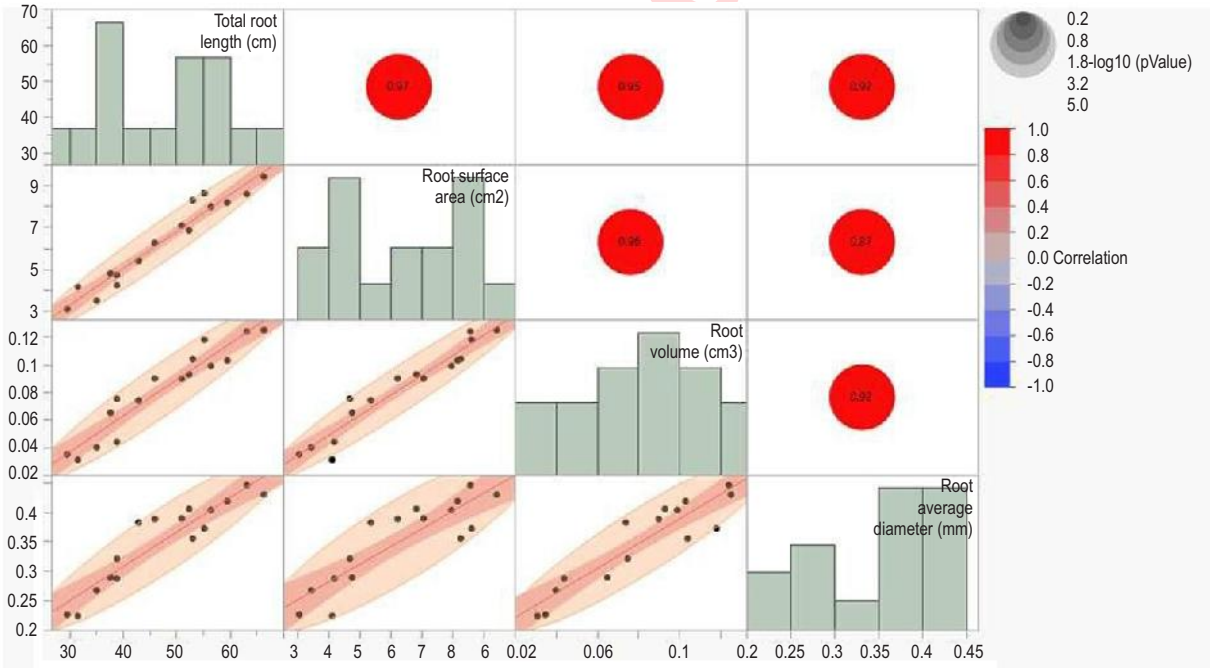


Fig. 6: Pearson correlation matrix among RSA traits-Total root length (cm), root surface area (cm²), root volume (cm³), and root average diameter (mm)-evaluated across all cultivars, packaging materials, and storage conditions at 3 months of storage.

strong interrelationship among RSA parameters further suggests that TRL, RSA, RV and RAD can serve as reliable indicators of seedling vigour during storage studies. Preservation of these traits under suitable storage conditions is therefore essential for maintaining robust seedling establishment and improving crop performance.

It should be noted that RSA assessments in this study were conducted on seedlings raised in cocopeat under controlled conditions, which provides a reproducible and uniform medium for root scanning but may not fully replicate root behaviour in actual field soils. Cocopeat is a low-density, low-resistance medium, and RSA traits measured therein may differ from those

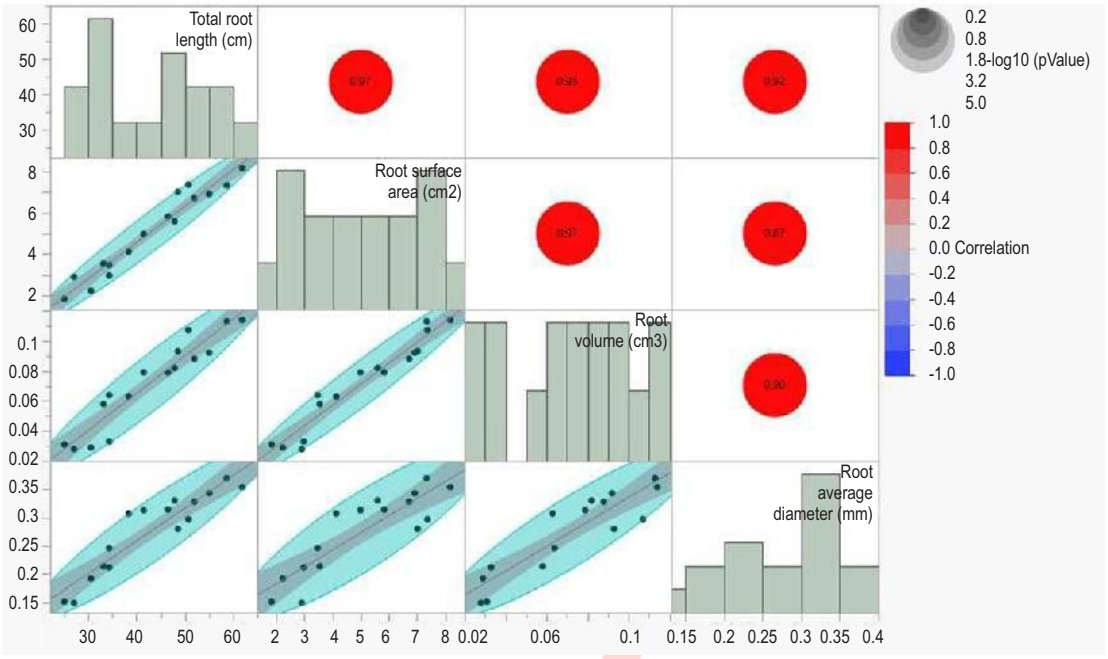


Fig. 7: Pearson correlation matrix among RSA traits-Total root length (cm), root surface area (cm²), root volume (cm³), and root average diameter (mm)-evaluated across all cultivars, packaging materials, and storage conditions at 6 months of storage.

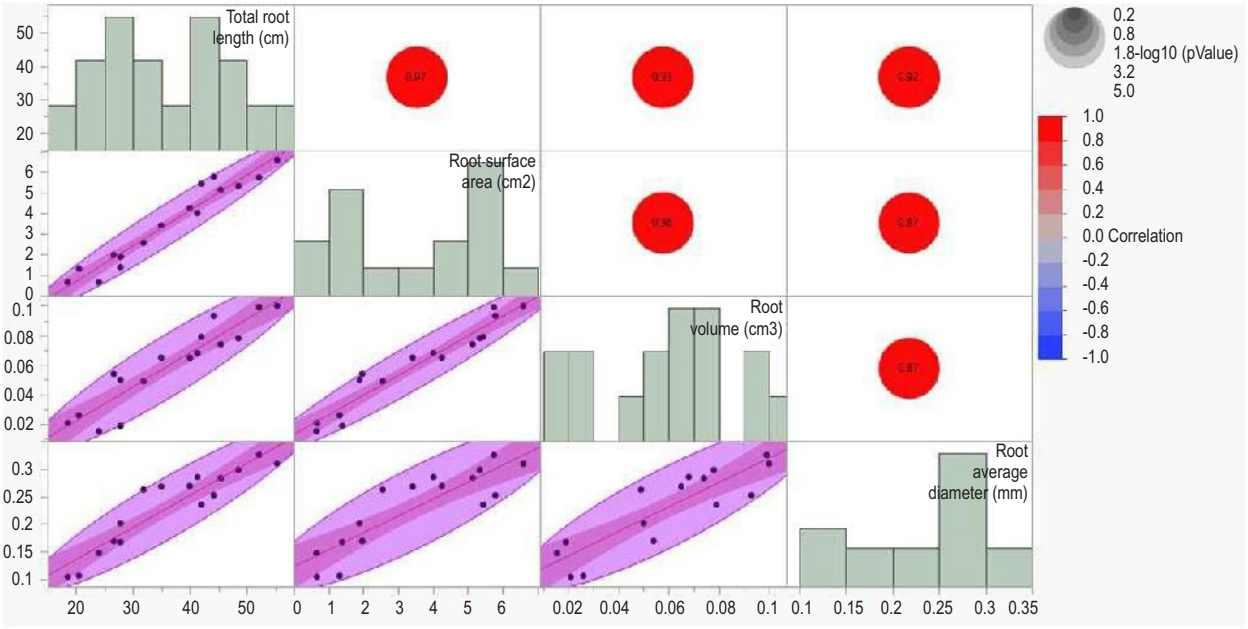


Fig. 8: Pearson correlation matrix among RSA traits-Total root length (cm), root surface area (cm²), root volume (cm³), and root average diameter (mm)-evaluated across all cultivars, packaging materials, and storage conditions at 9 months of storage.

expressed in field soils with varying texture, bulk density, and nutrient availability. However, RSA traits measured in standardized growth media are widely used as relative indicators of root development potential across treatments (Bouma *et al.,* 2000), and the comparative differences among treatments observed in this study are expected to reflect similar trends in field conditions. Future studies comparing seedling performance of stored seeds in both controlled media and field soils would further

validate the practical applicability of these findings. Overall, the combined use of moisture-proof packaging materials and low-temperature storage environments appear essential for preserving seed vigour, maintaining robust root system architecture, and ensuring improved seedling establishment during extended storage periods. These findings provide valuable insights for refining seed storage strategies and enhancing the quality and performance of papaya seeds under long-term storage conditions.

From a seed industry perspective, the findings of this study have direct relevance to seed packaging, quality control and distribution practices. The recommendation to use aluminium polypouches with controlled cold storage (5°C, 30% RH) for papaya seed storage provides actionable guidance for seed companies and public gene banks seeking to extend shelf life, while maintaining seedling vigour. Additionally, the identification of 'Shabnam' as astorage-tolerant cultivar offer breeders a genetic resource for developing varieties with improved seed longevity, a trait of increasing importance under changing climatic conditions that challenge conventional ambient storage.

Acknowledgments

We gratefully acknowledge the ICAR–Indian Agricultural Research Institute (IARI) New Delhi, India; National Phytotron Facility, IARI, New Delhi, India for their guidance and support in accomplishing the research activities.

Authors' contribution: B. Verma: Carried out manuscript drafting, experimental work and data recording; **K.K. Singh:** Experimental design formulation; **S.K. Lal:** Provided the facility of using root win-rhizo scanner; **D. Singh:** Helped in data analysis; **S.R. Jacob** and **A. Anand:** Helped in revision of the manuscript; **J Prakash:** Provide seed material for research work; **R. Choudhary:** Helped in laboratory work. **K.K. Singh and R. Choudhary have equally contributed to this research.**

Funding: Indian Council of Agricultural Research (ICAR), Indian Agricultural Research Institute.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that is there is no conflict of interest.

Data availability: The data mentioned are original and no external data were used.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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