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### Differential expression of *Arabidopsis* EJC core proteins under shortday and long-day growth conditions

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#### ABSTRACT

Exon junction complexes (EJCs) associate with mRNAs, mediate the pre-mRNA splicing and eventually gets displaced by ribosomes during the initial phase of translation. EJCs are involved in several critical physiological pathways. The functional nature of EJCs and the underlying molecular mechanism(s) still needs to be elucidated particularly in case of plants. Here, we report that the putative core protein factors of the EJC differentially express under short-day and long-day conditions. Since, plants are constantly exposed to biotic and abiotic factor(s), it would be significant to see how the EJCs respond to different stress inducing conditions. The protein levels of EJC core proteins under short-day conditions were 1.25 times higher relative to the protein levels under long-day conditions. Similar results were observed for the mRNA transcripts of the EJC core protein factors. These results signify that under short-day conditions, the EJC proteins are more activated and might be involved in few events which are yet to be revealed.

#### Introduction

The exon junction complex (EJC) comprising of three core protein factors is a multi-protein complex and interacts with the spliced messenger RNA (mRNA) to form mRNA-ribonucleoproteins (mRNPs). EIC manipulates the post-transcriptional events (1-4). Several associating proteins bind to the core EJC factors, depending on the cellular event that they will mediate. Essentially in a sequence-independent manner, the EJCs assemble and bind to the mRNAs in the region, primarily in the upstream region of exonexon junctions (5, 6). EJCs remain bound to the mRNA throughout their life cycle (7, 8). The central dogma is regulated by the EJC proteins, but the underlying molecular mechanism(s) in which they still remains blurred. The role of EIC in the splicing process is highly regarded and thought to play a central role in determining the fate of the mature mRNA (9). The EJC increases the fidelity, efficiency and productivity of the translation event (10, 11). Interestingly, how the EJC proteins respond to changes in plant growth conditions still remains to be revealed. Light, being an abiotic factor plays a significant role in regulating the growth of the plant. Flowering other and developmental stages are affected depending on the availability of light (12). It is intriguing to reveal the role of EJC when the plants are grown at different exposure to light. Plants invariably alter the key molecular processes when exposed to different stress conditions (13-15). Since, EJC is well involved in various physiological pathways; it would be significant to reveal the role of EJC when plants are grown at varied growth conditions (16, 17). Here, we provide evidence that the EJC core protein factors; Y14, MAGO and eIF4AIII gets differentially expressed under shortday and long-day growth conditions, which specifically highlights that the EJC proteins are more activated and get accumulated to respond to changed environmental conditions by modulating the key molecular events.

#### **Materials and Methods**

#### Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) seeds sown in soil. The plants were grown were simultaneously under short-day (12/12 hr light/dark) and long-day (16/8 hr light/dark) conditions. Temperature was maintained at 22 °C with70-80% relative humidity. blotting and semi-quantitative Western reverse transcriptase polymerase chain reaction (sq-RT PCR) assays were done using the leaves of Arabidopsis thaliana. The primer sequence resembling to TAGAATCAGAAGCAGTC ATGGCGAACA (forward primer) and GTAACGTCTTCTCGGACTTCTTGAAC

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(reverse primer) of the coding sequence of Y14 were used for amplifying the open reading frame using cDNA as template and similarly for amplifying the open frame of MAGO, ATGGCCGCGG reading AAGAAGCGACGGAGTTC (forward primer) and GATAGGCTTGATTTTGAAGTGCAG (reverse primer) primer sequences were used. ATGGCGACATCTGAAGCGGAATATG (forward primer) and CTTGCTAAAGGTCATCTCCGAGTAC (reverse primer) were used to amplify the coding sequence of eIF4AIII. PCR was done up to 25 cycles to amplify the gene products.

## Semi-quantitative reverse transcriptase polymerase chain reaction (sq-RT PCR)

Full-length cDNA of Y14, MAGO and eIF4AIII was obtained by RT-PCR using Col-0 RNA. For RNA extraction, twenty-one-day-old plants were used. Twenty-one-old plants display optimum development and most importantly bolting process does not get initiated, which would otherwise may affect the synthesis of desired transcripts. According to the manufacturer's instruction (TRIzol® reagent, Thermo Fisher Scientific) total RNA was extracted. Genomic DNA were removed using 2 units of DNase (TURBO DNase<sup>™</sup>, Thermo Fisher Scientific). The purified RNA after phenol/chloroform/isoamyl alcohol extraction, DEPC-treated water was used to solubilise the RNA. Gene-specific primers were used polymerase chain reactions. The primer for resembling sequence ATGGCGAACA to TAGAATCAGAAGCAGTC (forward primer) and GTAACGTCTTCTCGGACTTCTTGAAC (reverse primer) of the coding sequence of Y14 were used for amplifying the open reading frame using cDNA as template and similarly for amplifying the open reading frame of MAGO, ATGGCCGCGG AAGAAGCGACGGAGTTC (forward primer) and GATAGGCTTGATTTTGAAGTGCAG (reverse primer) primer sequences were used. ATGGCGACATCTGAAGCGGAATATG (forward CTTGCTAAAGGTCATCTCCGAGTAC primer) and

(reverse primer) were used to amplify the coding sequence of eIF4AIII. ATGGGTAAAGAGAAGTTTCACATC (forward primer) and CTTGGCACCCTTCTTGACTGCAGCCTT (reverse primer) were used to amplify the coding sequence of EF1 $\alpha$ . PCR was done up to 25 cycles to amplify the gene products. Reactions were performed in triplicate and the relative levels of the transcripts were normalized to the expression levels of genes used as internal controls, which included EF1 $\alpha$ . Since EF1 $\alpha$  is one of the constitutively expressed genes, it was used as an experimental control.

#### Protein extraction and Western blotting

Total proteins were isolated from 0.1 gm leaf of the twenty-one-day-old *Arabidopsis thaliana* Col-0 plants. The leaves were harvested from plants which were grown simultaneously under short-day (12/12 hr light/dark) and long-day (16/8 hr light/dark) conditions at 22 °C. The composition of the extraction buffer was 150 mM of NaCl, 30 mM of Tris-Cl (pH 8.0), 1 mM of PMSF, 1 mM of EDTA, and 1X proteinase inhibitor. Western blot analysis was done using protein-specific antibodies.

#### **Results and Discussion**

## Y14, MAGO and eIF4AIII are highly conserved proteins.

The core EJC proteins are highly conserved in several plant species as evident from the similarities they share for their protein sequences (Fig. 1, Fig. 2, Fig. 3). The web-based tool (UniProt) was used to generate the multalign images indicating the proteins across various plant species. The *Arabidopsis* EJC proteins harbour utmost number of fully conserved amino acid residues as compared to other plant species. It is also revealed that considerable fraction of amino acid residues of EJC core proteins of *Arabidopsis* share resemblance>70%. A small amount of amino acid residues represent non-conserved segment. Since, the *Arabidopsis* proteins,

F419J7 R0IJ97 A0A087H150 M4DCS1 V4KGL4 A0A067JH32	Y14 ARATH ROIJ97_9BRAS AOAO87H150 ARAAL M4DCS1_BRARP V4KG14_EUTSA AOAO67JH32_JATCU	1 1 1 1	MANIESEAVDFEPEEDDIMDEEGTAIDGADVSPRAGHPRIKSATAGANGES-AKKTKG MANIESEAVDFEPEEDDIMDEEGTAVDPADVSPRAGOPRIKSATAGANGES-APORKTKG MANIEAEAVDFEPEEDDIMDEESGAVDGGDVSPRAGLPRIRSATAGANGES-TKKTKG MANIEAEAVDFEPEEDDIMDEDVGASDISPRAGHPRIRSATAGANGES-A-NRKTKG MANMESEAVDFEPEEDDIMDEDAGAVDAGDVSPRAGHMRIRSATAGANGES-A-ORKTKG MANADAEAVDFEPEEDDIMDEDAGAV-DVDASPRAGHPRIKSATAGANGES-A-QRKTKG	57 59 57 55 58 58
F419J7	Y14 ARATH	58	RGFREEKDSDRQRRLSSRDFESLGSDGRPGPQRSVEGWIILVSGVHEETQEEDITNAFGD	117
R0IJ97	ROIJ97_9BRAS	60	RGFREERDSDRQRHLSSRDFESLGSDGGSGPQRSIEGWIILVSGVHEEAQEDDISNAFGD	119
A0A087H150	AOAO87H150 ARAAL	58	RGFREEKDSDRQRRLSSRDFESLGSDGGLGPQRSIEGWIILVTGVHEEAQEDDISNAFGD	117
M4DCS1	M4DCS1_BRARP	56	RGFREEKDSDRQRLSSRDFESLGSDGGHGPQRSIEGWIVLVTGVHEEAQEDDISNAFGD	115
V4KGL4	V4KG14_EUTSA	59	RGFREEKDSDRQRPLSSRDFESLGSDGGHGPQRSIEGWILVTGVHEEAQEDDISNAFGD	118
A0A067JH32	AOAO67JH32_JATCU	59	RGFREEADADRQNRLASRDFDSLGTDGGPGPQRSIEGWIILVTGVHEEAQEDDISNAFGD	118
F419J7	Y14 ARATH	118	FGEIKNLNLNLDRRSGYVKGYALIEYEKKEEAQSAISAMNGAELLTQNVSVDWAFSSGFS	177
R0IJ97	ROIJ97_9BRAS	120	FGEIKNLHLNLDRRTGFVKGYALIEYEKKEEAQKAISAMNGAELLTQNVSVDWAFSNGFI	179
A0A087H150	AOAO87H150 ARAAL	118	FGEIKSLHLNLDRRTGFVKGYALIEYEKSEEAQSAIKAMNGAELLTKNVSVDWAFSNGFS	177
M4DCS1	M4DCS1_BRARP	116	FGEIKSLHLNLDRRTGFVKGYALIEYEKSEEAQSAIKAMNGAELLTKNVSVDWAFSNGFS	175
V4KGL4	V4KGL4_EUTSA	119	FGQIKSLHLNLDRRTGFVKGYALIEYEKSEEAQSAIKAMNGAELITKNVSVDWAFSNGFS	178
A0A067JH32	AOAO67JH32_JATCU	119	FGEIKNLHLNLDRRTGFVKGYALIEYEKSEEAQSAIKAMNGAEILTKOVSVDWAFSNGFS	178
F4I9J7	Y14 ARATH	178	GGESYRRKNSRYGRSQRSRSPRRRY	202
R0IJ97	ROIJ97 9BRAS	180	AGGSQWRKNQRSGRRSRSPRRRY	202
A0A087H150	AOAO87H150 ARAAL	178	-VGSYRRKNLRSGRRSRSPKRRY	199
M4DCS1	M4DCS1 BRARP	176	NAGSYRRNMRSGRSQRSRSPRRF	200
V4KGL4	V4KGL4-EUTSA	179	-FGSYRRKNARSGRSQRSRSPRRRY	202
A0A067JH32	AOA067JH32_JATCU	179	KRKNMRSGRAHRSRSPRRY	198

**Fig. 1.** Protein sequence alignment of the YI4 of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (F4I9J7: *Arabidopsis thaliana*; R0IJ97: *Capsella rubella*; A0A087H150: *Arabis alpina*; M4DCS1: *Brassica rapa*; V4KGL4: *Eutrema salsugineum*; A0A067JH32: *Jatropha curcas*).

023676 R0GK06 A0A087HK06 M4EV35 V4LAQ3 A0A067K9B5	MGN_ARATH ROGRO6 9BRAS AOA087HK06 ARAAL M4EV35 BRARP V4LAQ3 EUTSA AOA067K9B5_JATCU	1 9 121 1 1 1	MAAEEATEFYLRYYVGHKGKEGHEFLEFEFREDGKLRYANNS REREREREAMAGEEENEFKLRYYVGHKGKEGHEFLEFEFRSDGKLRYANNS AKRERNRGAKQLDAFMKELAIPLKGYMESFPRKRIHPYERSLITIL-GDGKVE- MEENEFYLRYVGHKGKEGHEFLEFEFRPDGKLRYANNS MAAEENEFYLRYYVGHKGKEGHEFLEFEFRPDGKLRYANNS MAAAEDDEFYLRYYVGHKGKEGHEFLEFEFRPDGKLRYANNS MAAEDDEFYLRYYVGHKGKEGHEFLEFEFRPDGKLRYANNS	42 59 176 40 42 41
023676 R0GK06 A0A087HK06 M4EV35 V4LAQ3 A0A067K9B5	MGN ARATH ROGKO6_9BRAS AOAO87HKO6_ARAAL M4EV35_BRARP V4LAQ3_EUTSA AOAO67K9B5_JATCU	43 60 177 41 43 42	NYKNDTIIRKEVFL-TPAVLKECKRIVSESIILKEDDNNWPEPDRVGKQELEIVIGNEHI NYKNDTMIRKEVFL-TPAVLKECKRIVSESIMKEDDKNWPEPDRVGKQELEIVIGNEHI -LGQVDVLRKVISVGKEHASICAKALSKRPAEDRINEGVEKLEIVFQ NYKNDTMIRKEVFL-TPAVLKECKRIVSESIMKEDFKWPEPDRVGRQELEIVIGNEHI NYKNDTMIRKEVFL-TPAVLKECRRIVSESIMKEDANWPEPDRVGRQELEIVIGNEHI NYKNDTMIRKEVFL-TPAVLKECRRIIAESIMKEDANWPEPDRVGRQELEIVIGNEHI	101 118 223 99 101 100
023676 R0GK06 A0A087HK06 M4EV35 V4LAQ3 A0A067K9B5	MGN ARATH ROGKO6 9BRAS AOAO87HKO6 ARAAL M4EV35 BRARP V4LAQ3 EUTSA AOA067K9B5_JATCU	102 119 224 100 102 101	SFATSKIGSLVDCQSSNDPEGLRIFYYLVQDLKCLVFSLISLHFKIKPI SFATSKIGSLVDVQSSNDPEGLRIFYYLVQDLKCLVFSLISLHFKIKPI QHGSAVDDLSIAKLLSIAK SFATSKIGSLVDVQSSDPEGLRIFYYLVQDLXCLVFSLISLHFKIKPI SFATSKIGSLVDVQSSKDPEGLRIFYYLVQDLKCLVFSLISLHFKIKPI	150 167 247 159 150 149

**Fig. 2.** Protein sequence alignment of the MAGO of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (O23676: *Arabidopsis thaliana*; ROGKQ6: *Capsella rubella*; A0A087HK06: *Arabis alpina*; M4EV35: *Brassica rapa*; V4LAQ3: *Eutrema salsugineum*; A0A067K9B5: *Jatropha curcas*).

Q94A52 R0G584 A0A087HAZ9 M4E102 V4LZG1 A0A067L4G5	RH2_ARATH R0G584_9BRAS A0A087HAZ9_ARAAL M4E102_BRARP V41ZG1_EUTSA A0A067I4G5_JATCU	1 1 1 1	MATANPGRGGGRRGGGAMDDDKLVFETTDGIEPITSFNDMGIKEDVLRGVYEYGFEK MATANPGRGGGRRGGGPMDDDKLVFETTEGIEPITSFNDMGIKEDVLRGVYEFGFEK MATANVANPGRGGGRIDDDKLVFETTEGIEPITSFNDMGIKEDVLRGVYEYGFEK MAEANPGRGGGRRGGGPMDDDKLVFETTEGIEPITSFNDMGIKEDVLRGVYEYGFEK MAEASMSNPGRGGRRGGGPMDDDKLVFETTEGIEPITSFNDMGIKEDVLRGVYEFGFEK MAAAV-TSVVPAGRSARRAAAEDDKLVFETTEGIEPIASFDEMGIKNDLLRGIYAYGFEK	57 57 56 57 60 59
Q94A52 R0G584 A0A087HAZ9 M4E102 V4LZG1 A0A067L4G5	RH2 ARATH ROG384_9BRAS AOA087HAZ9_ARAAL M4E102_BRARP V4LZG1_EUTSA AOA067L4G5_JATCU	58 58 57 58 61 60	PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQVVDTSSREVQALILSPTRELAT PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQVVDTSSREVQALILSPTRELAS PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQVVDTSSREVQALILSPTRELAS PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQIVDTSSREVQALILSPTRELAS PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQIVDTSSREVQALILSPTRELAS PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQIVDTSSREVQALILSPTRELAS PSAIQQRAVMPILQGRDVIAQAQSGTGKTSMIALSVCQIVDTSSREVQALILSPTRELAS	117 117 116 117 120 119
Q94A52	RH2 ARATH	118	QTEKT QAIGLHANIQAHACIGGNSVGEDIRKLEHGVHVVSGTPGRVCDMIKRRSLRTRA	177
R0G584	ROG584 9BRAS	118	QTEKTIQAIGLHANIQAHACIGGNSVGEDIRKLEHGVHVVSGTPGRVCDMIKRRSLRTRA	177
A0A087HAZ9	AOAO87HAZ9 ARAAL	117	QTEITIKTIQAHACIGGKSVGEDIRNLENGVHVVSGTPGRVCDMIKRRSLRTRA	176
M4E102	M4E102 BRARP	118	QTEKTIQAIGLHANIQAHACIGGKSVGEDIRKLENGVHVVSGTPGRVCDMIKRRSLRTRA	177
V4LZG1	V41ZG1_EUTSA	121	QTEKSIQAIGLHASIQAHACIGGKSVGEDIRKLEHGVHVVSGTPGRVCDMIKRRSLRTRA	180
A0A067L4G5	AOA067L4G5_JATCU	120	QTEKVILAIGDYINIQAHACIGGKSVGEDIRKLEYGVHVVSGTPGRVCDMIKRRSLRTRA	179
Q94A52	RH2 ARATH	178	IKLLILDESDEMLSRGFKDQIYDVYRYLPPDLQVCLVSATLPHEILEMTSKFMTEPVKIL	237
R0G584	ROG584 9BRAS	178	IKLLILDESDEMLSRGFKDQIYDVYRYLPPDLQVCLVSATLPHEILEMTSKFMTEPVKIL	237
A0A087HAZ9	AOAO87HAZ9 ARAAL	177	IKLLILDESDEMLSRGFKDQIYDVYRYLPPDLQVCLVSATLPHEILEMTSKFMTDPVKIL	236
M4E102	M4E102 BRARP	178	IKLLILDESDEMLSRGFKDQIYDVYRYLPPDLQVCLVSATLPHEILEMTSKFMTDPVKIL	237
V4LZG1	V4LZG1 EUTSA	181	IKLLILDESDEMLSRGFKDQIYDVYRYLPPELQVVLISATLPHEILEMTSKFMTDPVKIL	240
A0A067L4G5	AOAO67L4G5_JATCU	180	IKLLVLDESDEMLSRGFKDQIYDVYRYLPPELQVVLISATLPHEILEMTSKFMTDPVKIL	239
Q94A52	RH2 ARATH	238	VKRDELTLEGIKQFFVAVEKEEWKFDTLCDLYDTLTITQAVIFCNTKRKVDYLSEKMRSH	297
R0G584	ROG584 9BRAS	238	VKRDELTLEGIKQFFVAVECEDWKFDTLCDLYDTLTITQAVIFCNTKRKVDWLSEKMRSN	297
A0A087HAZ9	A0A087HAZ9 ARAAL	237	VKRDELTLEGIKQFFVAVEKEEWKFDTLCDLYDTLTITQAVIFCNTKRKVDWLSEKMRSN	296
M4E102	M4E102 BRARP	238	VKRDELTLEGIKQFFVAVEKEEWKFDTLCDLYDTLTITQAVIFCNTKRKVDWLSEKMRSN	297
V4LZG1	V4LZG1 EUTSA	241	VKRDELTLEGIKQFFVAVEKEDWKFDTLCDLYDTLTITQAVIFCNTKRKVDWLSEKMRSN	300
A0A067L4G5	A0A067L4G5_JATCU	240	VKRDELTLEGIKQFFVAVEREEWKFDTLCDLYDTLTITQAVIFCNTKRKVDWLSEKMRSN	299
Q94A52	RH2 ARATH	298	NFTVSSMHGDMPQKERDAIMNEFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	357
R0G584	ROG384_9BRAS	298	NFTVSSMHGDMPQKERDEIMNQFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	357
A0A087HAZ9	AOA087HAZ9 ARAAL	297	NFTVSSMHGDMPQKERDEIMNQFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	356
M4E102	M4E102_BRARP	298	NFTVSSMHGDMPQKERDEIMNQFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	357
V4LZG1	V41ZG1_EUTSA	301	NFTVSSMHGDMPQKERDEIMNQFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	360
A0A067L4G5	AOA067L4G5_JATCU	300	NFTVSSMHGDMPQKERDAIMNQFRSGDSRVLITTDVWARGIDVQQVSLVINYDLPNNREL	359
Q94A52	RH2 ARATH	358	YIHRIGRSGRFGRKGVAINF <mark>V</mark> KSDDIKILRDIEQYYSTQIDEMFMNVADLI	408
R0G584	ROG384_9BRAS	358	YIHRIGRSGRFGRKGVAINFVKSDDIKILRDIEQYYSTQIDEMFLNVADLI	408
A0A087HAZ9	AOA087HAZ9 ARAAL	357	YIHRIGRSGRFGRKGVAINFVKSDDIKILRDIEQYYSTQIDEMFMNVADLI	407
M4E102	M4E102_BRARP	358	YIHRIGRSGRFGRKGVAINFVKSDDIKILRDIEQYYSTQIDEMFMNVADLI	408
V4LZG1	V4LZG1_EUTSA	361	YIHRIGRSGRFGRKGVAINFVKSDDIKILRDIEQYYSTQIDEMFMNVADLI	411
A0A067L4G5	AOA067L4G5_JATCU	360	YIHRIGRSGRFGRKGVAINFVKSDDIKILRDIEQYYSTQIDEMFMNVADLI	410

**Fig. 3.** Protein sequence alignment of the eIF4AIII of *Arabidopsis thaliana*. Residues that are conserved are represented in box shades. Figure was generated with UniProt. (Q94A52: *Arabidopsis thaliana*; R0G584: *Capsella rubella*; A0A087HAZ9: *Arabis alpina*; M4E102: *Brassica rapa*; V4LZG1: *Eutrema salsugineum*; A0A067L4G5: *Jatropha curcas*).

Y14, MAGO and eIF4AIII are extremely conserved in diverse plant species; it suggests that they all are equipped with these essential protein factors. The EJC core proteins are engaged in several numbers of physiological events and are thus indispensable in nature. Essentially The EJC maintains a stable, sequence-independent, hold on the mRNA until its removal during translation in the cytoplasm (18).

# The Y14, MAGO and eIF4AIII transcripts are differentially expressed under long-day and short-day conditions.

The core EJC protein factors are engaged in several key molecular events. Plants when exposed to varied environmental conditions acclimatize themselves according to the prevailing stimulus. We intended to see the expression pattern of the core EJC proteins at the transcript level under long-day and short-day growth conditions. Interestingly, it was observed that the expression levels of *Y14*, *MAGO*, *eIF4AIII* were upregulated for short-day grown plants relative to the long-day plants to 1.26, 1.25 and 1.09 times respectively (Fig. 4A, Fig. 4B). The transcript level of *ACTIN* represents the control of the experiment. Heat map of differential expression patterns of the EJC core genes under long-day and short-day growth conditions are represented in Fig. 4C.

## The protein products of Y14, MAGO and eIF4AIIIare higher at short-day growth conditions

The protein levels of the core EJC protein factors are differentially regulated under long-day and short-day growth conditions. Concurrently, it was observed that the protein levels of Y14, MAGO, eIF4AIII were upregulated for short-day grown plants relative to the long-day plants to 1.38, 1.21 and 1.1 times respectively (Fig. 5A, Fig. 5B). The protein level of ACTIN represents

the internal control of the experiment. Ponceau S staining represents RuBisCo and confrms equal protein loading. This result signifies that the EJC core proteins might accumulate under short-day growth conditions so that the plants can respond at a molecular level. The increase in protein levels of the Y14, MAGO, eIF4AIII also highlights that under short-day growth conditions these protein factors may be involved in molecular events which are yet to be exposed.

#### Conclusion

Revealing the functional and physiological role of the EJC is of great interest may unravel many hidden molecular mechanism(s). For instance, the EJC is indispensable for the growth of plants as the knockout of the core EJC protein factors results in embryo lethality conditions in *Arabidopsis*. Similarly in animals, knockout of both the core and peripheral EJC proteins significantly affects the



**Fig. 4.** Differential RNA level expression of the *Y14*, *MAGO*, *eIF4AIII* under long-day and short-day conditions. (A) The agarose-gel images were generated using primers specific for *Y14*, *MAGO* and *eIF4AIII*. *ACTIN* amplification represents the internal control of the experiment. (B) The expression levels of *Y14*, *MAGO*, *eIF4AIII* were upregulated for short-day grown plants relative to the long-day plants to 1.26, 1.25 and 1.09 times respectively. (C) Heat map of differential expression patterns of the EJC core genes under long-day and short-day growth conditions. Web-based tool Morpheus was used to generate the heat map.



**Fig. 5.** Differential protein level expression of the Y14, MAGO, eIF4AIII under long-day and short-day conditions. (A) The Western blots were generated using α-Y14, α-MAGO and α-EIF4AIII antibodies. Protein loading is shown by Ponceau S staining for RuBisCo. The protein level of ACTIN is used as an internal control. (B) The protein levels of Y14, MAGO, eIF4AIII were upregulated for short-day grown plants relative to the long-day plants to 1.38, 1.21 and 1.1 times respectively.

growth of the cell and mostly lead to hereditary diseases. It seems worthwhile to focus on the underlying molecular mechanism(s) through which EJC mediates a wide range of physiological events. The detailed mechanism(s) of the EJC-mediated events remains to be elucidated. Safeguarding the accurate spatial and temporal structure of nuclear compartments is critical for efficient mRNA processing and maintenance of genome integrity. The EJC links the different aspects of mRNA biogenesis, such as transcription, splicing, export and translation. The parallel exposition of the function of the EJC and its position with respect to nuclear architecture is essential to realize how the cell responds internal and external stimuli that they encounter. How EJC-mediated response is triggered in plants in response to stress conditions is not yet fully understood. Our results gives a prima facie evidence that the EIC core proteins are differentially expressed under short-day growth conditions in Arabidopsis and may be well involved in modulating different physiological processes necessary for the plant to resist the environmental changes. Future research on understanding the role of EJC would be significant.

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#### Authors' contributions

AS conceived the idea, performed the experiments, analyzed the results, wrote the manuscript, have read and approved the final manuscript before submission. KBS conceived the idea, analyzed the results, have read and approved the final manuscript before submission.

#### **Conflict of interests**

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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