Folia Hort. 36(4) (2024): 533–558

DOI: 10.2478/fhort-2024-0036

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Seed germination, seedling growth performance, genetic stability and biochemical responses of papaya (*Carica papaya* L.) upon pre-sowing seed treatments

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ABSTRACT

It is necessary to move towards using innovative, safe and environmentally friendly materials to break seed dormancy, increase germination percentage (GP) and obtain strong seedlings. This investigation was carried out during 2022/2023 and 2023/2024 seasons at the Faculty of Agriculture's nursery, Damietta University, Egypt, to study the influence of pre-sowing of papaya seeds (*Carica papaya* L.) cv. Red Lady for 24 hr in magnetic water (M.W) at 2000 Gauss, chitosan (CH) at 1.5% and gibberellic acid (GA_3) at 1000 ppm, as separate treatments or in combination, on seed germination, seedling growth parameters and genomic-DNA template stability. Among all the investigated treatments, the individual application of GA_3 ranked first followed by the M.W treatment resulting in the highest GP (100% and 86.36%), germination rate index (15.53 and 14.06) and seedling vigour index (883.33 and 490.30), respectively. As for seeds' biochemical content, the highest values of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (47.04% and 46.11%), total phenolic content (5.41 and 4.81 mg · g^{-1} DW), α-amylase enzyme (16.45 µmol · min^{-1} · mL and 12.37 µmol · min^{-1} · mL), proline content (3.87 mg · g^{-1} and 3.67 mg · g^{-1} DW) and total phosphorus content (0.18% and 0.13%) were highest in the GA_3- and M.W-treated seeds, respectively. Genome template stability percentage (GTS%) derived from papaya seed protein profiles using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique showed higher percentages if CH is added to any treatment. Nevertheless, the most significant adverse effect on GTS% was generally noticed in response to mixed treatments. Mixed treatments may up-regulate gene expression towards the formation of antioxidants and secondary metabolites rather than the formation of primary metabolites responsible for increasing plant growth parameters, which might be the reason for reduced growth parameters in these treatments. Similarity index obtained by unweighted pair-group method with arithmetic averages (UPGMA) showed the highest values with M.W and GA_3 treatments both sharing 28 bands, similar to the control by 80% and 75%, respectively, and inducing significant changes in the morphological growth parameters besides seed phytochemicals when compared to the control. By the end of this research, it could be concluded that soaking papaya seeds in a 1000 ppm GA_3 or 2000 Gauss M.W solution separately for 24 hr before sowing could significantly improve seed germination and development; while using mixed forms of the investigated concentrations, they had no significant effect or the germination process and seedling growth were negatively affected. Further studies are needed in this regard.

Keywords: antioxidant activity, gibberellic acid, ion leakage, magnetic water, protein profiles

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Abbreviations: CH, chitosan; GA_3, gibberellic acid; GTS%, genome template stability percentage; M, monomorphic band; M.W, magnetic water; MF, magnetic field; P, polymorphism; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

INTRODUCTION

Papaya (*Carica papaya* L.), a member of the Caricaceae family, is grown as a tree in tropical and subtropical climates. It is considered as one of the most promising sustainable fruit crops in Egypt due to its fast-growing and low-demanding nature. Papaya fruits are a great source of raw material for the food and pharmaceutical industrial sectors (Ali et al., 2011) as they are nutritious fruits high in minerals, antioxidants and vitamins A and C (Boshra and Tajul, 2013; Pinnamaneni, 2017). Papain, a unique fruit enzyme found in papaya, is a significant therapeutic ingredient (Priatni et al., 2020).

Seeds are the main commercial propagating method of papaya; yet, the slow and irregular germination of papaya seeds in addition to their high prices is one of the major challenges to papaya growers and nurserymen (Chadha, 1992; Bhardwaj et al., 2012). Furthermore, the presence of the gelatinous outer layer of seeds (sarcotesta) and the possible loss of seed viability under storage conditions have also proven to be other limiting factors of papaya seed germination (Bhattacharya and Khuspe, 2001; Yogeesha et al., 2007).

Papaya seed germination and seedling development have been reported to be affected by many factors such as removing the sarcotesta, the sowing and growing media, temperature and light changes around seeds during germination as well as the pre-sowing treatments of seeds with chemical substances including plant growth regulators and minerals (Bhattacharya and Khuspe, 2001; Yogeesha et al., 2007; Bhardwaj et al., 2012; Zanotti et al., 2014; Lanjhiyana et al., 2020; Hossain et al., 2023). Gibberellins (GAs) represent a class of phytohormones that modulate various aspects of plant growth and development which include, seed germination, stem cell elongation, flowering induction and fruit development (Lange and Lange, 2006; Takehara and Ueguchi-Tanaka, 2018). GA plays a significant role in managing and promoting seed germination through the induction of enzymes that soften the seed coverings, stimulating embryo cells expansion and initiating the biosynthesis of α-amylase within the germinating seeds (Peng and Harberd, 2002; Hartmann et al., 2014). Gibberellic acid (GA_3) is the most important commercial product of GAs used in agricultural practices.

Rana et al. (2020) documented a remarkable improvement in 'Surya' papaya seed germination after soaking the seeds in 200–500 ppm GA_3 solution for 24 hr or 48 hr. The highest germination percentage (GP) (96.0%) was obtained with the 400 ppm GA_3 solution for a soaking duration of 48 hr, and equally high results (93.3% and 95.3%) were obtained with 500 ppm GA_3 for the soaking durations of 24 hr and 48 hr, respectively. Bhattacharya and Khuspe (2001) investigated the effect of pre-sowing submersion of seeds obtained from 10 different papaya cultivars for 24 hr in (100 ppm and 200 ppm NAA, 100 ppm and 200 ppm GA_3 or 1.5% KNO_3) solutions and found that the 200 ppm GA_3 treatment was ranked first and increased germination to 12%–79% depending on the studied cultivar.

Overall, the most favourable concentration of GA_3 used for seed soaking is 50–150 ppm in different plants (Dissaanayake et al., 2010; Ansari et al., 2013; Shariatmadari et al., 2017), but Du et al. (2022) found that the optimal concentration of GA_3 for hempseed is 400 mg · L^{-1} and 600 mg · L^{-1}.

Applying a magnetic field (MF) to the water or the seeds enhanced seed germination and seedling growth of many plant species (Samarah et al., 2021). Seed germination, seedling growth and reproduction, and meristem cell proliferation were all positively impacted by magnetic treatment (Hozayn et al., 2014). These studies on sunflower (Asgharipour and Omrani, 2011), cucumber (Al-Shrouf, 2014) and tomato (El-Yazied et al., 2012) demonstrated that applying a MF to the irrigation water increases germination and plant growth and results in favourable outcomes for seedling vigour, dry weight and length (Hozayn et al., 2015). The fresh weight of the wheat seedling roots using DIW after 10 min and 15 min, as well as the fresh weight of the seedling shoots and seedling roots using TW after 25 min, all increased significantly with the magnetically treated water (Al-Akhras et al., 2024). Magnetic water (M.W) could be one of the most promising ways of applying a MF in the future to enhance agricultural production in an environmentally friendly way (Dobránszki and Teixeira Da Silva, 2014). Chitosan (CH), made by deacetylation of chitin, is a biodegradable composite found in crustacean scales, cell walls of fungi, epidermis from insects and some algae, and it is the second most common predominant biopolymer in the environment after cellulose (Uthairatanakij et al., 2007). CH, with its individual biological and physiological characteristics, has been used in several medicinal and agricultural applications (Lizárraga-Paulín et al., 2011). CH possesses promising possibilities for promoting seed germination and enhancing seedling growth through its ability to improve water absorption and nutrient uptake besides stimulating plant defence mechanisms; therefore, it has emerged as a potential environmentally friendly seed coating (Godínez-Garrido et al., 2022; Arif et al., 2023; Boamah et al., 2023; Riseh et al., 2024).

Seed proteins play a crucial role in both dormancy and germination. During dormancy, certain proteins are involved in maintaining the seed in a dormant state. For example, protein carbonylation and selective mRNA

oxidation are processes that help maintain dormancy. When conditions become favourable, these proteins undergo changes that allow the seed to break dormancy and begin germination. Proteins are the end products of genes; hence, any variation in gene could be obvious in the protein profile (Hayee et al., 2020; Mahmoud and Abd El-Fatah, 2020). At biochemical levels, various environmental stresses and chemicals cause changes in protein metabolism by changing the protein profile. Protein electrophoretic analysis by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is considered a biochemical technique used to establish the mutagenic potentiality of several treatments on the gene expression that delivers information about the structural genes and their regulatory systems that control protein biosynthetic pathways (Fayez, 2000; Fayez et al., 2013).

Limited studies have investigated the effect of GA_3 on papaya seed germination and seedling growth performance. However, the response of papaya seeds to M.W and the CH treatments and their combination with GA_3 has not been studied or documented yet. Thus, the purpose of this research was to evaluate the effect of pre-sowing seed submersion in M.W, CH and GA_3 solutions either separately or in combination on papaya seed germination process, the corresponding biochemical changes, seedling growth parameters and genetic stability and to obtain strong seedlings that can respond to unsuitable environmental conditions.

MATERIALS AND METHODS

Plant material and experimental site

This study was conducted at the nursery of Horticulture Department, Faculty of Agriculture, Damietta University, Egypt, during two successive seasons 2022/2023 and 2023/2024. Fruits of cv. Red Lady papaya (*Carica papaya* L.) were collected at stage five of maturity (>75% were mature with yellow-coloured peels; Pereira et al., 2009). The fruits were obtained from a commercial farm in Sadat City, Menoufia Governorate, Egypt (30°25′06″N and 30°34′29″E). Seeds were taken out of the fruits, mixed with dry sand and then shaken for 24 hr to remove the sarcotesta.

Treatments and seeds planting

Papaya seeds were immersed in tap water, magnetic water (2000 Gauss), chitosan (1.5%; 15 g powder of CH in 1000 mL of 1% acetic acid) and GA_3 (1000 ppm) separately or in combination for 24 hr in beakers compared to dry seeds (without treatment) arranged in nine treatments as follows: T1 = dry seeds (control); T2 = tap water (T.W); T3 = magnetic water (M.W); T4 = chitosan (CH) 1.5%; T5 = gibberellic acid (GA_3, 1000 ppm); T6 = M.W (2000 Gauss) + GA_3 (1000 ppm); T7 = M.W (2000 Gauss) + CH 1.5%; T8 = GA_3 (1000 ppm) + CH (1.5%) and T9 = M.W (2000 Gauss) + GA_3 (1000 ppm) + CH (1.5%) (1:1:1, w/v). The magnetised water used in the soaking treatment of papaya seeds was obtained by passing tap water through an electromagnetic field generator (Model, Delta Water 14000 Gauss) belonging to a local company called 'Delta Water', and the seeds were soaked in the magnetised water for 24 hr at room temperature. CH and GA_3 were purchased from Sigma, USA. The treated seeds were then air-dried in shade for about 20 min. The dried seeds were immediately planted by the end of October in cork trays of 20 cm × 11 cm size, containing a media of peat moss/sand (2:1, v/v) under a plastic tunnel. Germination parameters were recorded at the time of germination; chemical properties and protein electrophoresis were determined after soaking the seeds for 24 hr in the specified solutions; and seedling growth parameters were recorded after 30 days of planting. Trays were arranged in a randomised complete block design with three replicates per treatment, with 33 seeds in each replicate. The standard seed germination test was conducted for papaya as described by the International Seed Testing Association (Powell, 2009).

Germination parameters

Germination percentage (GP), germination rate index (GRI), the coefficient of velocity (CV) and seed vigour index (SVI) calculation formulas are presented in Table 1 and were estimated according to Taghizadeh and Solgi (2017).

Determination of biochemical compounds of papaya seeds

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity was estimated based on Kitts et al. (2000). Briefly, five dilutions of each ethanol extract were prepared.

Table 1. Formula used to calculate papaya seed germination parameters, according to Taghizadeh and Solgi (2017).

GP	$GP = \frac{Ng}{Nt} \times 100$	Ng = germinated seeds Nt = total sown seeds
GRI	$GRI = \frac{G1}{1} + \frac{G2}{2} + ... + \frac{Gi}{i}$	*Gi* = the germination on each day after number of days from sowing *i* = alternate day after sowing
CV	$CV = \frac{\Sigma Ni}{\Sigma Ni \times Ti} \times 100$	*Ni* = the number of germinated seeds *Ti* = the last day of germination
SVI	SVI = (PL + RL) × GP	PL = shoot length RL = root length GP = germination percentage

CV, coefficient of velocity; GP, germination percentage; GRI, germination rate; SVI, seed vigour index.

Concentrations ranged from 20 mg · mL^{-1} to 1 mg · mL^{-1}. An aliquot of 1 mL of the prepared concentrations of the tested extracts was added to 1 mL of DPPH (0.14 mM) and allowed to stand in darkness for 30 min at room temperature; the mixture was incubated; and then the absorbance was measured at 517 nm. A solution free of the sample was used as a blank. The antioxidant activity of the sample was calculated according to the equation of $[(A_1 - A_2/A_1)] \times 100$, where A_1 is the control absorbance and A_2 is the extract (sample) absorbance. The IC_{50} values were calculated using an inhibition curve where there was a correlation between the percentage of DPPH inhibition and the concentrations of the samples (Ebrahimzadeh et al., 2009).

Total phenol content

In a mortar with 10 mL of 80% methanol, a known dry weight of papaya seeds was ground and filtered; the filtrate was centrifuged at 10000 rpm for 20 min; the supernatant was removed and the pellet (residue) was re-extracted with 5 mL of 80% methanol. It was centrifuged and the supernatant was recovered. The supernatant was evaporated to dryness. The residue was dissolved in 5 mL of distilled water (d. H_2O). A 0.5 mL aliquot of each sample was taken into test tubes. The volume of each tube was made up to 3 mL with d. H_2O. A volume of 0.5 mL of Folin-Ciocalteu reagent was added to each tube. Folin-Ciocalteu reagent was prepared by dissolving 10 g sodium tungstate and 2.5 g sodium molybdate in 70 mL water. Of note, 5 mL of 85% phosphoric acid and 10 mL of concentrated hydrochloric acid were added and then refluxed for 10 hr. Then, 2 mL of 20% $NaCO_3$ was added to each tube. The tube was transferred into a boiling water bath for 1 min and cooled, and the optical density of the developed colour was assessed using a spectrophotometer at 725 nm. Different concentrations of catechol (100 mg · 100 mL^{-1} d. H_2O) were used to generate the standard curve. From the standard curve, the concentration of phenol in the test samples was calculated and expressed as milligram phenol equivalent per 100 g dry weight (Yadav, 2012). Phenol content in the extract was measured using Folin-Ciocalteu assay developed by Lin and Tang (2007) and expressed as milligram gallic acid equivalent per 1 g dried seeds.

Total flavonoid content

According to Chang et al. (2020), aluminium chloride calorimetric assay was used to assess flavonoid content in the extract with a slight modification in the assay developed by Rolim et al. (2005). About 10 g of dried seeds were dissolved in 100 mL of 80% aqueous methanol at 25°C. The solution was filtered, and the filtrate was transferred to a water bath holding in a crucible to evaporate it to dryness and weighed to constant weight. After the incubation, 3.4 mL NaOH (1 M) was added to this mixture and the absorbance was measured at 510 nm. The total amount of flavonoids present in seeds was expressed as mg · 100 g^{-1} of dry weight.

α-Amylase activity

With minor modifications, the α-amylase activity was estimated as described by Miller (1959). Of note, 1 mL of supernatant and 1 mL of solubilised starch solution were combined to create the reaction mixture, which was then incubated for 10 min at 60°C. A volume of 2 mL of the dinitro-salicylic acid (DNSA) reagent was added to stop the reaction. After cooling the mixture for 15 min in an ice water bath, it was centrifuged for 5 min at 5000 rpm and 4°C. Using a blank sample as a reference, the amount of enzyme was determined at 540 nm using a UV-VIS spectrophotometer. The quantity of amylase required to generate 1 μmol of maltose per 1 min in the assay conditions was designated as one unit of enzyme activity.

Reducing sugar content

DNSA method, according to the procedures of Krivorotova and Sereikaite (2014) with a slight modification, was used to determine RS content. Thawing 1 g of DNSA and 30 g of sodium–potassium tartaric acid in 80 mL of 0.5 N NaOH at 45°C to prepare DNSA reagent, the solution was kept at room temperature and diluted to 100 mL using d. H_2O. For the measurement, 2 mL of DNSA reagent was added to the plant extract of 1 mL (1 mg · mL^{-1}) in a clean test tube, kept at 95°C for 5 min and then cooled down to room temperature. About 7 mL of d. H_2O was added to the solution, and its absorbance was measured at 540 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800). The content of RS was calculated using the calibration curve of standard D-glucose (200–1000 mg · L^{-1}), and the results were stated as milligram D-glucose equivalent (GE) per gram dry extract weight.

Proline content

The proline content of papaya dried seeds was described in the Bates et al.'s (1973) method. Homogenised 500 mg of tissue in 5 mL of 3% H_2SO_4 forming the plant extract, 2 mL of extract and 2 mL of ninhydrin reagent plus glacial acetic acid were incubated together in a water bath at 100°C for 30 min and cooled, and then 4 mL of toluene was added. The mixture was vortexed, and the toluene containing chromophore absorption was measured at 520 nm using a UV-VIS spectrophotometer.

Total phosphorus content

In a muffle furnace, 2 g of papaya seeds were ignited at 550°C for 1 h. The residue was extracted with 1 M HCl for 16 hr in an extraction bottle. The organic phosphorus fraction was turned to inorganic forms of phosphorus by calcination. Thereafter, the total amount of phosphorus (inorganic and former organic) in seeds was extracted by quantitative method using HCl according to Pardo et al. (2003).

Seedling morphological measurements

From each replicate, randomly selected five seedlings of 19-day-old were used to measure seedling length (cm) and stem diameter (mm) at a height of 1 cm from the soil surface using a digital calliper 6 (0.01–150 mm; Digimess, São Paulo, SP, Brazil). The number of roots, root length (cm), number of leaves and leaf area (cm^2) were determined.

Soluble protein analysis using SDS-PAGE

Papaya seeds were ground to fine powder in cold mortar and pestle in liquid nitrogen and mixed with 2 mL of extraction buffer (1 M Tris HCl, pH 8.8, 0.25 M EDTA). Samples were transferred to Eppendorf tubes, allowed to stand overnight in refrigerator, then vortexed for 15 sec and centrifuged at 12000 rpm for 20 min. The supernatants were collected as total soluble protein extracts. SDS-PAGE technique was used to assess the effect of each treatment on papaya seed protein profile based on the method described by Laemmli (1970). Briefly, a volume of 50 µL of protein extract was added to the same volume of buffer (10% SDS, glycerol, 1 M Tris HCl, pH 8.8, 0.25 M EDTA) in Eppendorf tube; 10 µL of 2-mercaptoethanol was added to each tube and boiled in a water bath for 10 min, and then 10 µL bromophenol blue was added to each tube before sample loading. Protein bands were visualised by staining the gel in 0.25% Coomassie brilliant blue (R-250), then immersed in destaining solution I (50% methanol and 10% acetic acid) for 1 hr to obtain distinguishable protein bands and finally agitated in the destaining solution II (7% acetic acid and 5% methanol) until gel background was clear. The gel was photographed and each variable in SDS-PAGE was considered a locus, so scored as present (1) or absent (0) using scanning densitometer, Gel Doc 2000 and BIO-RAD. Molecular weights of different bands were calibrated with a wide-range molecular marker Sigma (10–250 kDa).

Polymorphism percentages were estimated: %P = (PB/TB) × 100, where PB is the number of polymorphic bands and TB is the total number of bands generated from protein profiles. The genetic similarity indices between treated papaya seeds and control were calculated based on the comparison of their protein profiles: % similarity = ½ × [(S/S + U) + (S/S + V)] × 100, where S indicates similar bands between treatments A and B; U indicates bands found in treatment A not B and V indicates bands found in treatment B not A (Akbar et al., 2012). Dendrogram was constructed using Dice's coefficient and unweighted pair group method with arithmetic averages (UPGMA) based on the Euclidean similarity index by the PAST software Version 1.91, Oslo, Norway (Hammer et al., 2001). Also, genome template stability percentage GTS% = [1 – (*a*/*n*)] × 100 was estimated to evaluate genetic variations, where *a* is the average number of PB bands observed in each treated sample and *n* is the number of total bands in the control (Osman et al., 2020).

Statistical analysis

Using the Co-Stat software package, Version 6.303 (789 Lighthouse Ave PMB 320, Monterey, CA, 93940, USA), the one-way analysis of variance (ANOVA) was performed on the data from three replications in a completely randomised block design. Levels of significance were compared using Duncan's new multiple range test at $p < 0.05$. To substantiate the whole results, further correlation analyses using Pearson's *r*-test statistic were performed**.**

RESULTS

Seed germination parameters

Germination percentage (GP), germination rate index (GRI), coefficient of velocity (CV) and seedling vigor index (SVI)

During the trial, GP was calculated starting on the 10th day of seed planting till the 26th day with a 4-day interval (Figure 1), and the individual applications of GA_3 (1000 ppm), CH (1.5%) or M.W (2000 Gauss) significantly improved papaya seed germination while the mixed treatments negatively affected the germination process. The highest values of GP (%), GRI and SVI were noted when papaya seeds were soaked for 24 hr in the 1000 ppm GA_3 solution followed by the 2000 Gauss M.W treatment, and the values were (100%, 15.53, 883.33) and (86.63%, 14.06, 454.84) respectively (Figures 1 and 2). As for the CV, CH, GA_3 and M.W treatments, all showed the highest results with no significant differences to one another nor to the control or the tap-water treatment; the lowest GP, germination rate index and CV and lowest seedling vigour index resulted from submerging papaya seeds for 24 hr in each of the mixture solutions M.W (2000 Gauss) + GA_3 (1000 ppm), M.W (2000 Gauss) + CH (1.5%), GA_3 (1000 ppm) + CH (1.5%) and M.W (2000 Gauss) + GA_3 (1000 ppm) + CH (1.5%) compared to the separate treatments and the control.

Chemical characteristics

Antioxidant activity and antioxidant compounds

Changes in antioxidant activity (DPPH%) along with antioxidant compounds such as total phenolic and total flavonoid contents, after submerging papaya seeds for 24 hr in M.W, CH and GA_3 solutions either individually or in combination, were investigated. The most effective treatment in this pattern was the submersion in 1000 ppm GA_3 solution, resulting in the highest value of antioxidant activity (47.04%), total phenolic content (5.41 mg · g^{-1} DW) and total flavonoid content (11.52 mg · g^{-1} DW) (Figure 3). By contrast, data showed the lowest value of antioxidant activity and total flavonoid content, with papaya seeds submerged in M.W (2000 Gauss) + GA_3 (1000 ppm), recording 12.90% and 4.14 mg · g^{-1} DW, respectively.

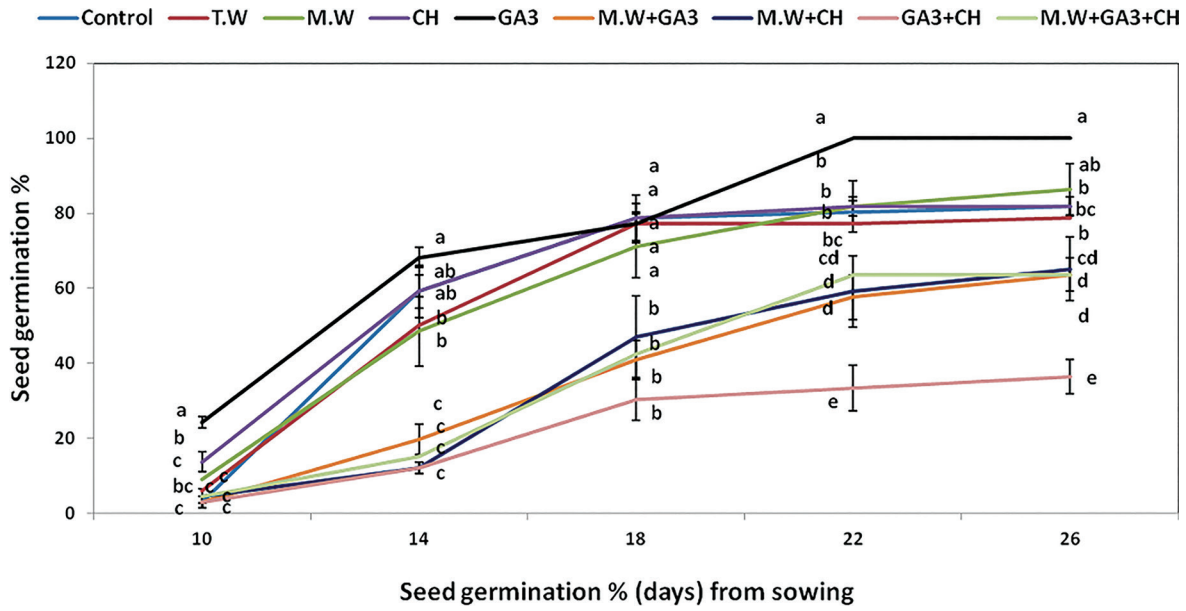

Figure 1. Effect of papaya seed pre-sowing treatments on seed GP. Data are means of two seasons (2022/2023 and 2023/2024) and three replicates (n = 3) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan. GP, germination percentage.

α-Amylase activity and reducing sugar content

Generally, the investigated individual treatments (M.W, CH and GA_3) significantly increased α-amylase activity of papaya seed embryos compared with the control and mixed treatments (Figure 4). Submerging papaya seeds in the mixed solutions led to a significant decrease in α-amylase activity. Among all treatments, the T6, M.W (2000 Gauss) + GA_3 (1000 ppm), expressed the lowest concentration of α-amylase activity. The highest concentration of α-amylase activity was shown in seeds submerged in the 1000 ppm GA_3 solution (16.45 μmol · min^{-1} · mL). All treatments significantly increased the reducing sugar (RS) content in papaya seeds compared with the dry seeds except for T6, M.W (2000 Gauss) + GA_3 (1000 ppm), where the slight increase was not significant. The maximum RS content 6.91 mg · g^{-1} DW and 6.56 mg · g^{-1} DW was obtained with M.W (2000 Gauss) and GA_3 (1000 ppm) treatments, respectively.

Proline and total phosphorus contents

As shown in Figure 5, the lowest proline content in papaya seeds was recorded in those submerged in the four mixture solutions (T6, T7, T8 and T9) while the increase caused by GA_3, CH or the M.W treatment was not significant compared with the proline content of the dry seeds and the tap-water treatment. On the contrary, the increase in total phosphorus content within the seeds submerged in the GA_3 solution or the M.W was significant when compared with other treatments. The highest concentration of total phosphorus (0.17%) was recorded with the GA_3 (1000 ppm) solution followed by the M.W (2000 Gauss) treatment (0.13%).

Morphological characteristics of papaya seedlings

According to data presented in Figures 6 and 7, the GA_3 (1000 ppm) treatment ranked first in improving the papaya seedlings' root system as well as the vigour growth and development. The improvement caused by this treatment was significant compared to the control in most of the studied morphological characteristics like seedling height (9 cm), stem diameter (0.15 mm), number of leaves (5) and leaf area (2.14 cm^2). The 2000 Gauss M.W treatment and the 1.5% CH solution significantly increased seedling stem diameter and leaf area compared to the control, tap-water and the mixed solution treatments. The lowest values of seedling height, root length, stem diameter and leaf area were recorded in seedlings of the papaya seeds submerged in those four mixed treatments.

Electrophoresis analysis of proteins

Electrophoresis analysis of total protein extracts using SDS-PAGE with a protein marker of molecular weight 10–250 kDa succeeded in showing variations among treated and untreated papaya seeds (Figure 8). Each treatment exhibited a distinctive electrophoretic pattern. In Table 2, the total number of bands were 32 with a molecular weight ranging from 10.24 kDa to 200.20 kDa, 2 of which were monomorphic with molecular weights

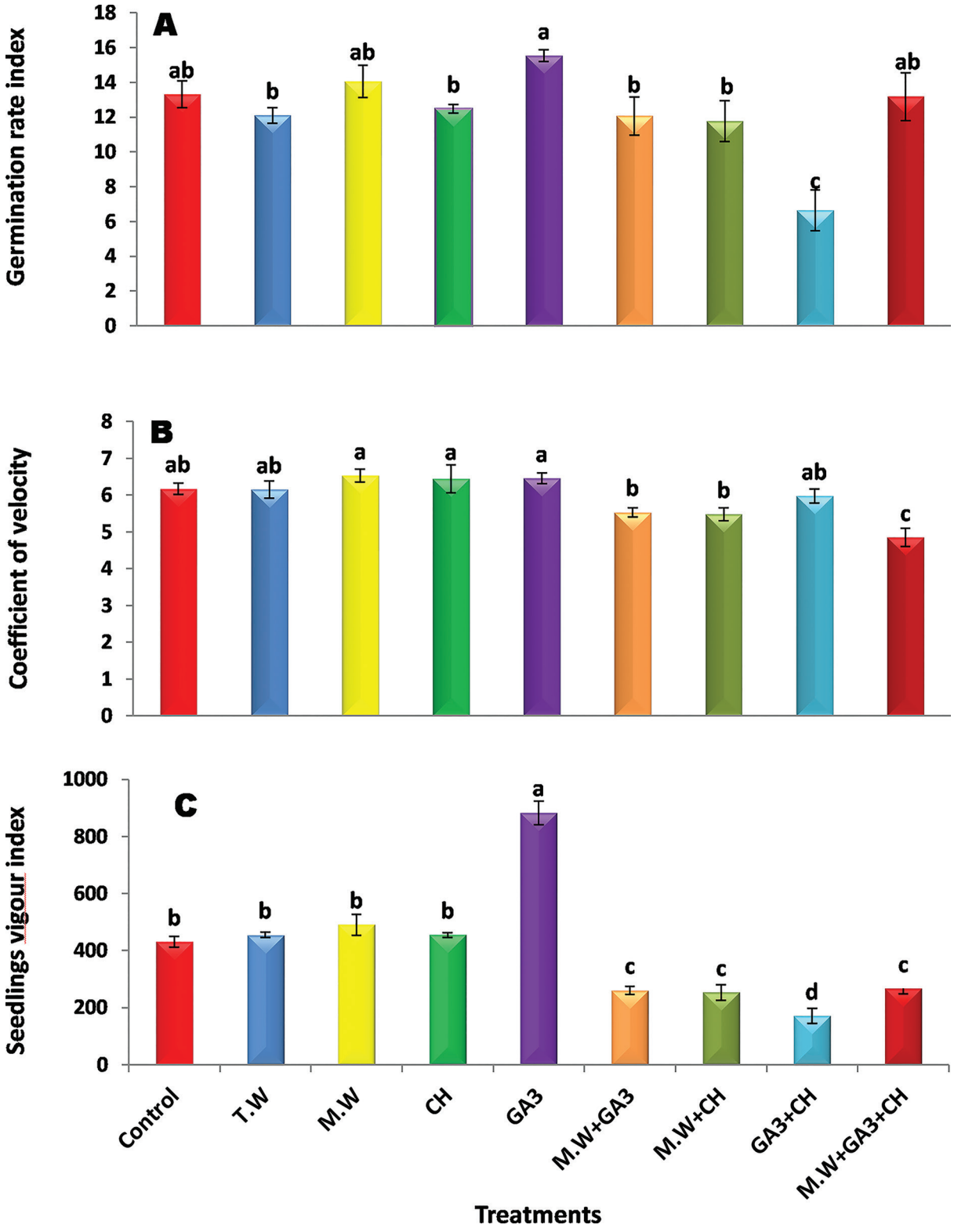

Figure 2. Effect of papaya seed pre-sowing treatments on germination rate index (A), CV (B) and SVI (C). Data are means of two seasons (2022/2023 and 2023/2024) and three replicates (n = 3) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan. CV, coefficient of velocity; GRI, germination rate index; SVI, seedling vigour index.

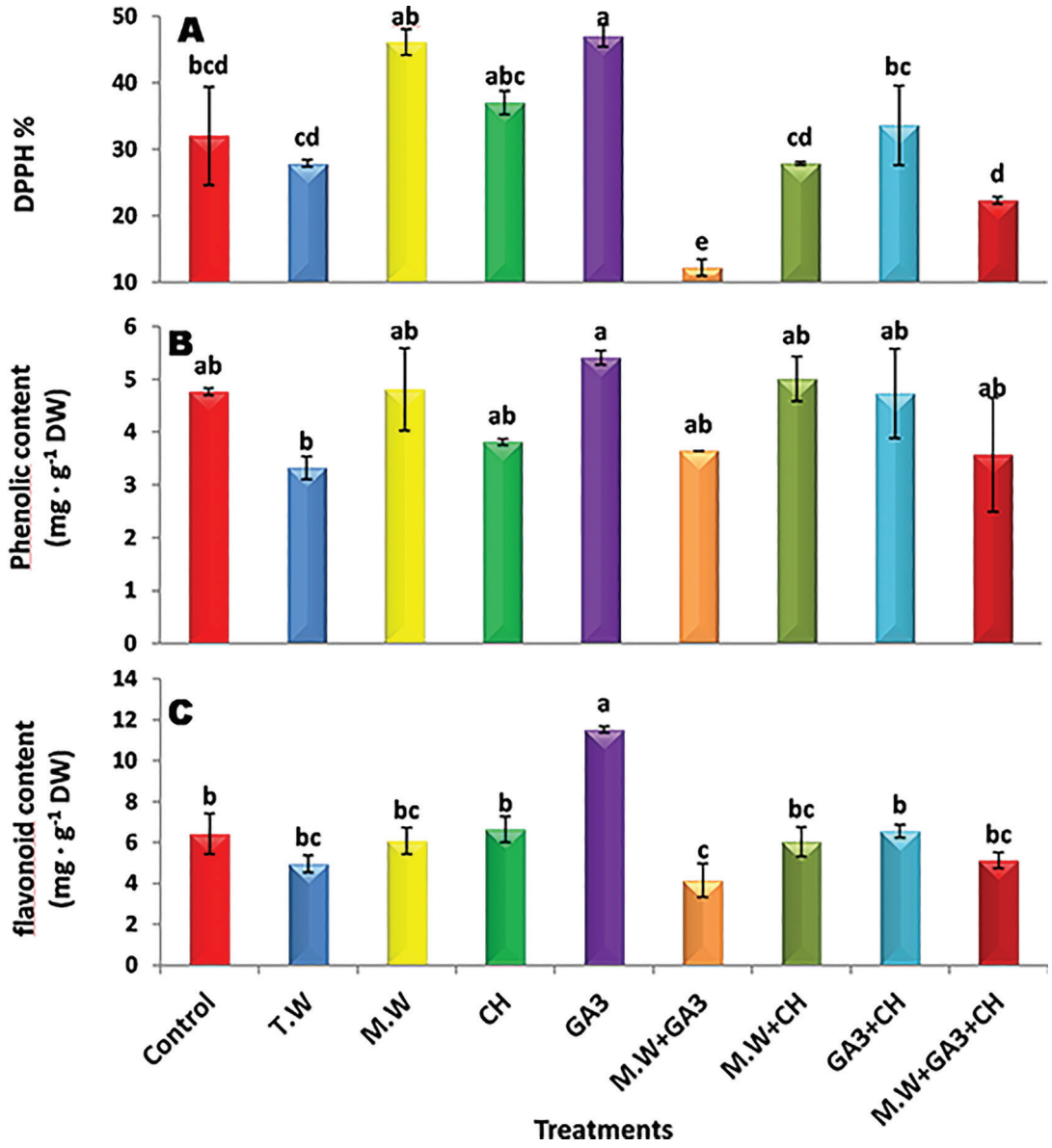

Figure 3. Effect of papaya seed pre-sowing treatments on seed antioxidant activity (DPPH%) (A), total phenolic content (mg · g^{-1} DW) (B) and total flavonoid content (mg · g^{-1} DW) (C). Data are means of two seasons (2022/2023 and 2023/2024) and three replicates ($n = 3$) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

of 10.46 kDa and 151.72 kDa and 30 bands of which were polymorphic; 4 negative unique markers (NUMs) and 13 positive unique markers (PUMs), with a net gel polymorphism equals 93.75%. The most obvious changes in the protein profiles were the emergence of new bands, for example, the band of molecular weight 33.42 kDa that could be used as PUM for M. W (2000 Gauss) pre-sowed papaya seeds. Also, the disappearance of some bands was observed as the bands with molecular weights 11.54 kDa and 24.13 kDa that both could be used as NUMs for M.W (2000 Gauss) + GA_3 (1000 ppm) pre-sowed papaya seeds.

The percentage of polymorphism in the treated seeds in contrast to the control showed a wide variation based on their protein electrophoretic banding pattern as shown in Table 2. The maximum percentage of polymorphism (84.62%) was recorded in response to the mixed treatment of M.W (2000 Gauss) + GA_3 (1000 ppm) + CH (1.5%), while the minimum percentage of polymorphism (77.78%) was in response to the single treatment of M.W (2000 Gauss) and the mixed treatment of M.W (2000 Gauss) + CH (1.5%).

Separate evaluation of protein band profiles discriminates the genetic similarity between treated

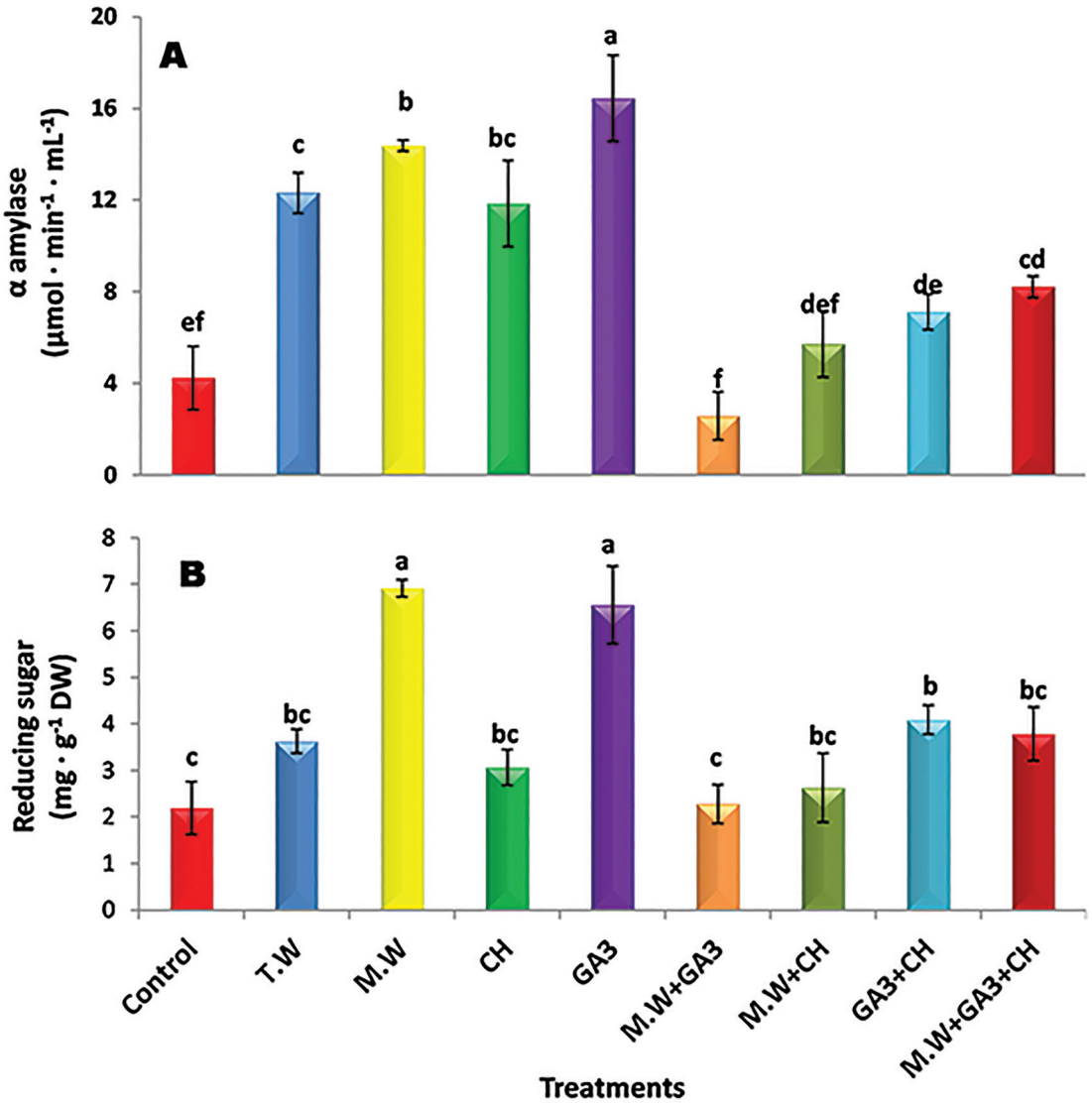

Figure 4. Effect of papaya seed pre-sowing treatments on α-amylase (µmol · min^{-1} · mL^{-1}) (A) and RS (mg · g^{-1} DW) (B). Data are means of two seasons (2022/2023 and 2023/2024) and three replicates (n = 3) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan. RS, Reducing sugar.

and untreated papaya seeds as presented in Table 3 and in a dendrogram constructed based on a similarity coefficient (Figure 9). A genetic similarity index was noticed in a high percentage, 93%, in M.W (2000 Gauss), GA_3 (1000 ppm) and GA_3 (1000 ppm) + CH (1.5%) papaya seed protein profiles and the lowest value, 72%, was recorded in control and T6 papaya seed protein profile. Moreover, a great similarity index was recorded between T6, M.W (2000 Gauss) + GA_3 (1000 ppm), and T8, M.W (2000 Gauss) + CH (1.5%), treated papaya seed protein profiles by about 86%; thus, the dendrogram exhibited two main groups: the first group had two subgroups consisted of T6 and T8 papaya seeds sharing 24 similar bands and the second group comprises the remaining treatments of seven subgroups in which genetic similarity index showed pronounced differences ranging from 72% to 93%.

As presented in Table 4 and Figure 10, genomic-DNA template stability percentage, GTS% values, reflecting changes in papaya seed protein-banding patterns showed considerable differences among the treated and the control papaya seeds; the maximum GTS% (35.71%) was in response to mixed treatments of M.W (2000 Gauss) + CH (1.5%) and M.W (2000 Gauss) + GA3 (1000 ppm) + CH (1.5%), while the minimum GTS% (7.14%) was in response to GA3 as a single treatment. However, the absolute genetic instability (100%) was recorded in response to submerging papaya

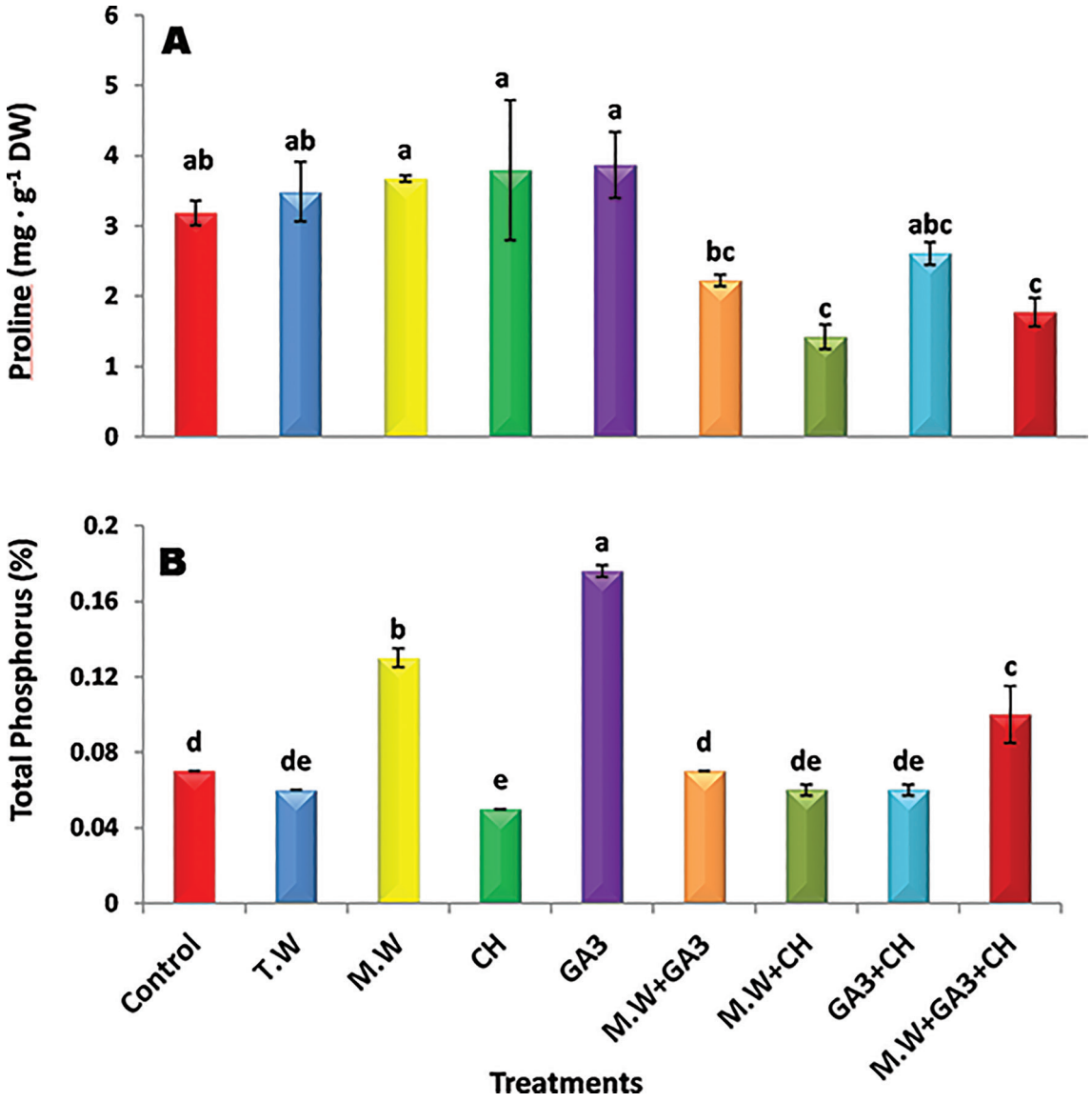

Figure 5. Effect of papaya seed pre-sowing treatments on proline content (mg · g^{-1} DW) (A) and total phosphorus (%) (B). Data are means of two seasons (2022/2023 and 2023/2024) and three replicates (n = 3) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan.

seeds in the mixed treatment of M.W (2000 Gauss) + GA3 (1000 ppm).

Table 5 shows the Pearson's correlation coefficient (r) between the genomic-DNA template stability deduced from the electrophoretic protein-banding pattern of untreated dry seed (control) and pre-sowed papaya seeds in M.W, 1.5% CH and GA_3 separately or in combination and low positive correlations were observed between GTS% induced by the treatments and the assessed antioxidants (DPPH, flavonoids and total phenolics) and proline. However, there is a negative correlation in between GTS% and biosynthetic α-amylase, RSs and vegetative growth parameters (seedling height, seedling vigour index, stem diameter, leaf area, number of roots and CV) but a high significant correlation coefficients was noticed in between the phosphorous content and GTS%.

DISCUSSION

For the successful production of papaya seedlings in nurseries, ensuring a high seed GP with an elevated germination rate and seedling vigour index is critical. In this study, papaya seeds of the Red Lady cultivar treated with GA_3 (1000 ppm) for 24 hr or M.W (2000 Gauss) for 24 hr have proven to be superior compared to other treatments in terms of GP after 10, 14, 18, 22 and 26 days of sowing, GRI, as well as SVI. The GA_3

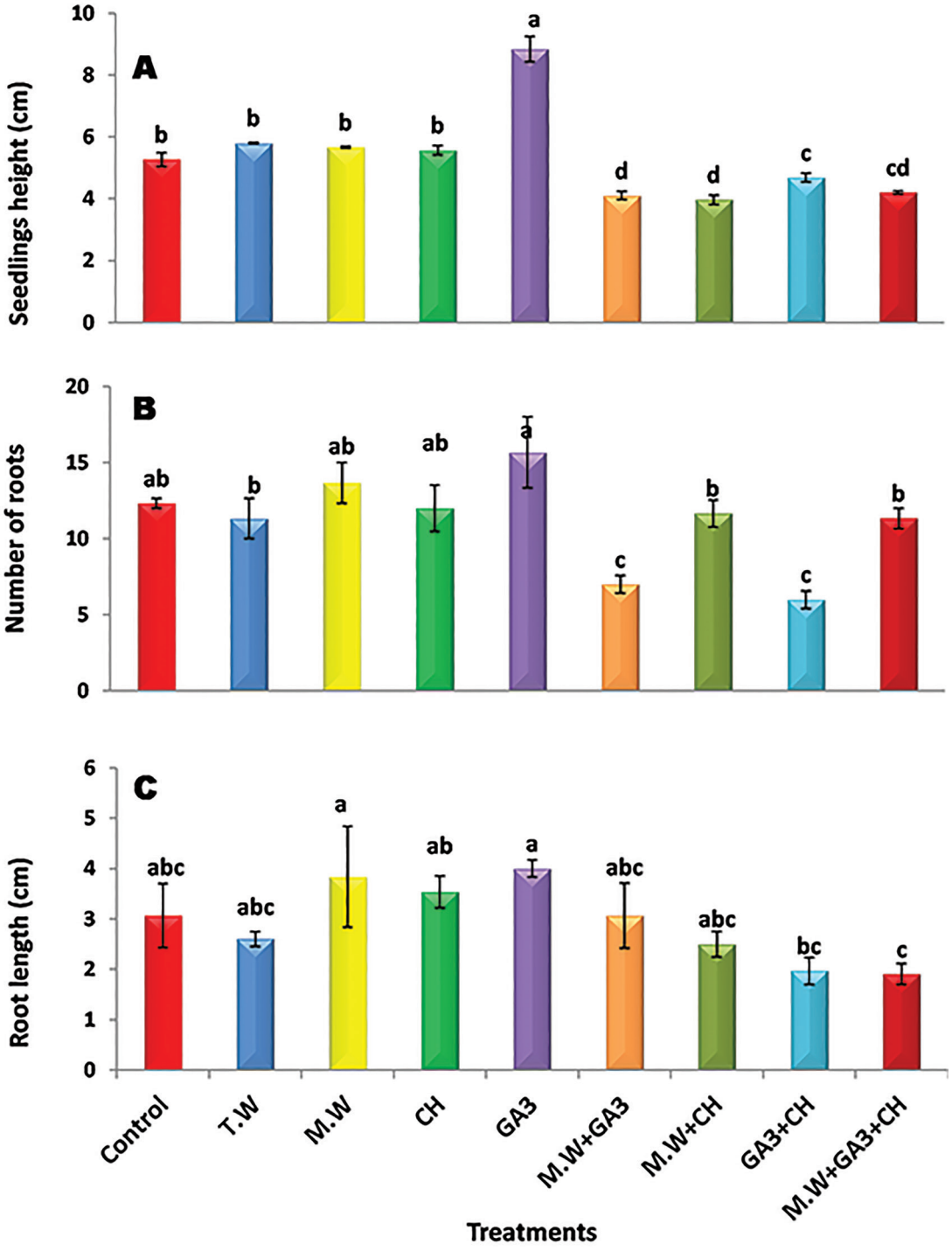

Figure 6. Effect of papaya seed pre-sowing treatments on seedling height (cm) (A), number of roots (B) and root length (cm) (C). Data are means of two seasons (2022/2023 and 2023/2024) and three replicates (n = 3) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan.

treatment showed the highest GP% (100%), GRI (15.53) and SVI (883.33). Most of the physical seedling growth parameters like seedling height, stem diameter and number of leaves were also significantly higher with the submersion of seeds in the GA_3 solution. Among many pre-sowing treatments given to enhance the germination of 10 Papaya cultivars, Bhattacharya and Khuspe (2001) documented that seed submersion in a 200 ppm GA_3

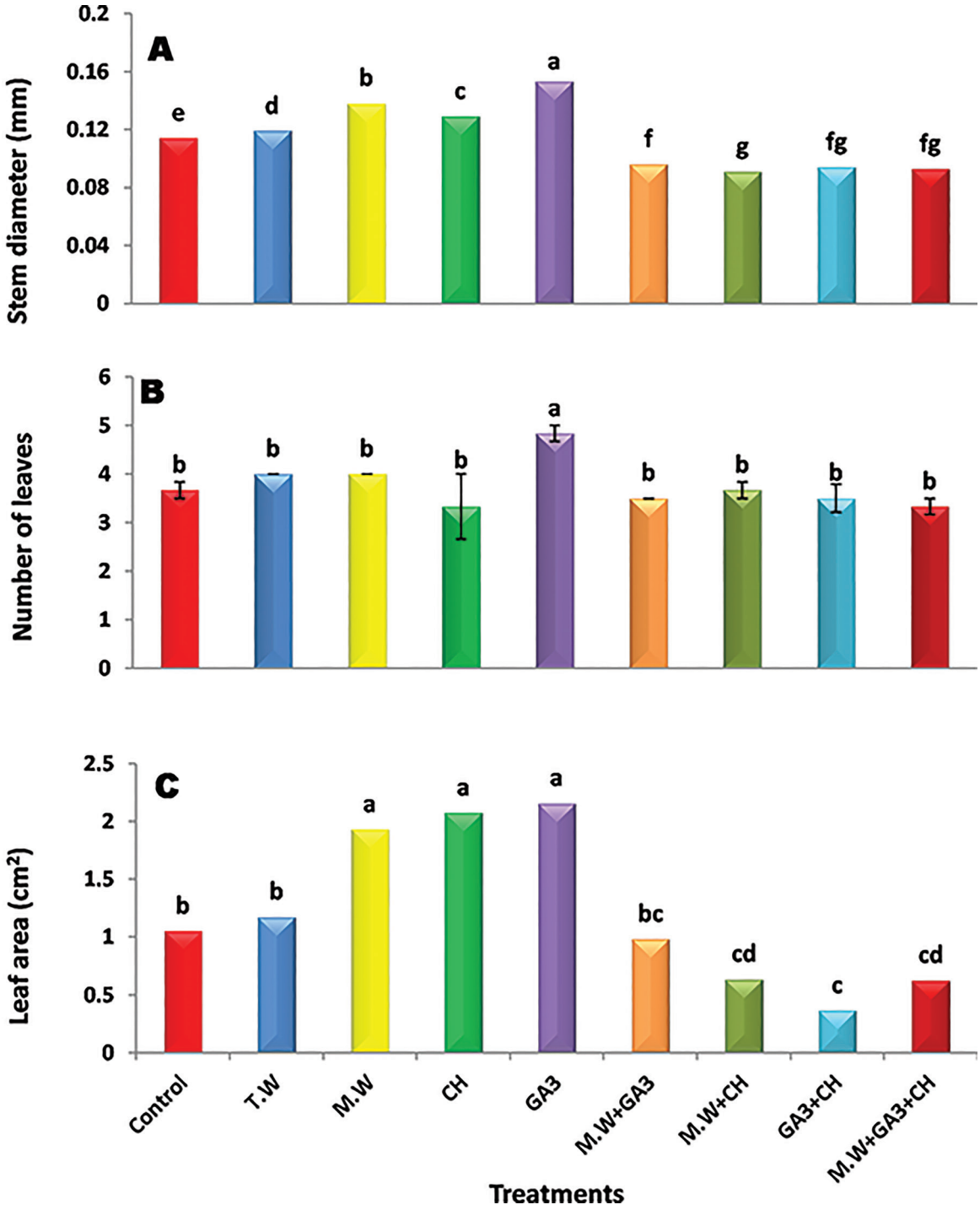

Figure 7. Effect of papaya seed pre-sowing treatments on stem diameter (A), number of leaves (B) and leaf area (cm^2) (C)**.** Data are means of two seasons (2022/2023 and 2023/2024) and three replicates ($n = 3$) each season. The mean values ± SE of each parameter followed by different alphabetical letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan.

solution for 24 hr was found to be the best treatment almost for all the investigated cultivars. Ramteke et al. (2015) also reported that the 'Coorg Honey Dew' papaya seeds submerged for 12 hr in a 200 ppm GA_3 solution showed the highest GP, seedling height, root length, number of leaves per seedling and leaf area in contrast to the 0 ppm and 100 ppm GA_3 treatments. The positive impact of GA_3 treatment on papaya seed germination and seedling growth parameters was also reported by other researchers (Desai et al., 2017;

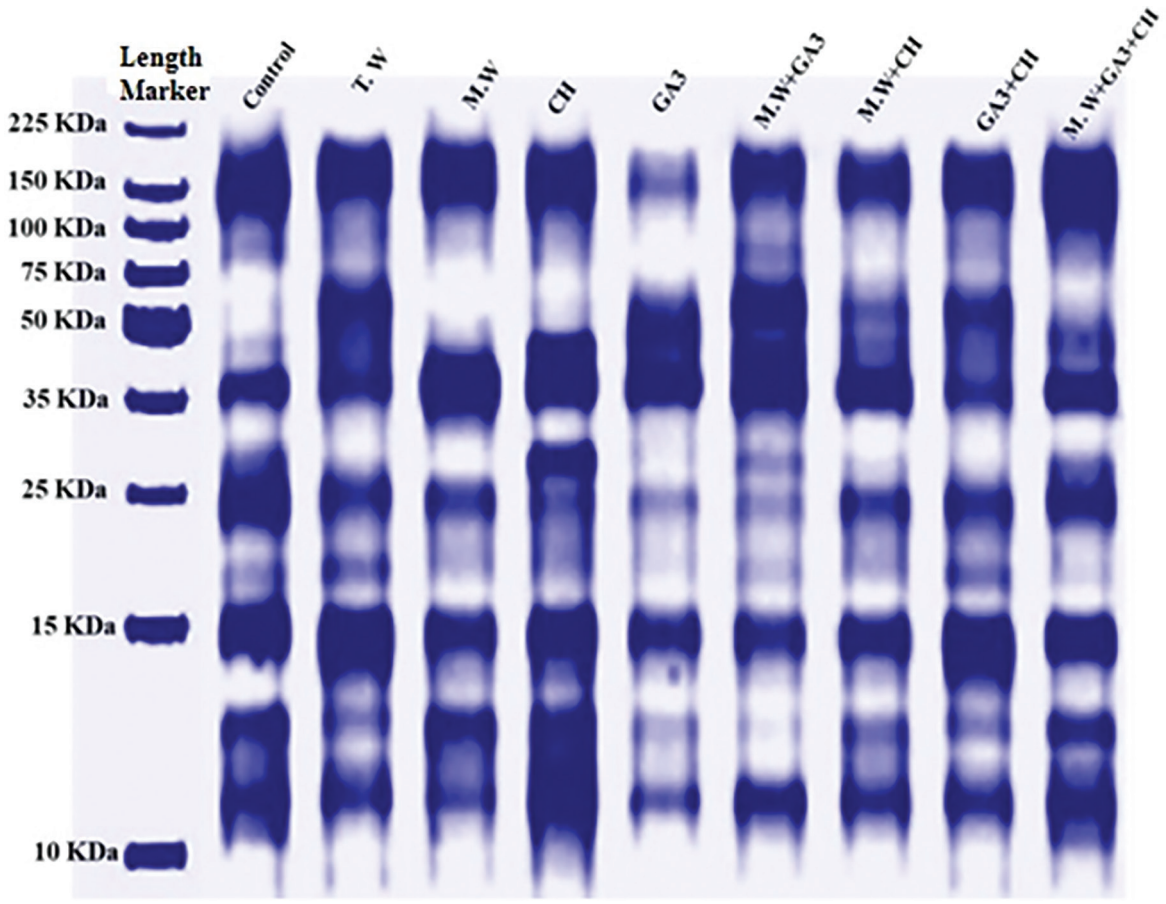

Figure 8. Electrophotograph generated by SDS-PAGE analysis of protein-banding pattern of untreated and submerged papaya seeds in magnetic water (2000 Gauss), M.W; chitosan (1.5%), CH and gibberellic acid (1000 ppm), GA_3 separately or in combination. M, marker; Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA_3, gibberellic acid (1000 ppm); M.W + GA_3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA_3 + CH, gibberellic acid + chitosan; M.W + GA_3 + CH, magnetic water + gibberellic acid + chitosan; K. Da, Kilo Dalton; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Hossain et al., 2023). GA_3 was also found to effectively improve seed germination and seedling growth of other crops (Mohamed et al., 2018; Dinesh and Padmapriya, 2022; Mohammed, 2023).

Phytohormone homeostasis is known to promote seed germination and seedling growth (Hartmann et al., 2014), though particularly GAs were found to play a significant role in seed germination and dormancy release by activating enzymes responsible for weakening of seed coverings making it easier for the radicle to emerge, initiating synthesis of proteases that hydrolyse proteins to amino acids and amylases that hydrolyse starch to sugar, thus facilitating mobilisation of endosperm storage reserves into the embryo axis, as well as stimulating cell expansion of the embryo (Karssen et al., 1989; Peng and Harberd, 2002; Finch-Savage and Leubener-Metzger, 2006; Hartmann et al., 2014).

Earlier studies documented similar results to ours showing that the exogenous application of GA_3 accelerated the germination rate of melon, flax, sesame and onion seeds, causing early germination and emergence of seedlings (Heikal et al., 1982; Edelstein et al., 1995). At similar work conducted by Desai et al. (2017), papaya seeds soaked for 12 hr in a 150 ppm GA_3 solution required the minimum number of days (8.33) for half of the investigated seeds to germinate. Dinesh and Padmapriya (2022) also reported the minimum duration for the germination of guava seeds with the 1000 ppm GA_3 treatment for 24 hr.

Numerous research investigations have examined the impact of MF on plants over a long time. One safe and reasonably priced physical pre-sowing treatment that can improve post-germination, plant development and yield to expose seeds to a MF (Flòrez et al., 2007). Plant traits such as seed germination, seedling growth rate, meristem cell proliferation and reproduction, and chlorophyll amounts were all impacted by MFs (Namba et al., 1995; Reina et al., 2001). The results of this study are corroborated by those of El-Sayed and Eata (2017) who found that seeds treated by magnetised water improved tomatoes, peppers, cucumbers and wheat seed germination and seedling emergence, and Ismail et al. (2020) who found that using magnetised water

Table 2. SDS-PAGE analysis of protein-banding patterns of untreated and submerged papaya seeds in tap water; T.W, magnetic water (2000 Gauss); M.W, chitosan (1.5%); CH, gibberellic acid (1000 ppm); GA_3 solutions separately or in combination; %P, % polymorphism.

No.	Molecular weight (kDa)	Control	Treatments								Band type
			T.W	M.W	CH	GA_3	MW + GA_3	GA_3 + CH	MW + CH	MW + GA_3 + CH	
1	200.20	1	1	0	0	0	0	0	0	0	P
2	178.12	1	0	1	1	1	1	1	1	1	P[NUM-2]
3	171.50	0	0	0	0	0	0	0	0	1	P[PUM-9]
4	151.72	1	1	1	1	1	1	1	1	1	M80
5	102.25	0	0	0	0	0	0	0	0	1	P[PUM-9]
6	65.42	0	1	0	0	0	1	0	0	0	P
7	56.02	0	1	0	0	0	1	1	1	0	P
8	50.85	0	0	0	0	1	0	0	0	0	P[PUM-5]
9	46.92	0	0	0	0	1	0	0	0	0	P[PUM-5]
10	43.73	1	0	0	1	0	0	0	0	1	P
11	38.87	0	0	1	1	1	1	0	0	0	P
12	36.64	0	1	1	1	1	0	1	0	0	P
13	35.60	1	0	0	0	0	1	1	1	1	P
14	33.42	0	0	1	0	0	0	0	0	0	P[PUM-3]
15	27.73	0	0	0	1	0	1	0	0	0	P
16	26.87	1	0	0	0	0	0	0	0	0	P[PUM-C]
17	25.20	1	0	0	0	0	0	0	0	0	P[PUM-C]
18	24.13	1	1	1	1	1	0	1	1	1	P[NUM-6]
19	23.22	0	0	0	0	0	1	0	0	1	P
20	19.46	0	0	0	0	1	0	0	0	0	P[PUM-5]
21	18.82	1	1	0	0	0	0	0	0	0	P
22	18.19	0	0	0	0	0	1	0	1	0	P
23	15.23	1	0	0	0	0	1	0	0	1	P
24	14.57	1	1	1	1	1	1	1	0	1	P[NUM-8]
25	14.16	0	0	0	0	0	0	0	1	0	P[PUM-8]
26	13.65	0	1	0	0	0	0	0	1	0	P

Continued

Table 2. Continued

No.	Molecular weight (kDa)	Control	Treatments								Band type
			T.W	M.W	CH	GA_3	MW + GA_3	GA_3 + CH	MW + CH	MW + GA_3 + CH	
27	11.54	1	1	1	1	1	0	1	1	1	P[NUM-6]
28	10.93	0	0	0	1	0	0	0	0	0	P[PUM-4]
29	10.58	0	0	0	1	0	0	0	0	0	P[PUM-4]
30	10.46	1	1	1	1	1	1	1	1	1	M
31	10.35	1	0	0	0	0	0	0	0	0	P[PUM-C]
32	10.24	0	0	0	0	0	0	0	0	1	P[PUM-9]
Total bands of each sample		14	11	9	12	11	12	9	10	13	Gel
P% in each sample		**85.71**	**81.81**	**77.78**	**83.33**	**81.82**	**83.33**	**77.78**	**80.00**	**84.62**	polymorphism % = 93.75%

M, monomorphic band; NUM, negative unique marker; P, polymorphic band; PUM, positive unique marker; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

improved the germination of *Pinus tropicalis* seeds. Hormones and enzymes contributing to germination and mobilisation of nutrients may be activated by seeds soaked in magnetised water prior to seed sowing.

In this research, soaking papaya seeds in a 1000 ppm GA_3 solution for 24 hr was found to significantly increase the antioxidant activity (DPPH) and flavonoid content in the investigated seeds compared to the non-treated ones. Several studies documented a positive effect of GA_3 treatments, stimulating the antioxidant enzymes activities and enhancing the whole antioxidant defence system of some plants, especially under stress conditions (Tuna et al., 2008; Siddiqui et al., 2011; Sardoei et al., 2014; Hu et al., 2018; Kaur et al., 2023). While in another study conducted by Ahmad et al. (2021) on pea seeds, GA_3 treatment (soaking in a 0.5 mM GA_3 solution for 6 hr before sowing) did not improve the pea seedling content of total phenolics and total flavonoids, as well as the percentage of DPPH inhibition (antioxidant activity). In our study, the M.W treatment has caused an increase in the antioxidant activity (DPPH inhibition) over the control. Similarly, Bhardwaj et al. (2012) reported a positive effect of MF treatment on cucumber seeds regarding antioxidant and enzyme activity. MFs possess an antioxidant activity via producing oxygen anion molecule ($OH^- + OH^- \rightarrow H_2O + O^-$) which can stop the free radical cycle and this may explain the changes in antioxidant activity (Kronenberg, 1985; Saikia and Upadhyaya, 2011).

The α-amylase enzyme activity and RSs in this research were found to be significantly higher in papaya seeds treated with GA_3 and M.W treatments compared to the control and the rest of treatments. The effectiveness of GA_3 seed treatment increasing total amylase activity and total soluble solid content in mug bean seedlings was also reported by Kaur et al. (2023). GAs are transferred to the aleurone layer and release α-amylase when seeds germinate. Exogenous GA_3 stimulates the synthesis and activation of seed protease and amylase, enhancing seed germination capacity (Jones and Carbonell, 1984). During seed germination, amylase in the seeds is essential for hydrolysing the starch found in the endosperm and turning it into soluble sugars, which provides energy for the seed germination and root and shoot development (Kaneko et al., 2002).

A change in enzyme activity in MF-treated seeds was reported by Rácuciu et al. (2008). Applying magnetic treatment may aid seed lots with poor GPs to perform better and if it could mitigate the negative effects of low temperature during germination. The increased activity of α-amylase can explain the rise in germination speed in magnetically treated seeds compared with unexposed controls. Wheat seeds treated at 150 mT and 100 mT showed significantly increased α-amylase activity than the controls according to Bhardwaj et al.'s (2012) observations. Reddy et al. (2012) reported that after 45 min of treatment, they found that *green gram* seeds α-amylase activity was considerably increased

Table 3. Number of DS and S bands recorded by separate evaluation of protein profiles of treated and untreated papaya seeds and their genetic similarity indices as computed according to Dice coefficient.

Treatments	Control	T. W	M.W	CH	GA_3	M.W + GA_3	GA_3 + CH	M.W + CH	M.W + GA_3 + CH
Control	$100\%^{0DS, 32S}$	$80\%^{11DS, 21S}$	$80\%^{11DS, 21S}$	$77\%^{12DS, 20S}$	$75\%^{13DS, 19S}$	$72\%^{14DS, 18S}$	$84\%^{9DS, 23S}$	$77\%^{12DS, 20S}$	$84\%^{9DS, 23S}$
T.W		$100\%^{0DS, 32S}$	$86\%^{8DS, 24S}$	$79\%^{11DS, 21S}$	$81\%^{10DS, 22S}$	$75\%^{13DS, 19S}$	$90\%^{26DS, 6S}$	$84\%^{9DS, 23S}$	$75\%^{13DS, 19S}$
M.W			$100\%^{0DS, 32S}$	$92\%^{5DS, 27S}$	$93\%^{4DS, 28S}$	$80\%^{11DS, 21S}$	$93\%^{4DS, 28S}$	$84\%^{9DS, 23S}$	$82\%^{10DS, 22S}$
CH				$100\%^{0DS,32S}$	$88\%^{7DS, 25S}$	$77\%^{12DS, 20S}$	$88\%^{7DS, 25S}$	$77\%^{12DS, 20S}$	$79\%^{11DS, 21S}$
GA_3					$100\%^{0DS, 32S}$	$75\%^{13DS, 19S}$	$90\%^{6DS, 26S}$	$79\%^{11DS, 21S}$	$77\%^{12DS, 20S}$
M.W + GA_3						$100\%^{0DS, 32S}$	$84\%^{9DS, 23S}$	$86\%^{8DS, 24S}$	$79\%^{11DS, 21S}$
GA_3 + CH							$100\%^{0DS, 32S}$	$92\%^{5DS, 27S}$	$86\%^{8DS, 24S}$
M.W + CH								$100\%^{0DS, 32S}$	$80\%^{11DS, 21S}$
M.W + GA_3 + CH									$100\%^{0DS, 32S}$

DS, dissimilar; S, similar; T.W. magnetic water (2000 Gauss); M.W, chitosan (1.5%); CH, gibberellic acid (1000 ppm); GA_3, solutions separately or in combination.

compared to the controls. Furthermore, numerous investigations have discovered proof in favour of the MF stimulation of α-amylase, which led to a steady drop in the levels of starch and oligosaccharides and a rise in the levels of RS in the early stages (48–96 hr) of seed germination (Paul et al., 1970).

Amino acids were expressed in seeds treated with GA_3, CH and M.W separately or in combination with proline content. Among the skeletal building blocks of proteins, amino acids, in addition to their involvement in the synthesis of the various proteins necessary for seed germination, also take part in various metabolic pathways such as those leading to the production of carbohydrates, nucleotides, hormones (ethylene, abscisic acid and GAs) and secondary metabolites (Guo et al., 2021). Plant glycolytic pathways go through pyruvate, and the synthesis of pyruvate is primarily due to the so-called 'phosphoenolpyruvate-pyruvate shunt'. The main provider of phosphoenolpyruvate is the product of the general amino acid deamination. Hence, the free amino acid pool plays a key role in the establishment of metabolic pathways connected to the energy carriers and also those related to the synthesis of sugars (Yang et al., 2020).

The GA_3 had the greatest effect on growth parameters such as seedling height, number of roots, root length, stem diameter, number of leaves and leaf area, followed by a significant effect by magnetised water. The enhancement in papaya seedling length and root length with GA_3 treatment might have transpired due to improved osmotic uptake of nutrients by this hormone which caused elongation of cells in the cambium tissue of internodal region (Miceli et al., 2019). The maximum stem diameter could be because of cell division and cell elongation promotion in the collar region by GA_3. The results are in agreement with Gupta and Corpas (2022). The increase in the number of leaves might be due to the greatest height of seedlings under GA_3 (1000 ppm) and may be due to the affected cell division and cell growth by the activity of GA_3 to the shoot apex which increases the new leaves, this obliges in the stimulation of the plant's physiological function and chemical stimulatory action which forms new leaves at a faster rate as suggested by Dilip et al. (2017).

The maximum leaf area might be due to increase in leaf length and width, which finally improves the leaf area of the plant. Current results are reinforced by Schrader et al. (2021). Magnetised irrigation water showed enhanced *Vicia faba* shoot length by 2.42% above the untreated tap water (Hussain and Abo Al-Saoud, 2024). Also, chickpea (*Cicer arietinum* L.) seedlings treated with magnetic irrigation water were 2.67 cm taller than the length of the seedlings irrigated with untreated tap water following the application of the treatment, 18 days after sowing (Sarraf et al., 2020).

Proteins are considered primary gene products of active structural genes; their size and amino acids

sequence are the direct results of nucleotide sequences of the genes. Therefore, any detected variation in protein systems is considered a reflection for genetic variations (Mohammed et al., 2021). Various exogenous substances can interact with a variety of different plant cellular components as proteins and DNA altering protein expression (Hossain et al., 2015). Electrophoresis of proteins by SDS-PAGE can be economically used to assess genetic variation (Megbowon, 2020). It is a well-established technique to measure differential gene expression after treatment with substances (Wang and Tang, 2021). Some studies used SDS-PAGE for the detection of protein profile alterations occurring during exposure to CH (Moradkhani and Jabbari, 2023), M.W (Elbeshehy and Almaghrabi, 2013) and GA_3 (Mahesh et al., 2023).

In this study, the electrophoretic papaya seed protein profile alternations revealed the ability of the different treatments to alter the gene expression in exposed plants. The protein profiles showed quantitative variations among the samples investigated as the appearance of new bands and disappearance of novel ones in comparison with that of the control plants. This could be due to mutational events at the regulatory genes that either activate transcription or suppress unexpected genes, respectively (Wang and Tang, 2021). Similar results were obtained by other authors (Salehi et al., 2021; Yang et al., 2021).

The SDS-PAGE succeeded to uncover the genetic variations among papaya seeds under different single treatments (M.W, CH and GA_3) by 71.43% or mixed treatments by 80.95% with net polymorphism equals 93.75%. Thirty-two protein bands (10.24–200.20 kDa) were identified, and only two were monomorphic (10.46 kDa and 151.72 kDa). The most observable changes in the SDS-PAGE patterns were the developed new bands and missing of others in relation to certain treatment. The distinction protein polymorphisms shown between treated papaya seeds in this study may result from insertions or deletions between mutated sites of protein bands and could be used as biomarkers for identification of the extent of harmful effect of the treatments if compared to control. The results were in accordance to those of Anju et al. (2015) who detected diminished polypeptide bands in papaya seed protein profiles and appearance of new ones in response to cadmium stress.

Genetic similarity index entitles a great relationship between the control and M.W + CH and M.W + GA_3 + CH treated papaya seed protein profiles sharing 23 similar protein bands which stabilise genome by higher percentage rather than other treatments; however, such genomic-DNA template stability does not ensure increased growth characteristics. Great similarity index (93%) was recorded in response to M.W and GA_3 treatment, both sharing 28 bands of 32 total bands which might trigger the DNA molecule to synthesise certain molecules that aid in plant growth as RSs, and this observation could be correlated to greatest growth characteristics developed by such treatments. Great genetic difference existed between control and M.W + GA_3 treated papaya seed protein profile sharing only 14 protein bands.

Genomic-DNA template stability was used to reflect changes in SDS profiles and to compare these changes with modification in papaya growth parameters and phytochemical characteristics. GTS% is related to the level of DNA damage, the efficiency of DNA repair and replication. The GTS% for untreated plant seedlings was defined as 100%, and there were significant decreases in the GTS of all treated plants. The obtained result indicated that the most negative significant changes in GTS% were induced by M.W (2000 Gauss) + GA_3 (1000 ppm) treatment. The highest GTS% was conserved by mixed treatments M.W (2000 Gauss) + CH (1.5%) and M.W (2000 Gauss) + GA_3 (1000 ppm) + CH (1.5%) followed by singular treatments; GTS% values of T.W and M.W (2000 Gauss) treatments were found to be 35.71% and 21.43%, respectively.

In comparison to control, T.W induced significant alteration in the gene expression and lowered the GTS% to 21.43%, and the same percentage was developed by M.W treatment, in which three bands were developed and eight protein bands disappeared. This result was in accordance with Hozayn and Abdul Qados (2010) who found that the M.W treatment of wheat cultivars showed an increase in the number of protein bands with accompanied increased growth parameters and total indole acetic acid in treated plants. Balouchi et al. (2007) confirmed that MF is known as an environmental factor which affects gene expression; therefore, by augmentation of biological processes that involve free radicals by stimulating the activity of proteins and enzymes, it influences the structures of cell membrane and increases the permeability and ion transport, which then affects some metabolic pathways. Single treatment by CH caused a decreased value of GTS%; it might be related to the fact that CH could create protein-CH conjugates that interact with histone protein and consequently affect the differential expression of many cellular proteins due to its gelling properties (Silva et al., 2014).

GA_3 treatment induced appearance of new five protein bands and disappearance of eight bands if compared to the control and this is in accordance with several reports indicating that GA treatment causes a significant increase in the rate of mRNA synthesis, GA_3, plant hormone, control plant growth via modulation of protein synthesis and mRNA levels. The relative rates of synthesis of many polypeptides were greatly altered in isolated barley aleurone layers treated with GA_3, increasing some, notably α-amylase, and decreasing others (Jones and Carbonell, 1984). Hence, in this study, GA_3 may exhibit a downregulated gene expression in the way to form new products that

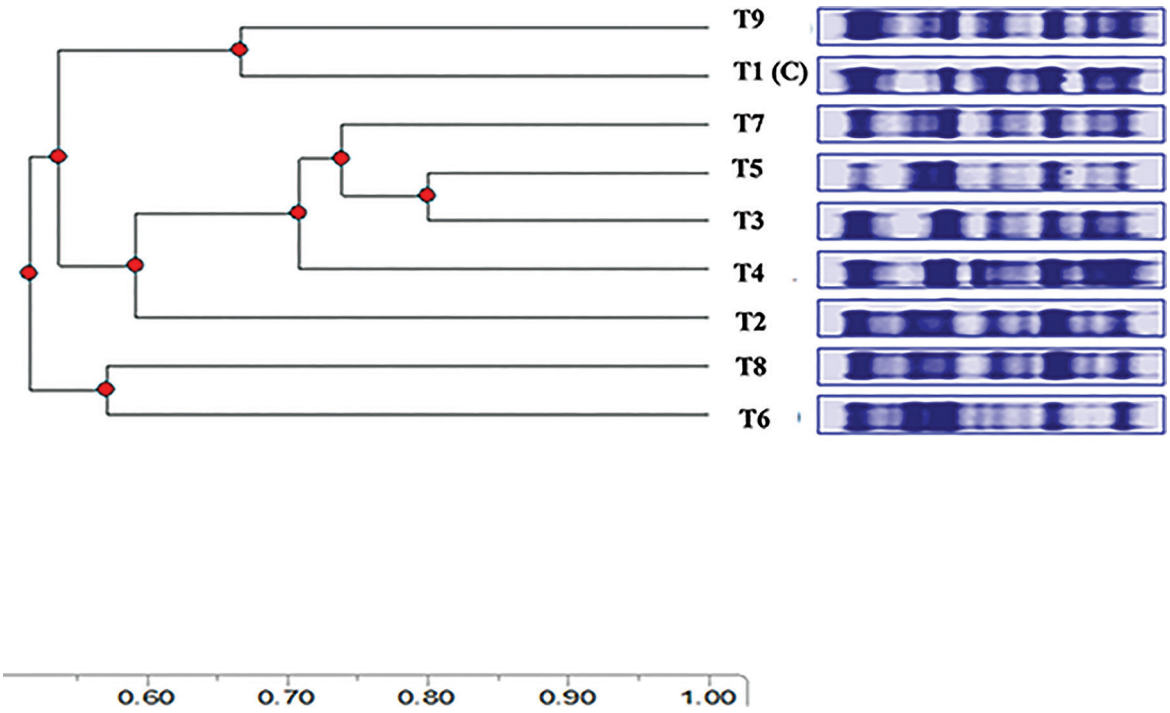

Figure 9. Dendrogram derived from separate evaluation of SDS-PAGE protein profiles of eight treated and untreated papaya seeds constructed data using UPGMA and similarity matrices computed according to Dice coefficient. T1 (C), Control (dry seeds); T2, tap water (T.W); T3, magnetic water (M.W, 2000 Gauss); T4, chitosan (CH, 1.5%); T5, gibberellic acid (GA3, 1000 ppm); T6, M.W (2000 Gauss) + GA3 (1000 ppm); T7, M.W (2000 Gauss) + CH (1.5%); T8, GA3 (1000 ppm) + CH (1.5%); T9, M.W (2000 Gauss) + GA3 (1000 ppm) + CH (1.5%). SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; UPGMA, unweighted pair-group method with arithmetic averages.

Table 4. GTS% based on changes in SDS-PAGE protein electrophoretic banding pattern of untreated and submerged papaya seeds in magnetic water (2000 Gauss), M.W; chitosan (1.5%), CH; gibberellic acid (1000 ppm), GA_3; separately or in combination. *a*, number of newly developed bands; *b*, number of diminished bands; *a* + *b*, polymorphic bands

Control total bands		Treatments															
		T.W		M.W		CH		GA_3		M.W + GA_3		M.W + CH		GA_3 + CH		M.W + GA_3 + CH	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
14		4	7	3	8	5	7	5	8	6	8	2	7	4	8	4	5
a + b		11		11		12		13		14		9		12		9	
GTS%	100	21.43		21.43		14.29		7.14		0.00		35.71		14.29		35.71	

GTS%, genome-template stability percentage; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

enhances plant growth and this might be the reason of increased T5 papaya growth parameters (1000 ppm GA_3 treatment) if compared to other treatment and the control.

The genomic-DNA is completely unstable in response to T6, M.W (2000 Gauss) + GA_3 (1000 ppm), treatment due to appearance of new six bands and disappearance of eight bands. The existence of such specific protein bands in treated papaya seeds might be explained based on the potentiality of T6 to trigger the expression of specific genes along DNA molecule. A process appears to play a key role in regulating a cascade of biochemical reactions which consequently might determine the ultimate appearance growth pattern of the produced plants via alteration of DNA-binding protein receptors mechanism which might amplify the signal-transduction pathway (Hooley et al., 1990).

It was obvious that the addition of CH to any treatment enhances the plant genome template stability; M.W (2000 Gauss) + CH (1.5%) exhibited 35.71% as derived from protein profile of its treated plant while M.W alone exhibited 21.34%. Also, its addition doubles the stability of plant genome as seen between GA_3 (1000 ppm) and GA_3 (1000 ppm) + CH (1.5%) treatments. Finally, it was also observed that CH addition to T6, M.W (2000 Gauss) + GA_3 (1000 ppm), results in a

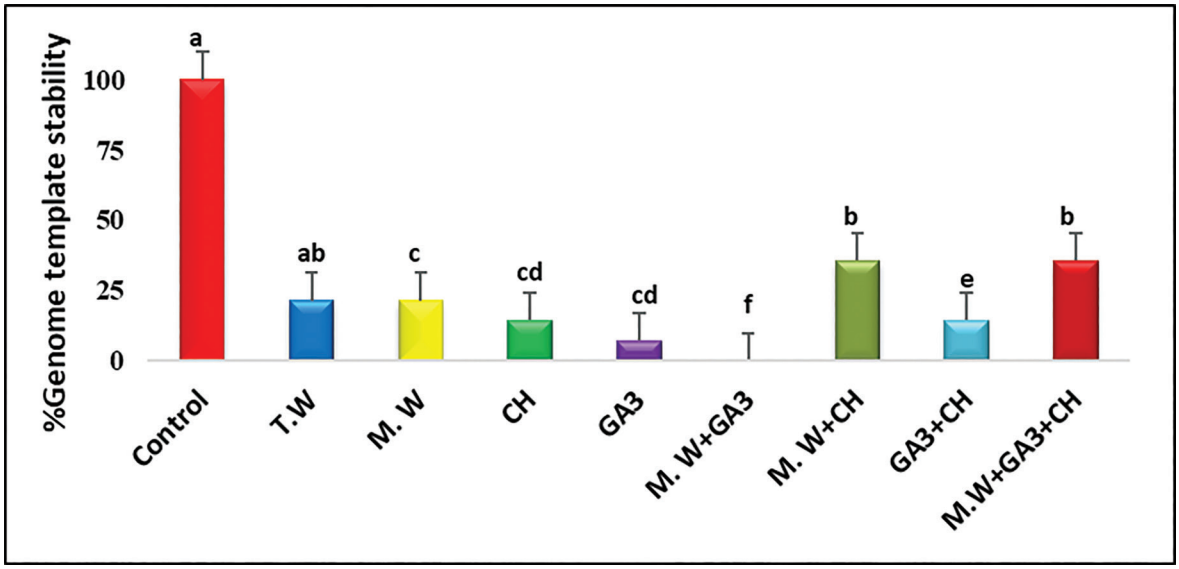

Figure 10. GTS% based on changes in SDS-PAGE protein electrophoretic banding pattern of untreated and pre-sowed papaya seeds in magnetic water (2000 Gauss), M.W; chitosan (1.5%), CH and gibberellic acid (1000 ppm), GA3 separately or in combination. Vertical bars represent GTS% ± standard error. Control, dry seeds; T.W, tap water; M.W, magnetic water (2000 Gauss); CH, chitosan (1.5%); GA3, gibberellic acid (1000 ppm); M.W + GA3, magnetic water + gibberellic acid; M.W + CH, magnetic water + chitosan; GA3 + CH, gibberellic acid + chitosan; M.W + GA3 + CH, magnetic water + gibberellic acid + chitosan. GTS%, genome template stability percentage. SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

complete enhancement to plant genome stability in T9, M.W (2000 Gauss) + GA_3 (1000 ppm) + CH (1.5%). CH induces plant defence mechanisms by indirectly enhancing secondary metabolite synthesis and interacts with cellular chitin synthase activating various genes such as genes of the reactive oxygen species pathway (Singh et al., 2019). Hence, CH addition may upregulate gene expression towards formation of antioxidants and secondary metabolites rather than formation of primary metabolites aid in increasing plant growth parameters, and it might be the reason for reduced growth parameters in mixed treatments.

Low positive correlations were observed between GTS% induced by the treatments and the assessed antioxidants; however, there is negative correlation between GTS% and biosynthetic α-amylase and RSs. Hence, it is thought that the mixed treatments with higher values of GTS% might direct its metabolic pathway to form antioxidants that ameliorate the stress exerted from its heavy components rather than formation of the main compounds; amylases and RSs enhancing plant growth could be the reasons of increased GTS% coupled with noticeable decreased papaya germination parameters and vegetative growth parameters and healthiness in response to mixed treatments. This result was in accordance with Hong et al. (2022) who revealed that upregulated proteins are usually those related to antioxidant and stress tolerance, whereas downregulated proteins are those usually responsible for cell proliferation and division.

Apparently, protein electrophoresis is a powerful biochemical method to assess the genetic stability among different treatments. Several studies revealed the reliability of this technique in identification of the genetic variations in date palm and harmful effects developed in plants among many other plants (Pardhe, 2021; Sun et al., 2021; Roy et al., 2022). Also, a high significant positive correlation coefficient between GTS% and phosphorous ion content was obvious and it might be due to adverse effects caused by reacting with phosphate groups of adenosine- diphosphate (ADP) or triphosphate (ATP) and by replacing essential ions. Harmful effects could be summarised by causing phytotoxicity by changing cell membrane permeability or by reacting with active groups of various enzymes regulating plant metabolism progression (Santos et al., 2022).

CONCLUSIONS

From the findings of this study, it could be concluded that papaya seed treatments either with submersion in a 1000 ppm GA_3 solution or M.W (2000 Gauss) for 24 hr before sowing could significantly improve seed germination and development in contrast to the untreated seeds, while submerging in the 1.5% CH solution or the mixed forms of the investigated concentrations either had no significant effect or negatively affected the germination process and seedling growth parameters. Also, it was revealed that genomic-DNA template stability percentages deduced from electrophoretic protein-banding patterns of papaya seeds were lowered in all treatments either separate or in combination and this could be due to the trigger of specific gene

Table 5. Genomic-DNA template stability (GTS) and correlated % growth parameters and phytochemical changes among untreated and submerged papaya seeds.

GTS-Growth parameters											
	GTS%	Seedling height	SVI	Stem diameter	Number of leaves	Root length	Leaf area	Root number	% Germination	CV	
GTS%	1.00	–0.17	–0.09	–0.16	–0.13	–0.10	–0.09	0.22	0.10	–0.05	
GTS-Phytochemicals											
	GTS	DPPH		α-Amylase	Proline	RSs	Total phenolics	Flavonoids	Phosphorous		
GTS%	1.00	0.29		–0.15	0.29	–0.11	0.22	0.10	0.38*		

CV, coefficient of velocity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GTS%, genome template stability percentage; RSs, Reducing sugars; SVI, seedling vigour index.

expression that aid in formation of antioxidants and also in increasing seedling health and performance. However, it is suggested that the addition of biopolymer CH to mixed treatment (GA_3 + M.W) ameliorates its harmful effects on genomic-DNA stability percentage. The obtained results are considered applicable and can be easily used by agricultural producers in nurseries to break the dormancy of papaya seeds and enable them to produce seedlings throughout the year. In addition, the materials used, whether magnetised water, CH or gibberellic, are safe, environmentally friendly and inexpensive. However, this result deserves further study.

ACKNOWLEDGEMENTS

I would like to thank the Department of Horticulture—Faculty of Agriculture—Damietta University for providing their laboratory and their moral support to us. Also, I would like to thank the management of the research farm at the Faculty of Agriculture, Mansoura University, for providing the place to conduct the research experiment. The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-109).

FUNDING

This research was funded by Taif University, Saudi Arabia, Project No. TU-DSPP-2024-109.

AUTHOR CONTRIBUTIONS

M.S.A.—methodology, formal analysis, investigation, software, data curation, writing—original draft, writing—review & editing, validation, visualisation. E.A.E—investigation, validation, visualisation, data uration, methodology. A.M.A.A.—resources, data curation, editing, visualisation, formal editing, biochemical analysis. E.A.E., H.G.A.E., S.I.—investigation, software, data curation, methodology, validation. M.A., B.S.A.—investigation, software, review. L.M.A.—chemical analysis, editing and visualisation. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received: November 21, 2024; accepted: January 21, 2025