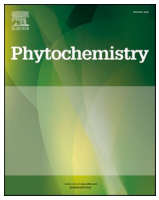
Phytochemistry 203 (2022) 113381

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem



The pivotal role of cultivar affinity to arbuscular mycorrhizal fungi in determining mycorrhizal responsiveness to water deficit

Paola Ganugi [a], Shamina Imran Pathan [b,*], Leilei Zhang [a], Paola Arfaioli [b], Stefano Benedettelli [b], Alberto Masoni [c], Giacomo Pietramellara [b], Luigi Lucini [a]

[a] Department for Sustainable Food Process, Research Centre for Nutrigenomics and Proteomics, Università Cattolica del Sacro Cuore, Piacenza, Italy
[b] Department of Agriculture, Food, Environment and Forestry, University of Florence, P.le delle Cascine 28, Firenze, 50144, Italy
[c] Department of Biology, University of Florence, Sesto Fiorentino, 50019, Italy

ARTICLE INFO

Keywords:
T. turgidum subsp. Durum (Desf.) Husn
Poaceae
UHPLC-ESI/QTOF-MS
Multivariate analysis
Differential metabolites
Mycorrhizal affinity
Water stress

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have gained remarkable importance, having been proved to alleviate drought stress-induced damage in wheat due to their ability to ameliorate plant water use efficiency and antioxidant enzyme activity. However, despite the current relevance of the topic, the molecular and physiological processes at the base of this symbiosis never consider the single cultivar affinity to mycorrhization as an influencing factor for the metabolic response in the AMF-colonized plant. In the present study, the mycorrhizal affinity of two durum wheat species (*T. turgidum* subsp. *durum* (Desf.)) varieties, Iride and Ramirez, were investigated. Successively, an untargeted metabolomics approach has been used to study the fungal contribution to mitigating water deficit in both varieties. Iride and Ramirez exhibited a high and low level of mycorrhizal symbiosis, respectively; resulting in a more remarkable alteration of metabolic pathways in the most colonised variety under water deficit conditions. However, the analysis highlighted the contribution of AMF to mitigating water deficiency in both varieties, resulting in the up- and down-regulation of many amino acids, alkaloids, phenylpropanoids, lipids, and hormones.

1. Introduction

The potential role of arbuscular mycorrhizal fungi (AMF) in low-input agriculture and sustainable agricultural management has emerged recently (Basu et al., 2018). Several authors have highlighted the ability of AMF to colonise plant roots and have shown the beneficial effects of this symbiosis in terms of soil nutrient uptake and protection from biotic and abiotic stresses (Pozo et al., 2010; Begum et al., 2019; Latef et al., 2016). Among abiotic stresses, AMF-triggered benefits have been observed under drought conditions, emphasising the potential use of mycorrhizae as an eco-friendly solution for marginal soils (dos Santos Pereira et al., 2018). Current climate change, characterised by increased temperatures, unpredictable precipitation, and reduced water availability, has led researchers to deepen their understanding of the processes underlying drought tolerance in AMF-inoculated plants (Subramanian et al., 2006; Ortiz et al., 2015).

Several studies have been conducted on durum wheat (*Triticum turgidum* subsp. durum (Desf.)) This crop is widely cultivated in different regions under varied climatic conditions, ranging from the Arctic Circle to the Equator, and from sea level to the high mountains in Tibet (Tadesse et al., 2020). Given the substantial yield losses caused by water scarcity (Farooq et al., 2014), AMF have been proposed to alleviate drought-induced damage in wheat because of their ability to ameliorate plant water-use efficiency and antioxidant enzyme activity (Augé et al., 2015). AMF colonisation can provide both direct and indirect benefits to durum wheat, including the uptake and translocation of nutrients, mitigation of abiotic stress-related negative effects, resistance to pathogens, and amelioration of the structure of soil aggregates (Ganugi et al., 2019). Moreover, recent metabolomic studies have revealed that mycorrhizal colonisation significantly influences the metabolomic pathways and root exudation patterns in wheat roots (Saia et al., 2015; Lucini et al., 2019). AMF have been shown to modulate both primary (sugar, organic acid, and amino acid content) and specialized metabolites, with phenylpropanoids showing the strongest modulation in mycorrhizal plants (Kaur and Zhawar, 2015). Bernardo et al. (2019) studied the regulation of specialized metabolism in mycorrhized wheat

* Corresponding author.
E-mail address: shamina.pathan@unifi.it (S.I. Pathan).

https://doi.org/10.1016/j.phytochem.2022.113381
Received 11 April 2022; Received in revised form 10 August 2022; Accepted 14 August 2022
Available online 27 August 2022

under water depletion conditions, which resulted in lipid, carbohydrate, oxidative stress, and brassinosteroid modulation. Furthermore, its impact on proteins related to sugar metabolism, cell wall rearrangement, cytoskeletal organisation, and sulfur-containing proteins has also been reported (Bernardo et al., 2017).

AMF responsiveness indicates the changes induced in plants by AMF colonisation which includes changes in morphology and/or vigour and/or performance and/or yields in response to a process generally defined, the amplitude of which depends on the plant-AMF strain combination, in addition to edaphic, agronomic, and stress conditions. Plant genetic polymorphisms underpin the differences in AMF responsiveness, and significant differences have been observed among accessions of the same species for various crops (Sawers et al., 2017; Berger and Gutjahr , 2021). However, previous research did not take into account the different metabolic responses of mycorrhized wheat which may significantly depend on the specific cultivar affinity to AMF, which can vary significantly depending on the ploidy number, geographic origin, nutrient use efficiency, and year of release of the genotype, as well as specific chromosomal regions associated with mycorrhization symbiosis (Hetrick et al., 1992; Zhu et al., 2001; Yücel et al., 2009; De Vita et al., 2018). Despite its pivotal role in determining the actual AMF-triggered relief from drought stress, the specific colonisation affinity of plants to mycorrhiza has been disregarded to date. Recently, the link between the genetic diversity of durum wheat and root affinity to AMF was investigated through a genome-wide association study, which allowed identification of the chromosome regions involved in the symbiosis (Ganugi et al., 2021). In the aforementioned study, 127 accessions of durum wheat were genotyped using 35 K single nucleotide polymorphisms, resulting in 6 clusters with different affinities to *Funneliformis mosseae* and *Rhizoglomus irregulare*. This suggests that the mycorrhizal affinity of a given plant cultivar to mycorrhiza may play a pivotal role in determining AMF responsiveness, even though it has been primarily underestimated to date. Therefore, this study aimed to comparatively investigate the root responses of 2 durum wheat species (*T. turgidum* subsp. durum (Desf.)) varieties, showing either high or low mycorrhizal affinity, respectively; under well-watered and water-deficient conditions. We hypothesised that high and low mycorrhizal affinities of host plants could have different effects on root metabolomics. Moreover, a larger alteration was observed under water-deficit conditions. Therefore, an untargeted metabolomics approach was chosen to comprehensively investigate the intricate crosstalk occurring during the AMF-root-stress interaction (Villate et al., 2021). Indeed, a broad metabolic reprogramming is elicited in roots following AMF colonisation, during drought, and even more so during their combination (Bernardo et al., 2019).

2. Results

2.1. Characterisation of both varieties

Ultrahigh-pressure liquid chromatography accurate-mass quadrupole time-of-flight mass spectrometry with electrospray ionisation (UHPLC-ESI/QTOF-MS) analysis of Iride and Ramirez samples provided chemical characterisation of the root metabolomic profile of both varieties.

Overall, 3294 putative metabolites were revealed in the Iride samples, as provided in the supplementary material, together with the individual abundance and composite mass spectra (Table S1). The heat map, obtained with fold-change-based clustering, identified 2 main clusters, with drought stress providing a hierarchically prominent role in shaping the metabolite profile (Fig. 1). Indeed, the [+AMF + STRESS] and [-AMF + STRESS] samples were gathered and markedly separated from the [+AMF-STRESS] and [-AMF-STRESS] roots. Within these two clusters, the effect of AMF treatment was visible; since for both

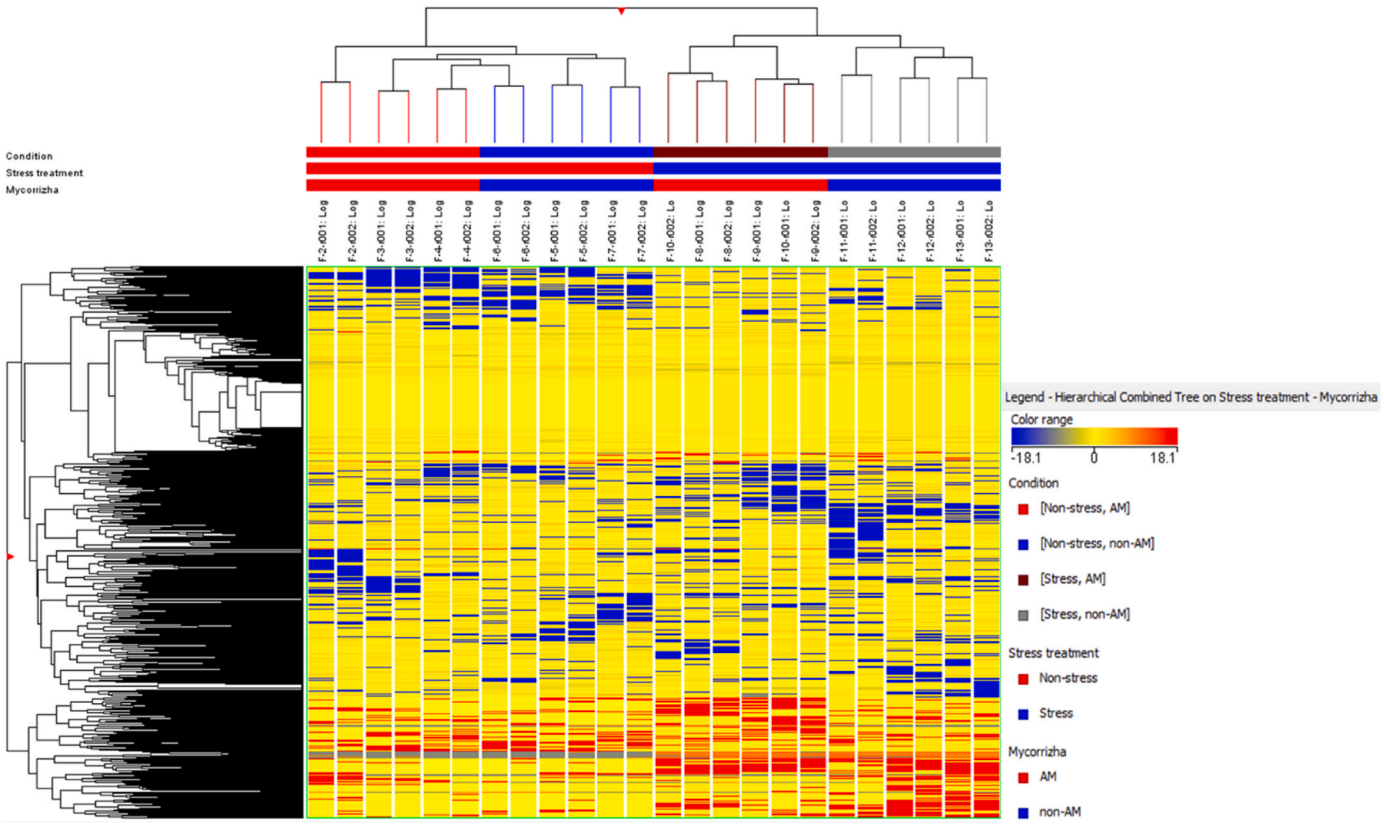


Fig. 1. Unsupervised hierarchical cluster analysis of metabolite profile in Iride durum wheat roots, either mycorrhized or not, under drought or well-watered conditions.

[+STRESS] and [-STRESS] conditions, mycorrhized samples appeared distinct from the non-mycorrhized samples.

Regarding Ramirez, the same untargeted metabolomic approach showed 3396 putative metabolites (Table S2), based on which the similarity/dissimilarity among conditions was identified through unsupervised hierarchical clustering analysis. Specifically, the fold-change-based heat map distinctly highlighted the same 2 clusters as Iride, identified by the [+STRESS] and [-STRESS] samples (Fig. 2). However, within these groups, the effect of mycorrhizal treatment was relevant only in the presence of drought stress, because a clear separation between [+AMF] and [-AMF] roots was not achieved within the [-STRESS] cluster.

To identify compounds that discriminate different metabolic profiles between conditions better, OPLS-DA Orthogonal projection to latent structures discriminant analysis) supervised analysis was performed. This multivariate approach generated a score plot that provided insights into the separation between experimental groups, starting from the metabolic data.

Concerning Iride, non-stressed samples were grouped together, regardless of the presence or absence of AMF colonisation, while a significant effect of AMF was identified, reflecting the separation between the [+AMF + STRESS] and [-AMF + STRESS] conditions (Fig. 3a). The predictivity of the model was established by goodness-of-fit R2Yand goodness-of-prediction Q2Y values of 0.986 and 0.88, respectively. Moreover, cross-validation CV-ANOVA ($p < 0.001$) was adequate, outlier absence was confirmed by Hotelling's T2 using both 95% and 99% confidence limits (suspect and strong outliers, respectively), and the permutation test except for overfitting.

Similar discrimination was achieved with the same multivariate analysis conducted on Ramirez samples, with $R2Y = 0.974$ and $Q2Y = 0.727$ as the model fitting parameters (Fig. 3b). The CV-ANOVA coefficient ($p < 0.001$) was acceptable, and permutation testing excluded overfitting. The OPLS-DA scatter plot showed a clear separation between conditions according to stress treatment, whereas no distinction between [+AMF] and [-AMF] samples was achieved in non-stressed roots.

The compounds with a variable importance in projection (VIP) score ≥ 1, representing the variables that mainly determined the discrimination in the OPLS-DA score plot projection, were annotated for both varieties (Table S3). Among these discriminant compounds, alkaloids, flavonoids, terpenoids, and amino acids were the most represented metabolic classes in both, the Iride and Ramirez cultivars.

2.2. Evaluation of AMF affinity

The level of AMF affinity assessed by dissecting microscopy confirmed the presence of root symbiosis in the mycorrhized samples and the absence of fungi in non-mycorrhized samples, for both varieties and under both stress conditions. The two varieties showed significantly different affinities for mycorrhization, with Iride and Ramirez roots presenting a high (mean = 64.94%) and a low (17.36%) percentage of AMF affinity (Fig. 4).

2.3. Metabolomics on the different conditions

Based on previous results of chemical characterisation and mycorrhizal affinity, volcano analysis was performed to determine root metabolite enrichment and depletion patterns under the given treatments. Metabolites with fold changes higher than 1.2 (p-value < 0.05, 216 and 96 compounds for Iride and Ramirez, respectively) were selected for this analysis (Tables S4 and S5). A Venn diagram was constructed to identify the shared and unique metabolites between the Iride and Ramirez varieties. The results showed that only 4.1% of the metabolites were common between the two (Fig. 5). The compounds listed in Tables S5 and S6 were loaded onto the PlantCyc Pathway Tool, where the principal metabolic pathways involved in the wheat varieties' response to water deficit and mycorrhizal conditions were identified (Figs. 6 and 7).

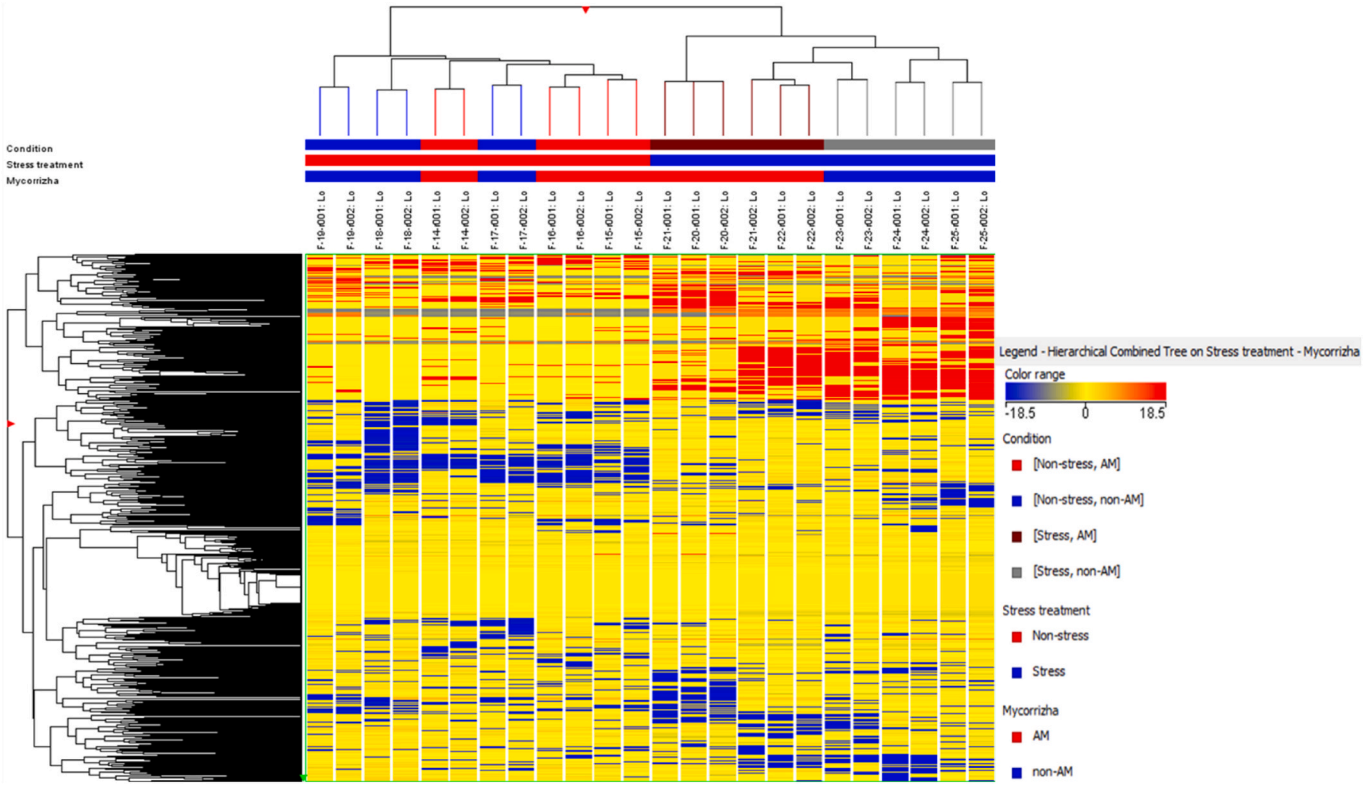


Fig. 2. Unsupervised hierarchical cluster analysis of metabolite profile in Ramirez durum wheat roots, either mycorrhized or not, under drought or well-watered conditions.

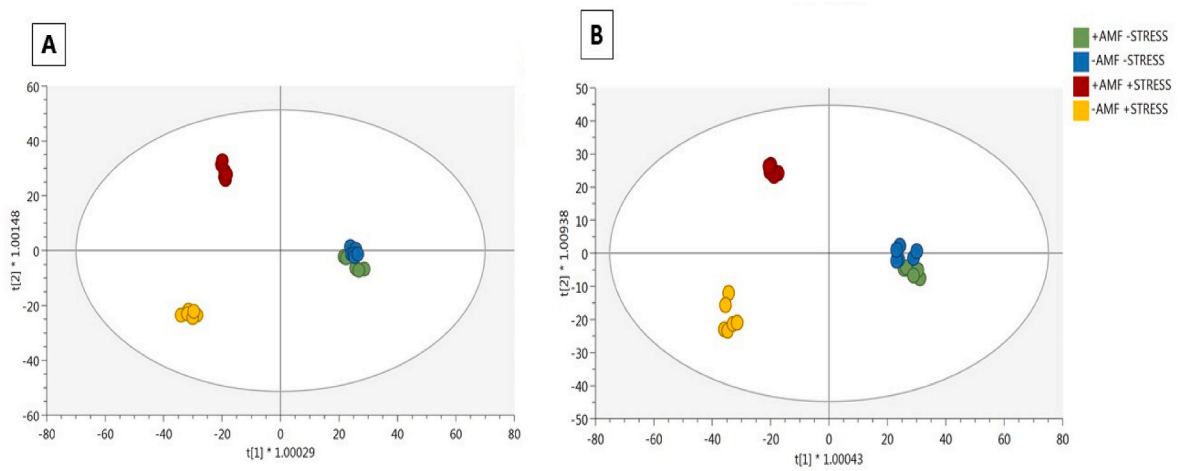


Fig. 3. Orthogonal projection to latent structures discriminant analysis (OPLS-DA) supervised modeling of Iride (A) and Ramirez (B) root metabolomic profiles, either mycorrhized or not, under drought or well-watered conditions.

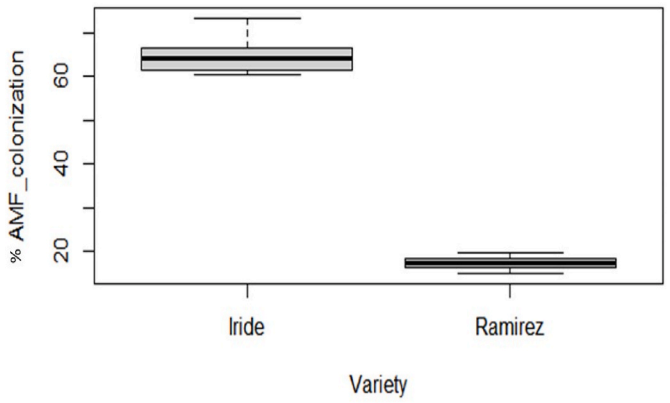


Fig. 4. Boxplot representation of arbuscular mycorrhiza fungal root affinity (AMF %) of roots from Iride and Ramirez varieties, irrespective from the watering regime.

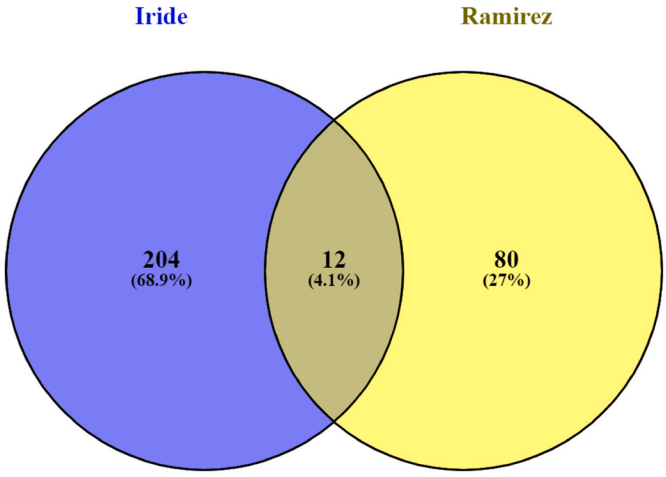


Fig. 5. Biosynthetic metabolic processes (A) and synthesis of specialized metabolites (B) modulated in [-STRESS + AMF], [+STRESS + AMF] and [+STRESS-AMF] Iride roots. Volcano Plot analysis ($p < 0.05$, fold change ≥ 1.2) was performed on the metabolomics dataset, and differential metabolites were loaded into the PlantCyc Pathway Tool (https://www.plantcyc.org/). Metabolic subcategories are represented by the x-axis, while the y-axis stand for the cumulative log fold change (FC). The large dots correspond to the average (mean) of all FCs for the different metabolites in the class, while the small dots represent the individual log FC.

With respect to primary metabolism, amino acid and lipid biosynthesis were mostly affected by mycorrhizal inoculation and water deficit in both varieties. Iride samples showed elicitation of amino acid accumulation by mycorrhizal inoculation, especially for l-methionine and l-histidine biosynthesis. Moreover, amino acid biosynthesis was modulated in Ramirez by the AMF-drought interaction, particularly with respect to L-citrulline and L-proline content. However, lipid biosynthesis was negatively modulated in both varieties. In Iride, phospholipid desaturation was notably reduced, with decreased levels of glycerolipids, including 1-oleoyl-2-(3E)-hexadecenoyl-phosphatidylglycerol, 1-linoleoyl-2-(3E)-hexadecenoyl-phosphatidylglycerol, and 1-α-linolenoyl-2-palmitoyl-phosphatidylglycerol. However, this reduction was mild for the [+ AMF + STRESS] samples. In Ramirez, palmitoleate was downregulated by water deficit and by the combined effects of AMF and water deficit.

Interestingly, extensive modulation of specialized metabolism was observed in both the varieties (Figs. 6 and 7). Among the N-containing compounds, alkaloids represented the most intensely modulated class of compounds, resulting in accumulation in the mycorrhizal water-stressed and non-stressed roots of both varieties. Under drought stress, (13S, 14R)-13-*O*-acetyl-1,8-dihydroxy-N-methylcanadine and vindolidine accumulated in Iride [-AMF] roots, whereas increased levels of ecgonine methyl ester, (S)-tetrahydropapaverine, theobromine, 6-hydroxyprotopine, and 7,8-dihydroberberine were observed after AMF inoculation. Lastly, 6-hydroxy-allocryptopine and noroxopluviine concentrations increased by AMF under well-watered conditions. In Ramirez, (S)-nandinine, (S)-stylopine, and noscapine appeared to increase under water scarcity, while the last compound, together with betaxanthine, showed high levels under AMF treatment.

Phenylpropanoid content was positively modulated by drought in both varieties. In Iride, higher levels of lignans (5′-demethylyatein, thujaplicatin, and 6-methoxypodophyllotoxin) and flavonoids (3,7,4′-trimethylquercetin, isorhamnetin 3-sulfate, rotenolone, genistein, 6-*C*-glucosyl chrysin, and dalcochinin) were detected. Whereas, in Ramirez, higher levels of flavonoids (kaempferol 3-*O*-(3″-*O*-p-coumaroyl, 6″-*O*-feruloyl)-glucoside, patulitrin, cyanidin 3-*O*-[2″-*O*-(xylosyl)-6″-*O*-(p-coumaroyl) glucoside] 5-*O*-glucoside, naringin and luteolinidin) were observed. In contrast, accumulation of phenylpropanoid compounds following mycorrhizal root colonisation was observed only in Iride samples.

Terpenoid levels showed opposite trend between the two cultivars, which were considered to surge and decline in Iride and Ramirez,

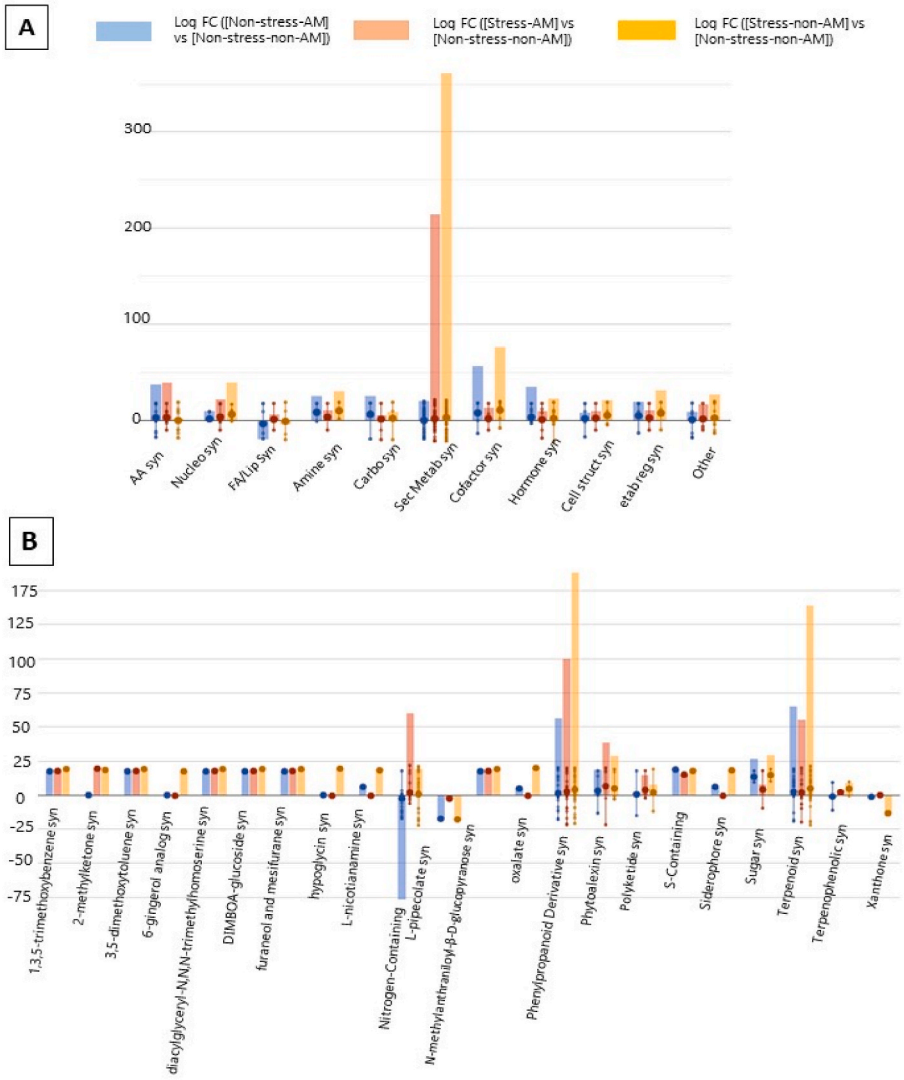


Fig. 6. Biosynthetic metabolic processes (A) and synthesis of specialized metabolites (B) modulated in [-STRESS + AMF], [+STRESS + AMF] and [+STRESS-AMF] Ramirez roots. Volcano Plot analysis ($p < 0.05$, fold change ≥ 1.2) was performed on the metabolomics dataset, and differential metabolites were loaded into the PlantCyc Pathway Tool (https://www.plantcyc.org/). Metabolic subcategories are represented by the x-axis, while the y-axis stand for the cumulative log fold change (FC). The large dots correspond to the average (mean) of all FCs for the different metabolites in the class, while the small dots represent the individual log FC.

respectively. This effect was the result of both, the action of water deficit and the coupled effect of drought and AMF inoculation. Higher levels of taxunin, desoxyhemigossypol-6-methyl ether, gypsogenate-28-beta-D-glucoside, and soyasapogenol B were observed under low water availability, whereas AMF induced an increase in the concentrations of (4S)-4-(5,5-dimethylcyclohex-1-en-1-yl)cyclohex-1-ene-1-carbaldehyde and 3-hydroxylubimin. In contrast, under water deficit conditions, Ramirez mycorrhizal and non-mycorrhizal roots showed decreased levels of isolipitenone, valencene, and (3E)-4,8-dimethylnona-1,3,7-triene.

Finally, the phytohormone profile was significantly modulated in both varieties. More specifically, gibberellin content exhibited a marked reduction in both Iride and Ramirez mycorrhized and water-stressed roots, whereas cis-tuberonic acid (a precursor of jasmonate) was negatively modulated only in the high-affinity variety colonised by AMF.

3. Discussion

Host plant affinity to AMF is highly variable and depends on many factors, including root anatomy, environmental characteristics (temperature, soil moisture, and fertility), and inoculum potential (Sharda and Koide , 2010; Eissenstat et al., 2015; Turrini et al., 2016). Moreover, significant variation in wheat genotype affinity to AMF has been reported (Singh et al., 2012; De Vita et al., 2018) and was confirmed by our experiment. Iride samples showed high levels of AMF symbiosis (64.94% on average), whereas a low rate of mycorrhization was observed in Ramirez roots (17.36%).

Despite the extensive research carried out and great progress made to date, much work is still needed to clarify the mechanics and implications of plant-AMF symbiosis. Interestingly, recent studies have investigated the molecular basis of wheat species variability for mycorrhizal affinity and 11 putative quantitative trait loci (QTLs) linked to plant-AMF symbiosis, located on chromosomes 1A, 2A, 2 B, 5A, 6A, 7A and 7B, were detected (De Vita et al., 2018; Ganugi et al., 2021). De Vita et al. (2018) reported that markers associated with root colonisation are mainly co-located with genes coding proteins involved in stress responses, plant growth and developmental processes, cellular homeostasis, biosynthesis of specialized metabolites, and pathogen resistance.

In our study, metabolic regulation after AMF treatment in both varieties was evident only under water deficiency, while no visible mycorrhizal effect occurred under well-watered conditions. Conversely, the affinity to AMF of the two varieties, reported a markedly different plant response to fungal inoculum, reflecting the cultivar-specific ability to recruit AMF and promote effective root-mycorrhizal symbiosis. Consequently, cultivar-specific responses to AMF were observed by metabolomics under water deficiency, indicating the differences at the biochemical level, with a more remarkable alteration of metabolic pathways for Iride samples. This conclusion is in agreement with the analyses of Mäder et al. (2000), who suggested the potential use of AMF

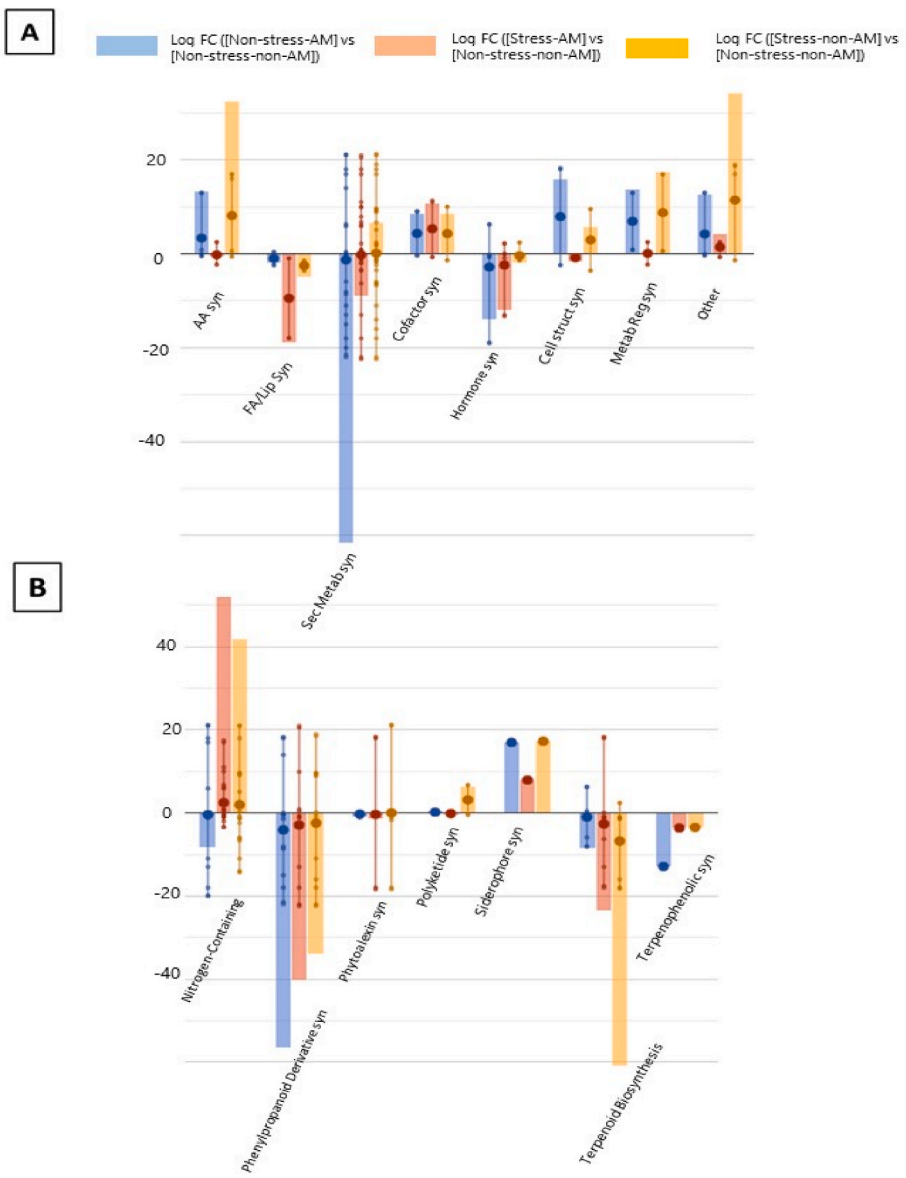


Fig 7. Biosynthetic metabolic processes (A) and synthesis of secondary metabolites (B) modulated in [-STRESS+AMF], [+STRESS+AMF] and [+STRESS-AMF] Ramirez roots. Volcano Plot analysis ($p < 0.05$, fold change $> = 1.2$) was performed on the metabolomics dataset, and differential metabolites were loaded into the PlantCyc Pathway Tool (https://www.plantcyc.org/). Metabolic subcategories are represented by the x-axis, while the y-axis stand for the cumulative log fold change (FC). The large dots correspond to the average (mean) of all FCs for the different metabolites in the class, while the small dots represent the individual log FC.

inoculation in dryland agriculture.

Water scarcity negatively affects plant developmental processes, such as photosynthesis, transpiration, and water and nutrient uptake, depending on the stress level, plant genotype and growth stage (Kapoor et al., 2020). Usually, following limited water availability, plants start to produce reactive oxygen species (ROS), altering carbohydrate/protein synthesis and lipid metabolism, and can cause cell death and oxidative damage (Sharma et al., 2012). However, plants have evolved adaptive mechanisms to overcome water shortages, including the initial limitation of transpiration through stomatal closure and subsequent cytoplasmic accumulation of osmoprotectant solutes. In addition, plants activate many processes to cope with the deleterious effects of water scarcity, including greater water uptake, improved stomatal conductance, and increased water-use efficiency.

In our study, this process of osmotic adjustment involved the modulation of amino acids, alkaloids, phenylpropanoids, lipids, and hormones, confirming the findings from previous studies (Shao et al., 2009; Marček et al., 2019). Moreover, metabolic analysis revealed that AMF contributes to mitigating stress, resulting in the increased and decreased accumulation of numerous biochemical compounds and activation or deactivation of physiological pathways, as previously suggested (Bernardo et al., 2019).

Concerning amino acids, the accumulation of citrulline in drought-stressed Ramirez samples supported the results of Akashi et al. (2008), and Kusvuran et al. (2013), , who reported that higher levels of citrulline in response to environmental stresses in the vegetative tissues of watermelon, melon, and chickpea, respectively. Thus, the increase in citrulline content, synthesised by plants from ornithine and carbamoyl phosphate, may play a key role in protecting cellular enzymes from oxidative damage. Similarly, the higher levels of 5-amino-2-oxopentanoate could suggest an osmolarity increment during water limitation because proline biosynthesis, the biochemical pathway in which this compound is involved, is known to accumulate in dehydrated plant tissues of many species, including maize, wheat, and pea (Rampino et al., 2006; Charlton et al., 2008; Witt et al., 2012). Under the same water deficit conditions, Ramirez plants inoculated with AMF showed a downregulation of proline biosynthesis and a lower accumulation of citrulline than the non-AMF plants, indicating that AMF plants experienced less stress under drought conditions.

Increased levels of these organic compounds were observed in both varieties under water scarcity, which is in agreement with previous experiments on wheat (Guo et al., 2020; Stallmann et al., 2020). It is

known that alkaloids play a role in protecting plants from attacks by vertebrate and invertebrate herbivores (Shymanovich et al., 2015). Nevertheless, the accumulation of these compounds in plants has been reported to be involved in drought stress tolerance in many species, including *Senecio longilobus* (Briske and Camp, 1982), *Catharanthus roseus* (Jaleel et al., 2007), and *Papaver somniferum* (Szabó et al., 2003). Several alkaloids were found in higher quantities in AMF-colonized roots, as previously reported for tomato, ragwort roots (Rivero et al., 2015; Hill et al., 2018) and some medicinal plants, including *Camptotheca acuminata* and *Camptotheca. Roseus* (Yu et al., 2010; de la Rosa-Mera et al., 2011). Moreover, the greatest alkaloid accumulation by AMF was observed under low water availability, occurring more markedly in the Iride samples, which showed a higher number of upregulated alkaloids.

Increased concentrations of phenylpropanoids were observed in Iride samples under water deficit conditions, highlighting the pivotal role of these specialized metabolites in mitigating plant water scarcity, as reported previously for wheat (Ma et al., 2014; Kaur and Zhawar, 2015) and tomato (Sánchez-Rodríguez et al., 2011). AMF inoculation under drought conditions revealed the combined effects of mycorrhizae and stress on osmoprotectant production. In contrast, mycorrhizal roots of Ramirez wheat, both well-watered and under stressed conditions, showed decreased phenylpropanoid concentrations. Apparently, the low AMF affinity of this wheat variety could be the reason for decreased phenylpropanoid accumulation, which seems to play a crucial role in plant–microorganism interactions. For example, evidence supports the importance of flavonoids in mycorrhizal formation, including hyphal growth and spore germination (Mierziak et al., 2014). Elevated flavonoid biosynthesis during mycorrhizal development has been found in white clover, melon roots and *Medicago truncatula* (Akiyama et al., 2002; Ponce et al., 2004; Schliemann et al., 2008). Interestingly, increased levels of these compounds emphasise the improved ability of mycorrhizal plants to resist stress (Harrison and Dixon, 1993).

Accumulation of terpenoids in plants under drought stress conditions has been widely demonstrated (Munné-Bosch et al., 1999; Vaughan et al., 2015), revealing their antioxidant potential against oxidative damage. However, water shortage modulated the terpenoid content in Iride and Ramirez, exhibiting increased and decreased concentrations of these specialized metabolites, respectively. Similarly, under both well-watered and stress conditions, AMFinoculation did not lead to an increased terpenoid concentration in Ramirez samples, whereas higher levels of isoprenoids were achieved in Iride, supporting previous results concerning lettuce and sweet potato (Farmer et al., 2007; Baslam et al., 2013).

However, the dissimilar mycorrhizal modulation of terpenoid content in the 2 durum wheat varieties could be ascertained to factors that have been found to lead to variability in AMF-associated terpenoid accumulation. As reviewed by Welling et al. (2016), decreased terpenoid accumulation in Ramirez could be affected by the wheat genotype and the specificity of AMF. Moreover, decline in symbiosis between plants and AMF, resulting in low mycorrhizal affinity, could have limited terpenoid accumulation in the host plant.

Significant changes in the lipid content of the 2 wheat varieties were observed in roots exposed to water deficiency. In particular, glycerolipids, which are involved in phospholipid and fatty acids biosynthesis, decreased in the stressed samples. Glycolipids and phospholipids are important membrane components and are primary targets of environmental stress (Hou et al., 2016). Drought stress activates distinct phospholipases that preferentially hydrolyse membrane phospholipids, leading to their degradation (Wang et al., 2020). Nevertheless, evidence suggests that AMF inoculation has evolved the ability of plants to positively modulate lipid composition to enhance stress tolerance (Begum et al., 2019). Interestingly, the reduction of phospholipid desaturation under water scarcity appeared to be greatly reduced in the mycorrhized roots of the Iride variety, probably revealing an increase in unsaturated lipids, which play an essential role in plant membrane stabilisation (Zhang et al., 2005). Moreover, the level of phosphatidylethanolamine (PE), a non-bilayer-forming lipid, was markedly increased relative to that of the bilayer-forming lipid phosphatidylcholine (PC), thus contributing to overcoming drought stress by enhancing membrane flexibility (Vigh et al., 1986). In the mycorrhized roots of Ramirez, palmitoleate (16:1) concentration under water stress notably decreased, whereas it appeared to increase under well-watered conditions. Palmitoleate (16:1) is synthesised by the fungus in intraradical mycelia starting from palmitic acid, which, in turn, is acquired from the host plant. Nevertheless, plant lipid transfer to AM fungi appears to be reduced by drought stress (Trépanier et al., 2005). Consequently, the low water availability and the limited contribution of mycorrhization to overcome this deficiency related to the low AMF affinity of Ramirez could explain the reduction in palmitoleic acid concentration.

Finally, substantial evidence has emerged for water deficit and mycorrhizal modulation of wheat hormone levels. First, low water availability negatively modulated gibberellin content in both varieties (gibberellin A36 and A19 in Iride; A9, and A19 in Ramirez), suggesting their crucial role in conferring abiotic stress tolerance to plants, as reported by Colebrook et al. (2014). This result agrees with the results of Wang et al. (2008) and Krugman et al. (2011), who revealed, reduced gibberellin levels in maize and a decrease in GA 2-beta dioxygenase (GA2OX1) expression involved in gibberellin biosynthesis in wild accessions of emmer. In addition, water deficiency led to the down-regulation of 6-deoxocastasterone in Iride samples, a plant hormone involved in brassinosteroid biosynthesis. Brassinosteroids are steroidal hormones that regulate many physiological responses such as seed germination, cell elongation, and root development (Wei and Li, 2016). Nevertheless, despite the additional role of these hormones in increasing drought tolerance in many species (Haubrick et al., 2006; Kagale et al., 2007), plant responses to stress are not mediated by changes in brassinosteroid content (Jager et al., 2008).

4. Conclusions

Iride and Ramirez wheat varieties showed high and low levels of AMF symbiosis, respectively, reflecting the cultivar-specific affinity to mycorrhization which translated into a more remarkable alteration of metabolic pathways in the Iride samples. Nevertheless, AMF inoculation under water deficiency led to a broad metabolic reprogramming in both varieties, highlighting the contribution of fungi to mitigating stress.

The results of this study emphasise the potential for further -omics based approaches exploiting the variability between durum wheat varieties and the mechanisms underlying the cultivar-specific affinity to mycorrhization.

5. Experimental

5.1. Experimental design, plant growth conditions, and AMF inoculation

The experiment was conducted in a growth chamber at the Department of Agriculture (DAGRI), University of Florence, Italy. We used 2 varieties of *Triticum turgidum* ssp. *durum*, “Iride” and “Ramirez”, which we had previously determined to differ in the extent of AMF affinity (Ganugi et al., 2021). In particular, Iride shows high mycorrhizal affinity, whereas Ramirez shows low mycorrhizal affinity (Ganugi et al., 2021). Both selected varieties were modern accessions and were genetically uniform (all seeds within the variety belonged to the same genotypes). Silty-clay agricultural soil (Table S6) was collected from a farm located in Grosseto, Italy (42°53′04″N11° 16′17″ N). The soil was sampled at a depth between 0 and 10 cm at 3 different points, 20 m away from each other, along the diagonal of a 500 m^2 plot, to obtain 3 independent replicates. Subsequently, it was transferred to the laboratory to be air-dried, crushed, sieved (pore size: 2 mm), and then mixed with silica sand (0.2–1 mm size) and peat (Terriccio universal, Tuttofiori, TerComposti, Brescia, Italy) (3.5:3:3.5, w/w) to improve aeration and

drainage. The resulting soil mixture (Table S6) was homogenized and 1 kg of soil was placed in square pots (22 cm × 10.5 cm).

Subsequently, all pots were transferred to a climatic chamber with a cycle of 16 h light and 8 h dark, at a temperature of 15 °C during the light cycle and 25 °C during the night cycle. The experiment was conducted with 2 mycorrhizal ([+AMF] and [-AMF] for AMF-inoculated and non-inoculated plants, respectively) and 2 stress conditions ([+ STRESS] and [-STRESS] for well-watered and water-deficit plants, respectively). The experiments were arranged in a completely randomised design, with 4 seeds per pot and 9 replicates per condition ([+AMF + STRESS], [+AMF-STRESS], [-AMF + STRESS], and [-AMF-STRESS]), amounting to a total of 72 pots (36 for Iride and 36 for Ramirez). The plant seeds were subjected to mycorrhizal treatments before sowing. Seeds were inoculated with the commercial product, MICOSAT F® SEMI wp (CCS AOSTA Srl. Italy) with 2 a.m. fungal species (*Rhizoglomus irregulare* and *Funneliformis mosseae*) at a concentration of 460 sp/g (230 sp/g of each). Furthermore, the commercial product also had beneficial rhizosphere bacteria (1×10^7 CFU/g) and saprophytic fungi (*Trichoderma koningii*; 3×10^8 sp/g). One gram of the product (for each cultivar) was dissolved in 250 mL of sterilised water as per manufacturer's instructions, 108 seeds of each cultivar were tanned, and 3 seeds were sown into each pot. After 3 months of growth and regular watering (60% of field water holding capacity), watering was stopped in [+AMF + STRESS] and [-AMF + STRESS] only. Sampling was performed to detect the first mild phenotypic symptoms of water stress in some plants which occurred after the 15th day of irrigation interruption. All root samples from each pot were immediately frozen in liquid nitrogen and stored at −80 °C for downstream analysis. Root samples from 3 plants growing in each pot were pooled together as one sample. All root samples were subjected to mycorrhizal colonisation. The samples that did not show mycorrhizal colonisation under the [+AMF] treatment, and samples that showed mycorrhizal colonisation under [-AMF] conditions were discarded before further analysis. Thus, 3 individual replicates were selected for further metabolomic and mycorrhizal count analyses. The percentages of AMF root symbiosis were estimated using the gridline intersect method with a dissecting microscope (Wild, Leica, Milano, Italy) at 25x or 40x magnification, after clearing with 10% KOH and staining with 0.05% trypan blue in lactic acid (Giovannetti and Mosse, 1980).

5.2. Metabolomic analysis

Root tissues were crushed to a fine powder with a mortar and pestle and immediately stored at −80 °C for subsequent analysis. Two root aliquots (0.5 g) per sample (a total of 6 technical replicates per condition) were extracted in 5 mL of 0.1% formic acid in an aqueous solution of 80% methanol using Ultra-Turrax (Ika T-25, Staufen, Germany), as previously reported (Ganugi et al., 2021). The extracts were centrifuged (12,000×*g*) and the obtained supernatants were transferred into vials and stored at −20 °C until the analysis. The screening of root metabolites was carried out using liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry, with an electrospray ionisation source (UHPLC-ESI/QTOF-MS), according to a previously reported metabolomic analysis (Rocchetti et al., 2019). Briefly, reverse-phase chromatographic separation was performed using an Agilent pentafluorophenyl (PFP) column (2.0 × 100 mm, 3 μm) (Santa Clara, CA, USA) and a mobile phase of acetonitrile in water (6%–94% in 33 min) with a flow rate of 200 μL min^{-1}. The mass spectrometer was run in positive SCAN mode (range of 100–1000 m/z, 1 Hz, 30,000 FWHM). The Agilent Profinder B.07 software (Santa Clara, CA, USA) was then used to process the spectral data through the "find-by-formula" algorithm. Alignment was performed for both mass and retention time, and the compounds were annotated using the PlantCyc 12.6 database (Schläpfer et al., 2017) by means of the monoisotopic mass, isotope ratio, and isotope accurate spacing, according to Level 2 with reference to the COSMOS Metabolomics Standards Initiative (Salek et al., 2013). Only compounds present in 100% of the replicates within at least one condition were retained and analysed.

5.3. Statistical analysis and data interpretation

Metabolomic data analysis was performed using Agilent Mass Profiler Professional B.12.06 (Agilent Technologies) (Lucini et al., 2019). The abundance was Log2 transformed, normalised at the 75th percentile, and normalised to the median in the control. Based on fold change values, unsupervised hierarchical cluster analysis (HCA) was performed using the Ward agglomerative algorithm of the Euclidean distances to describe phytochemical patterns. Subsequently, the raw dataset was loaded into SIMCA 16 (Umetrics, Malmo, Sweden), pareto-scaled, and then elaborated in a supervised manner according to orthogonal projections to latent structures discriminant analysis (OPLS-DA). ANOVA of the cross-validated residuals (CV-ANOVA) ($p < 0.01$) and permutation testing ($N = 200$) were conducted to validate and exclude model overfitting. Subsequently, the goodness-of-fit R2Y and goodness-of-prediction Q2Y were also calculated. Based on the OPLS-DA model and adopting 95% and 99% confidence limits for the suspected and strong outliers, Hotelling's T2 distance was used to investigate the outliers. Metabolites with a variable importance in projection (VIP) score ≥1 were recorded. Volcano analysis ($p < 0.05$, Benjamini-Hochberg correction; fold change >1.2) was performed to identify the differential compounds, and the output was analysed using the Omic Viewer Pathway Tool of PlantCyc software (Stanford, CA, USA) to identify the related biochemical pathways (Paley et al., 2017). Finally, a Venn diagram was created using the online tool Venny 2.1, to identify the differential metabolites shared between Iride and Ramirez.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by the project "PIF AGRO: "GRANT SOILBIOFERT - Tecniche agronomiche e modelli di selezione per ottenere frumenti adatti alle condizioni pedoclimatiche della Toscana" CUP ARTEA 839335-finanziato dalla Regione Toscana, PSR 2014/2020. The activity at Università Cattolica del Sacro Cuore received financial support from the Emilia-Romagna region, grant 5202721 (project BettER-Bio, Programma Di Sviluppo Rurale 2014–2020, operazione 16.1.01 - year 2020-FA 3A).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2022.113381.

References

Akashi, K., Yoshimura, K., Nanasato, Y., Takahara, K., Munekage, Y., Yokota, A., 2008. Wild plant resources for studying molecular mechanisms of drought/strong light stress tolerance. Plant Biotechnol. 25, 257–263. https://doi.org/10.5511/plantbiotechnology.25.257.

Akiyama, K., Matsuoka, H., Hayashi, H., 2002. Isolation and identification of a phosphate deficiency-induced C-glycosylflavonoid that stimulates arbuscular mycorrhiza formation in melon roots. Mol. Plant Microbe Interact. 15, 334–340. https://doi.org/10.1094/MPMI.2002.15.4.334.

Augé, R.M., Toler, H.D., Saxton, A.M., 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered

conditions: a meta-analysis. Mycorrhiza 25, 13–24. https://doi.org/10.1007/s00572-014-0585-4.
Baslam, M., Esteban, R., García-Plazaola, J.I., Goicoechea, N., 2013. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. Appl. Microbiol. Biotechnol. 97, 3119–3128. https://doi.org/10.1007/s00253-012-4526-x.
Basu, S., Rabara, R.C., Negi, S., 2018. AMF: the future prospect for sustainable agriculture. Physiol. Mol. Plant Pathol. 102, 36–45. https://doi.org/10.1016/j.pmpp.2017.11.007.
Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., Zhang, L., 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front. Plant Sci. 10, 1–15. https://doi.org/10.3389/fpls.2019.01068.
Berger, F., Gutjahr, C., 2021. Factors affecting plant responsiveness to arbuscular mycorrhiza. Curr. Opin. Plant Biol. 59, 101994 https://doi.org/10.1016/j.pbi.2020.101994.
Bernardo, L., Morcia, C., Carletti, P., Ghizzoni, R., Badeck, F.W., Rizza, F., Lucini, L., Terzi, V., 2017. Proteomic insight into the mitigation of wheat root drought stress by arbuscular mycorrhizae. J. Proteonomics 169, 21–32. https://doi.org/10.1016/j.jprot.2017.03.024.
Bernardo, L., Carletti, P., Badeck, F.W., Rizza, F., Morcia, C., Ghizzoni, R., Rouphael, Y., Colla, G., Terzi, V., Lucini, L., 2019. Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. Plant Physiol. Biochem. 137, 203–212. https://doi.org/10.1016/j.plaphy.2019.02.007.
Briske, D.D., Camp, B.J., 1982. Water stress increases alkaloid concentrations in threadleaf groundsel (Senecio longilobus). Weed Sci. 30, 106–108. https://doi.org/10.1017/s0043174500026278.
Charlton, A.J., Donarski, J.A., Harrison, M., Jones, S.A., Godward, J., Oehlschlager, S., Arques, J.L., Ambrose, M., Chinoy, C., Mullineaux, P.M., Domoney, C., 2008. Responses of the pea (Pisum sativum L.) leaf metabolome to drought stress assessed by nuclear magnetic resonance spectroscopy. Metabolomics 4, 312–327. https://doi.org/10.1007/s11306-008-0128-0.
Colebrook, E.H., Thomas, S.G., Phillips, A.L., Hedden, P., 2014. The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. 217, 67–75. https://doi.org/10.1242/jeb.089938.
de la Rosa-Mera, C.J., Ferrera-Cerrato, R., Alarcón, A., de Jesús Sánchez-Colín, M., Muñoz-Muñiz, O.D., 2011. Arbuscular mycorrhizal fungi and potassium bicarbonate enhance the foliar content of the vinblastine alkaloid in Catharanthus roseus. Plant Soil 349, 367–376. https://doi.org/10.1007/s11104-011-0883-y.
De Vita, P., Avio, L., Sbrana, C., Laidò, G., Marone, D., Mastrangelo, A.M., Cattivelli, L., Giovannetti, M., 2018. Genetic markers associated to arbuscular mycorrhizal colonization in durum wheat. Sci. Rep. 8, 1–12. https://doi.org/10.1038/s41598-018-29020-6.
dos Santos Pereira, E., Vinholes, J., Franzon, R.C., Dalmazo, G., Vizzotto, M., Nora, L., 2018. Psidium cattleianum fruits: a review on its composition and bioactivity. Food Chem. 258, 95–103. https://doi.org/10.1016/j.foodchem.2018.03.024.
Eissenstat, D.M., Kucharski, J.M., Zadworny, M., Adams, T.S., Koide, R.T., 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol. 208, 114–124. https://doi.org/10.1111/nph.13451.
Farmer, M.J., Li, X., Feng, G., Zhao, B., Chatagnier, O., Gianinazzi, S., Gianinazzi-Pearson, V., van Tuinen, D., 2007. Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China. Appl. Soil Ecol. 35, 599–609. https://doi.org/10.1016/j.apsoil.2006.09.012.
Farooq, M., Hussain, M., Siddique, K.H.M., 2014. Drought stress in wheat during flowering and grain-filling periods. CRC Crit. Rev. Plant Sci. 33, 331–349. https://doi.org/10.1080/07352689.2014.875291.
Ganugi, P., Masoni, A., Pietramellara, G., Benedettelli, S., 2019. A review of studies from the last twenty years on plant–arbuscular mycorrhizal fungi associations and their uses for wheat crops. Agronomy 9. https://doi.org/10.3390/agronomy9120840.
Ganugi, P., Masoni, A., Sbrana, C., Dell'Acqua, M., Pietramellara, G., Benedettelli, S., Avio, L., 2021. Genetic variability assessment of 127 Triticum turgidum L. accessions for mycorrhizal susceptibility-related traits detection. Sci. Rep. 11, 1–11. https://doi.org/10.1038/s41598-021-92837-1.
Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.
Guo, X., Xin, Z., Yang, T., Ma, X., Zhang, Y., , Wang, Ren, Y., Lin, T., 2020. Metabolomics response for drought stress tolerance in Chinese wheat genotypes (Triticum aestivum). Plants 9, 1–15. https://doi.org/10.3390/plants9040520.
Harrison, M.J., Dixon, R.A., 1993. Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of Medicago truncatula. Mol. Plant Microbe Interact. 6, 643–654. https://doi.org/10.1094/MPMI-6-643.
Haubrick, L.L., Torsethaugen, G., Assmann, S.M., 2006. Effect of brassinolide, alone and in concert with abscisic acid, on control of stomatal aperture and potassium currents of Vicia faba guard cell protoplasts. Physiol. Plantarum 128, 134–143. https://doi.org/10.1111/j.1399-3054.2006.00708.x.
Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S., 1992. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. 70, 2032–2040. https://doi.org/10.1139/b92-253.
Hill, E.M., Robinson, L.A., Abdul-Sada, A., Vanbergen, A.J., Hodge, A., Hartley, S.E., 2018. Arbuscular mycorrhizal fungi and plant chemical defence: effects of colonisation on aboveground and belowground metabolomes. J. Chem. Ecol. 44, 198–208. https://doi.org/10.1007/s10886-017-0921-1.
Hou, Q., Ufer, G., Bartels, D., 2016. Lipid signalling in plant responses to abiotic stress. Plant Cell Environ. 39, 1029–1048. https://doi.org/10.1111/pce.12666.
Jager, C.E., Symons, G.M., Ross, J.J., Reid, J.B., 2008. Do brassinosteroids mediate the water stress response? Physiol. Plantarum 133, 417–425. https://doi.org/10.1111/j.1399-3054.2008.01057.x.
Jaleel, C.A., Manivannan, P., Kishorekumar, A., Sankar, B., Gopi, R., Somasundaram, R., Panneerselvam, R., 2007. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in Catharanthus roseus exposed to water deficit. Colloids Surfaces Biointerface 59, 150–157. https://doi.org/10.1016/j.colsurfb.2007.05.001.
Kagale, S., Divi, U.K., Krochko, J.E., Keller, W.A., Krishna, P., 2007. Brassinosteroid confers tolerance in Arabidopsis thaliana and Brassica napus to a range of abiotic stresses. Planta 225, 353–364. https://doi.org/10.1007/s00425-006-0361-6.
Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., Sharma, A., 2020. The impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production. Appl. Sci. 10 https://doi.org/10.3390/app10165692.
Kaur, L., Zhawar, V.K., 2015. Phenolic parameters under exogenous ABA, water stress, salt stress in two wheat cultivars varying in drought tolerance. Indian J. Plant Physiol. 20, 151–156. https://doi.org/10.1007/s40502-015-0156-5.
Krugman, T., Peleg, Z., Quansah, L., Chagué, V., Korol, A.B., Nevo, E., Saranga, Y., Fait, A., Chalhoub, B., Fahima, T., 2011. Alteration in expression of hormone-related genes in wild emmer wheat roots associated with drought adaptation mechanisms. Funct. Integr. Genomics 11, 565–583. https://doi.org/10.1007/s10142-011-0231-6.
Kusvuran, S., Dasgan, H.Y., Abak, K., 2013. Citrulline is an important biochemical indicator in tolerance to saline and drought stresses in melon. Sci. World J. https://doi.org/10.1155/2013/253414.
Latef, A.A.H.A., Hashem, A., Rasool, S., Abd-Allah, E.F., Alqarawi, A.A., Egamberdieva, D., Jan, S., Anjum, N.A., Ahmad, P., 2016. Arbuscular mycorrhizal symbiosis and abiotic stress in plants: a review. J. Plant Biol. 59, 407–426. https://doi.org/10.1007/s12374-016-0237-7.
Lucini, L., Colla, G., Miras Moreno, M.B., Bernardo, L., Cardarelli, M., Terzi, V., Bonini, P., Rouphael, Y., 2019. Inoculation of Rhizoglomus irregulare or Trichoderma atroviride differentially modulates metabolite profiling of wheat root exudates. Phytochemistry 157, 158–167. https://doi.org/10.1016/j.phytochem.2018.10.033.
Ma, D., Sun, D., Wang, C., Li, Y., Guo, T., 2014. Expression of flavonoid biosynthesis genes and accumulation of flavonoid in wheat leaves in response to drought stress. Plant Physiol. Biochem. 80, 60–66. https://doi.org/10.1016/j.plaphy.2014.03.024.
Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U., 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biol. Fertil. Soils 31, 150–156. https://doi.org/10.1007/s003740050638.
Marček, T., Hamow, K.Á., Végh, B., Janda, T., Darko, E., 2019. Metabolic response to drought in six winter wheat genotypes. PLoS One 14, 1–23. https://doi.org/10.1371/journal.pone.0212411.
Mierziak, J., Kostyn, K., Kulma, A., 2014. Flavonoids as important molecules of plant interactions with the environment. Molecules 19, 16240–16265. https://doi.org/10.3390/molecules191016240.
Munné-Bosch, S., Schwarz, K., Alegre, L., 1999. Enhanced formation of α-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiol. 121, 1047–1052. https://doi.org/10.1104/pp.121.3.1047.
Ortiz, N., Armada, E., Duque, E., Roldán, A., Azcón, R., 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. J. Plant Physiol. 174, 87–96. https://doi.org/10.1016/j.jplph.2014.08.019.
Paley, S., Parker, K., Spaulding, A., Tomb, J.F., O'Maille, P., Karp, P.D., 2017. The omics dashboard for interactive exploration of gene-expression data. Nucleic Acids Res. 45, 12113–12124. https://doi.org/10.1093/nar/gkx910.
Ponce, M.A., Scervino, J.M., Erra-Balsells, R., Ocampo, J.A., Godeas, A.M., 2004. Flavonoids from shoots and roots of Trifolium repens (white clover) grown in presence or absence of the arbuscular mycorrhizal fungus Glomus intraradices. Phytochemistry 65, 1925–1930. https://doi.org/10.1016/j.phytochem.2004.06.005.
Pozo, M.J., Jung, S.C., López-Ráez, J.A., Azcón-Aguilar, C., 2010. Impact of arbuscular mycorrhizal symbiosis on plant response to biotic stress: the role of plant defence mechanisms. In: Koltai, H., Kapulnik, Y. (Eds.), Arbuscular Mycorrhizas: Physiology and Function. Spinger, Dordrecht, pp. 193–207. https://doi.org/10.1007/978-90-481-9489-6.
Rampino, P., Pataleo, S., Gerardi, C., Mita, G., Perrotta, C., 2006. Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environ. 29, 2143–2152. https://doi.org/10.1111/j.1365-3040.2006.01588.x.
Rivero, J., Gamir, J., Aroca, R., Pozo, M.J., Flors, V., 2015. Metabolic transition in mycorrhizal tomato roots. Front. Microbiol. 6, 1–13. https://doi.org/10.3389/fmicb.2015.00598.
Rocchetti, G., Blasi, F., Montesano, D., Ghisoni, S., Marcotullio, M.C., Sabatini, S., Cossignani, S., Lucini, L., 2019. Impact of conventional/non-conventional extraction methods on the untargeted phenolic profile of Moringa oleifera leaves. Food Res. Int. 115, 319–327. https://doi.org/10.1016/j.foodres.2018.11.046.
Saia, S., Ruisi, P., Fileccia, V., Di Miceli, G., Amato, G., Martinelli, F., 2015. Metabolomics suggests that soil inoculation with arbuscular mycorrhizal fungi decreased free amino acid content in roots of durum wheat grown under N-limited, P-rich field conditions. PLoS One 10, 1–15. https://doi.org/10.1371/journal.pone.0129591.
Salek, R.M., Steinbeck, C., Viant, M.R., Goodacre, R., Dunn, W.B., 2013. The role of reporting standards for metabolite annotation and identification in metabolomic studies. GigaScience 2 (2), 13. https://doi.org/10.1186/2047-217X-2-13.
Sánchez-Rodríguez, E., Moreno, D.A., Ferreres, F., Rubio-Wilhelmi, M.D.M., Ruiz, J.M., 2011. Differential responses of five cherry tomato varieties to water stress: changes

on phenolic metabolites and related enzymes. Phytochemistry 72, 723–729. https://doi.org/10.1016/j.phytochem.2011.02.011.

Sawers, R.J.H., Svane, S.F., Quan, C., Grønlund, M., Wozniak, B., Gebreselassie, M.N., González-Muñoz, E., Chávez Montes, R.A., Baxter, I., Goudet, J., Jakobsen, I., Paszkowski, U., 2017. Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate transporters. New Phytol. 214, 632–643. https://doi.org/10.1111/nph.14403.

Schläpfer, P., Zhang, P., Wang, C., Kim, T., Banf, M., Chae, L., Dreher, K., Chavali, A.K., Nilo-Poyanco, R., Bernard, T., Kahn, D., Rhee, S.Y., 2017. Genome-wide prediction of metabolic enzymes, pathways, and gene clusters in plants. Plant Physiol. 173, 2041–2059. https://doi.org/10.1104/pp.16.01942.

Schliemann, W., Ammer, C., Strack, D., 2008. Metabolite profiling of mycorrhizal roots of Medicago truncatula. Phytochemistry 69, 112–146. https://doi.org/10.1016/j.phytochem.2007.06.032.

Shao, H.B., Chu, L.Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R., Shao, M.A., 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit. Rev. Biotechnol. 29, 131–151. https://doi.org/10.1080/07388550902869792.

Sharda, J.N., Koide, R.T., 2010. Exploring the role of root anatomy in P-mediated control of colonization by arbuscular mycorrhizal fungi. Botany 88, 165–173. https://doi.org/10.1139/B09-105.

Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M., 2012. Reactive oxygen species oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J. Bot., Le 2012, 1–26. https://doi.org/10.1155/2012/217037.

Shymanovich, T., Saari, S., Lovin, M.E., Jarmusch, A.K., Jarmusch, S.A., Musso, A.M., Charlton, N.D., Young, C.A., Cech, N.B., Faeth, S.H., 2015. Alkaloid variation among epichloid endophytes of sleepygrass (achnatherum robustum) and consequences for resistance to insect herbivores. J. Chem. Ecol. 41, 93–104. https://doi.org/10.1007/s10886-014-0534-x.

Singh, A.K., Hamel, C., DePauw, R.M., Knox, R.E., 2012. Genetic variability in arbuscular mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services and cropping systems in Canada. Can. J. Microbiol. 58, 293–302. https://doi.org/10.1139/W11-140.

Stallmann, J., Schweiger, R., Pons, C.A.A., Müller, C., 2020. Wheat growth, applied water use efficiency and flag leaf metabolome under continuous and pulsed deficit irrigation. Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-66812-1.

Subramanian, K.S., Santhanakrishnan, P., Balasubramanian, P., 2006. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci. Hortc. 107, 245–253. https://doi.org/10.1016/j.scienta.2005.07.006.

Szabó, B., Lakatos, Á., Koszegi, Botz, L., 2003. HPTLC and HPLC determination of alkaloids in poppies subjected to stress. J Planar Chromatogr 16, 293–297. https://doi.org/10.1556/JPC.16.2003.4.9.

Tadesse, W., Sanchez-Garcia, M., Thabet, A.S., Tawkaz, S., El Hanafi, S., Skaf, P., ElBaouchi, A., Eddakir, K., El-Shamaa, K., Assefa, S., Baum, M., 2020. Wheat Breeding Handbook at. ICARDA, Beirut, Lebanon.

Trépanier, M., Bécard, G., Moutoglis, P., Willemot, C., Gagné, S., Avis, T.J., Rioux, J.A., 2005. Dependence of arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. Appl. Environ. Microbiol. 71, 5341–5347. https://doi.org/10.1128/AEM.71.9.5341-5347.2005.

Turrini, A., Sbrana, C., Avio, L., Njeru, E.M., Bocci, G., Bàrberi, P., Giovannetti, M., 2016. Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol. Fertil. Soils 52, 643–653. https://doi.org/10.1007/s00374-016-1106-8.

Vaughan, M.M., Christensen, S., Schmelz, E.A., Huffaker, A., Mcauslane, H.J., Alborn, H.T., Romero, M., Allen, L.H., Teal, P.E.A., 2015. Accumulation of terpenoid phytoalexins in maize roots is associated with drought tolerance, Plant Cell. Environ. Times 38, 2195–2207. https://doi.org/10.1111/pce.12482.

Vigh, L., Huitema, H., Woltjes, J., van Hasselt, P.R., 1986. Drought stress-induced changes in the composition and physical state of phospholipids in wheat. Physiol. Plantarum 67, 92–96. https://doi.org/10.1111/j.1399-3054.1986.tb01268.x.

Villate, A., San Nicolas, M., Gallastegi, M., Aulas, P.A., Olivares, M., Usobiaga, A., Etxebarria, N., Aizpurua-Olaizola, O., 2021. Review: metabolomics as a prediction tool for plants performance under environmental stress. Plant Sci. 303, 110789 https://doi.org/10.1016/j.plantsci.2020.110789.

Wang, C., Yang, A., Yin, H., Zhang, J., 2008. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. J. Integr. Plant Biol. 50, 427–434. https://doi.org/10.1111/j.1774-7909.2008.00638.x.

Wang, Y., Zhang, X., Huang, G., Feng, F., Liu, X., Guo, R., Gu, F., Zhong, X., Mei, X., 2020. Dynamic changes in membrane lipid composition of leaves of winter wheat seedlings in response to PEG-induced water stress. BMC Plant Biol. 20, 84. https://doi.org/10.1186/s12870-020-2257-1.

Wei, Z., Li, J., 2016. Brassinosteroids regulate root growth, development, and symbiosis. Mol. Plant 9, 86–100. https://doi.org/10.1016/j.molp.2015.12.003.

Welling, M.T., Liu, L., Rose, T.J., Waters, D.L.E., Benkendorff, K., 2016. Arbuscular mycorrhizal fungi: effects on plant terpenoid accumulation. Plant Biol. 18, 552–562. https://doi.org/10.1111/plb.12408.

Witt, S., Galicia, L., Lisec, J., Cairns, J., Tiessen, A., Araus, J.L., Palacios-Rojas, N., Fernie, A.R., 2012. Metabolic and phenotypic responses of greenhouse-grown maize hybrids to experimentally controlled drought stress. Mol. Plant 5, 401–417. https://doi.org/10.1093/mp/ssr102.

Yu, Y., Yu, T., Wang, Y., Yan, X.F., 2010. Effect of inoculation time on camptothecin content in arbuscular mycorrhizal Camptotheca acuminate seedlings. Chin. J. Plant Ecol. 34, 687–694. https://doi.org/10.3773/j.issn.1005-264x.2010.06.008.

Yücel, C., Özkan, H., Ortaş, I., Yağbasanlar, T., 2009. Screening of wild emmer wheat accessions (*Triticum turgidum* subsp. *dicoccoides*) for mycorrhizal dependency. Turk. J. Agric. For. 33, 513–523. https://doi.org/10.3906/tar-0902-47.

Zhang, M., Barg, R., Yin, M., Gueta-Dahan, Y., Leikin-Frenkel, A., Salts, Y., Shabtai, S., Ben-Hayyim, G., 2005. Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. Plant J. 44, 361–371. https://doi.org/10.1111/j.1365-313X.2005.02536.x.

Zhu, Y.G., Smith, S.E., Barritt, A.R., Smith, F.A., 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. Plant Soil 237, 249–255. https://doi.org/10.1023/A:1013343811110.