Brief Communication

Use of microRNA-encoded peptides to improve agronomic traits

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MicroRNAs (miRNAs) are small regulatory RNA molecules (21–24 nt) regulating the expression of target genes at the post-transcriptional level, by cleaving their mRNA. Because these target genes are mainly regulatory genes, miRNAs are involved at the crossroads of several biological processes (Liu et al., 2018). miRNAs are transcribed as long primary transcripts (pri-miRNAs) (Xie et al., 2005). Recent findings revealed that plant pri-miRNAs encode regulatory peptides called miRNA-encoded peptides (miPEPs) (Lauressergues et al., 2015; Sharma et al., 2020). miPEPs specifically enhance the transcription of their pri-miRNA, leading to phenotypes consistent with the functions of their cognate miRNA. Only a few miPEPs have been found as useful tools in agronomy. Indeed, soybean miPEP172c was shown to increase nodulation, aiming for a better yield (Couzigou et al., 2016), and grapevine miPEP171d increased adventitious root formation (Chen et al., 2020). In both cases, the role of the corresponding miRNA was previously known. Here, we questioned whether it would be feasible to screen a whole set of miPEPs for a particular phenotype in Arabidopsis thaliana, and whether it was possible to transpose these data to plants of agronomic interest.

We focused our study on the identification of A. thaliana miPEPs modulating root development. In the perspective to transpose our results from A. thaliana to other species of agronomic interests, we narrowed our analysis on miRNAs present in most plant species (conserved miRNAs). The first step consisted of identifying A. thaliana pri-miRNAs, by crossing the genome, and we next searched the corresponding miPEPs in families of agronomic interest, we searched the corresponding pre-miRNA on the genomes of the wild cabbage Brassica oleracea, and Barbarea vulgaris, a weed, which can be a seed contaminant (MacDonald and Cavers, 1991). We were able to identify only one miRNA homolog in each of the corresponding genome, and we next searched the corresponding miPEPs in these pre-miRNAs and defined miPEPs in B. oleracea and B. vulgaris by the identification of the first ORF (Fig. 1a, b, c). We next cultivated B. oleracea and B. vulgaris seedlings in vitro, treated or not with the corresponding miPEP and monitored their root development. Barbarea vulgaris BvmiPEP164b was able to decrease root development (Fig. 1f) while B. oleracea BomiPEP397a was able to increase cabbage root development (Fig. 1h), showing that miPEPs exhibit a remarkable functional conservation of their function between species.

The overall development of a plant is closely subordinated to root development, and the modulation of root growth directly affects the development of aerial parts accordingly. So, we tested the effect of BomiPEP397a and BvmiPEP164b on cabbage and B. vulgaris growth, respectively. We found that watering B. vulgaris plants with BvmiPEP164b led to a 21% decrease in the foliar surface (Fig. 1g, j), while watering cabbage plants with...
BomiPEP397a led to a 36% increase in the foliar surface (Fig. 1i, k). Finally, we validated that BvmiPEP164b and BomiPEP397a were able to increase the expression of their respective pri-miRNA while decreasing the expression of the corresponding target genes (Fig. 1l, m).

The identification of a whole set of miPEPs in certain plant species can be difficult due to the lack of strong genomic and transcriptomic data. We show here that a screening of a high number of miPEPs in A. thaliana followed by the identification of homologs in plants of interest is an efficient method to identify miPEPs active on a particular phenotype. In that way, we show that watering plants with miPEPs which modulate root growth can lead to an overall change in plant development, thus being a suitable alternative to the use of chemicals in agronomy.

Weeds are one of the major problems of world agriculture. While some countries try to strongly restrict the use of chemicals, more and more plants are starting to be resistant to these chemicals. Since miPEPs are very specific (Lauressergues et al., 2015), we can imagine the use of a cocktail of several peptides to improve the development of crops and their resistance to stresses (pathogens, starvation…) while reducing weed growth.

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Conflict of interest

Authors declare no conflict of interest.

Author contributions

JPC designed the research; JPC, BG and MO performed the molecular biology and plant experiments; HSC performed bioinformatics analysis; BG analysed screening data; JPC, PT and SP wrote the paper.

References

Chen, Q.-j., Deng, B.-H., Gao, J., Zhao, Z.-Y., Chen, Z.-i., Song, S.-R., Wang, L. et al. (2020) A miRNA-encoded small peptide, wmi-miPEP171d1, regulates adventitious root formation. Plant Physiol. 183, 656–670.

Couzigou, J.M., André, O., Guillotin, B., Alexandre, M. and Comber, J.P. (2016) Use of microRNA-encoded peptide miPEP172c to stimulate nodulation in soybean. New Phytol. 211, 379–381.

Couzigou, J.M., Lauressergues, D., André, O., Gutjahr, C., Guillotin, B., Bécard, G. and Comber, J.P. (2017) Positive gene regulation by a natural protective miRNA enables arbuscular mycorrhizal symbiosis. Cell Host Microbe, 21, 106–112.

Lauressergues, D., Couzigou, J.M., Clemente, H.S., Martinez, Y., Dunand, C., Bécard, G. and Comber, J.P. (2015) Primary transcripts of microRNAs encode regulatory peptides. Nature, 520, 90–93.

Liu, H., Yu, H., Tang, G. and Huang, T. (2018) Small but powerful: function of microRNAs in plant development. Plant Cell Rep. 37, 515–528.

MacDonald, M.A. and Cavers, P.B. (1991) The biology of Canadian weeds. 97. Barbarea vulgaris R. Br. Can. J. Plant Sci. 71, 149–166.

Sharma, A., Badola, P.K., Bhatia, C., Sharma, D. and Trivedi, P.K. (2020) Primary transcript of miR858 encodes regulatory peptide and controls flavonoid biosynthesis and development in Arabidopsis. Nat. Plants, 6, 1262–1274.

Wang, S., Quan, L., Li, S., You, C., Zhang, Y., Gao, L., Zeng, L. et al. (2019) The PROTEIN PHOSPHATASE54 complex promotes transcription and processing of primary microRNAs in Arabidopsis. Plant Cell, 31, 486–501.

Xie, Z., Allen, E., Fahlgren, N., Calamara, A., Givan, S.A. and Carrington, J.C. (2005) Expression of Arabidopsis MiRNA genes. Plant Physiol. 138, 2145–2154.

Zhang, Q.-i., Su, L.-y., Zhang, S.-T., Xu, X.P., Chen, X.-H., Li, X., Jiang, M.Q. et al. (2020) Analyses of microRNA166 gene structure, expression, and function during the early stage of somatic embryogenesis in Dimocarpus longan Lour. Plant Physiol Biochem. 147, 205–214.