

# IDENTIFICAÇÃO DOS MECANISMOS ENVOLVIDOS NA RESILIÊNCIA DA VIDEIRA À SECA – ESTUDO FOCADO EM CASTAS PORTUGUESAS

## UNRAVELLING THE MECHANISMS INVOLVED IN GRAPEVINE RESILIENCE TO DROUGHT STRESS – A FOCUS ON PORTUGUESE CULTIVARS



### RESUMO

A videira (*Vitis vinifera* L.) é uma cultura bem adaptada ao clima mediterrânico, no qual as plantas são frequentemente expostas a períodos de seca. No entanto, o aumento da frequência e severidade de ondas de calor e episódios de seca pode resultar em impactos negativos na produção e qualidade da uva. Por esse motivo, os investigadores têm reunido esforços para entender a resposta da videira à seca, com o objetivo de identificar e selecionar genótipos mais resilientes.

Este estudo visa contribuir para esta linha de investigação através de uma abordagem que integra diferentes ómicas (transcriptómica, proteómica, metabolómica e metagenómica) em variedades portuguesas com comportamentos diferentes sob condições de seca. Seis cultivares portuguesas utilizadas na produção de vinho tinto (três com elevada tolerância e três identificadas como sensíveis à seca), selecionadas do campo ampelográfico das vinhas do Esporão, serão usadas para todas as análises integradas no projeto Vine&Wine – BioGrapeSustain.

Para cada cultivar, o mRNA e miRNA serão analisados por RNAseq e os dados serão validados por RT-qPCR. Para análise funcional, será seguida uma abordagem transgênica por silenciamento dos miRNA e/ou do seu alvo. Para a análise do proteoma, será utilizada eletroforese bidimensional (2-DE) para investigar diferenças ao nível das proteínas entre cultivares com alta e baixa tolerância à seca. Proteínas diferencialmente expressas serão posteriormente identificadas por LC-ESI-MS/MS. Metabolitos extraídos de folhas e frutos serão analisados por ESI-FT-ICR-MS. Uma análise estatística permitirá identificar os compostos mais relevantes na resposta à seca, em particular os metabolitos que poderão interferir na qualidade do vinho. Após seleção dos metabolitos de interesse, será realizada uma análise LC-MS/MS para quantificação dos mesmos. Adicionalmente, será realizada uma análise metagenómica a nível dos frutos para estudar o microbioma da videira, com o objetivo de identificar comunidades bacterianas e fúngicas que possam estar associadas à resiliências das plantas à seca, e que poderão ser usadas para melhorar a tolerância dos genótipos sensíveis.

### ABSTRACT

Grapevine (*Vitis vinifera* L.) is well-adapted to the Mediterranean climate in which plants are often exposed to periods of drought. Nevertheless, the increase in the frequency and severity of heat waves and drought events can have negative impacts on grape yield and quality. Therefore, researchers have been gathering efforts on understanding grapevine response to drought stress, aiming to identify and select more resilient genotypes.

This study aims to contribute to this research line by following an integrative multiomics approach (transcriptomics, proteomics, metabolomics and metagenomics) in Portuguese cultivars that behave differently upon drought stress. Six Portuguese grapevine cultivars used for red wine production (three with high tolerance and three identified as sensitive to drought stress), selected from the ampelographic field of Esporão's vineyards, will be used for all analysis integrated in the project Vine&Wine – BioGrapeSustain.

For each cultivar, mRNA and miRNA will be analysed through RNAseq, and data will be validated by RT-qPCR. A transgenic approach by silencing the miRNA and/or its target will be followed for functional analysis. For proteome analysis, two-dimensional gel electrophoresis (2-DE) will be used to investigate differences at protein levels between high and low drought tolerant cultivars. Differentially expressed proteins will be further identified by LC-ESI-MS/MS. Metabolites extracted from leaves and fruits will be analysed by ESI-FT-ICR-MS. Statistical analysis will allow the identification of the compounds that are most relevant in drought stress response, particularly the metabolites that could interfere in wine quality. After selecting the interesting metabolites, an LC-MS/MS analysis will be performed for quantification. Additionally, a metagenomic analysis will be performed at fruit level to study grapevine microbiome, with the goal of identifying bacterial and fungal communities that could be related with plant resilience to drought stress, which could be further used to improve tolerance of drought sensitive genotypes.

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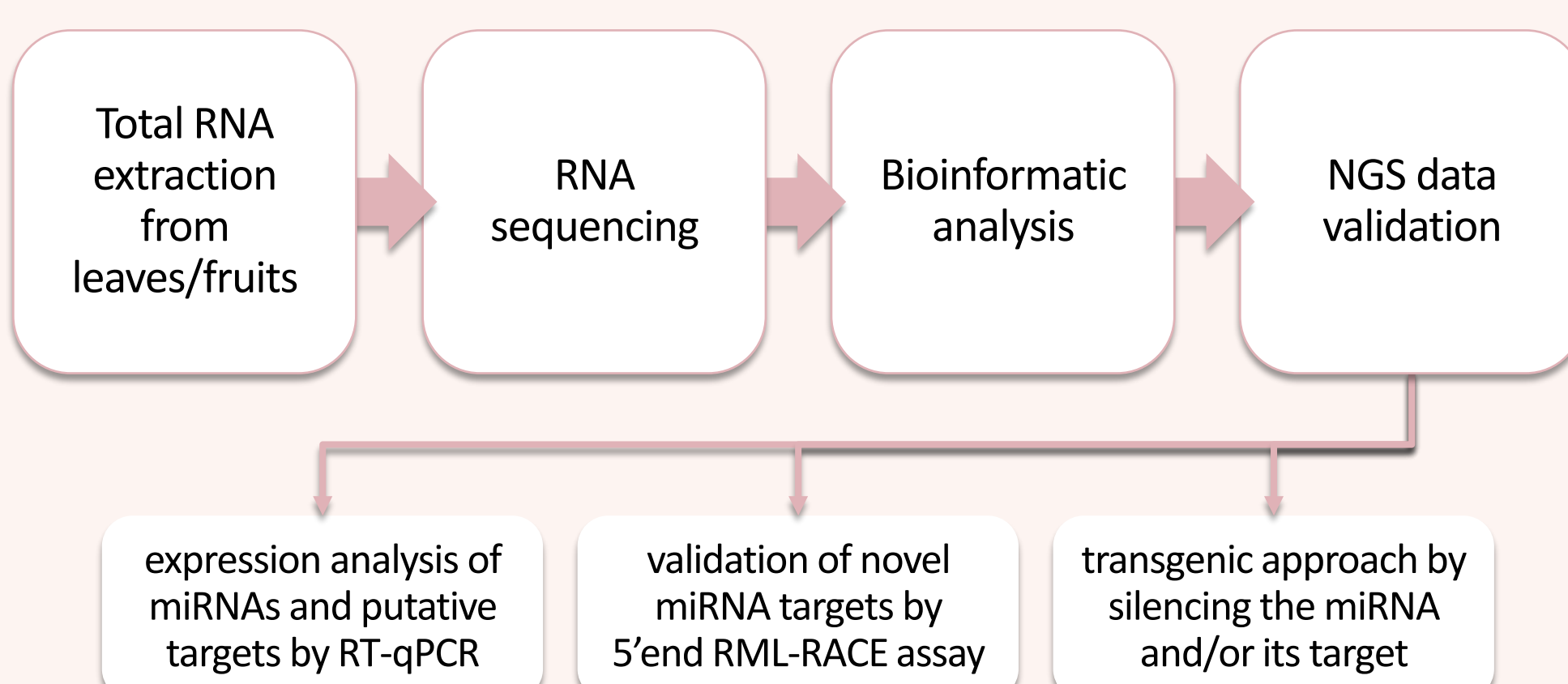
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### OBJECTIVE AND METHODS

This research plan is directed to understand the mechanisms of high plasticity over environmental stresses presented by existing grapevine genotypes. The main goal is to investigate the mechanisms involved in plant acclimatization, contributing for the improvement of Portuguese grapevine cultivars by increasing drought stress tolerance.

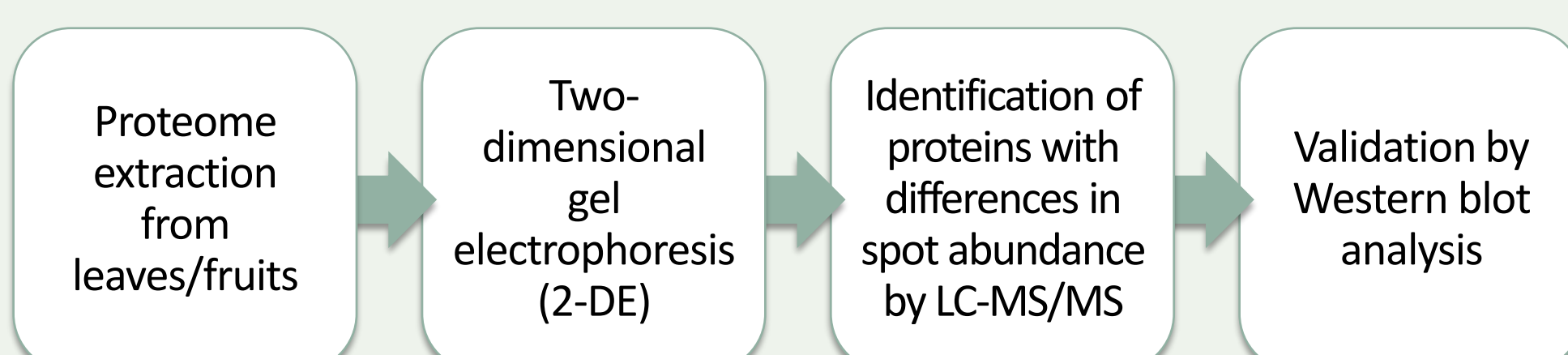
#### Transcriptome Analysis

To identify miRNA-mRNA pairs associated to a differential response to drought stress among grapevine cultivars



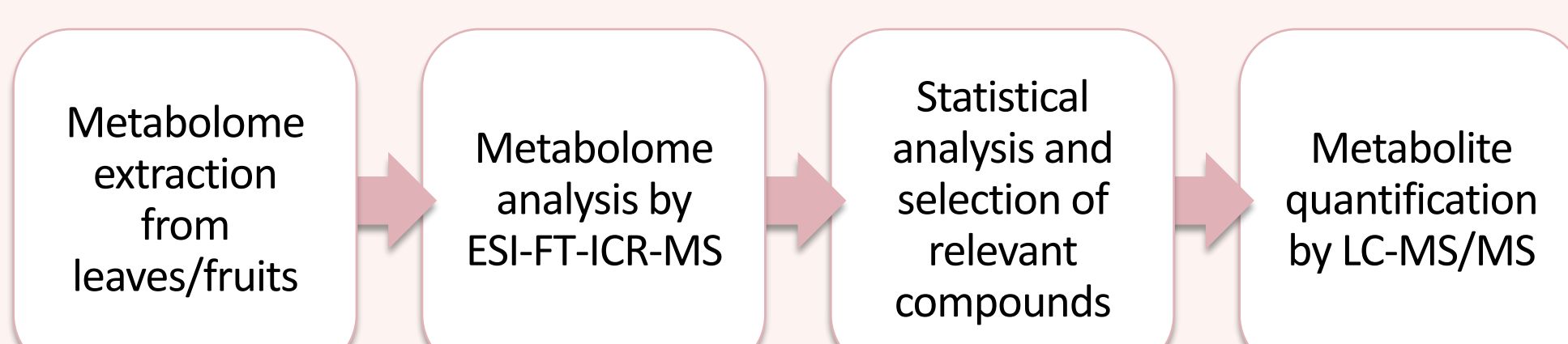
#### Proteome Analysis

To identify biomarkers associated with drought stress tolerance in grapevine



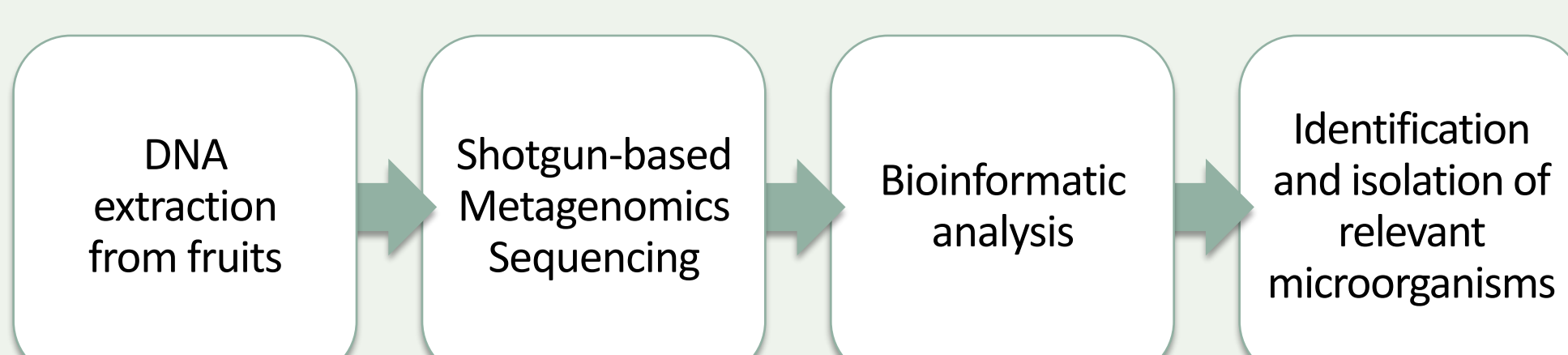
#### Metabolome Analysis

To detect the activation of specific metabolic pathways related to drought stress



#### Microbiome Analysis

To identify bacterial and fungal communities associated with drought stress tolerance in grapevine



### EXPECTED RESULTS

- By comparing the results obtained for drought tolerant and drought sensitive cultivars, an integrative analysis will allow to characterize molecular mechanisms associated with grapevine resilience to drought stress, which can be further used to improve the tolerance of drought sensitive genotypes.
- The results from the microbiome analysis will allow to identify bacterial/fungal endophytes associated with plant resilience upon drought stress and wine typicity, which will be further tested for application in nurseries to assess their ability to protect sensitive plants against drought stress.
- Overall, this research will provide important knowledge about grapevine response mechanisms and how they can be used as a tool to improve drought stress tolerance in sensitive genotypes, thereby contributing for the preservation of endogenous cultivars and the sustainability of this agronomical sector.

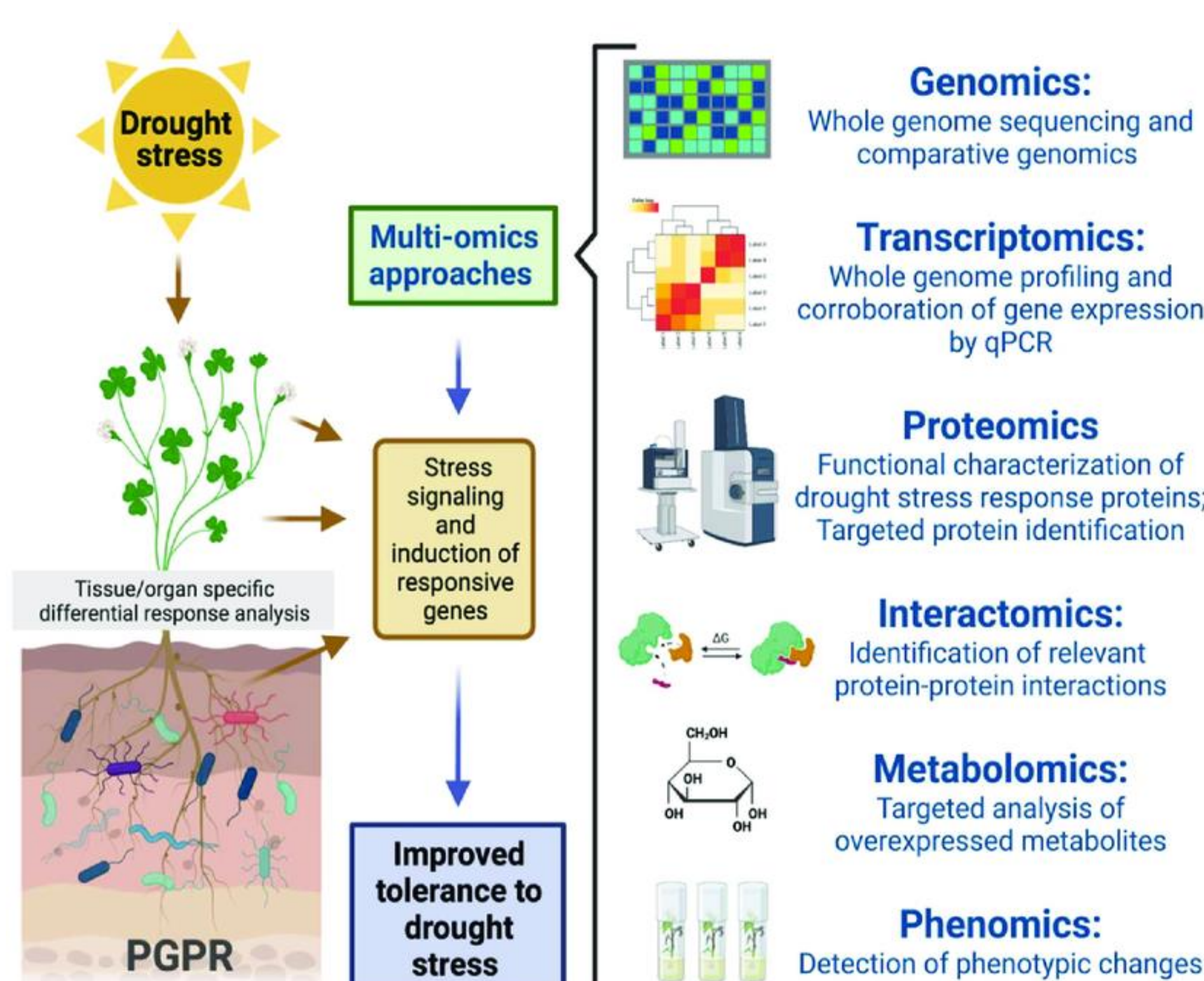
### ADDITIONAL INFORMATION

#### References

Fadiji, A. E., Orozco-Mosqueda, M. del C., Santos-Villalobos, S. de los, Santoyo, G., & Babalola, O. O. (2022). Recent Developments in the Application of Plant Growth-Promoting Drought Adaptive Rhizobacteria for Drought Mitigation. *Plants* 2022, Vol. 11, Page 3090, 11(22), 3090. <https://doi.org/10.3390/plants11223090>

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(Fadiji et al., 2022)

