Tracing Mobile RNA Motifs: Insights from k-mer Analysis in Grafted Plants

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Abstract

Mobile RNAs, including long RNAs (messenger RNA and long non-coding RNA) and small RNAs (small interfering RNA and micro RNA), are systematic signaling molecules that move across plant cells to execute essential functions such as growth, defense from pathogens, and reproduction. During grafting, the scion (shoot) and rootstock are cut and joined, re-establishing the connection of vascular tissue. This research creates tools for the exploration of specific sequences and structures that may enable mobile RNA molecules to escape the confines of their cell of origin to have a systemic impact within the plant. The reported results highlight a program created using Python to analyze and visualize mobile RNA in short sequences, namely k-mers. Using this program, we can analyze frequency distributions across k-mers of various lengths to detect what motifs are conserved in their plant of origin post graft, which motifs are transferred to the grafted plant, and, by extension, what phenotypes are expressed by this transfer. New knowledge acquired within the framework of this work further develops previous research on RNA-based signaling in plants by proposing that conserved RNA motifs could be leveraged for bioengineering applications. With this knowledge, we could manipulate the systemic mobility of mRNA in enhancing resilience in crops from pathogens, improving adaptation to various environments, and increasing yield in agriculture, all at a fraction of the cost and time of traditional plant breeding, revolutionizing the field of agricultural biotechnology.

Background

Mobile RNA signals are nucleic acids that cross cells, tissues, and between different organisms capable of regulating or signaling key biological processes. There are two major classes of mobile RNAs: long RNAs, including long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs), and small RNAs, including small interfering RNAs (siRNAs) and regulatory microRNAs (miRNA). Small RNAs have paved the way for understanding long-distance mobile signaling; however, the movement dynamics and mechanisms for the long RNA counterparts are far less understood.

Small RNAs are considered essential regulators of the expression of genes. In response to environmental or biological stress, vascular mobile small RNAs initiate regulation mechanisms meant to modulate the organism's response. This class of RNAs has demonstrated utility in communication across different tissues, organisms, and even between species, most notably in interactions with pathogens and parasitic species (Li et

al., 2024; Yan et al., 2022). Host-induced gene silencing illustrates the effectiveness of mobile signaling, where small RNAs are trafficked between a host and parasite to suppress critical genes within the parasite genome, providing a means for plants to protect against pathogen attack. These mechanisms position sRNAs as viable, sustainable alternatives to traditional pesticides for agricultural disease management (Koch et al., 2019). Additionally, sRNAs influence other vital functions, including stress response and nutrient allocation, which underscores the importance of mobile RNA studies, especially amid environmental challenges from climate change (Banerjee et al., 2006; Haywood et al., 2005). Despite our understanding of small RNAs in both functionality and mechanisms of mobility, the characterization of mobile mRNAs of plants remains poorly understood. A small handful of studies have functionally characterized and associated mRNA movements through tissues in grafted plants to support non-cell-autonomous regulation, the process by which a cell influences the behavior, function, or development of other cells through signaling mechanisms (Miller et al., 2020).

Tomato and eggplant plants were chosen for the grafting procedure due to their evolutionary link and graft compatibility. Both species are appropriate models for researching RNA mobility in grafted plants because they are members of the Solanaceae family. The two plants' divergence evolutionarily creates a diversity in their genomes that makes it possible to identify species-specific motifs that represent physiological or ecological adaptations and conserved motifs that are thought to support universal mechanisms of RNA mobility. Choosing these two specific nightshades allowed for cross-species comparisons of RNA mobility patterns by transferring mobile RNAs from the donor plant into the recipient plant through the graft junction. The work uses these features to explore the universality and specificity of sequence patterns that control RNA transport, a unique and pioneering endeavor in the field of plant biology and genetics.

The grafting process is an example of a selective mechanism of genetic material transfer, involving the transfer of only a subset of the donor's (either plant's) mobile mRNAs to the recipient plant rather than the entire transcriptome. This transfer is believed to be dependent on sequence-specific and structural features, with a significant role played by conserved k-mers identified in this study. These k-mers act as signals for mobility (Kirk et al., 2018). The movement mechanism occurs through the phloem, where RNA-binding proteins or transport complexes recognize these 'postal codes' and facilitate their systemic transport (Foster et al., 2002; Haywood et al., 2005). Only

mRNAs essential for inter-organ signaling, such as those governing stress responses, nutrition, or developmental timing, are mobilized thanks to the transfer's selectivity. Non-mobile transcripts, meanwhile, stay inside the cell from whence they originated. In order to maximize the graft's physiological compatibility and functional advantage without overpowering the recipient's genomic expression, this precision most likely represents an evolutionary adaptation to incorporate certain donor features (Yang et al., 2015). Emphasizing selective enrichment mechanisms offers a framework for bioengineering applications, such as creating synthetic RNAs with improved mobility to solve problems in agriculture.

Many open questions remain on the precision and accuracy of mobile mRNA targeting to functional destinations. Studies of the transport of small RNAs have revealed a canonical route of transport and function for these mobile signals. The movement mechanisms and functional impact of numerous putatively mobile mRNAs remain less clear. Studies in the past two decades have begun addressing these questions, providing evidence for the influence of mRNA sequence and structural motifs on their transport. The influence of these motifs provides evidence for a hypothesis that there are selective mechanisms to localize mRNAs in plants (Kanehira et al., 2010; Foster et al., 2002). Despite evidence for these motifs being sufficient for mobilizing previously non-mobile transcripts, bioinformatic identification of these motifs from published lists of known mobile mRNAs has been exceedingly difficult. to identify and leaves room for new methodology to identify and categorize these motifs. Issues in determining the factors that govern mRNA movement between tissues, in turn, result in an inability to screen for signaling function in a high throughput manner, i.e. the lack of data on the path of mRNA result in an inability to test for mRNA functionality. The nature of the function of mobile RNAs in plant signaling is complex, resulting in only a small handful of these mobile signals being robustly studied. A key question is the mechanistic basis of how the action of mobile RNAs can influence signaling and regulatory processes throughout the plant and how such mechanisms can be leveraged to address pressing needs in agriculture and bioengineering. Although much of the research on mobile sRNAs has identified how they participate in defense, stress tolerance, and developmental control, there are significant further steps regarding the mobile mRNA population. Mobile mRNAs, with their ability to circulate through plant tissues, may be well suited for developing new bioengineering strategies involving long-distance signaling. Manipulating these signaling pathways and applying targeted delivery of engineered signals has the potential to improve precision breeding,

which would, in turn, provide a system to revolutionize crop development, increase disease resilience, and improve plant adaptability in our ever changing climate. Even though a deep understanding of mRNA mobility may transform agriculture, mobile mRNAs' behavioral and functional mechanisms have not been studied as much depth as those of sRNAs (Zhang et al., 2016; Haywood et al., 2005).

This will be achieved by drawing parallels of our present knowledge of sRNAs by identifying mRNAs, a small but significant percent of which are mobile, and by dissecting pathways and factors conferring mobility. The discovery of the sequence motifs or structural properties responsible for systemic mRNA movement in plants will allow us to use these mechanisms to develop novel crop lines with improved systemic responses against abiotic stressors such as drought, nutrient deficiencies, and biotic stressors such as pathogen infection.

The knowledge derived would provide a base for the next generation in crop engineering: targeting the modulation of mobile mRNA pathways to underpin the improvement in growth, productivity, and stress tolerance, which are key needs for modern agriculture. Equally significant was the realization that the study of mobile mRNAs had far-reaching implications beyond agriculture into the core of innovations in bioengineering, ranging from sustainable agriculture to ecosystem restoration and even environmental monitoring. In developing the framework for understanding the behavior of mobile mRNAs, one moves beyond the realm of purely theoretical explorations into practical applications, triggering an avalanche of studies to further basic scientific knowledge and industry-relevant innovation.

mRNA mobility in grafted plants opens new perspectives in crop engineering, especially for improving tolerance against abiotic stresses such as drought and nutrient deficiencies. Knowledge of how mobile mRNAs mediate systemic signals over graft junctions will enable targeting specific gene expression toward improving drought tolerance, optimizing nutrient uptake, and modulating developmental responses under variable growing conditions. This will perhaps offer a means for mRNA to manipulate crops so that they intrinsically alter their physiological processes in response to environmental changes, supporting their sustainability and productivity. While technologies like CRISPR are exact methods of genetic modification, embedding mRNA mobility into such technologies brings another innovative route toward enhancing agricultural resilience.

While the enhancement of crops through mRNA manipulation does, at the same time, raise ethical concerns due to their possible impact on ecosystems and biodiversity,

these questions need critical investigation via well-conducted research. Using mobile RNAs as systemic regulators in grafted plants is something to watch out for, including undesirable results, such as changes to ecological equilibriums. Our analysis utilizes the Seekr Python library to pursue k-mers and pattern visualization within the mobile mRNA sequences, elucidating new insights that would further refine the current understanding of mRNA behavior in grafted plant systems. This approach will underpin practical outcomes for mobile RNA and provide the potential for revolutionary advances in the field of plant bioengineering consistent with the commitment to sustainable and adaptable agricultural solutions.

Materials and Methods

Tomato (*Solanum lycopersicum*) and eggplant (*Solanum melongena*) were selected for grafting due to their graft compatibility and genetic distance within the Solanaceae family, which provides a model for studying RNA mobility using inter species grafting. Their economic importance as grafted crops and established compatibility in previous grafting studies make them ideal candidates for investigating the movement of mRNAs in heterografted systems. Additionally, the use of inter species grafts allows for the identification of putatively conserved and species-specific RNA motifs associated with mobility, offering insights into potential mechanisms underlying systemic RNA transport in vascular plants

As part of a wet lab procedure in the lab of Dr. Frank, homografted (self-grafted) and heterografted (inter-species grafted) plants underwent total RNA isolation from whole-shoot or whole-root tissue every 4 hours for 48 hours under long-day growing conditions. This RNA was poly-A selected, to ensure only lncRNA and mRNA were captured, and sequenced using 150 bp paired-end Illumina RNA sequencing techniques. To ensure the highest quality of data only samples with over 5 million reads were retained and individual reads underwent quality control and trimming using the program Fastp. Mobile RNA was identified using heterografted samples. Computationally, this was done by finding species-specific sequences that could provide transcript identity and species of origin.

Using the transcript sequences for both species and the stand-alone program SeekR (Kirk et al., 2018) iterated over k-mer sizes of 2-mer to 6-mers enriched k-mers were identified in the pool of mobile RNAs relative to the total transcriptome. This was done for mobile RNAs identified in tomato (Solanum lycopersicum cv. M82) compared

to the same reference transcriptome and eggplant (Solanum melongena cv. BARI06) compared to the same reference transcriptome.

After this procedure was independently completed by Ms. Heeney, I performed Mobile RNA sequence analysis using a custom-built graphical user interface (GUI) developed in Python. This GUI, designed with user-friendliness in mind, integrated and analyzed the Pearson correlation matrix output generated by SeekR. To generate the GUI, I leveraged the Python package Tkinter (Lundh, 1999), allowing direct user interaction with the analysis tool. The GUI included error handling to ensure that both a test and reference RNA sequence file were selected before the analysis could proceed. If either file was missing, an error message was displayed using the messagebox.showerror() function. For all numerical computations, array manipulations, and data storage, the packages NumPy (Harris et al., 2020) and Pandas (McKinney, 2010) were used.

Initially, I loaded coding sequences, genomic sequences that contain spliced transcripts, from FASTA files using the Biopython SeqIO module with the function load_sequences, which converted the text file of sequences into a list format for analysis. The core analysis involved k-mer counting and comparison. Using the BasicCounter class from seekr.kmer_counts, the function kmer_analysis calculates k-mer counts for each sequence. These counts were then subjected to Pearson correlation analysis through seekr.pearson, comparing k-mers in test sequences to those in reference sequences. Significant motifs were identified with the function find_significant_motifs, which extracted k-mers that exceeded a user-defined correlation threshold using the line:

To begin the analysis, all motifs with correlation coefficients greater than the user-defined significance threshold (default = 0.5) were considered significant. The indices of significant motifs were extracted from the correlation matrix, and the corresponding k-mer motifs were identified. The significant motifs were listed in a non-editable text box, providing users with a clear and readable format to view the results. Finally, k-mer enrichment and depletion in the test set was visualized via a plot of summed-z-scored against k-mer identity faceted by k-mer size, ensuring a comprehensive understanding of the data.

Results

Comparing the set of mobile transcripts in eggplant to the set of all transcripts in eggplant I iterated over k-mer sizes of 2-mer to 6-mers (**Figure 1**). This was done to

find an optimal k-mer size that shows both enrichment and depletion of specific motifs.

```
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt

figure, axis = plt.subplots(1, 5)
for i in range(2,7):
    data = pd.read_csv("t" + str(i) + "sum_log_none.csv")
    axis[i-2].bar(data.iloc[:,0], data.iloc[:,1])
    #axis[i-2].hist(data.iloc[:,1])
    axis[i-2].set_title(str(i) + "-mer")
```

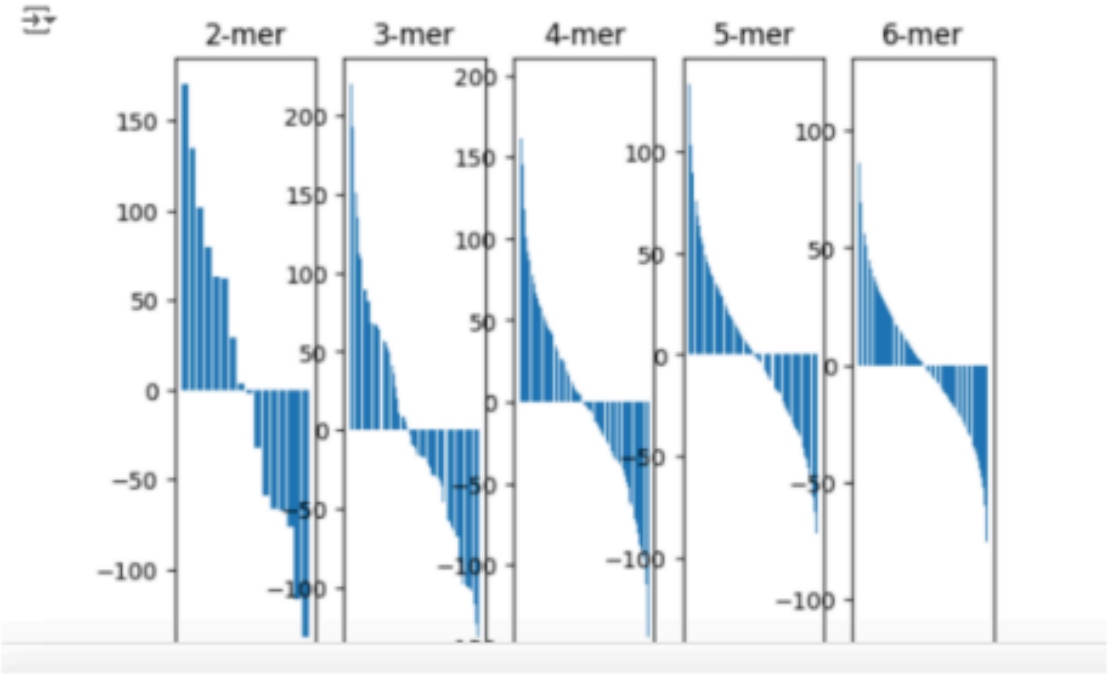

Figure 1: *Summed z-score against k-mer identity for eggplant k-mers enriched and depleted in mobile eggplant transcripts faceted by k-mer size. Enrichment distributions across different k-mer lengths, ranging from 2-mer to 6-mer. The x-axis in each faceted subplot represents specific k-mers (or sequences of length k), and the y-axis indicates the summed z-score for each k-mer, with positive and negative values illustrating the enrichment or depletion of the given k-mer in the mobile set compared to the reference set. The segment of code shown is part of the program that produced this output, given the Seek Pearson correlation matrix. The Python code identified specific k-mers as highly enriched in mobile RNAs, suggesting their association with mobility—a finding not previously reported in existing datasets. Conversely, it revealed certain sequences as significantly depleted, indicating potential inhibitors of mobility. Previous studies lacked this level of granularity in motif detection. K-mer size of 4-mer and 5-mer was selected for downstream analysis to optimally capture biologically relevant motifs. Image created by student researcher.*

Figure 1 gives the overview of k-mer frequency distribution from 2-mer to 6-mer in the dataset of mobile RNA sequences. In each faceted subplot, the k-mer identity is plotted along the x-axis. The y-axis is the summed z-score from the Pearson correlation matrix representing overall k-mer enrichment or depletion from the expected

frequency of 0. All k-mer sizes showed a similar distribution of enriched and depleted k-mers when abstracting the variable of k-mer identity (**Figure 1**). All k-mer sizes showed enrichment of some motifs above a summed z-score of 50 and depletion of some motifs below a summed z-score of -50. This indicates k-mers exist that are putatively associated with mobility and k-mers that are putatively negatively associated with mobility.

Figure 2 presents a significant comparison of k-mer enrichment between mobile eggplant transcripts (blue) and mobile tomato transcripts (orange). The y-axis, representing the overall k-mer enrichment or depletion from the expected frequency of 0, is a key indicator of the findings. The presence of overlapping enriched bars, in addition to the significance and Pearson correlation coefficient seen in **Table 1** for each of these species-specific k-mer sets, indicates the potential for evolutionarily conserved motifs that are associated with mobility with Solanaceous plants, and perhaps vascular plants generally. The presence of bars enriched in one species but depleted in the other indicates the potential for species-specific motifs that are associated with mobility within a given plant as well. These findings underscore the significance of RNA sequence on functional mobility, thus increasing our knowledge of k-mer enrichment and depletion patterns in different plant species.

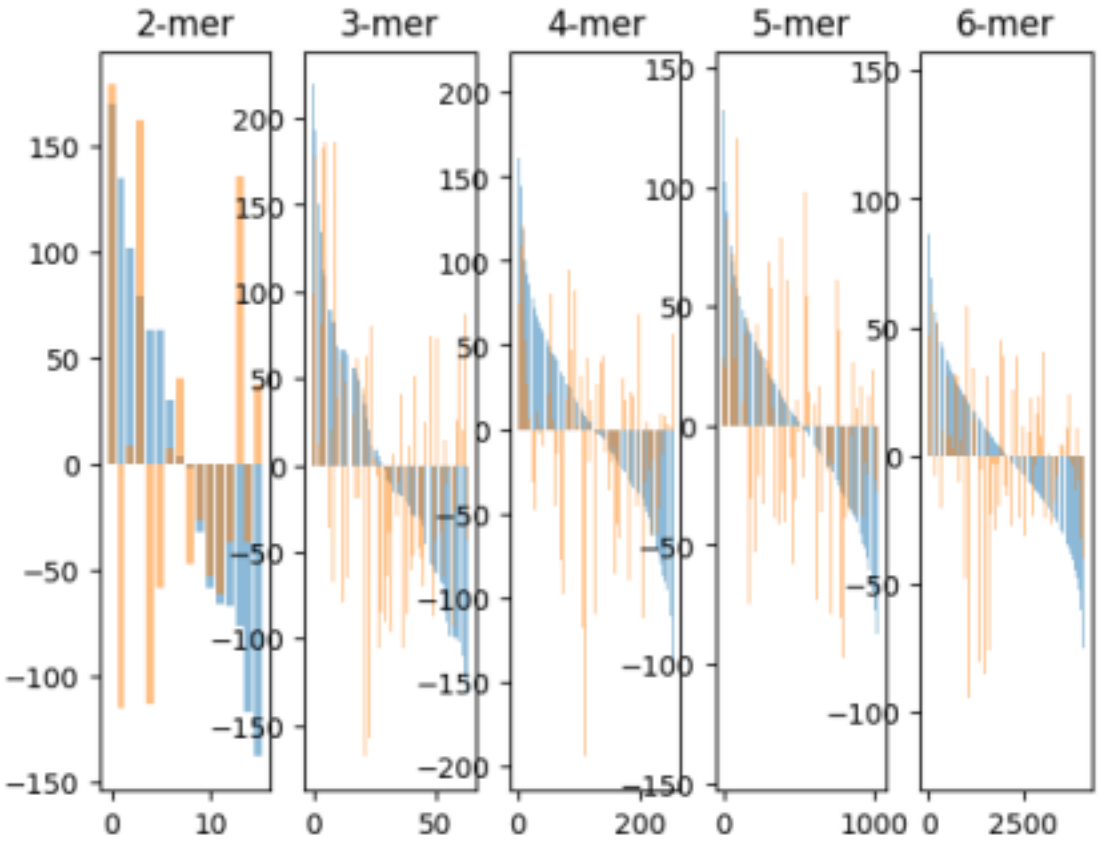

Figure 2:
Comparative enrichment distributions across different k-mer lengths, ranging from 2-mer to 6-mer, for both eggplant (blue) and tomato (orange) mobile transcripts. The x-axis in each faceted subplot represents specific k-mers (or sequences of length k), and the y-axis indicates the summed z-score frequency of occurrence for each k-mer, with positive and negative values illustrating the enrichment or depletion of the given k-mer in the mobile set compared to the reference set. K-mer size of 4-mer and 5-mer was selected for downstream analysis when considering comparative k-mer analysis. Comparative bar plots of k-mer frequency distributions for 2-mer to 6-mer sequences, with two datasets overlaid for each k-mer length. The two sets of bars, represented in different colors (blue and orange), indicate the frequency counts of k-mers in each dataset, while the x-axis reflects specific k-mers and the y-axis displays their frequency deviation. This comparative capability provided by the code is novel, as previous approaches were limited to single-species analyses and did not identify both conserved and species-specific mobility motifs. Image created by student researcher.

k-mer	*Pearson coeff.*	*p-value*
2-mer	*0.140*	*0.604*
3-mer	*0.362*	*3.24e-3*

4-mer	*0.425*	*1.18e-12*
5-mer	*0.399*	*1.62e-40*
6-mer	*0.286*	*3.39e-78*

Table 1. Given the overlaying k-mer distributions, as visualized in Figure 2, the Pearson coefficient and p-value associated with the correlation between eggplant and tomato mobile k-mer enrichment and depletion are given. As k-mer size increases p-value increases in significance. 2-mer remains insignificant, and 6-mer drops in Pearson correlation coefficient, making optimal k-mer size for downstream analysis 4-mer or 5-mer.

Discussion

Solanaceous plants, specifically eggplant and tomato, were selected for this experiment due to their graft compatibility. Because these species are 15 million years diverged genomically, observation of cross-species RNA mobility based on genetic distance between graft partners is possible. Moreover, Solanaceous plants have been used to study gene expression and grafting due to their economic and agricultural value as grafted crops. The Solanaceous family provides a robust framework for studying mRNA transport in grafted plants (L. Yang et al., 2023).

The comparative analysis of k-mers between eggplant and tomato mobile transcripts revealed overlapping enrichment patterns for specific k-mers, particularly at lengths of 4-mers and 5-mers. These conserved enriched k-mers suggested the existence of evolutionarily conserved motifs that are hypothesized to play a role in facilitating RNA mobility between organs within the plant. The relatively strong Pearson correlation coefficients for 4-mers (0.425) and 5-mers (0.399) and their highly significant p-values support this theory. Furthermore, non-overlapping motifs, as seen in distinct enrichment or depletion patterns between eggplant and tomato, indicate species-specific mobility signatures may exist. These unique motifs may reflect adaptations to each species' physiological or ecological needs, providing a line of evidence supporting both conserved and species-specific factors shape RNA mobility mechanisms.

The selection of 4-mer and 5-mer lengths for downstream analysis emerged as a critical finding. While 2-mers failed to show significant enrichment and 6-mers demonstrated reduced correlation coefficients, the intermediate lengths effectively captured biologically relevant motifs while maintaining computational efficiency. I hypothesize that these enriched motifs serve as ‘postal codes’ for RNA mobility, likely

serving as recognition signals for RNA-binding proteins or targeting guides during cell-to-cell transport. Enriched motifs in the mobile RNA pool but not in the total transcriptome strengthen the hypothesis that selective mechanisms enable specific RNAs to escape their cell of origin and move systemically.

The k-mer enrichment and depletion patterns observed in the study support the observation of non-random nature of mRNA mobility events, potentially governed by sequence- or structure-specific recognition processes. This selective enrichment of mobile RNAs aligns with the hypothesis that conserved motifs facilitate RNA mobility. The identification of species-specific k-mers highlights the complexity of these processes and suggests that long-distance transport may be tailored to unique environmental or physiological contexts. These findings provide a strong foundation for future experimental work and inspire further investigation into the direct interaction of the identified conserved motifs with RNA-binding proteins or other transport machinery components.

The study demonstrated that k-mer analysis is a valuable tool for uncovering the sequence features underlying mRNA mobility in plants. By identifying conserved and species-specific motifs, the research supports the hypothesis that certain sequences may enable RNA mobility. Additionally, this study created a foundation of motifs that can be biologically tested for their ability to mobilize traditionally non-mobile transcripts like CRISPR-CAS9, which can be leveraged for genome editing in agricultural biotechnology. Further applications include designing synthetic RNA molecules with enhanced mobility, ultimately contributing to developing resilient, high-yield crops. This potential offers hope for the future of precision plant breeding in our ever-changing world due to climate change.

Traditionally, the inability to define determinants controlling mRNA movement between tissues has hindered the analysis of signaling functions in a high-throughput manner largely because methods for following mobile RNAs and assessing their effectiveness were generally lacking. This paper has kept such challenges in focus while integrating interspecies grafting with advanced sequencing methodologies that take advantage of the unique genetic properties of both tomato and eggplant to identify and profile mobile mRNAs crossing graft junctions. This framework allows for a large-scale, high-resolution study of the sequence motifs, so-called k-mers, associated with mobility, unlike traditional methods, such as in situ hybridization or fluorescence tagging, that face severe limitations in throughput and scalability. It directly links motif enrichment to

functional RNA movement, therefore identifying conserved and species-specific motifs while establishing a generally extensible framework for testing RNA function that could be used for more effective bioengineering.

Addressing our central question, specific sequences, and structural features enable the systemic movement of mRNA molecules in grafted plants. We have gone a long way in deciphering the mechanisms underlying RNA translocation. Data from our k-mer analytical program unveiled distinctive motifs highly represented in mobile RNAs, showing that a sequence intrinsically could carry the property of being mobile within mRNA. The present finding has added to our understanding of sequence-dependent transport mechanisms that, so far, need to be better defined. Further testing will strengthen our findings by incorporating identified motifs into non-mobile RNAs and observing whether they confer mobility. We will further explore the interaction of these motifs with RNA-binding proteins to understand the molecular pathways involved. Future experiments will yield more information on the mechanisms of RNA mobility and enhance our capabilities for pattern recognition, which will assist in plant bioengineering and provide new information in the agricultural sector.

Conclusion

Our experimental approach identified motifs with SeekR and optimized parameters such as k-mer length to reveal the optimal k-mer size to balance computational resource use, as this positively scales with the k-mer size and relevance of k-mers identified. This approach filters out ubiquitous sequences to leave only those motifs that are enriched in the pool of bioinformatically identified mobile RNAs. This selectivity strengthens our inference by removing the significance of sequences ubiquitous across the transcriptome. At the same time, one needs to be aware of the important limitations of the approach adopted in this work:

1. The results here are preliminary and concern a minimal range of lengths of k-mers studied here, i.e., from 2-mer to 6-mer, and not including all possible k-mers.
2. Though SeekR is a potent tool in the analysis of k-mer enrichment, other algorithms or complementary methods (i.e., long short-term memory recurrent neural network) might give a more holistic outlook on the sequence motif associated with the mobility of RNA.

3. Although the input data was sampled over diurnal time, ensuring 80% of the transcriptome was expressed endogenously, further studies across developmental time and under combinatorial stress conditions would reveal a deeper set of mobile RNAs.
4. This study is limited to RNAs mobile in Solanaceous crop species. Expanding this body of work would be meaningful because the phenomenon of vascular-mobile RNA signaling is hypothesized to occur in all vascular plants.

Further research is warranted: repeating our experiment with a wider variety of plant species under different conditions will extend the range of applicability of our results. Comparisons can be drawn through further research aimed at diversities within mobile RNA motifs across different plant genera, especially those adapted to different environmental conditions. In this regard, further studies may help ascertain if certain k-mers confer increased mobility under particular environmental conditions, such as stress responses like high salinity or acute temperature levels.

Another route of further experimentation is expanding on insights generated from this analysis. Although our data indicate a potential correlation between mobility and k-mer presence, they do not suggest a plausible mechanism for how those conserved sequences enabled mobility.

Further studies are necessary to establish if specific k-mers, or combinations of multiple k-mer sequences, represent the sequence-specific signals sufficient for RNA mobility. One such experiment could be done by tagging synthetically generated non-mobile RNAs combinatorially with a library of enriched k-mer sequences and detecting the presence of these synthetic RNAs in different organ systems. This would provide evidence for sequences or structures that are sufficient to mobilize a previously non-mobile transcript. Further studies of the protein interaction partners of these RNAs will provide insight on potential mechanisms of movement.

Future analyses may ask whether enriched k-mers also serve functions shielding RNAs from macromolecule degradation.

Pairing the hypotheses gendered from this study with *in vivo* RNA imaging techniques, like single-molecule FISH, can visualize the cellular distribution of these RNAs or of all RNAs containing these motifs, potentially highlighting the precision and accuracy of targeted delivery from specific motif combinations.

Once we understand the dynamics and mechanisms controlling the mobility of

transcripts, we can begin to engineering novel signals to increase plant tolerance to abiotic and biotic stresses (Ali et al., 2022; Bhattacharjee et al., 2024), including working beyond basic research to applied work in crop improvement and sustainable agriculture.

Further, the findings from this study may lead to an improvement in the approaches to managing pests through HIGS, based on the design of mobile RNAs, which interfere with genes whose functions are crucial for pest survival (Koch et al., 2019.) Conserved k-mers can be exploited based on RNA mobility to develop RNA molecules that can be better translocated by the plants across their tissues toward the pests, where they can more effectively reduce the use of chemical pesticides. This application is in step with the aims of sustainable agriculture, providing an efficient, safe adjunct to the classical methods of plant pest control.

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