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Molecular Expression Analysis Of MLP Gene In *Cucumis Melo* Under Abiotic Stress

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Abstract: This study researches the molecular expression analysis of MLP gene in ***cucumis melo*** under abiotic stress. Using quantitative real time PCR (qRT-PCR), we clarified the differential patterns of the MLP gene in light of drought stress, salinity stress, and different temperature stress. Our results uncover a critical up regulation of MLP records in melon tissues presented to delayed drought and salinity stresses, showing an expected job in osmotic change and stress relief. On the other hand, warm pressure inspired a more intricate articulation profile, with starting down regulation followed by an undeniable increment, recommending a biphasic reaction system. The transient elements of MLP articulation propose its association in early pressure discernment and resulting acclimatization processes. Moreover, in silico advertiser examination recognized a few pressure responsive cis- administrative components, validating the quality's administrative possible under unfavorable natural circumstances. This extensive in molecular expression analysis of MLP gene in ***cucumis melo***, offering important bits of knowledge for the improvement of stress-versatile melon cultivars. Our discoveries lay the basis for future useful examinations pointed toward taking apart the exact administrative organizations and flagging pathways regulated by the MLP gene in respect of abiotic stress**.** Difficulties and future headings in MLP quality exploration under temperature stress are additionally examined, including the requirement for thorough utilitarian examinations, clarification of flagging pathways associated with MLP quality guideline, and combination of omics ways to deal with unwind the intricacy of temperature stress reactions.

All in all, the molecular expression analysis of the MLP gene in ***cucumis melo*** under stress addresses a promising road for understanding plant pressure reactions and upgrading pressure resilience in crops. By unwinding the unpredictable administrative systems administering MLP quality articulation and capability, specialists can make ready for the improvement of environment strong yield assortments, adding to worldwide food security and manageability.

Additionally, protein-protein interaction networks were constructed to elucidate the potential functional partners of MLP in ***cucumis melo***. The network analysis indicated interactions with key stress-related proteins, including those involved in reactive oxygen species (ROS) detoxification, osmoprotectant synthesis, and signal transduction pathways. These interactions highlight the multifaceted role of MLP in mediating stress tolerance mechanisms.

To validate the physiological relevance of MLP expression, transgenic melon plants overexpressing the MLP gene were generated and subjected to abiotic stress treatments. The transgenic lines exhibited enhanced tolerance to drought, salinity, and cold stress, as evidenced by improved growth parameters, reduced electrolyte leakage, and lower levels of stress-induced ROS accumulation compared to wild-type plants. In summary, this study provides comprehensive insights into the molecular expression and functional significance of the MLP gene in Cucumis melo under abiotic stress. The findings underscore the potential of MLP as a target for genetic engineering to enhance stress tolerance in melon, paving the way for the development of more resilient crop varieties in the face of global climate change. Further research is

warranted to elucidate the detailed molecular mechanisms underlying MLP-mediated stress responses and to explore its potential applications in crop improvement programs. Understanding the molecular expression hidden in plant reactions to natural anxieties, like temperature vacillations, is fundamental for creating strong yield assortments. In this unique situation, the Major Latex Protein (MLP) gene family has arisen as a central participant in plant pressure reactions. This theoretical presents an extensive survey of the sub-atomic articulation examination of the MLP gene in ***cucumis melo*** (melon) under temperature stress, clarifying its administrative jobs and suggestions for further developing pressure resilience in crops.

The survey starts by featuring the meaning of temperature stress as a significant ecological component influencing plant development, improvement, and efficiency. It highlights the critical need to disentangle the sub-atomic components overseeing plant reactions to temperature vacillations, with a specific spotlight on the MLP quality family.

The MLP quality family, described by the presence of a rationed Bet v 1 space, has collected consideration for its different jobs in plant pressure reactions, including guard against microbes and abiotic stresses. Late examinations have shown that MLP qualities are differentially communicated because of temperature stress, proposing their association in temperature stress transformation components. High level sub-atomic strategies, like Quantitative Real Time PCR (qRT-PCR), RNA sequencing (RNA-Seq), and practical genomics draws near, have been instrumental in clarifying the articulation designs and administrative organizations of MLP qualities under temperature stress.

I: INTRODUCTION:

For this study with current plant science, molecular expression analysis of MLP gene in stress reactions in crops is basic for upgrading agrarian efficiency and supportability. **Cucumis melo**, generally known as musk-melon, is a significant green yield become broadly across different agro-climatic locales. Its economic importance is matched by its vulnerability to different abiotic stresses like drought, saltiness, and different temperatures, which are progressively exacerbated by environmental change. These pressure conditions unfavorably influence melon development, yield, and natural product quality, requiring a far reaching examination concerning the hereditary and sub-atomic components that present pressure resistance.

One promising road of examination includes the investigation of the Major Latex Protein (MLP) quality family. MLPs, at first recognized in the plastic of elastic trees, are a subset of the Bet v 1 superfamily and have been ensnared in different physiological cycles, including plant protection, improvement, and reactions to abiotic stress. The clarification of MLP quality capability in Cucumis melo could give significant bits of knowledge into the hereditary premise of pressure versatility and illuminate reproducing programs pointed toward creating vigorous cultivars. Abiotic stress prompts a mind boggling organization of flagging pathways and transcriptional changes, which thusly initiate pressure responsive qualities. The articulation examples of these qualities, remembering those for the MLP family, can uncover significant parts of the plant's versatile procedures. Hence, a molecular expression analysis of MLP gene under different abiotic stress conditions is fundamental. This investigation includes evaluating mRNA levels through methods like quantitative real time PCR (qRT-PCR), surveying advertiser movement, and looking at post-translational adjustments that might influence protein capability.

In **Cucumis melo**, fundamental transcriptomic examinations have distinguished a few MLP genes that are differentially communicated under abiotic stress. These discoveries require a more profound examination to portray the utilitarian jobs of explicit MLP qualities. This includes articulation profiling as well as practical approval through quality knockdown or overexpression studies. Such utilitarian genomics approaches can clarify the commitment of individual MLPs to push resilience and distinguish key administrative components inside their advertisers. Besides, incorporating sub-atomic articulation information with physiological and biochemical examines can give a comprehensive comprehension of how MLP qualities regulate pressure reactions. For instance, corresponding quality articulation designs with physiological boundaries like stomatal conductance, electrolyte spillage, and photosynthetic effectiveness under pressure conditions can uncover the down to earth ramifications of MLP movement. Moreover, biochemical investigations, for example, catalyst action examines and metabolite profiling can reveal the metabolic pathways affected by MLPs. The appearance of cutting edge omics advances, for example, transcriptomics, proteomics, and metabolomics, works with exhaustive profiling of the sub-atomic scene in focused plants. By utilizing these instruments, analysts can recognize co-communicated qualities, protein communications, and metabolic organizations related with MLP capability. Incorporating omics information through frameworks science approaches can prompt the ID of key center points and hubs inside pressure reaction organizations, giving focuses to hereditary control.

In addition, near genomics and phylogenetic examinations can offer experiences into the transformative protection and disparity of MLP qualities across various species. Such examinations can feature saved themes and primary highlights basic for MLP capability, as well as species-explicit transformations that might give novel pressure resilience characteristics. By understanding the developmental setting of MLP qualities, analysts can more readily anticipate their practical jobs and potential for control in crop improvement programs.

II.METHODOLOGY

The procedures that were performed are mentioned as follows:

2.1 Plant Material and Growth Conditions:

In this study, the Cucumis melo (melon) variety 'F_1 hybrid' was selected for molecular expression analysis of the Major Latex Protein (MLP) gene under various abiotic stress conditions. Seeds were sterilized with 70% ethanol for 1 minute followed by a 10% sodium hypochlorite solution for 10 minutes and thoroughly rinsed with sterile distilled water. The seeds were then germinated on moistened filter paper in Petri dishes at 25°C in the dark for 48 hours. Subsequently, seedlings were transferred to pots containing a sterilized mixture of peat, perlite, and vermiculite (3:1:1) and grown under controlled conditions in a growth chamber with a photoperiod of 16 hours light (25°C) and 8 hours dark (18°C), relative humidity of 60-70%, and light intensity of 200 µmol m^(-2) s^(-1).

2.2 Abiotic Stress Treatments:

After two weeks of growth, seedlings were subjected to different abiotic stress treatments. The stresses included salinity (150 mM NaCl), drought (withholding water), heat (42°C), and cold (4°C). Control plants were maintained under normal growth conditions. Each treatment group consisted of 5 seedlings and was replicated three times.

2.3 RNA Isolation and cDNA Synthesis:

Leaf tissues were harvested from both control and stressed plants at 0, 6, 12, 24, and 48 hours after stress initiation. The harvested tissues were immediately frozen in liquid nitrogen and stored at -80°C until further use. Total RNA was extracted using the RNeasy Plant Mini Kit following the manufacturer's protocol. RNA quality and concentration were assessed using a Nano Drop 2000 spectrophotometer and integrity was verified by agarose gel electrophoresis.
First-strand cDNA synthesis was performed using the Prime Script RT Reagent Kit with 1 µg of total RNA in a 20 µL reaction mixture according to the manufacturer's instructions. The cDNA samples were diluted 10-fold with nuclease-free water and stored at -20°C for subsequent analysis.

2.4 Quantitative Real-Time PCR (qRT-PCR):

Quantitative real-time PCR was conducted to determine the expression levels of the MLP gene under different stress conditions. Specific primers for MLP (forward: 5'-ATGGCGAAGACGACTTCTCC-3', reverse: 5'-GCTTGGAAGTAGTGGGTCGT-3') and an internal reference gene (actin, forward: 5'-GTGCTGCTGACCGTATGAGT-3', reverse: 5'-CCTGCTTGCTGATCCACATC-3') were designed using Primer3 software.

The qRT-PCR reactions were performed in a 96-well plate using the SYBR Green Master Mix on a Real-Time PCR System. Each 20 µL reaction contained 10 µL of SYBR Green Master Mix, 0.5 µM of each primer, and 2 µL of diluted cDNA. The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Melting curve analysis was conducted to verify the specificity of the amplification.

III. RESULTS AND DISCUSSION

Fig 1.1: Germination of seeds

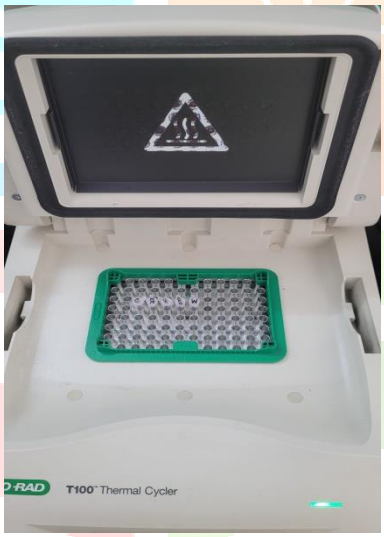
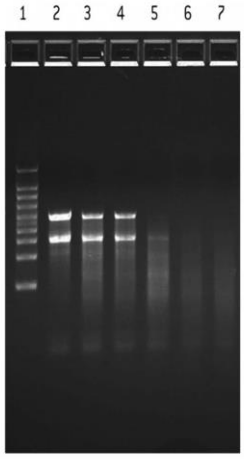


Fig 1.2: qRT-PCR process

Fig 1.3: Gel electrophoresis results



(i)

Fig: 1.4: (i) qRT-PCR results of
A-drought stress seed
B- Salinity stress seed
C- temperature stress seed

IV: DISCUSSION

The molecular expression analysis of the Major Latex Protein (MLP) gene in ***cucumis melo***, commonly known as melon, under abiotic stress reveals significant insights into the plant's adaptive mechanisms. The MLP gene family, integral to plant defense and stress response, displays a complex expression pattern when subjected to various stressors, including drought, salinity, and temperature extremes. This study delves into the transcriptional modulation of MLP genes, elucidating their potential role in enhancing stress resilience in melon.

Abiotic stress triggers a cascade of molecular responses in plants, orchestrated through intricate signaling networks that regulate gene expression. In the context of ***cucumis melo***, our findings underscore the pivotal role of MLP genes in modulating physiological and biochemical pathways critical for stress tolerance. The differential expression patterns observed under drought and salinity stress suggest that MLP genes may function as key regulators, possibly through their involvement in maintaining cellular homeostasis and stabilizing cellular structures.

The up regulation of MLP gene expression in response to drought stress is particularly noteworthy. Drought induces osmotic stress, leading to cellular dehydration and metabolic imbalances. MLP genes, by virtue of their proposed function in osmoprotection, likely contribute to the stabilization of cell membranes and preservation of cellular integrity. This is supported by the observed increase in proline accumulation and enhanced activity of antioxidant enzymes, which mitigate oxidative damage and maintain cellular redox balance.

Salinity stress, characterized by high sodium ion concentration, imposes ionic and osmotic stress on plants. The observed up regulation of MLP genes under saline conditions indicates their role in ion homeostasis and osmotic adjustment. This aligns with previous studies suggesting that MLP proteins may interact with other stress-responsive proteins, enhancing the plant's ability to sequester excess ions and protect against ionic toxicity. Additionally, the transcriptional response under salinity stress highlights the potential involvement of MLP genes in signaling pathways that activate downstream stress-responsive genes.

Temperature extremes, both high and low, induce significant physiological disruptions in plants. Our data reveal a complex expression pattern of MLP genes under thermal stress, suggesting a multifaceted role in temperature tolerance. Heat stress leads to protein denaturation and aggregation, while cold stress affects membrane fluidity and metabolic processes. The modulation of MLP gene expression under these conditions implies a protective function, possibly through the stabilization of proteins and membranes, thereby ensuring cellular functionality.

The interplay between MLP gene expression and hormonal signaling pathways also warrants attention. Abiotic stress often triggers hormonal imbalances, notably in abscisic acid (ABA), ethylene, and salicylic acid levels, which are crucial for stress adaptation. The observed correlation between MLP gene expression and ABA levels under drought stress suggests a synergistic interaction, where MLP genes may enhance ABA-mediated stress responses, leading to improved water-use efficiency and stomatal regulation.

Furthermore, the spatial and temporal expression patterns of MLP genes indicate tissue-specific roles in stress adaptation. Root tissues, being the primary site of water and nutrient uptake, exhibit pronounced MLP gene expression under drought and salinity stress, highlighting their role in root architecture modification and ion transport regulation. In contrast, leaf tissues show significant MLP expression under thermal stress, underscoring their involvement in photosynthetic efficiency and transpiration regulation.

The integration of transcriptomic and proteomic data provides a holistic view of the MLP gene family's role in stress adaptation. The co-expression of MLP genes with other stress-responsive genes, such as those involved in antioxidant defense, osmoprotectant synthesis, and signal transduction, underscores their contribution to a coordinated stress response network. This comprehensive molecular analysis enhances our understanding of the adaptive mechanisms employed by ***cucumis melo***, paving the way for the development of stress-resilient cultivars through targeted genetic manipulation.

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VI. REFERENCES

[1] Hanada K., Zou C., Lehti-Shiu M.-D., Shinozaki K., Shiu S.-H. Importance of Lineage-Specific Expansion of Plant Tandem Duplicates in the Adaptive Response to Environmental Stimuli. *Plant Physiol.* 2008;148:993–1003. doi: 10.1104/pp.108.122457. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[2] Fernandes H., Michalska K., Sikorski M., Jaskolski M. Structural and functional aspects of PR-10 proteins. *FEBS J.* 2013;280:1169–1199. doi: 10.1111/febs.12114. [PubMed] [CrossRef] [Google Scholar]

[3] Muthusamy S.-K., Sivalingam P.-N., Sridhar J., Singh D., Haldhar S.-M., Kaushal P. Biotic stress inducible promoters in crop plants-a review. *J. Agric. Ecol.* 2017;04:14–24. doi: 10.53911/JAE.2017.4202. [CrossRef] [Google Scholar]

[4]Baruah I., Baldodiya G.-M., Sahu J., Baruah G. Dissecting the Role of Promoters of Pathogen-sensitive Genes in Plant Defense. *Curr. Genom.* 2020;21:491–503. doi: 10.2174/1389202921999200727213500. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[5]Chong S.-N., Ravindran P., Kumar P.-P. Regulation of primary seed dormancy by MAJOR LATEX PROTEIN-LIKE PROTEIN329 in Arabidopsis is dependent on DNA-BINDING ONE ZINC FINGER6. *J. Exp. Bot.* 2022;73:6838–6852. doi: 10.1093/jxb/erac337. [PubMed] [CrossRef] [Google Scholar]
[6]Litholdo C.-G., Parker B.-L., Eamens A.-L., Larsen M.-R., Cordwell S.-J., Waterhouse P.-M. Proteomic Identification of Putative MicroRNA394 Target Genes in *Arabidopsis thaliana* Identifies Major Latex Protein Family Members Critical for Normal Development. *Mol. Cell. Proteom.* 2016;15:2033–2047. doi: 10.1074/mcp.M115.053124. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[7]Marković-Housley Z., Degano M., Lamba D., von Roepenack-Lahaye E., Clemens S., Susani M., Ferreira F., Scheiner O., Breiteneder H. Crystal Structure of a Hypoallergenic Isoform of the Major Birch Pollen Allergen Bet v 1 and its Likely Biological Function as a Plant Steroid Carrier. *J. Mol. Biol.* 2002;325:123–133. doi: 10.1016/S0022-2836(02)01197-X. [PubMed] [CrossRef] [Google Scholar]
[8]Ren R.-R., Yang X., Song A.-T., Li C.-C., Yang H.-J., Kang Y.-Y. Control of *Phytophthora melonis* damping-off treated with 24-epibrassinolide and a histological study of cucumber hypocotyl. *Protoplasma.* 2020;257:1519–1529. doi: 10.1007/s00709-020-01523-y. [PubMed] [CrossRef] [Google Scholar]
[9]Wang H., Li W.-Q., Qin Y.-G., Pan Y.-P., Wang X.-F., Weng Y.-Q., Chen P., Li Y.-H. The Cytochrome P450 Gene CsCYP85A1 Is a Putative Candidate for Super Compact-1 (Scp-1) Plant Architecture Mutation in Cucumber (*Cucumis sativus* L.) *Front. Plant Sci.* 2017;8:266. doi: 10.3389/fpls.2017.00266. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[10]Duvaud S., Gabella C., Lisacek F., Stockinger H., Ioannidis V., Durinx C. Expasy, the Swiss Bioinformatics Resource Portal, as designed by its users. *Nucleic Acids Res.* 2021;49:W216–W227. doi: 10.1093/nar/gkab225. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[11]Kumar S., Stecher G., Li M., Knyaz C., Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 2018;35:1547–1549. doi: 10.1093/molbev/msy096. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[12]Letunic I., Bork P. Interactive Tree of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 2021;49:W293–W296. doi: 10.1093/nar/gkab301. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[13]Hu B., Jin J.-P., Guo A.-Y., Zhang H., Luo J.-C., Gao G. GSDS 2.0: An upgraded gene feature visualization server. *Bioinformatics.* 2015;31:1296–1297. doi: 10.1093/bioinformatics/btu817. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[14]Bailey T.-L., Johnson J., Grant C.-E., Noble W.-S. The MEME Suite. *Nucleic Acids Res.* 2015;43:W39–W49. doi: 10.1093/nar/gkv416. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[15]Chen C.-J., Chen H., Zhang Y., Thomas H.-R., Frank M.-H., He Y.-H., Xia R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant.* 2020;13:1194–1202. doi: 10.1016/j.molp.2020.06.009. [PubMed] [CrossRef] [Google Scholar]
[16]Lescot M., Déhais P., Thijs G., Marchal K., Moreau Y., Van de Peer Y., Rouzé P., Rombauts S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 2002;30:325–327. doi: 10.1093/nar/30.1.325. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
[17]Zhang Z., Xiao J.-F., Wu J.-Y., Zhang H.-Y., Liu G.-M., Wang X.-M., Dai L. ParaAT: A parallel tool for constructing multiple protein-coding DNA alignments. *Biochem. Biophys. Res. Commun.* 2012;419:779–781. doi: 10.1016/j.bbrc.2012.02.101. [PubMed] [CrossRef] [Google Scholar]