



Plant Metabolic Networks Under Stress: a Multi-species/Stress Condition Meta-analysis

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Abstract

Plant stress acclimation depends on metabolic changes. However, the knowledge concerning the modularity and robustness of plant metabolic networks under stress conditions remains fragmented. Here we carried out a multi-species/stress condition meta-analysis using previously published metabolite profiling data from plants under water deficit, cold, salt and nitrogen deprivation stress conditions. We carried out extensive network and multivariate analyses aiming to identify stress biomarkers and to investigate how plant metabolic network is altered in terms of topology and connectivity by stress conditions. Partial least squares discriminant analysis (PLS-DA) was effective in differentiating stressed from non-stressed plants at a metabolic level. However, no general pattern in both density and heterogeneity of the metabolic network was observed after stress imposition. Integrative analysis identified metabolic markers for multiple stresses in plants, including asparagine, shikimate, fructose and raffinose. This analysis further highlights that amino acid metabolism is a major hub for plant stress acclimation. This idea is supported by the fact that several amino acids related to photorespiration and that are used as substrates for alternative plant respiration pathways or the synthesis of secondary metabolites were found as key for the structure and modulation of the network under stress. Our results collectively suggest that stress-induced changes in metabolic network topology are species/stress level-specific. However, several hubs related to amino acid metabolism emerge as key nodes in the network when plants are subjected to stress conditions, highlighting that our approach can be used by systems-driven plant breeding programs toward plant stress tolerance improvement.

Keywords Biological networks · Hubs · Metabolomics · Plant metabolism · Plant systems biology

1 Introduction

The global population is expected to increase up to 9.5 billion by 2050 (USCB, 2015), which will require substantial increases in primary foodstuff production (Zandalinas et al. 2021). According to FAO, the production of staple cereal crops must double by 2050 to meet the global food demand (FAO 2017). In parallel, it has been shown that stressful periods triggered by the climate change scenario are negatively affecting the yield of several crops cultivated worldwide (Birami et al. 2020; Bisbis et al. 2018; Rosenzweig et al.

2014; Trisos et al. 2020; Ye and Fan 2021). The increase in demand for primary foodstuffs is thus outstripping increases in crop yield, given that yield improvements are slowing or stagnating as the approaches of the green revolution reach their biological limits (Long et al. 2015). It is thus important to find strategies to improve plant productivity in a highly stressful environment (Evans and Lawson 2020; Yoshida and Yamaguchi-Shinozaki 2021). To achieve this, understanding the mechanisms that regulate plant stress responses is key for the development of new strategies to simultaneously improve crop yield and stress tolerance through metabolic engineer, synthetic biology and/or de novo domestication of wild crop relatives (Fernie and Yan 2019; Kubis and Bar-Even 2019; Wurtzel et al. 2019; Zsögön et al. 2018). However, unfortunately, our knowledge concerning plant stress responses has been limited by the failure to consider the regulation in a systemic perspective (Neto et al. 2021; Souza et al. 2016; Sweetlove and Fernie 2005). Our understanding about structure, modulation and robustness of plant networks

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is thus very scarce, which explains the lack of success in obtaining stress-tolerant crops (Flexas 2016).

As sessile organisms, plants are constantly subjected to stress conditions (Galviz et al. 2022). A common phenomenon observed in plants under stress is the generation of different strains, i.e. when a stress factor generates different types of stress responses in plant cells (Kranner et al. 2010; Lichtenthaler 1998). Furthermore, different types of (a)biotic stress lead to common responses in plants (Zandalinas et al. 2022; Zandalinas and Mittler 2022). For instance, stomatal closure has been reported as one of the primary plant responses to an internal osmotic stress (Jones 1998), which can be induced by water deficit, excess of salt, extreme temperatures and other adverse environmental conditions (Gago et al. 2020). Given that the stomatal pore is the first barrier for the influx of CO₂ for photosynthesis (Lima et al. 2018), the stress-induced stomatal closure reduces the amount of substrate for CO₂ assimilation and consequently for the entire metabolism (Auler et al. 2022). It is expected therefore that certain metabolic changes may be similarly altered between different types of stress. Indeed, it has been shown that the accumulation of sugars, amino acids and polyols is commonly observed in plants under stress (Avin-Wittenberg et al. 2015; Barros et al. 2017; Batista et al. 2019; Domingues-Junior et al. 2019; Fonseca-Pereira et al. 2019; Merchant et al. 2006; Merchant and Richter 2011; Obata and Fernie 2012; Pires et al. 2016). However, given the complexity of plant metabolism (Sweetlove and Fernie 2013), reductionist approaches have failed in unveiling how these metabolic changes regulate plant stress acclimation. This is because some emergent properties are fundamental to understand the functioning of complex biological systems, such as plants (Barabási 2009; Bertolli et al. 2014; Lüttge 2021; Neto et al. 2021; Souza et al. 2004). This implies that it is not possible to understand stress acclimation mechanisms by only looking at the parts, highlighting the need for systemic analysis (Neto et al. 2021).

Systems biology approaches have been successfully used to understand the modulation of networks of a wide range of biological systems (Barabási 2009; Mitchell 2006), especially microorganisms that served as a basis for the establishment of network theories (Albert and Barabási 2002; Almaas et al. 2004; Jeong et al. 2001). For instance, the lethality and centrality theory of network biology suggests that nodes of the yeast protein–protein interaction network with a higher degree of connections (known as hubs) have a high probability to be essential to the organism, i.e. mutation in hubs has a high probability to be lethal to the organism (Jeong et al. 2001). However, plant protein–protein interaction network analysis demonstrates that hubs of the plastidial redox network

are in fact important for plant growth, but not essential (Souza et al. 2019). In fact, plants may have fewer lethal nodes given their higher genetic and biochemical plasticity when compared to animals and microorganisms, which is a fundamental characteristic acquired by a sessile organism along evolution (Daloso 2014; Leitch and Leitch 2008; Valladares et al. 2002). The higher biochemical plasticity is evidenced by the fact that plants have several isoforms of enzymes that carry high metabolic control coefficients (Araújo et al. 2012) coupled to a complex, highly connected and spatially distributed redundant and compensatory systems responsible for post-translational regulation of plant metabolic pathways (Friso and van Wijk 2015; Geigenberger et al. 2017; O’Leary and Plaxton 2020; Souza et al. 2019; Sweetlove et al. 2017). These characteristics aid plants to grow and survive in highly stressful environments, but it makes it difficult to manipulate plant metabolism toward a desired state such as plants with improved growth and stress resilience (Razaghi-Moghadam and Nikoloski 2021; Sweetlove et al. 2014, 2017).

Biological systems are known to have scale-free networks, in which the number of nodes and links follow a power law distribution, i.e. few nodes have a high number of links (Albert 2005; Barabási et al. 2003; Barabási 2009; Broido and Clauset 2019). The hubs are important to the maintenance of the network structure, but they are also more vulnerable to external attacks (Albert et al. 2001; Albert and Barabási 2002; Barabási and Oltvai 2004). Therefore, hubs have been pointed out as important elements for the stability of the networks (Souza and Lüttge 2015), setting them as prominent players of biological networks. However, the controllability of real networks, i.e. the capacity to control a system to a desired final state by, for example, modifying the expression of a certain gene, is not driven by the hubs of the network (Liu et al. 2011). Thus, it is not clear which nodes of the network should be the focus of systems-driven plant breeding programs. This highlights the need to improve our knowledge regarding the modulation of networks under stress, especially concerning plant metabolic networks in which information is scarce. Taking this into account, we have investigated how the topology and the connectivity of metabolic networks are altered when plants are subjected to stress conditions. We carried out a meta-analysis of previously published metabolite profiling data from Arabidopsis, rice, grapevine, soybean and sugarcane under water deficit, eucalyptus under cold stress, sugarcane cell suspension under nitrogen (N) starvation and *Egletes viscosa* (L.) Less under salt stress. Metabolomics data was used to construct correlation-based networks, which, coupled to multivariate analyses, allowed us to identify stress metabolic markers as well as to investigate how stress imposition alters the topology and connectivity of metabolic networks.

2 Material and Methods

2.1 Meta-analysis Approach

We carried out a meta-analysis of previously published metabolite profiling data obtained from plants under different stress conditions. No new experiment has been performed in this work. Our meta-analysis approach is then solely based in previously performed experiments. In Sects. 2.2 and 2.3, we briefly describe how the plants were grown, how the stress treatments were imposed and how the metabolite profiling was carried out.

2.2 Plant Material, Growth Conditions and Stress Treatments

Rice (*Oriza sativa* cv. Nipponbare) plants were grown under greenhouse conditions (natural sunlight photoperiod, 28 ± 3 °C, relative humidity 60%) and submitted to five hydric treatments using the soil matric potential (SMP, kPa) as soil moisture measurement. Plants were kept under well-watered (0 kPa, control), mild drought (-9.8 kPa), moderate drought (-31 kPa), heavy drought (-309.9 kPa) and severe drought conditions (Sds, severe dehydration stress in soil containers, in which the irrigation was interrupted) (Todaka et al. 2017).

Grapevine (*Vitis vinifera* L.) “Grenache” plants were grown under field natural conditions (natural sunlight photoperiod, maximum of $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 32 ± 5 °C, relative humidity 55.57%) and submitted to two hydric treatments, well-watered (WW, control) and water deficit (WD) (Gago et al. 2017).

Sugarcane (*Saccharum* spp.) plants were cultivated under greenhouse conditions (natural sunlight photoperiod, average temperature 25.8 °C) and subjected to three hydric treatments using the leaf water potential (LWP, MPa) as plant hydration measurement. Plants were kept under well-watered (control), moderate drought (-0.5 MPa) and severe drought (-1 MPa) conditions (Vital et al. 2017).

Arabidopsis thaliana plants were grown under growth chamber conditions (8/16 h light/dark photoperiod, $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 19 ± 3 °C, relative humidity $67 \pm 7\%$) and submitted to six hydric treatments, well-watered (control), one or two drought cycle events, rehydration of control and after one or two drought cycle events (Fonseca-Pereira et al. 2019).

Two eucalyptus plant species (*Eucalyptus globulus* and *E. Grandis*) were grown in greenhouse and acclimated under growth chamber conditions (12/12 h light/dark photoperiod, $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, average temperature

25 °C). After acclimation, they were subjected to two thermic treatments, ambient temperature of 25 °C (control) and 10 °C (cold) (Domingues-Junior et al. 2019).

Egletes viscosa L. plants were cultivated under greenhouse conditions (12/12 h photoperiod, 29 ± 3 °C, relative humidity 63.5%) and subjected to three treatments, irrigated only with water (control), irrigated with doses of 40 or 80 mM NaCl (Batista et al. 2019).

Soybean (*Glycine max* L.) plants were grown under greenhouse conditions (natural sunlight photoperiod, 25 ± 10 °C, relative humidity $75 \pm 10\%$) and submitted to four hydric treatments using LWP as plant water status measurement, well-watered (control), moderate drought (-1 MPa), severe drought (-1.5 MPa) and rehydration after severe drought (Mesquita et al. 2020).

Cell cultures of sugarcane were cultivated in MS medium (Murashige and Skoog 1962) supplemented with vitamins, 3% sucrose and 3 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and subjected to three nitrogen treatments, non-stressed (full MS, control), slightly nitrogen deficient (MS with only 12 mmol L^{-1} of nitrate (NO^{-3}); N 30%) and completely nitrogen deficient (MS without NO^{-3} , N 0%) (Bottcher et al. 2021).

2.3 Metabolomics Analysis

Shoots from rice, whole rosettes from *Arabidopsis thaliana* and leaves from grapevine, eucalyptus, sugarcane, eucalyptus, soybean and *Egletes viscosa* were collected and immediately frozen in liquid nitrogen for metabolite profiling analysis. The extraction, derivatization and gas chromatography coupled to mass spectrometry analysis of polar metabolites were performed as described previously (Avin-Wittenberg et al. 2015; Lisec et al. 2006). Metabolite analysis and annotation were carried out using TagFinder® and the Golm Metabolome Database (<http://gmd.mpimp-golm.mpg.de/>), as previously described (Kopka et al. 2005; Luedemann et al. 2008).

2.4 Multivariate Statistical Analysis

Metabolomics data was analysed by biomarker analysis and partial least squares discriminant analysis (PLS-DA) using the Metaboanalyst platform (Chong et al. 2018). Biomarker analysis is used to identify potential single or multiple biomarkers from two conditions based on receiver operating characteristic (ROC) curves using well-established methods (Xia et al. 2013). PLS-DA is a biased, scale-reduction analysis largely used in metabolomics studies (Worley and Powers 2015). Beyond differentiating sample groups, PLS-DA also provides variable importance in projection (VIP) scores of the main parameters (metabolites) responsible for the discrimination found between the

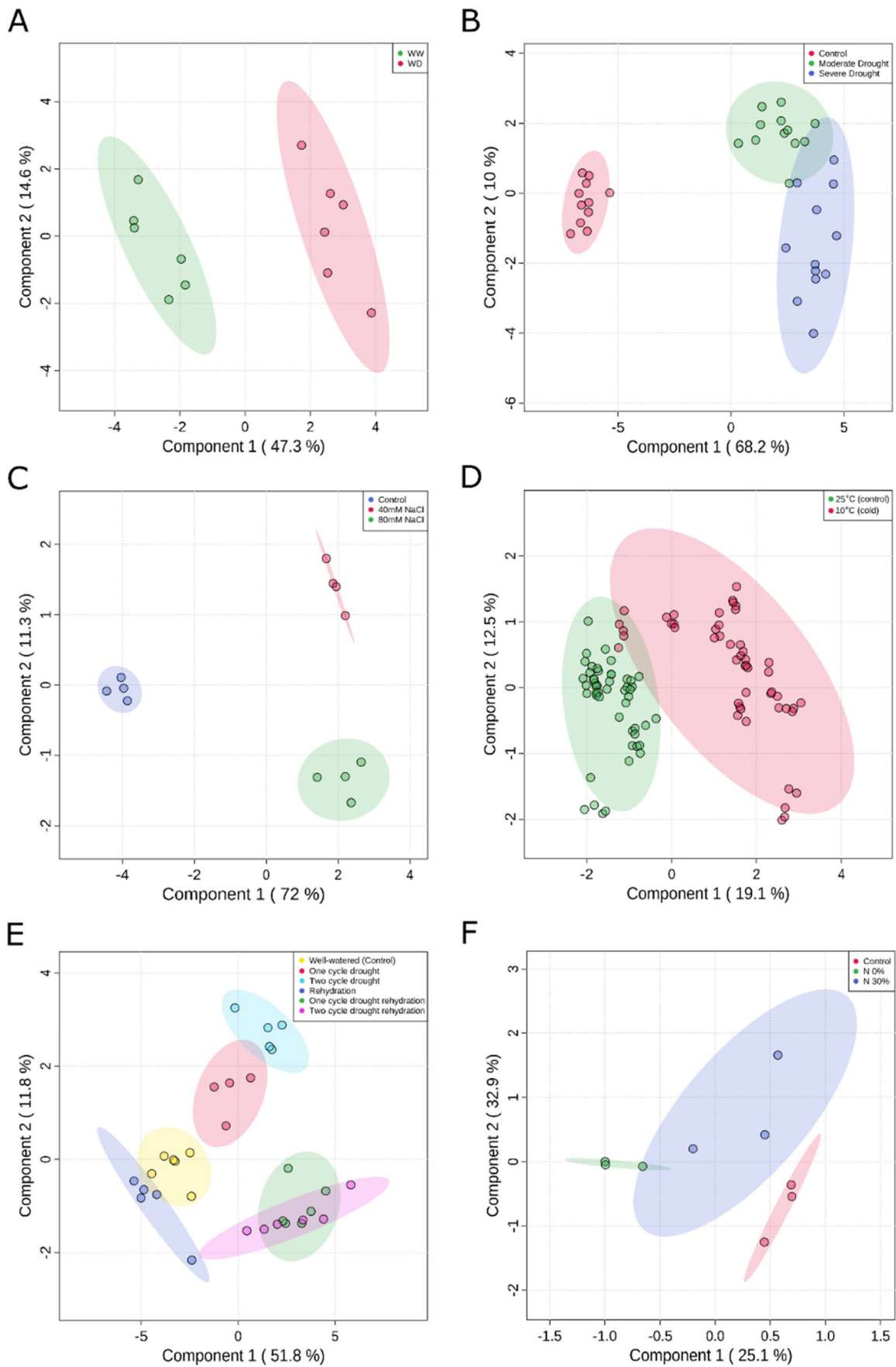


Fig. 1 Partial least squares discriminant analysis (PLS-DA) using metabolite profiling data from stressed and non-stressed plants. **A** *Vitis vinifera* L. Grenache under well-watered (WW) and water-deficit (WD) conditions. **B** Sugarcane under WW (control) and moderate and severe drought conditions. **C** *Egletes viscosa* under non-stressed (control) and excess of NaCl (40 mM and 80 mM NaCl) conditions. **D** Eucalyptus under ambient temperature of 25 °C (control) and 10 °C (cold). **E** *Arabidopsis thaliana* under well-watered (control), one or two drought cycles, rehydration of well-watered control, rehydration after one or two drought cycle conditions. **F** Sugarcane under non-stressed (control), completely nitrogen deficient (N 0%) and slightly nitrogen deficient (N 30%) conditions. These analyses were carried out using Metaboanalyst (Chong et al. 2018)

two major components of the PLS-DA. Metabolites with VIP scores higher than 1 are considered great representatives of the PLS-DA (Xia and Wishart 2011). The metabolite profiling data was normalized by cube root transformation and Pareto scale options in the Metaboanalyst® 5.0 platform (Pang et al. 2021).

2.5 Network Analysis

Correlation-based networks were created between the relative metabolite content found in all species under all treatments using each data separately using. The nodes in all networks represent the metabolites and the edges (links) correspond to the strength of the correlation among them. The correlation-based networks were created by restricting the connections to a specific limit of debiased sparse partial correlation (DSPC) coefficient (r) value ($-0.5 > r > 0.5$) (Freire et al. 2021). DSPC analysis was carried out by using the CorrelationCalculator software (Basu et al. 2017). Correlation-based metabolic networks were generated by using Cytoscape® (Shannon et al. 2003).

Network parameters such as network heterogeneity and network density were obtained using the NetworkAnalyzer on Cytoscape®, as described earlier (Assenov et al. 2008). Other parameters such as the number of hubs (N°Hubs), preferential attachment and new hubs were obtained as described in our previous work (Freire et al. 2021). The relationship between N°Hubs and the network was analysed by linear regression and Pearson's correlation using the software SigmaPlot® 14 (Systat Software Inc., San Jose, CA, USA).

In order to investigate the existence of a pattern of changes in network parameters after stress imposition, the relative changes in network heterogeneity, network density and N°Hubs were obtained under stress conditions of each study, normalized by those found in their respective controls, and a heatmap was constructed. A hierarchical clustering analysis (HCL) was carried out in the heatmap created, using the MeV® software.

2.6 Integrative Analysis

We next integrated the data derived from PLS-DA, biomarker and network analyses. We combined all metabolites with VIP scores higher than 1 from PLS-DA, all significant biomarkers ($P < 0.05$) and the hubs of the metabolic networks under stress conditions. The information were grouped in a Venn diagram in order to observe shared metabolites among the three approaches used. Venn diagram was constructed using a web platform (<https://bioinformatics.psb.ugent.be/webtools/Venn/>).

3 Results

3.1 Stress Imposition Substantially Alters Plant Metabolism

In order to obtain better insights on common and specific metabolic changes induced by different abiotic stress conditions, we collected previously published metabolite profiling data and analysed them by different systemic approaches. Firstly, we analysed to which extent the level of stress imposed altered plant metabolism by partial least squares discriminant analysis (PLS-DA). In general, PLS-DA clearly discriminated stressed from non-stressed plants (Fig. 1A–F), with exception of plants under modest stress levels. This is the case of rice plants under mild and moderate water deficit (WD), which were clustered together with well-watered (WW) plants (Fig. 2A), and soybean plants under moderate WD, which were partially clustered with WW plants (Fig. 3A).

Beyond discriminating different groups of data, the PLS-DA further provides a list of metabolites, based on the variable importance in projection (VIP) score, which mostly contributed to the discrimination observed in the PLS-DA model (Xia and Wishart 2011). The number of metabolites included in the VIP score lists ranges from 9 in Eucalyptus under cold stress to 20 in sugarcane under WD conditions (Figs. S1–S2). Several amino acids related to osmotic adjustment (e.g. Pro), photorespiration (e.g. Gly, Ser), synthesis of secondary metabolites (Trp, Phe) and alternative pathways of respiration (Val, Leu, Ile) have VIP scores higher than 1. Furthermore, sugars and organic acids were also highly representative in the VIP score lists (Figs. S1–S2). In general, these metabolites have higher levels in the most stressful conditions, as clearly observed in rice and soybean plants under four and two levels of drought, respectively (Figs. 2B, B).

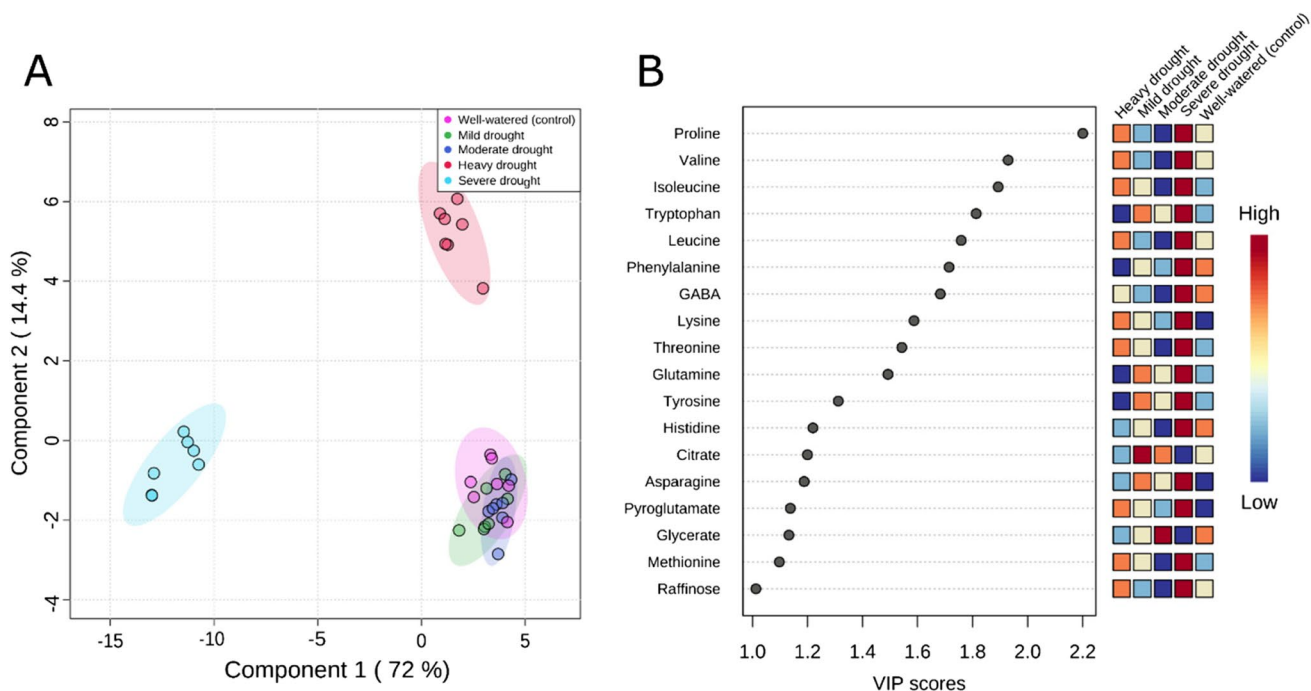


Fig. 2 Partial least squares discriminant analysis (PLS-DA) (A) and variable importance in projection (VIP) score (B) using metabolite profiling data from *Oryza sativa* L. under well-watered (WW) or dif-

ferent levels of drought. These analyses were carried out using Meta-boanalyst (Chong et al. 2018)

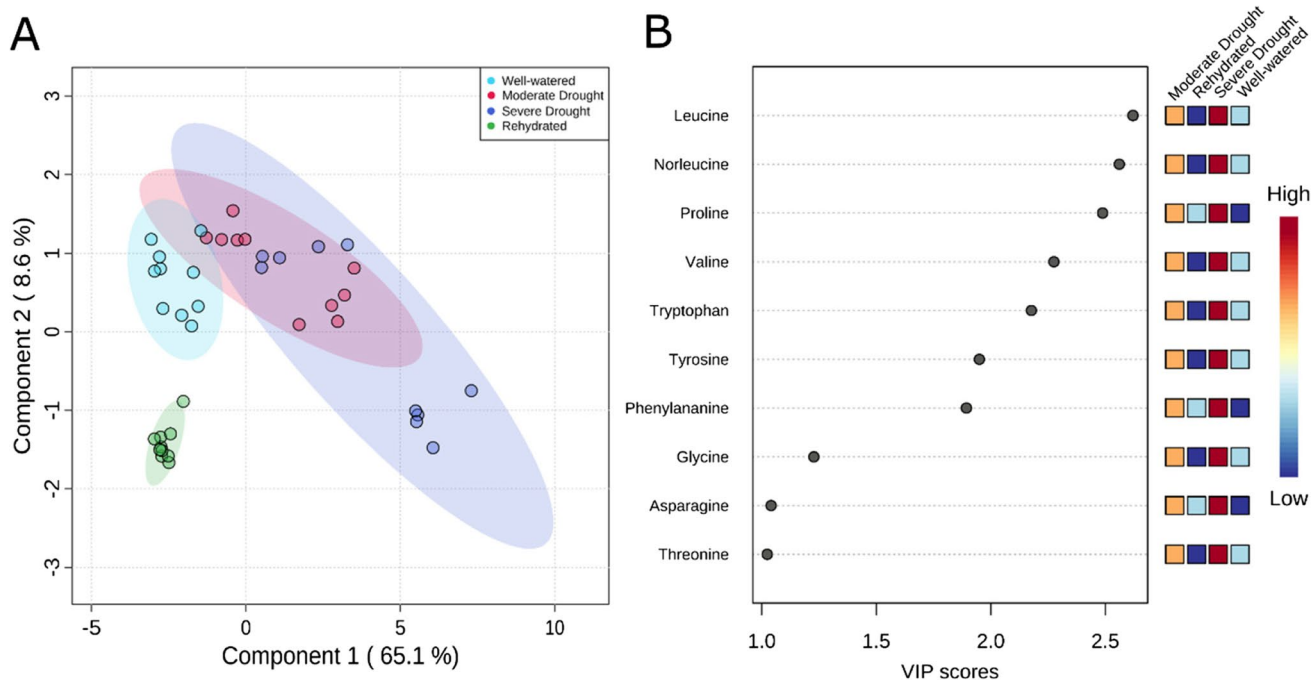


Fig. 3 Partial least squares discriminant analysis (PLS-DA) (A) and variable importance in projection (VIP) score (B) using metabolite profiling data from *Glycine max* L. under well-watered (WW), mod-

erate and severe drought conditions and after rehydration. These analyses were carried out using Metaboanalyst (Chong et al. 2018)

3.2 Network Analysis Reveals that the Changes Induced by Abiotic Stress Imposition Are Species/Stress Level–Specific

We next created correlation-based metabolic networks to investigate the effect of stress at the network level. No general pattern of response in network density, heterogeneity and number of hubs. However, the number of hubs (nodes with a high degree of connection) increased under stress in all species, which is related either to the appearance of new hubs (i.e. nodes that are only considered hubs under stress) or to the preferential attachment (Supplementary Dataset S1). Hubs are defined here as nodes with a higher degree of connection (number of links) than the average of links found in the network under non-stress conditions, whilst preferential attachment is observed when a node is considered a hub under both control (non-stress) and stress conditions (Freire et al. 2021). In the next sections, we describe the network results in each species.

3.2.1 Rice Under Drought

Network density increased 1.8 times in moderate drought when compared to control, thus presenting a greater connection. Regarding heterogeneity, this was approximately 2 times lower in moderate drought and 5 times lower in severe drought, when compared to the control. Moderate, heavy and severe drought conditions presented 2.7, 1.6 and 1.1 times more hubs than the control. Among the drought treatments, moderate and heavy drought presented a bigger hub appearance and preferential attachment values. Among the identified metabolites as hubs, arginine presented 8 connections, trehalose, putrescine 7 and mannose and tryptophan 6 (Fig. 4; Supplementary Dataset S1).

3.2.2 Grapevine Under Drought

Metabolic network density in plant under water deficit (WD) was 1.3 times higher than the control, presenting, therefore, a more connected network (Fig. 5A–B). Regarding heterogeneity, plants under WD presented a value of 0.352, being less than the control treatment (0.425). Regarding the number of hubs, WD presented 34 metabolites defined as hubs, whilst the control presented only 21. Among these, alanine, *myo*-inositol and proline have a higher number of links (12, 12 and 11, respectively). Nineteen metabolites were classified as new hubs under WD and fifteen were classified as hubs in both control and WD conditions (Supplementary Dataset S1).

3.2.3 Eucalyptus Under Cold Stress

Metabolic networks in stressed plants presented density 1.2 times bigger than the control, whilst heterogeneity was 1.1 times bigger in the control. In contrast, the number of hubs in cold was 23, being 1.8 times bigger than the control, which had only 13. The number of hub appearances in cold was 11, whilst the other 12 metabolites were and remained classified as hubs in both conditions. Among the hubs, 3,6-anidrogalactose, trans-2-hidroxi-cinamic acid and glycerol presented degree of connectedness 5, followed by tryptophan and glucose, both with 4 connections (Fig. 5C–D; Supplementary Dataset S1).

3.2.4 Arabidopsis thaliana Under Water Deficit

Arabidopsis metabolic networks under WD presented lower density than the control (Fig. 6A–C). However, heterogeneity was greater and lesser in plants under one or two cycles of WD, respectively, compared to the control. Curiously, plants under one cycle of WD did not present hubs in the metabolic network, whilst plants under two cycles of WD had just 2 metabolites classified as hubs, against 13 in the control treatment. It should be noted that these two hubs were already classified as hubs in watered plants, which means that hub appearance after one or two cycles of WD was null (Supplementary Dataset S1).

3.2.5 Sugarcane Under Water Deficit

Sugarcane metabolic networks under moderate drought presented lower density when compared to the control treatment (Fig. S2). However, severe drought presented a slight decrease (1.1) in density, as compared to the control. Regarding heterogeneity, stressed plants presented 1.4 and 1.1-times higher values in moderate and severe drought, respectively, when compared to the control. The highest number of hubs was in severe drought with 17, whilst the control and moderate drought presented 15 and 18 hubs, respectively. Among these, alanine, aspartate and fructose were the metabolites with more connections in moderate drought with 3, 3 and 2 connections, respectively, whilst in severe drought were valine, aconitate and fructose presenting 4, 3 and 3 connections respectively. It should be noted that leucine and fructose were identified as hubs in both drought conditions (Supplementary Dataset S1).

3.2.6 Egletes viscosa Under Salt Stress

Density and heterogeneity of the metabolic networks presented low variations between the control plants and the ones submitted to salt stress (Fig. 7A–C). However, network heterogeneity was 2.1 times lower in the 80 mM NaCl

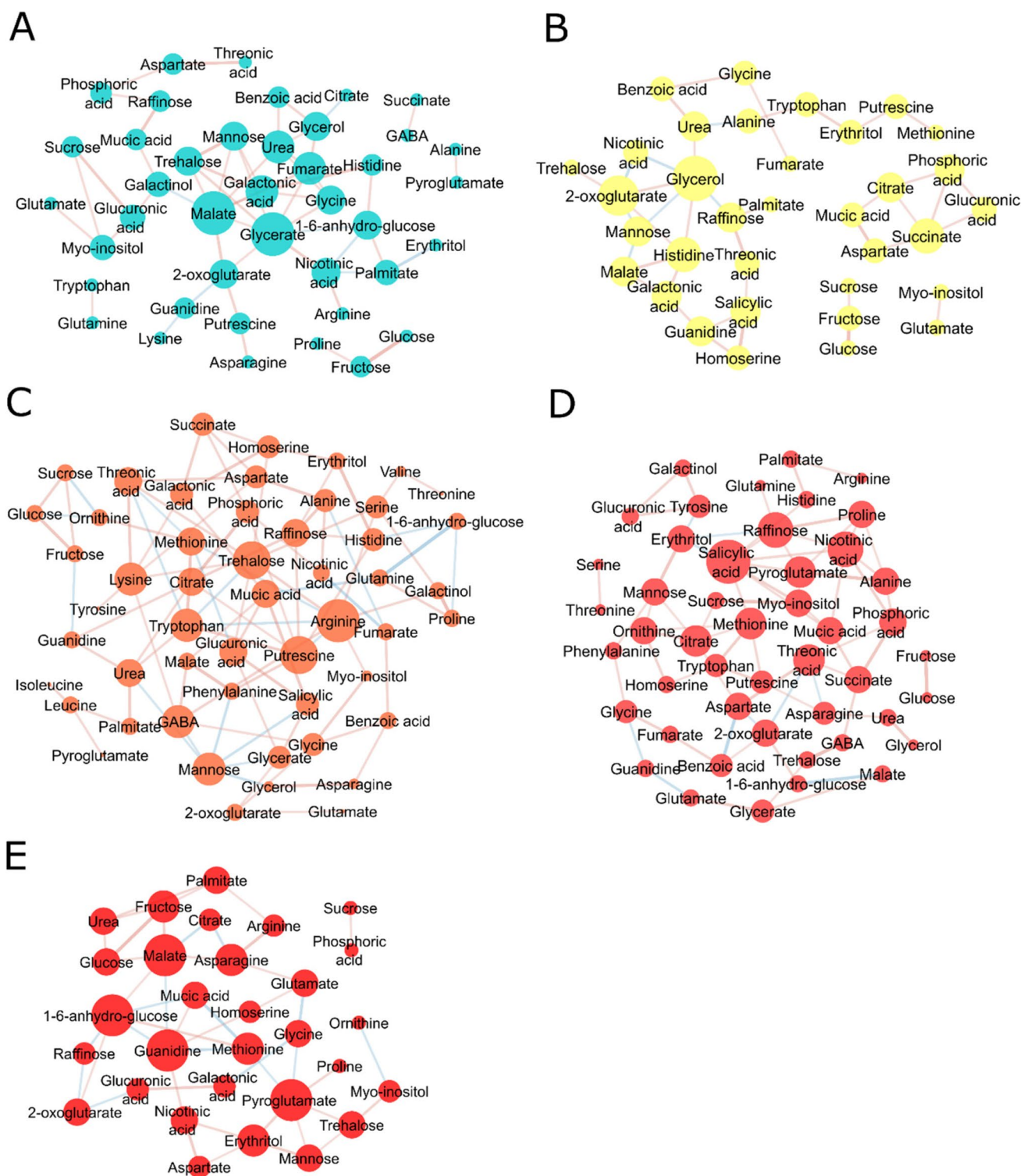


Fig. 4 Correlation-based metabolic networks from rice (*Oryza sativa* L.). The data was obtained from shoot metabolite profiling of *O. sativa* plants under well-watered (A), mild drought (−9.8 kPa) (B), moderate drought (−31 kPa) (C), heavy drought (−309.9 kPa), (D) and severe drought (Sds, severe dehydration stress in soil containers) (E) conditions. Bigger nodes indicate higher degree of connections,

i.e. nodes with higher number of links. The links (i.e. lines connecting two nodes) represent the strength of correlation between them, measured by debiased sparse partial correlation (r). Two nodes are connected only if $-0.5 > r > 0.5$. Thicker lines indicate higher r , in module. Blue and red lines represent positive and negative correlations, respectively

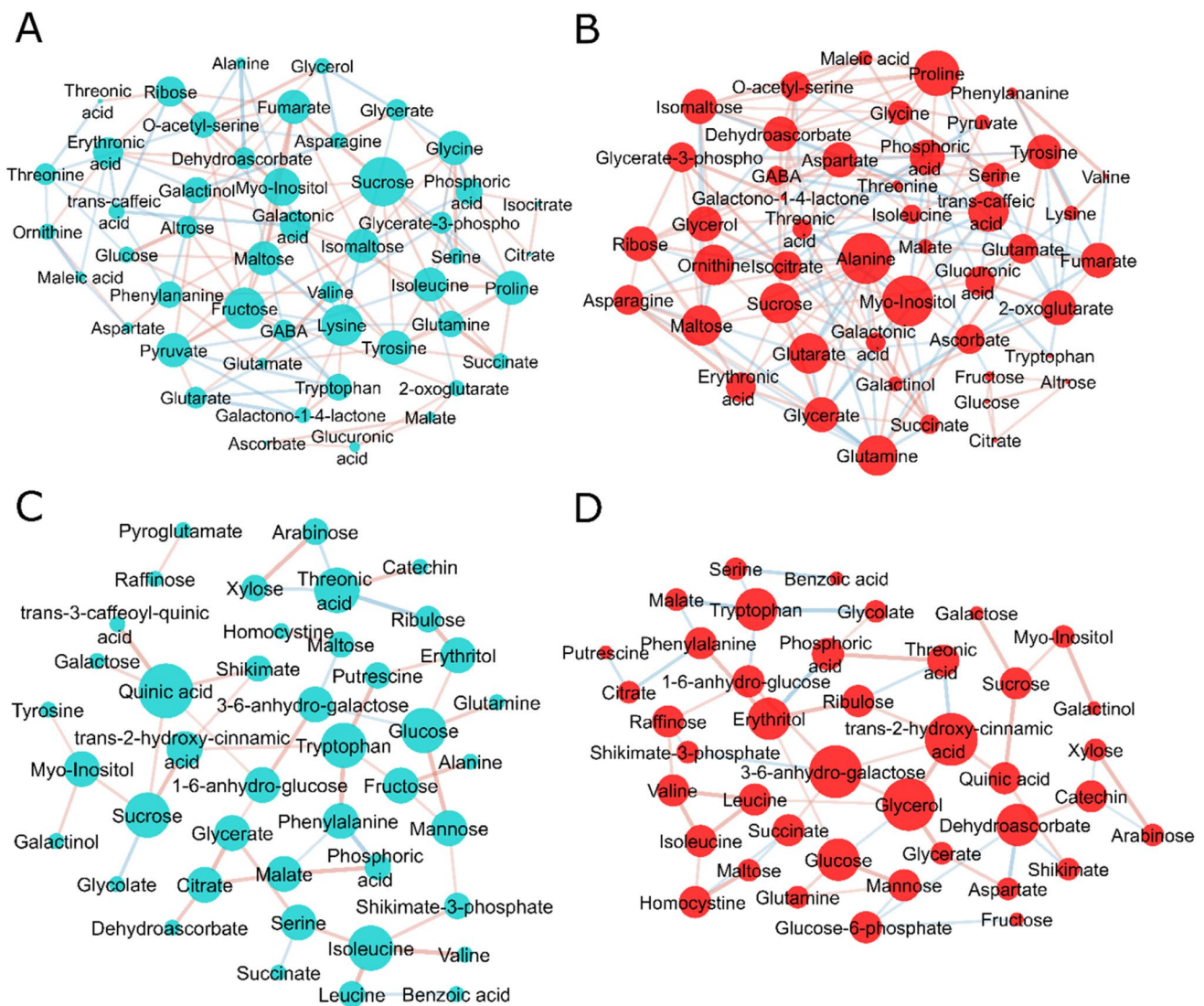


Fig. 5 Correlation-based metabolic networks from *Vitis vinifera* L. “Grenache” (A–B) and *Eucalyptus* (*E. globulus* and *E. Grandis*) (C–D). The data from *V. vinifera* and *Eucalyptus* were obtained from leaf metabolite profiling of *V. vinifera* plants under well-watered (A) and water-deficit (B) conditions and *Eucalyptus* plants under ambient temperature (25 °C) (C) and cold stress (10 °C) (D) conditions.

Bigger nodes indicate higher degree of connections, i.e. nodes with higher number of links. The links (i.e. lines connecting two nodes) represent the strength of correlation between them, measured by debiased sparse partial correlation (r). Two nodes are connected only if $-0.5 > r > 0.5$. Thicker lines indicate higher r , in module. Blue and red lines represent positive and negative correlations, respectively

treatment than in the control. This treatment (80 mM NaCl) also presented a substantial increase (2.2 times) in the number of hubs, having 19 new hubs. Valine, norleucine and phosphoric acid were the metabolites that presented a higher degree of connections, with 20, 19 and 18, respectively (Supplementary Dataset S1).

3.2.7 Soybean Under Water Deficit

The network density was 1.6 times lower in severe drought when compared to the control, whilst there was no variation in network density in plants under moderate drought

or rehydrated (Fig. S3). Network heterogeneity increased in all treatments, compared to the control, being the biggest difference reported for severe drought treatment, which presented an increase of 1.5 times compared to the control. Among the treatments, moderate drought presented the highest number of hubs (22), in which 11 were new hubs and 11 were already classified as hubs in control situations and that remained highly connected in stress situations, evidenced by the value of preferential attachment. Metabolites with the highest degree of connection in moderate drought were transcafeic acid, galactonic acid and glycolate with 4, 3 and 3 degrees, respectively, whilst in severe drought were

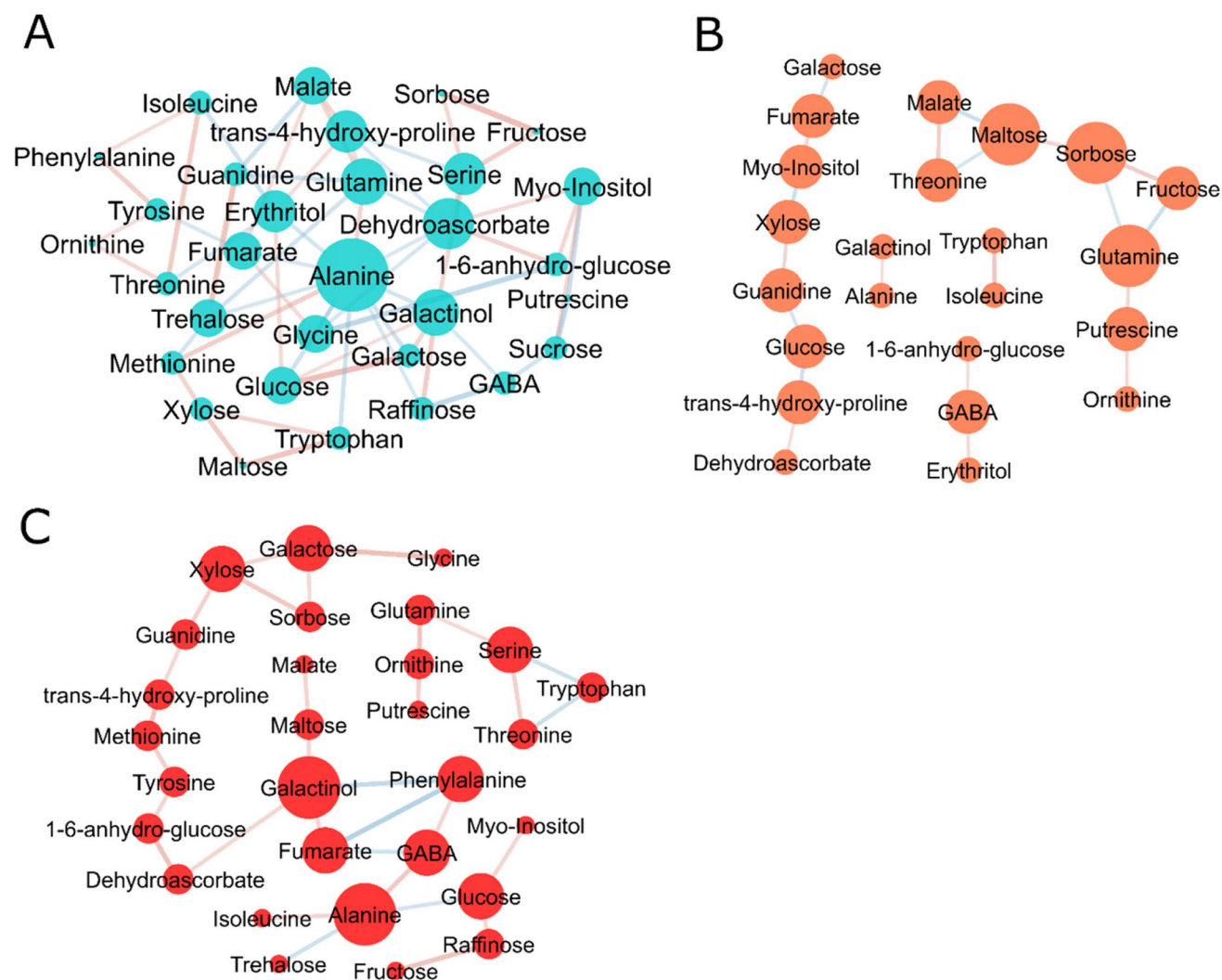


Fig. 6 Correlation-based metabolic networks from *Arabidopsis thaliana*. The data was obtained from whole rosette metabolite profiling of *A. thaliana* plants under well-watered (A) and one (B) or two drought cycle events (C) conditions. Bigger nodes indicate higher degree of connections, i.e. nodes with higher number of links. The links (i.e.

lines connecting two nodes) represent the strength of correlation between them, measured by debiased sparse partial correlation (r). Two nodes are connected only if $-0.5 > r > 0.5$. Thicker lines indicate higher r , in module. Blue and red lines represent positive and negative correlations, respectively

dehydroascorbate, ethanolamine and lactate presenting 1 connection and fructose and fumarate with 2 connections each (Supplementary Dataset S1).

3.2.8 Sugarcane Under N Starvation

Slight decreases in metabolic network density for both N deprivation conditions were observed (Fig. S4). However, heterogeneity was 2.4 and 1.7 times smaller in conditions of N 0% and N30%, respectively. The number of hubs was also not altered after stress, although 13 and 15 new hubs were identified in N 0% and N 30% treatments, respectively. Among the new hubs, sucrose stands out presenting 35 and

36 connections in N 0% and 30% treatments, respectively (Supplementary Dataset S1).

3.3 Integrating Network and Multivariate Analyses

We next used integrative approaches to obtain better insights concerning plant stress metabolic responses. First, we combined all network analyses and investigated the relative changes in the network parameters of each study. For this, the values of heterogeneity, density and number of hubs (N°Hubs) obtained under stress conditions were normalized by dividing them by the values found in the respective control of the experiment (i.e. non-stressed plants). The objective of this analysis was to investigate

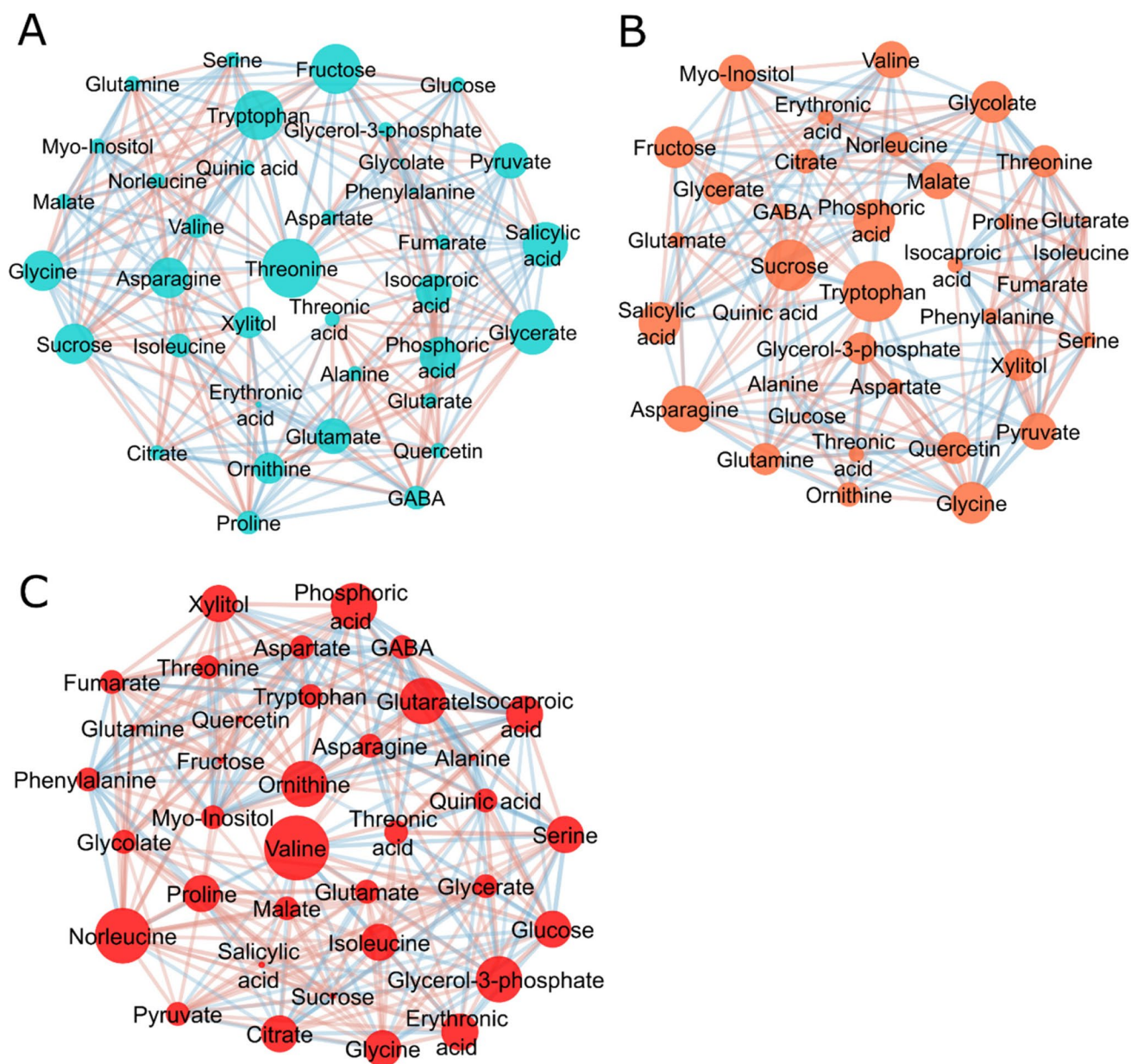


Fig. 7 Correlation-based metabolic networks from *Egetes viscosa*. The data was obtained from leaf metabolite profiling of *E. viscosa* plants under non-stressed (**A**) and saline conditions by irrigation with 40 (**B**) or 80 mM NaCl solution (**C**). Bigger nodes indicate higher degree of connections, i.e. nodes with higher number of links. The

links (i.e. lines connecting two nodes) represent the strength of correlation between them, measured by debiased sparse partial correlation (r). Two nodes are connected only if $-0.5 > r > 0.5$. Thicker lines indicate higher r , in module. Blue and red lines represent positive and negative correlations, respectively

if there is a pattern of the response of these parameters after stress imposition. However, no pattern was observed, neither in relation to the type of stress (e.g. drought) nor among the level of stress within the species (Fig. 8A). The hierarchical clustering analysis (HCL) grouped different species and treatments according to the decrease in density and N° Hubs, whilst another large group was grouped according to the increase in these parameters. An intermediate group containing sugarcane under severe drought and

soybean under moderate drought were clustered separately, as they showed small increases in all parameters (Fig. 8A). HCL analysis also highlighted that the density and N° Hubs parameters are highly correlated. We then investigated the relationship between these parameters through regression analysis. The N° Hubs was positively correlated with the density of the network ($R^2 = 0.82$; $P < 0.001$) (Fig. 8B), highlighting the power of the N° Hubs parameter to provide

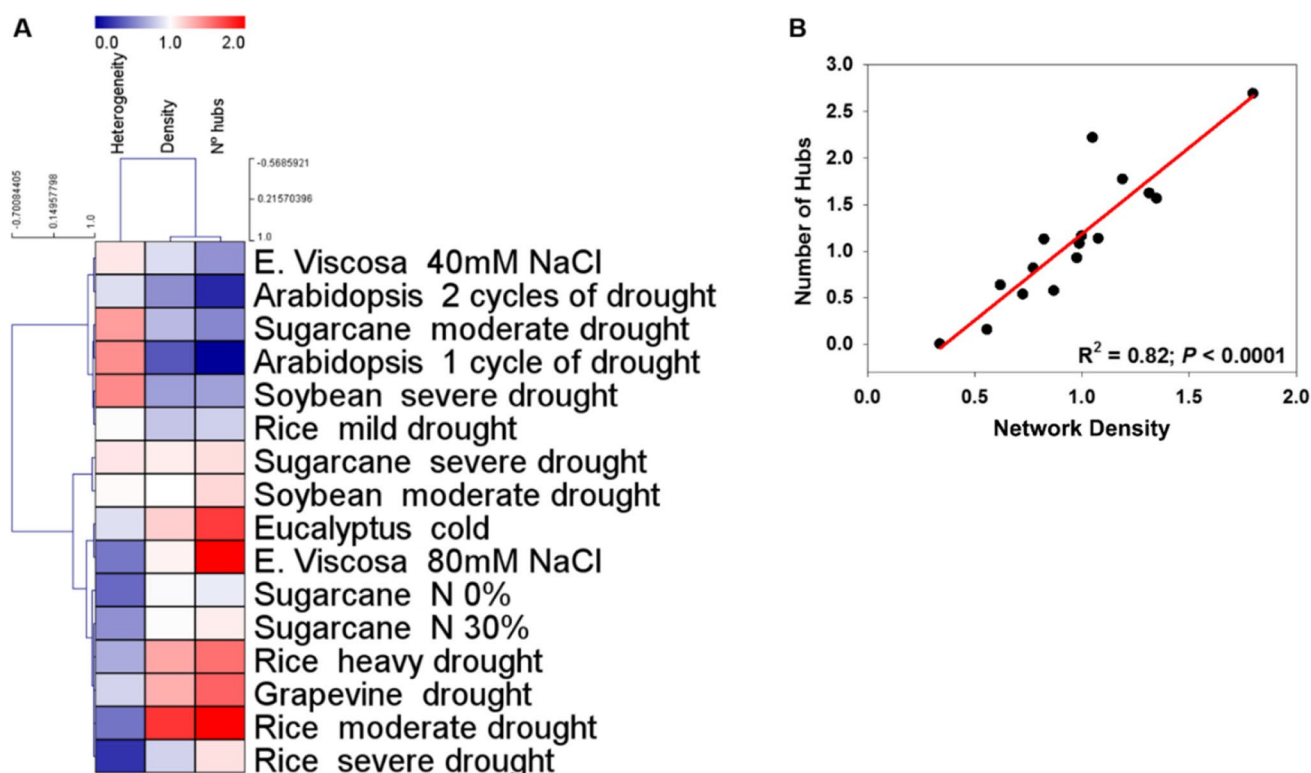


Fig. 8 Comparative analysis of the parameters obtained in the metabolic network analysis of plants under several abiotic stress conditions. **A** Hierarchical clustered heat map demonstrating the relative changes in network heterogeneity, density and number of hubs. The values obtained under stress conditions were normalized according to their respective controls. Similar trends were grouped by hierarchical

clustering analysis (HCL) using the MeV® software. **B** Relationship between number of hubs and network density. Linear regression was carried out using data from all species/stress conditions and the SigmaPlot® software. Both R^2 and P value of the regression analysis are highlighted in the graph

direct information concerning the major hubs of the network and indirectly about the density of the network.

Afterwards, we carried out a biomarker analysis using the Metaboanalyst platform (Pang et al. 2021). This analysis indicates which metabolites are markers of each stress level condition (Xia et al. 2013). Thus, in experiments in which more than one stress level was used, biomarkers were obtained for each stress level. After obtaining the list of metabolites that were considered markers for each stress level (Supplementary Dataset S1), we then combined this with the list of metabolites with VIP scores higher than 1 in the PLS-DA model and the hubs found in each stress level condition in Venn diagrams (Fig. 9A). The construction of Venn diagrams allowed us to identify metabolites from each experiment and stress level that are at the intersection of the three approaches used (biomarkers, PLS-DA and networks). Subsequently, we combined the information from all experiments in order to verify which metabolites appeared most at these intersections (Fig. 9B). The metabolites with the highest number of intersections in the different treatments are shown in Table 1. Among these, asparagine had the highest number of intersections (6), including three different types

of stress (water deficit, salt stress and N limitation). Furthermore, several amino acids (Val, Leu, Ile, Phe, Trp, Gly and Pro), glycerate, shikimate and the sugars raffinose and fructose also showed two or more intersections, with many of these intersections involving different types of stress (Table 1). This analysis highlight that different metabolic pathways are involved in plant stress acclimation, but amino acid metabolism is a major hub for it.

4 Discussion

4.1 Unveiling the Complexity of Plant Stress Metabolic Responses at Network Level

In the face of the climate change scenario, in which more severe and prolonged droughts and extreme temperatures are predicted to be more common, it is important to understand how plants acclimate to abiotic stress conditions to maintain or improve crop yield under stressful conditions (Cattivelli et al. 2008; Zandalinas et al. 2021). In this vein, the use of systemic approaches is fundamental to unravel the

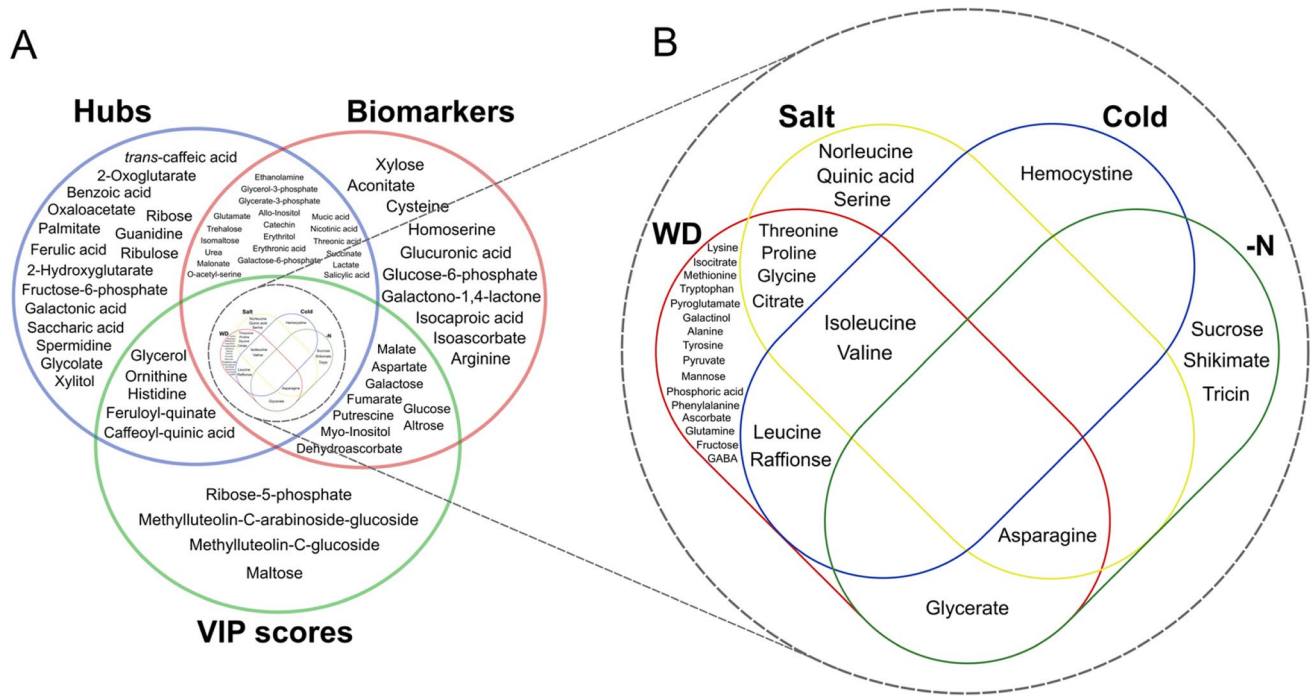


Fig. 9 Integration of multivariate and network analyses. **A** Venn diagram integrating the metabolites found as stress biomarkers, the hubs from network analyses and metabolites from the variable importance

in projection (VIP) scores list of the partial least squares discriminant analysis (PLS-DA). **B** Venn diagram highlighting the intersections of those metabolites in each stress condition

Table 1 Metabolites found in the intersections of the Venn diagrams from biomarkers, network and PLS-DA analyses. The number of intersections column indicates the number of studies in which the metabolite was found in the intersection of the Venn diagram. The column types of stress highlight under which stress condition the

metabolite was found in the intersection of the Venn diagram. The last column demonstrates the metabolism or metabolic pathway which the metabolite is related. Abbreviations: *ETF/ETFQO*, electron-transfer flavoprotein/ electron-transfer flavoprotein:ubiquinone oxidoreductase; *-N*, nitrogen deprivation stress; *WD*, water deficit

Metabolites	Number of intersections	Type of stress	Metabolism/metabolic pathway
Asparagine	6	WD, salt, -N	Amino acids
Valine	4	WD, cold, salt	Amino acids/substrate <i>ETF/ETFQO</i>
Isoleucine	4	WD, cold, salt	Amino acids/substrate <i>ETF/ETFQO</i>
Leucine	3	WD, cold	Amino acids/substrate <i>ETF/ETFQO</i>
Raffinose	3	WD, cold	Sugars/Osmoprotection
Glycine	3	WD, salt	Photorespiration
Phenylalanine	3	WD	Amino acids/secondary metabolism
Fructose	3	WD	Sugar/signalling/Osmoprotection
Glycerate	2	WD, -N	Photorespiration
Proline	2	WD, salt	Amino acids/Osmoprotection
Tryptophan	2	WD	Amino acids/secondary metabolism
Shikimate	2	-N	Secondary metabolism

mechanisms of plant acclimation and adaptation to abiotic stresses (Evans and Lawson 2020; Neto et al. 2021). Indeed, network analyses are routinely used to better understand the abundance of information originated from large-scale data analysis, especially those from transcriptomics and interactome studies (Gutiérrez et al. 2007; Jeong et al. 2001; Jones et al. 2014; Kuhalskaya et al. 2020; Souza et al. 2019).

However, it is not clear how and to which extent network and multivariate statistical analyses can be used in conjunction in metabolomics studies and which emergent properties are obtained by combining these approaches. Here, we carried out a meta-analysis using data from previously published metabolite profiling of plants under different stress conditions, aiming to identify common metabolic responses

among the studies and which emergent properties arise from the integration of different systemic analysis.

Previous studies suggest that plants under stress have higher connected photosynthetic networks (Daloso et al. 2014; Souza et al. 2005b, 2009). Higher network connectivity has been proposed to be associated to a higher stability of the system (Souza et al. 2005a; Souza and Lüttge 2015). We then hypothesized that stress conditions would lead to higher connected metabolic networks, i.e. with higher network density. However, no clear pattern of changes in network density and topology among species/stress conditions was observed. Whilst the network density increased in some species under stress, the opposite was observed in other studies or under a different intensity of stress. Our results suggest therefore that plant metabolic network responses are specific to the level of stress condition in which the plant is subjected. These results highlight the difficulty to identify stress metabolic markers for multiple species and to choose biotechnological targets to improve stress tolerance in plants through systems-driven metabolic engineering (Bertolli et al. 2014; Flexas 2016). It is noteworthy that the responses to stress vary according to the level of stress in which the plant is subjected (Galviz et al. 2022). Thus, the lack of pattern in network parameters among the different stress/species investigated here could be due to the intrinsic limitation of our meta-analysis, which was based on different studies.

Although metabolic network analysis was highly variant among the studies, network analysis of distinct plant species under stress allowed us to further evaluate the power of the parameter number of hubs (N°Hubs) recently established in our group (Freire et al. 2021). This parameter was created aiming to identify the hubs in metabolic networks, highlighting the nodes (metabolites) that are key for the topology of the network under different conditions. Here, we use this approach to identify the hubs that are prominent in plant metabolic networks under non-stress and/or stress conditions. Interestingly, the N°Hubs was positively correlated to network density ($R^2=0.82$; $P<0.001$), which is a well-established and widely used network parameter. However, network density provides an idea only about network connectivity per se, whereas, in addition to it, the N°Hubs provides which nodes most influence network topology according to the conditions in which the plant is submitted. Furthermore, beyond unveiling the major connected nodes of the network, N°Hubs offer a possibility to be integrated into other analyses in order to have better insights concerning the plasticity of metabolic networks.

4.2 Metabolism of Amino Acids as a Major Hub for Plant Stress Metabolic Responses

The integration of network, PLS-DA and biomarker analyses enable the identification of metabolites that mostly

contributed to plant acclimation, in which several of them are common to different species/stress conditions. In general, PLS-DA using metabolite profiling data clearly distinguished stressed from non-stressed plants, similar to other studies that have used PLS-DA or principal component analysis (PCA) (Filippou et al. 2021; Fonseca-Pereira et al. 2019; Obata et al. 2015; Todaka et al. 2017). Moreover, the intersections of Venn diagrams obtained by combining the VIP score list of the PLS-DA and network and biomarker analyses identified which metabolites mostly contributed to plant stress acclimation. Several metabolites appeared in the Venn diagram intersections in more than one type and level of stress, ranging from one in Arabidopsis under drought to thirteen in sugarcane under severe drought. Among these, asparagine stands out in 6 intersections, in which its content increased in five out of six stress conditions compared to its respective control. Asparagine is a metabolite belonging to the glutamine/asparagine synthetase pathway, being an important source of nitrogen for this and other pathways (Gaufichon et al. 2016). This result reinforces the importance of nitrogen metabolism for plant stress acclimation (Batista-Silva et al. 2019; Hildebrandt 2018).

Plant stress responses involve the activation of complex signalling networks that leads to substantial metabolic alterations throughout the plant (Fàbregas and Fernie 2019; Zandalinas et al. 2022; Zandalinas and Mittler 2022). Among the pathways activated under stress, amino acid catabolism is of paramount importance for metabolic homeostasis (Hildebrandt et al. 2015). For example, plants activate alternative pathways to provide substrate to mitochondrial respiration under stress-induced carbon starvation. This occurs through the degradation of proteins and branched-chain amino acids (Leu, Ile and Val) and activation of the energetic metabolism through the alternative ETF/ETFQO pathway of respiration (Araújo et al. 2010, 2011), which contribute to alleviating damage triggered by stress conditions (Barros et al. 2020; Brito et al. 2022; Pires et al. 2016). Additionally, the degradation of aromatic amino acids (Tyr, Trp and Phe) further helps plants cope with stress by providing substrates for the synthesis of hormones and secondary metabolites (Saito et al. 2013). Here, several of these amino acids were identified in the Venn diagram intersections, i.e. different approaches indicate that they are good candidates to discriminate plants under stress to those under non-stress conditions. It is noteworthy that amino acid metabolism has been implicated in modulating both local and systemic responses, suggesting that these metabolites are not the only source of carbon and nitrogen for different pathways but are also good stress signal messengers that aid the entire plant to acclimate to stress conditions (Balfagón et al. 2022; Choudhury et al. 2018). These results collectively pinpoint amino acid metabolism as a major hub for plant stress responses.

The other metabolites with a high number of intersections in the Venn diagrams are osmoprotectants (Pro, fructose and raffinose) and related to photorespiration (Gly and glycerate), two mechanisms highly documented of contributing to stress acclimation (Gomes Silveira et al. 2003; Guilherme et al. 2019; Timm and Hagemann 2020), especially under drought (Fàbregas and Fernie 2019; Obata and Fernie 2012). Recent temporal metabolomics analyses demonstrated that the osmoprotectants Pro, raffinose, trehalose and sucrose progressively accumulate in *Arabidopsis* plants under drought (Lozano-Elena et al. 2022). Furthermore, the accumulation of these metabolites plus fructose, glycerate and other sugars, amino acids and organic acids are exacerbated in plants overexpressing brassinosteroid insensitive-like 3 (BRL3), which has been previously shown to be more drought-tolerant (Fàbregas et al. 2018). Lozano-Elena and collaborators further demonstrated that several transcripts related to both jasmonic (JA) and abscisic (ABA) acids are upregulated in response to drought (Lozano-Elena et al. 2022). These findings coupled to our results suggest that metabolic responses to drought involve a complex interplay between signalling pathways coordinated by JA, ABA and brassinosteroids, highlighting the challenge to elect targets for genetic manipulation toward plant drought tolerance improvement (Flexas 2016).

5 Conclusions

The integration of network, PLS-DA and biomarker analyses allowed us to identify which metabolites are possible markers of the abiotic stresses tested here, among which asparagine, amino acids (Val, Ile, Leu, Gly, Phe, Pro and Trp), sugars (fructose and raffinose) and shikimate appears as key for plant stress acclimation. Our results highlight that the metabolism of amino acids is a major hub for plant stress responses. Our meta-analysis provides a systemic data analysis strategy for analysing metabolite profiling data from plants under stress, in which different tools are used to identify metabolic markers. We further demonstrated that the parameter number of hubs has great potential in unveiling the major hubs of metabolic networks, which facilitate the evaluation of the plasticity of the metabolic network and its major hubs under different conditions.

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Author Contribution DMD idealized the work. LLC and FBSF performed the analysis, with supervision of DMD. Data analysis and interpretation and the establishment of the figures were carried out by LLC, FBSF and DMD. All authors contributed to writing the manuscript. DMD obtained funding and is responsible for this article.

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Declarations

Conflict of Interest The authors declare no competing interests.

References

- Albert R (2005) Scale-free networks in cell biology. *J Cell Sci* 118:4947–4957. <https://doi.org/10.1242/jcs.02714>
- Albert R, Barabási A-L (2002) Statistical mechanics of complex networks. *Rev Mod Phys* 74:47–97. <https://doi.org/10.1103/RevModPhys.74.47>
- Albert R, Jeong H, Barabasi A-L (2001) Error and attack tolerance of complex networks. *Nature* 409:542–542. <https://doi.org/10.1038/35054111>
- Almaas E, Kovács B, Vicsek T, Oltvai ZN, Barabási AL (2004) Global organization of metabolic fluxes in the bacterium *Escherichia coli*. *Nature* 427:839–843. <https://doi.org/10.1038/nature02289>
- Araújo WL, Ishizaki K, Nunes-Nesi A, Larson TR, Tohge T, Krahnert I, Witt S, Obata T, Schauer N, Graham IA, Leaver CJ, Fernie AR (2010) Identification of the 2-hydroxyglutarate and isovaleryl-CoA dehydrogenases as alternative electron donors linking lysine catabolism to the electron transport chain of *Arabidopsis* mitochondria. *Plant Cell* 22:1549–1563. <https://doi.org/10.1105/tpc.110.075630>
- Araújo WL, Tohge T, Ishizaki K, Leaver CJ, Fernie AR (2011) Protein degradation - an alternative respiratory substrate for stressed plants. *Trends Plant Sci* 16:489–498. <https://doi.org/10.1016/j.tplants.2011.05.008>
- Araújo WL, Nunes-Nesi A, Nikoloski Z, Sweetlove LJ, Fernie AR (2012) Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. *Plant, Cell Environ* 35:1–21. <https://doi.org/10.1111/j.1365-3040.2011.02332.x>
- Assenov Y, Ramírez F, Schelhorn S-E, Lengauer T, Albrecht M (2008) Computing topological parameters of biological networks. *Bioinformatics* 24:282–284. <https://doi.org/10.1093/bioinformatics/btm554>
- Auler PA, Freire FBS, Lima VF, Daloso DM (2022) On the role of guard cells in sensing environmental signals and memorising stress periods. *Theor Exp Plant Physiol*. <https://doi.org/10.1007/s40626-022-00250-4>
- Avin-Wittenberg T, Bajdzienko K, Wittenberg G, Alseikh S, Tohge T, Bock R, Giavalisco P, Fernie AR (2015) Global analysis of the role of autophagy in cellular metabolism and energy homeostasis in *Arabidopsis* seedlings under carbon starvation. *Plant Cell Online* 27:306–322. <https://doi.org/10.1105/tpc.114.134205>
- Balfagón D, Gómez-Cadenas A, Rambla JL, Granell A, de Ollas C, Bassham DC, Mittler R, Zandalinas SI (2022) γ -Aminobutyric acid plays a key role in plant acclimation to a combination of high light and heat stress. *Plant Physiol*. <https://doi.org/10.1093/plphys/kiac010>
- Barabási AL (2009) Scale-free networks: a decade and beyond. *Science* (80-) 325:412–413. <https://doi.org/10.1126/science.1173299>
- Barabási A, Dezso Z, Ravasz E (2003) Scale-free and hierarchical structures in complex networks. *AIP Conf ...* 661:1–16. <https://doi.org/10.1063/1.1571285>

- Barabási AL, Oltvai ZN (2004) Network biology: understanding the cell's functional organization. *Nat Rev Genet* 5:101–113. <https://doi.org/10.1038/nrg1272>
- Barros JAS, Cavalcanti JHF, Medeiros DB, Nunes-Nesi A, Avin-Wittenberg T, Fernie AR, Araújo WL (2017) Autophagy deficiency compromises alternative pathways of respiration following energy deprivation in *Arabidopsis thaliana*. *Plant Physiol* 175:62–76. <https://doi.org/10.1104/pp.16.01576>
- Barros JAS, Siqueira JAB, Cavalcanti JHF, Araújo WL, Avin-Wittenberg T (2020) Multifaceted roles of plant autophagy in lipid and energy metabolism. *Trends Plant Sci* 1–13. <https://doi.org/10.1016/j.tplants.2020.05.004>
- Basu S, Duren W, Evans CR, Burant CF, Michailidis G, Karnovsky A (2017) Systems Biology Sparse Network Modeling and Metascape-Based Visualization Methods for the Analysis of Large-Scale Metabolomics Data 33:1545–1553. <https://doi.org/10.1093/bioinformatics/btx012>
- Batista VCV, Pereira IMC, de Paula-Marinho SO, Canuto KM, de Pereira RCA, Rodrigues THS, de Daloso DM, Gomes-Filho E, de Carvalho HH (2019) Salicylic acid modulates primary and volatile metabolites to alleviate salt stress-induced photosynthesis impairment on medicinal plant *Egletes viscosa*. *Environ Exp Bot* 167:103870. <https://doi.org/10.1016/j.envexpbot.2019.103870>
- Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun H, Hildebrandt TM (2019) The role of amino acid metabolism during abiotic stress release. *Plant Cell Environ* 42:1630–1644. <https://doi.org/10.1111/pce.13518>
- Bertolli SC, Mazzafera P, Souza GM (2014) Why is it so difficult to identify a single indicator of water stress in plants? A proposal for a multivariate analysis to assess emergent properties. *Plant Biol* 16:578–585. <https://doi.org/10.1111/plb.12088>
- Birami B, Nägele T, Gattmann M, Preisler Y, Gast A, Arneith A, Ruehr NK (2020) Hot drought reduces the effects of elevated CO₂ on tree water-use efficiency and carbon metabolism. *New Phytol* 226:1607–1621. <https://doi.org/10.1111/nph.16471>
- Bisbis MB, Gruda N, Blanke M (2018) Potential impacts of climate change on vegetable production and product quality – a review. *J Clean Prod* 170:1602–1620. <https://doi.org/10.1016/j.jclepro.2017.09.224>
- Bottcher A, Domingues-Junior AP, de Souza LP, Tohge T, Araújo WL, Fernie AR, Mazzafera P (2021) Sugarcane cell suspension reveals major metabolic changes under different nitrogen starvation regimes. *Bragantia* 80. <https://doi.org/10.1590/1678-4499.2021-0009>
- Brito DS, Quinhones CGS, Neri-Silva R, Heinemann B, Schertl P, Cavalcanti JHF, Eubel H, Hildebrandt T, Nunes-Nesi A, Braun H-P, Araújo WL (2022) The role of the electron-transfer flavoprotein: ubiquinone oxidoreductase following carbohydrate starvation in *Arabidopsis* cell cultures. *Plant Cell Rep* 41:431–446. <https://doi.org/10.1007/s00299-021-02822-1>
- Broido AD, Clauset A (2019) Scale-Free Networks are rare. *Nat Commun* 10:1–10. <https://doi.org/10.1038/s41467-019-08746-5>
- Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *F Crop Res* 105:1–14. <https://doi.org/10.1016/j.fcr.2007.07.004>
- Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J (2018) MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* 46:W486–W494. <https://doi.org/10.1093/nar/gky310>
- Choudhury FK, Devireddy AR, Azad RK, Shulaev V, Mittler R (2018) Local and systemic metabolic responses during light-induced rapid systemic signaling in *Arabidopsis*. *Plant Physiol* submitted. <https://doi.org/10.1104/pp.18.01031>
- Daloso DM (2014) The ecological context of bilateral symmetry of organ and organisms. *Nat Sci* 6:184–190. <https://doi.org/10.4236/ns.2014.64022>
- de Daloso DM, Antunes WC, Santana TA, Pinheiro DP, Ribas RF, Sachetto-Martins G, Loureiro ME (2014) *Arabidopsis* gun4 mutant have greater light energy transfer efficiency in photosystem II despite low chlorophyll content. *Theor Exp Plant Physiol* 26:177–187. <https://doi.org/10.1007/s40626-014-0025-z>
- Domingues-Junior AP, de Daloso DM, Machado M, Rosado-Souza L, de Souza LP, Fernie AR, Mazzafera P (2019) A cold change: how short low temperature exposure affects primary metabolism in leaves and stems of two eucalyptus species. *Theor Exp Plant Physiol* 8:429–444. <https://doi.org/10.1007/s40626-019-00156-8>
- Evans JR, Lawson T (2020) From green to gold: agricultural revolution for food security. *J Exp Bot* 71:2211–2215. <https://doi.org/10.1093/jxb/eraa110>
- Fàbregas N, Fernie AR (2019) The metabolic response to drought. *J Exp Bot* 70:1077–1085. <https://doi.org/10.1093/jxb/ery437>
- Fàbregas N, Lozano-Elena F, Blasco-Escámez D et al (2018) Overexpression of the vascular brassinosteroid receptor BRL3 confers drought resistance without penalizing plant growth. *Nat Commun* 9:1–13. <https://doi.org/10.1038/s41467-018-06861-3>
- FAO F and AO (2017) The future of food and agriculture – trends and challenges. Rome
- Fernie AR, Yan J (2019) De novo domestication: an alternative route toward new crops for the future. *Mol Plant* 12:615–631. <https://doi.org/10.1016/j.molp.2019.03.016>
- Filippou P, Zarza X, Antoniou C, Obata T, Villarroel CA, Ganopoulos I, Harokopos V, Gohari G, Aidinis V, Madesis P, Christou A, Fernie AR, Tiburcio AF, Fotopoulos V (2021) Systems biology reveals key tissue-specific metabolic and transcriptional signatures involved in the response of *Medicago truncatula* plant genotypes to salt stress. *Comput Struct Biotechnol J* 19:2133–2147. <https://doi.org/10.1016/j.csbj.2021.04.018>
- Flexas J (2016) Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: why so much little success? *Plant Sci* 251:155–161. <https://doi.org/10.1016/j.plantsci.2016.05.002>
- Fonseca-Pereira P, Daloso DM, Gago J, De Oliveira Silva FM, Condori-Apfata JA, Florez-Sarasa I, Tohge T, Reichheld JP, Nunes-Nesi A, Fernie AR, Arajo WL (2019) The mitochondrial thioredoxin system contributes to the metabolic responses under drought episodes in *Arabidopsis*. *Plant Cell Physiol* 60:213–229. <https://doi.org/10.1093/pcp/pcy194>
- Freire FBS, Bastos RLG, Bret RSC, Cândido-Sobrinho SA, Medeiros DB, Antunes WC, Fernie AR, Daloso DM (2021) Mild reductions in guard cell sucrose synthase 2 expression leads to slower stomatal opening and decreased whole plant transpiration in *Nicotiana tabacum* L. *Environ Exp Bot* 184:104370. <https://doi.org/10.1016/j.envexpbot.2020.104370>
- Friso G (2015) van Wijk KJ (2015) Update: post-translational protein modifications in plant metabolism. *Plant Physiol* 169:01378. <https://doi.org/10.1104/pp.15.01378>
- Gago J, Fernie AR, Nikoloski Z, Tohge T, Martorell S, Escalona JM, Ribas-Carbó M, Flexas J, Medrano H (2017) Integrative field scale phenotyping for investigating metabolic components of water stress within a vineyard. *Plant Methods* 13:1–14. <https://doi.org/10.1186/s13007-017-0241-z>
- Gago J, Daloso DM, Carriqui M, Nadal M, Morales M, Araújo WL, Nunes-Nesi A, Perera-Castro AV, Clemente-Moreno MJ, Flexas J (2020) The photosynthesis game is in the “inter-play”: mechanisms underlying CO₂ diffusion in leaves. *Environ Exp Bot* 178:104174. <https://doi.org/10.1016/j.envexpbot.2020.104174>
- Galviz Y, Souza GM, Lüttge U (2022) The biological concept of stress revisited: relations of stress and memory of plants as a matter of space–time. *Theor Exp Plant Physiol* 34:239–264

- Gaufichon L, Rothstein SJ, Suzuki A (2016) Asparagine metabolic pathways in Arabidopsis. *Plant Cell Physiol* 57:675–689. <https://doi.org/10.1093/pcp/pcv184>
- Geigenberger P, Thormählen I, Daloso DM, Fernie AR (2017) The unprecedented versatility of the plant thioredoxin system. *Trends Plant Sci* 22:249–262. <https://doi.org/10.1016/j.tplants.2016.12.008>
- Gomes Silveira JA, De Almeida VR, Almeida Da Rocha IM, De Oliveira Monteiro Moreira AC, De Azevedo Moreira RD, Abreu Oliveira JT (2003) Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. *J Plant Physiol* 160:115–123. <https://doi.org/10.1078/0176-1617-00890>
- Guilherme EA, Carvalho FEL, Daloso DM, Silveira JAG (2019) Increase in assimilatory nitrate reduction and photorespiration enhances CO₂ assimilation under high light-induced photoinhibition in cotton. *Environ Exp Bot* 159:66–74. <https://doi.org/10.1016/j.envexpbot.2018.12.012>
- Gutiérrez AR, Lejay LV, Dean A, Chiaromonte F, Shasha DE, Coruzzi GM (2007) Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in Arabidopsis. *Genome Biol* 8:R7. <https://doi.org/10.1186/gb-2007-8-1-r7>
- Hildebrandt TM (2018) Synthesis versus degradation: directions of amino acid metabolism during Arabidopsis abiotic stress response. *Plant Mol Biol* 98:121–135. <https://doi.org/10.1007/s11103-018-0767-0>
- Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP (2015) Amino acid catabolism in plants. *Mol Plant* 8:1563–1579. <https://doi.org/10.1016/j.molp.2015.09.005>
- Jeong H, Mason SP, Barabási AL, Oltvai ZN (2001) Lethality and centrality in protein networks. *Nature* 411:41–42. <https://doi.org/10.1038/35075138>
- Jones H (1998) Stomatal control of photosynthesis and transpiration. *J Exp Bot* 49:387–398. https://doi.org/10.1093/jexbot/49.suppl_1.387
- Jones AM, Xuan Y, Xu M et al (2014) Border control - a membrane-linked interactome of Arabidopsis. *Science* 344:711–716. <https://doi.org/10.1126/science.1251358> ((80))
- Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E, Dörmann P, Weckwerth W, Gibon Y, Stitt M, Willmitzer L, Fernie AR, Steinhauser D (2005) GMD@CSB.DB: the Golm metabolome database. *Bioinformatics* 21:1635–1638. <https://doi.org/10.1093/bioinformatics/bti236>
- Kranter I, Minibayeva FV, Beckett RP, Seal CE (2010) What is stress? Concepts, definitions and applications in seed science. *New Phytol* 188:655–673. <https://doi.org/10.1111/j.1469-8137.2010.03461.x>
- Kubis A, Bar-Even A (2019) Synthetic biology approaches for improving photosynthesis. *J Exp Bot* 70:1425–1433. <https://doi.org/10.1093/jxb/erz029>
- Kuhalskaya A, Ahchige MW, de Souza LP, Vallarino J, Brotman Y, Alseikh S (2020) Network analysis provides insight into tomato lipid metabolism. *Metabolites* 10. <https://doi.org/10.3390/metabo10040152>
- Leitch AR, Leitch IJ (2008) Genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483. <https://doi.org/10.1126/science.1153585> ((80-))
- Lichtenthaler HK (1998) The stress concept in plants: an introduction. *Ann N Y Acad Sci* 851:187–198. <https://doi.org/10.1111/j.1749-6632.1998.tb08993.x>
- Lima VF, Medeiros DB, Dos Anjos L, Gago J, Fernie AR, Daloso DM (2018) Toward multifaceted roles of sucrose in the regulation of stomatal movement. *Plant Signal Behav* 0:1–8. <https://doi.org/10.1080/15592324.2018.1494468>
- Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc* 1:387–396. <https://doi.org/10.1038/nprot.2006.59>
- Liu YY, Slotine JJ, Barabási AL (2011) Controllability of complex networks. *Nature* 473:167–173. <https://doi.org/10.1038/nature10011>
- Long SP, Marshall-Colon A, Zhu XG (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell* 161:56–66. <https://doi.org/10.1016/j.cell.2015.03.019>
- Lozano-Elena F, Fàbregas N, Coletto-Alcudia V, Caño-Delgado AI (2022) Analysis of metabolic dynamics during drought stress in Arabidopsis plants. *Sci Data* 9:1–12. <https://doi.org/10.1038/s41597-022-01161-4>
- Luedemann A, Strassburg K, Erban A, Kopka J (2008) TagFinder for the quantitative analysis of gas chromatography - mass spectrometry (GC-MS)-based metabolite profiling experiments. *Bioinformatics* 24:732–737. <https://doi.org/10.1093/bioinformatics/btn023>
- Lüttge U (2021) Integrative emergence in contrast to separating modularity in plant biology: views on systems biology with information, signals and memory at scalar levels from molecules to the biosphere. *Theor Exp Plant Physiol* 33:1–13. <https://doi.org/10.1007/s40626-021-00198-x>
- Merchant A, Richter AA (2011) Polyols as biomarkers and bioindicators for 21st century plant breeding. *Funct Plant Biol* 38:934–940. <https://doi.org/10.1071/FP11105>
- Merchant A, Tausz M, Arndt SK, Adams MA (2006) Cyclitols and carbohydrates in leaves and roots of 13 Eucalyptus species suggest contrasting physiological responses to water deficit. *Plant, Cell Environ* 29:2017–2029. <https://doi.org/10.1111/j.1365-3040.2006.01577.x>
- Mesquita RO, Coutinho FS, Vital CE, Nepomuceno AL, Rhys Williams TC, de Oliveira J, Ramos H, Loureiro ME (2020) Physiological approach to decipher the drought tolerance of a soybean genotype from Brazilian savanna. *Plant Physiol Biochem* 151:132–143. <https://doi.org/10.1016/j.plaphy.2020.03.004>
- Mitchell M (2006) Complex systems: network thinking. *Artif Intell* 170:1194–1212. <https://doi.org/10.1016/j.artint.2006.10.002>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* 15:473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Neto MCL, Carvalho FEL, Souza GM, Silveira JAG (2021) Understanding photosynthesis in a spatial-temporal multiscale: the need for a systemic view. *Theor Exp Plant Physiol* 33:113–124. <https://doi.org/10.1007/s40626-021-00199-w>
- O’Leary B, Plaxton WC (2020) Multifaceted functions of post-translational enzyme modifications in the control of plant glycolysis. *Curr Opin Plant Biol* 55:28–37. <https://doi.org/10.1016/j.pbi.2020.01.009>
- Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. *Cell Mol Life Sci* 69:3225–3243. <https://doi.org/10.1007/s00018-012-1091-5>
- Obata T, Witt S, Lisec J, Palacios-Rojas N, Florez-Sarasa I, Yousfi S, Araus JL, Cairns JE, Fernie AR (2015) Metabolite profiles of maize leaves in drought, heat, and combined stress field trials reveal the relationship between metabolism and grain yield. *Plant Physiol* 169:2665–2683. <https://doi.org/10.1104/pp.15.01164>
- Pang Z, Chong J, Zhou G, De Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques PÉ, Li S, Xia J (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 49:W388–W396. <https://doi.org/10.1093/nar/gkab382>
- Pires MV, Pereira Júnior AA, Medeiros DB, Daloso DM, Pham PA, Barros KA, Engqvist MKM, Florian A, Krahnert I, Maurino VG, Araújo WL, Fernie AR (2016) The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in Arabidopsis. *Plant Cell Environ* 39:1304–1319. <https://doi.org/10.1111/pce.12682>

- Razaghi-Moghadam Z, Nikoloski Z (2021) GeneReg: a constraint-based approach for design of feasible metabolic engineering strategies at the gene level. *Bioinformatics* 37:1717–1723. <https://doi.org/10.1093/bioinformatics/btaa996>
- Rosenzweig C, Elliott J, Deryng D et al (2014) Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. *Proc Natl Acad Sci U S A* 111:3268–3273. <https://doi.org/10.1073/pnas.1222463110>
- Saito K, Yonekura-Sakakibara K, Nakabayashi R, Higashi Y, Yamazaki M, Tohge T, Fernie AR (2013) The flavonoid biosynthetic pathway in Arabidopsis: structural and genetic diversity. *Plant Physiol Biochem* 72:21–34. <https://doi.org/10.1016/j.plaphy.2013.02.001>
- Shannon P, Markiel A, Owen Ozier 2, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2498–2504. <https://doi.org/10.1101/gr.1239303.metabolite>
- Souza GM, Lüttge U (2015) Stability as a phenomenon emergent from plasticity–complexity–diversity in eco-physiology. In: Lüttge U, Beyschlag W (eds) *Progress in Botany*, 1st edn. Springer-Verlag, Berlin, pp 211–239
- Souza GM, Ribeiro RV, Pincus SM (2004) Changes in network connectance and temporal dynamics of gas exchange in *Citrus sinensis* under different evaporative demands. *Brazilian J Plant Physiol* 16:119–130. <https://doi.org/10.1590/S1677-04202004000300001>
- Souza GM, Pincus SM, Monteiro JAF (2005) The complexity–stability hypothesis in plant gas exchange under water deficit. *Brazilian J Plant Physiol* 17:363–373. <https://doi.org/10.1590/S1677-042005000400004>
- Souza GM, Ribeiro RV, De ORF, Machado EC (2005) Network connectance and autonomy analyses of the photosynthetic apparatus in tropical tree species from different successional groups under contrasting irradiance conditions. *Rev Bras Botânica* 28:47–59. <https://doi.org/10.1590/S0100-84042005000100005>
- Souza GM, Ribeiro RV, Prado CHBA, Damineli DSC, Sato AM, Oliveira MS (2009) Using network connectance and autonomy analyses to uncover patterns of photosynthetic responses in tropical woody species. *Ecol Complex* 6:15–26. <https://doi.org/10.1016/j.ecocom.2008.10.002>
- Souza GM, Prado CHBA, Ribeiro RV, Barbosa JPRAD, Gonçalves AN, Habermann G (2016) Toward a systemic plant physiology. *Theor Exp Plant Physiol* 28:341–346. <https://doi.org/10.1007/s40626-016-0071-9>
- Souza PVL, Lima-Melo Y, Carvalho FE, Reichheld JP, Fernie AR, Silveira JAG, Daloso DM (2019) Function and compensatory mechanisms among the components of the chloroplastic redox network. *CRC Crit Rev Plant Sci* 38:1–28. <https://doi.org/10.1080/07352689.2018.1528409>
- Sweetlove LJ, Fernie AR (2005) Regulation of metabolic networks: understanding metabolic complexity in the systems biology era. *New Phytol* 168:9–24. <https://doi.org/10.1111/j.1469-8137.2005.01513.x>
- Sweetlove LJ, Fernie AR (2013) The spatial organization of metabolism within the plant cell. *Annu Rev Plant Biol* 64:723–746. <https://doi.org/10.1146/annurev-arplant-050312-120233>
- Sweetlove LJ, Obata T, Fernie AR (2014) Systems analysis of metabolic phenotypes: what have we learnt? *Trends Plant Sci* 19:222–230. <https://doi.org/10.1016/j.tplants.2013.09.005>
- Sweetlove LJ, Nielsen J, Fernie AR (2017) Engineering central metabolism – a grand challenge for plant biologists. *Plant J* 90:749–763. <https://doi.org/10.1111/tpj.13464>
- Timm S, Hagemann M (2020) Photorespiration – how is it regulated and regulates overall plant metabolism? *J Exp Bot*. <https://doi.org/10.1016/j.jbiomac.2015.10.079>
- Todaka D, Zhao Y, Yoshida T et al (2017) Temporal and spatial changes in gene expression, metabolite accumulation and phytohormone content in rice seedlings grown under drought stress conditions. *Plant J* 90:61–78. <https://doi.org/10.1111/tpj.13468>
- Trisos CH, Merow C, Pigot AL (2020) The projected timing of abrupt ecological disruption from climate change. *Nature*. <https://doi.org/10.1038/s41586-020-2189-9>
- Valladares F, Balaguer L, Martínez-Ferri E, Pérez-Corona E, Manrique E (2002) Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytol* 156:457–467. <https://doi.org/10.1046/j.1469-8137.2002.00525.x>
- Vital CE, Giordano A, de Almeida SE, Rhys Williams TC, Mesquita RO, Vidigal PMP, de Santana LA, Pacheco TG, Rogalski M, de Oliveira Ramos HJ, Loureiro ME (2017) An integrative overview of the molecular and physiological responses of sugarcane under drought conditions. *Plant Mol Biol* 94:577–594. <https://doi.org/10.1007/s11103-017-0611-y>
- Worley B, Powers R (2015) Multivariate analysis in metabolomics Bradley. *Curr Metabolomics* 1:92–107. <https://doi.org/10.2174/2213235X11301010092.Multivariate>
- Wurtzel ET, Vickers CE, Hanson AD, Millar AH, Cooper M, Voss-Fels KP, Nikel PI, Erb TJ (2019) Revolutionizing agriculture with synthetic biology. *Nat Plants* 5:1207–1210. <https://doi.org/10.1038/s41477-019-0539-0>
- Xia J, Wishart DS (2011) Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. *Nat Protoc* 6:743–760. <https://doi.org/10.1038/nprot.2011.319>
- Xia J, Broadhurst DI, Wilson M, Wishart DS (2013) Translational biomarker discovery in clinical metabolomics: an introductory tutorial. *Metabolomics* 9:280–299. <https://doi.org/10.1007/s11306-012-0482-9>
- Ye CY, Fan L (2021) Orphan crops and their wild relatives in the genomic era. *Mol Plant* 14:27–39. <https://doi.org/10.1016/j.molp.2020.12.013>
- Yoshida T, Yamaguchi-Shinozaki K (2021) Metabolic engineering: towards water deficiency adapted crop plants. *J Plant Physiol* 258–259:153375. <https://doi.org/10.1016/j.jplph.2021.153375>
- Zandalinas SI, Mittler R (2022) Plant responses to multifactorial stress combination. *New Phytol*. <https://doi.org/10.1111/nph.18087>
- Zandalinas SI, Fritschi FB, Mittler R (2021) Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends Plant Sci* 26:588–599. <https://doi.org/10.1016/j.tplants.2021.02.011>
- Zandalinas SI, Balfagón D, Gómez-Cadenas A, Mittler R (2022) Responses of plants to climate change: metabolic changes during abiotic stress combination in plants. *J Exp Bot*. <https://doi.org/10.1093/jxb/erac073>
- Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weigl S, Fritschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. *Nat Biotechnol* 36:1211–1216. <https://doi.org/10.1038/nbt.4272>

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