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Methyl jasmonate improves rubber production and quality in *Lactuca Serriola*

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The increase in demand for natural rubber has led to the search for alternative sources. *Lactuca serriola* is emerging as a promising candidate, as the quality of the natural rubber it produces is comparable to that of the Pará Rubber Plant, *Hevea brasiliensis*. This study examines the effect of methyl jasmonate (MeJA), a known elicitor, on the expression of key rubber biosynthesis pathway genes (*HMGR1*, *HMGS1*, *CPT2*, and *SRPP1*) in the latex of *L. serriola* plants. The expression levels of these genes increased significantly after the foliar application of 200 and 400 µM MeJA. The highest relative expression level for *HMGR1*, *HMGS1*, *CPT2* and *SRPP1* was 3.74, 18.56, 11.91and 16.59 fold respectively. Furthermore, the rubber content in *L. serriola* showed a significant rise post-treatment compared to the control with increasing the level of MeJA (6.19%, 7.24% and 7.85% which correspond to 0, 200 and 400 µM). Gel permeation chromatography revealed an augmentation in the molecular weight of extracted natural rubber from treated plants. Samples treated with 400 µM of MeJA had the highest molecular weight (1570 kg mol^{-1}) compared to control (1186 kg mol^{-1}). This study has demonstrated that MeJA, through the regulation of rubber biosynthesis genes, is capable of enhancing the quality and quantity of natural rubber extracted from alternative sources, such as *L. serriola*.

Keywords Cis-polyisoprene, *Lactuca serriola*, Natural rubber biosynthesis, Gene expression, Methyl Jasmonate, Rubber molecular weight

Natural rubber (NR) is sourced from latex, a unique cytoplasmic substance found in rubber-producing plants. Latex is synthesized within either the laticiferous cell network or individual parenchymal cells across an extensive range of approximately 12,500 plant species[1]. Scientifically referred to as cis-1,4-polyisoprene, NR is used in a diverse array of products including a variety of industrial and medical products[2]. It possesses exceptional qualities such as resilience, impact resistance, elasticity and efficient heat dispersion[3]. Because of these characteristics, NR is irreplaceable in various applications including medical devices and heavy-duty tires[4–7]. There are over 2500 known rubber-producing plants, however, the majority exhibit low rubber yields and inadequate molecular weight[8,9]. Currently, *Hevea brasiliensis*, is the primary source of commercially feasible NR[2,10], but its particular cultivation conditions and sensitivity to fungal infections make cultivation challenging.

With recent rapid economic growth, the demand for NR has surged, prompting search for alternative sources. In light of potential NR shortages and economic predictions, there is an immediate need for improvements in production[11]. This involves exploring possible rubber-producing species[2] and employing genetic engineering techniques[8]. One such source is *Lactuca serriola* L., commonly known as Prickly lettuce—a plant species identified as a potential source of NR[11]. This species belongs to the *Asteraceae* family and is widely distributed across Europe, Asia, and North America. Cornish et al.[12] demonstrated that *L. serriola* yields high molecular weight (Mw) rubber that is comparable to NR from Pará rubber trees. The plant is an annual that yields substantial biomass with concentrated rubber content and has a short growth cycle, which makes it easy to work with[11,13].

The NR molecule is a polymer composed of units of isoprene derived from isopentenyl diphosphate (IPP) in the cis- configuration. The surface of rubber particles within laticifer cells serves as the site for NR metabolism[14]. Laticifers are specialized tissues for biosynthesis and storage in most rubber producing plants[12].

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The process of rubber biosynthesis involves both the mevalonate (MVA) pathway, which converts pyruvic acid into isopentenyl diphosphate (IPP) and predominantly takes place within the cytosol, as well as the 2C-methyl-d-erythritol 4-phosphate (MEP) pathway, which transforms pyruvic acid and glycerate 3-phosphate into IPP and occurs in plastids[15,16]. IPP is a critical substrate for the production of various terpenes, including NR[17]. The MVA pathway is especially important, with key enzymes such as hydroxymethylglutaryl-CoA reductase (*HMGR*) and hydroxymethylglutaryl-CoA synthase (*HMGS*)[5] (Fig. 1).

The incorporation of IPP units into a prenyl chain is an essential step in the process, which requires initiators such as farnesyl pyrophosphate (FPP)[12]. The synthesis of NR involves the use of IPP precursors through the collaboration of various enzymes and proteins. These include farnesyl pyrophosphate synthase (*FPS*), cis-prenyltransferase (*CPT*), small rubber particle protein (*SRPP*), rubber elongation factor (*REF*), and other unidentified proteins working in synergy[18].

One approach to cultivating alternative rubber crops involves the identification of essential regulatory genes in the processes of rubber biosynthesis. The regulation of gene expression is achieved by utilizing elicitors such as jasmonates within the laticiferous tissues, where NR synthesis occurs[19]. Jasmonates and its variants such as methyl jasmonate (MeJA) are cellular regulators that originate from lipids and play a vital role in developmental processes and help plants respond to biotic and abiotic stressors[19]. They can trigger secondary metabolism by activating transcription[20].

Jasmonic acid (JA) plays a crucial role in encouraging the differentiation of laticiferous cells[12]. Transcriptional responses to JA, ethylene, and MeJA, were indicated in a cell line expressing laticifer-specific genes. Moreover, the induction of secondary laticifer differentiation, responsible for latex production in *H. brasiliensis*, is attributed to linolenic acid and JA[21,22]. In small-scale field trials this resulted in increased latex production[23]. The present study investigates the application of exogenous MeJA to *L. serriola* plants to induce secondary metabolism and understand its effects on the expression of rubber biosynthesis genes (*HMGR*, *HMGS*, *CPT* and *SRPP*). The objective is to assess changes in the expression of key rubber biosynthesis genes, quantifying and characterizing the rubber extracted. It is the first time that transcriptomics is being investigated in *L. serriola* with the plant specific designed primers.

Materials and methods

Plant materials and MeJA application

L. serriola seeds were gathered from Karaj, Iran (35°46′ N, 51°00′ E). To create working stock and eliminate environmental effects, a single seed from the collection was grown in the research greenhouse of the Iranian

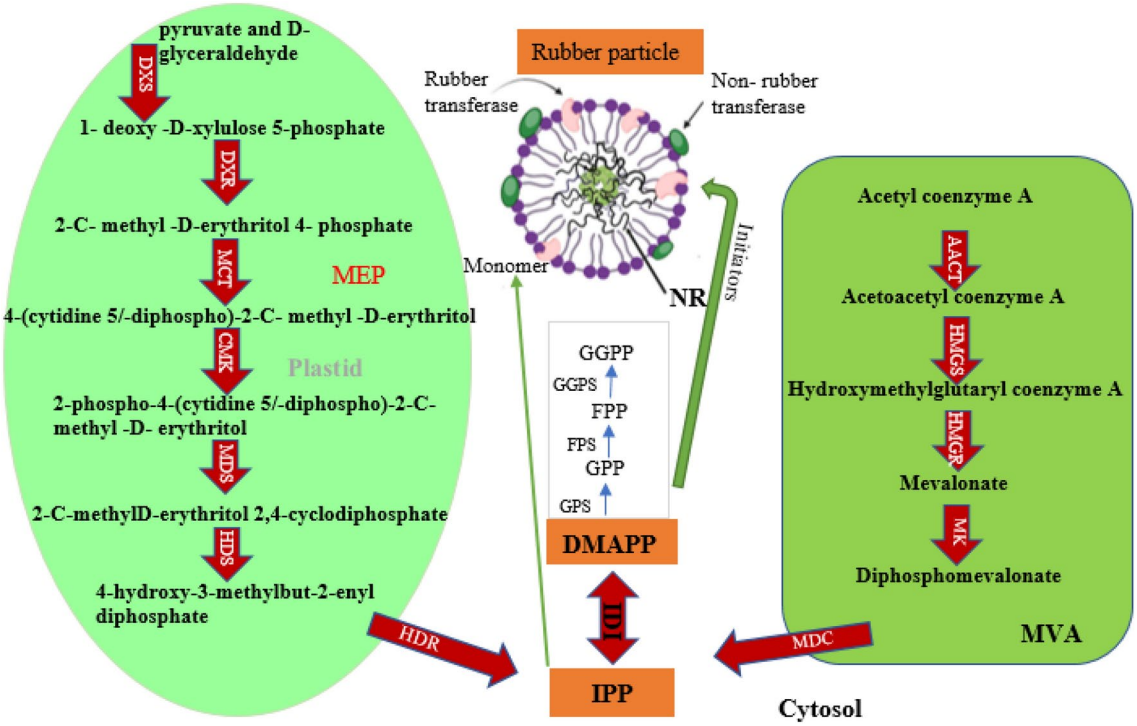

Fig. 1. The biosynthesis pathway of natural rubber (cis-1,4-polyisoprene). AACT: acetyl coenzyme A acetyltransferase; *HMGS*: hydroxymethylglutaryl coenzyme A synthase; *HMGR*: hydroxymethylglutaryl coenzyme A reductase; *MK*: mevalonate kinase; *MDC*: diphosphomevalonate decarboxylase; *DXS*: 1- deoxy -D-xylulose 5-phosphate synthase; *DXR*: 1- deoxy -D-xylulose 5-phosphate reductoisomerase; *MCT*: 2-C- methyl -D-erythritol 4-phosphate cytidylyltransferase; *CMK*: 4-(cytidine 5/-diphospho)-2-C- methyl -D-erythritol kinase; *MDS*: 2- C- methyl -D-erythritol 2,4-cyclodiphosphate synthase; *HDS*: 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; *HDR*: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase.

Biological Research Center. Plants were initiated by seeding the working stock collections in plastic pots filled with potting media (peat moss, coco peat and perlite) and maintained at temperatures of 26 °C during the day and 22 °C at night. Additional sodium vapor lighting was used to supplement natural light, providing a 14-h daytime photoperiod that matched the temperature profile. Plants were given sub-irrigation as required.

MeJA (Sigma-Aldrich) was prepared in a 0.1% (v/v) ethanol solution at three different concentrations (0, 200, and 400 µM) in 1000 mL volumetric flasks. The solution was applied to the plants approximately two weeks before the bolting stage using a sprayer until the runoff stage was reached (30 ml per plant). To ensure uniform application, the same number of sprayer cycles was used for each plant. The spraying process was repeated three times with three replicates, with one-week intervals, leading up to the latex harvest. Control plants were sprayed with distilled water containing 0.1% (v/v) ethanol[24]. The plants had an average height of approximately 150 cm, with stem diameters up to 12 mm. Latex was collected from the bolting stem of each individual in three replicates. For molecular analysis, latex was obtained at three separate time intervals following treatment: 6, 12, and 24 h in three biological replicates. In the assessment of rubber quality and quantity, latex sampling took place one week after the final MeJA application to examine the impact of MeJA concentration on rubber characteristics and the extracted latex was stored at − 20 °C for further analysis.

Latex sampling and rubber extraction

The proportions of water, resin, rubber, and insoluble material in the latex of each plant were assessed through gravimetric analysis[13]. Latex collection involved making 8 mm diagonal incisions on the stem using a razor, and the dripping latex was gathered in a pre-weighed microcentrifuge tube. The tube was then capped and re-weighed to determine the initial weight of the collected latex. The latex was dried for 48 h at 35 °C under vacuum to remove water. The rubber extraction was conducted exactly according to the method of Bell et al.[13].

Gel permeation/size exclusion chromatography

The rubber component of the latex was analyzed using gel permeation size exclusion chromatography (GPC/SEC) with refractive index detection to determine the length of polymer chains. The dried rubber fractions were dissolved in tetrahydrofuran at a concentration of approximately 5 mg mL^{-1} at room temperature and left overnight. The procedure was done according to the method of Ramirez-Cadavid et al.[25].

Fourier transform infrared analysis

For FTIR analysis, 15 to 20 mg of extracted *L. serriola* rubber was used. The functional groups of the samples were determined using KBr cells on an ALPHA II Bruker device. FTIR spectra were acquired in 16 scans over a range of 4000 to 400 cm^{-1} and a resolution of 4 cm^{-1}.

Bioinformatics and plant specific primer design

No sequences for the target genes in *L. serriola* were available, therefore, existing RNA sequences for designing our target plant-specific primers in databases were used. Paired-end short reads from total RNA-seq data of *L. serriola* were obtained from the NCBI-SRA (Sequence Read Archive). To ensure the accuracy of the transcriptome assembly, initial quality assessment of raw reads in all samples was performed using FastQC software (Version 0.11.9;[26]). Initially, substandard bases, reads, and potential adapter impurities were removed using trimmomatic software. This involved setting the minimum phred score (TRAILING) to 20 and the minimum length (MINLEN) to 50[27]. Following trimming, the resulting high-quality reads, denoted as "clean reads," served as the primary input sequences for transcript assembly.

The transcript assembly was carried out using Trinity software (version 2.8.5), employing the de Bruijns graph-based strategy, following the methodology described by[26]. Functional annotation of the transcriptome was conducted to unveil functionality using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UniProt (https://www.uniprot.org/). After selecting the best transcripts, primer designing was performed using Primer 3 software (version0.4.0). The designed primers were specific for the *HMGR1*, *HMGS1*, *CPT2*, and *SRPP1* genes. The sequences of primers utilized for qRT-PCR are listed in Table 1.

Real-time RT-PCR

For RNA isolation and transcription analysis approximately 100 mg of latex was mixed with 1 ml of TRIzol reagent (Invitrogen). The RNA extraction procedure was according to manufacturer guidelines. To eliminate genomic DNA contamination, all RNA samples underwent treatment with the RNase-Free DNase Set (Fermentas, Waltham, MA). The quality and quantity of RNA were assessed through agarose gel electrophoresis and nanodrop ND-1000, respectively.

For cDNA synthesis, 3–5 µg of purified total RNA was employed in accordance with the manufacturer's instructions using the Easy cDNA Synthesis Kit (Pars Tous, Iran). Plant-specific primer pairs were used for quantitative real-time polymerase chain reaction (RT-PCR). The RT-PCR analysis of NR biosynthesis genes' relative expression was conducted using SYBR green Master Mix 2X (Pars -Tous, Iran) on a QIAGEN real-time PCR system.

The RT-PCR cycling conditions were adjusted as follows: an initial denaturation at 95 °C for 10 min, followed by 40 three-step cycles of 95 °C for 20 s, 20 s at an annealing temperature ranging from 54 to 58 °C (based on the individual annealing temperature required for each primer pair), and an extension at 72 °C for 20 s. The tubulin gene served as the internal control. All reactions were conducted in three biological replicates and three technical replicates. The results are expressed as mean ± SE and the gene expression relative changes were quantified by the comparative CT method[28].

Target genes	Primer sequencing (sequence in 5'-3' direction)	Fragment size (bp)
CPT2	F: CCAAAACCGCCTGAACAAGA	153
	R: GAGTGACACCTGACCCTGAT	
HMGR1	F: TCCCCTCTATCCAGTTCACG	129
	R: TGGTGTCAAAGGGTGTTCAA	
HMGS1	F: ATGGCTCCTCAAAACGTCGG	248
	R: CTTCCTACTTCCAAGCGACCA	
SRPP1	F: AGTTGTCCTTCGCATACCCA	145
	R: GCCGAAAACGATGTTCCTGT	
Tubulin	F: CCATAAGTTTGATCTCATGTATGC	101
	R: CAAGGTCCTCACGAGCCT	

Table 1. Primer nucleotide sequences used in RT-PCR.

Statistical analysis

Statistical analysis was carried out using SAS 9.4. The research followed a factorial design based on a completely randomized setup with three replications. Mean comparisons were conducted using Fisher's least significant differences at either the 0.05 or 0.01 probability thresholds. Furthermore, the standard error was calculated to provide a measure of precision. Data correlation and cluster analysis (heatmap) was performed using R package to assess relationships between variables.

Results

Gene expression analysis

To assess the impact of MeJA treatment on gene expression within the rubber biosynthesis pathway, quantitative real-time (qRT-PCR) analysis was conducted. The relative expression levels of *HMGR1*, *HMGS1*, *CPT* 2 and *SRPP1* genes were analyzed at 6, 12 and 24 h after treatment with MeJA at three different concentration levels (0, 200 and 400 µM). Statistically significant differences were observed in the expression levels of all genes in response to the elicitor treatments (Fig. 2).

In *HMGR1,* after treatment with 400 µM MeJA the transcription value increased about 3.5-fold at 6 h, before dropping to around threefold at 12 h and 0.8-fold at 24 h. With 200 µM MeJA, the transcription value doubled at 12 h, but at 6 h and 24 h, no statistical increase was observed (Fig. 2A).

The *HMGS1* results demonstrated a substantial increase at all time points, with a particularly notable 12-fold rise at 12 h after exposure to 200 µM MeJA, compared to the control. At the highest MeJA concentration of 400 µM, the expression of *HMGS1* displayed a contrasting response, with a substantial increase at 6 h (10.9-fold) reaching its highest peak (18.6-fold) at 12 h post treatment (Fig. 2B).

For *CPT2* transcription results at 200 µM MeJA, a significant increase occurred, peaking at 4.1-fold at 12 h (Fig. 2C). For 400 µM MeJA, a significant raise was observed at 6 h post-treatment (11.9-fold), then dropping to 7.59-fold at 12 h but still significantly higher compared to the controls, and further declined at 24 h (0.84-fold).

At 200 µM MeJA, a striking increase in expression level of *SRPP1* was observed at 24 h (16.2-fold) and 6 h (7.8-fold), while it only moderately increased at 12 h (1.9-fold). At the 400 µM MeJA concentration, the expression pattern commenced with a significant increase of tenfold at 6 h, followed by another notable rise of threefold at 12 h, relative to the control. Additionally, a more moderate increase of twofold was observed at 24 h (Fig. 2D).

Effect of methyl jasmonate on rubber content and molecular weight

The analysis of rubber content and molecular weight provides insights into the influence of MeJA on rubber biosynthesis in *L. serriola*. Our findings reveal significant alterations in both rubber content and molecular weight, demonstrating the regulatory role of MeJA in rubber production and quality.

Impact of methyl jasmonate on rubber content

Three concentrations of MeJA (0 µM, 200 µM, and 400 µM) were administered to *L. serriola* plants to examine the impact of MeJA on the extracted rubber and other latex components. A substantial increase in rubber content was observed in treated plants compared to the control plants (Fig. 3). The rubber content in the control group was 6.2%, whereas the post-treatment rubber content for 200 µM and 400 µM was 7.2% and 7.8%, respectively. Treatment with MeJA resulted in a 14–16% increase in rubber yield, which was statistically significant ($p < 0.01$). Additionally, the acetone-soluble resin material of latex was also quantified in the treatment groups (Table 2). This change demonstrates the potential of MeJA to modulate the rubber biosynthesis pathway.

Influence of methyl jasmonate on molecular weight

The molecular weight (M_w) of treated and non-treated rubber from a mixture of three biological replicates were measured at the bolting stage. MeJA treatment had a distinct impact on the molecular weight of the synthesized rubber. Notably the M_w of rubber produced in the MeJA-treated plants exhibited a marked increase compared to the control group. The M_w of control group was 1186 kg/mol, while these figures for the treated plants with 200 µM and 400 µM were 1510 kg/mol and 1570 kg/mol, respectively (Table 2). The rubber samples investigated

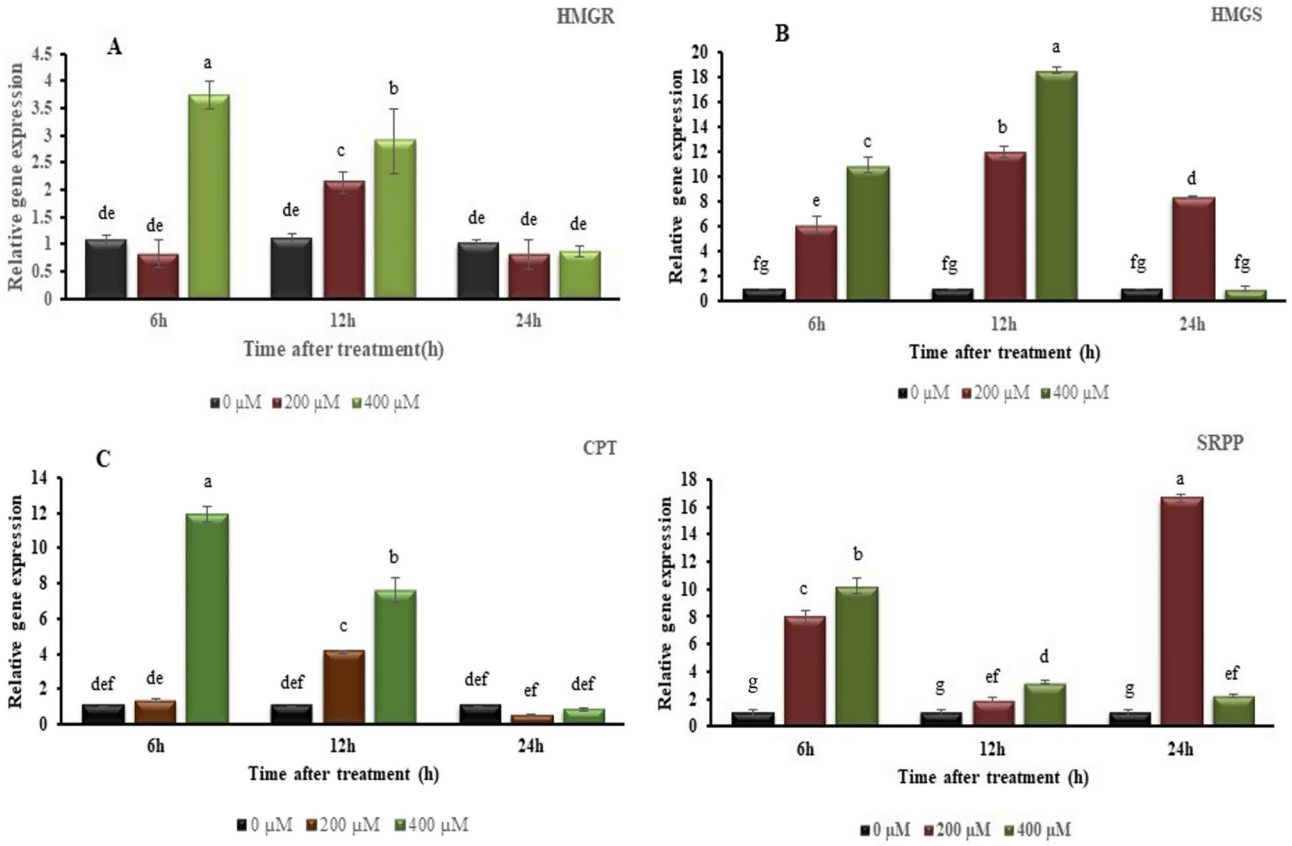

Fig. 2. Effect of different concentrations of methyl jasmonate at different exposure times on expression levels of (**A**) *HMGR1*, (**B**) *HMGS1*, (**C**) *CPT* 2 and (**D**) *SRPP1* genes in rubber biosynthesis pathway in *L. serriola*. Error bars are shown as ± SE (n = 3). Means followed by the same letter are not significantly different according to the Duncan's multiple range test at p = 0.01.

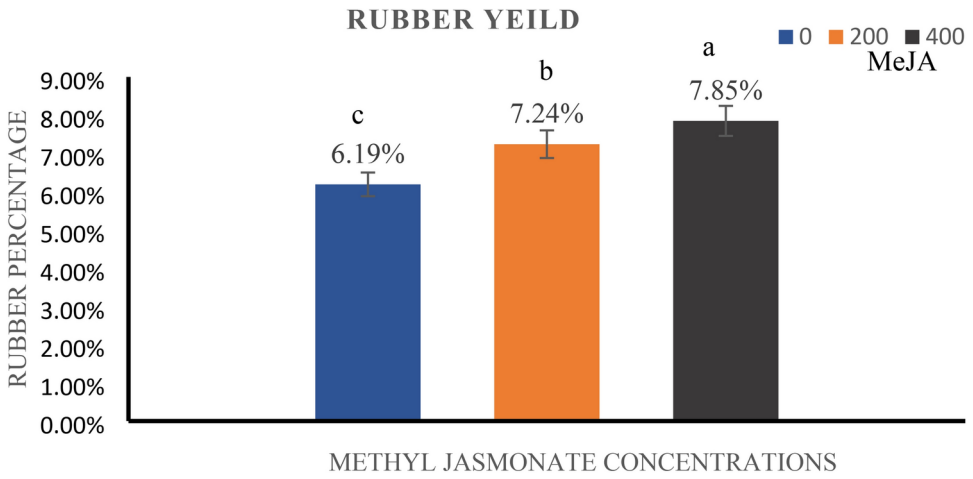

Fig. 3. Effect of different concentrations of methyl jasmonate on the extracted rubber content in *L. serriola*. Error bars are shown as ± SE (n = 3). Means followed by the same letter are not significantly different according to the Duncan's multiple range test at 0.01 probability level.

in this study had M_ws above 1000 kg/mol, a characteristic of a high-quality rubber. The polydispersity values were 1.34, 1.34, and 1.28 for the control, 200 µM treatment, and 400 µM treatment, respectively (Table 2; Fig. 4).

Samples	M_w (kg/mol)	M_w/M_n (PDI)	Latex Water (%wt/wt)	Latex Resin (%wt/wt)	Latex Insolubles (%wt/wt)
Control	1186	1.34	$41^{**} \pm 0.15$	$17.25^{**} \pm 0.20$	$35.5^{ns} \pm 0.18$
MeJA 200 µM	1510	1.34	$38.84^{**} \pm 0.16$	$18.13^{**} \pm 0.15$	$35.79^{ns} \pm 0.39$
MeJA 400 µM	1570	1.28	$37.6^{**} \pm 0.24$	$18.78^{*} \pm 0.10$	$35.77^{ns} \pm 0.18$

Table 2. Effect of different concentrations of methyl jasmonate on some attributes of latex and its extracted rubber in *L. serriola*. ** and * are significant at $P < 0.01$ and $P < 0.05$ respectively. ns is not significant. n = 3.

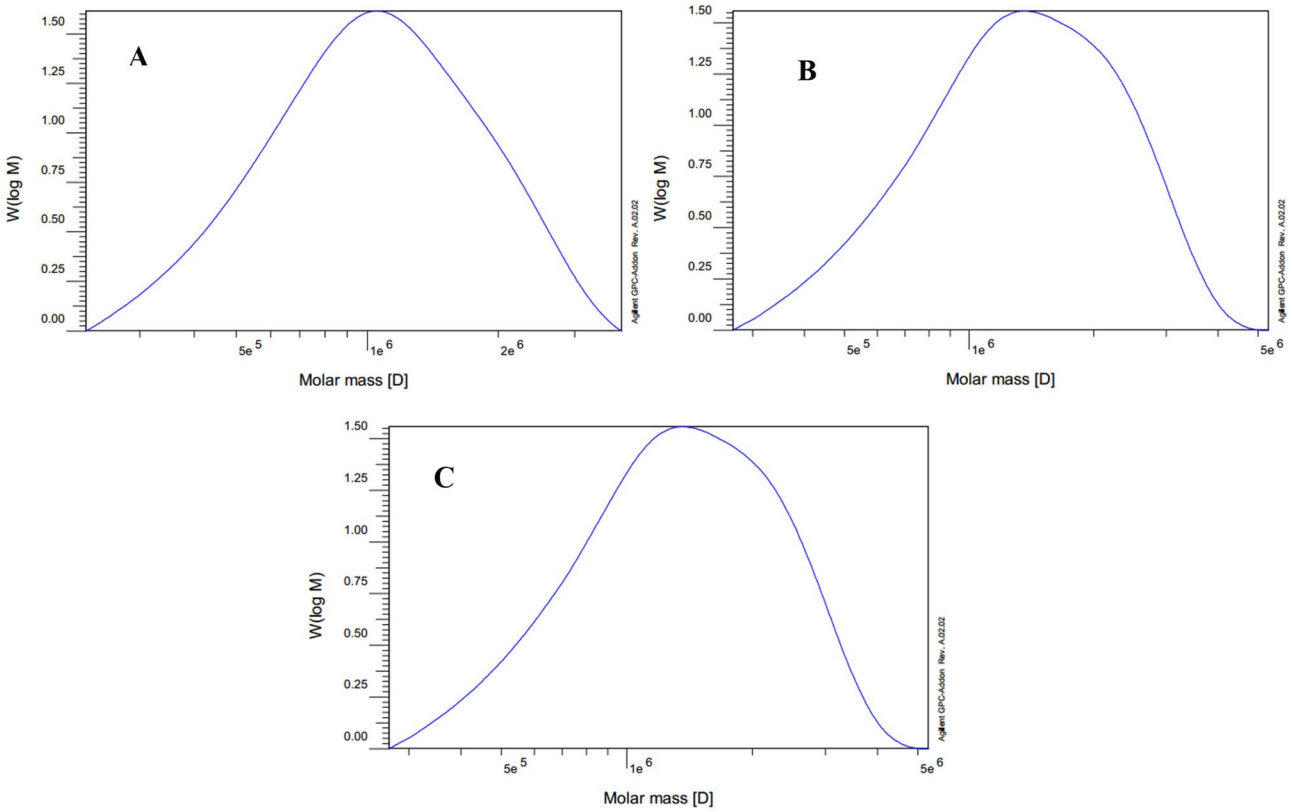

Fig. 4. GPC/SEC chromatograms of extracted rubber from latex of methyl jasmonate treated *L. serriola*. (**A**) control plants, (**B**) 200 µM of MeJA, (**C**) 400 µM of MeJA.

FTIR analysis

The FTIR analysis of NR extracted from *L. serriola* showed distinctive chemical structures spanning the range of 500–4000 cm^{-1}. Notably, distinct peaks were observed at the wavelengths 2963, 2924, and 2853 cm^{-1} (Fig. 5). Additionally, a prominent sharp peak at 1730 cm^{-1} and a characteristic polyisoprene peak at 860 cm^{-1} were evident in the infrared spectrum of NR. These apparent peaks are associated with specific compounds as shown in Fig. 5.

Mutual correlations among rubber biosynthesis gene expression levels and rubber yield in *L. serriola*

To study potential correlations and cluster analysis between the expression profile of studied genes (*HMGR1*, *HMGS1*, *CPT2*, and *SRPP1*) and rubber yield, heatmap and correlation analysis were conducted. The findings suggest that treatment with MeJA led to a rise in the expression levels of the examined genes and an increase in rubber yield. These characteristics exhibited positive correlations with each other, with the exception of yield and *SRPP1* at specific MeJA concentrations (Fig. 6). Cluster analysis identified that *HMGS1* and yield were grouped together in one cluster, followed by *SRPP1* in a subsequent level of classification. Furthermore, *HMGR1* showed correlation with *CPT2* in the subsequent tier of classification. Moreover, MeJA treatments at different levels (200 µM and 400 µM) were clustered together.

The correlation analysis (Fig. 7) aimed to determine whether these genes collaborate to co-regulate rubber synthesis and accumulation. The temporal gene expression levels of *HMGR1*, HMGS*1* and *CPT2* exhibited highly significant positive associations. *SRPP1* did not show any correlation with *HMGR1* and *CPT2*.

The HMGR1, *HMGS1*, and CPT2 expression levels all showed a strong correlation with rubber yield. However, a weaker correlation was observed between *SRPP1* and rubber yield.

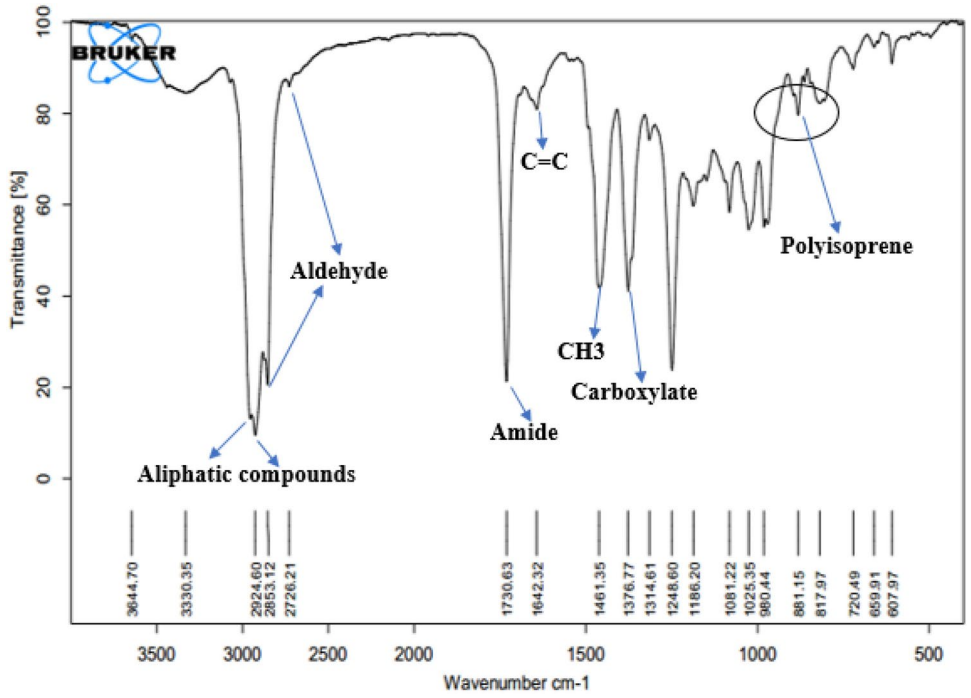

Fig. 5. FTIR spectrum of cis-1,4-polyisporene derived from of *L. serriola*.

Discussion

The rubber biosynthesis pathway, a complex network of biochemical processes crucial for NR production in rubber-producing plant species, was the focal point of this study. The impact of MeJA on the expression of four rubber biosynthesis genes—*HMGR1*, *HMGS1*, *CPT2*, and *SRPP1*—in *L. serriola* and effect on rubber yield and quality were investigated.

When plants are treated with exogenous JA or MeJA, they stimulate their own synthesis by metabolizing α-linolenic acid (α-LeA). The internal JA serves as the substrate, undergoing a transformation through a process which activates the pathway of JA signaling, consequently, the regulation in expression of JA-responsive genes and also downstream genes linked to the biosynthesis of NR, occurs due to the transcription factors' release[18], ultimately leading to an increase in rubber content[29].

The investigated genes in this study, exhibited clear upregulation in response to MeJA treatment and implies a positive regulatory role in reinforcing rubber production in *L. serriola* (Fig. 2). *HMGR1*, *HMGS1* and *CPT2* had higher expression levels after application of 400 µM MeJA, but *SRPP1* had much higher expression at 200 µM MeJA. The genes responsible for vital enzymes and proteins in natural latex metabolism showed inducible expression in latex derived from *L. serriola* plants at least at one-time point. These genes expression levels in plants exposed to exogenous MeJA, showed variability dependent on the stimuli concentration (200 µM, 400 µM), duration of induction (6, 12 and 24 h), and gene type[29]. The variation implies a potential association between increased expression of the genes and production of latex, potentially contributing to a systematic defense mechanism within laticifers against plant stress[30].

HMGS1 and *HMGR1*, displayed significant changes in expression levels after MeJA treatment. Sirinupong et al.[31] demonstrated that there is a positive correlation between content of latex rubber with *HMGS* activity. *HMGS*, which catalyzes the irreversible conversion of acetoacetyl-CoA to HMG-CoA, appears to play a crucial role and possibly acts as a key regulatory point in the MVA pathway in plants[32]. Rate-limiting *HMGR* in the MVA pathway, results in diminished IPP availability when its activity decreases, resulting in a reduced rate of rubber synthesis. Therefore, a strong correlation between the rubber production rate and *HMGR* expression level is possible[33]. Our results are consistent with those of Du et al.[34], who investigated the identification of multiple *HMGR* genes, revealing their evolutionary relationships and expression patterns in response to 1 mmol/L MeJA and ethylene treatments over four time points (0, 3, 6, 12, and 24 h). They found that the expression of *HMGR* genes was significantly influenced by MeJA, which is known to regulate plant defense responses and secondary metabolite production. Notably, *HMGR1* expression in roots of *Taraxacum- koksaghyz* peaked 12 h after MeJA treatment.

CPT, responsible for the production of cis-polyisoprene, exhibited a marked increase in expression at both MeJA concentrations, suggesting its involvement in rubber biosynthesis regulation through catalyzing the condensation of an allylic prenyl diphosphate to IPP. According to Cherian et al.[8], *CPT* genes were reported to be essential for rubber biosynthesis in T. kok-saghyz[35], L. sativa[36], and H. brasiliensis[37]. The application of RNAi to inhibit *CPT* in *T. brevicorniculatum* resulted in a decrease in levels of cis-1,4-polyisoprene leading to the virtual elimination of extended chain molecules[33], indicating the involvement of *CPT* in regulating molecular weight.

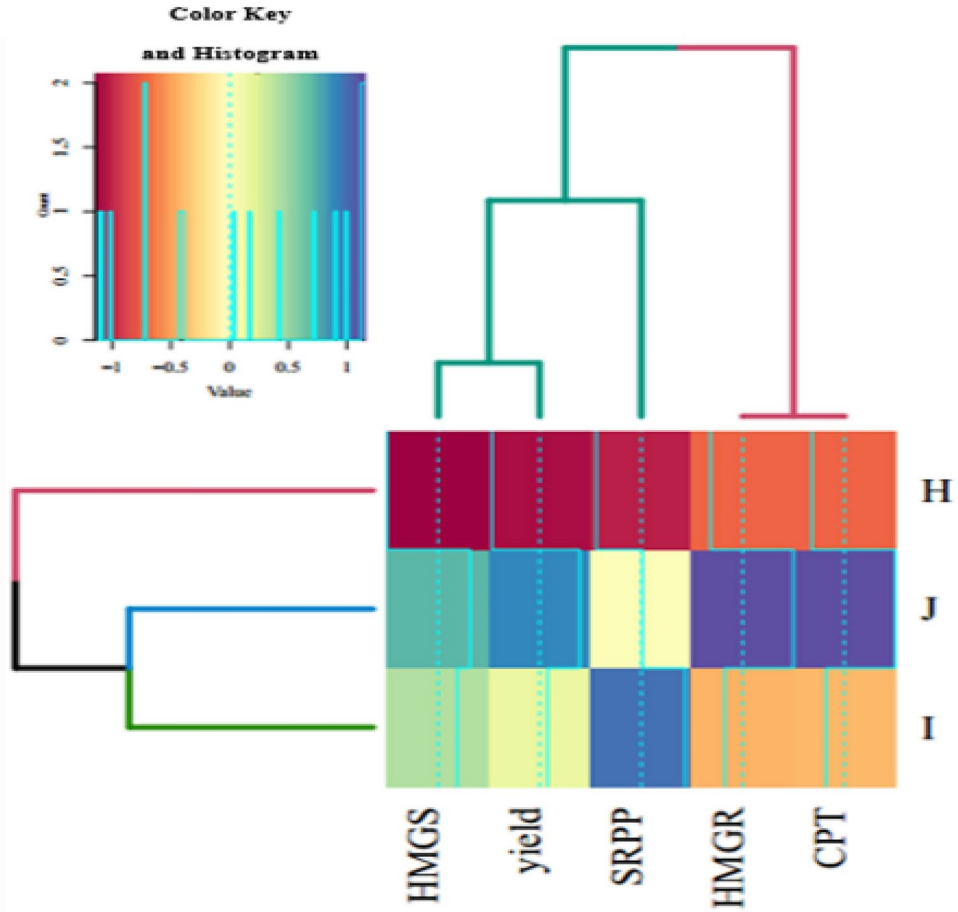

Fig. 6. Shows the heatmap and cluster analysis of the expression levels of rubber biosynthesis genes (*HMGR1*, *HMGS1*, *CPT2* and *SRPP1*) and rubber yield under 3 different levels of MeJA (H:0 µM, I: 200 µM and J:400 µM). The visualization combines a heatmap and cluster analysis to show how MeJA affects NR yield and rubber biosynthesis genes in plants. Darker colors in the heatmap indicate higher values for those parameters, while lighter colors represent lower values. The clustering helps identify groups of parameters that respond similarly to MeJA. The distance between dendrograms on the top and left sides of the heatmap reflects the degree of similarity between parameters or MeJA treatments.

SRPP, a protein associated with rubber particle formation, demonstrated the most significant changes in expression, particularly at 200 µM MeJA and 24 h post-treatment. This suggests a potential link between *SRPP* and rubber particle dynamics influenced by MeJA. There is substantial evidence confirming *SRPP*'s role in latex coagulation, contributing to the sealing of wound sites and preventing potential infections by fungal or bacterial pathogens[38–40]. The involvement of *SRPP* in producing rubber with high molecular weight, rather than in the rubber biosynthesis process itself, is supported by lack of *SRPP* homologs observed in *Ficus carica* and *Ficus benghalensis*[8,41,42]. This suggests that *SRPP* influences both rubber quantity and the stability of rubber molecular weight in plants[43]. However, Chakrabarty et al.[44] established that the homologs of *SRPP* in lettuce did not play rate-limiting catalytic roles in the biosynthesis of natural rubber.

The role of *SRPP* is also crucial in MVA, MEP, and FPP synthesis, as well as in integrating IPP units into the prenyl chain. In particular, *SRPP* has been suggested to enhance the stability of rubber particles without compromising the integrity of cellular membrane.

To determine the presence of a synergistic effect among the rubber biosynthesis genes and rubber yield, a correlation analysis was conducted. The significant positive associations among *HMGR1*, *HMGS1* and *CPT2* indicate a coordinated regulation of these genes implicated in rubber accumulation throughout the latex metabolic process (Figs. 6 and 7). The high and significant correlation observed between *HMGR1*, *HMGS1*, and *CPT2* with rubber yield suggests that these genes likely play important roles in rubber synthesis in L. serriola. However, *SRPP* may not act as a limiting factor[29]. Despite the increase in the number of *SRPP1* transcripts, weaker correlation with rubber yield was evident. Additionally, there was no observed synergistic effect between *SRPP1* and two other studied genes (*HMGR1* and *CPT2*). This contrast might be explained by the concept that *SRPP* may play a role in the regulation of rubber molecular weight rather than direct rubber biosynthesis[8].

Besides its potential involvement in regulating the molecular weight of rubber, *SRPP*s might be triggered by stress, thereby enhancing stress tolerance in plants[45]. The higher expression levels could be a response to elicitor stress, and it is plausible that *SRPP* transcript levels are post-transcriptionally regulated. Our findings align with

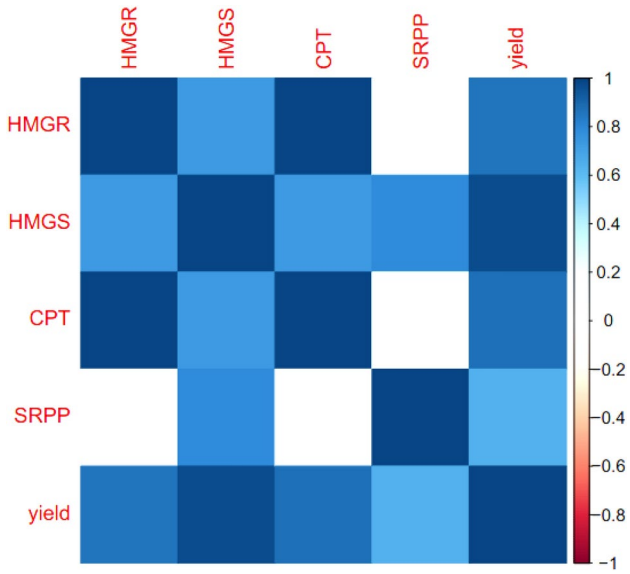

Fig. 7. Correlation among the expression levels of rubber biosynthesis pathway genes and rubber yield in *L. serriola*. Darker colors indicate higher values, while lighter colors represent weaker correlations.

those of Wu et al.[29], who reported similar results in *H. brasiliensis*. They noted a strong correlation between *HMGR*, HMGS, and *CPT* with dry rubber yield, but that *SRPP* showed no significant correlation.

The potential of MeJA as a signaling molecule to stimulate the production of diverse secondary metabolites by triggering the expression of relevant genes is well established (e.g., Refs.[46–48]). According to Wei et al.[49], MeJA significantly upregulates genes involved in terpene biosynthesis, underscoring its role as a potent elicitor of secondary metabolites. This upregulation is driven by the activation of transcription factors that regulate biosynthetic gene expression, a mechanism also observed in other plants like *Medicago truncatula* and *Taxus*. The analysis of rubber content and molecular weight provides insights into the influence of MeJA on rubber biosynthesis in *L. serriola*. Our findings reveal significant alterations in both rubber content and molecular weight, indicating the regulatory role of MeJA in rubber production.

Regarding rubber content, our results indicate that treatment with MeJA (400 µM) significantly increased rubber content by approximately 16% compared to the control samples (Fig. 3). Analysis of the expression profiles of rubber biosynthesis genes revealed elevated relative expression levels of *CPT2*, *HMGR1*, and *HMGS1*, demonstrating a correlation with the induced rubber accumulation.

Application of exogenous MeJA not only stimulates the density of laticifer cells but also triggers the JA signaling pathway responsible for regulating rubber biosynthesis in H. brasiliensis[18,50]. In addition, inducing the differentiation of secondary laticifers is a promising strategy for increasing rubber yield, as the number of laticifers directly affects latex production. While genetically controlled, laticifer formation can be induced by external signals, including exogenous JA and MeJA. Studies have shown that JA and MeJA promote secondary laticifer differentiation, with mechanical damage during tapping and reduced turgor pressure also rapidly increasing JA levels[51].

Our findings align with the discoveries of Saeedi et al.[52], who examined the impact of MeJA concentrations (300 µM and 600 µM) on rubber content in *Taraxacum kok-saghyz* plants. They demonstrated the substantial and positive effects of MeJA treatment, which resulted in heightened rubber yields. In a separate study, Wu et al.[29] also reported an increase in rubber yield as a result of the effect of MeJA on rubber trees.

According to Bell et al.[13], the rubber content among the investigated biotypes ranged from 2 to 12%, with the most frequent percentages being between 4 and 5%, aligning with our findings. However, our research outcomes showed a significant difference with the results reported by[11], who reported a rubber content of 54% in *L. serriola* plants. This disparity in rubber content can be attributed to our rubber extraction method, which involved extraction from the whole latex, leading to a lower rubber percentage.

Molecular weight (M_w) stands out as a crucial parameter in determining the quality of rubber, exhibiting a positive correlation between quality and molecular weight. The capacity to transform rubber into a high-quality product characterized by exceptional resistance to abrasion and tensile strength is linked to the Mw of the initial rubber polymer chain[53].

The rubber samples investigated in this study had all M_ws above 1000 kg/mol, which is one of the characteristics of a high-quality rubber. A M_w of 1000 kg/mol or higher stands as the primary and most significant determinant of usable rubber[1]. *L. serriola* naturally synthesizes a high molecular weight NR, but it could be influenced by substrate concentration and type and the ratio of the initiator FPP to IPP[5]. These can be attributed to the modulation of enzyme activity and modifications in the levels of gene expression related to chain elongation and termination of rubber, which were enhanced after application of MeJA in this study. Our findings reveal an increase in the molecular weight of rubber following treatment with MeJA (Table 2 and Fig. 4). This heightened

molecular weight in response to MeJA is likely attributed to the influence of MVA pathway enzymes, such as *HMGR1* and *HMGS1*, which play a direct role in IPP production.

Genes such as *SRPP1*, *CPT2*, and *HMGR1* could be involved in the regulation of M_w, as their expression levels responded to MeJA application. Previous studies have demonstrated the pivotal role of *CPT*, *SRPP*, and *HMGR* in regulating M_w across various rubber-producing plants[33,43].

M_w uniformity is assessed by the polydispersity index[54]. Values of polydispersity were calculated through the division of the weight-averaged molecular weight by the number-averaged molecular mass (Mn). Elevated rate indicates either an uneven distribution of M_w or a blend of molecules of rubber varying in size[55]. In this investigation, all analyzed rubber samples exhibited a narrow M_w range (Table 2). These are consistent with the polydispersity value of 1.1 for rubber extracted from *L. serriola* determined by Bushman et al. (2015). It is commonly assumed that, while keeping other molecular features unchanged, reduced polydispersity indices lead to advantageous characteristics, including heightened tensile strength, softening point and impact strength[56].

The FTIR analysis of NR from *L. serriola* in this study (Fig. 5) showed distinct chemical structures in the range of 500–4000 cm^{-1} associated with saturated aliphatic compounds, aligning with prior research methodologies[57]. Notably, in the spectral range of 1738 to 2850 cm^{-1}, a weak band at 2726 cm^{-1} was observed, assigned to C-H stretching mode linked to aldehyde compounds[58]. The sharp peak at 1730 cm^{-1}, indicating the symmetric stretching mode of C=O (amide), corroborated our observations and aligned with prior studies[59]. Peaks at 1642, 1461, and 1376 cm^{-1} corresponded to C=C stretching, CH3 symmetric deformation, and carboxylate functionalities, respectively consistent with earlier research[52,60]. The distinctive polyisoprene peak observed at 881 cm^{-1}, associated with the C-H out-of-plane bending vibration of cis-1,4-polyisoprene units, aligns with the results reported by Aielo et al.[61] which reported nearly the same results. Our FTIR results contribute to understanding NR composition, aligning with prior research[60]. However, due to lack of references for FTIR of *L. serriola* natural rubber, the polyisoprene peak wave number is little higher (881 cm^{-1}) which could be the plant characteristic.

This research highlights the influence of MeJA in modulating gene expression in rubber biosynthesis pathway, consequently impacting both the quantity and quality of the produced rubber. Subsequent research is warranted to clarify the precise molecular processes behind these observed responses and their relevance in enhancing rubber production in rubber-producing plant species.

Conclusions

This study provides valuable insights into the potential application of MeJA as a strategic tool to enhance rubber yield in non-traditional rubber-producing plants, exemplified by *L. serriola*. The research effectively illustrates the modulatory effect of MeJA in influencing the expression of rubber biosynthesis genes and its consequential impact on both the quantity and quality of rubber in *L. serriola*. MeJA demonstrated a notable increase in the expression of critical rubber biosynthesis genes (*HMGR1*, *HMGS1*, *CPT2*, and *SRPP1*), resulting in a concurrent elevation of rubber yield and molecular weight. To deepen our understanding, future research should delve into the intricate molecular mechanisms underlying the MeJA-mediated upregulation of these genes.

Furthermore, it is essential to conduct thorough investigations into the long-term effects of MeJA treatment on both the rubber yield and the quality of the produced rubber. MeJA appears to be a promising tool with the potential to significantly enhance rubber yield in *L. serriola* by skillfully regulating the expression of key rubber biosynthesis genes. This research not only sheds light on the molecular intricacies involved but also unveils new pathways for improving rubber production in non-traditional rubber-producing plants. The implications of such advancements could potentially influence practices in the broader rubber industry and contributing to more sustainable rubber sourcing.

Data availability

The data that support the findings of this study are available within the paper. Any other supporting data are available from the corresponding author upon request.

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Author contributions

All authors contributed to the study conception and design. The initial idea was presented by MR.N. and M.A. The design of the experiments and the execution of the experiments were carried out by M.A and A.F., P.A and R.G. Data analysis was done by A.F and M. Z. M.A., MR.N. and A.F. wrote and edited the manuscript. All authors discussed the results and contributed to the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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